

**Quantitative assessment of yield traits between family groups of  
the cultured abalone, *Haliotis midae*, during the process of canning**

by

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## Abstract

The species *Haliotis midae* is of great commercial value to the South African abalone industry and is mainly exported to Asian markets, specifically China. Up to 50% is sold as canned products with *H. midae* registering an average canning yield of approximately 35%. The species is presently genetically undomesticated and breeding programmes are being introduced to improve a range of production traits of which growth and yield is of primary importance.

The objective of the study was to determine genetic parameters such as heritability, genotypic and phenotypic correlations of yield-related traits to assess the potential genetic improvement through selective breeding. A series of yield-related parameters were identified that is of relevance to the standard abalone canning procedure.

Low to moderate heritabilities were recorded for most traits, including pre-shuck/live weight ( $0.20 \pm 0.06$ ), post-shuck weight ( $0.15 \pm 0.05$ ), post-gut weight ( $0.15 \pm 0.05$ ), post-brine weight ( $0.19 \pm 0.06$ ), pre-canning weight ( $0.19 \pm 0.06$ ), post-canning weight ( $0.21 \pm 0.06$ ), shell weight ( $0.16 \pm 0.05$ ), canning yield percentage ( $0.08 \pm 0.03$ ) and shell weight to post-gut weight ratio (SW: PGW) ( $0.09 \pm 0.04$ ). Weight related parameters are phenotypically highly correlated ( $0.86 \leq r \leq 0.99$ ) but show negative correlation with canning yield percentage ( $-0.38 \leq r \leq 0.04$ ). The nett yield of abalone shows a relatively strong positive correlation with the live weight ( $r = 0.66$ ). Shell length is highly heritable ( $h^2 \approx 0.48$ ) and show a strong positive correlation with live weight ( $r = 0.94$ ). Shell weight is also highly correlated with live weight ( $r = 0.80$ ) and the SW: PGW ratio does not show a significant correlate with live weight ( $r = 0.03$ ).

Weight-related traits show heritability values ranging from 0.15 to 0.20 that could allow a positive genetic response. Shell length (as a linear growth parameter) shows a high heritability ( $h^2 \approx 0.48$ ) and a strong positive correlation with live weight ( $r = 0.94$ ) which also makes it suitable for use as a selection criterion in breeding programmes for improved growth rate. Direct selection for canning yield is compromised by the destructive nature of measurement and the low heritability ( $h^2 < 0.10$ ). The negative correlations between yield as a percentage and growth traits ( $-0.38 \leq r \leq 0.04$ ) further complicate its use as a direct breeding objective. Although the canning yield as a percentage shows a decrease with an increase in live weight, the nett canning yield increases ( $r = 0.66$ ) with the live weight. It is therefore recommended to use shell length as a criterion for selection for increased growth rate and nett yield, thereby optimising profitability.

## Opsomming

Die spesie *Haliotis midae* is van groot kommersiële waarde tot die Suid-Afrikaanse perlemoenindustrie en word meestal uitgevoer na markte in Asië, spesifiek China. Tot 50% van die perlemoen wat in Suid-Afrika geproduseer en uitgevoer word, word verblik en huidiglik is die verblikingsopbrengspersentasie ongeveer 35%. *Haliotis midae* is tans geneties onderontwikkeld en die gebruik van teelprogramme word nou geïmplementeer met die doel om 'n verskeidenheid eienskappe te verbeter, waarvan groei en opbrengs van primêre belang is.

Die doelwit van die studie was om genetiese parameters soos oorerflikheid en ook die genotipiese en fenotipiese korrelasies van obrengsverwante eienskappe te bepaal om sodoende die potensiële genetiese verbetering as gevolg van selektiewe teeling te assesseer. 'n Reeks obrengsverwante eienskappe is geïdentifiseer wat relevant is binne bestaande en standaard kommersiële perlemoenverblikingsprotokolle.

Lae tot matige oorerflikheidswaardes is waargeneem en sluit in lewende, of voorontskulpingsgewig ( $0.20 \pm 0.06$ ), na-ontskulpingsgewig ( $0.15 \pm 0.05$ ), na-oopvlekkingsgewig ( $0.15 \pm 0.05$ ), na-pekelgewig ( $0.19 \pm 0.06$ ), voor-verblikingsgewig ( $0.19 \pm 0.06$ ), na-verblikingsgewig ( $0.21 \pm 0.06$ ), skulpgewig ( $0.16 \pm 0.05$ ), verblikingsopbrengspersentasie ( $0.08 \pm 0.03$ ) en 'n skulpgewig tot na-oopvlekkingsgewig verhouding (SW: PGW) ( $0.09 \pm 0.04$ ). Gewigsverwante parameters is fenotipies hoogs gekorreleer met mekaar ( $0.86 \leq r \leq 0.99$ ) maar toon 'n negatiewe korrelasie met die verblikingsopbrengspersentasie ( $-0.38 \leq r \leq 0.04$ ). Die netto opbrengs van perlemoen dui op 'n relatiewe sterk positiewe korrelasie met lewende gewig ( $r = 0.66$ ). Skulplengte is hoogs oorerflik ( $h^2 \approx 0.48$ ) en toon 'n sterk positiewe korrelasie met lewende gewig ( $r = 0.94$ ). Skulpgewig is ook hoogs gekorreleer met lewende gewig ( $r = 0.80$ ) en die SW: PGW verhouding toon geen beduidende korrelasie met lewende gewig nie ( $r = 0.03$ ).

Gewigsverwante eienskappe toon oorerflikheidswaardes wat varieer tussen 0.15 en 0.20 en kan 'n moontlike genetiese respons lewer. Skulplengte (as 'n liniêre groeiparameter) toon 'n hoë oorerflikheid ( $h^2 \approx 0.48$ ) en 'n sterk positiewe korrelasie met lewende gewig ( $r = 0.94$ ) wat dit gepas maak vir gebruik as 'n seleksiekriterium in 'n teelprogram met verbeterde groeitempo as doel. Direkte seleksie in terme van verblikingsopbrengs word ingeboet danksy die destruktiewe natuur van die metingsmetodiek asook 'n lae oorerflikheid ( $h^2 < 0.10$ ). Die negatiewe korrelasies tussen verblikingsopbrengs (uitgedruk as 'n persentasie) en groeieienskappe ( $-0.38 \leq r \leq 0.04$ ) dien as 'n verdere komplikasie in die gebruik van dié eienskap as direkte teeldeelwit. Alhoewel die verblikingsopbrengs 'n afname toon soos lewende gewig toeneem, is daar steeds 'n positiewe korrelasie tussen die netto verblikingsopbrengs en die lewende gewig ( $r = 0.66$ ). Dit word dus aangeraai om skulplengte as seleksiekriterium vir verbeterde groeitempo en netto opbrengs te gebruik om sodoende wins te maksimaliseer.

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“In quietness and trust is your strength.” Isaiah 30:15

# Contents

<b>Declaration</b> .....	<b>i</b>
<b>Abstract</b> .....	<b>ii</b>
<b>Opsomming</b> .....	<b>iii</b>
<b>Acknowledgements</b> .....	<b>iv</b>
<b>Contents</b> .....	<b>v</b>
<b>List of figures</b> .....	<b>vii</b>
<b>List of tables</b> .....	<b>viii</b>
<b>Abbreviations and Symbols</b> .....	<b>ix</b>
<b>1. Introduction</b> .....	<b>1</b>
1.1 <i>Overview of global fisheries and aquaculture industry</i> .....	1
1.2 <i>Global abalone industry</i> .....	2
1.3 <i>South African abalone industry</i> .....	3
1.3.1 <i>Problem statement and objectives</i> .....	5
1.4 <i>Summary</i> .....	6
<b>2 Literature Review</b> .....	<b>7</b>
2.1 <i>Introduction</i> .....	7
2.1.1 <i>The South African Abalone (<i>Haliotis midae</i>)</i> .....	7
2.1.2 <i>Taxonomy</i> .....	8
2.1.3 <i>Distribution and habitat</i> .....	8
2.1.4 <i>Diet (natural versus artificial feeding)</i> .....	9
2.1.5 <i>General Biology</i> .....	10
2.1.6 <i>Circulatory system</i> .....	12
2.1.7 <i>Immunology</i> .....	12
2.1.8 <i>Life cycle and Reproduction</i> .....	13
2.1.8.1 <i>Seasonality and its effect on chemical composition and yield of abalone meat</i> .....	14
2.1.8.2 <i>Artificial conditioning and spawning</i> .....	18
2.2 <i>Genetic improvement</i> .....	19
2.2.1 <i>Selective breeding</i> .....	19
2.2.2 <i>Heritability</i> .....	22
2.2.2.1 <i>Traits of economic value</i> .....	25
2.2.3 <i>Inbreeding, Heterosis and Hybridization</i> .....	27
2.3 <i>Canning effects</i> .....	30
2.4 <i>Summary</i> .....	35

<b>3</b>	<b>Materials and Methods</b> .....	<b>36</b>
3.1	<i>Brood stock collection</i> .....	36
3.2	<i>Location</i> .....	36
3.3	<i>Experimental design</i> .....	36
3.4	<i>Family structure</i> .....	36
3.5	<i>Canning trial</i> .....	39
3.5.1	Sampling.....	39
3.5.2	Tagging of abalone.....	39
3.5.2.1	Tagging methods.....	39
3.5.2.3	Identification of individual abalone .....	41
3.5.2.4	Transportation and purging .....	41
3.5.3	Canning protocol .....	41
3.5.4	Measurement of abalone.....	43
3.6	<i>Statistical analysis</i> .....	44
<b>4</b>	<b>Results and Discussion</b> .....	<b>46</b>
4.1	<i>Analysis of Variance (ANOVA)</i> .....	46
4.1.1	Effect of Location.....	46
4.1.2	Effect of Gender .....	48
4.1.4	Effect of Sires .....	49
4.1.5	Effect of Dams.....	49
4.1.6	Effect of Families .....	50
4.2	<i>Correlation studies</i> .....	54
4.2.1	Phenotypic correlation.....	54
4.2.2	Genotypic correlation .....	59
4.3	<i>Heritability estimation</i> .....	62
<b>5</b>	<b>Conclusion</b> .....	<b>64</b>
	<i>Recommendation for further work</i> .....	66
	<b>References</b> .....	<b>67</b>

## List of figures

<b>Figure 1:</b> Abalone in a: canned form; b: live fresh form; c: fresh frozen form, d: dried form (Photos: Arnold Vlok). .....	2
<b>Figure 2:</b> Percentage and value (Rand) contribution of each sub-sector to the total production value of South Africa's mariculture industry (Dept. of environmental affairs and tourism, 2009). .....	4
<b>Figure 3:</b> Location of farms along the natural distribution of <i>H. midae</i> around the South African coastline (Adapted from Troell <i>et al</i> , 2006). .....	9
<b>Figure 4:</b> External abalone features (No shell) (FAO, 2009). .....	11
<b>Figure 5:</b> Spawning seasons of different abalone species divided into three groups (Adapted from Shepherd & Laws, 1974). .....	16
<b>Figure 6:</b> From left to right: Cross-section of raw abalone muscle tissue, cross-section of cooked abalone muscle tissue (Adapted from Gao <i>et al</i> , 2001). .....	33
<b>Figure 7:</b> Display of the sampling method (Photo: Arnold Vlok). .....	39
<b>Figure 8:</b> Initial tagging method through respiratory hole of shell (Photo: Danie Brink). .....	40
<b>Figure 9:</b> Final tagging method through adductor foot muscle (Photo: Danie Brink). .....	40
<b>Figure 10:</b> Standard canning protocol where a: shucking and gutting of before brining process; b: removal of facial components and cleaning after brining and overnight refrigeration; c: tagged abalone ready to be canned; d: abalone are placed in cans containing a standard brine solution; e: cans are sealed mechanically; f: cans are placed in a pressure cooker to cook and sterilize for long term preservation (Photos: Danie Brink). .....	42
<b>Figure 11:</b> Measuring weight (g) of abalone at the post-gut stage (Photo: Danie Brink). .....	43
<b>Figure 12:</b> Gender determination of females (left) and males (right) (Photo: Danie Brink). .....	43
<b>Figure 13:</b> The live weight (g) of abalone in relation to canning yield (%) and nett yield (g) based on family averages. .....	53
<b>Figure 14:</b> The relationship between the family averages of the mean shell weight and the mean canning yield. ( $R^2$ = Coefficient of determination).....	55
<b>Figure 15:</b> The relationship between the family average of the mean live weight and the mean canning yield. ( $R^2$ = Coefficient of determination).....	56

## List of tables

<b>Table 1:</b> Indication of product specificity and use in different market regions. (Adapted from Oakes & Ponte, 1996).....	3
<b>Table 2:</b> Estimated supply volume and price structure for major abalone-producing countries (Adapted from Oakes & Ponte, 1996).....	4
<b>Table 3:</b> Taxonomic classification of the abalone species <i>Haliotis midae</i> (Adapted from WoRMS, 2012).....	8
<b>Table 4:</b> Comparison of periods of maximum growth of <i>H. laevigata</i> and <i>H. rubra</i> with periods of maximum food supply, gonad production and sea temperature at two different sites around the Australian coast (Adapted from Shepherd & Hearn, 1983). .....	18
<b>Table 5:</b> Heritability estimates of morphological traits at different growth stages from a variety of abalone species. ....	24
<b>Table 6:</b> Heritability components estimated for the dressing percentage of ectotherms (Adapted from Gjedrem, 1997). ....	26
<b>Table 7:</b> Carcass components of <i>H. midae</i> , before the canning process commences (Adapted from Sales <i>et al</i> , 1999). ....	31
<b>Table 8:</b> Family groups created from unique and shared parents, as full/half sib families with indication of location where animals were reared (Adapted from Van Schalkwyk, 2011). ....	37
<b>Table 9:</b> The number of families allocated to each of the four locations. ....	38
<b>Table 10:</b> Evaluation of the location effect on the means of shell weight, live weight and canning yield of full sib families. ....	47
<b>Table 11:</b> Evaluation of the effect of locations on the means of shell weight, live weight and canning yield. ....	48
<b>Table 12:</b> Means of live weight and canning yield of male and female abalone. ....	48
<b>Table 13:</b> Mean canning yield from offspring with different sires. ....	49
<b>Table 14:</b> Mean canning yield of offspring from different dams. ....	50
<b>Table 15:</b> Family averages of shell weight, live weight, shell weight to post-gut ratio and canning yield percentage. ....	51
<b>Table 16:</b> Phenotypic correlation between different canning stages or parameters measured during the canning process. ....	58
<b>Table 17:</b> Approximations of heritability values $\pm$ SE (on the diagonal) of, and genotypic correlation $\pm$ SE (above the diagonal) between the different canning stages or parameters that was measured during the canning trial. ....	61

## Abbreviations and Symbols

AMP	Adenosine monophosphate
ATP	Adenosine triphosphate
Ca	Calcium
R <sup>2</sup>	Coefficient of Determination
F	Coefficient of Inbreeding
CV	Coefficient of Variation (expressed as a percentage)
°C	Degrees Celsius
°C h <sup>-1</sup>	Degrees Celsius per hour
F <sub>1</sub>	First generation progeny
FAO	Food and Agricultural Organization
g	Grams
IUCN	International Union for Conservation of Nature
>	Larger than
LSD	Least Square Difference
Ltd.	Limited
m	Meters
mm	Millimeters
μ	Mu
pH	Negative logarithmic value of Hydrogen ion concentration
pp.	Pages
r	Pearson's correlation's coefficient
%	Percentage
p	Probability as a statistically significant limit
(Pty.) Ltd.	Property limited
QTL	Quantitative Trait Locus
®	Registered trademark
n	Sample size
<	Smaller than
TM	Trademark
US	United States
12L: 12D	12 hours light: 12 hours dark

## 1. Introduction

### 1.1 Overview of global fisheries and aquaculture industry

The fishing industry involves harvesting of fish (marine and freshwater), shellfish and other aquatic animals and is followed by processing, marketing, distribution and sales of products. Capture fishery includes the harvesting of a wide range of natural resources and is extremely diversified. The type of capture fishing depends on the species selection and availability, location, financial capacity, and access to the necessary equipment. It ranges from recreational fishing, culture-based fishing and small-scale or artisanal fishing to capital-intensive industrial fishing which makes use of large vessels with advanced navigational systems and high degrees of mechanization to allow maximum production capacity (FAO, 2012). Capture fisheries have however reached a maximum potential and many of the marine species have already been overexploited and could become endangered or extinct, if not already (Subasinghe *et al*, 2009). The most caught species include anchoveta, tuna, herring, pollock and mackerel (FAO, 2011) with more than 1400 species of fish that has been included on the Red list of endangered species by the International Union for Conservation of Nature (IUCN) (Pumphrey, 2012). One such species, *Thunnus maccoyii*, also known as the Southern Bluefin Tuna, is listed as being critically endangered with a population trend that is currently on the decrease (IUCN, 2012).

Capture fisheries and aquaculture supplied approximately 142 million tons of fish in 2008 of which 115 million tons were used for human consumption (FAO, 2010). Global levels of capture fishing have remained stable over the last decade, partly due to legislative action, with the aim of restoring overexploited fisheries. Aquaculture production has however showed a steady growth rate of 6.1% in the same period (FAO, 2011). This farming of marine or freshwater organisms in land- or ocean-based systems, is currently the fastest growing animal-food-producing sector in the world (FAO, 2010). Spread across all the world continents, aquaculture now produces 46% of the world's fish supply (FAO, 2010). China is the largest fish-producing country with a production of recorded 47.5 million tons in 2008, (FAO, 2010) supplying 62.5% of the fish, crustaceans and mollusc to global markets. India, Vietnam, Indonesia, Thailand and Bangladesh are also some of the top producers in aquaculture (FAO, 2010).

With an ever growing demand for human food production aquaculture could contribute substantially to meet future needs (Hew & Fletcher, 2001) and the industry now receives recognition for its potential to contribute to food security and economic growth in global terms (Subasinghe *et al*, 2009).

## 1.2 Global abalone industry

The international abalone industry is increasing its contribution to aquaculture production with an estimated 350 000 tons of abalones, winkles and conchs produced in 2009; this value translating to 672 million US dollars (FAO, 2011). Asia, and more specifically, China is the largest abalone producer in the world with a gross production of 4 500 tons per annum (Troell *et al*, 2006) from more than 300 abalone farms (Cook & Gordon, 2010). Other countries with a significant contribution to the global abalone supply include Japan, Korea, Thailand, United States, Mexico, Chile, Australia, New Zealand and South Africa (Cook & Gordon, 2010; Oakes & Ponte, 1996) with most of the abalone sold into the Asian countries China and Japan, the USA and Europe (Cook & Gordon, 2010). The different abalone species have very unique sensory properties (Chiou *et al*, 2004) and the preferred species, product form and quality depends on intended consumer destinations, preferences and cultural traditions (Oakes & Ponte, 1996). *Haliotis midae* specifically, is exported in different forms with unequal proportions; namely live export (35%), canned (50%), dried (10%) or fresh frozen (5%) (Figure 1).



**Figure 1:** Abalone in a: canned form; b: live fresh form; c: fresh frozen form, d: dried form (Photos: Arnold Vlok).

The market tends to differentiate based on the quality in terms of product size, texture and meat colour (Malcolm *et al*, 2008). In some markets, price and quality depends on the

species whereas brand names of the processed products determine the quality in other markets (Oakes & Ponte, 1996). South African canneries mainly target the Chinese market, as the largest consumer world-wide with a preference for canned products (Table 1) (Oakes & Ponte, 1996). Japan, on the other hand, is the largest consumer of live, fresh and frozen abalone. The fresh meat is used mainly for Japanese sushi and sashimi and premium-quality abalone is therefore used (Oakes & Ponte, 1996; Malcolm *et al*, 2008)

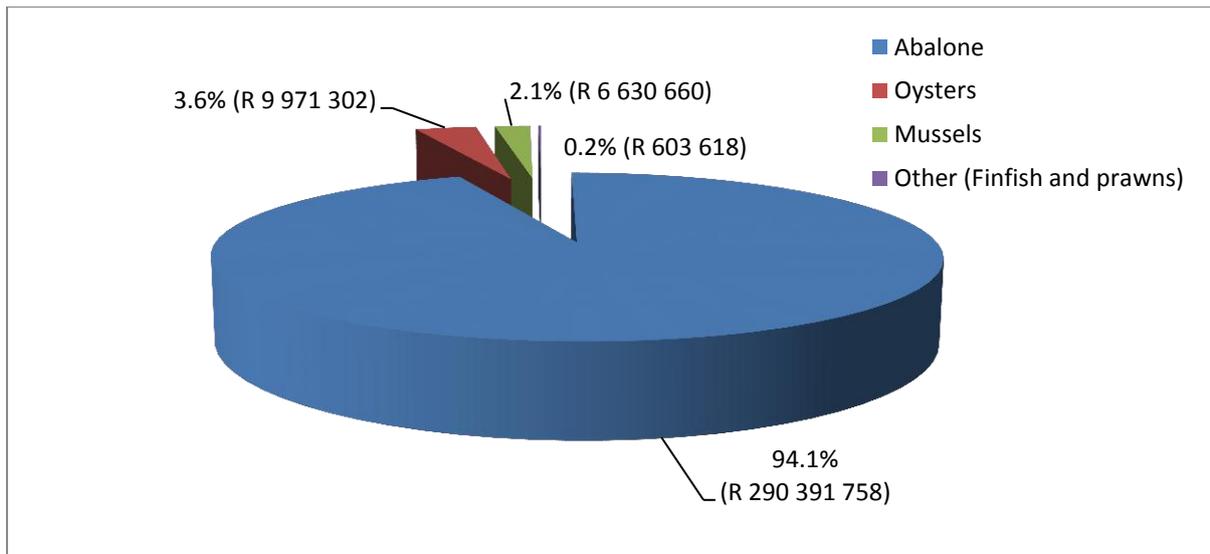
**Table 1:** Indication of product specificity and use in different market regions. (Adapted from Oakes & Ponte, 1996).

<b>Product form (Derived from <i>H. rufescens</i>)</b>	<b>Size of whole abalone (g)</b>	<b>Market region</b>	<b>Product use</b>
Live quality	100 - 110	Japan	Sushi, Sashimi
Live premium	85 - 100	USA, Hong Kong	Grilled, Steamed
Live petite	75 - 85	USA, Hong Kong	Traditional Asian cuisine
Premium fillet (fresh / frozen)	85 - 100	USA, France	Traditional USA restaurant cuisine
Processed whole foot (canned)	65 - 95	China, Hong Kong, Southeast Asia	

There is an increasing demand for abalone products and, specifically the species *Haliotis midae*, indigenous to South Africa, is very much a sought after product and therefore very valuable. South Africa has become one of the largest abalone producers outside of Asia which now results in an increased development of land-based abalone farms in the country (Cook & Gordon, 2010).

### **1.3 South African abalone industry**

Abalone farming started in the early 1990's and production has grown at a steady pace since then. South Africa accounts for 27% of Africa's mariculture production and is the main African producer of farmed abalone (Dept. of environmental affairs and tourism, 2009). It is the most valuable maricultural resource in South Africa with a 94.1% contribution, approximately R290.4 million generated towards the mariculture industry in 2008 (Figure 2).



**Figure 2:** Percentage and value (Rand) contribution of each sub-sector to the total production value of South Africa's mariculture industry (Dept. of environmental affairs and tourism, 2009).

From a total South African mariculture production of approximately 2014.62 tons, abalone production contributed 1037.11 tons in 2008 and clearly indicates the value of the industry (Dept. of environmental affairs and tourism, 2009). In terms of the international price structure, *H. midae* along with *Haliotis fulgens* (Table 2) is also one of most valuable abalone species when specifically processed as canned goods.

**Table 2:** Estimated supply volume and price structure for major abalone-producing countries (Adapted from Oakes & Ponte, 1996).

Country	Species	Annual supply (metric tons)	1993 price (US\$/metric ton)
Mexico	<i>H. fulgens</i> , <i>H. rufescens</i> , <i>H. cracherodii</i>	2 000	24 000
<b>South Africa</b>	<b><i>H. midae</i></b>	<b>600</b>	<b>25 000</b>
Australia	<i>H. laevigata</i> , <i>H. rubra</i> , <i>H. roei</i>	6 300	21 850
Hong Kong	<i>H. diversicolor supertexta</i> , <i>H. diversicolor diversicolor</i>	567	22 200
Japan	<i>H. discus hannai</i>	4 000	66 000
USA	<i>H. rufescens</i> , <i>H. fulgens</i> , <i>H. sorenseni</i>	350	25 000

There are currently 14 commercial farms which include 12 hatcheries and one experimental sea cage farm (Dept. of environmental affairs and tourism, 2009). The commercial farms are land-based and are equipped with pump ashore technology and recirculation culture systems (Shipton & Britz, 2007). Research has so far, mainly been focused on developing efficient production systems, artificial feeds and nutrition, controlling disease and optimizing reproduction (Elliott, 2000; Shipton & Britz, 2007). Abalone can spawn, and are reared successfully in captivity (Troell *et al*, 2006) and technology that is needed for the successful artificial spawning of the species has also been developed in recent years. Spat are reared in land-based hatcheries and grown out in tank systems where optimal conditions are provided (Shipton & Britz, 2007). These environmental factors have received abundant attention but the additive genetic components that determine the performance potential of abalone must also be investigated to improve traits with economic value.

### **1.3.1 Problem statement and objectives**

South Africa currently has a successful and growing abalone industry although the farmed indigenous species, *Haliotis midae* is still genetically undomesticated. Brood stock used by farms have so far been obtained from wild populations and considered as unimproved genotypes (Brink, 2005). *H. midae* carries high economic value and successful development and domestication of the specific species within the industry can ensure sustainability of economic viability and maintain global competitiveness - genetic improvement within the species, is therefore vital (Brink, 2005). Profitability is currently dependent on live weight of fresh abalone. Processed products are sold with a fixed weight (g/can) but, abalone with increased canning yields will result in an increased number of cans filled and sealed which allows increased profitability.

Currently, 50% of the total abalone production on farms is processed, canned and exported to be sold into Asian markets. The industry average for canning yield of the *H. midae* species is, at present, around 35%.

A national breeding program was initiated in 2006 and focuses on the improvement of growth rate of abalone. An improved growth rate increases production in terms of increased turnover rate and results in a decreased growth period to reach the preferred market size. Selection based on growth rate can increase live weight of abalone but a heavier/larger abalone does not necessarily increase the canning yield which means that the current breeding strategy could be less than ideal.

An investigation to identify biological traits of commercial value, specifically with regard to the canning industry, is necessary to enable adjustments within the breeding program based on specific genetic parameters.

Genetic parameters should include genotypic and phenotypic variance, correlation studies and heritability estimations of quantitative traits with possible economic value to the industry and will enable identification and development of strains and genotypes with superior genetic characteristics to increase the production efficiency of *H. midae*.

Improved utilization of possible additive genetic variance and increased genetic response through possible adjustments to the breeding strategy could enable a substantial improvement in canning yield or yield-related trait performances and will be of great financial benefit to commercial producers of abalone.

The satisfaction of the above mentioned objectives will evaluate the degree to which the rate of genetic gain in the breeding programme can be maximised (Franchini *et al*, 2011). Improved productivity, profitability and sustainability of the South African abalone industry can then be insured. An industry with financial strength and future stability can also contribute to providing sustained economic growth, job creation and foreign trade benefits in South Africa (Brink, 2005).

#### **1.4 Summary**

As a result of an increasing demand for human food production aquaculture has expanded globally with a steady growth rate. The species, *H. midae*, has proven to be of great economic value and competes well within global markets. The South African abalone industry aims to target the largest consumer of abalone, China, which has shown to have a preference for canned products. Half of South Africa's abalone produce is canned and the genetic domestication of *H. midae*, possibly specifically aimed at increasing the canning yield, is necessary to ensure economic sustainability. Establishment of successful breeding programmes can enable this and a canning trial was therefore initiated to identify possible yield-related traits of economic value that can affect the canning yield of abalone in commercial production systems. Selection of these traits is based on genetic parameters that aim to describe the effect of the traits on the canning yield accurately and can then be included in a breeding strategy which allows a positive genetic response and improves production performance. A breeding programme with specific breeding goals to increase the canning yield of canned abalone could be beneficial to the South African abalone industry by increasing profitability and ensuring sustainability.

## 2 Literature Review

### 2.1 Introduction

The literature review of the species *Haliotis midae* will provide a general description in terms of its taxonomic classification, natural distribution and habitat. These factors determine the species' diet which influences aspects such as growth rate, feed conversion efficiency and yield. The general appearance of abalone is described to gain a better understanding of the species anatomy with regard to growth rate and post-canning yield. Knowledge of the abalone life cycle, initially developing from spat into juveniles to finally become sexually mature animals are also important to allow maximal reproductive manipulation, specific to the species. Successful artificial conditioning and spawning are also only possible if reproductive cycles are well understood. The effects of seasonality on different species' play a significant role and can allow artificial manipulation of the reproduction cycle to ensure optimal supply of offspring with maximum growth rates and canning yields throughout the year. An understanding as to how abalone respond to physiological stressors such as temperature, salinity, dehydration and osmotic stress and the effect of these factors on the yield of abalone during the canning process is vital and a brief overview of the cardiovascular and immunological systems will therefore also be discussed.

Different approaches to genetic improvement such as selective breeding, inbreeding, heterosis and hybridization will be reviewed to determine whether the utilization of these selection methods could be valuable to enable a change in canning yield. The particular stages of the canning process will be presented to highlight the physiological effects that they have on *H. midae*. These effects influence the final yield of canned products and can either be adapted to reduce environmental variance or abalone can be manipulated genetically through selection to handle these stressors more efficiently. Knowledge of the physiological effects that the canning process induces on abalone could assist with the identification of potential quantitative traits that can be included in a selection programme to increase the canning yield, production efficiency and profitability of commercial farms.

#### 2.1.1 The South African Abalone (*Haliotis midae*)

South Africa is home to five indigenous Haliotid species (*Haliotis midae*, *Haliotis parva*, *Haliotis spadicea*, *Haliotis queketti* and *Haliotis alfredensis*). *Haliotis midae* is the only species with commercial significance (Sales & Britz, 2001) and this is due to an abundance in large populations around the South African coast (Franchini *et al*, 2011). In their natural environment *H. midae* can grow up to a maximum shell length of 200 mm but this occurs over a time period of approximately 20 years (Sales & Britz, 2001).

### 2.1.2 Taxonomy

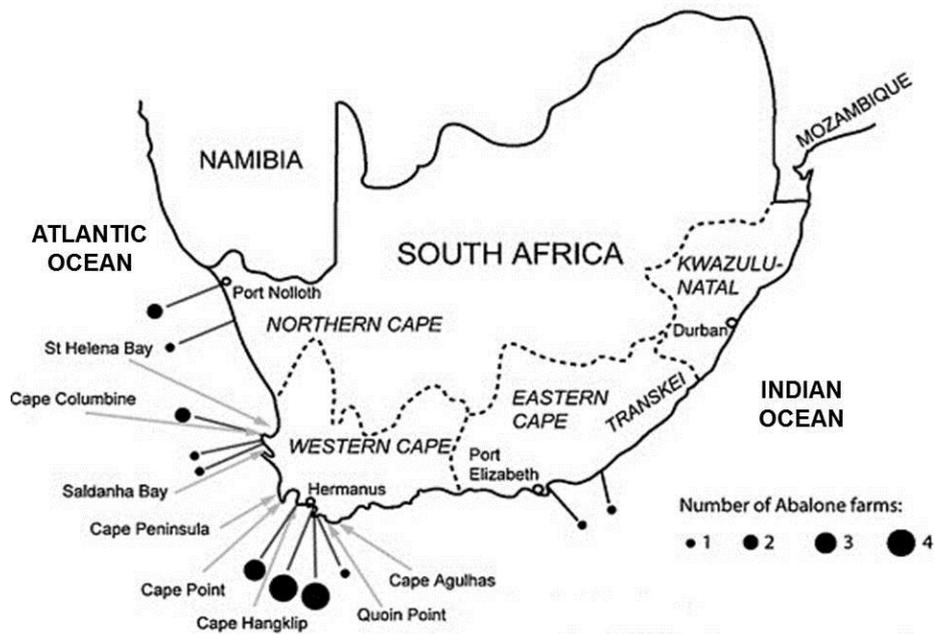
Also known as *Haliotids*, abalone are part of the phylum Mollusca and can be classified as *Gastropoda*. They share the subclass *Prosobranchia* with limpets and snails and belong to the family of Haliotidae (Table 3) (Schoonbee, 2007).

**Table 3:** Taxonomic classification of the abalone species *Haliotis midae* (Adapted from WoRMS, 2012).

<b>Kingdom</b>	Animalia
<b>Phylum</b>	Mollusca
<b>Class</b>	Gastropoda
<b>Superfamily</b>	Haliotoidea
<b>Family</b>	Haliotidae
<b>Genus</b>	<i>Haliotis</i>
<b>Species</b>	<i>midae</i>

### 2.1.3 Distribution and habitat

*Haliotis midae* has a natural distribution that reaches from Port St John (Indian Ocean) to Port Nolloth (Atlantic Ocean) (Sales & Britz, 2001). Figure 3 depicts this by indicating locations of all the commercial farms along the South African coast to illustrate the species distribution.



**Figure 3:** Location of farms along the natural distribution of *H. midae* around the South African coastline (Adapted from Troell *et al*, 2006).

Optimum water depth and optimal temperature are species dependent and both factors vary dependent on location. Abalone can be found at depths of up to 30 meters, (Kilburn & Rippey, 1982) but maximum population densities do however occur at depths between 3-10 meters due to abundance of natural food (FAO, 2009). The optimal temperature range for *H. midae* is between 12 - 20°C. Temperatures reaching outside this range have negative effects on growth rates by affecting the food conversion efficiency (Sales & Britz, 2001).

#### 2.1.4 Diet (natural versus artificial feeding)

*Haliotis midae* is a generalist, opportunistic, slow-feeding, herbivorous species (Sales & Britz, 2001; Troell *et al*, 2006) with a nocturnal feeding pattern (Day & Cook, 1995) and therefore relatively inactive during daytime hours. Abalone species feed on a variety of algae but *H. midae* appears to prefer kelp *Ecklonia maxima* (Sales & Britz, 2001). Juveniles prefer microalgae and diatoms which are found on the surfaces they adhere to (Elliott, 2000).

The necessity, under commercial conditions to ensure that feed is of optimal quality to enable maximal growth is vital. Good growth rates enable abalone to reach the appropriate preferred market size within an economically viable time frame (Schoonbee, 2007). It takes *H. midae* approximately 4 years to reach a market size of  $\pm 90$  mm (Troell *et al*, 2006) and an average shell length of 100 mm over five years is also ideal for commercial production (Sales & Britz, 2001).

Only 5% of energy obtained from feed contributes towards reproduction and growth, the rest is spent on excretion and respiration (Schoonbee, 2007). The animals prefer fresh kelp but it has been shown to contain a low protein content of 15% with an unbalanced amino acid profile (Troell *et al*, 2006). Feed-alternatives that exist are seaweed-based pellets, natural fresh kelp and formulated feeds such as Abfeed<sup>TM</sup> (Troell *et al*, 2006). Protein is important to support a gain in meat weight (instead of excessive shell growth) and kelp alone cannot provide sufficient protein to sustain this, so the general practice on farms is to feed a mixed diet of pellets, Abfeed<sup>TM</sup> and fresh kelp. This has shown to produce the highest growth rates (Troell *et al*, 2006).

### 2.1.5 General Biology

The word mollusc is derived from Latin and means 'soft' which describes the tissue structures found inside the protective shell of abalone (Kilburn & Rippey, 1982). The body of abalone consists of four regions; namely the head, the mantle complex, the adductor muscle or foot and the vital viscera/systems such as the digestive tract, circulatory, reproductive and respiratory systems (Elliott, 2000) (Figure 4).

Abalone are univalved (Elliott, 2000) invertebrates and have flattened calcified shells that provide necessary form, structure and protection (Heaseman & Savva, 2007). The shell is ear-shaped and consists of three layers, namely the cuticle, the horny layer and the pearl layer. The cuticle (periostracum) is the most outer layer and comprises of a very thin layer of tanned protein known as concholin (Kilburn & Rippey, 1982). The horny layer is composed of calcium carbonate embedded in concholin whereas the pearl (nacreous) layer has chemical components which are similar to that of the horny layer but it is also exposed to secretions from the mantle. Horizontal plates of aragonite produce the sparkling lustre that is associated with mother-of-pearl when light is refracted by this layer (Kilburn & Rippey, 1982). Shell growth occurs as a result of mantle secretion of calcium and concholin and the activity causes concentric lines, known as growth lines, observed on the edge of the shell. The shell has a row of respiratory pores (*tremata*) (FAO, 2009) and as growth occurs, older respiratory pores and fractures close as the necessary calcium is secreted (FAO, 2009, Kilburn & Rippey, 1982). Various pigments are produced by mantle cells to allow a variety of colour patterns on the shell (Kilburn & Rippey, 1982). The shell can grow up to a certain length after which it only thickens still (Bevelander, 1988).

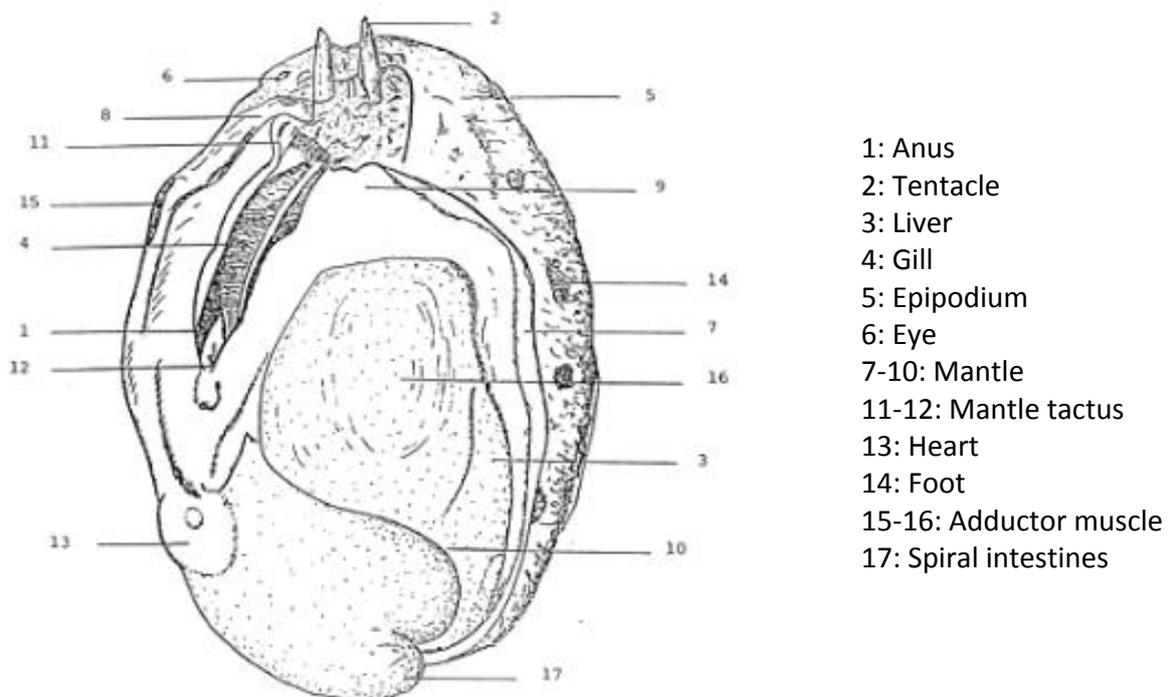
The foot consists of muscular tissue and contains a blood cavity which functions as a hydrostatic skeleton and enables protrusion, retraction, expansion or contraction (Kilburn and Rippey, 1982). It is a well-developed structure with a large surface to volume ratio which

enables it to adhere to surfaces very effectively. The epipodes occupy the lower part of the foot with branch-shaped tentacles which are typically found at the ends (FAO, 2009).

The head lies in front of the foot and is bilaterally symmetrical. It has a mouth, tentacles and eyes at the end of eye stalks (Heaseman & Savva, 2007). The mouth has a radula, a long rasping tongue (Heaseman & Savva, 2007) which allows it to tear algae into 1-2 mm fragments which can then be digested more easily (Day & Cook, 1995).

The mantle complex is made up of the mantle itself and some underlying viscera (Kilburn & Rippey, 1982). A chamber forms and contains the gills, the reproductive and the excretory organs. A constant through-current is maintained by cilia which beat to circulate fresh water through the chamber and assists to remove excrement and allows gaseous exchange (Kilburn & Rippey, 1982).

A hump-like mass is situated above the foot and the viscera are contained within. Internal reproductive organs, digestive organs and the heart can be found here (Kilburn & Rippey, 1982). Blood is generally colourless but can show a slight tinge of blue due to the respiratory pigment, haemocyanin, a copper-containing protein assisting in oxygen transportation (Kilburn & Rippey, 1982).



**Figure 4:** External abalone features (No shell) (FAO, 2009).

### 2.1.6 Circulatory system

The circulatory system of molluscs is defined as an open system which implies that it lacks well defined capillaries and therefore rather percolates through a system of sinuses and lacunae. This makes it very inefficient compared to the closed system of mammalian animals. The heart consists of two auricles and a single ventricle which allows circulation through the rest of the body via the gills and the kidneys. Haemolymph returning from the body circulates through the right kidney after which it is oxygenated in the ctenidia to finally enter the auricles. The single ventricle finally pumps the haemolymph via two vessels, the pallial artery and the aortic bulb, to supply the whole body with oxygenated haemolymph via arterial sinuses (Bourne *et al*, 1990). There is no definite distinction between the blood and the interstitial fluid. This results in circulatory fluid of gastropods being known as haemolymph.

The heart plays an integral part in urine formation. Contractions of the heart chambers create pressure that allows ultra-filtration of haemolymph through the pericardium. The filtrate is then altered by the right kidney which has a secretory function, and reabsorption of glucose occurs via the left kidney (Harrison, 1962). The heart and kidneys play no further role in the maintenance of water balance and remaining filtrate is excreted as urine (Vosloo & Vosloo, 2006). As dehydration occurs, urine production ceases (Vosloo & Vosloo, 2006) and has no further effect on weight loss as a result of fluid loss. There is thus no expected effect on the canning yield.

### 2.1.7 Immunology

Elements of immunity ensure survival and are detectable in almost all living organisms. Gastropods do however only rely on their innate immunity for host survival, with no adaptive immunity to their disposal (Ellis *et al*, 2011; Roch, 1999). The invertebrate immune response consists of cellular and humoral components and each of these components have afferent and efferent arms (Ellis *et al*, 2011). The predominant mechanism of internal defence is phagocytosis but release of cytotoxins is also used alongside the initiation of an inflammatory response. Competent cells are recruited throughout the body and a wide variety of molecules exist to defend against foreign material, pathogenic or non-pathogenic, as well as self-modified cells (Roch, 1999).

The immune system of gastropods is very sensitive to environmental perturbation. Factors such as sensitivity to seasonal variation, temperature, salinity, seawater pH, air exposure, hypoxia, and exposure to differing concentrations of ammonia and nitrite have all shown to affect the efficiency of immunity significantly. Other stressors such as mechanical disturbances, pollution via contaminants and metals have also been identified. The effect is

a lowered immune response with a depressed effect on phagocytosis and related bacteria-clearing adaptations and affects the overall physiological status of the animals which results in disease outbreaks and an increased mortality rate is observed (Ellis *et al*, 2011; Hooper *et al*, 2007; Malham *et al*, 2003).

The response to stress is ultimately to divert energy away from non-essential processes (e.g. reproduction and growth) towards bio-energetic processes such as mobilization of energy substrates and increased oxygen uptake to ease adaptation to the specific threat (Ellis *et al*, 2011; Malham *et al*, 2003). Long-term diversion of energy due to sustained stress-causing biotic and abiotic conditions, especially under artificially created environmental conditions, could affect growth rate to produce abalone of sub-standard market sizes with a decreased canning yield. The aim, from a commercial perspective, is therefore to prevent this diversion of energy at all costs and so doing optimizing growth and/or reproduction which ultimately maximize efficiency and profitability. A thorough understanding of molluscan immunology is necessary to allow identification and exploration of all the stressors that affect it. Optimal management of environmental stressors can then be implemented to ensure energetic efficiency of metabolic processes from a physiological and immunological perspective.

### **2.1.8 Life cycle and Reproduction**

Abalone are dioecious animals (FAO, 2009). Male and female individuals are therefore needed to reproduce. Gonads are visible when the muscle foot is deflected away from the shell. Mature male gonads (testes) have a creamy appearance and female gonads (ovaries) are and olive green colour originating from the developing oocytes found inside (Heaseman & Savva, 2007). They are broadcast spawners and spat is released through respiratory pores, into the surrounding seawater, after which fertilization can take place (Heaseman & Savva, 2007). They are very fecund and females can produce between 3 and 4 million eggs from one spawning (Vorster, 2003).

Eggs that are fertilized hatch into first-stage swimming larvae, known as trochophores. Trochophores are microscopic, barrel-shaped larvae with cilia belts around their middle and tufts of cilia on top and below their bodies (Kilburn & Rippey, 1982). They develop into shelled veliger larvae after 20 hours of growth. The larvae then continue to develop and grow to settle and transform into post-larvae, otherwise known as early juveniles. This stage is reached after a period of approximately six days. During the settlement stage larvae suffer heavy mortalities but the juveniles that survive continue to develop, progressively adapting to an adult diet (Heaseman & Savva, 2007). As the abalone grow beyond the length of 5 mm,

nocturnal behaviour develops and abalone begin to emerge to feed only at night (Heaseman & Savva, 2007).

Wild adult *H. midae* start to show sexual maturity at the age of approximately seven years. This age is however shortened to three years on the warmer, eastern coast of South Africa, and also when the abalone are reared under cultured conditions (Sales & Britz, 2001). Other examples of abalone species where sexual maturity is reached at approximately three years, are found in Southern Australia and include the species *Haliotis laevigata*, *Haliotis rubra* and *Haliotis scalaris*. It does however appear that the animals attain sexual maturity but only show substantial spawning volumes after another year of growth (Shepherd & Laws, 1974).

Capinpin Jr. *et al*, (1998) established that sexual maturity can also be reached much earlier when reared under hatchery conditions. Hatchery-reared male and female abalone reached sexual maturity at the smaller shell length of respectively 35.0 mm and 35.9 mm, whereas wild-caught males and females only reached sexual maturity at the same length of 40.6 mm. The difference in shell length is almost insignificantly small, but equates to a significant age difference. This trend could however be due to the provision of a constant food supply in hatcheries which will result in abalone with better body condition and therefore a potential for better growth rates.

#### **2.1.8.1 Seasonality and its effect on chemical composition and yield of abalone meat**

Extensive research has been done on seasonality of reproduction in abalone species and temperature appears to be the most important factor that affects abalone reproduction. Sudden temperature fluctuation acts as stimulus for spawning and also plays a part in the gonadal maturation (Shepherd & Laws, 1974). Tropical regions have water temperatures that show less seasonal variation which result in abalone having longer lasting spawning periods. *Haliotis asinina*, a tropical abalone species spawn throughout the year except during the summer months of May and June, which appears to be a resting stage (Capinpin Jr. *et al*, 1998). A spawning peak is however observed in October when the water temperatures are lower in the northern hemisphere (Capinpin Jr. *et al*, 1998). Temperate abalone, on the other hand, have definite annual spawning seasons; as few as once or twice a year and this is, once again due to definite seasonal variation (Capinpin Jr. *et al*, 1998). *Haliotis midae* is a temperate species and spawns twice annually in their natural habitat, during spring and autumn (Sales & Britz, 2001). Similarly to *Haliotis rubra*, it does however show variability in timing and duration of spawning season in different locations (Shepherd & Laws, 1974) and this could be due to varying abiotic environments such as the difference in sea temperature around the South African coast. The species of *Haliotis* can be divided into three different groups based on their spawning patterns (Figure 5). The three groups include

abalone that spawn during summer months, abalone that spawn in all months excluding the summer months and lastly abalone that spawn all year round.

Research shows that the effect of seasons has a significant effect on the chemical composition of all the different tissues of abalone. A study was done in Japan where summers occur during June, July and August. Hatae *et al*, (1995) harvested adductor muscle samples from *H. discus* abalone in the months of February, April, June, July, August, October, November and December with the aim of determining the chemical composition in the meat, and the effect of a change in chemical composition on the textural properties of abalone.

Adenosine triphosphate (ATP) and ATP-related compounds showed an increase during summer and a decrease during winter. This is due to the enzymatic activity of deaminase and adenosine-deaminase decreasing which causes an accumulation of AMP concentration. A significant ( $p < 0.05$ ) increase in protein content, specifically amino acids Arginine, Taurine, Glycine, Glutamine and Glutamic acid were also detected during summer months. Adenosine monophosphate (AMP) works via synergism with Glutamic acid to stimulate the “umami” taste and this is assumed to be the reason why the preferred season to eat fresh abalone in Japan is summer.

			SPRING			SUMMER			AUTUMN			WINTER		
Month (northern):			M	A	M	J	J	A	S	O	N	D	J	F
Month (southern):			S	O	N	D	J	F	M	A	M	J	J	A
1	<i>H. cyclobates</i>	Tipara Reef	_____			_____								
	<i>H. laevigata</i>	Tipara Reef	_____			_____								
		West I.	_____			_____			_____			_____		
	<i>H. pustulata</i>	Gulf of Suez				_____								
	<i>H. corrugata</i>	Baja California				_____			_____					
	<i>H. cracherodii</i>	Santa Monica				_____			_____					
	<i>H. fulgens</i>	Baja California				_____			_____					
2	<i>H. ruber</i>	West I.	_____			_____			_____			_____		
		Tipara Reef	_____			_____			_____			_____		
		Beachport, S. Aust	_____			_____			_____			_____		
		Tasmania	_____			_____			_____			_____		
		Jervis Bay, NSW	_____			_____			_____			_____		
	<i>H. discus hannai</i>	Miyage, Japan				_____			_____			_____		
		Southern Japan				_____			_____			_____		
		Southern Japan				_____			_____			_____		
	<i>H. midae</i>	Stony Point, S. Africa	_____			_____			_____			_____		
		Sea Point, S. Africa	_____			_____			_____			_____		
	Dassen I, S. Africa	_____			_____			_____			_____			
<i>H. australis</i>	Kaikoura N.Z.	_____			_____			_____			_____			
3	<i>H. roei</i>	West I.	_____			_____			_____			_____		
	<i>H. scalaris</i>	Tipara Reef	_____			_____			_____			_____		
	<i>H. rufescens</i>	California	_____			_____			_____			_____		

**Figure 5:** Spawning seasons of different abalone species divided into three groups (Adapted from Shepherd & Laws, 1974).

*Haliotis discus hannai* abalone spawn naturally in November and December. Carbohydrate concentration is the highest in July and August and lowest in October - just before spawning occurs (Hatae *et al*, 1995). This trend can be explained by the fact that gonadal growth and development is needed to allow successful spawning – proteins and carbohydrates will therefore accumulate in advance and are catabolised prior to spawning to allow the maintenance of this rapid growth period. Fish and shellfish are known to have a high lipid content and use the lipids as an energy source. It was however found that abalone do not follow this trend and has a low lipid content of only 0.4%. Glycogen concentration in abalone decreases before spawning occurs which implies that abalone has an alternative energy source to lipids when energy is needed for gonadal growth (Hatae *et al*, 1995).

Hatae *et al*, (1995) also found a decrease in collagen content during summer months with an expected increase during winter months. Fibre networks within the muscle are less compacted during summer time which improves the texture by making the meat tender. The hypothesis exists that collagen can be important in energy storage and can have an effect on muscle metabolism prior to spawning to support gonadal growth.

These chemical alterations should affect the canning yield. During the period of gonadal growth before spawning, more energy will be invested in reproductive growth so the expected effect would be that the canning yield would decrease during this time of the year. The resting phase, more easily observed in temperate species, would allow the most growth of non-essential tissues such as muscle and would therefore produce the largest canning yield. This assumption could however be opposed by the research that Shepherd & Laws, (1974) did to investigate the effect of seasonal variation on gonadal size. They calculated a gonadal index to measure the relationship between body size and gonad size under the assumptions that a spent gonad will be smaller than a ripe gonad and also that the size of the gonad will be proportional to the size of the abalone itself. Results showed that the gonadal index decreased during spawning and increased once again as the gonad started to develop. There is however no way of knowing whether the increase in gonadal index is due to a significant increase in gonadal size or whether the index only reflects an actual body size decrease or dystrophy which would still show a proportional increase in gonadal size as a result of the seasonal variation. The increase in gonadal index reflects the change in gonadal size compared to the body size. Body size could therefore still be increasing with a constant growth rate during months of spawning and canning yield would not be affected by gonadal development.

During the canning process, gonads are removed and the canning yield would remain unaffected if a constant growth rate of meat was maintained. Shepherd & Hearn, (1983) did however show that certain factors affect growth rate of abalone. The effect of seasonality is included and affects growth rate by channelling energy into gonadal development and away from growth. Results in Table 4 shows that periods of maximum growth rate do not coincide well with months when gonadal development occurs and growth rate is therefore lower during this period. The period of gonadal production does however coincide well with periods of maximum food supply showing that the species naturally only spend energy to reproduce when their own survival in terms of food shortages isn't at stake. Seasonal gonadal development therefore affects the growth rate of abalone in their natural environment which will certainly affect the canning yield negatively over a constant time period.

**Table 4:** Comparison of periods of maximum growth of *H. laevigata* and *H. rubra* with periods of maximum food supply, gonad production and sea temperature at two different sites around the Australian coast (Adapted from Shepherd & Hearn, 1983).

Species and site	Period of max growth	Period of max food supply	Period of gonad production
<i>H. laevigata</i>			
West Island	Aug - Feb	June - Dec	Apr - Sept
Tipara Reef	May - Nov	May - Sept	Apr - Sept
<i>H. rubra</i>			
West Island	Aug - Jan	June - Jan	Apr - Sept
Tipara Reef	July - Dec	June - Sept Dec - Feb	July - Mar

Abalone that are part of the normal production system are still subjected to seasonal variation and the canning yield will be affected. The severity to which it affects production is as of yet unknown. Using species with discreet seasonal reproductive behaviour could therefore be beneficial, as gonadal growth would only occur during specific months and the negative influence on canning yield will be minimalized to a few months in a year. On-land abalone farms only select a few breeding pairs which are exposed to artificial conditioning and making use of artificial conditioning to allow spawning throughout the year, could reduce seasonal variation and allow an increase in commercial production by reproducing more regularly. Fecundity and reproductive efficiency can however decrease as biorhythms are artificially removed.

#### **2.1.8.2 Artificial conditioning and spawning**

It has been found that *H. midae* show an unwillingness to spawn when caught from the wild, even when in a ripe condition. Hatcheries have therefore developed artificial methods to condition the abalone to allow successful spawning (Sales & Britz, 2001).

Artificial conditioning in hatcheries allows regular spawning and this is done by closely regulating the physio-chemical environment of the brood stock abalone (Grubert & Ritar, 2005). Factors that are of great importance include regulation of temperature (16 - 18°C), high levels of dissolved oxygen in the water, low levels of nitrites and ammonia and a pH ranging between 7.5 - 8.5 (Grubert & Ritar, 2005). A high-quality diet (formulated feed) is

also vital and may exceed the necessary daily intake (Grubert & Ritar, 2005). An artificial photoperiod is maintained (12L: 12D) with light intensities of 90-100 lux measured at the bottom of the tanks when filled with seawater (Grubert & Ritar, 2005). Spawning is induced by simultaneous exposure to ultra-violet irradiated seawater and a temperature increase of 4°C at a rate of 1°C h<sup>-1</sup> (Grubert & Ritar, 2005). Close regulation of these factors optimizes gonad growth and development (Grubert & Ritar, 2005). Temperature is the main factor that affects gonad development and it has been found that spawning rates are higher at 18°C but significantly more gametes are produced at 16°C (Grubert & Ritar, 2005). The temperature can therefore be adjusted according to the needs of the breeder.

## **2.2 Genetic improvement**

A sustainable and cost effective way to increase production is to focus on genetic improvement which increases production of cultured abalone through the manipulation of the genetic variance present in any specific species (Elliott, 2000). The utilization of genetics has allowed great improvement in domestic animal production during the late 20<sup>th</sup> century (Goyard *et al*, 2008). The use of genetics via the implementation of breeding programs in the aquaculture industry is a new development and the benefits could still be more than initially expected (Goyard *et al*, 2008). Production of cultured abalone can be increased by enlarging farm size, improving management practices and marketing strategies, altering nutrition or lastly, as mentioned, by exploiting the genetic material of the species. Applied breeding programs can reduce the production costs under differing marketing and management systems and it is important to determine what the associated cost reduction with a positive genetic response in terms of specific traits of importance can be (Dickerson, 1973). Production gains ranging from 5 - 10% per generation have been recorded with the implementation of genetic improvement programmes and reports of 50 - 100% increase in the growth rate of *Haliotis rufescens* show that genetic improvement is economically viable if such results are obtained (Elliot, 2000). Several breeding methods or strategies have been applied, developed and tested in aquaculture. These methods allow determination of the rate of genetic improvement of species-specific traits and accentuate the potential to be economically efficient and viable – with the benefit of progressive success and development in aquaculture. Such methods include selective breeding, inbreeding and crossbreeding (heterosis), hybridization and genome manipulation (Vandeputte, 2003).

### **2.2.1 Selective breeding**

Selection with the aim of genetic improvement occurs when animals with phenotypic superiority are chosen to be used as future parents to improve traits that are of economic value to the industry (Vorster, 2003). Directional selection is applied and the normal

distribution within the population for a specific trait is disrupted. To improve the production efficiency genetically, traits of economic value should be identified and evaluated to establish potential for a viable genetic response, attainable via breeding programs. Measurement of genetic variation of traits of value within populations, and heritability calculation of these traits enable prediction of genetic response rate and identification of effective breeding strategies (Kube *et al*, 2007). Short generation interval and high selection intensities also allow an optimal genetic response but these parameters are dependent on the biological species that are being researched (Robinson *et al*, 2010). Different selection methods exist to ensure that breeding can commence with the most genetic improvement, specific to a species (Lymberg *et al*, 2000).

Breeding selectively aims to increase homozygosity amongst genes of desirable traits (Wada & Jerry, 2008) and allows uniformity in expression throughout a population and fixation of genes. There is however a risk of reducing genetic diversity within populations when selective breeding programmes are applied to increase homozygosity without proper management. Populations face losing the capacity to adapt to the ever-changing environments that they are exposed due to a loss in phenotypic variation (Katsuhiko & Dean, 2008). Loss of genetic diversity is normally prevalent in aquaculture populations that are based on small stock founder numbers which have been in a closed breeding system for several generations (Katsuhiko & Dean, 2008). This can be due to the accelerated genetic drift that occurs in these smaller populations (Machado-Schiaffino *et al*, 2007). The occurrence of genetic bottleneck effects also appears when too few mating pairs are used which decreases the effective population size and causes genetic erosion (Machado-Schiaffino *et al*, 2007). Unbalanced sex ratios also influence loss of genetic variation (Machado-Schiaffino *et al*, 2007). This can be avoided with proper management which prevents inbreeding and a decrease in fitness characteristics as a response to the inbreeding depression (Katsuhiko & Dean, 2008).

The aquaculture industry generally lags behind the livestock animal industry in term of utilizing well-designed breeding programmes to improve commercial production (Gjedrem *et al*, 2012). Despite relatively high heritabilities for economically valuable traits, a typically high fecundity and short generation intervals, aquaculture species have not benefited sufficiently from the recent modern developments in animal breeding (Elliot, 2000; Gjedrem *et al*, 2012; Lymberg *et al*, 2000). There are however many breeding programs that are improving production successfully amongst fish and shellfish species such as carp, Atlantic salmon, shrimp, sea bream, tilapia, catfish and oysters, mussels and abalone and papers show that the average production gain per generation ranges between 5 – 10% (Elliott, 2000; Gjedrem

*et al*, 2012; Goyard *et al*, 2008; Robinson *et al*, 2010; Vandeputte, 2003; Van Schalkwyk, 2011).

Growth rate is normally the trait that attracts the most attention in selection programmes. Faster growing animals reduces production time and also expenses (Elliott, 2000). Hara & Kikuchi, (1992) showed that third generation mass selection of *H. discus hannai* with shell lengths of 20-30 mm resulted in an increase of 21% in daily growth rate. An even larger 65% increase was observed in animals that had larger shells (30-70 mm). Kawahara *et al*, (1997) reported a 10 - 15% increase in growth rate per generation through selection using the same abalone species. Their research also revealed a significant correlation between juvenile (13 months) shell length and shell length and whole body weight at 84 months. This correlation enables selection of abalone during juvenile stages before full growth potential has been established.

In New South Wales, Australia a breeding programme to improve the growth rate of the Sydney rock oyster, *Saccostrea glomerata*, began as a response to a 40% decline in production rates. The initial aims were to improve growth rates by reducing the time to reach market size by one month per generation without compromising meat yield. An increase in growth rate, or an average weight for age advantage of 18% was achieved after only two generations of selection. This equated to reducing the growth period by three months, which exceeded the initial breeding goals with a significant improvement in production rates (Nell *et al*, 2000).

Another study was done by Langdon *et al*, (2003) to identify top performing families within three lines of the cultures Pacific oyster, *Crassostrea gigas*. Selection was applied based on live weight and yield of meat when harvested. Best performing oysters were used as brood stock within the three lines to create a full sib family structure. Non-selected families were also created to act as controls. The progeny that arose from crossing the selected parental families showed a positive response to selection for higher yield and the improvement ranged from 0.04 - 25.5%. Offspring from control families showed statistically significant lower yields.

There are however many different quantitative (polygenic) traits of economic value, and include body length and weight, shape, food conversion efficiency, resistance against diseases, dropsy and hypoxia, cortisol stress response, gonad weight, fat content and even percentage deformed larvae (Elliott, 2000; Vandeputte, 2003). The common carp, *Cyprinus carpio*, is one of the most cultured aquaculture species and many different selective breeding programmes have been implemented to improve its commercial production. Literature reports that selection for disease and cold resistance show positive genetic

responses whereas selection based on growth rate in carp isn't as successful. The cold tolerant Amur wild carp and a fast growing Galician carp was crossbred to create a fast growing, cold tolerant carp known as the Ropsha carp. Many inter-crosses and backcrosses were done to create a cold resistant carp which was then mass selected for growth rate over five generations. An approximate improvement in cold tolerance was made which resulted in a 30 – 40% increase in winter survival (Vandeputte, 2003). Another breeding program that was implemented in Russia selected for a dropsy-resistant strain in Krasnodor carp (Kirpichnikov *et al*, 1993). Fish that possessed the strain were mass selected with an intensity of 30 - 35% over nine generations. Mass selection also occurred for growth rate and improvement was reported to be successful but absolute growth rate figures could not be given (Kirpichnikov *et al*, 1993). Disease resistance and cold resistance are therefore preferred as selection traits when breeding with carp as the selection response based on growth rate seems to be inconsistent (Vandeputte, 2003).

The genetic response that these examples showed to selection acts as compelling evidence to prove that genetically superior strains can be developed when directional selection is applied. It is however important to realise that the outcome of genetic selection will always be dependent on the species, the specific trait of interest and its ability to respond to selection in terms of the amount of additive variance that is present within the sample population. It is therefore important to take the heritability of canning yield, as a quantitative indication of the selection response into account when selection strategies are revised to improve production. There are, as of yet, no literature presented that can report possible heritability values for canning yield. Other yield-related traits such as length, width and weight parameters (Section 2.2.2., Table 5) have however been researched and can be indicative of the magnitude to which a selection response in canning yield will be possible.

### **2.2.2 Heritability**

Genetic improvement is only possible if genetic variation for a specific trait between individuals within a population is sufficient (Vorster, 2003). Phenotypic variance is normally recorded in quantitative studies and is determined by environmental and genetic components of variation (Vorster, 2003). Genetic variance consists of additive and non-additive components of which the additive variation is heritable and is used to calculate heritability. The non-additive components of genetic variance include interactive variance and variance due to dominance (Vorster, 2003). Heritability reflects the proportion of observed phenotypic variance that is explained by additive variance (Falconer & Mackay, 1996). It depends on the magnitude of all the different components of variance and these are affected by gene frequencies which can differ from population to population – it is

therefore important to know that heritability differs accordingly (Falconer & Mackay, 1996). It is difficult to estimate heritability but it plays an important predictive role by expressing the reliability of recorded phenotypic values as a guide to their breeding values which determines the individual's genetic influence in the following generation (Falconer & Mackay, 1996). Higher heritability values indicate a greater parent-offspring resemblance and allow performance prediction of offspring based on the performance of parents (Vorster, 2003) which is of great value to breeders during the selection process. Traits that are closely related to reproductive fitness generally have lower heritability whereas traits that are not related to reproductive fitness have higher heritability (Falconer & Mackay, 1996). A study by Mousseau & Roff, (1987) compared heritability between endotherms and ectotherms comparing four categories of characteristics. The aim was to determine whether lower heritability values are closely related to or associated with fitness characteristics or not. The categories included behavioural, life history, morphological or physiological traits and it was found that life history traits, which included fecundity, viability, survival and developmental rate, had the lowest heritability values and those are the traits that affect fitness directly. Morphological traits, which included body size measurements, had the highest heritability values and were more loosely associated with fitness related traits. Table 5 shows heritability values of different morphological traits which tend to reflect moderate to higher heritability.

The tabularised heritability estimates (Table 5) of some morphological traits show that research has been done to establish the extent to which these traits could improve production when included as part of a selection programme. The heritability of the specific traits seems to increase as the abalone age while the accuracy of selection and genetic improvement is increased with higher heritability ( $h^2$ ) values. This trend therefore indicates that it will be better to apply selection when abalone are older. The study done by Li *et al*, (2005) does however suggest otherwise. It could be explained by the possibility that various, and different genes are expressed during different stages of growth. The low heritabilities of shell weight and length reported by Li *et al*, (2005) could therefore be an indication of the selection response of a completely different set of quantitative trait loci (QTL's) when compared to earlier growth stages, and the low  $h^2$  values must be acceptable for the specific traits at those ages (Adeleke *et al*, 2011). Kube *et al*, (2007) reported a heritability value of 0.04 for shell length at a 38 month period (approximately 3.2 years) which falls within the range that was given by Li *et al*, (2005) and provides further evidence that the decrease in heritability, and deviance from the above mentioned trend could be due to the expression and measurement of different active QTL's during that growth stage.

**Table 5:** Heritability estimates of morphological traits at different growth stages from a variety of abalone species.

<b>Genetic parameter</b>	<b>Abalone Species</b>	<b>Source</b>	<b>h<sup>2</sup> Estimate</b>
<b>Shell length</b>			
10, 20 and 30 days	<i>H. discus hannai</i>	Deng <i>et al</i> , 2007	0.23 - 0.26
420 days	<i>H. diversicolor</i>	You <i>et al</i> , 2010	0.23
12 months	<i>H. asinina</i>	Lucas <i>et al</i> , 2006	0.48
18 months	<i>H. refuscens</i>	Jonasson <i>et al</i> , 1999	0.27
24 months	<i>H. refuscens</i>	Jonasson <i>et al</i> , 1999	0.34
38 months	<i>H. laevigata</i>	Kube <i>et al</i> , 2007	<b>0.04</b>
1,2,3 and 4 years	<i>H. rubra</i>	Li <i>et al</i> , 2005	<b>0.07 - 0.02</b>
<b>Shell width</b>			
10, 20 and 30 days	<i>H. discus hannai</i>	Deng <i>et al</i> , 2007	0.21 - 0.32
420 days	<i>H. diversicolor</i>	You <i>et al</i> , 2010	0.25
<b>Total weight</b>			
420 days	<i>H. diversicolor</i>	You <i>et al</i> , 2010	0.29
12 months	<i>H. asinina</i>	Lucas <i>et al</i> , 2006	0.36
38 months	<i>H. laevigata</i>	Kube <i>et al</i> , 2007	0.10
<b>Meat weight</b>			
38 months	<i>H. laevigata</i>	Kube <i>et al</i> , 2007	0.10
<b>Shell weight</b>			
38 months	<i>H. laevigata</i>	Kube <i>et al</i> , 2007	0.16
1,2,3 and 4 years	<i>H. rubra</i>	Li <i>et al</i> , 2005	0.09 - 0.01

Another observation made from Table 5 shows that the heritability values of linear body measurements such as length and width are higher compared to heritabilities of traits that measured weight. This shows that variation still remains, even within the previously mentioned group, morphological traits. Canning yield or dressing percentage is determined through weight measurement. As stated, heritability of weight-related traits are lower than those of linear traits and decreases the possible selection response and genetic improvement that can be gained from selection for specific weight traits to increase canning yield. Other traits could however be used to increase canning yield via indirect selection

under the condition that they show strong positive correlation with weight. The mass of volume at a constant density is a function of weight which depends on the length, width and height of an object. The prediction that linear measurements such as length and width, could correlate well with weight would therefore be a valid hypothesis and could reap more success in a selection program due to higher heritability values. Other traits of economic value, independent of a correlation with weight, could also be used as selection criteria to increase canning yield.

### **2.2.2.1 Traits of economic value**

The main quantitative traits that receive the most attention are growth rate, live weight, shell length and shell width but as these improve, breeders realise that other traits are also important and can also be economically beneficial and important to include as part of a selection index. Such traits include higher survival rates, disease resistance, more efficient food conversion efficiency or even highly sought-after market traits such as colour, taste or tenderness of meat (Elliott, 2000; Lafarga-de la Cruz *et al*, 2012).

Growth rate is easily measurable and reduces production costs by allowing animals to reach market-size much faster (Gjedrem, 1983; Hayes *et al*, 2007). Most of the available research that has been done on abalone species was to estimate growth rate by measuring related growth traits such as shell width and length- and body weight. Heritability estimates of 0.30 and 0.36 were calculated for growth rate in different abalone species (Jonasson *et al*, 1999; Lucas *et al*, 2006) and these moderate to high estimates should allow a successful selection response. There is a strong genetic correlation between growth rate and food conversion ratio in livestock. Fish and shell fish are however poikilothermic and therefore utilize less energy specifically for maintenance. The genetic variation for a trait such as the basic metabolic rate of fish or shellfish could be low and indicate that a selection response is unlikely (Gjedrem, 1983). Under artificial rearing conditions undomesticated marine animals experience continuous stress which would raise their energy requirements to fulfil basic maintenance needs and could increase food conversion efficiency. Interestingly enough it has been reported that rainbow trout grow faster with an increased growth rate accompanied by a proportional increase in food consumption, but no change in food conversion ratio is detected (Gjedrem, 1983). This observation supports the hypothesis that a lack of genetic variation in basic metabolic rates reduces the possibility of a positive selection response. The heritability of the young trout that were studied by Gjedrem, (1983) was high ( $0.31 \pm 0.11$ ) with a low coefficient of variation (CV) of 6%.

Kinghorn, (1983) also quantified the heritability of food conversion efficiency ( $0.03 \pm 0.10$  and a CV of 8.3%) in rainbow trout and concluded that selection to improve the food

conversion efficiency will be of little economic value. Henryon *et al*, (1999) estimated the heritability of food conversion efficiency with the aim to evaluate whether the inclusion of the trait in a marron breeding program would be of commercial value. Heritability values of 0.05, 0.03, and 0.05 were determined from brood stock, juvenile and mature marron populations respectfully. They concluded that, whilst food conversion efficiency is important, the growth rate of the marron tail specifically is, economically, up to 7900 times more valuable. The low heritability values would also not contribute sufficiently to allow a positive selection response. These reports show that even though a genetic correlation between growth rate and food conversion efficiency exists, a guaranteed success with artificial selection isn't always possible.

There are currently no heritability estimates in the literature to determine whether canning yield of abalone should be included as part of the selection criteria to improve production. As part of the canning process all viscera are removed. The brining process also causes loss of haemolymph and consequently extreme weight loss follows. Canning yield is the same as dressing percentage which is a term normally used in livestock studies. Heritability of dressing percentages for similar traits can give an indication as to what the canning yield could be. Utrera & van Vleck, (2004) reviewed carcass traits, specifically dressing percentages of cattle in general and found that dressing percentage had an average estimated heritability of 0.36. This estimation is moderate to high and suggests that a selection response is possible.

Ectotherms have proved to have lower heritability values compared to endotherms (Mousseau & Roff, 1987). This suggests that heritability of dressing percentages in endotherms could be used as an indication of the canning yield of abalone but it will be an overestimation (Mousseau & Roff, 1987). Table 6 show large variation in ectotherm heritability estimates but they are lower compared to reported endothermic heritability values as expected, according to Mousseau & Roff, (1987).

**Table 6:** Heritability components estimated for the dressing percentage of ectotherms (Adapted from Gjedrem, 1997).

Species	Source	h <sup>2</sup> Estimates	
		h <sup>2</sup> <sub>S</sub>	h <sup>2</sup> <sub>D</sub>
Rainbow trout	Gjerde & Gjedrem, 1984	0.01	0.14
	Gjerde & Schaeffer, 1989	0.34	0.42
Atlantic salmon	Gjerde & Gjedrem, 1984	0.03	0.02
	Rye & Gjerde, 1996	0.20	0.18

S = sire component of variance, D = dam component of variance

### 2.2.3 Inbreeding, Heterosis and Hybridization

Inbreeding occurs when mating of individuals that share the same ancestry takes place (Falconer & Mackay, 1996). The consequence of sharing a common ancestor is an increased chance of carrying replicate alleles of ancestral genes in the offspring. Alleles that are identical by descent are known as autozygous alleles and produces homozygous allele pairs which causes inbreeding. The coefficient of inbreeding,  $F$ , is the measure of inbreeding and is known as the probability that any two genes at any locus are identical by descent (Falconer & Mackay, 1996). Two hypotheses exist that might explain the effect of inbreeding. The hypothesis of overdominance states that the heterozygous genotype at certain loci claims superiority to either homozygous genotypes and does this independently of deleterious recessive mutations in the homozygotes (Comings & MacMurray, 2000; Charlesworth & Charlesworth, 1999; Dickerson, 1973). Research indicates that overdominant loci have a much larger effect on population variance compared to the alternative hypothesis of dominance (Crow, 1952) which states that inbreeding depression is caused by recessive alleles or those that are partially deleterious recessive (Charlesworth & Charlesworth, 1999). Detrimental recessive alleles that are therefore brought into the hybrid zygote from one parent are rendered ineffective by dominant alleles from the other parent (Crow, 1948). Inbreeding depression causes a reduction in the mean phenotypic value of various fitness components (Falconer & Mackay, 1996) by reducing heterozygosity and also by allowing random drift in gene frequencies due to factors such as a small population size of brood stock (Dickerson, 1973). Reduction in heterozygosity increases the probability of deleterious recessive alleles being expressed (Nakadate *et al*, 2003) and produces fixation of genes that are not necessarily favourable (Falconer & Mackay, 1996). There is however the alternative, where favourable genes are fixed in a homozygous state by chance, which will be beneficial despite the presence of an inbreeding depression.

Hybrid vigour, also known as heterosis, is on the other hand, the opposite of inbreeding depression (Falconer & Mackay, 1996; Nakadate *et al*, 2003) where the progeny of crosses show an increase in mean phenotypic value for certain traits (Falconer & Mackay, 1996). Increased expression or number of genes with favourable effects and increased frequency of heterozygosity relative to the parental brood stock can be obtained through positive heterosis (Dickerson, 1973). The outcome is however dependent on the effectiveness of natural or artificial selection that is applied to create the specific lines within breeds, (Dickerson, 1973) genetically diverged populations (Nakadate *et al*, 2003) or different breeds of the same species. Progeny are generally considered to have a combination of the best traits from the parental strains (Comings & MacMurray, 2000) which improves overall fitness

by increasing reproductive capacity, physiological efficiency (Falconer & Mackay, 1996) and other traits such as disease resistance (Comings & MacMurray, 2000).

Intra-specific hybridization, also known as crossbreeding with different populations or specific lines created within the same species are used within commercial breeding programs to exploit the effects of intentional inbreeding and heterosis (Vorster, 2003). This phenomenon allows reshuffling of alleles in the next generation without altering gene frequencies and can be used as a short-term selection strategy to improve the phenotypic performance of traits with low heritability (Vorster, 2003). Crosses between genetically different populations reduce genetic load by increasing heterozygosity and restores fitness characteristics via heterosis (You *et al*, 2009). Effects of inbreeding are removed immediately and a significant difference; whether positive or negative, is already visible in the F<sub>1</sub> progeny (Vorster, 2003). You *et al*, (2009) found that by crossbreeding three different populations of *Haliotis diversicolor* reciprocally, significant improvement in growth rate and survival rates were achieved which made a substantial difference to the mortality rate that was previously spiralling out of control due to a loss in genetic variance within the studied populations.

Using genetically different domesticated brood stocks to produce intra-specific hybrids with no inbreeding effects and the presence of heterosis can thus be used as an alternative to selectively breeding within one composite population to increase allelic variability with the maintenance of related fitness traits and improved production (Goyard *et al*, 2008). Goyard *et al*, (2008) showed that biomass production is 1.4 times higher when F<sub>1</sub> hybrid populations of the Pacific blue shrimp *Penaeus (Litopenaeus) stylirostris* were created instead of using pure bred populations. Growth rates were also 37% faster with better survival rates during poor environmental and sanitary conditions.

With the effects of intra-specific hybridization in mind, effects of inter-specific hybridization should also be evaluated. Hybridization is being used as a breeding method in many fish and shellfish to improve and manipulate production characteristics such as survival rates, growth rates, sex ratios and disease resistance and allows this by facilitating adaptation to new environmental culture conditions (Lafarga-de la Cruz *et al*, 2012). Inbreeding effects such as larval deformity and low survival rates could also be reduced (Lafarga-de la Cruz *et al*, 2012). Research reveals that genetic improvement have been successfully brought about by introducing non-native genetic material through hybridization by moving species to troubled aquaculture areas that are in need of genetic revitalization (Guo, 2009). Despite the potential benefits of using hybridization as a standard breeding strategy, general trends have not been established. Different hybrid crosses exhibit different heterosis effects (Guo, 2009)

and only two interspecies hybrids are currently commercially produced (Lafarga-de la Cruz *et al*, 2010). The black abalone hybrid is a cross between *Haliotis discus hannai* and *Haliotis discus discus* and the Australian hybrid, the tiger abalone, is a cross between *Haliotis laevigata* and *Haliotis rubra* (Guo, 2009; Lafarga-de la Cruz *et al*, 2010). The tiger abalone shows an increased growth rate with a combination of various favourable traits from both species which makes it an improved species choice for commercial farmers to use (Guo, 2009). Additional hybrid crosses have been tested with the hope to diversify and improve the aquaculture industry but the risk of implementing hybrids in commercial production systems are still very high. A Chilean hybrid cross between *Haliotis rufescens* and the Japanese *Haliotis discus hannai* was tested, cultivated and measured under commercial conditions. Lafarga-de la Cruz *et al*, (2012) found that the fertilization rates of hybrids were significantly lower ( $42.50 \pm 3.10\%$ ) when compared to non-hybrids ( $84.30 \pm 3.10\%$ ), the survival rate of 28.60% at day 461 was intermediate and the hybrid cross also had a significant lower shell length ( $34.10 \pm 0.80$  mm) compared to its purebred Japanese offspring ( $38.10 \pm 0.80$  mm). Only one of the reciprocal crosses between the species was successful. Final yield recovery rates of the hybrids were however 3.00% at day 461 which proved to be the highest compared to purebred crosses. Many of the important rearing characteristics that are important in a commercial production system didn't show much improvement with the hybrids. Whether the small increase in the yield recovery rate is financially significant remains to be seen and explains why hybrids are not being used more often by commercial farmers.

Inbreeding, heterosis and hybridization can be used as methods of selection and genetic manipulation within the on-farm abalone populations to improve canning yield by possibly creating specific lines that are selected according to specific traits which can be crossbred to gain the effect of heterosis to improve canning yield if heritability of specific yield-related traits are too low to allow a positive genetic response. There is however the risk of creating bottlenecks with increased inbreeding coefficients which will not be beneficial and the fitness of abalone within the populations could consequently suffer and will effect commercial production negatively.

### 2.3 Canning effects

The canning process involves several steps which include the removal of the shell and various organ systems and is followed by brining and thermal processing. These steps have a large impact on the yield and the quality of the final product. Chemical, physical and sensory changes occur when abalone meat is canned to allow preservation (Chiou *et al*, 2004). Characteristics that are affected by the canning process include sensory properties such as taste, texture, odour and colour of meat and size of the abalone and, as previously mentioned, the international markets are very end-product specific and target consumer groups also has to be accounted for (Chiou *et al*, 2004; Malcolm *et al*, 2008).

It must be noted that the quality of abalone before the canning process is initiated has an influence on the quality and yield of the canned abalone. Living muscles normally have a pH of approximately 7.2 (Sales *et al*, 1999). As the abalone die, glycogen in the muscle is converted to lactic acid which lowers the pH to approximately 5.5 (Sales *et al*, 1999). Warne & Brown, (1984) suggested that abalone should be stored at temperatures between 5 – 8 °C before canning proceeds. Temperatures lower than this has the effect of lowering flesh pH by inducing anaerobic glycolysis which causes an increased weight loss upon canning. The increased weight loss is due to poor water-holding properties induced by anaerobic glycolysis that occurs too rapidly (Sales *et al*, 1999). The same study also showed that higher pH values produced abalone meat that was tenderer. The effect of glycogen concentration on the taste of abalone is however unclear at present and does not have an effect on the canning yield (Malcolm *et al*, 2008).

The physical removal of the shell and organ systems also affects the yield. The meat to shell weight ratio is preferred to be higher (Malcolm *et al*, 2008) as less of the total weight is lost by removing the shell. The table below shows average weights of *H. midae* where Sales *et al*, (1999) separated and weighed different carcass components of abalone (n = 18) to get an indication of the different anatomical weight distributions. The sampled abalone were captured from the wild and the individual ages were not reported. Based on the live weight average the assumption could be made that the animals were older than the normal acceptable age which the markets prefer in terms of size. The preferred, higher meat to shell ratio is however reflected in Table 7 and the wet weight yield before further processing is 34.80% which is an overestimation of the final percentage canning yield as a further percentage weight loss will occur due to brining and cooking of the abalone muscle.

**Table 7:** Carcass components of *H. midae*, before the canning process commences (Adapted from Sales *et al*, 1999).

Characteristics	Mean (g) ± Std Dev	Proportion (%) of live weight (g)
Live weight	492.20 ± 44.03	
Muscle weight	171.30 ± 15.18	34.80
Viscera weight	137.90 ± 26.45	28.00
Shell weight	183.00 ± 15.93	37.20

Once the abalone are exposed to air and removed from their natural habitat, weight loss commences due to physiological reactions to prevent or limit dehydration. Weight loss can be due to tissue loss, water loss in the form of evaporation, urine production and mucus production. The foot of the abalone cannot be retracted into a shell and there is no operculum that can close off as physical adaptations to prevent water loss in air. Within their natural habitat abalone are in an osmotic equilibrium and water exchange occurs freely.

Approximately 65% of *H. midae*'s body mass consists of water. Total body weight loss is attributed to water loss instead of tissue loss as the contribution of tissue to the total weight stays the same during dehydration. Vosloo & Vosloo, (2006) showed that water loss, as evaporation, is significant and leads to increased osmotic concentrations in the tissue. Once exposed to air the water turnover rate immediately decrease to less than 2.4% of what it would be in an aquatic environment to prevent water loss (Vosloo & Vosloo, 2006). Haemolymph volume also exhibits a decrease when exposed to air but there is only redistribution of fluid within the tissues and weight loss cannot be attributed to this factor (Edney, 1977). As the volume decreases, haemolymph pressure decreases which is necessary for ultra-filtration in the atrial walls of the pericardium to take place. Ultra filtration is the first step in urine production which ceases completely once dehydration occurs as the haemolymph pressure cannot be maintained (Vosloo & Vosloo, 2006). Urine production is therefore also not responsible for water loss during dehydration.

Abalone produces large amount is mucus during dehydration. Mucus production helps to maintain the osmotic concentrations within the tissue by eliminating important ions such as  $\text{Ca}^{2+}$  and  $\text{SO}_4^{2-}$  and is necessary to help abalone to adhere to surfaces (Vosloo & Vosloo, 2006). It does however have a water content of 90% and is therefore the main source of water loss, along with evaporation, during aerial exposure (Vosloo & Vosloo, 2006).

The duration of brining time and the salt concentration that is used affects the yield, but research shows that this effect becomes insignificant after thermal processing (Warne & Browne, 1982). Literature shows that making use of proteolytic enzymes after brining to clean flesh, alongside with addition of phosphate additives, have no significant influence on the canning yield (Warne & Browne, 1982).

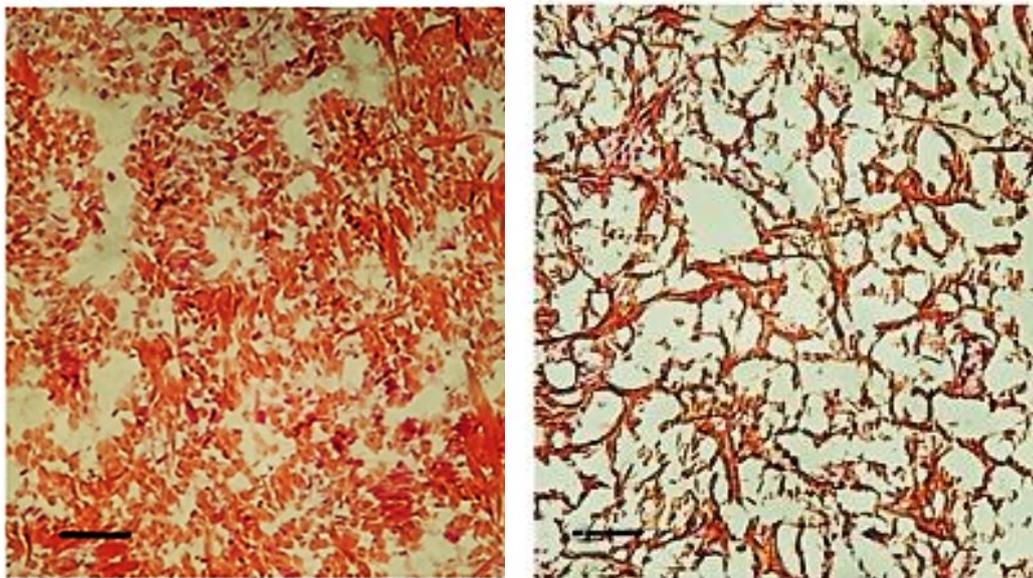
The extent of thermal processing is dependent on the temperature and the duration exposed to that temperature. Cooking abalone meat has a profound effect on the yield. Malcolm *et al*, (2008) showed that cooking abalone for six hours resulted in a meat yield of only 60%. Thermal processing of abalone at 80°C for up to two hours proved to have a significant effect on the taste, texture and appearance (Chiou *et al*, 2004). A balance of free amino acids and nucleotides is thought to be the major factor that characterizes the taste of abalone meat (Malcolm *et al*, 2008). Preferred properties have been proven to be associated with an abundance in the abalone-specific free amino acids Glutamic acid, Glycine, Adenosine Monophosphate and Glycinebetaine which are referred to as taste-active components (Chiou *et al*, 2004). This association between the increased specific free amino acids and a preferred taste/flavour was proved by Hatae *et al*, (1995) when the above mentioned free amino acids increased in abalone meat after it was cooked between 15 - 60 minutes. They suggested that this occurred due to hydrolysis of proteins by enzymes before enzyme inactivation occurred.

The process of cooking abalone meat affects coloration as the duration of cooking increases. Chiou *et al*, (2004) showed that little change occurred at 80°C but colouration did appear when cooked at 98°C. Their conclusion was that this could not be due to enzymatic reactions as a result of enzyme denaturation at that temperature. The preferred colour of canned goods should show no localized discolouration and has a creamy to yellow appearance (Chiou *et al*, 2004).

Textural and rheological properties of abalone meat are also affected by thermal processing (Chiou *et al*, 2004). Raw meat normally has a crisp and firm texture which changes into a soft, chewy and tender texture after being cooked (Chiou *et al*, 2004; Gao *et al*, 2001). Canned abalone is preferred to have a soft and chewy texture (Malcolm *et al*, 2008). Gao *et al*, (2001) found that meat shrank, water was lost and also components that were water-soluble and this caused weight loss when exposed to prolonged cooking. Three hours of cooking resulted in abalone that showed a reduction in water content from 85% to 77%. The individual height measurement of the abalone was also 38% lower compared to original measurements by Chiou *et al*, (2004) who conducted a study where small abalone were

cooked and resulted in weight loss ranging between 11 - 20% as abalone was cooked between temperatures 80 - 98°C for up to 2 hours.

A decrease in toughness, or conversely an increase in meat tenderness, is attributed to heat denaturation of myofibril fibres and gelation of rich collagen fibres (Chiou *et al*, 2004; Gao *et al*, 2001). There is a correlation between the toughness of abalone meat and collagen content with a lower collagen content being the tenderer product preferred by sensory panels (Chiou *et al*, 2004; Olaechea *et al*, 1993). Weight of the whole animal also shows a positive correlation with collagen content, where smaller abalone contain less collagen, hence once again more tender (Olaechea *et al*, 1993). After one hour of cooking abalone meat Chiou *et al*, (2004) showed that 41% of collagen fibres were converted to gelatine (Figure 6). As an increase in temperature causes gelatinization of collagen fibres large voids between denatured myofibrils are formed as fluid gelatine leaves muscle tissue (Gao *et al*, 2001).



**Figure 6:** From left to right: Cross-section of raw abalone muscle tissue, cross-section of cooked abalone muscle tissue (Adapted from Gao *et al*, 2001).

A study done by Sales *et al*, (1999) did however find that abalone that were cooled and stored at 16°C showed greater tenderness compared to cooked abalone. This conclusion to the study contradicts other research and no explanation could be given for the observation.

The weight loss that is associated with the canning process is mainly due to the loss of water via evaporation and mucus production, and other water-soluble components such as gelatine which is converted from collagen when heat is applied (Gao *et al*, 2001; Malcolm *et*

*a/*, 2008). Thermal processing plays a vital role in weight loss and effects the textural and sensory properties of the meat by altering the chemical composition of the free amino acid profile and the micro structure of muscular tissue.

## 2.4 Summary

*Haliotis midae* is one of five indigenous abalone species found along the coast of South Africa and is the only species with commercial value and significance. They are herbivorous, nocturnal animals with a preference of kelp *Ecklonia maxima* although commercial farms combine kelp with artificial feed alternatives to increase growth rates through provision of a diet with a balanced nutrient profile. The animals are univalved and mainly consist of a shell, muscular tissue and soft tissue viscera. When abalone are canned the shell and viscera are removed and is of less economic significance. Haliotids are dioecious with a high fecundity and a long generation interval which slows the rate of a genetic response. They are broadcast spawners and spawning occurs twice-yearly during spring and autumn. Sexual maturity is age dependent but can be shortened to approximately three to four years by providing optimal nutrition under the preferred or optimal environmental conditions. *Haliotis midae* is a temperate species and seasonal variation is clearly detectable. Seasonality plays an important role in affecting the chemical composition of abalone meat. Specific consumer preferences to certain species and product forms have developed as a result and these are based on the change in sensory and textural properties that is observed. The full extent of the effects of seasonality on the canning yield has not yet been established. Abalone are very sensitive to environmental fluctuation and factors such as sensitivity to seasonal variation, temperature, salinity, seawater pH, air exposure or dehydration and affect the efficiency of their immunity which must be avoided to prevent a negative effect on the general physiological state of the animals. Diversion of energy away from non-essential biochemical processes decreases growth rate and increases mortality rates and therefore negatively affects the canning yield. The canning process consists of different stages by causing weight loss due to various reasons. These include removal of the shell and vital organ systems, but weight loss is also ascribed to mucus production and evaporation of water and water-soluble components before and during thermal processing which decreases the canning yield significantly.

Genetic improvement via artificial selection methods can be of great value to allow commercial domestication of *H. midae*. Some success with selection programmes have been achieved in aquaculture but the global industry has however, as of yet, not really made full use of the possible benefits that quantitative genetics and genomics have to offer. According to published literature heritability values for yield-related traits tend to be low to moderate which could make the use of inbreeding and heterosis an alternative way to increase canning yield but the risks involved with inbreeding and crossbreeding must be considered as well.

### **3 Materials and Methods**

#### **3.1 Brood stock collection**

A total of 800 sexually mature abalone brood stock animals were collected from the Walker Bay region in the Western Cape in December 2006. These animals were subsequently divided into random groups ( $n = 180$ ) between the farms participating in a collaborative selective breeding programme. The animals were placed in separate holding tanks for the purpose of conditioning and induced spawning.

#### **3.2 Location**

The farms participating in the trial included Aquafarm Development Company (Pty.) Ltd, HIK Abalone (Pty.) Ltd. Roman Bay (Pty.) Ltd. and Irvin & Johnson (Pty.) and are all situated in the Hermanus and Gansbaai region on the southwestern coast of South Africa. These land based farms provided standard commercial ongrowing facilities that were used during the respective growth phases of the experiment. A commercial cannery, Abagold Cannery (Pty.) Ltd, in Hermanus, was used to conduct the processing and canning of the experimental material.

#### **3.3 Experimental design**

A randomized incomplete block was used as experimental design, implicating that there were less blocks than the number of applied treatments (Hinkelmann & Kempthorne, 2005). The variance due to block effects could therefore not be eliminated. Treatments, consisting of different sire dam combinations could not be spread evenly across each block due to a lack of control over the artificial spawning of brood stock. At the completion of the growth trial at the age of 62 months, each family on each location was distributed over four baskets (random repeats). For the subsequent yield trial a random sample ( $n=16$ ) were collected from each of the four baskets per family. Due to mortality or tag loss, there wasn't always enough abalone left from each basket to take a full sample.. The design was therefore also unbalanced because in three (Families 11,18 and 31) of the 24 families sampling of as few as 17 tagged individuals, as supposed to the standard 64, could be secured.

#### **3.4 Family structure**

The family structure was established through induced spawning of individual animals during the period of November to December 2007. Through controlled fertilization a number of half and full sib family groups were produced, each with a minimum of 1000 offspring per family. Eggs from one female were fertilized with the sperm from a male that spawned at the same time. The success rate of induced spawning amongst commercial brood stock, male and female, varies around the level of 40 percent (Elliot, 2000).

The obtained family structure consists of 18 sires and 11 dams which were randomly paired to create 19 full sib families of which nine are half sib families (Table 8). The choice of parents used for fertilization was based purely on the synchronized nature of induced spawning and the number of gametes produced. Sires were selected to be used as unique parents to create half sib families in the structure whereas dams that spawned sufficiently were used as shared parents. More sires were used as males responded better to artificial spawning than females, and also spawned more regularly with sufficient volumes of gametes. Family groups ( $n \geq 1000$ ) were kept in separate holding units (tanks) through the larval stages until weaning at the age of six months. At the six months period each full sib family was randomly divided into four groups ( $n = 250$ ) as repeats and kept in separate holding units (baskets) throughout the duration of the 62 months grow-out trial.

**Table 8:** Family groups created from unique and shared parents, as full/half sib families with indication of location where animals were reared (Adapted from Van Schalkwyk, 2011).

Full Sib Families	Half Sib Families	Location & Family	Shared parent	Unique parent
1	1	AF3	D	S 468
2		AF6		S 342
3	2	AF10	S	D B10B
4		I&J34		D B11B
5	3	RB15	D	S C30B
6		I&J36		S C28B
7	4	RB19	D	S M44
8		RB24		S M33
9	5	RB20	D	S C29F
10		I&J37		S C24F
11	6	RB25	D	S M34
12		I&J30		S M38
13	7	HIK46	D	S M4
14		HIK48		S M35
15	8	HIK49	D	S M13
16		HIK50		S M25
17	9	HIK51	D	S M26
18		HIK53		S M40
19		HIK52	D	F22 S M36

D = dam, S= sire

A total of 24 families, as indicated in Table 9 were randomly allocated to the four locations with five of these families being represented on more than one location. On each of the locations, the respective farms applied their own managerial practices in terms of stocking densities, holding units and feeding regimes.

**Table 9:** The number of families allocated to each of the four locations.

<b>Location</b>	<b>Number of families held/location</b>
Aquafarm	3
HIK	9
Irvin & Johnson	6
Roman Bay	6

The aim to include a unique full sib family as an internal reference group with each of the 19 families at all four locations could not be realised due to the lack of sufficient spawning volumes from any one such mating pair. A unique full sib reference group was however created per location and was included with each of the four baskets per family. The effect of location on family performance was difficult to establish but the five full sib families that were distributed amongst more than one farm could however be used to gain some information regarding this effect.

## 3.5 Canning trial

### 3.5.1 Sampling

Random sampling at each interval was ensured by devising a method through which bias was prevented. A piece of string was positioned to create an intersect across the abalone retrieved from each basket and the first 16 abalone that touched the intersect were selected (Figure 7).



**Figure 7:** Display of the sampling method (Photo: Arnold Vlok).

During the growth trial, the parameters of individual shell length and body weight were measured every six months. At the end of the 62 months growth trial, a random sample of 16 animals was collected from each of four family repeats, equal to 64 individuals per family that were pooled into a single family group to determine the canning data.

Each family group per location was then divided anew into random groups to act as repeats per location during the yield trial. Animals from a particular location were processed on the same day due to logistical reasons and biosecurity (HACCP) regulations.

### 3.5.2 Tagging of abalone

#### 3.5.2.1 Tagging methods

Step-wise weight measurements of each individual abalone were taken at consecutive stages during processing and that required individual identification and tagging. Measures were taken to prevent tag loss. Each abalone received two identification tags before the first stage of processing i.e. removal of the shell, commenced. An initial tag was placed through

an intact respiratory hole in the shell of the abalone, identifying the location, the family and the specific individual as shown in Figure 8.



**Figure 8:** Initial tagging method through respiratory hole of shell (Photo: Danie Brink).

Another tag was placed through the adductor foot muscle just before the shell (including the first tag) was removed (shucked) during the canning process (Figure 9). As soon as the second tag, in the form of a cable tie, is placed through the foot muscle, the animal starts to lose weight due to mucus production, as a reaction to stress and blood loss.



**Figure 9:** Final tagging method through adductor foot muscle (Photo: Danie Brink).

### **3.5.2.3 Identification of individual abalone**

Tags of different colours were prepared by labelling them with a permanent marker to identify a specific individual within a family on each of the four locations. The example in Figure 9 shows the farm HIK as location, 50 as the family identification followed by 34 as the individual identification from a total sample size of 64 (16x4) within the family.

### **3.5.2.4 Transportation and purging**

After sampling and tagging repeats per location was placed in separate mesh bags and transported to the Abagold cannery in Hermanus. All the farms are within an hour's drive from Abagold cannery. Aerial exposure that occurs during transit causes drip loss which was standardized by keeping animals from all locations in transit conditions for one hour. After the one hour transit period all animals were placed in holding tanks overnight to be purged. Feed was withheld to clear the digestive tract in preparation for the canning process.

### **3.5.3 Canning protocol**

The canning trial was conducted at the Abagold Cannery in Hermanus where the standard commercial canning protocol was followed. The abalone received from each location was processed in separate batches, each over a four day period. Weight measurement of individual animals is time-consuming and it would not be possible to use standardised handling protocols if animals from more than one location at a time had to be measured. Animals sampled from each of the locations were therefore processed separately to prevent unwanted environmental variance.

The protocol entails the tagged animals entering the cannery after an overnight (12 hours) period of purging. Thereafter they are shucked by removing the shells (Figure 10). The animals are then gutted by removing the viscera which includes the respiratory, digestive and reproduction organs. The adductor foot muscle remains and is then brined for approximately three hours in a standard saline solution. After the process is completed the abalone are kept in walk-in refrigerators overnight (12 hours). Facial components that include the tentacles, eyes and mouth parts are removed the following day and the abalone are then washed and scrubbed by hand to remove any remaining debris. The abalone are sorted according to size on a conveyer belt and placed in cans which are filled with a brine solution. Cans are sealed with the use of machinery and the cans are placed in a pressure cooker to cook the meat and also sterilize the contents. The cans are then left to cool down after which they can be labelled for marketing. Quality control is also done according to normal commercial procedures.



**Figure 10:** Standard canning protocol where a: shucking and gutting of before brining process; b: removal of facial components and cleaning after brining and overnight refrigeration; c: tagged abalone ready to be canned; d: abalone are placed in cans containing a standard brine solution; e: cans are sealed mechanically; f: cans are placed in a pressure cooker to cook and sterilize for long term preservation (Photos: Danie Brink).

### 3.5.4 Measurement of abalone

Step-wise measurements of individual weights (grams) at six specified stages were taken during the canning process – namely pre-shuck, post-shuck, post-gut, post-brine, pre-canning and post-canning weights. These six weight measurements were manually recorded per individual abalone and later captured in a Microsoft Excel datasheet. The same electronic scale (A&D EK 3000i) was used throughout the trial and was cleaned and calibrated at regular intervals to ensure accuracy (Figure 11). The weight of the tag, as can be seen in Figure 11, was compensated for by measuring all abalone with their tags included in the measurement. The effect of the tag's weight can therefore be nullified as every animal received the same treatment. The tags are a standard size and weigh approximately 1.00 gram.



**Figure 11:** Measuring weight (g) of abalone at the post-gut stage (Photo: Danie Brink).

The gender of the abalone was recorded in order to assess differences in yield between males and females. Gender is determined based on the colour of the gonads. Male spermatozoa have a creamy white appearance whereas the ova within the female gonads have an olive green appearance (Figure 12).



**Figure 12:** Gender determination of females (left) and males (right) (Photo: Danie Brink).

### 3.6 Statistical analysis

Raw data was recorded in the cannery by hand, then captured using Microsoft® Office Excel® 2010 whilst SAS Enterprise Guide 4 and Statistica 10 software packages (StatSoft, Inc. 2012) were used to analyse the data statistically. AS Reml was used to calculate approximations of genotypic correlations, as well as heritability estimates of canning yield and other related traits.

Analysis of variance (ANOVA) was done to assess the effect that location, gender, different sires, different dams, and unique parent combinations i.e. families had on the absolute means of pre-shuck weight, henceforth also referred to as live weight, and calculated shell weight, canning yield (expressed as a percentage), the ratio of shell weight to the total wet weight and the nett yield in grams of the each abalone.

- Shell weight was calculated using the following equation:

$$\text{Shell weight (g)} = A - B$$

Where A = Pre-shuck/live weight (g)

B = Post-shuck weight (g)

- Canning yield percentage (E) was calculated using the following equation:

$$\text{Canning yield (\%)} = 100 - \left( \frac{A-C}{A} * 100 \right)$$

Where A = Pre-Shuck/live weight (g)

C = Post-Canning weight (g)

- Shell weight to total weight ratio (SW: PGW) was calculated using the following equation:

$$\text{Shell weight to total weight ratio} = \frac{(A-B)}{D}$$

Where A = Pre-Shuck/live weight (g)

B = Post-Shuck weight (g)

D = Post-Gut weight (g)

- Nett canning yield was calculated using the following equation:

$$\text{Nett canning yield (g)} = \frac{(A * E)}{100}$$

Where A = Pre-Shuck/live weight (g)

E = Canning yield percentage

Six discreet age groups were created within a range of 109 days. This occurred due to biological limitations i.e. the lack in synchronization of induced spawning and caused the age of the different families and the four locations to be confounded. Variability due to the respective effects was therefore difficult to distinguish or separate. Age was included as a covariate to the percentage canning yield.

A correlation analysis was done by calculation of a Pearson's correlation coefficient. Statistica 10 software was used to calculate the phenotypic correlations between traits that were recorded and calculated during the trial. These include the six weight measurements, percentage canning yield, the shell weight ratio of shell to post-gut weight (SW: PGW) and the nett canning yield. The correlations were evaluated based on family averages.

AS Reml was used to calculate approximations for heritability. Between-family variance ratios were calculated to estimate these approximations and covariance ratios were used to calculate approximations for genetic correlations. The animals that were sampled are the first generation to be bred in captivity and no pedigree was available to allow an animal model to be used. A half-sib model could also not be run as the structure that existed did not allow gametes from sires to fertilize more than one female and females also did not spawn sufficiently to be fertilized by more than one sire. This implies that sire and dam effects could not be separated and sire-dam combinations i.e. families were used instead. A mixed model was used to fit sire and dam effects as a single random effect and age was included as a covariate to compensate for weight and yield differences due to a maximum age difference of 109 days.

The model that was fitted:

$$Y_{ijkl} = \mu + F_j + L_k + G_l + b_{(age)} + e_{ijkl}$$

Where

$Y_{ijk}$  was the observation on the  $i^{th}$  trait ( $Tr_a, Tr_b, \dots Tr_n$ ) where the respective traits included the six weight measurements that were taken, the percentage canning yield and the ratio of shell weight to post-gut weight (SW: PGW),

$\mu$  is the overall mean,

$F_j$  is the random effect of the  $j^{th}$  full sib family,

$L_k$  is the fixed effect of the  $k^{th}$  location,

$G_l$  is the fixed effects of gender where  $l$  represents males and females,

$b_{(age)}$  is the regression of the  $i^{th}$  trait on the age and

$e_{ijkl}$  is the residual term.

## 4 Results and Discussion

To conduct analysis of variance, existing assumptions have to be met to validate the results that are obtained. These assumptions are mainly that equal variances (homoscedasticity) and normality must exist in the data. Shapiro-Wilk's  $W$  test was used to test for normality and Levene's Test was used to test for homoscedasticity. The appropriate tests for homoscedasticity were done on canning yield with different families and gender as independent variables. Both tests were not significant with  $p$ -values of 0.2031 and 0.1276 respectively. The assumption of homoscedasticity is therefore valid. Normality testing showed normal bell-shaped distributions with a  $p$ -value (0.0769) larger than 0.05 which allows parametric testing. The sample size is large enough to provide statistical accuracy and quantile-quantile plots show that there are no outliers that could be responsible for the non-normal distribution.

### 4.1 Analysis of Variance (ANOVA)

Different statistical models are used to calculate to partition observed variance into components that exist due to different sources of variation. Analysis of variance is used to determine whether means of different groups are equal or significantly different. This method was used to determine whether the gender, location, family or a unique parent, may it be sire or dam, have a significant effect on the canning yield of abalone.

The null hypothesis that was tested:

$H_0: \mu_1 = \mu_2 = \dots = \mu_n$ , where  $\mu_i$  is equal to the mean canning yield percentage of different genders; different locations; different sires; different dams and different parent combinations i.e. families.

The alternative hypothesis that was tested:

$H_a: \mu_1, \mu_2, \dots, \mu_n$  are not equal.

#### 4.1.1 Effect of Location

Five full sib families were each randomly allocated to two different locations. The effect of the two locations on the absolute weight and canning yield percentage can be evaluated to establish whether the incomplete block design can statistically reduce variance. Significant differences that occur due to the effect of different locations can be attributed to environmental variance in the form of managerial practices, protocols and abiotic conditions that are different. Absolute live weight comparisons in Table 10 show significant differences between all four locations ( $p < 0.05$ ). The mean shell weight also differs significantly between all four locations ( $p < 0.05$ ).

Based on canning yield percentage (Table 10), the significance of differences between locations supports the rejection of the null hypothesis for all comparisons except for family 7a and 7b which were located respectively on Aquafarm and Roman Bay. Aquafarm and Roman Bay are managed by the same holding company and this result could be explained by their similarity of managerial practices which reduces difference in environmental variance.

**Table 10:** Evaluation of the location effect on the means of shell weight, live weight and canning yield of full sib families.

Family	Location	Shell Weight (g) ± Std Err	Live Weight (g) ± Std Err	Canning Yield (%) ± Std Err
7a	AF	27.72 ± 1.459*	94.87 ± 3.412*	30.33 ± 0.966
7b	RB	32.98 ± 1.459*	110.78 ± 3.412*	29.69 ± 0.966
29a	RB	33.46 ± 1.471*	111.34 ± 3.439*	27.88 ± 0.974*
29b	I&J	52.21 ± 1.482*	177.31 ± 3.439*	24.76 ± 0.974*
30a	RB	30.52 ± 1.495*	107.43 ± 3.439*	30.59 ± 0.974*
30b	I&J	47.52 ± 1.459*	165.05 ± 3.412*	23.23 ± 0.966*
35a	I&J	47.59 ± 1.471*	161.12 ± 3.439*	25.46 ± 0.974*
35b	HIK	30.90 ± 1.459*	92.06 ± 3.412*	28.70 ± 0.966*
37a	I&J	46.05 ± 1.459*	158.94 ± 3.412*	24.47 ± 0.966*
37b	HIK	40.39 ± 1.495*	117.38 ± 3.412*	29.61 ± 0.966*

\* Location comparison statistically significant according to LSD test ( $p < 0.05$ ).

AF = Aquafarm, RB = Roman Bay, I&J = Irvin & Johnson, HIK = HIK Abalone.

When the absolute shell weight, live weight and canning yield per location are evaluated and compared to establish location effects as shown in Table 11, the significance of differences between all the locations is once again confirmed. Mean canning yield percentage per location indicates again that Aquafarm and Roman Bay are significantly similar but another significant similarity between Roman Bay and HIK is also detected. Despite the similarity that Aquafarm and HIK share with Roman Bay, they are still significantly different from each other in terms of canning yield percentage.

**Table 11:** Evaluation of the effect of locations on the means of shell weight, live weight and canning yield.

<b>Location</b>	<b>Shell Weight (g) ± Std Err</b>	<b>Live Weight (g) ± Std Err</b>	<b>Canning Yield (%) ± Std Err</b>
<b>AF</b>	29.83 ± 0.943 <sup>d</sup>	97.49 ± 2.234 <sup>d</sup>	30.30 ± 0.212 <sup>a</sup>
<b>RB</b>	33.59 ± 0.685 <sup>c</sup>	113.19 ± 1.615 <sup>c</sup>	29.25 ± 0.457 <sup>ab</sup>
<b>HIK</b>	43.04 ± 0.523 <sup>b</sup>	124.46 ± 1.235 <sup>b</sup>	28.63 ± 0.328 <sup>b</sup>
<b>I&amp;J</b>	48.74 ± 0.684 <sup>a</sup>	167.42 ± 1.617 <sup>a</sup>	24.89 ± 0.421 <sup>c</sup>

Means with different superscripts differ significantly ( $p < 0.05$ ).

The null hypothesis is rejected ( $p < 0.05$ ). The experimental design of an incomplete block did not allow for variability to be reduced and families from different locations can therefore not be compared with accuracy.

#### 4.1.2 Effect of Gender

The results as presented in Table 12 show that there is no significant gender effect on the canning yield of abalone. Males and females proportionally, lose equal amount of weight when viscera, specifically reproductive organs, are removed and the rest of the canning process is completed. Absolute live weight values were also used to evaluate the effect of gender and show the same statistically insignificant results ( $p > 0.05$ ). The null hypothesis that the group means are equal cannot be rejected and there is no reason to consider gender as a trait in a selection process. Seasonality of abalone affects males and females equally and no significant difference is observed.

**Table 12:** Means of live weight and canning yield of male and female abalone.

<b>Gender</b>	<b>Live Weight (g) ± Std Err</b>	<b>Canning Yield (%) ± Std Err</b>
<b>Female</b>	128.71 ± 0.99 <sup>a</sup>	28.01 ± 0.30 <sup>a</sup>
<b>Male</b>	130.53 ± 1.50 <sup>a</sup>	27.68 ± 0.30 <sup>a</sup>
<b>p-value</b>	0.3522	0.5990

Means with different superscripts differ significantly ( $p < 0.05$ ).

#### 4.1.4 Effect of Sires

The results from Table 13 indicate that sires 468, C30B, 342, M40, M4 and M26 are the highest performers in terms of delivering the highest percentage canning yield relative to their own live weight, with significant differences from the remaining sires. When comparing the results with that of van Schalkwyk, (2011) there were no similarities amongst sires in terms of drip loss and canning yield.

**Table 13:** Mean canning yield from offspring with different sires.

Sires	Canning yield (%) ± Std Err
<b>468</b>	31.89 ± 0.98 <sup>a</sup>
<b>C30B</b>	30.49 ± 0.98 <sup>ab</sup>
<b>342</b>	30.01 ± 0.70 <sup>ab</sup>
<b>M40</b>	29.99 ± 0.99 <sup>abc</sup>
<b>M4</b>	29.34 ± 0.98 <sup>abcd</sup>
<b>M26</b>	29.29 ± 0.99 <sup>abcd</sup>
<b>M13</b>	28.30 ± 0.98 <sup>eb</sup>
<b>M44</b>	27.94 ± 0.98 <sup>eb</sup>
<b>M25</b>	27.94 ± 0.98 <sup>eb</sup>
<b>C29F</b>	27.93 ± 1.91 <sup>ae</sup>
<b>M35</b>	27.39 ± 0.98 <sup>ec</sup>
<b>B27F</b>	27.36 ± 0.60 <sup>ed</sup>
<b>M36</b>	27.13 ± 0.98 <sup>ed</sup>
<b>C28B</b>	27.02 ± 0.70 <sup>ed</sup>
<b>M34</b>	26.88 ± 0.70 <sup>e</sup>
<b>C24F</b>	26.72 ± 0.98 <sup>ed</sup>
<b>M33</b>	26.32 ± 0.70 <sup>e</sup>
<b>M38</b>	24.08 ± 1.97 <sup>e</sup>

Means with different superscripts differ significantly ( $p < 0.05$ ).

#### 4.1.5 Effect of Dams

Results from Table 14 indicate that offspring from dams 462 and F60 showed the best canning yield percentage and dam 462 differed significantly from all the other female brood stock that was used. F60, F28, C10F, F50 and B10B were significantly similar in performance and fall in the group second to best. The above named six females are the best performing females and can be used for future selection based on canning yield percentage.

When comparing results to what van Schalkwyk, (2011) found when investigating the same females for mean drip loss percentage, it can be reported that the offspring from the similar females also showed the smallest volume of drip loss. Although different individuals were sampled, though still originating from the same parent, the results indicates that there could be underlying genetic correlation between these traits and within the families.

**Table 14:** Mean canning yield of offspring from different dams.

Dams	Canning Yield (%) ± Std Err
<b>462</b>	30.64 ± 0.56 <sup>a</sup>
<b>F60</b>	29.64 ± 0.84 <sup>ab</sup>
<b>F28</b>	28.36 ± 0.84 <sup>cb</sup>
<b>C10F</b>	28.19 ± 0.71 <sup>cb</sup>
<b>F50</b>	28.12 ± 0.20 <sup>cb</sup>
<b>B10B</b>	28.09 ± 0.38 <sup>cb</sup>
<b>F22</b>	27.13 ± 0.31 <sup>c</sup>
<b>B11B</b>	27.09 ± 0.28 <sup>c</sup>
<b>C10B</b>	26.97 ± 1.35 <sup>c</sup>
<b>F66</b>	26.86 ± 0.45 <sup>c</sup>
<b>F62</b>	26.57 ± 0.80 <sup>c</sup>

Means with different superscripts differ significantly ( $p < 0.05$ ).

#### 4.1.6 Effect of Families

Results from Table 15 show that families 4, 30a, 16, 7a, 48, 7b, 37b, 41 and 46 are statistically similar and the best performers with the highest canning yield percentage. The full sib family pair 7a and 7b (Table 10) are both in this group and show once again that their performance was not statistically affected by different locations. It does however also emphasize the fact that the other full sib family pairs show significantly different canning yields but this can be due to the location effect rather than genetic performance which reduces accuracy of the results.

According to Table 15, family 18 is also one of the top performers in terms of canning yield percentage. The family does however show a very large standard error resulting from a small sample size of only 17 abalone that were sampled due to loss of animals during the grow-out phase. The family should therefore rather be grouped according to its mean and not included as one of the top performers. The same is true for families 11 and 31 where

small sample sizes result in relatively large standard errors and grouping still occurs based on respective means instead.

**Table 15:** Family averages of shell weight, live weight, shell weight to post-gut ratio and canning yield percentage.

Family	Shell Weight (g) ± Std Err	Live Weight (g) ± Std Err	SW: PGW ± Std Err	Canning Yield (%) ± Std Err
4	23.38 ± 1.46 <sup>n</sup>	86.26 ± 3.41 <sup>k</sup>	0.57 ± 1.40 <sup>b</sup>	31.89 ± 0.97 <sup>a</sup>
30a	30.52 ± 1.47 <sup>lm</sup>	107.43 ± 3.44 <sup>j</sup>	3.82 ± 1.41 <sup>ab</sup>	30.59 ± 0.97 <sup>ab</sup>
16	37.58 ± 1.47 <sup>ij</sup>	127.62 ± 3.41 <sup>f</sup>	2.23 ± 1.40 <sup>ab</sup>	30.49 ± 0.97 <sup>abc</sup>
7a	27.72 ± 1.46 <sup>m</sup>	94.87 ± 3.41 <sup>k</sup>	0.63 ± 1.40 <sup>b</sup>	30.33 ± 0.97 <sup>abc</sup>
48	43.51 ± 1.50 <sup>efh</sup>	127.15 ± 3.44 <sup>fg</sup>	4.03 ± 1.41 <sup>ab</sup>	29.99 ± 0.97 <sup>abcd</sup>
7b	32.98 ± 1.50 <sup>kl</sup>	110.78 ± 3.44 <sup>ij</sup>	0.64 ± 1.40 <sup>b</sup>	29.69 ± 0.97 <sup>abcde</sup>
37b	40.40 ± 1.50 <sup>hi</sup>	117.38 ± 3.44 <sup>hi</sup>	5.61 ± 1.41 <sup>a</sup>	29.61 ± 0.97 <sup>abcde</sup>
41	42.80 ± 1.50 <sup>fh</sup>	117.72 ± 3.41 <sup>gh</sup>	5.55 ± 1.40 <sup>a</sup>	29.34 ± 0.97 <sup>af</sup>
46	42.38 ± 1.47 <sup>gh</sup>	128.84 ± 3.44 <sup>ef</sup>	2.34 ± 1.41 <sup>ab</sup>	29.29 ± 0.97 <sup>af</sup>
35b	30.90 ± 1.46 <sup>lm</sup>	92.06 ± 3.41 <sup>k</sup>	2.31 ± 1.40 <sup>ab</sup>	28.70 ± 0.97 <sup>fb</sup>
44	46.55 ± 1.46 <sup>df</sup>	137.64 ± 3.41 <sup>de</sup>	0.79 ± 1.40 <sup>b</sup>	28.30 ± 0.97 <sup>fbg</sup>
11	41.47 ± 1.70 <sup>hi</sup>	116.34 ± 3.98 <sup>ij</sup>	0.88 ± 1.64 <sup>b</sup>	28.09 ± 1.13 <sup>fbgh</sup>
45	50.63 ± 1.46 <sup>bc</sup>	145.25 ± 3.41 <sup>d</sup>	0.82 ± 1.40 <sup>b</sup>	27.94 ± 0.97 <sup>fbgh</sup>
21	35.48 ± 1.46 <sup>ik</sup>	115.71 ± 3.41 <sup>ij</sup>	0.69 ± 1.40 <sup>b</sup>	27.94 ± 0.97 <sup>fbgh</sup>
18	25.56 ± 2.83 <sup>mn</sup>	85.23 ± 6.62 <sup>k</sup>	0.64 ± 2.72 <sup>ab</sup>	27.93 ± 1.87 <sup>afghi</sup>
29a	33.46 ± 1.47 <sup>kl</sup>	111.34 ± 3.44 <sup>ij</sup>	2.27 ± 1.41 <sup>ab</sup>	27.88 ± 0.97 <sup>fcgh</sup>
43a	44.09 ± 1.46 <sup>dfh</sup>	127.42 ± 3.41 <sup>f</sup>	2.39 ± 1.40 <sup>ab</sup>	27.39 ± 0.97 <sup>fdghj</sup>
47	46.00 ± 1.46 <sup>dfg</sup>	126.73 ± 3.41 <sup>fgh</sup>	0.86 ± 1.40 <sup>b</sup>	27.13 ± 0.97 <sup>feghi</sup>
38	48.01 ± 1.46 <sup>cd</sup>	167.58 ± 3.41 <sup>c</sup>	0.65 ± 1.40 <sup>b</sup>	26.72 ± 0.97 <sup>fghi</sup>
35a	47.59 ± 1.47 <sup>cde</sup>	161.12 ± 3.44 <sup>c</sup>	0.63 ± 1.41 <sup>b</sup>	25.46 ± 0.97 <sup>kh</sup>
29b	52.21 ± 1.48 <sup>ab</sup>	177.31 ± 3.44 <sup>b</sup>	2.24 ± 1.41 <sup>ab</sup>	24.76 ± 0.97 <sup>kij</sup>
37a	46.05 ± 1.46 <sup>dfg</sup>	158.94 ± 3.41 <sup>c</sup>	3.76 ± 1.40 <sup>ab</sup>	24.47 ± 0.97 <sup>ki</sup>
31	58.25 ± 2.92 <sup>a</sup>	196.08 ± 6.82 <sup>a</sup>	0.65 ± 2.80 <sup>ab</sup>	24.08 ± 1.93 <sup>kg</sup>
30b	47.52 ± 1.46 <sup>cde</sup>	165.05 ± 3.41 <sup>c</sup>	0.64 ± 1.40 <sup>b</sup>	23.23 ± 0.97 <sup>k</sup>

Means with different superscripts differ significantly ( $p < 0.05$ ).

Similar to the results reported by van Schalkwyk, (2011) on live export weights, families with heavier mean total weights delivered a smaller percentage canning yield. Family 4 produced

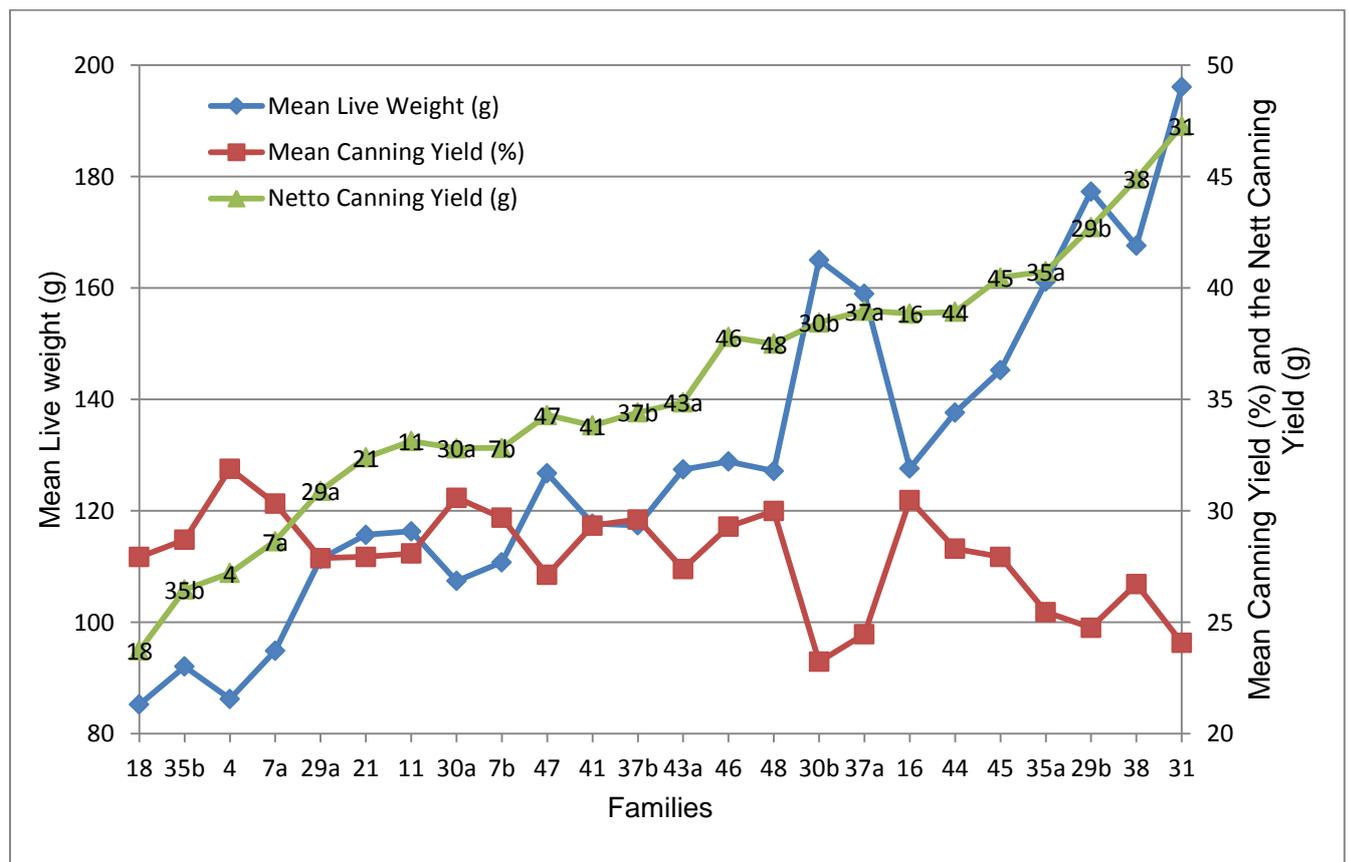
the lowest mean live weight of  $86.26 \pm 3.41$  and still produced the highest canning yield percentage of  $31.89 \pm 0.97$ . The best performing families according to mean live weight are families 38, 35a, 29b, 37a, 31 and 30b in an ascending order; these being the poorest performing families in terms of mean canning yield percentage. This concludes that heavier abalone produce lower canning yields. This trend could be explained by a hypothesis that abalone are heavier due to larger shells and/or visceral mass, that once removed, result in lower than expected canning yields. Table 15 shows families with the highest mean shell weight correspond to those with the highest live weight and once again the lowest canning yield percentage.

Further inspection reveals that mean live weight and mean post-shuck weight are highly correlated ( $r = 0.95$ ) and also, to a smaller extent, mean live weight and mean shell weight ( $r = 0.80$ ). In section 4.2.1, Table 16 also reveals that the mean post-shuck weight (shell removed) and mean post-gut weight are highly correlated ( $r = 0.92$ ). The correlation between the mean post-shuck weight (adductor foot and viscera) and the mean shell weight is however much lower ( $r = 0.57$ ). Once the viscera is removed, the correlation between the mean post-gut weight and the mean shell weight is higher ( $r = 0.62$ ). This indicates that the presence of the viscera, which include the respiratory system, the digestive system and the reproductive system, is responsible for lowering the correlation due to variation that is caused by one or more of the visceral systems. The data that was collected does not allow further investigation to distinguish which system causes the most variation. The digestive and respiratory systems will however vary proportionally as the live weights of abalone vary. The reproductive system could be the main source of variation due to age difference (range 109 days) which affects sexual maturity of the gonads. The canning trial was completed during August which corresponds to the start of *H. midae*'s spawning season. The gonads of all abalone are expected to respond to the incumbent spawning season. The fact that the degree of sexual maturity could vary due to an age difference could however explain the possible variation which could lead to a decreased correlation. As discussed in Section 4.1.2 there is no significant difference between gender in terms of final canning yield percentage. Those results do however not indicate the degree of variation of visceral weight within the genders male and female separately. Large variation in visceral weight could therefore result in the overestimation of canning yield.

A ratio between the shell weight and post-gut weight (SW: PGW) of the individual abalone has also been included in Table 15 to establish whether a heavy shell can be misleading in terms of the canning yield percentage. There is however no clear trend to suggest that the ratio increases as the mean shell weight and mean live weight of the different families increases. Also no trend is detectable when the ratio is compared to the canning yield

percentage. The use of a ratio as selection criterion to predict canning yield percentage is therefore of no use as families that produce higher canning yields can contain abalone with heavy or light shells.

Abalone are commercially sold per kilogram which means the nett yield should also be taken into consideration before decisions on selection strategies are made only based on the canning yield percentage. The nett yield was calculated based on the family averages of live weight and canning yield percentage presented in Table 15. The graphical depiction in Figure 13 clearly indicates that a higher live weight produces a lower canning yield percentage, in as much as the two curves are almost exactly symmetrical. Based on these results only, the suggestion would be, to rather select for families that have smaller abalone and produce higher canning yields, as previously mentioned. The nett canning yield in grams is however much higher in families that have higher live weights, even though the canning yields are lower. This trend shows that selecting for heavier live weights would still be more profitable compared to selecting for higher canning yields, as the nett yield of abalone meat is still higher.



**Figure 13:** The live weight (g) of abalone in relation to canning yield (%) and nett yield (g) based on family averages.

## 4.2 Correlation studies

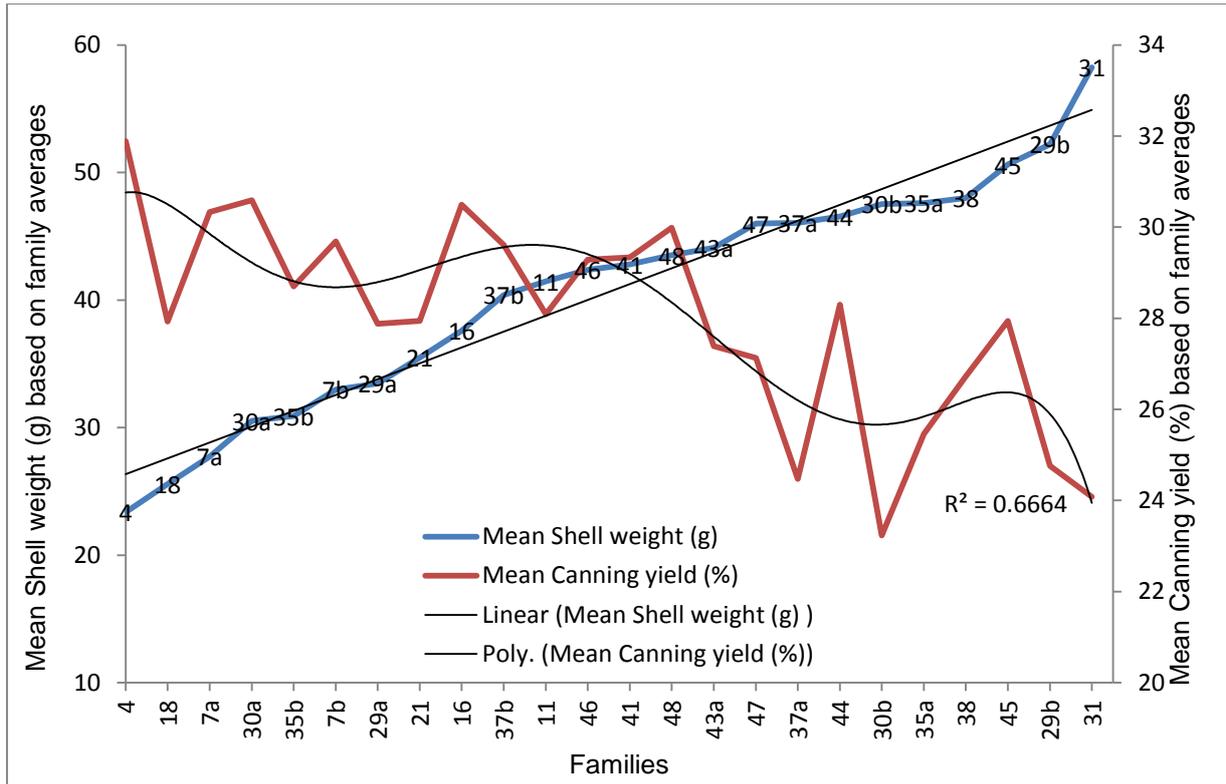
### 4.2.1 Phenotypic correlation

Phenotypic correlation can be explained as an estimated association between two observed traits within the same animal or a population and is a combination of genetic and environmental effects (Lewer, 2005; Searle, 1961). Correlation between traits was calculated to establish whether significant relationships and trends exist. Pearson's correlation coefficient was used to calculate the values. Under the null hypothesis that there is no correlation between the traits investigated, probabilities less than 0.05 will be statistically significant so that correlations, positive or negative will exist.

The results summarized in Table 16 indicate that there are very strong, statistically significant, positive correlations between the six weight measurements that were recorded during the canning trial. Live weight measurements correlate well with all the steps of the canning process with  $r$ -values of above 0.90. Live weight can therefore be predictive of the post-canning weight of the abalone as a correlation of 0.90 suggests that both values will be high, relative to each other.

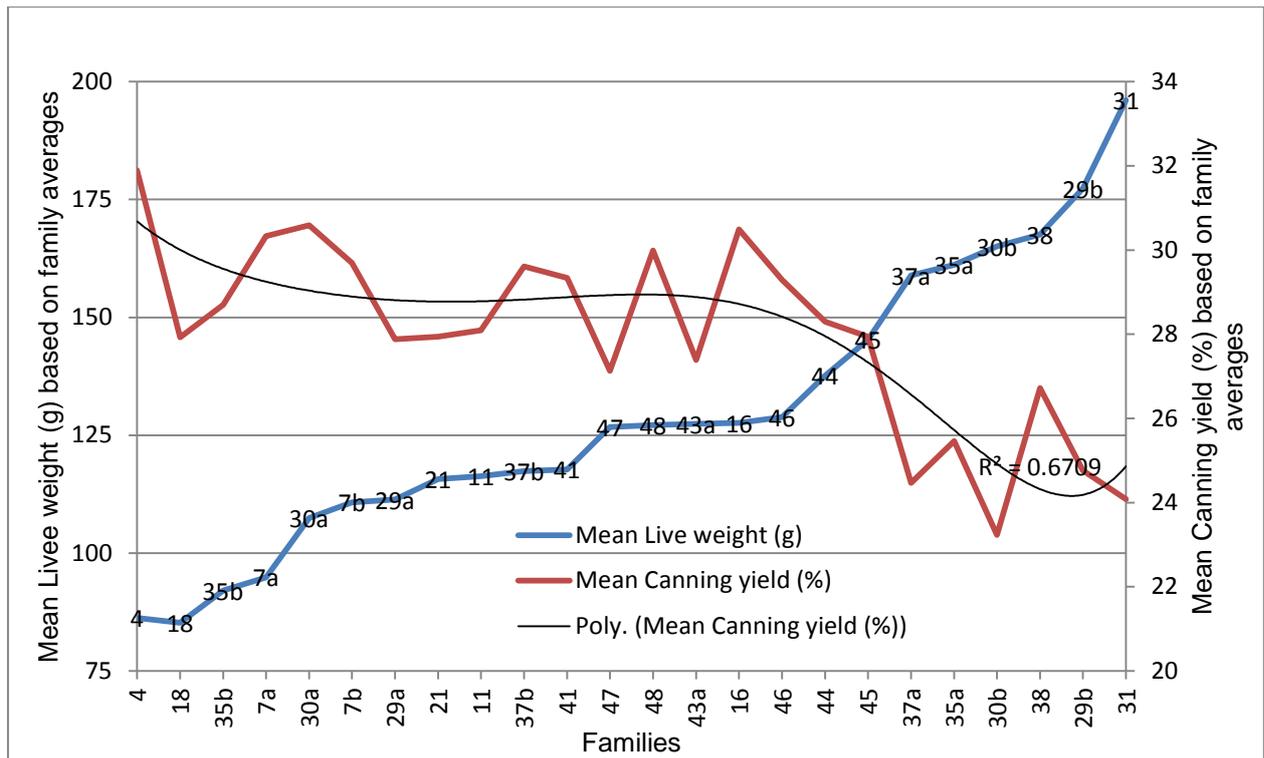
Mean shell weight correlates well ( $r = 0.80$ ) with mean live weight and confirms the trend that was identified amongst the families in Table 15 (Section 4.1.5). The lack of trend present in the SW: PGW ratio is also confirmed by a correlation of 0.03. Further evidence is presented in Table 15 (Section 4.1.5) where no trend is apparent between mean family live weights and the mean SW: PGW ratios. Other correlations that involved the SW: PGW ratio were either weakly negative or statistically insignificant. The use of a ratio as part of selection index to improve canning yield is therefore not beneficial.

The final trend that was observed in Table 15 (Section 4.1.5) is also confirmed by correlation studies. There is a relatively low, yet significant, negative correlation between canning yield percentage and both, shell weight ( $r = -0.24$ ) and live weight ( $r = -0.38$ ). This confirms that heavier abalone have lower canning yields. Determining a maximum threshold, in terms of either both the shell weight and the live weight which allows maximum canning yields could be beneficial. This would imply that the canning yield should show a quadratic response (bell-shaped curve) as the shell weight or the live weight increase. Graphical figures were used to establish the possible nature of such a curvature. The coefficient of determination ( $R^2$ ) was used as an indicator of the goodness of fit. The curve that produced the highest  $R^2$  value was included in the graphical illustrations.



**Figure 14:** The relationship between the family averages of the mean shell weight and the mean canning yield. ( $R^2$  = Coefficient of determination).

In both Figures 14 and 15 no sign of a quadratic curve is detected. Polynomial equations best described the response of mean canning yield by producing the highest possible  $R^2$  values of 0.6664 and 0.6709 respectively. This indicates that a threshold value to limit shell weight or live weight will not be useful as an absolute indicator that canning yield will only decrease from a specific point onwards. Absolute mean canning yields still show too much variation even though a relative decrease in canning yield is observed that will reduce the accuracy of this selection method.



**Figure 15:** The relationship between the family average of the mean live weight and the mean canning yield. ( $R^2$  = Coefficient of determination).

Van Schalkwyk, (2011) reported a strong positive correlation ( $r = 0.94$ ) between shell length and live weight. Kawahara *et al*, (1997) also confirmed this correlation with research that they did on *H. discus hannai* where 13 month juvenile shell length correlated well with the shell length and whole body weight of abalone at the age of 84 months. Length is a function of weight so that the same uniform object/material with a constant density will increase in weight when the surface area (length x width) increases. Shell length can therefore be used for selection purposes to adjust shell weight which is sufficiently correlated with live weight to enable a selection response in the canning yield. Shorter shell lengths, which produce lower live weights, would rather be suitable to increase the canning yield percentage as can be seen by the performance of family 4 in Table 15 (Section 4.1.5). Other families that reflect the same trend include families 16, 7a and 30a.

Due to the strong positive correlation between shell length and shell weight it can be assumed that the mean canning yield response to an increasing mean shell length will be similar to what is depicted in Figure 14 and a threshold value as part of a selection strategy will not help to increase canning yield percentages. Selecting for shell length to increase live weight will not have a positive effect on canning yield percentage. A longer shell length increases live weight which decreases canning yield. Van Schalkwyk, (2011) suggest this with a correlation of  $r = 0.83$  between shell length and canning loss percentage. Shell length

does however yield the highest heritability value ( $0.48 \pm 0.15$ ) when compared to other yield-related traits studied in this canning trial and must, for this reason, still be considered (Lucas *et al*, 2006).

If the results are evaluated without the aim to increase the canning yield percentage, a different conclusion can be made. As discussed in Section 4.1.5, when rather focussing on the mean nett yield of the families (Section 4.1.5., Figure 13), the negative correlation between the live weight and the canning yield percentage is less important. A statistically significant positive correlation ( $r = 0.66$ ) allows nett yield to increase as the live weight increases, despite the significant decrease in canning yield. This correlation is of moderate strength, though much higher and more advantageous compared to the respective correlations of live weight, canning yield and the SW: PGW ratio with one another.

The strength of the correlation between nett yield and the weight parameters measured during the canning process also increases, with post-canning weight having the highest correlation ( $r = 0.87$ ) (Table 16). The high phenotypic correlation ( $r = 0.90$ ) between live weight and post-canning weight (Table 16) also supports this interpretation by predicting a high final dressing weight.

**Table 16:** Phenotypic correlation between different canning stages or parameters measured during the canning process.

	<b>SW</b>	<b>PrS</b>	<b>PtS</b>	<b>PG</b>	<b>PB</b>	<b>PrC</b>	<b>PtC</b>	<b>SW:PGW</b>
Pre Shuck (PrS)	0.80*							
Post Shuck (PtS)	0.57*	0.95*						
Post Gut (PG)	0.62*	0.91*	0.92*					
Post Brine (PB)	0.70*	0.93*	0.90*	0.89*				
Pre Canning (PrC)	0.67*	0.93*	0.90*	0.89*	0.99*			
Post Canning (PtC)	0.64*	0.90*	0.87*	0.86*	0.98*	0.99*		
Nett Yield (NY)	0.58*	0.66*	0.68*	0.66*	0.74*	0.72*	0.87*	
Shell weight to post-gut weight ratio (SW:PGW)	0.01	0.03	-0.25*	-0.24*	-0.05	-0.05*	-0.06*	
Canning Yield percentage (% CY)	-0.24*	-0.38*	-0.32*	-0.25*	-0.08*	-0.06*	0.04	-0.26*

\* Pearson's correlation coefficient statistically significant ( $p < 0.05$ ). SW = Shell weight.

#### 4.2.2 Genotypic correlation

Genotypic correlation is the estimated association between the breeding values of two traits within the same animal, a family or a whole population. It is indicative of the proportion of genes that affect the two traits, negatively or positively (Lewer, 2005). It is difficult to measure genetic correlations but it is important to allow the necessary understanding of coordinate evolution through correlated responses to selection (Cheverud, 1988). Genotypic correlation arises due to the existence of pleiotropy, where single genes have effects on multiple traits, and also linkage disequilibrium, where the association between alleles at two different loci appear non-randomly (Cheverud, 1988). Genotypic approximations were calculated using AS Reml software. Covariance ratios were used to approximate these genotypic correlations between the relevant parameters and are displayed in Table 17.

The six weight parameters, live/pre-shuck, post-shuck, post-gut, post-brine, pre-canning and post-canning weight are all very highly correlated with each other and range from  $r = 0.97$  to  $r = 0.99$ . This is expected, as expression from the same polygenic group is responsible for a single quantitative trait, in this case weight, measured in a step-wise fashion to create six related parameters. Comparing these values of genotypic correlation with the corresponding phenotypic values shows that they are more strongly correlated with less variance of absolute values. Phenotypic correlations (Section 4.2.1., Table 16) show more variance and are not always so strongly correlated due to environmental effect that interacts with the genotype. The strong positive genotypic correlation (Table 17) between live weight and post-canning weight ( $r = 0.97$ ) also provide additional accuracy to the prediction that a higher live weight will deliver a higher post-canning weight and this allows selection for larger abalone to commence, even though a negative correlation with the canning yield exists.

When looking at the correlation of the six weight parameters with the shell weight to post-gut ratio, the correlation values range between  $r = 0.45$  and  $r = 0.58$  which are moderately positive correlations. The standard error of these correlations are however very high. Correlation values fall, with a 95 % confidence, within the largest interval of  $[\chi \pm 0.48]$ , where  $\chi$  represents the different parameters, and this reduces the accuracy of the correlation values and accepts such a range of values that a very strong positive trend could be mistaken for a very weak positive trend. The phenotypic correlations (Section 4.2.1., Table 16) could once again shed some light on the situation by firstly supporting the former and secondly indicating that the genotypic correlations could be an overestimation, even with the lowering effect that environmental interaction has on the genotype. These correlations would

therefore not be useful in as much that the SW: PGW ratio would not be advisable to include as a trait for selection to increase canning yield.

When comparing all the parameters in Table 17 to the canning yield percentage it is clear that there are no correlations that could have a significant positive effect on the canning yield percentage. Once again, the standard errors are high and reduces the validity of the values. The genotypic approximations can however be used to confirm trends that were identified phenotypically (Section 4.2.1). There is a negative correlation between the live weight and the canning yield percentage. The remaining weight parameters in Table 17 show weak positive correlations with the canning yield percentage, which could be misleading, were it not for the large standard errors which again indicate that the negative phenotypic correlations in Table 16 (Section 4.2.1) are still accepted. The genotypic approximation of the correlation between the mean SW: PGW and the canning yield percentage is a strong negative correlation and confirms the theory that a larger SW:PGW will decrease the canning yield percentage even though no conclusive evidence of such a trend could be found in this sampled population of *H. midae*.

**Table 17:** Approximations of heritability values  $\pm$  SE (on the diagonal) of, and genotypic correlation  $\pm$  SE (above the diagonal) between the different canning stages or parameters that was measured during the canning trial.

	<b>PrS</b>	<b>PtS</b>	<b>SW</b>	<b>PG</b>	<b>PB</b>	<b>PrC</b>	<b>PtC</b>	<b>SW:PGW</b>	<b>% CY</b>
Pre Shuck (PrS)	0.20 $\pm$ 0.06	0.99 $\pm$ 0.01		0.97 $\pm$ 0.02	0.98 $\pm$ 0.01	0.98 $\pm$ 0.01	0.97 $\pm$ 0.02	0.58 $\pm$ 0.20	-0.11 $\pm$ 0.29
Post Shuck (PtS)		0.15 $\pm$ 0.05		0.97 $\pm$ 0.02	0.99 $\pm$ 0.01	0.99 $\pm$ 0.01	0.98 $\pm$ 0.01	0.47 $\pm$ 0.24	0.02 $\pm$ 0.30
Shell weight (SW)			0.16 $\pm$ 0.05						
Post Gut (PG)				0.15 $\pm$ 0.05	0.97 $\pm$ 0.02	0.98 $\pm$ 0.01			0.10 $\pm$ 0.29
Post Brine (PB)					0.19 $\pm$ 0.06	0.99 $\pm$ 0.00			0.11 $\pm$ 0.29
Pre Canning (PrC)						0.19 $\pm$ 0.06	0.99 $\pm$ 0.00	0.45 $\pm$ 0.24	0.11 $\pm$ 0.29
Post Canning (PtC)							0.21 $\pm$ 0.06	0.45 $\pm$ 0.24	0.14 $\pm$ 0.29
Shell weight to post-gut weight ratio (SW:PGW)								0.09 $\pm$ 0.04	-0.74 $\pm$ 0.15
Canning Yield percentage (% CY)									0.08 $\pm$ 0.03

### 4.3 Heritability estimation

Approximations which represent heritability estimates are displayed on the diagonal in Table 17, Section 4.2.2. These estimates were calculated by using between-family variance ratios.

The heritability of canning yield percentage is the lowest of all the estimated values at 0.08 (Table 17, Section 4.2.2). The potential to allow a genetic response is therefore limited. Compared to other studies (Table 6) done on ectotherms, this value seems to be within in range and can be accepted although the heritabilities that were estimated in those studies were specifically sire and dam components of heritability and cannot be compared directly (Gjedrem, 1997). The heritability value of 0.08 is also, as predicted by Mousseau & Roff, (1987) lower than what would be expected in endotherms. The large amount of variance could however be due to the fact that three different species are being compared. Selection based on canning yield could be beneficial but would prove difficult as canning yield can only be determined after abalone with genetic potential have been processed and are no longer able to reproduce. It would therefore be more beneficial to use genetic parameters such as genotypic and phenotypic correlations to find yield-related traits that are predictive of canning yield, with a high degree of accuracy i.e. strong positive correlations. Reproduction can therefore occur before selected abalone are committed, and lost, to the canning process.

The seven approximations of heritability to represent the weight-related traits range between 0.15 and 0.21. Approximations made by Van Schalkwyk, (2011) show similar results with a range between 0.15 and 0.20. These values are low to moderate heritabilities and can show genetic response to selection. The parameters with the highest estimates of heritability are the live weight ( $0.20 \pm 0.06$ ) and the post-canning weight ( $0.21 \pm 0.06$ ) which are the first and the last stages of measurements that were recorded during the canning trial. The other parameters have lower heritability estimates and would therefore not be chosen and included as part of a breeding programme. The post-canning weight has the highest heritability approximation but would not be the best parameter to include as a direct selection trait. If selection is done based on the post-canning weight of abalone, already processed abalone that remain unselected would only be an unnecessary financial expenditure.

Of all the mentioned parameters the live weight would be the trait to use in a selection programme. Comparing the standard error of these heritability values to the standard errors reported with genetic correlation provides a much increased accuracy and improves the credibility of the results. An interval, with 95% confidence, of  $[\chi \pm 0.12]$  can be assumed based on the standard error associated with the live weight. Lucas *et al*, (2006) did report a heritability of  $0.36 \pm 0.13$  for live weight as a trait. Even though the absolute heritability

values show a large difference, the standard error indicates that the values, from a statistical viewpoint, could be much closer and similar. Other statistical parameters such as the coefficient of variation (CV) could also be used to determine whether enough variance is available within this specific trait to allow a reasonable genetic response when selection pressure is applied. Van Schalkwyk, (2011) does however report heritability approximations for weight from two different trials (a growth trial and live export drip loss trial) of  $0.15 \pm 0.05$  and  $0.11 \pm 0.04$ . The variation of heritability values for this trait is relatively large and therefore does not necessarily promote the idea that the trait should be used as a selection tool to increase the commercial production in a structured breeding programme.

As mentioned before, live weight and shell length is highly correlated. It could therefore be beneficial to use shell length as selection trait to improve production efficiency. Based on heritability values presented in Table 5 (Section 2.2.2) a greater genetic response to selection is possible if shell length is used as an indirect selection trait. Lucas *et al*, (2006) reported a value of 0.48 which makes shell length a highly heritable trait. It must however be said that this value was calculated from a sample population with a mean age of 12 months. When looking at values that were presented by Kube *et al*, (2007) the heritability of shell length decrease with age and was finally measured at 0.04 at a growth period of 32 months. This observation is discouraging as this canning trial used abalone that were reared and grown over a period of 62 months which could mean that the mentioned value could be lower. Shell length could therefore be a valuable selection tool but age could be an important factor that needs consideration.

The mean SW: PGW ratio produced a low heritability of 0.09. This means that this parameter will also be an ineffective way to increase abalone production through genetic selection. Phenotypic and genotypic correlation established that the ratio shows no trend and cannot be the reason why heavier abalone yield smaller canning yield percentages. This parameter would also be difficult to estimate as animals would have to be committed to the canning process to measure different components of the ratio. The only found literature that relates to this trait was published by Kube *et al*, (2007) and they determined the heritability of meat weight of abalone after a growth period of 38 months. This meat weight trait, with a heritability of 0.10, (Table 5, Section 2.2.2), lies close to the post-gut approximation of heritability ( $h^2 = 0.15$ ) that was determined during this canning trial (Table 17, Section 4.2.2). The other component, the mean shell weight's heritability was also determined during this canning trial and values at 0.16. Although these traits are components of the ratio and cannot be compared directly to the ratio, they help to reflect on the accuracy of the approximations.

## 5 Conclusion

The South African farmed abalone, *Haliotis midae*, has become a highly valuable and well established product on international markets. The need for the genetic improvement of this undomesticated species is becoming increasingly important to ensure sustainability of the industry and competitiveness on global markets.

To date all emphasis in terms of genetic work has been placed on improvement of growth characteristics. As up to 50% of product is exported in a canned form, canning yield has been identified as a further criterion with direct economic impact. A canning trial was subsequently initiated to determine the genetic parameters for a series of traits related to canning yield. This was done to assess the feasibility of the inclusion of canning yield into the genetic improvement strategy.

The following conclusions were made:

- No significant difference was observed in the live weight and canning yield between male and female abalone, therefore no particular gender offers any advantage over the other in terms of canning yield. The observed variation in canning yield within-gender could be attributed to variation in gonadal size which is affected by the age and sexual maturity of the individuals. Removal of internal viscera and the reproductive system reduces the variance observed in the canning yield within the two genders.
- Significant differences were observed in the canning yield of families, based on sire and dam phenotypic averages. Specific individuals who produced progeny with statistically significant phenotypic superiority could be identified. Sire and dam components of heritability could however not be separated due to the impairment caused by shallow genealogical histories of parental individuals. Superior families could therefore be identified to supply future brood stock to commercial hatcheries but only on the basis of family averages.
- The phenotypic ( $0.95 \geq r \geq 0.66$ ) and genotypic ( $0.98 \geq r \geq 0.97$ ) correlations between live weight (PrS) and the subsequent canning traits (Table 16 and 17) remain positive throughout. An increase in live weight will therefore lead to an increase in nett yield. Live weight, however, displays a negative phenotypic ( $r = -0.38$ ) and genetic correlation ( $r = -0.11$ ) with canning yield as a percentage (Table 16 and 17). It raises the question whether heavier animals develop a larger shell, relative to meat ratio. An analysis of the shell to meat ratio (SW: PGW) reveals no trend in relation to an increase in live weight

(Figures 14 and 15). This means that there is no conflict with the improvement of canning traits in relation to live weight.

- All heritability values that were determined in the canning trial (Table 17) are low to moderate values and are within range when compared to other studies. They are however statistical approximations. The results indicate that selection for improved growth rate, i.e. increased live weight is expected to lead to a correlated improvement in nett canning yield (Figure 13).
- Throughout the South African abalone industry shell length is used as a managerial norm and an indicator of growth performance. The trait is genetically highly correlated with live weight ( $r = 0.94$ ) and literature reports a high heritability value of 0.48. Selection based on shell length will therefore lead to an increase in live weight and this approach is practically feasible in terms of operating procedures during commercial production.
- Canning yield percentage is not recommended as a trait for selection to improve commercial production and profitability due to its low heritability. Shell length should be used as a selection criterion due to strong positive correlations with growth-related traits and this will indirectly improve the nett canning yield. It is more important to increase the nett canning yield as appose to increasing the percentage canning yield of the species.

## Recommendation for further work

A base population (n=800) of sexually mature males and females was collected from their natural habitat and entered a conditioning period with the aim to spawn sufficiently to create a large family structure with many half sib families of similar size. The collection from a natural habitat meant that there was no information regarding the pedigree, genealogy or genetic origin of the individuals that were collected. An imposed time limit resulted in a lack of dams that could spawn sufficient volumes of gametes and a classical half sib crossing structure could not be created. Some half sib families were lost during the 62 month growth trial and, in some families, individual abalone were lost due to tag loss or mortality. These mentioned factors all contributed to the fact that a standard animal model could not be completed with the use of AS Reml. A half sib analysis was also not possible which affects the accuracy of the results obtained from the canning trial. The experimental design was unbalanced due to abalone losses which affected the standard error and the accuracy of the results. Location effects were significantly different but the inclusion of reference groups only allowed for variation between groups within a location and not across all the locations. The location effect could therefore not be reduced and environmental variance could not be eliminated completely. Different age groups were created as brood stock spawned and these groups were not spread across locations. This resulted in a confounding effect of age and location which means the variance due to these effects cannot be separated.

The ideal experimental design must allow a full pedigree, with sufficient half-sib families of the same size. The effects of location, in terms of diet and managerial practices, and age must be reduced to a minimum. A full sib reference group should be used which is large enough to accompany all the half sib families that are created, and all the families, including the reference group, must be the same age. The use of a reference group of sufficient size is of significant importance, especially if the location effect cannot be eliminated completely. The main reason to repeat this experiment would be to determine why higher live weight yields leads to lower dressing percentages. More attention can also be paid to weight loss during the gutting process by separating the different organ systems to see what the effect of; specifically the reproductive system is on the canning yield.

An assessment of environmental factors could also shed light on other non-genetic factors that affect the canning yield. Understanding and altering the components of nutrition could affect the ratio (SW: PGW) positively. Increasing protein anabolism could increase the mean post-gut weight of abalone so that the canning yield is increased. The effect of nutrition on calcium metabolism and shell formation can be also be investigated to see whether it is possible to spend less energy on this component of anabolism to lower the SW: PGW ratio and so increase the canning yield percentage. Abalone shells are however still vital to the wellbeing of the animal and the integrity of this protective structure can only be compromised within limits.

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