# Assessment of yield traits between family groups of the cultured abalone (*Haliotis midae*) in South Africa

by

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# Declaration

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# Abstract

The abalone *Haliotis midae* is the most important aquaculture species in South Africa. The industry is dependent on export to Far Eastern markets in a variety of forms, including live, frozen, canned or dried. The species is considered undomesticated in the sense that the current commercial broodstock has been obtained from natural populations through a process of random collection. Global competition has necessitated the South African industry to introduce a genetic improvement program to increase biological productivity and financial profitability.

The objective of this study was to assess the genetic variation and to estimate key parameters in terms of growth and yield related traits, between family groups that form part of the breeding program. The study reports on heritability estimates of growth rate (0.14 ± 0.05), canning yield (0.08 ± 0.03), and drip loss during live export (0.03 ± 0.02). The high genetic correlation (0.94 ± 0.34) between shell length and live weight enables industry to utilise either weight or shell length as a criteria during operational practices such as sorting, grading and harvesting. The correlation of 0.85 ± 0.01 between live weight and canning loss indicates that animals that weigh more have a lower dressing percentage.

Based on these low heritability values obtained for yield related traits it is recommended not to include these traits in the selection program at this stage. The findings of the study were however, compromised by the availability of a limited number of family groups, the age differences between families and the effect of different locations on the variance in phenotypes. Further investigation is needed to confirm the credibility of the results.

# Opsomming

Die perlemoen *Haliotis midae* is die belangrikste akwakultuurspesie in Suid Afrika. Die industrie is afhanklik van uitvoere na markte in die Verre Ooste. ‘n Verskeidenheid van produkte word uitgevoer, insluitend lewendige, gevriesde, gedroogte en verblikte perlemoen. Die spesie word as ongedomestikeerd beskou aangesien die huidige teeldiere op ‘n lukrake wyse uit natuurlike populasies versamel is. Globale kompetisie het die Suid Afrikaanse industrie genoodsaak om ‘n genetiese verbeteringsprogram in werking te stel om sodoende die biologiese produktiwiteit en finansiële winsgewendheid te verbeter.

Die studie poog om genetiese variasie in groei en opbrengsverwante kenmerke tussen familiegroepe wat deel uitmaak van die teelprogram te ondersoek. Oorerflikheid van groeitempo (0.14 ± 0.05), opbrengs na verblikking (0.08 ± 0.03), en vogverlies na lewendige uitvoer (0.03 ± 0.02) is beraam. Die hoë korrelasie (0.94 ± 0.34) tussen gewig en skulplengte stel die industrie in staat om beide massa en skulplengte as kriteria te geruik tydens operasionele praktyke van sortering, groepering en oes. Die korrelasie van 0.85 ± 0.01 tussen gewig en verlies na verblikking dui aan dat swaarder diere ‘n laer uitslagpersentasie het.

As gevolg van die lae oorerflikheidswaardes vir opbrengsverwante kenmerke word daar aanbeveel dat hierdie kenmerke nie op hierdie stadium ingesluit word in die seleksieprogram nie. Resultate is egter beïnvloed deur ‘n beperkte aantal familie groepe, ouderdomsverskille tussen families en die effek van verskillende

lokaliteite op die variansie in die fenotipes. Verdere ondersoeke is nodig om die geloofwaardigheid van die bevindinge te bevestig.

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# Contents

# List of Figures

# List of Tables

# Abbreviations and Symbols

| | |
|---|---|
| % | Percentage |
| (Pty.) Ltd. | Property Limited |
| < | Smaller than |
| ® | Registered Trademark |
| ℃ | Degrees Celsius |
| AFASA | Abalone Farmers Association of South Africa |
| FAO | Food and Agriculture Organization |
| HACCP | Hazard analysis and critical control points |
| Ltd. | Limited |
| MCM | Marine and Coastal Management |
| p | Probability as a statistically significant limit |
| pp. | Pages |
| QTL | Quantitative Trait Locus |

# Chapter 1 Introduction

## 1.1 South African Marine Aquaculture

Marine aquaculture is defined as the farming and culture of marine aquatic organisms, including fish, molluscs, crustaceans, and plants, in controlled or selected marine aquatic environments. Some form of intervention takes place in the rearing process to enhance production, such as regular stocking, feeding, and protection from predators (Department of Environmental Affairs and Tourism, 2007).

The South African coastline stretches over a distance of 2 500 km from the mouth of the Orange River on the west coast to Kosi Bay on the east coast, incorporating the Atlantic an Indian Ocean (Figure 1.1) (Harrison, 2002). The natural resources thus provides the potential to culture a wide variety of marine species in the diverse climatic conditions, ranging from the colder west coast, to the more tropical east coast (Shipton & Britz, 2007).

The availability of natural resources, increasing demand for fish products, overexploited fish stocks, and decreasing commercial fishery quotas, (Vosloo & Vosloo, 2006) are driving forces for the development of aquaculture in South Africa. Aquaculture is expected to contribute to socio-economic development of coastal areas through skills-based employment and income, as well as food security.

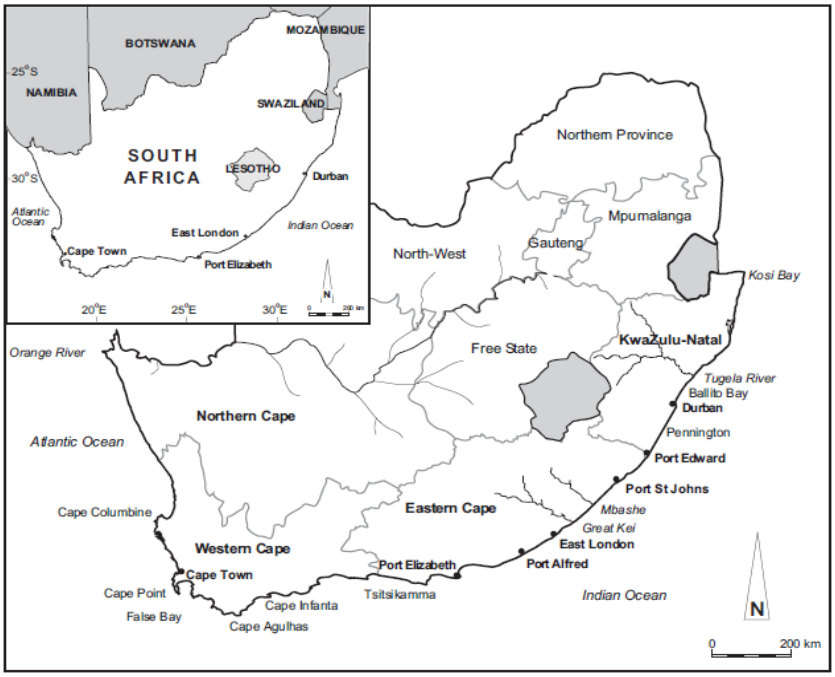


**Figure 1.1 Map of South Africa indicating the extended coastline (Adapted from Harrison, 2002).**

Aquaculture production in South Africa started in the 1980's with the establishment of oyster, mussel and prawn farming (Department of Environmental Affairs and Tourism, 2007). The South African aquaculture industry is mainly based on the culture of high value mollusc species such as abalone, oysters and mussels; as well as finfish species such as dusky kob, silver kob and yellowtail, together with prawns and seaweed (Shipton & Britz, 2007).

## 1.2 Abalone culture

Abalone have high commercial value and are important fishery resources worldwide. Aquaculture farming has been developed in 16 countries for 12 different *Haliotis* species to compensate for the high demand (Franchini *et al.*, 2011). In South Africa, the development of a viable mariculture abalone industry is the foremost success story of aquaculture in the country (Vosloo & Vosloo, 2006).

Abalone are inactive animals and easily accessible which has made illegal fishing a concerning factor for all abalone species (Franchini *et al.*, 2011). This fact, together with the strong demand and high prises on Asian markets, leads to overexploitation and a severe decrease in wild populations.

Abalone fisheries in South Africa have existed since 1949, but successful spawning of captured specimens only started in 1981 (Sales & Britz, 2001). Abalone farming started in the early 1990s (Sales & Britz, 2001) and by 1996 a few small operators had entered the industry. Production increased from a modest beginning of less than one ton in 1993 to about 900 tons in 2009 (Figure 1.2), 51 % of the total marine aquaculture production in South Africa (Department of Environmental Affairs and Tourism, 2007) and worth 31.1 million US dollars (Figure 1.3) (FAO, 2011). The South Africa abalone industry is still expanding and features as one of the top producers of farmed abalone, outside China.

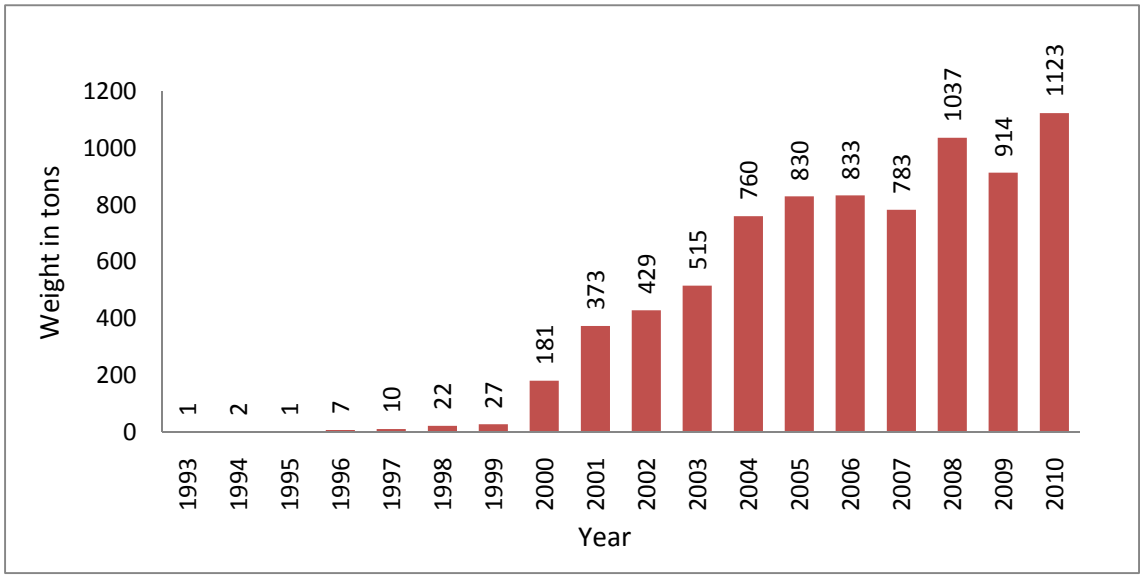


**Figure 1.2 Weight, in tons per annum, of farmed abalone in South Africa (Graph constructed by using data from the FAO, 2011).**

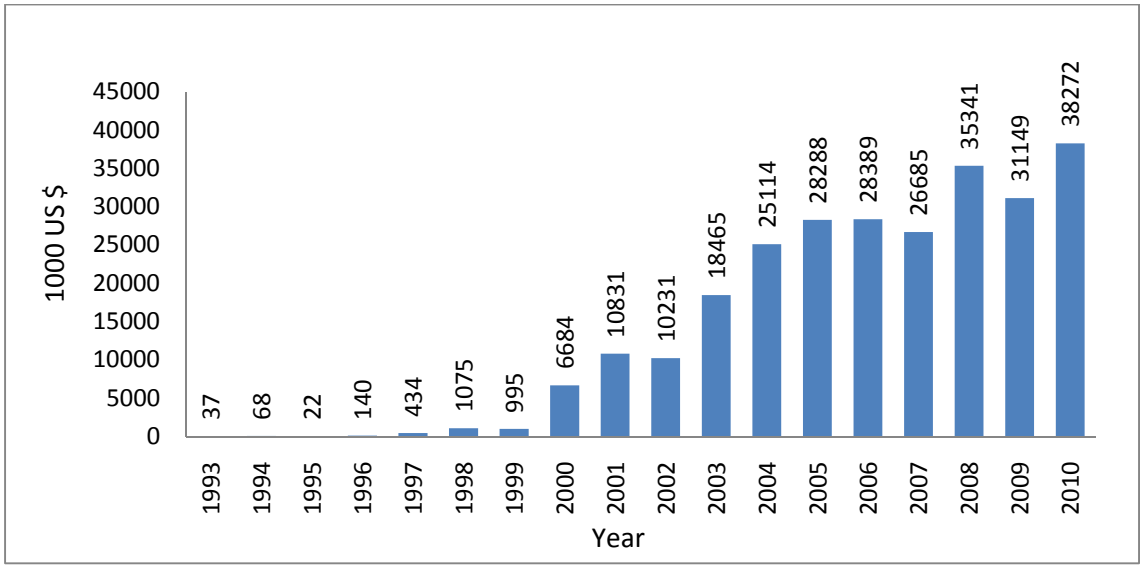


**Figure 1.3 Value, in US $ per annum, of farmed abalone in South Africa (Graph constructed by using data from the FAO, 2011).**

Most farms are located in the Western Cape. The Southern coastal area between Hermanus and Danger Point, and the Saldanha Bay / Sint Helena Bay area on the West coast, are most popular for abalone farming. Nonetheless, farms are located as far north as Port Nolloth in the Northern Cape, and as far east as Haga-Haga in the Eastern Cape.

Abalone culture in South Africa is land based and employs pump-ashore technology, combined with intensive recirculation culture systems. Considerable efforts to develop suitable culture technologies for the species have been made in the past two decades. Notable research efforts have addressed issues concerning system design, reproduction, nutrition, artificial feed development, and disease control (Britz, 1996; Bolton *et al.*, 2009; Mouton & Gummow, 2011).

The technology for artificial spawning of the animals has been developed. Spat are hatchery reared and grown out in tank systems. Initially, spat are cultivated on algal films. Once they are large enough they are weaned onto a diet

consisting of macro algae. Abalone are harvested after three to four years at about 85 mm to 100 mm shell length (Shipton & Britz, 2007).

Existing Infrastructure in South African abalone aquaculture includes HACCP certified processing facilities; pelleted feed; an air route for the live transport of abalone; a health management programme; production infrastructure; producers of purpose built tanks and baskets; a South African Molluscan Shellfish Monitoring Program through MCMs laboratories; research support from universities together with research initiatives on farms; and a well established producer association, AFASA, which represents farmers' collective interests. With this infrastructure in place, together with concerted efforts between the private sector and government-backed research institutions, South African abalone culture technology develops continually (Sales & Britz, 2001).

Abalone are one of the most highly valued seafoods in the world (Oakes & Ponte, 1996) and the biggest consumption is from Asian countries (Sales & Britz, 2001). Quality of the product depends on size, texture, and colour. Abalone species with lighter pigmentation of the foot command the highest prices; for sushi, a firm crisp texture is desired; while size preferences vary between consumers and product forms. Specific market needs can be met by abalone culturing, regarding size and shell colour (Oakes & Ponte, 1996). Abalone meat has a reputation for toughness, but when frozen immediately after shucking[1], it is tender, in comparison with red meat (Sales & Britz, 2001). Meat products include fresh, frozen, canned or dried forms of the muscle foot. Farmed abalone can be harvested at standardized sizes to meet specific

[1] "Shucking" is the process by which the shell of the abalone is removed from the soft body parts.

market needs and can be cultured for specific shell colour (Oakes & Ponte, 1996). The meat is used in traditional cuisine and ceremony, while shells are often used for jewellery (Sales & Britz, 2001).

Nucleation of abalone to produce pearls is not widely done, because of the haemophilic nature of the animals. However, with careful seeding, abalone can have no internal damage and produce rare pearls (Aquilina & Roberts, 2000).

## 1.3 Aims of this study

A consortium of producers within the South African abalone industry, in collaboration with Stellenbosch University, has started the first genetic improvement program for the local species in 2006. This was done in response to rising costs of production and increased global completion, amongst other Japan, Korea, China, Mexico, USA, Australia, New Zealand, Chile and other Asian countries which also started abalone culture in the nineties (Sales & Britz, 2001). The genetic improvement program is currently focussing on the improvement of growth rate as a trait of primary economic importance.

Traits of economic importance in abalone production, such as growth rate, feed conversion, meat yield, survival, and meat quality, are quantitative in nature controlled by a large number of genes, each with a small cumulative contribution to the phenotype. No quantitative genetic studies have to date been conducted on the indigenous species, *Haliotis midae,* and there is a need to evaluate these traits in terms quantitative genetic parameters such as phenotypic and additive genotypic variation, heritability and correlations upon which genetic improvement programs are based (Newkirk, 1980).

Artificial selection shifts the population mean into the desired direction, however it can only act when variance exists among individuals in the population. Selective breeding is based on controlled mating to increase the frequency of individuals with a desirable commercial trait in farm stocks (Falconer & MacKay, 1996).

As mentioned, the first genetic improvement program for the species was introduced in 2006 with the aim to exploit the inherent biological potential (Elliott, 2000), in particular to improve the growth rate of the South African abalone *Haliotis midae* under commercial culture conditions.

Apart from of growth rate, improvement of yield characteristics can also play a major role in the profitability of abalone farming. The objective of this study was therefore to assess the nature of differences between family groups in terms of a variety of yield traits and to estimate the key genetic parameters such as heritability, and phenotypic and genetic correlations that could form the basis of the structuring of a genetic improvement program.

Other traits that may be investigated in future include texture, taste, disease and stress resistance, survival rates, fecundity, and age at maturity in order to improve the economic viability of the commercial abalone industry.

## 1.4 Summary

Abalone aquaculture in South Africa is a well established industry and abalone is the most important aquaculture product in the country. The cultured species is considered undomesticated since the current generation of commercial broodstock is obtained from natural populations. This study is part of a strategy that aims to improve the productivity of the local industry through genetic

enhancement, by means of a selective breeding program. When significant heritability exists for a trait, selective breeding will shift the mean phenotype of the offspring in the desired direction, by breeding with superior individuals. This is of particular importance for the local industry to remain competitive on global markets, more so in view of recent genetic enhancement programs introduced by competitors such as Australia, Japan, China, and Taiwan. Australia is a leader in the field of selective breeding programs of abalone with great success (Lucas *et al.*, 2006, Kube *et al.* 2007; Li, 2008; Hamilton *et al.*, 2009).

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# Chapter 2 Literature review

## 2.1 The South African Abalone (Haliotis midae)

### 2.1.1 Evolutionary Classification

Abalone are marine gastropods from the only genus in the Haliotidae family, *Haliotis*. *Haliotis* is part of the largest phylum in marine waters, Mollusca, which include 23 % of marine animals. It is the second largest phylum, after Arthropoda in the animal kingdom. Mollusca is a widespread phylum and comprise of between 50 000 and 80 000 living species and 35 000 fossil species from marine, freshwater and terrestrial environments (Van der Merwe, 2010). The word 'Gastropoda' is derived from Ancient Greek with the literal meaning 'stomach foot'. The class bears this anthropomorphic name owing to the appearance that the animals move on their stomachs. However, the viscera of gastropods is usually dorsally situated and often covered by the shell. Table 2.1 presents the taxonomic classification of *Haliotis midae*. (Elliott, 2000; Ragg, 2003; Van der Merwe, 2010).

**Table 2.1 Taxonomic classification of the South African abalone (Van der Merwe, 2010; Ragg, 2003).**

| | |
|---|---|
| **Kingdom** | Animalia |
| **Phylum** | Mollusca |
| **Class** | Gastropoda |
| **Family** | Haliotidae |
| **Genus** | *Haliotis* |
| **Species** | *Midae* |

### 2.1.2 Habitat, Distribution, Ecology

Abalone of 56 extant species are found in both tropical and temperate waters (Elliott, 2000; Franchini *et al*., 2011). Six *Haliotis* species are found in Southern African waters. Figure 2.1 illustrates the distribution of the six species (*H. midae, H. parva L., H. spadicea, H. queketti, H. speciosa* and *H. postulata*) around the Southern African coastline (Sales & Britz, 2001; Van der Merwe, 2009). *H. midae* is the only local species with acceptable market characteristics such as size, colour, and taste that occur in numbers sufficient to be commercially exploited (Sales & Britz, 2001; Franchini *et al*., 2011).

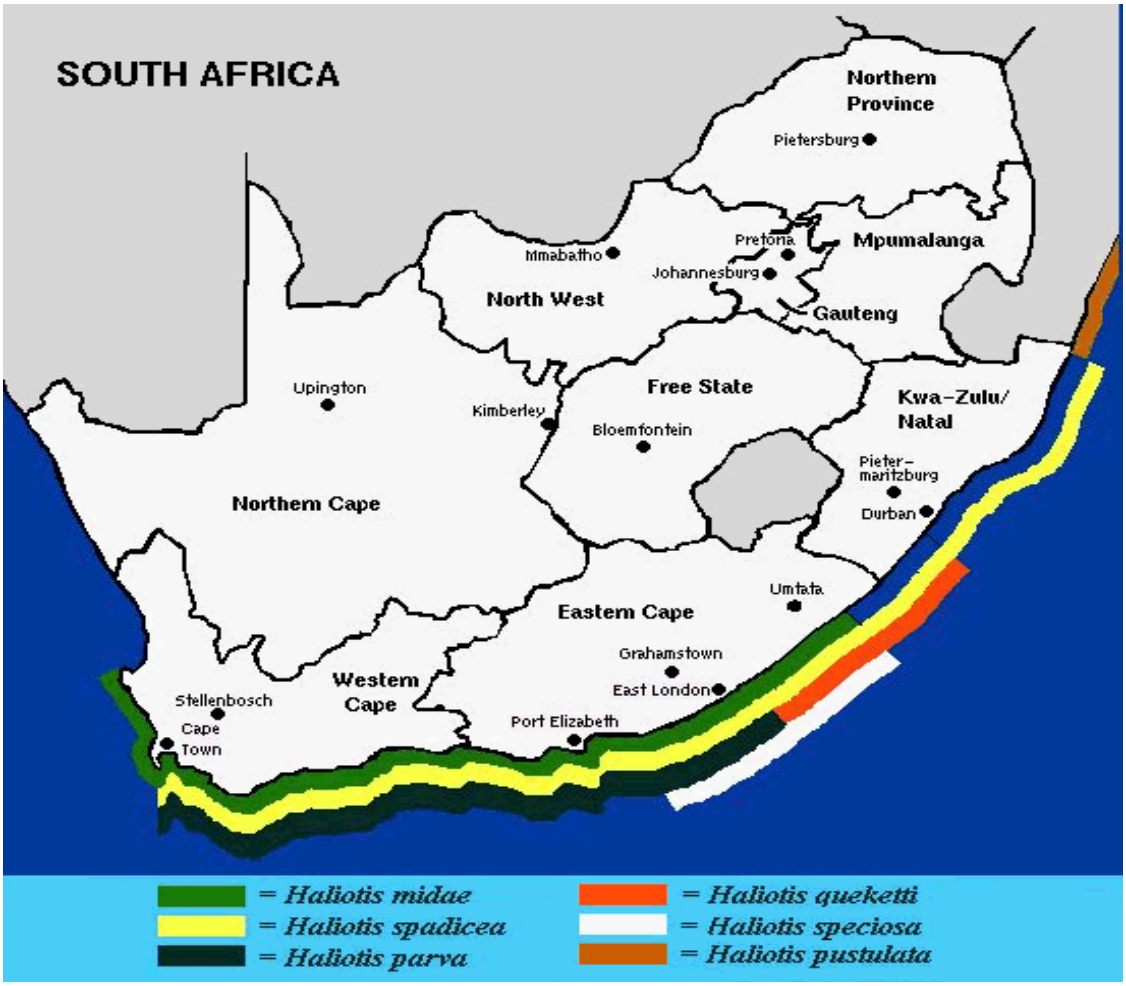


**Figure 2.1 Map of Southern Africa, indicating the distribution of the six different indigenous abalone species (Van der Merwe, 2009).**

The distribution of *H. midae* spans the confluence of the Atlantic and Indian Oceans over a wide temperature range from 12 ℃ to 21 ℃. They are found from the cold waters of the Benguela upwelling system on the Western Cape coast to the warmer Eastern Cape coast, which is influenced by the southward flowing Agulhas current (Sales & Britz, 2001; Vosloo & Vosloo, 2006). Their natural ecological niche is the high energy, sub tidal zone, where they live on rocks or in kelp forests, permanently covered with sea water. They exhibit nocturnal grazing patterns and mostly remain inactive during the day (Sales & Britz, 2001). In the wild, their sedentary lifestyle along shallow, rocky coastlines exposes them to be captured easily (Franchini *et al.*, 2011).

### 2.1.3 Biology and anatomy

Abalone (Figure 2.2) are univalve (single-shelled) marine gastropods (Elliott, 2000). The shells of abalone are characterised by a low and open structure that spirals outwards from the apex.

**Figure 2.2 South African abalone, Haliotis midae (Photograph: Gert le Roux) (Van der Merwe, 2009).**

Several respiratory pores of increasing size, from the posterior side (at the apex) to the anterior side, exist on the outer edge of the shell and new pores are formed as the animal grows. Abalone are permanently attached to their shells in the centre. The shell is formed in the larval stage and serves as protection against predators throughout life. The inner layer of the shell consists of colourful mother-of-pearl.

On the dorsal side is the very strong, flat muscle foot which is used for movement and to attach to surfaces. Abalone have the ability to pull their shell down rapidly and tightly onto the substratum if they are disturbed (Sales & Britz, 2001). It is then extremely hard to remove them from the surface. Along the edge of the muscle foot, the epipodium is found. When the animal is relaxed, the epipodium and epipodial tentacles are often visible around the shell edge. These are no longer seen when the abalone is disturbed with its shell clamped down.

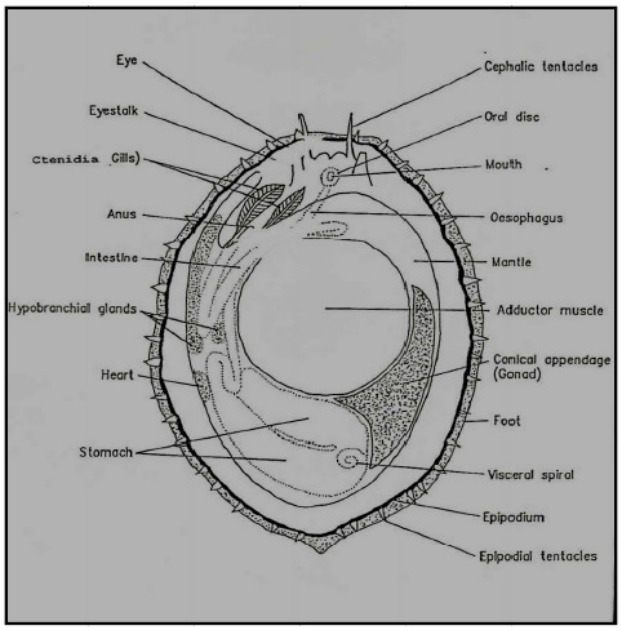


**Figure 2.3 Ventral view of organs and soft body parts of the abalone (Van der Merwe, 2010).**

The digestive, respiratory, circulatory and reproductive organs are circled under the shell, around the muscular foot (Figure 2.3). The head and mouth is on the side of the apex and the anus is under the last open hole, at the end of an opening in the mantle (Anderson, 2003).

The abalone possesses two kidneys with different functions. Ultra-filtration through the artrial walls is only possible when haemolymph pressure outweighs the colloidal osmotic pressure of the haemolymph. Therefore, a reduction in haemolymph pressure will decrease urine production until it stops completely. After filtration through the artrial walls into the pericardium, the primary filtrate is altered by active secretion of the right kidney and reabsorption of the left kidney (Vosloo & Vosloo, 2006).

Abalone have sensitive oral tentacles and light sensitive eyes to sense the surroundings. Inside the mouth, a large number of slender teeth are placed in each transverse row on the radula and the abalone ingest with a licking action. The radula is protruded and the toothed surface is pressed against the algal food source, which scrapes off fine particles. The radula is then pulled back into the mouth (Purchon, 1977).

Reproductive organs are internal. The eggs of the females are coloured green, while male sperm are beige. This colour is visible in sexually mature animals when the epipodium is lifted away from the shell. Abalone are broadcast spawners and reproduce by releasing copious amounts of gametes into the ocean (Anderson, 2003).

Sperm and eggs leave the reproductive organs through a small duct that leads to the open holes of the shell. This duct opening is close to the anus. In

addition, gills are located behind the head near the shell holes. Furthermore, directly under all the open holes in the shell is a slit in the mantle, which is lined with beating cilia that create continuous water flow from the head, over the gills, past the anus and out through the shell holes. This process provides clean oxygenated water for the gills, and constantly removes waste from the anus without delay. Gametes of reproducing abalone are also released in this area and washed out through the shell holes by this respiratory current (Anderson, 2003).

Gastropods have an open circulatory system (Hooper *et al.*, 2007) with clear haemolymph (Jorgensen *et al.*, 1984). The haemolymph is pumped by muscular contraction of the heart and circulates through the body and gills and transport oxygen and carbon dioxide. An open circulation system exists, but circulation is tissue specific. Haemolymph cannot coagulate and wounds are usually fatal (Ragg & Taylor, 2006).

Gas exchange takes place in the body between tissue cells and haemolymph; and in the gills, gas exchanges between water and haemolymph to keep the abalone oxygenated (Anderson, 2003). Abalone has the ability to respire in air and can survive emersion for long time periods, despite the inability to dampen their gills (Wells and Baldwin, 1995; Bubner *et al.*, 2009). Although respiration is not optimal during aerial exposure, it is a valuable trait for the live abalone export market, where abalone are exposed to air for up to 40 hours (Vosloo & Vosloo, 2006).

### 2.1.4 Immune system and stress

Marine invertebrates have no acquired immune system and therefore rely entirely on their effective and robust innate immune systems (Tincu & Taylor, 2004). The immune system of abalone has both humoral and cellular components. Antimicrobial factors in the haemolymph of molluscs are synthesized by haemocytes (Hooper *et al.*, 2007). Cellular responses are also associated with the spontaneously moving haemocytes, which phagocytise microbes (Tincu & Taylor, 2004). Humoral and cell mediated immunity are consequently linked (Hooper *et al.*, 2007).

Haemocytes form the internal defence lineage and have the most important role in the immune system of molluscs. They are responsible for chemotaxis, antigen and pathogen recognition (Tincu & Taylor, 2004), attachment followed by agglutination, phagocytosis, and elimination and encapsulation of invaders by respiratory burst or exocytosis of antimicrobial peptides. Molluscan haemocytes also perform physiological functions such as the transport and digestion of nutrients, and excretion; and play a role in wound or shell reparation (Hooper *et al.*, 2007; Travers *et al.*, 2009). Haemocytes migrate, attach to the invader, phagocytose, and finally kill in successful immune responses (Travers *et al.*, 2009).

Momentary haemacytopania (reduced haemocyte count) is observed in stressed abalone but it is not clear whether they undergo lysis or leave the haemolymph in a coordinated response (Hooper *et al.*, 2007). Hooper *et al.* (2011) examined immune parameters (haemocyte counts), to evaluate sensitivity of two year old hybrids of *H. laevigata* and *H. rubra* farm stock to anaesthesia and handling stressors. Humoral immunity (antibacterial levels in

the cell free haemolymph) and cellular immunity (phagocytic rates) were measured by means of bioassays. One control and three treatment groups were used in the study. Three repeats per treatment group were evaluated after the treatment. The three repeats for the control group were not disturbed prior to sampling. The first treatment group was manually chipped with a metal spatula and the second group was anaesthetised without handling before sampling, while the third group received anaesthetics, where after they were moved in the customary manner, and then sampled. The immune assays showed the greatest changes occurred in abalone subjected to both handling and anaesthetics, the group who received anaesthetics but no handling was less effected; while the least effect was seen on manually chipped abalone moved without anaesthesia. Hooper *et al.* (2011) advocate that anaesthesia could be the most stressful component of stocks movement.

Abalone are subjected to many stressors before and during live export. If these factors challenge the immune system too severely, high mortalities can be expected during and short after export.

### 2.1.5 Life cycle and reproduction

*H. midae* is found to be 100 % sexually mature at around seven years in the wild. However, maturity is reached much earlier on the warmer east coast and cultured abalone reach maturity as early as three years of age. Depending on locality, spawning can occur twice a year, usually in spring and autumn. Two discrete groups of ova are found within a ripe ovary. These are released on consecutive spawnings (Sales & Britz, 2001).

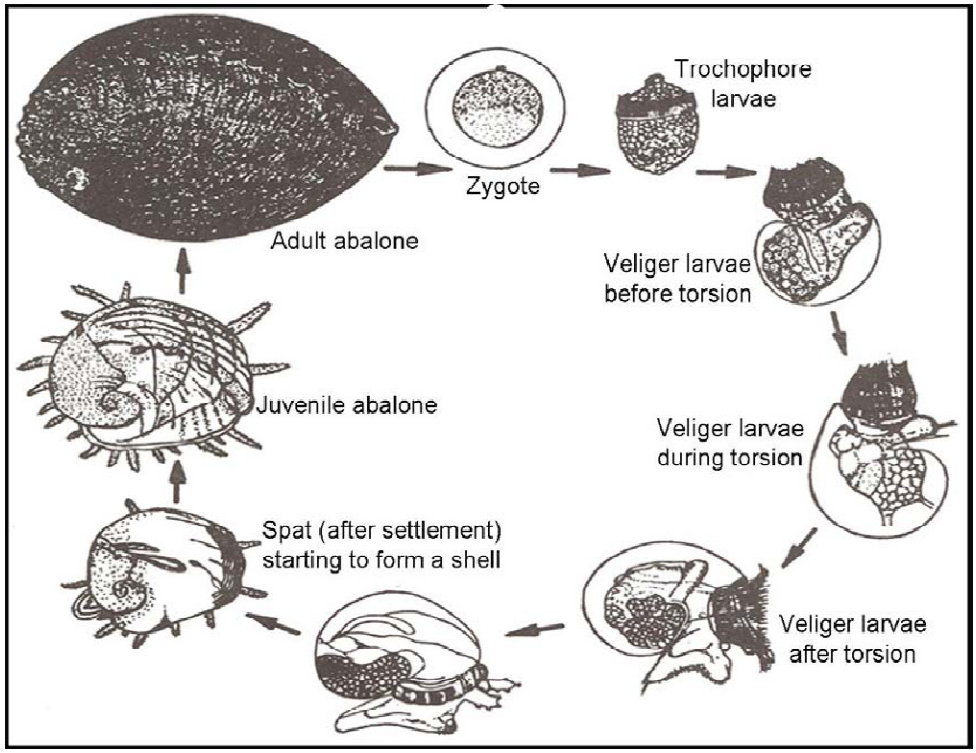


**Figure 2.4 Illustration of the abalone life cycle (Rhode, 2010).**

After fertilization and repeated cleavage the lecithotrophic (planktonic-dispersal larvae that lives off yolk supplied by the egg), trochophore (free-swimming planktonic marine larvae) larvae develops and grows further into planktonic, non-feeding bentic juveniles (Lemay & Boulding, 2009). This larval period continues for five days at 20 ℃, or seven days at 17.5 ℃. Hereafter the larvae settle down in shallow water, develop into abalone and feed on benthic diatomes (Sales & Britz, 2001).

### 2.1.6 Feeding and growth

*H. midae* is a nocturnal, herbivorous species. On farms they are reared on a formulated diet and macro algae such as harvested kelp (*Ecklonia maxima*) and cultured *Gracilaria* spp. (*Gracilaria verrucosa*), or combinations (Sales & Britz, 2001).

Once the five to seven days temperature dependant larval phase is completed, the newly metamorphosed abalone prefer prostrate diatomes, such as Hendey, Hustedt and Agardh *(Cocconeis sublittoralis, Amphora proteoides* and *Achmenthes brevipes*, respectively). At four to six millimetres shell length; they are weaned onto seaweed or formulated feed, whereas matured *H. midae* prefer kelp. Growth rates can be improved by addition of *Porhpyra, Ulva* or *Aeodes* species. In wild *H. midae*, feed intake averaged 8.1 % of soft body weight per day at 14 ℃ and 11.4 % at 19 ℃ (Sales & Britz, 2001), consistent with expected metabolic rates.

*Haliotis midae* can reach a maximum size of about 200 mm shell length. This size is reached at an age of over 30 years. Farm production is concentrating on an average size of 100 mm after 5 years. Growth rates of 0.08 % to 4.5 % of body weight per day have been reported for abalone with 10 mm to 17 mm shell length. These growth rates were obtained with formulated feed with a corresponding feed conversion ratio of 0.9 to 2.4. Growth rate, feed conversion ratio and protein efficiency ratio are optimal between 12 ℃ and 20 ℃ (Sales & Britz, 2001).

Sufficient comprehension of growth characteristics is important for the efficiency and sustainability of aquaculture and management strategies for wild abalone sources. Addressing biotic and abiotic factors, such as culture diets, ambient temperatures, and stocking densities, have the possibility of exhibiting a rapid increase in growth rate. The effect of genetics and breeding techniques make valuable improvements in traits such as growth rate (Huang & Hseu, 2010).

## 2.2 Genetic improvement

Genetic variances and correlations lie at the centre of the quantitative evolutionary theory. For estimations to be made, large samples of related individuals are required (Cheverud, 1988). A standard measure for the proportion of genetic variation in a population is the heritability which can be used to predict rates of genetic gain to find optimal breeding strategies (Falconer & MacKay, 1996). High individual variability is found in the growth rates of wild animals (Sales & Britz, 2001). This provides the material on which selective breeding can operate (Falconer & MacKay, 1996).

When related individuals have offspring, the progeny are called inbred and are almost always less fit than progeny of non-relatives. As the homozygosity within a population increases, the fitness will decline. Decreased vigour in terms of growth, survival, or fertility after one or more generations is also often observed. This can lead to a decrease in ability to reproduce and transmit genetic material to the next generation, relative to other populations. This ability to contribute to future generations is also called fitness (Stearns, 1992).

When the heterozygous phenotype is the superior phenotype it is called overdominance (Falconer & MacKay, 1996). Inbreeding depression can be caused by decreases in heterozygous overdominance genes, or by an increase in homozygous recessive deleterious genes in homozygous offspring (Park *et al.*, 2006). This is an important aspect in hatchery management. In the study of Araki and Schmid (2010), they evaluated the fitness of hatchery bred fish in the wild. Hatchery bred populations of different marine species were released in the ocean to restore and enhance wild stocks. After a summary of 50 years' data

they have found that a reduction of genetic variation and reproductive fitness were common in a variety of fish species, although some successful stockings did occur where no or little negative effects were found. Without considering the effect of hatchery fish on stocked populations, it is still an indication that hatchery populations can have lower fitness because of inbreeding.

Heterosis or hybrid vigour, on the other hand, is the opposite of inbreeding depression. Fitness lost by inbreeding can be restored by crossing (Falconer & MacKay, 1996). Cross breeding is expected to decrease genetic load by reducing the loss of alleles and consequently increase fitness through hybrid vigour as a result of increased heterozygosity (You *et al*., 2009). Cross breeding thus uses dominance to result in superior hybrid offspring by suppression of undesirable recessive alleles of one parent by dominant alleles form the other parent which leads to fewer under-expressed genes than in the parents, while overdominance improves offspring viability by reducing the amount of homozygous harmful recessives and shows an over-expression of some genes in the heterozygous offspring compared to the homozygous parents.

Ahmed *et al.* (2008) studied the effectiveness of interspecies hybrids of Japanese abalone species *Haliotis discus discus*, *Haliotis gigantea* and *Haliotis madaka* for aquaculture. They characterised the genetic background and studied gonad development of hybrids. Hybridization has been proposed as a method to increase growth rate among abalone species. Crosses between *H. discus discus*, and *H. madaka* was easier to carry out than crosses involving *H. gigantea*, but all hybrids developed normally, produced viable gametes and successfully produced a $F_2$ generation and backcrossed individuals. The crossing of different populations can also be beneficial. This was proven by You

*et al.* (2009) when they studied crosses of three populations of *H. diversicolor*. A comparison between performances in growth rate and survival at early juvenile, later juvenile, and grow-out stages were tested among six reciprocal cross lines and three parental lines. It exhibited that crossbreeding between different populations can benefit the abalone industry by increasing heterozygosity, reducing the effect of recessive lethal genes, and improving fitness, resulting in hybrid vigour.

*H. midae* has a long generation interval and reaches sexual maturity only after four to five years. It will thus take a long time before the effects of traditional methods of genetic improvement such as selective breeding, cross-breeding and hybridization can be seen. Genetic improvement strategies for abalone can therefore benefit from modern techniques like transgenesis. One example would be to add growth hormone genes to the genetic composition of *H. midae.* This could result in the abalone growing faster in a much shorter time compared to offspring of selective breeding stock (Roux *et al*., 2008). The technology is however still new, and consumers' sensitivities have to be taken into account.

Artificial selection drives the development of farm stocks and results in domestication. Like natural selection, it allows differential reproductive success to individuals with different traits. Only individuals with desirable characteristics are allowed to reproduce. In evolutionary biology fitness describes the average contribution of an individual of a given genotype to the gene pool of the next generation, relative to another genotype. Natural selection always favours the fitter individuals to reproduce, while artificial selection specifically chooses individuals with the desired trait. Variation in the phenotype is needed for selection to act and continuous variation in phenotypes is visible as reaction

norms (Stearns, 1992). The reaction norm is the set of different phenotypes found in the same genotype as the summed effect of many genes across a range of environmental conditions. The environment can differ quantitatively in distribution of phenotypes when several genotypes are assessed. A plot of reaction norms of different genotypes can be made with distribution of environments as the independent variable and distribution of phenotypes as the dependant variable. When we see a slope in the reaction norm the phenotype is called plastic. Phenotypic plasticity is therefore described as 'the ability of a genotype to produce distinct phenotypes when exposed to different environments throughout its ontogeny' (Pigliucci, 2005). The steeper the slope of a particular genotype is, the greater the plasticity of the phenotype.

When reaction norms of genotypes are not parallel, genotype by environment (G x E) interactions are present. The variance in phenotypes of individuals in different environments reduces to zero in the event where reaction norms cross. When a population is assessed in only one environment for phenotypic variances and its associated genetic components to estimate heritability, it can be possible that the population is evaluated very close to or at the crossing point of the reaction norms (Stearns, 1992). Variances will be low, which imply a low estimated heritability, while the possibility exists that in other environments bigger variances between related groups of a population may occur, with fewer variances within these groups. This has to be taken into account when the estimated heritability value is interpreted.

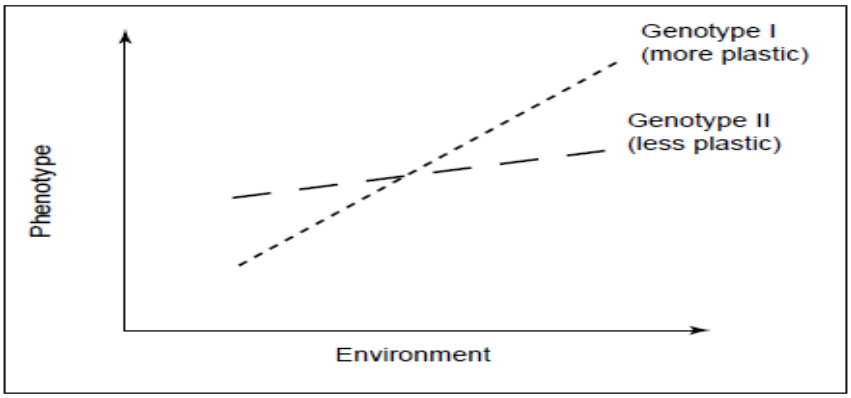


**Figure 2.5 Reaction norms of a species that show phenotypic plasticity as well as genotype by environment (G x E) interaction. (Adapted from Pigliucci, 2005)**

Artificial selection has produced highly successful results visible in everyday life, but can be of great risk to a population when it causes the increase in frequency of traits that cause lower fitness within the population. Fitness and the life history theory are interdependent. Major examples of life history traits include age at first reproductive event, reproductive lifespan and aging, and number and size of offspring. Enormous variations are found among different species of organisms, balancing time, effort and energy usage in a way to ensure survival of the species (Stearns, 1992; Pigliucci, 2005).

Mousseau and Roff (1987) did an experiment to test if traits directly connected to fitness have a tendency to have lower estimates of heritability than traits more loosely associated with fitness as proposed by Falconer & McKay (1996). The hypothesis was tested using 1120 narrow sense heritability estimates for wild, outbred animal populations. It can be expected that traits closely related to fitness will show less additive genetic variance as a consequence of natural selection. Falconer & McKay (1996) stated that characters with the lowest estimates of heritability are the traits closest related to fitness, and traits with high heritability can be considered the ones which are least important to natural

selection. Significant amounts of additive genetic variance may exist for traits closely associated with fitness and can happen because of mechanisms such as mutation, heterozygote advantage, frequency dependence, fluctuating environments and migration. Heritabilities for fitness characteristics can also be zero. This is due to negative genetic correlation between fitness components. This phenomenon is also called antagonistic pleiotropy (Stearns, 1992).

In the study of Mousseau and Roff (1987), heritability estimates were derived from wild, outbred stock, which were not screened or selected. Four categories of traits were used: a) behavioural traits such as alarm reaction, activity level, and sensitivity to conditioning; b) life history traits including characters that are directly connected to fitness such as fecundity, viability, survival and development rate; c) morphological traits include body size and other metric characters; and d) physiological traits, such as oxygen consumption, body temperature and resistance to heat stress. Their study confirmed what is accepted in literature that life history traits tend to have lower heritability than morphological traits.

They also investigated whether patterns of heritability differ between taxa. They compared invertebrate versus vertebrate and ectotherms versus endotherms for morphological and life history traits. The only significant difference was found in heritability of morphological traits between ectotherms and endotherms, where ectotherms exhibited lower heritability than endotherms. Behavioural and physiological traits showed heritabilities similar to life history traits rather than morphological traits. This could be because behavioural and physiological traits are subject to limitations similar to those influencing fitness traits. The magnitude of heritability indicates, however, that even for traits closely

associated with fitness, variance is maintained in most natural populations (Mousseau & Roff, 1987).

### 2.2.1 Selective Breeding strategies

Selective breeding programmes have played a key role in successful development of aquaculture industries worldwide. Aquaculture species which have benefitted from selective breeding approaches in the past includes marine and fresh water species like Atlantic salmon, rainbow trout, carp, sea bass, sea bream, shrimp, catfish, tilapia, scallop and oysters. The beneficial response of selective breeding is limited by the breeding program, the trait at hand and biological limitations of the species. The volume of genetically improved stock grown together, and the quest that benefits should outweigh costs, will determine the economic viability of the breeding program (Robinson & Hayes 2010).

Quantitative variation is influenced by Quantitative Trait Loci (QTL) and the environment. It characterises economically important traits, like growth rate, meat quality and disease resistance in farmed animals. The rate of genetic gain from selective breeding programs can be maximised by the use of Quantitative Trait Loci (QTL) (Franchini *et al*., 2011). QTL studies was not part of this study, but was investigated as part of the overall improvement strategy.

Selective breeding in *Haliotidae* spp.

A selective breeding initiative needs to make strong genetic improvement, limit inbreeding, create large benefits for stakeholders and be a commercially viable entity. An example of inbreeding in the industry is the *H. diversicolor* population in China, introduced from Taiwan, as discussed by You *et al.* (2009). It was

cultured for 10 generations and accumulated inbreeding and no new genetic material was introduced. A decreased growth rate and vast mortalities at post-larval and grow-out stages occurred. Production decreased from 2385 tons in 2001 to 639 tons in 2006.

The effects of a breeding program are not instantaneously visible and it is undesirable to invest a large amount of money and time on a suboptimal process. Simulations of biological and genetic improvement are potentially valuable, cost effective ways to predict the outcome of a breeding program and evaluate the possible improvements that can be gained by it. Traits like disease resistance is difficult to select for and simulations play a particularly useful role when these traits are examined. This eliminates any possible biosecurity risks associated with disease resistance experiments (Robinson *et al*., 2010).

Robinson *et al.* (2010) simulated a selective breeding model investigating family breeding value for disease resistance and best performing individuals according to growth rate as selection criteria in blacklip and greenlip abalone (*Haliotis rubra* and *Haliotis laevigata*, respectively). They simulated disease resistance and reported that the lower the quantity of families used, the higher the variability in performance of each selective breeding program. Under the most successful case, using 33 families had a similar improvement rate compared to breeding programs with larger family numbers such as 100 or more, but in the worst case, very little improvement was made. Selective breeding programs using larger numbers of families achieved a greater genetic response on average. However, this improvement factor diminishes as the number of families increase. Similar genetic responses were obtained during the

simulations with 250, 200 and 150 families. Fewer families in the breeding program also increase the risk for inbreeding.

In the study of Park *et al.* (2006), the occurrence of inbreeding depression is seen in deformity and survival rates in Pacific abalone. When creating a breeding program of abalone seeds, inbreeding needs to be avoided and the effective population size maintained at levels that ensure enough heterozygosity.

In Australia, selective breeding programs have been introduced for greenlip (*H. leavigata*) abalone. Kube *et al.* (2007) reported a trend of genetic variation for growth rate. Gains for growth rate in the region of 10 % per generation were predicted.

South African abalone producers are experiencing increased competition on world markets, which makes it essential to lower production costs and increase product quality and yield, therefore the need to introduce their own genetic improvement program.

### 2.2.2 Traits of interest

Provision of an optimum environment with minimum stress for the abalone is the key to successful mariculture (Hooper *et al*., 2011), but in a farm set up, important daily husbandry practices can cause stress. Two main limitations described by abalone farmers in Australia are the period from grow-out to market size and the stress tolerance of the animals. Stress induced by sustained high summer water temperatures, disease and live export are problematic for production (Robinson *et al*., 2010).

In molluscs, haemocytes produce the stress response molecules. Stress response often evokes immune responses and is generally a non-specific pattern of neuroendocrine reactions to a situation that threatens homeostasis. Very little research has been done on abalone stressors and stress responses, but it seems that in abalone, as in other molluscs, the stress and immune responses are centred on the haemocyte. These blood cells produce mediators of stress and the main immune responses. It has been reported that after mechanical shaking of *H. tuberculata* an increase in the stress hormones adrenaline and nor-adrenaline is observed in the haemolymph. The elevated levels returned to baseline values again. An initial decrease in haemocyte levels was observed followed by an increase in haemocyte count, migratory activity, phagocytosis and superoxide production after the stress. These immune responses peaked after two to four hours and then decreased to basal levels. Exposure of *H. diversicolor* to low levels of the stressor nitrate increased haemocyte counts for 72 hours followed by a decreased count, whereas for low ammonia levels, the haemocyte count increased for 24 hours, followed by significant decreases. Higher concentration of nitrate and ammonia decreased haemocyte counts in *H. diversicolor*. Different effects are thus caused by mild stressors and severe stressors. The stress responses are similar across many phyla. The same cascade of responses from the same molecules is observed. This includes corticotrophin releasing hormone (CRH) stimulating the release of adrenocorticotrophic hormone (ACTH), which stimulates the release of biogenic amines. Elevated temperature, nitrate, and ammonia; and reduced dissolved oxygen and salinity, outside the normal ranges were applied as stressors to *H. diversicolor*. Haemocyte counts, superoxidase production and phenoloxidase

measurements varied and could not be used as reliable indicators of immunosuppression in abalone. Abalone subjected to these stressors showed depression of phagocytosis and efficiency in clearing bacteria (Hooper *et al.*, 2007).

Most farm procedures are potentially stressful events but anaesthesia and handling are considered to be leading stressors (Hooper *et al.*, 2011). Anaesthesia is often used to prevent injury during removal from tanks in the farming setup because of the reaction of abalone to pull their shell down quickly and firmly onto the substratum when disturbed (Sales & Britz, 2001). Removal and handling therefore requires anaesthesia in order to prevent injuries as the abalone haemolymph has no coagulating properties and most wounds would be fatal (Ragg & Taylor, 2006). In the study of Hooper *et al.* (2011) it was concluded that anaesthesia causes even more severe stress than handling. More research on methods of handling without anaesthesia or alternative methods of anaesthesia could be beneficial for the industry.

Growth

The von Bertalanffy model was used by Huang *et al.* (2010) to estimate and express growth rate. The model for length and weight, respectively, is:

$$L_t = L_\infty\left(1 - e^{-k(t-t_0)}\right)$$

$$W_t = W_\infty\left(1 - e^{-k(t-t_0)}\right)^\beta$$

Where $L_t$ and $W_t$ are shell and body weight at time *t*; $L_\infty$ and $W_\infty$ the hypothetical asymptotic length and weight, *k* is the growth coefficient; $t_0$ is the

hypothetical age at zero length; and $\beta$ is derived from the allometric growth model:

$$W = \alpha L^{\beta}$$

Huang *et al.* (2010) reported that after a decade of selection of animals with well-matured gonads during the reproductive season, cultured population of abalone had smaller shell length ($L_{\infty}$) and a larger growth coefficient (*k*) than wild populations. This phenomenon is also seen in the von Bertalanffy model of fishes, and other abalone species. Both these factors thus exhibit a hereditary factor (Huang & Hseu, 2010).

In the simulated study of Robinson *et al.* (2010), genetic response of 7 % to 13 % per generation in early generations of selection, were close to realized responses reported in the literature for these traits in other aquaculture species. The estimated responses in molluscs range from 9 % to 20 %.

The tropical abalone *H. asinna* has the fastest recorded natural growth rate and reaches sexual maturity within one year. Heritability estimates of growth related traits were calculated for 12 months old animals. Heritability estimates for shell length were 0.48 ± 0.15, 0.38 ± 0.13 for shell width and 0.36 ± 0.13 for weight. Genetic correlations between weight and shell size were more than 98 %, which indicates that animals can be selected on shell length (Lucas *et al.*, 2006). In a study on *H. diversicolor* heritability estimates of growth related traits from post-larva to market size ranged between 0.15 to 0.37 for shell length, and 0.18 to 0.42 for shell width (You *et al.*, 2010). In addition, Kube *et al.* (2007) reports estimates of heritability for growth traits in three year old *H. laevigata* to be 0.10, 0.10 and 0.04 respectively for total weight, meat weight and shell length.

Although these estimates are relatively low, they still predict up to five percent gain in total weight through selective breeding and regard this slight increase as economically important.

Chronic stress from, for example, increased stocking densities of farmed abalone and increased metabolic wastes, especially ammonia, increase the risk of disease outbreak and decrease growth rate (Hooper *et al*., 2007; Hooper *et al*., 2011). As in mammals, there is a neuroendocrine link between stress and immune functions, and therefore it is likely that there may also be a link between stress and the neuroendocrine system directly affecting growth hormones (Hooper *et al*., 2007).

Yield

No published literature related to canning yield in abalone could be found. Canning yield can also be described as the dressing percentage of the abalone. The process involves removing of the shell, guts and mouth parts, washing, brining and cooking. Haemolymph constantly drains from the meat once the animals are shucked. During the brining process haemolymph is drawn from the meat by osmotic pressure. Altogether, these factors cause for major weight loss.

Abalone and fish are ectotherms. Dressing percentage can be described as a morphological trait. Based on the study of Roff and Mousseau (1987) where it was found that ectotherms have lower heritability for morphological traits compared to endotherms we expect abalone to have a lower dressing percentage heritability than endotherms such as sheep or cattle.

Sheep and goats exhibit high heritability for dressing percentages. Sen *et al.* (2004) reported heritability estimates of 55.78 ± 0.32 and 53.50 ± 1.43, respectively, for sheep and goats. Gall (2008) listed heritability estimates for dressing percentage of cattle as 0.20 – 0.35, while Pariacote *et al.* (1998) reported dressing percentage heritability of 0.49 ± 0.19 in African Short Horn beef cattle. In contrast, trout, Atlantic salmon and Channel catfish exhibit heritability estimates for dressing percentage of 0.01, 0.03, and 0.00, respectively (Gjedrem, 1983).

Live abalone fetch higher prices and about 60 % of farmed abalone in South Africa are destined for the live export market. The prices of abalone ranges between US $ 32 and US $ 35 per kg live mass (Vosloo & Vosloo, 2006). Live abalone are traded on the basis of landed mass, hence methods to reduce water loss during export is an important consideration for the South African abalone industry. In particular in view of the long distance travelled, and time to reach markets, compared to competitors such as Korea, Japan, New Zealand and Australia.

Abalone are transported in polystyrene boxes during live export. The animals are packed in plastic bags containing 100 % oxygen and are humidified with seawater (Vosloo & Vosloo, 2006). Ice packs are put on top of the bags. This is done to reduce hypoxic stress since low temperature reduces the metabolism and therefore lowers the oxygen demand (Bubner *et al.*, 2009). Animals are deprived of food before transport to prevent them from producing faeces inside the boxes (Sales & Britz, 2001). Supplementation with ice and oxygen proved to be highly significant in decreasing mortalities (Bubner *et al.*, 2009).

Animals lose between 4 % and 15 % of their mass during exportation (Vosloo & Vosloo, 2006; Bubner *et al*., 2009), which can also contribute to mortalities during and after export (Bubner *et al*., 2009). This contributes to substantial reduction in the income for exporters who are paid on landed mass (Vosloo & Vosloo, 2006).

Other gastropods such as pacific oysters (*Crassostrea gigas*) are well adapted to aerial exposure. Pacific oysters are immobile bivalves. Their natural habitat is the intertidal zone and they are cyclically challenged with hypoxic conditions. Some bivalves open their shells during aerial exposure and utilize atmospheric oxygen while a variety of other bivalves obtain energy anaerobically through glycolysis (Kawabe *et al*., 2010).

Aerial exposure is, however, an unnatural and extreme stressor for abalone and cause water loss through several mechanisms (Vosloo & Vosloo, 2006). In some intertidal gastropods the foot can be retracted into the shell and an attached opercular disc, dorsally to the upper surface of the posterior part of the foot, closes of during aerial exposure. The sub tidal natural habitat of abalone, however, does not require adaptation to aerial exposure, and abalone do not have opercular discs to protect them from losing moisture.

Vosloo and Vosloo (2006) compare mucous production of *H. midae* during aerial exposure with the tropical limpet *Cellana grata* and the common limpet, *Patella vulgata*. Mucous of *C. grata* contains 90 % water and *P. vulgata* produced mucous at an increased rate during aerial exposure. This had large effect on the respective species in terms of water balance. The study was done to investigate mechanisms of water loss in *H. midae*. They found that the

reduction in mass is completely attributed to water loss. All measurable ions had elevated levels in dehydrated *H. midae* entailing that osmotic concentrations increase proportional to decreasing body fluids. Water loss can be attributed to evaporation and dehydration. Moreover, dehydration is caused by urine and mucous production and is responsible for about 55 % of total water loss. Urine production, however, contributed to an insignificantly small amount, which makes mucous production the main mechanism of water loss. The water content of mucous is 90 % and thus is mucous production is responsible for about 50 % of all water loss during intense dehydration (Vosloo & Vosloo, 2006).

Bubner *et al.* (2009) reported a reduction in the pH of haemolymph of all abalone which underwent a transport simulation. Groups treated with higher concentrations of oxygen had a smaller decrease in haemolymph pH compared to animals treated with lower oxygen levels or ambient air in transportation bags. A low pH reduces the rate of oxygen delivery to the tissues. This is associated with the reverse Bohr Effect; under which haemocyanin (the respiratory pigment in abalone haemolymph) increases its affinity for oxygen.

Mucus production in *H. tuberculata* uses between 23 % and 29 % of the total energy budget (Vosloo & Vosloo, 2006). Elevated rates of mucous production, together with unfavourable and stress inducing conditions is energy draining and can lead to mortalities in abalone during exportation. Stresses in abalone that are exported alive include various factors such as fasting, anaesthesia, handling, aerial exposure, temperature fluctuations, and oxygen deprivation.

*H. iris* lose large amounts of haemolymph during aerial exposure and handling as part of the defence response to reduce volume of the body and clamp tightly to the substrate. By doing this they can protect themselves with their shells (Bubner *et al.*, 2009). Mucous is also needed to attach to substrate and forms a protective barrier, which is needed in high stocking density conditions such as during rearing of young animals and during exportation.

## 2.3 Summary

South African abalone species are members of the phylum gastropoda and found along the extended coastline in more temperate waters. *H. midae* was found to be the only species, among the six species which are indigenous to the Southern African coast, with commercial value. The species reproduce by means of broadcast spawning and has a complex life cycle with many larval stages. *H. midae* is nocturnal and herbivorous. The species has been cultured for the past 20 years without domestication. Consideration of a breeding program is the next step to improve production of the industry.

Genetic improvement by artificial selection during a breeding program can be described as manipulation of the genetic variation present within a particular species, which will result in production gain. Two basic genetic phenomena form the basis of a breeding program: Firstly, the resemblance between relatives; and secondly, inbreeding depression or oppositely hybrid vigour. Quantitative genetics aims to show how the degree of resemblance between relatives of different types can be used to predict the outcome of selective breeding. Inbreeding usually decreases fitness (Falconer & MacKay, 1996). Selective breeding is therefore about finding the right balance between the

extremes of too much variation in traits, which is not economically optimal, and a severely inbred population with reduced fitness, which can also be harmful to the industry in extreme cases.

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# Chapter 3 Materials and Methods

## 3.1 Experimental design

In random complete block designs each block contains all treatments. This design strategy improves the accuracy of the comparisons by eliminating the variability among blocks. In experiments where it is not possible to run all the treatment combinations in one block, a randomized incomplete block design can be applied (Montgomery, 2005). All treatment combinations, in other words sire and dam combinations, could not be made due to biological limitations, therefore the experimental design used was the balanced incomplete block design. Biological limitations include a lack of adequate control over artificial spawning of abalone broodstock.

## 3.2 Base population

A base population of the abalone *Haliotis midae* was established in 2005 at the start of the genetic improvement program. It consisted of 800 sexually mature individuals that were randomly collected from the Walker Bay region, stretching from Betties Bay to Franskraal on the South Coast of South Africa. Five commercial abalone farms located in the Overstrand Region of the Southern Cape are participating in the selective breeding program. Three farms: Abagold (Pty.) Ltd.; HIK Abalone (Pty.) Ltd. (HIK); and Aquafarm Development Company (Pty.) Ltd. (AF), are situated at Hermanus, while Irvin & Johnson Ltd., Abalone Division (I&J) is sited at Danger Point; and Roman Bay Sea Farm (Pty.) Ltd. (RB) is located near Gansbaai. Predominantly, *H. midae* do not spawn readily after they are collected from the wild. Consequently, broodstock conditioning protocols were developed (Sales & Britz, 2001). The animals were randomly distributed amongst the participating farms to

serve as broodstock and were subsequently conditioned for controlled spawning that started in December 2006. Animals kept on Abagold (Pty.) Ltd. were not used for the purposes of this thesis as a result of accidental size sorting that distorted the mean growth measurement within those family groups.

### 3.2.1 Family groups

Males and females were kept individually and in separate containers during conditioning and spawning. A series of full and half sib family groups were established through combining sperm and eggs obtained from individual males and females after induced spawning. Sexually mature abalone are highly fecund with females producing between 0.1 to 8.0 x $10^6$ eggs, depending on body size (Elliott, 2000; Hamilton *et al.* 2009). The newly collected broodstock, however, first had to be conditioned before spawning could be induced on a regular basis by means of chemical methods. This included adaptation to their new environment at a set temperature of 18 °C and photoperiod regime of 12 hours light and 12 hours darkness.

The number of individual families that could be produced was determined by the success of synchronized spawning and the availability of facilities for hatching and settlement of the separate family groups (Hamilton *et al.* 2009). Not all animals could be spawned on the same day, consequently spawning dates were recorded for each full sib family, with ages ranging over a 109 day period. At the end of the spawning cycle in February 2006, a total of 19 full sib families which comprise of nine half sib families were successfully reared and subscribed to an extended growth trial period of 62 months. The families were bred from 18 sires and 11 dams. The traditional hierarchal family structure of one sire mated to more than one dam could not be

followed due to low volumes of viable sperm that were available. The eventual family structure is presented in Table 3.1.

**Table 3.1 The family structure consisting out of full and half sib families of abalone as subscribe to a 62 month growth trial period.**

| Full Sib Families | Half Sib Families | Location & Family number | Shared parent | | Unique parent | |
|---|---|---|---|---|---|---|
| 1 | 1 | AF 4 | D | 462 | S | 468 |
| 2 | | AF 7a; RB 7b | | | S | 342 |
| 3 | 2 | AF11 | S | B27F | D | B10B |
| 4 | | I&J 35a; HIK 35b | | | D | B11B |
| 5 | 3 | RB 16 | D | C10F | S | C30B |
| 6 | | I&J37a; HIK 37b | | | S | C28B |
| 7 | 4 | RB 21 | D | F66 | S | M44 |
| 8 | | RB 29a; I&J 29b | | | S | M33 |
| 9 | 5 | RB 18 | D | C10B | S | C29F |
| 10 | | I&J 38 | | | S | C24F |
| 11 | 6 | RB 30a; I&J 30b | D | F62 | S | M34 |
| 12 | | I&J 31 | | | S | M38 |
| 13 | 7 | HIK 41 | D | F28 | S | M4 |
| 14 | | HIK 43 | | | S | M35 |
| 15 | 8 | HIK 44 | D | F50 | S | M13 |
| 16 | | HIK 45 | | | S | M25 |
| 17 | 9 | HIK 46 | D | F60 | S | M26 |
| 18 | | HIK 48 | | | S | M40 |
| 19 | | HIK 47 | D | F22 | S | M36 |

D = dam, S = sire, AF = Aquafarm Development Company (Pty.) Ltd., I&J = Irvin & Johnson Ltd. Abalone Division, RB = Roman Bay Sea Farm (Pty.) Ltd., HIK = HIK Abalone (Pty.) Ltd.

The various families were randomly allocated to the different farms, i.e. locations, were they were treated according to standard managerial practices applied by the particular farms in terms of type of holding units, stocking densities, flow rates and feeding practices. The experimental groups, i.e. four repeats, were however, never subjected to any treatment that could affect the random nature of these groups, such

as size sorting. The difference in rearing methods applied by the participating farms could however contribute to location effects on growth and yield traits and had to be taken into account during data analysis and interpretation of results.

No single full sib family group could be generated that was sufficiently large in numbers to allow for such a full sib reference group to placed as an internal reference group, within families at all localities. To compensate for this short coming, a different full sib reference group was located on each farm, whilst five full sib families were large enough to be split into repeats hosted at two different localities. The latter contributed significantly to the evaluation of the effect of location in the data analysis.

Each full sib family were raised in a singular container through the larval, settlement and weaning stages. At the stage of weaning, six months after fertilization, at an average shell length of 10 mm, each family group were randomly divided into four repeats, with each repeat kept in a separate rearing basket from there onwards.

## 3.3 Growth Measurements

The body weight (grams) and shell length (millimetre) of families were recorded through non-destructive randomly sampling at six-monthly intervals, from the age of six months to 62 months. Before measurements were taken, animals were anesthetized with the use of $MgSO_4$ or $CO_2$, and transferred from rearing baskets to mesh baskets to facilitate draining of excess water before animals were measured. Individual body weight of a random sample of 16 animals per repeat was recorded in grams by means of an AND EK-300i electronic balance. Individual shell lengths were measured, with a Mitutoyo IP67 digital calliper, as the distance between the posterior and anterior extremities of the shell (O'Omolo *et al.*, 2003; Vlok, Unpublished).

The growth trail was completed after 62 months, which corresponds to completion of a commercial production cycle. The family groups were then used to analyse yield traits associated with live export and canning, two of the main forms in which the products are sold.

## 3.4 Simulation of live export procedures

The objective of the trial was to assess the nature of genetic differences between the family groups in relation to weight (drip) loss during live export, as well as to determine correlations between related traits and body measurements.

At the age of 62 months 16 animals were randomly selected from all four repeats of each of the 24 family groups. Each of the sampled animals received a unique identification tag that contained information of the individual, family and location, inserted through a breathing hole in the shell (Figure 3.1).

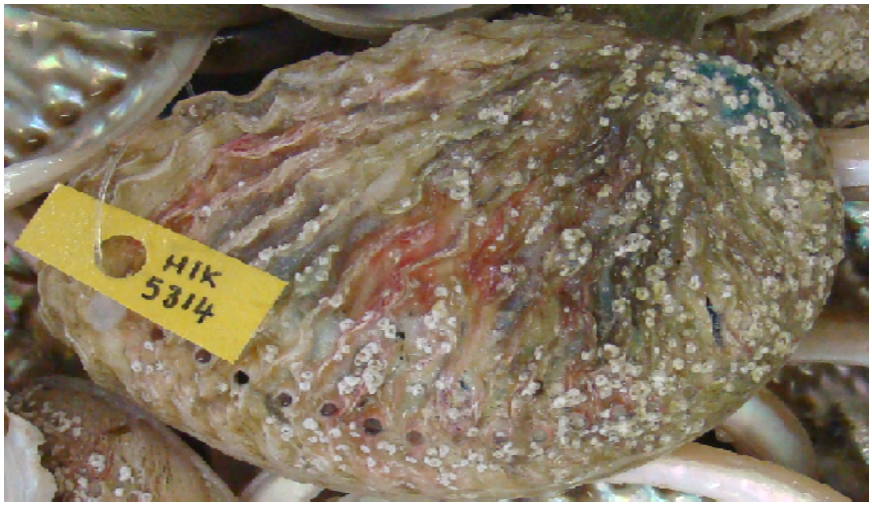


**Figure 3.1 Individual tagging system used for abalone (*H. midae*) during assessment of weight loss during simulation of live export conditions (Photograph: Prof. D. Brink).**

After initial random sampling from the respective family repeats and tagging, all animals were pooled over families and randomly underwent the subsequent treatment regimes, simulating live export conditions. Consequently, all families

received on average the same treatment for the same duration. The experimental treatment simulated conditions to which commercial animals are exposed to during standardized live export procedures followed by Aquafarm Development Company (Pty.) Ltd., which is similar to that applied in a previously study on live transport of animals conducted by the Research and Development unit of Aquafarm in 2009.

The treatment procedure can be described in terms of the following steps:

Purging

Sampled animals were randomly divided into standard mesh purging bags with an equal number of animals from each family per bag. The bags were then submerged overnight for 16 hours at 15 ℃ water temperature into a 1000 litre (L) holding tank supplied with aeration and a continuous flow through of seawater at a rate of 0.1 L per second. This allowed for the purging of the digestive systems prior to packing and transportation.

Packing

After purging overnight, the mesh-bags were removed from the tank and suspended in air for 15 minutes to drain off excess water. The initial body weights (in grams) were recorded on an individual basis with an electronic balance (AND EK-300i) at the start of the period of aerial exposure. The abalone were then packed in 25 L polystyrene boxes according to standard live export packing procedures at the Aquafarm packing room. Animals are transported alive in plastic bags containing 100 % oxygen, humidified with seawater, with ice packs on top of the bags. The polystyrene containers are taped shut and only opened by the receiving party between 30 and 42 hours after sealing (Sales & Britz, 2001; Vosloo & Vosloo, 2006).

The temperature inside the boxes was monitored and found to be in the in the preferred range of five to seven degrees Celsius. Temperature inside boxes may increase from four degrees Celsius to ambient temperatures of between 16 and 23 ℃ depending on the destination and duration of the export process (Vosloo & Vosloo, 2006).

Transport

The transport process during live export was simulated in terms of temperature, duration and handling conditions as recorded during standard overseas export hauls to Hong Kong, as one of the primary market destinations. The transport simulation can be described according to the following steps:

Step 1 – After packing, the transport boxes were kept at ambient temperatures of 19 to 22 ℃ at Aquafarm for a period of three hours, simulating the time it takes from packing to collection and transport of the abalone to Cape Town International Airport.

Step 2 – The boxes were then moved into cold storage facilities at Sea Plant Products (SPP), Hermanus and kept at six degrees Celsius for two hours, simulating holding conditions in cold rooms at Cape Town Airport, prior to custom clearance.

Step 3 – The boxes were taken out of the cold-room and left at ambient temperature for three hours to simulate the time it would take for handling during custom clearance.

Step 4 – Then the boxes were returned to the cold-room conditions and held at six degrees Celsius for 20 hours to simulate the duration and conditions on the plane during the flight from Cape Town to Malaysia Airport, on route to Hong Kong.

Step 5 – The boxes were then again removed from the cold room and kept at ambient temperature for two hours and twenty minutes to simulate the transfer of freight between flights at Malaysia Airport.

Step 6 – The boxes were placed back in the cold storage at six degrees Celsius for five hours to simulate the duration and conditions on the flight from Malaysia to Hong Kong.

Step 7 – The boxes were then removed from cold storage and kept at ambient temperature for an hour to simulate offloading at Hong Kong Airport and transfer to the client's holding facility.

Step 8 – At the end of the 39 hour procedure animals were placed on a mesh surface to remove excess mucous and the final body weight were recorded on an individual basis as before.

## 3.5 Canning yield

A separate yield trial was conducted on the same families to assess variation in weight loss between families during the commercial canning process, as well as the correlation between the respective traits and measurements.

Results from the canning trial and the growth assessment were used for a comparison between the performance of the respective family groups regarding drip loss, canning yield and growth.

The weight of all individuals was recorded at five stages during the canning process: initial weight after purging (Pre shuck); weight after removal of the shell (Post shuck); weight after removal of the guts (Post gut); weight after the brining process of the abalone meat (Post brine); weight before canning (Pre can); the weight after canning

and cooking (Post can). Canning yield is expressed by post canning weight as a percentage of the initial weight. Canning loss is consequently 100 minus the percentage canning yield (Gerber, unpublished).

## 3.6 Statistical analysis

### 3.6.1 Microsoft Excel

The data recorded as individual live weight before and after live export treatment was processed in a Microsoft® Office Excel® 2007 format, with drip loss calculated as a percentage of initial weight. Family correlations and ranking of families for the different characteristics were also considered.

### 3.6.2 Statistica

The data was then analysed with the use of the Statistica 10 software package (StatSoft, Inc. 2011). The residual plots displayed circular patterns, which do not correspond well with the straight line of normally distributed residuals; hence the percentage drip loss data was then transformed by the logarithmic function with base number 10.

Sires, dams and families were also grouped by the non-parametric exploratory algorithm, Classification and Regression Tree. This test does not test any formal hypothesis, but shows trends in the data in the form of rules. This type of analysis, however, holds a risk that random patterns may appear as trends. Therefore, the data was split into a "training set" (70 %) and a "test set". The analysis is done in the training set, and applied to the test set. If the test set results correspond to the training set, it can be said with more certainty that it is not random patterns. The existence of phenotypic correlations between traits was also considered. Furthermore, correlations of the rankings of the families, based on family solutions

obtained in ASReml were evaluated to search for trends of correlated responses between the different traits with the Spearman-R function.

### 3.6.3 ASReml

Narrow sense heritability is defined as the ratio of additive genetic variance to phenotypic variance and is estimated from the degree of resemblance between relatives. For classical balanced experimental designs variance components are simple linear functions of the observed covariance between relatives. However, maximum likelihood statistical procedures do not require balanced designs. Restricted maximum likelihood procedures takes the loss of degrees of freedom from estimating fixed effects from the data into account and reduce the bias compared to ordinary maximum likelihood procedures (Falconer & MacKay, 1996). ASReml software (Gilmour *et al*., 2006) was used to estimate the variance components of weight, length, drip loss and canning loss.

Correlations between weight and length, weight and drip loss, and weight and canning loss and the heritability estimates of the traits were the main points of interest in this analysis. Drip loss, canning loss and length were measured on three different random sample groups, together with the corresponding initial weight of every group, within the 19 families. Correlations between drip loss and canning loss (yield), drip loss and length, and canning loss (yield) and length was evaluated on family averages.

Due to the fact that the assessed animals were the first generation captive bred population, no pedigree information beyond the sire and dam of every animal was known. This eliminated the option of determining heritability with an animal model. The half sib structure that existed in this data was also not sufficient to run a half sib

model. The latter requires that every sire's gametes fertilized a number of females, or that every female's eggs get fertilized by a number of sires.

Owing to the high confounding level between sire and dam effects, sires and dams were combined as sire-dam combinations, referred to as families (n=19). These effects were fitted as a single random effect in a mixed model, using ASReml software (Gilmour *et al.*, 2006). The random effects of sires (n=18) and dams (n=11) were then fitted as individual effects, with or without the interaction of sires with dams fitted additionally. None of the additional random effects over and above families resulted in an improvement in the log likelihood ratios. The between-family variance ratio was thus used to act as a proxy for heritability in this study.

The mixed model fitted was:

$$Y_{ijk} = \mu + F_i + L_j + G_k + e_{ijk}$$

Where $Y_{ijk}$ is the observation on weight, drip loss or canning loss, $\mu$ is the overall mean, $F_i$ is the random effect of the i-th full sib family, $L_j$ is the fixed effect of the *j*-th location, $G_k$ is the fixed effects of gender[2], with k = male or female, and $e_{ijk}$ is the random residual term. Age was included as a linear covariate to account for changes in weight and yield traits associated with an increased age of the animals. The between family variance ratios were used as proxies for heritability estimates and covariance ratios were used as proxies for genetic correlations.

## 3.7 Summary

Sexually mature animals were randomly collected from a natural population and conditioned as broodstock for synchronised spawning. Full sib and half sib families

[2] Gender was only included in the model of animals evaluated for canning loss.

were created as an outcome of the controlled spawning. Thereafter the family groups were reared at different participating farms.

The growth rate of the family groups were recorded at six month intervals up to the age of 62 months, at which stage a series of yield related traits were determined. A canning trail assessed the percentage yield of after canning, while a live export simulation trail was conducted to determine weight loss during conditions of live export. The simulation accounted for temperature, and handling stressors and the duration exposed to stressful conditions. A series of correlations and heritability estimates were calculated for the respective growth and yield related traits.

## 3.8 References

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# Chapter 4 Results and discussion

To fulfil the assumption of ANOVAs, the data was tested for normality and homogenous variances. After the log transformation of the data, the residual plots were found to be well within bound of the normality assumption of analysis of variance (ANOVAs). The second assumption of ANOVAs, that variances of different samples are equal, was tested by Levene's test for homogeneity of variances. All p-values obtained were <0.05, which indicates non-homogenous variances. This weakened the strength with which conclusions were drawn from the ANOVAs. Nevertheless, non-parametric methods displayed the same ranking of sires, dams and families for drip loss percentage. The means for drip loss were calculated by back transformation of the log transform.

## 4.1 Analysis of variance

Analysis of variance methods were applied to determine if there were differences between animals from different locations, animals fathered by different sires, animals mothered by different dams, and animals which have different parent combinations, thus differences between different families.

The null hypothesis tested in the ANOVAs was:

$H_0$: $\mu_1 = \mu_2 = \cdots = \mu_n$ , where $\mu_i$ equals the mean $\log_{10}$ (Drip loss %) of the different farms; from different sires; from different dams; or from different families.

With the alternative hypothesis:

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

The majority of the p-values were < 0.0001. The differences in means are tabularised in Tables 4.1 – 4.5.

### 4.1.1 Evaluation of location

The effect of location was first assessed on five full sib families, which were hosted on two locations each. The location and the rearing methods on respective farms were the only differences between the treatments of the two groups of each full sib family.

**Table 4.1 Evaluation of the effect of different locations due to farm specific rearing protocols.**

| Family | Location | Mean Pre export weight | p-value |
|---|---|---|---|
| **7a** | AF | 103.65 ± 28.45 | 0.83 |
| **7b** | RB | 102.59 ± 26.97 | |
| **29a** | RB | 100.81 ± 17.07 | <0.0001 |
| **29b** | I&J | 166.96 ± 35.87 | |
| **30a** | RB | 103.39 ± 25.45 | <0.0001 |
| **30b** | I&J | 163.60 ± 41.91 | |
| **35a** | I&J | 143.13 ± 35.23 | <0.0001 |
| **35b** | HIK | 85.46 ± 35.22 | |
| **37a** | I&J | 157.18 ± 27.99 | <0.0001 |
| **37b** | HIK | 116.39 ± 22.02 | |

When only the five full sib families, which were hosted at two locations, are assessed, we observe that mean weight values for animals from Aquafarm and from Roman Bay are not significantly different. This trend is consistent in the next analysis, which includes all families. The mean drip loss percentage is a back transformation of the log values used in the ANOVAs.

**Table 4.2 Mean Weight and Drip Loss Percentages of animals from different locations.**

| Location | Mean Pre export weight | Mean log10(Drip loss %) | Mean Drip Loss % |
|---|---|---|---|
| **AF** | 98.12 ± 2.09 | 0.93 ± 0.02 [ab] | 8.47 ± 1.04 |
| **RB** | 107.83 ± 1.28 | 0.90 ± 0.01 [b] | 8.02 ± 1.03 |
| **I&J** | 160.09 ± 1.94 | 0.95 ± 0.01 [a] | 8.91 ± 1.03 |
| **HIK** | 121.81 ± 1.21 | 0.84 ± 0.01 [c] | 6.93 ± 1.02 |

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

The differences in weight of all the animals hosted at different sites showed the same pattern as in Table 4.1, where only the full sib families that were hosted on two locations were assessed. All farms differed significantly, with all p-values <0.0001, except for one p-value. The exception was a p-value of 0.025 for the null hypothesis that mean weights of Roman Bay and Aquafarm were not significantly different. The p-value is statistically significant and the null hypothesis is still rejected, but the lower factor of rejection is consistent with what we found in Table 4.1 for these two locations. The similarities observed between these two farms can be explained by the fact that they are owned by the same holding company and therefore share in a similar managerial approach.

Different management regimes at the respective locations had a large effect on mean weight of the animals. Factors like stocking densities, feeding and handling as well as natural environmental differences between the different locations are nested in the component of variance in mean weight and drip loss percentage caused by environmental effects on the animals. Animals reared on I&J were kept at lower stoking densities and a higher mean weight is observed.

### 4.1.2 Evaluation of dams

Animals with F22, F50, B11B and F60 as dams did not differ significantly, and similarly did animals of dams B11B, F60 and F28 not differ significantly. Offspring of

these five dams were the best performers according to the regression tree grouping (See Chapter 3; 3.6.2).

**Table 4.3 Mean Drip Loss Percentages of animals from different dams.**

| Dam | Mean $log_{10}$(Drip loss %) | Mean Drip Loss % | Regression tree group |
|---|---|---|---|
| **F22** | 0.79 ± 0.03 $^{e}$ | 6.20 ± 1.06 | 1 |
| **F50** | 0.81 ± 0.02 $^{e}$ | 6.52 ± 1.04 | 1 |
| **B11B** | 0.85 ± 0.02 $^{de}$ | 7.02 ± 1.04 | 1 |
| **F60** | 0.85 ± 0.02 $^{de}$ | 7.12 ± 1.04 | 1 |
| **F28** | 0.87 ± 0.02 $^{cd}$ | 7.44 ± 1.04 | 1 |
| **C10F** | 0.91 ± 0.02 $^{bc}$ | 8.07 ± 1.04 | 2 |
| **462** | 0.91 ± 0.02 $^{bc}$ | 8.18 ± 1.04 | 2 |
| **F66** | 0.92 ± 0.02 $^{bc}$ | 8.23 ± 1.04 | 2 |
| **F62** | 0.95 ± 0.02 $^{ab}$ | 8.89 ± 1.04 | 3 |
| **B10B** | 0.96 ± 0.03 $^{ab}$ | 9.06 ± 1.07 | 3 |
| **C10B** | 0.98 ± 0.02 $^{a}$ | 9.66 ± 1.06 | 3 |

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

### 4.1.3 Evaluation of sires

Animals sired by sires M13, M36 and M40 did not display significant differences in drip loss percentage, and likewise animals sired by sires M36, M40, M4, M44 did not perform significantly different. Offspring from these five sires and sire M25 fall in the best performing regression tree group.

Note that sire 468 performed better than sire M25, but M25 is in regression tree group 1, while sire 468 is in regression tree group 2. This is because the variance in drip loss percentage of the offspring of sire 468 is almost twice the amount of variance for the offspring of sire M25. This is not visible in the standard errors of Table 4.3 because of rounding and transformations.

**Table 4.4 Mean Drip Loss Percentage of animals from different sires.**

| Sire | Mean $log_{10}$(Drip loss %) | Mean Drip Loss % | Regression tree group |
|---|---|---|---|
| **M13** | 0.75 ± 0.03 $^{h}$ | 5.68 ± 1.06 | 1 |
| **M36** | 0.79 ± 0.03 $^{gh}$ | 6.20 ± 1.06 | 1 |
| **M40** | 0.82 ± 0.03 $^{fgh}$ | 6.67 ± 1.06 | 1 |
| **M4** | 0.84 ± 0.03 $^{dfg}$ | 6.86 ± 1.06 | 1 |
| **M44** | 0.85 ± 0.03 $^{dfg}$ | 7.05 ± 1.06 | 1 |
| **M25** | 0.88 ± 0.03 $^{bdef}$ | 7.54 ± 1.06 | 1 |
| **468** | 0.86 ± 0.03 $^{bdfg}$ | 7.28 ± 1.06 | 2 |
| **B27F** | 0.88 ± 0.02 $^{bdf}$ | 7.52 ± 1.04 | 2 |
| **M26** | 0.88 ± 0.03 $^{bdef}$ | 7.61 ± 1.06 | 2 |
| **C28B** | 0.89 ± 0.02 $^{bde}$ | 7.80 ± 1.04 | 2 |
| **M35** | 0.91 ± 0.03 $^{bcd}$ | 8.06 ± 1.06 | 2 |
| **C30B** | 0.94 ± 0.03 $^{ace}$ | 8.65 ± 1.06 | 2 |
| **342** | 0.94 ± 0.02 $^{ace}$ | 8.67 ± 1.04 | 2 |
| **C29F** | 0.95 ± 0.05 $^{abd}$ | 8.84 ± 1.13 | 2 |
| **M34** | 0.95 ± 0.02 $^{ac}$ | 8.84 ± 1.04 | 3 |
| **M33** | 0.95 ± 0.02 $^{ac}$ | 8.88 ± 1.04 | 3 |
| **M38** | 0.97 ± 0.05 $^{ab}$ | 9.34 ± 1.13 | 3 |
| **C24F** | 0.99 ± 0.03 $^{a}$ | 9.86 ± 1.06 | 3 |

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

### 4.1.4 Evaluation of families

It appears from the regression tree group analysis that the families with the least fluid loss were families 44, 47, 35b, 48, with no significant difference. Families 41 and 21 also fall in the best performing group.

In the ASReml model, the effect of sires and dams seperately on weight had no significant improvement on the log likelihood values, and these effects were thus not assessed in the model.

**Table 4.5 Mean Drip Loss Percentages of animals from different families.**

| Family | Mean Pre export weight | Mean $log_{10}$(Drip loss %) | Drip Loss % | Regression tree group |
|---|---|---|---|---|
| **44** | 130.89 ± 3.40 | 0.75 ± 0.03 $^{j}$ | 5.68 ± 1.06 | 1 |
| **47** | 125.46 ± 3.26 | 0.79 ± 0.03 $^{ij}$ | 6.20 ± 1.06 | 1 |
| **35b** | 85.46 ± 4.15 | 0.82 ± 0.03 $^{hij}$ | 6.54 ± 1.06 | 1 |
| **48** | 129.66 ± 3.12 | 0.82 ± 0.03 $^{ghij}$ | 6.67 ± 1.06 | 1 |
| **41** | 119.21 ± 2.43 | 0.84 ± 0.03 $^{eghi}$ | 6.86 ± 1.06 | 1 |
| **21** | 113.85 ± 2.41 | 0.85 ± 0.03 $^{eghi}$ | 7.05 ± 1.06 | 1 |
| **4** | 87.58 ± 2.09 | 0.86 ± 0.03 $^{ceghi}$ | 7.28 ± 1.06 | 2 |
| **45** | 139.66 ± 3.13 | 0.88 ± 0.03 $^{cefgh}$ | 7.54 ± 1.06 | 2 |
| **35a** | 143.13 ± 4.40 | 0.88 ± 0.03 $^{cefgh}$ | 7.54 ± 1.06 | 2 |
| **37b** | 116.39 ± 2.75 | 0.88 ± 0.03 $^{cefgh}$ | 7.57 ± 1.06 | 2 |
| **46** | 127.14 ± 3.26 | 0.88 ± 0.03 $^{cdegh}$ | 7.61 ± 1.06 | 2 |
| **30a** | 103.39 ± 3.21 | 0.89 ± 0.03 $^{cdeg}$ | 7.83 ± 1.06 | 2 |
| **7b** | 102.59 ± 3.37 | 0.90 ± 0.03 $^{bce}$ | 8.03 ± 1.06 | 2 |
| **43a** | 122.91 ± 2.87 | 0.91 ± 0.03 $^{bce}$ | 8.06 ± 1.06 | 2 |
| **37a** | 157.18 ± 3.50 | 0.91 ± 0.03 $^{bce}$ | 8.04 ± 1.06 | 3 |
| **29a** | 100.81 ± 2.13 | 0.93 ± 0.03 $^{abc}$ | 8.43 ± 1.06 | 3 |
| **16** | 119.90 ± 2.62 | 0.94 ± 0.03 $^{abdf}$ | 8.65 ± 1.06 | 3 |
| **18** | 102.00 ± 6.49 | 0.95 ± 0.05 $^{abce}$ | 8.84 ± 1.13 | 3 |
| **7a** | 103.65 ± 3.80 | 0.96 ± 0.03 $^{abd}$ | 9.07 ± 1.07 | 3 |
| **31** | 179.12 ± 8.33 | 0.97 ± 0.05 $^{abc}$ | 9.34 ± 1.13 | 4 |
| **29b** | 166.96 ± 4.52 | 0.97 ± 0.03 $^{ab}$ | 9.37 ± 1.06 | 4 |
| **11** | 104.57 ± 4.43 | 0.97 ± 0.03 $^{ab}$ | 9.40 ± 1.07 | 4 |
| **38** | 165.07 ± 3.75 | 0.99 ± 0.03 $^{a}$ | 9.86 ± 1.06 | 4 |
| **30b** | 163.60 ± 5.24 | 1.00 ± 0.03 $^{a}$ | 9.93 ± 1.06 | 4 |

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

The six families in the best performing regression tree group weigh on average 38.44 ± 2.31 grams less than families 31, 29b, 11, 38, and 30b, which form the regression tree group with the most fluid loss. A trend that heavier individuals loose more moisture during live transport is in consequence observed. This is not always true, as we can see from family 11. Smaller animals have, in general, higher metabolic rates than their larger counterparts (Vosloo & Vosloo, 2006). In addition smaller

animals have a larger surface area relative to their volume than bigger animals. One would thus expect that smaller animals loose more water due to evaporation and dehydration. This is however not the case as we can clearly see that heavier animals have most of the time, a higher percentage drip loss in comparison with lighter animals. This should be taken into account in marketing strategies. This is in contrast with the findings of Wells *et al.* (1995) for *H. australis*, which displayed lower susceptibility to anaerobic stress in larger animals compared to smaller ones (Wells & Baldwin, 1995).

## 4.2 Correlations between traits

Correlations between traits in the three different trails were considered. This included the two traits of the drip loss trail, the two traits of the growth trail, and the six weight traits of the canning loss trail.

**Table 4.6 Correlation of rankings of different traits.**

| | Pre export weight | DL | Weight | Length | Pre shuck weight | Post shuck weight |
|---|---|---|---|---|---|---|
| **DL** | 0.08 | | | | | |
| **Weight** | 0.62* | -0.19 | | | | |
| **Length** | 0.60* | -0.01 | 0.94* | | | |
| **Pre shuck weight** | 0.76* | 0.04 | 0.74* | 0.69* | | |
| **Post shuck** | 0.79* | -0.06 | 0.81* | 0.72* | 0.96* | |
| **Can loss** | 0.18 | 0.13 | -0.09 | -0.18 | 0.06 | 0.16 |

*Correlations with statistically significant correlation, ($p < 0.05$). Pre export weight refers to the weight measured before the simulation of the group of abalone that underwent the transport simulation; log(DL): logarithm with base 10 of drip loss percentage; DL: Percentage moisture (drip) loss; Weight: weight of abalone in the growth trail; Length: Length of shells of abalone in the growth trail; Pre shuck: Weight before shucking of animals in the canning trail; Post shuck: Weight after shucking; Post gut: Weight after guts was removed; Post brine: weight after the brining process of the abalone meat; Pre can: Weight before canning; Post can: Weight after canning; Can loss: percentage weight loss after canning.

The rankings were done on family solutions obtained from ASReml. The correlation of family rankings for weight is not very high. The correlation between ranking of weight of the drip loss trail and weight of the growth trail is 0.62, while the correlation between weight of the drip loss trail and weight of the canning trail is 0.76. Correlation between weight of the growth trail and weight of the canning trail was 0.74. This indicates the consistency in performances of families relative to each other. Although these correlations are not very high, all of them are significant on the 5 % level. Family performances over the three different trails were thus well correlated.

Shell length and live weight seems to be highly correlated traits. The correlation of the family ranking according to length with family ranking according to weight in the growth trail is 0.94. This is evidence that shell weight does not differ significantly between families. Animals selected for total weight will still be the heaviest after shucking.The high significant correlation (0.96) between pre shuck weight and post shuck weight shows that selection for shell length will improve weight and vice versa. Selection for increased growth rate and increased post shuck weight can both be made on the overall weight of the animals. However, no significant correlation is seen in the ranking of families when the relationship between canning yield and weight is assessed.

### 4.2.1 Phenotypic correlations

Spearman correlation coefficients are non-parametric coefficients. P-values smaller than 0.05 are considered to be statistically significant. Since the animals had an age variation of 109 days, the data was manipulated by calculating the growth per day and multiplying it with the average age of the whole sample as an additional

observation. According to the results from the ASReml output, age had no significant effect on the data.

**Table 4.7 Phenotypic correlations of different traits, based on family evaluations. Traits on statistically significant level of up to 10 % are displayed.**

| Trends of significant phenotypic correlations | Correlation | p-value |
|---|---|---|
| Age and Drip loss % | 0.41 | 0.05 |
| Length and Drip loss % | 0.39 | 0.07 |
| Weight and Drip loss % | 0.40 | 0.06 |
| Length and Drip loss % corrected for age | 0.46 | 0.03 |
| Length and Canning loss % | 0.72 | 0.01 |
| Weight and Canning loss % | 0.68 | 0.01 |
| Weight and Drip loss % corrected for age | 0.46 | 0.03 |

It was assumed that the weight loss after 39 hours was mainly due to drip loss. Drip loss and canning loss correlates well with length and weight. This finding points to a similar trend as before in that bigger animals have bigger losses relative to smaller animals. Smaller abalone will thus have a higher percentage yield compared to bigger animals.

### 4.2.2 Genotypic correlations

> 'In the genetics of continuous variation, the genetic correlation coefficient between any two characters plays a part in the discussion of correlated responses under selection, and of the combination of different measurements to secure maximum improvement, the so called selection index' (Robertson, 1959).

Table 4.6 contains proxies for heritability and genetic correlations. The between family variance ratio was used as a proxy for heritability. This can be found on the diagonal. Covariance ratios were used as proxies for genetic correlations. Traits

which were not measured on the same animals are shaded grey and phenotypic correlations based on family means are displayed.

**Table 4.8 Proxies for heritability and correlations of traits.**

| | Weight (export) | log(DL) | DL | Weight | Length | Pre shuck | Post shuck | Post gut | Post brine | Pre can | Post can | Can loss |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Weight (export)** | 0.15 ± 0.05 | | | | | | | | | | | |
| **log(DL)** | 0.19 ± 0.31 | 0.04 ± 0.02 | | | | | | | | | | |
| **DL** | 0.45 ± 0.20 | 0.95 ± 0.02 | 0.03 ± 0.02 | | | | | | | | | |
| **Weight** | 0.95 | 0.44 | 0.53 | 0.11 ± 0.04 | | | | | | | | |
| **Length** | 0.94 | 0.42 | 0.51 | 0.94 ± 0.34 | 0.14 ± 0.05 | | | | | | | |
| **Pre shuck** | 0.97 | 0.32 | 0.44 | 0.96 | 0.96 | 0.19 ± 0.06 | | | | | | |
| **Post shuck** | 0.96 | 0.40 | 0.52 | 0.95 | 0.93 | 0.99 ± 0.01 | 0.15 ± 0.05 | | | | | |
| **Post gut** | 0.95 | 0.38 | 0.51 | 0.94 | 0.93 | 0.97 ± 0.02 | 0.97 ± 0.02 | 0.15 ± 0.05 | | | | |
| **Post brine** | 0.93 | 0.22 | 0.32 | 0.95 | 0.93 | 0.98 ± 0.01 | 0.99 ± 0.01 | 0.97 ± 0.02 | 0.18 ± 0.06 | | | |
| **Pre can** | 0.93 | 0.23 | 0.33 | 0.94 | 0.93 | 0.98 ± 0.01 | 0.99 ± 0.01 | 0.98 ± 0.01 | 0.99 ± 0.01 | 0.19 ± 0.06 | | |
| **Post can** | 0.90 | 0.22 | 0.31 | 0.92 | 0.91 | 0.97 ± 0.01 | 0.97 ± 0.01 | 0.98 ± 0.01 | 0.99 ± 0.01 | ~1 | 0.20 ± 0.06 | |
| **Can loss** | 0.86 | 0.30 | 0.45 | 0.81 | 0.83 | 0.85 ± 0.01 | 0.85 ± 0.02 | 0.86 ± 0.01 | 0.75 ± 0.01 | 0.73 ± 0.02 | 0.69 ± 0.02 | 0.08 ± 0.03 |

Weight (export) refers to the weight measured before the simulation, of the group of abalone that underwent the transport simulation; log(DL): logarithm with base 10 of drip loss; DL: Percentage moisture loss; Weight: weight of abalone in the growth trail; Length: Length of shells of abalone in the growth trail; Pre shuck: Weight before shucking; Post shuck: Weight after shucking of the canning trail animals; Post gut: Weight after guts was removed; Post brine: weight after the brining process of the abalone meat; Pre can: Weight before canning; Post can: Weight after canning; Can loss: percentage weight loss after canning.

Very low heritability is observed for drip loss (0.03 ± 0.02) and canning loss (0.08 ± 0.03). Weight (on average 0.15 ± 0.05) and length (0.14 ± 0.05) show a low to medium heritability. Selection may therefore not be very effective, especially for drip loss and canning loss, but even a slight increase can be economically important (Kube *et al.*, 2007).

Lucas *et al.* (2006) estimated the heritability of shell length and width, and wet weight in *H. asinina*. Substantially higher estimates of heritability were approximated, with heritability of shell length 0.48 ± 0.15 and wet weight heritability 0.36 ± 0.13. *H. asinina* has the fasted growth rate of all abalone. In addition, Kube *et al.* (2007) estimated lower heritability for growth traits in three year old *H. laevigata* with heritably of 0.10, 0.10, and 0.04 respectively for total weight, meat weight, and shell length. It was reported that the latter population was small. Literature thus presents a high variation in heritability estimates of growth rate between different species.

Mucous production can be described as a physiological trait. The heritability estimate of drip loss is very low (0.03 ± 0.02). Physiological traits display lower estimates of heritability compared to morphological traits, according to Moussaeu *et al.* (1987). The low heritability can be an indication that the trait is closely related to fitness and has therefore little genotypic variance as the result of natural selection. This gives some evidence for our suspicion that mucous production is an important mechanism of defence to abalone and should rather not be tempered with.

Dressing percentage of cattle has moderate heritability estimates and is expected to be in the range of 0.20 – 0.35 (Gall, 2008). Heritability for dressing

percentage in African Short Horn beef cattle had a high estimate of 0.49 ± 0.19 (Pariacote *et al.*, 1998). Sheep and goats exhibit dressing percentages of 55.78 ± 0.32 and 53.50 ± 1.43, respectively (Sen *et al.*, 2004). However, dressing percentages in trout, Atlantic salmon and Channel catfish were estimated to be 0.01, 0.03, and 0.00, respectively (Gjedrem, 1983). Our estimate of 0.08 ± 0.03 is thus somewhat higher compared to the mentioned fish species. The low heritability of dressing percentage of abalone and fish, compared to cattle is consistent with the findings of Moussaeu *et al.* (1987) that ectotherms have lower heritability for morphological traits than endotherms.

**Table 4.9 Initial weight and canning loss for different genders.**

| Gender | Pre Shuck Weight (g) | Canning loss (%) |
|---|---|---|
| **Female** | 128.37 ± 35.11[a] | 72.51 ± 3.38[§] |
| **Male** | 130.79 ± 39.36[a] | 72.67 ± 3.23[§] |
| **Unknown** | 104.22 ± 38.65[b] | 73.72 ± 3.15[§] |

Means with different superscripts differ significantly, (P<0.05).

Gender had no significant effect, as shown in Table 4.9. This is consistent with our expectation, since morphology between sexes does not differ as much as in mammals. Heifers usually have up to two percent lower dressing percentage than steers at a similar fat level, because of waste fat in the udder, around the internal organs and on the carcass (The beef site, 2006). Note that the sexes of 52 smaller animals were not clearly visible as neither green ova nor beige sperm could be distinguished through the mantle of the gonads possibly due to sexual immaturity. The number of male and female animals was respectively 684 and 670. A slightly lower dressing percentage is observed for the animals with unknown gender. Based on the genotypic correlation, weight is positively correlated with canning loss. A conclusion that lighter animals have lower

dressing percentage can however not be made based on the phenotypic values as shown in Figure 4.1, where we detect that the highest dressing percentage (lowest canning loss percentage) is associated with the lightest family.

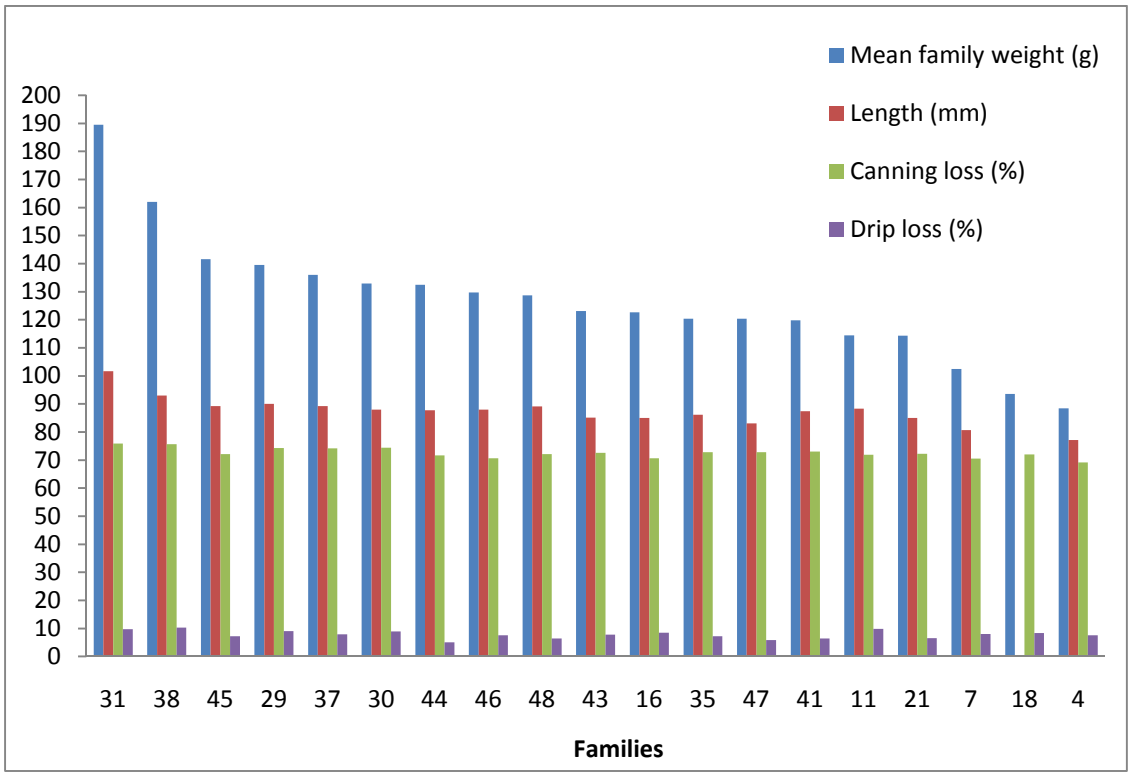


**Figure 4.1 Family relationships of Length, Canning loss and Drip loss relative to the mean family weight across the three experiments.**

Data presented in Figure 4.1 indicates that growth displays the most variation amongst the traits considered. The high correlation (genetic correlation: 0.94 ± 0.34) between length and weight is also evident. The large standard deviation, however weakens the power with which conclusions can be drawn. The same trend was also found in *H. asinna* (Lucas *et al.*, 2006) where they found the correlation between shell length and weight to be higher than 0.98. This together with the correlations of ranking (Table 4.5) confirms what was suggested after the correlations of rankings. We conclude that animals with a longer shell will also be heavier, and selection can be done on size rather than weight for practicality during the sorting procedure on farms. The high genetic

correlation of 0.85 ± 0.01 between weight and canning loss shows that animals that weigh more have a lower dressing percentage.

## 4.3 Summary

The heritability and correlations of drip loss, growth and canning yield for *H. midae* were estimated. Farm specific rearing protocols had a significant effect on the mean weight of families over locations. This, together with the fact that every parent was involved in a maximum of two crosses, was the main factors that complicated data analysis and questions the accuracy of the outcomes.

Of the three traits, growth rate has the highest heritability and provide a sufficient basis for genetic improvement through selection either on body weight, or shell length. Dressing percentage has a lower heritability, with a general trend that heavier animals have a lower dressing percentage. Very low heritability was estimated for drip loss. This can be an indication that the trait is closely related to fitness and has low variances between families due to natural selection. The heritability estimates may be different with an improved family structure. To assure that the estimation of heritability is more or less spot on, it could be useful to do similar trails over a range of temperatures to see if any G x E interaction and phenotypic plasticity is observed. Phenotypic plasticity will show a gradient of the phenotype as a function of the environment. If different genotypes show different phenotypic values with a change in environment, this will typically result in non-parallel or crossing functions, also known as G x E interactions (Stearns, 1992).

Selection for growth and dressing percentage does not seem to have risks, but at the end, if selection would have an effect on mucous production of *H. midae* due to significant heritability of the trait, decisions should be made with cautious consideration of the effect that selection for less drip loss, i.e. less mucous production, might have on the overall fitness of the farmed population.

We recommend that selection for growth should be continued on the basis of a sufficiently high heritability for both weight and shell length. Given the low heritability values recorded for drip loss and dressing percentage, it is recommended not to incorporate them into the selection program but rather to conduct further assessments. It is also recommended that additional research is conducted on the physiological process during stress and areal exposure, as well as the effect of nutrition on yield of both live exported and canned abalone.

## 4.4 References

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# Chapter 5 Conclusion

Abalone are univalve marine gastropods (Elliott, 2000). Six species are indigenous to the Southern African coastline and 52 species are recognised worldwide (Elliott, 2000; Franchini *et al*., 2011). Abalone inhabit the intertidal zone, where they feed nocturnally on kelp and other seaweeds (Sales & Britz, 2001; Franchini *et al*., 2011). They reach sexual maturity from three years of age in warmer water, while it can take up to seven years in colder temperatures (Sales & Britz, 2001). Abalone are broadcast spawners and possess a complex life cycle with several larval stages (Lemay & Boulding, 2009). *Haliotis midae* is the only farmed species in South Africa (Franchini *et al*., 2011).

Abalone farming is the most valuable sector in the relatively underdeveloped South African aquaculture industry (Shipton & Britz, 2007). Abalone are an international valued commodity, which led to sharp decreases in natural populations due to over-exploitation and poaching. Abalone farming has been developed in many countries for a variety of species (Franchini *et al*., 2011) to meet the high demand and to supplement natural stocks. The success of the local industry is due to good cooperation between industry and research organizations that have developed technologies in support of the local industry and its progression (Shipton & Britz, 2007). Different fields of research such as nutrition, genetics, biotechnology, reproduction and post-harvest characteristics are combined to improve the knowledge systems and technologies for the cultured of *H. midae*.

This study forms part of a project aimed at the genetic improvement of *H. midae* through selective breeding, the first of its kind for the species. It had the

objective to estimate heritability and genotypic and phenotypic correlations regarding growth rate, canning yield, and drip loss during live export. The outcome of the study is to direct the inclusion of these traits in the genetic improvement strategy for the species.

The study estimated the heritability for growth rate in terms of live weight (0.14 ± 0.05) and shell length (0.14 ± 0.05) that justify the inclusion of the trait in the selection program, also taking into account that growth rate is a key determinant of financial viability of abalone farming. The high genetic and phenotypic correlations between live weight and shell length allow for both of these traits to be considered as selection criteria. The low heritability estimates obtained for drip loss (0.03 ± 0.02) and canning yield (0.08 ± 0.03) however indicate that there is insufficient evidence for the inclusion of these traits in the selection program at this stage. Due to the weaknesses in the current experimental structure as a result of a limited number of families which differ in age and were reared at different locations, it is recommended that additional assessments of genetic parameters for these traits are conducted in order to inform their future inclusion in the selection program.

## 5.1 Recommendations for future work

For this study a new base population (N = 800) was established through the collection of sexually mature animals from the natural population, in line with recommendation of Robinson, *et al*. (2010). This approach then required that the broodstock be conditioned for induced spawning in order to create a series of full and half sib families. Pedigree data was not available for this wild stock, and therefore an animal model could not be used in ASReml to analyse the data.

Problems experienced with conditioning and synchronised spawning of the broodstock, as well as the loss of a number of family groups on one of the participating farms, reduced the number of crossings per sire and/or dam to a level that were not sufficient for half a sib analysis with ASReml, and consequently proxies had to be used.

It is therefore recommended that an additional set of half sib families be created as a basis for further assessment and analysis of genetic parameters within the framework of the selective breeding program.

The fact that families were kept at different locations, and exposed to different management regimes also complicated the model. The low spawning success of newly conditioned broodstock prevented the inclusion of a single full sib reference group across all locations. Subsequently, conditioning of the broodstock have improved to levels were a single full sib reference group can be obtained. It is nevertheless recommended that during future analysis all family groups that form part of such an analysis should be kept at the same location for the duration of the trial in order to reduce environmental variation.

The simulation of live transport procedures accounted for actual timelines, temperature regimes and handling stressors. Improvements in the simulation protocol and experimental procedures can however be achieved in standardizing of the weighing protocols, specifically with regard to removing of access moisture and mucous with cognisance of the impact of exposure time as an additional stressor.

## 5.2 References

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