

**A Quantitative Genetic Analysis of the Effect of Crossbreeding on the Growth Rate of  
the South African Abalone, *Haliotis midae*.**

**Gysbert Vorster**

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at the University of Stellenbosch.

Supervisor: Mr. Danie Brink

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## DECLARATION

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and that I have not previously in its entirety or in part submitted it at any university for a degree.

Signature:.....

Date:.....

## ABSTRACT

The genetic status of *H. midae* broodstock in the South African aquaculture industry reflects that of random samples originating from undomesticated wild populations. The nature of growth in abalone is very slow, taking between three and four years to reach a marketable size of between 60 to 100 grams. It is therefore of paramount importance to improve this trait in order to ensure global competitiveness and economic viability within the industry. Improving this negative characteristic through conventional selection methods is a long-term venture and alternative means that would yield instantaneous results had to be considered. Crossbreeding was identified as an alternative, short-term strategy to improve growth rate.

A crossbreeding experiment was performed between two populations of the abalone, *Haliotis midae*, from the East (E) and West (W) Coast of South Africa. This was done to investigate the occurrence of heterosis for growth among the crossbred genotypes (East x West, West x East). Fifteen males and females from both the East and the West Coast populations were mated in a complete diallele crossbreeding experiment to produce four progeny groups (WW, EE, EW and WE). Progeny groups were evaluated for weight ( $b_w$ ) and length gain ( $b_L$ ) over a specific growth period of 9 months.

The results provided no evidence of significant differences in weight ( $P = 0.085$ ) or length gain ( $P = 0.244$ ) between the four progeny groups, giving no indication of significant heterosis for weight and length gain among the crossbred progenies of these East and West Coast populations. It is recommended that further efforts to obtain improved growth rate in the abalone, *Haliotis midae*, through crossbreeding only be considered in light of clear evidence of substantial genetic differentiation between such populations.

## UITREKSEL

Die huidige status van perlemoen, soos dit voorkom in akwakultuur bedrywighede in Suid Afrika, weerspieël dié van 'n ewekansige monster vanuit wilde, natuurlike populasies. Perlemoen is inherent 'n stadig groeiende organisme wat tussen drie en vier jaar neem om tot 'n bemarkbare grote van 60 tot 100 gram te groei. Dit is dus uiters noodsaaklik om hierdie eienskap te verbeter ten einde die bedryf ekonomies lewensvatbaar en mededingend op wêreld markte te maak. Konvensionele seleksie as 'n metode om hierdie negatiewe eienskap te verbeter is 'n langtermyn onderneming wat die identifisering van 'n korttermyn metode, wat onmiddellike resultate lewer, noodsaak. Kruisteelt is geïdentifiseer as geskikte korttermyn oplossing aangesien dit onmiddellike resultate lewer.

'n Kruisteel eksperiment is uitgevoer tussen twee populasies van die perlemoen, *Haliotis midae*, van die Ooskus (E = East) en die Weskus (W = West) van Suid Afrika. Dit is gedoen om die omvang van heterose vir groeitempo in die gekruisde nageslag (East x West, West x East) te bepaal. Fyftien mannetjies en wyfies van beide die Oos- en Weskus populasies is met mekaar gepaar in 'n volledige dialleel kruising om vier nageslag groepe (WW, EE, EW en WE) te vorm. Die nageslag is geëvalueer ten opsigte van massa ( $b_w$ ) en lengte ( $b_L$ ) toename oor 'n spesifieke groei tydperk van 9 maande.

Die eksperimentele resultate dui daarop dat die vier nageslag groepe nie betekenisvol van mekaar verskil het ten opsigte van massa ( $P = 0.085$ ) en lengte ( $P = 0.244$ ) toename nie en dat daar dus geen aanduiding van heterose vir massa en lengte toename in die nageslag van kruisings tussen die Ooskus en Weskus populasies bestaan nie. Daar word aanbeveel dat kruisteling as 'n metode van genetiese verbetering van groeitempo in *Haliotis midae* slegs oorweeg word in die lig van nuwe molekulêre bewyse van genoegsame genetiese differensiasie tussen sulke populasies.

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## CHAPTER ONE

### 1. INTRODUCTION

#### 1.1 Overview of abalone production in South Africa

The abalone, *Haliotis midae*, is one of five species of abalone indigenous to the South African coastline (excluding *H. pustulata* occurring along the far North East Coast of South Africa). It forms the basis of the commercial abalone industry being the only species that is harvested commercially. Commercial abalone harvesting has seen an annual commercial catch that peaked at 2800 tons in 1965, but declined steadily to 600 tons in 1995 after the annual commercial catch was limited by quotas in 1970 (Tarr, 1995). In 2001, the Department of Environmental Affairs and Tourism (Department of Environmental Affairs and Tourism, 2001) allocated fishing rights for abalone to 212 successful applicants over a period of two years and reduced the Total Allowable Catch (TAC) for the fishery from 496 tonnes to 475 tonnes.

Further reductions, if not a complete ban, is expected due to overexploitation of the natural resource through commercial and recreational fishing, as well as illegal harvesting. Illegal harvests have reached levels of alarming proportions with authorities being unable to exert effective control over the situation. This phenomenon of overexploitation of natural abalone resources extends worldwide and the increasing demand has given rise to inflated prices on global markets, fuelling the situation even further. Concerns from various stakeholders over the status of the natural resource and the sustainability of natural harvests sparked interest in the artificial cultivation of abalone, including *H. midae*. Artificial cultivation holds additional benefits in terms of consistency of supply, environmental control, economic growth and supplementation of natural stocks.

Artificial cultivation was initiated in the 1980's and Genade *et al.* (1988) recorded the first event of induced spawning of abalone, *H. midae*, at the Aquaculture Unit of the Fisheries

Development Corporation of South Africa Ltd. at Knysna in 1982. Further research into the biological and economic feasibility of commercial cultivation of *H. midae* gave indication of encouraging results in terms of growth rate and food conversion efficiency. Continuation of research and development by the Council for Scientific and Industrial Research (CSIR) and other research and industrial institutions led to the establishment of several commercial abalone farms along the South African coast during the period of 1990 to 2000.

## **1.2 Genetic status of farmed stocks**

Initial research and development on abalone focussed primarily on aspects such as the development of production methods, husbandry systems, nutritional requirements and feeding, water quality, environmental parameters and disease management, with little attention being given to the management and development of the genetic resources. The local abalone culture industry has reached a stage where all aspects of husbandry and cultivation need to be addressed in order to maintain competitiveness on global markets.

Hatcheries acquired their initial brood stock or founding populations from the wild through a process of random collection of sexually mature individuals, often from geographical areas adjacent to the farming operation. The fecundity of *H. midae* is exceptionally high, with from 3-4 million eggs produced from a single female spawning. This implies that only a small number of broodstock, as little as 8 to 20 individuals, are required to produce adequate numbers of seed stock for commercial farming operations. Most abalone farms are permitted to collect and keep 200 abalone from natural populations for breeding purposes. The cohorts are mostly sub-divided into smaller breeding groups with an effective population size of between 50 and 70 individuals that are spawned together. These groups usually comprise a male to female ratio of 1:3 to 2:3. Although few commercial units, if any, have implemented genetic improvement programmes, a sense of urgency to start such programmes was noted (personal survey).

The current status of breeding groups in commercial abalone hatcheries therefore tends to reflect that of a random wild sample with a relative small effective population size, due to the high natural fecundity. The absence of proper genetic management systems such as individual or group identification systems, record keeping, mating designs, uneven sex ratios, etc. has led to the exposure of farmed population to unknown levels of inbreeding. This creates a situation where the status of commercial broodstock in the South African abalone culture industry can be considered as “undomesticated”, resembling the genetic characteristics of wild populations, with its genetic integrity under threat due to inadequate genetic management practices. This situation necessitates the implementation of genetic improvement and management strategies in order to ensure improved genetic quality and economic competitiveness on global markets.

## CHAPTER TWO

### 2. REVIEW OF GENETIC IMPROVEMENT STRATEGIES FOR ABALONE

The most common methods of genetic improvement of farmed organisms are through selection, crossbreeding of same species or hybridization of different species. Other methods of improvement include chromosome manipulation, genetic engineering and marker assisted selection techniques.

#### 2.1 Improvement through Breeding

Aquaculture is a recent development and the majority of farmed aquatic species are in an undomesticated genetic state compared to other terrestrial farmed animals such as cattle, sheep, pigs, chickens etc. Evolutionary development has ensured adaptation of these aquatic species to a variety of natural environments where the ability to survive and reproduce is of paramount importance. The transfer of the species from the wild to the commercial captive environment imposes new challenges to which the species is less adapted. These include nutrition, feeding behaviour, stocking densities, health and disease risks, handling, transport, processing and quality. Under these new husbandry conditions, a process of accelerated domestication through the implementation of genetic improvement strategies is required to ensure optimal performance in terms of economic important criteria such as rate of growth, feed conversion efficiency, survival, yield, product quality, etc. Most of the historically farmed animal species have gone through the process of natural adaptation to farming environments spanning thousands of years.

There are two conventional ways through which a breeder can change the genetic properties of the population. The first, through the choice of the individuals to be used as parents, which constitutes selection and the second, through controlling the way in which the parents are mated, which embraces inbreeding and crossbreeding (Falconer, 1989). This process of domestication and genetic improvement through selection and breeding is normally

associated with a reduction in genetic variation. As a result, the genetic variability of economic and fitness traits is quite low in most of the common farm animal populations.

Undomesticated species such as *H. midae*, as with many other aquaculture species such as mussels (Innes & Haley, 1977; Newkirk, 1980), oysters (Newkirk, 1980), and various finfish species that still resemble the wild population profiles, are however expected to demonstrate significant levels of genetic variation that may form the basis of genetic improvement strategies. Fish and shellfish often have higher genetic variance compared to farmed land animals. Gjedrem (1997) notes the genetic variation for growth rate is seven to ten percent in farmed land animals and 20-35 percent in fish and shellfish. The extent of variation along with other genetic parameters such as heritability, correlations, heterosis effects, and genotype/environment interactions needs to be determined for economically important traits before embarking on any long-term breeding programme. These parameters will give an indication of the expected significance of selection in breeding programmes, what type of selection strategy to use as well as the best strains to be used for a specific environment. The way in which these breeding strategies are applied will to a large extent determine the rate at which genetic change can be brought about. General indications are that genetic improvements in traits of economic importance in mollusc species of between 10 to 20 percent per generation can be expected on the basis of reported genetic parameters (Newkirk 1980).

### **2.1.1 Improvement through Selection**

Selection is the practice whereby superior individuals in a population are identified, often on the basis of phenotypic performance, to be used as parents of future generations in order to improve certain characteristics of economic importance. The improvement in the mean value of a specific trait within the population in successive generations is referred to as the realised response to selection. The expected response to selection for a specific trait in a specific



population can also be predicted on the basis of Equation 1 from Falconer (1989) if the relevant parameters are known:

$$R = (i.h^2.\sigma_p) / L \quad (1)$$

where:

**R** = the expected response or genetic improvement in the value of the trait under selection

**I** = the selection intensity, indicating the proportion of the population that was selected as parents, expressed in terms of units of standard phenotypic deviations of the trait under selection,

**h<sup>2</sup>** = the heritability of the trait under selection,

**σ<sub>p</sub>** = the standard phenotypic deviation of the trait under selection, and

**L** = the generation interval of the species, i.e. age at sexual maturity

A change in any of the parameters determining genetic response has a direct effect on genetic improvement. An understanding of the parameters that determine genetic improvement in an individual, allows the breeder to obtain improved results more efficiently by focussing on the parameter in question.

#### **2.1.1.1 Intensity of Selection**

The proportion of the population included in the selected group is termed the intensity of selection (*i*) (Falconer, 1989). Selection intensity increases as the proportion of the population selected as broodstock decreases. It is clear from equation 1 that the higher selection intensity is, the higher response to selection will be. The South African abalone, *Haliotis midae*, is a dioecious, broadcast spawner with a very high fecundity (Wood & Buxton, 1996). This

characteristic of abalone makes allowance for achieving extraordinary high levels of selection intensities in comparison to other terrestrial farmed species.

### 2.1.1.2 Genetic variation and Heritability ( $h^2$ )

Genetic variation between individuals within a population forms the basis for improvement of a trait through selection. The formation of a breeding unit with a broad genetic base that incorporates a large amount of genetic variation is essential in achieving genetic improvement through selection. Phenotypic variation ( $V_P$ ), observed as differences in phenotypic characteristics, is determined both by genetic ( $V_G$ ) and environmental ( $V_E$ ) variances as expressed in Equation 2 (Falconer, 1989):

$$V_P = V_G + V_E \quad (2)$$

where,

$V_P$  = the observed phenotypic variation

$V_G$  = the variation due to genetic effects

$V_E$  = the variation due to environmental effects

Reports on genetic variation indicate the presence of significant levels of genetic variation for economically important traits in blue mussel, *Mytilus edulis*, (Innes & Haley, 1977; Mallet *et al.*, 1986) and Pacific oyster, *Crassostrea gigas* (Lannan, 1972). Jönasson *et al.* (1999) reported large phenotypic variation in rate of survival between families of the red abalone, *Haliotis rufescens*, ranging between 0.02% and 31.5%. Mallet and Haley (1984) observed significant additive genetic effects in oyster larval viability at day 12 and larval shell length at day 2. Jones *et al.* (1996) detected significant phenotypic variation (CV=7.2-10.85%) in the larval giant scallops, *Placopecten magellanicus*.

The genetic component of variation can in turn be described in terms of heritable or additive variance ( $V_A$ ) and the non-heritable or non-additive components of dominance variance ( $V_D$ ) and interactive variance ( $V_I$ ) as explained by equation 3 (Falconer, 1989):

$$V_G = V_A + V_D + V_I \quad (3)$$

where:

$V_G =$  the variation due to genetic effects  
 $V_A =$  the variation of breeding values

$V_D =$  the variation due to dominance effects of alleles

$V_I =$  the variation due to interaction between alleles

The assessment of the genetic and environmental control of measurable characteristics is defined by the concept of heritability. The heritability ( $h^2$ ) of a characteristic refers to the proportion of the observed phenotypic variation ( $V_P$ ) of that characteristic in a particular population that is explained by additive genetic variance ( $V_A$ ) among individuals within the population, as expressed by equation 4 (Falconer, 1989):

$$h^2 = V_A/V_P. \quad (4)$$

The heritability of a trait is therefore a characteristic of the species and the population in which it is measured. The greater the additive genetic portion of total variance, the greater the degree of resemblance between parent and offspring will be and the greater the value for heritability. High values for heritability indicate the potential for genetic improvement through selection since response to selection increases with high values for heritability (Equation 1). Heritability allows the breeder to predict the performance of the offspring based on the performance of the parent. It also indicates the accuracy of selection.

Table 1 Heritability values ( $h^2$ ) for economically important quantitative traits in different shellfish species.

Species	Trait	$h^2$	Author(s)
Abalone			
<i>Haliotis rufescens</i>	Survival	0.11 (0.33)	Jónasson <i>et al.</i> , 1999
	Shell length: 8 months	0.08	
	10 months	0.06	
	18 months	0.27	
	24 months	0.34	
Oyster			
<i>Saccostrea cucullata</i>	Total weight	0.28 ± 0.01	Jarayabhand & Thavornmyutikarn, 1995
<i>Ostrea chilensis</i>	Shell height	0.34 ± 0.12	Toro & Newkirk, 1991
	Live weight	0.43 ± 0.18 - 0.69 ± 0.11	Toro <i>et al.</i> , 1995
<i>Crassostrea virginica</i>	Larval growth rate	0.25 - 0.50	Newkirk <i>et al.</i> , 1977
	Larval Growth	0.24	Longwell, 1976
	Length: Juvenile	0.29 - 0.71	Losee, 1978

<i>Crassostrea gigas</i>	Length:	12 months	0.81±0.07	Lannan, 1972
	Width:	12 months	1.17±0.05	
	Height:	18 months	0.10±0.12	
		12 months	0.81±0.27	
	Total weight:	18 months	0.19±0.003	
	Meat weight:	18months	0.33±0.19	
Scallop			0.37 ± 0.06	
<i>Argopecten circularis</i>	Total Weight		0.33 ± 0.08 - 0.5 ± 0.13	Ibarra <i>et al.</i> , 1999
	Shell Width		0.10 ± 0.07 - 0.18 ± 0.08	Ibarra <i>et al.</i> , 1999
<i>Argopecten irradians</i>	Shell Length		0.21 to 0.37	Crenshaw <i>et al.</i> , 1991
<i>Placopecten magellanicus</i>	Shell Length:	4day	1.10 ± 0.171	Jones <i>et al.</i> , 1996
		14day	1.24 ± 0.399	
		21day	1.21 ± 0.384	
Mussel				
<i>Mytilus edulis</i>	Length:	Larval	0.11±0.02	Mallet <i>et al.</i> , 1986
		Larval	0.16; 0.29	Innes & Haley, 1977
		Larval	0.12 - 0.62	Newkirk, 1980

	Juvenile	0.62 ± 0.06	Mallet <i>et al.</i> , 1986
	Adult	0.22 ± 0.07 - 0.92 ± 0.27	Mallet <i>et al.</i> , 1986
Clam			
<i>Mercenaria mercenaria</i>	Growth rate: Juvenile	0.37	Rawson and Hilbish, 1990
	Growth rate: 2 years	0.42 ± 0.01	Hadley <i>et al.</i> , 1991

Jónasson *et al.* (1999) assessed the heritability estimates of survival and size of a synthetic population of the red abalone, *Haliotis rufescens*, imported from California to Iceland. Heritability estimates for survival at age 4 months was reported as 0.11 and for shell length at age 8, 10, 18 and 24 months respectively as 0.08, 0.06, 0.27 and 0.34.

Realized heritability for many economically important traits in several shellfish species have been reported. Realized heritability is calculated as the realized response (R) over the selection differential (S), selection differential being the difference between the means of the selected parents and the whole population before selection (Falconer, 1989). Ibarra *et al.* (1999) estimated realized heritability for shell width ( $0.10 \pm 0.07$  to  $0.18 \pm 0.08$ ) and total weight ( $0.33 \pm 0.08$  to  $0.59 \pm 0.13$ ) in the catarina scallop, *Argopecten circularis*, while Crenshaw *et al.* (1991) estimated realized heritability for shell length in the bay scallop, *Argopecten irradians*, as 0.21 to 0.37. Jones *et al.* (1996) estimated heritability of larval shell length on day 4, 14, and 21 in the giant scallop, *Placopecten magellanicus*, as  $1.10 \pm 0.171$ ,  $1.24 \pm 0.399$  and  $1.21 \pm 0.384$  respectively. The estimates were found to be not significantly different among the three age groups. These estimates were made from full-sib families and it should be noted that the values for  $V_A$  might be inflated with non-additive genetic, maternal and common environmental components of variation.

Lannan (1972) estimated the realized heritabilities for, among other traits, total weight and meat weight in the oyster *Crassostrea gigas* as  $0.33 \pm 0.19$  and  $0.37 \pm 0.20$  respectively while Losee (1978) estimated heritability for juvenile length at between 0.29 and 0.71. Jarayabhand and Thavornytikarn (1995) estimated realized heritability for total weight at commercial size in the tropical oyster, *Saccostrea cucullata*, to be  $0.28 \pm 0.01$ . Heritability for shell height in the Chilean oyster, *O. chilensis*, has been estimated as  $0.34 \pm 0.12$  (Toro and Newkirk 1991) and heritability for live weight as  $0.43 \pm 0.18$  -  $0.69 \pm 0.11$  (Toro *et al.*, 1995). Newkirk *et al.* (1977) assessed the heritability of larval growth rate in *Crassostrea virginica*

to be between  $h^2=0.25$  and  $h^2=0.50$  while Longwell (1976) estimated heritability for larval growth rate at  $h^2=0.24$ .

Mallet *et al.* (1986) estimated heritability for larval length in the blue mussel, *Mytilus edulis*, at  $0.11 \pm 0.02$ , for juvenile length at  $0.62 \pm 0.06$  and for adult length at between  $0.22 \pm 0.07$  and  $0.92 \pm 0.27$ . Innes & Haley (1977) estimated heritability for larval length at between  $h^2=0.16$  and  $h^2=0.29$  while Newkirk (1980) estimated heritability for larval length to be between  $h^2=0.12$  and  $h^2=0.62$ .

Rawson and Hilbish, (1990) estimated growth rate in juveniles of the clam, *Mercenaria mercenaria*, to be 0.37, while Hadley *et al.* (1991) estimated growth rate of two year olds to be  $0.42 \pm 0.01$ . The accuracy of the estimate of heritability increases as the number of groups on which it is based increases. The heritability estimates for most of the economically important traits of the aquaculture species listed above are high overall. Although many of the estimates for heritability have not been made at market size and are based on small populations, the potential for genetic change through long-term directional breeding programmes is still evident. Jónasson *et al.* (1999) concluded that selection for growth in the red abalone should be made as close to market size as possible on the basis of heritability estimates ranging from as low as 0.08 at age eight months through 0.27 to 0.38 at age 24 months (Table 1). Although the cost of housing more animals increases over time, a more accurate value for heritability is obtained when selection is postponed until market size.

No estimates for genetic variation or heritability in quantitative traits of *Haliotis midae* have been reported in literature to date. It can be assumed to be significant, however, since no artificial selection, hence no reduction in natural levels of genetic variation, has taken place.



### **2.1.1.3 Phenotypic variation and Standard deviation**

Phenotypic variability is a property of the trait and the population in which it is measured and can be expressed in terms of the standard deviation. The phenotypic standard deviation also describes the units in which the selection response is measured (Falconer, 1989).

### **2.1.1.4 Generation interval**

Generation interval is defined as the length of time between corresponding stages in the life cycle of successive generations (Falconer, 1989). The effect of selection, which is genetic gain, is cumulative in nature from one generation to the next. Response over time can however be influenced by the generation interval associated with a particular species. The longer the generation interval of a particular trait or species, the lower the response through selection expressed in a period of time.

Abalone species, in general, display long generation intervals of between four and five years in comparison with other aquaculture species like oysters (one year), mussels (one year), trout and salmon (two years) and tilapia (six months). The relatively long generation interval of *Haliotis midae* of about five years reduces the efficiency of selection as a strategy to achieve short-term genetic improvement of economic important traits. Due to the cumulative nature of selection response, it should however still be considered as a long-term strategy for genetic improvement. Hara and Kikuchi (1989) reported a response of 21 percent in daily growth in the abalone, *H. discus hannai*, up to the 20-30mm stage and 65 percent up to the 30-70mm, after three generations of selection. Accessible literature on the subject of selection in abalone was found to be rare even though artificial cultivation of abalone has been practiced for some time in the Far East. This could be due to the shift in emphasis from mere propagation to improvement with greater knowledge of breeding and inheritance as well as the infancy of the industry outside of Japan.

Selection for resistance to summertime mortality in the Pacific oyster, *Crassostrea gigas*, was initiated after Lipovsky and Chew (1972) exposed laboratory animals to elevated water temperatures to induce stress and eventually mortality. Survivors of these experiments were subsequently used (Beattie *et al.*, 1978), as was their progeny (Beattie *et al.*, 1980), as parents to produce successive generations of *C. gigas* with an elevated resistance to high summer water temperatures. The result was a selected line with a dramatically higher resistance to summertime mortality compared to the original control groups (Hershberger *et al.*, 1984). Ward *et al.* (2000) reported a response of eight percent in growth rate in the first generation from a mass selection in *Crassostrea gigas*. In other tests performed on Pacific oysters by Langdon *et al.* (2003) the average live weight yield of progeny from selected lines was 9.5 percent greater than that of non-selected control families. Nell *et al.* (1999) reported a genetic gain of nine percent increased growth rate in Sydney rock oysters (*Saccostrea commercialis*). Toro *et al.* (1996) obtained similar results in the Chilean oyster (*Ostrea chilensis*). In the hard-shell clam or quahaug (*Mercenaria mercenaria*), a genetic gain of nine percent per generation of selection for growth rate has been estimated.

## **2.1.2 Methods of Selection**

Heritability estimates is of value in choosing which selection method to use under particular circumstances. When the heritability is low, family selection offers an improved response while with-in family selection is preferred when the heritability for a trait is high (Falconer, 1989).

### **2.1.2.1 Mass Selection (Individual Selection)**

Individual or mass selection is the simplest form of selection requiring the least amount of management. Individual selection can be applied under conditions where the relationship between animals is unknown, i.e. in the absence of identification techniques. Individuals are

selected according to their own phenotypic performance irrespective of familial origin. Individual selection may yield better results than family and within family selection across a broad range of conditions such low heritability (Ward *et al.*, 2000). In experiments performed on *C. virginica*, Haley *et al.* (1975) reported that mass selection of adult oysters gave an apparent strong response to selection for growth rate. Ward *et al.* (2000) reported a response of eight percent in growth rate in the first generation from a mass selection in *Crassostrea gigas*.

#### **2.1.2.2 Family Selection**

This selection method refers to the procedure where whole families are used or discarded on the basis of their mean performance. All individuals in the selected families, or a random sample taken equally from each, are used as future parents. Family selection is recommended when the heritability of a trait is low and common environmental effects are small. The efficiency of family selection compared with individual selection increases noticeably as the number per family increases, in particular when the heritability is below 0.4. The high fecundity of most aquaculture species allows for large families, which make family selection based on full- and half-sib testing a much more sensible option, as it contains none of the disadvantages associated with progeny testing. There is for example no increase in generation interval when using family selection. A trait of economic importance can be recorded with an optimal degree of accuracy.

Family selection is more complicated, however, than individual selection as it requires large numbers of full sib families to be included in the selection programme in order to obtain an efficient intensity of selection. The efficiency of family selection also decreases as the correlation among half-sibs increases. Another concern is inbreeding, which becomes a problem when only using a few large families. Extensive facilities to raise and test full sib families are required to prevent this. Family selection would therefore be recommended for a

breeding program involving abalone if the heritability for a specific trait is less than 0.4. Due to the planktonic larval stage of abalone, separate rearing of individual families is required until marking is possible. The result is that the number of families that can be raised at a normal breeding facility is limited.

### **2.1.2.3 Within Family Selection (Sib selection)**

In the case of sib-selection, the phenotypic values of siblings are used to determine the breeding value of a member of the family. The advantage of sib selection, seen as response to selection ( $R$ ), increases as the intra-class correlation ( $t$ ), or heritability ( $h^2$ ) decreases. Sib selection is superior to individual selection in situations where  $t$  is larger than 0.25. The efficiency of sib selection is improved when half-sib groups are used instead of full-sib groups, because of the removal of the effect of common environmental effects. Sib selection has great potential as selection method for fish species. It has most of the advantages of progeny testing, does not result in an increase in generation interval and the number of sibs required for a high correlation between the breeding value of the family member under selection and the mean performance of the sib group, is not beyond the normal rearing capabilities of most breeding facilities. In addition, sib selection can be combined with individual selection without any additional resource input. Sib selection is also useful in terms of traits where destructive sampling methods are required in order to determine the performance, e.g. fillet yield, chemical composition, etc.

### **2.1.2.4 Combined Selection**

Combined selection is a term used when information from both the individual and family is used in order to determine breeding value, i.e. to identify superior individuals. Combined selection is the most effective method of selection, though often more complicated in terms of management and execution. Combined selection is always most efficient when heritability is

approaching  $h^2=0.5$ . When the heritability is lower than  $h^2=0.5$ , family selection is more efficient while individual selection is more efficient when heritability is higher than  $h^2=0.5$ . When common environmental effects are present the above holds true at lower values for heritability. Newkirk *et al.* (1977) suggested combined selection to improve length in the American oyster, *Crassostrea virginica*, because of the high variability between sibs in a common environment, as well as intermediate values for heritability. The complication regarding management is emphasized, however.

#### **2.1.2.5 Index Selection**

Often, more than one trait is included in the breeding goal, for example growth rate and disease resistance. Each trait is valued corresponding to its economic importance and included in an index. Breeding candidates are then ranked based on a breeding value related to their total performance.

#### **2.1.3 Mating strategies**

The objective of a breeding programme is to improve the phenotypic performance of a given population through genetic improvement strategies, which include selection procedures and mating programmes. A breeder can obtain different results through different breeding designs depending on the genetic parameters of the selected broodstock. Additive genetic variation is exploited through selection, whilst both additive and non-additive genetic variation could be exploited through mating procedures. Different mating designs are therefore used for different purposes or objectives.

##### **2.1.3.1 Inbreeding**

Inbreeding refers to the mating of individuals with a common ancestry, of which the genetic relationship is closer than that of randomly selected individuals in the population. Inbreeding

typically reduces the level of genetic variation within a population through an increase in the frequency of homozygous genotypes and a reduction in the frequency of heterozygous genotypes (Falconer, 1989). As a result, the inbred offspring usually demonstrates a high degree of uniformity, depending on the number of genes involved in determining the trait.

While the isolation of commercial stock from wild stocks presents an environment favourable for domestication and genetic improvement, it also creates the risk of a potential loss in genetic variation and preservation of alleles due to inbreeding that occur in small closed populations. Inbreeding depression is a phenomenon caused by inbreeding and is associated with a decrease in the performance of inbred populations. Inbreeding depression is mostly evident in traits associated with fitness, such as decline in fecundity and survival, although production traits such as growth rate and feed conversion may also be affected (Kincaid, 1976a, b). The detrimental effects of inbreeding are well documented and can result in decreases of 30 percent or greater in growth production, survival and reproduction.

Broodstock populations consisting of limited numbers of individuals and improper mating systems are the most common cause of inbreeding in aquaculture systems. The high fecundity associated with many aquaculture species implies that only a small number of broodstock is required to provide sufficient numbers of offspring. Insufficient numbers of broodstock along with unequal sex ratios and high selection intensities are also often applied to broodstock management, which contribute toward a reduction in the effective population size ( $N_e$ ) and an increased rate of inbreeding. Genetic drift and inbreeding depression is further compounded due to the unequal contribution of parental gametes of the progeny generation during mass spawns, unintentional selection of inferior siblings or half-siblings and the random survival of families within stocks (Hedgecock & Sly, 1990). A study determining the effect of mating system on inbreeding levels and selection response in salmonid aquaculture found that factorial mating systems produced significantly less inbreeding than single pair mating and nested mating systems with the same number of

parents (McKay *et al.*, 1992). Special precaution should be taken in species such as abalone where only a few females are able to produce enough eggs (15,000,000) and juveniles (150,000) (Tarr, 1995) to supply a standard commercial unit. For example: 10 females could produce 40 million ova to supply 200,000-400,000 spat for seeding of the grow-out. This, however, is too small a number of individuals to form an effective population size and would result in high levels of inbreeding.

An effective population size of 50 individuals, with an equal sex ratio, is required to ensure acceptable levels of inbreeding of less than 1 percent per generation. The respective number of male and female broodstock, which contributes to the next generation, determines the effective population size ( $N_e$ ) as explained by equation 5 (Falconer, 1989):

$$N_e = \frac{4 \cdot N_m \cdot N_f}{N_m + N_f} \quad (5)$$

where,

$N_m$  = number of males that contribute to the next generation

$N_f$  = number of females that contribute to the next generation

The rate of inbreeding or inbreeding coefficient ( $F$ ) can be defined as the probability that the two alleles at a gene in an individual are identical by descent (Weaver and Hedrick, 1995). An inbreeding coefficient of 1 percent therefore implies that an increment of 1 percent of the population share alleles from a common ancestor with every generation.

With every generation of breeding more and more individuals share alleles originating from a common ancestor. The rate of inbreeding is therefore dependent on the breeding design as well as the population size as expressed in equation 6 (Falconer, 1989):

$$\text{Inbreeding coefficient (F)} = 1/2N_e \quad (6)$$

An indication of the extent to which the coefficient of inbreeding (F) is affected by the number of individuals and the sex ratio within a breeding unit, is presented in Table 2.

Table 2 Different levels of inbreeding, expressed in terms of the inbreeding coefficient, F, associated with number of individuals and sex ratio within a breeding unit.

Number of	Male	Female	Male	Female	Male	Female	Male	Female
Sex ratio	25	25	10	40	10	10	5	15
Total number, N	50		50		20		20	
Effective Population Size, Ne	50		32		20		15	
Inbreeding Coefficient, F	1.00%		1.56%		2.50%		3.33%	

Controlled inbreeding, used in a selection programme, can be a valuable tool in genetic improvement strategies as is illustrated in the commercial production of plants. Highly specialized inbred lines, with no apparent value in itself, are established, with optimal benefit being realised in the progeny when these inbred lines are crossed with each other. This technique has also been implemented in animal breeding but with less success, the best example of which, is found in the poultry industry.

The potential for using inbred lines in aquaculture is considerable due to the high fecundity of aquaculture species and the ease of producing gynogenetic offspring. The use of inbred lines for outcrossing has been reported in rainbow trout for traits such as hatchability, survival in different phases and growth rate in freshwater as well as in sea cages (Kincaid, 1983). Hedgecock (1996) reported on the omnipresence of non-additive gene action and heterosis in crosses between more than 50 inbred lines of the Pacific oyster. Bentsen (1990), however, observed that the resources and time needed to produce, maintain and



replace inbred lines might be better utilized by increasing the efforts to improve the additive genetic performance of purebred lines.

Although inbreeding can be used to the advantage of the breeder, most often it is not and takes its course in an uncontrolled fashion. As mentioned, inbreeding depression not only affects traits associated with fitness, i.e. fecundity and survival, but also negatively affects production traits such as growth rate and feed conversion efficiency. This is probably due to the effect of recessive alleles when dominant alleles disappear. Wada & Komaru (1994) illustrated the negative effect of inbreeding depression in specimens of the Japanese pearl oyster, *Pinctada fucata martensii*. Selection for the favoured white shell colouration led to the development of highly inbred lines due to small breeder populations. White, hatchery produced individuals were crossed with the common brown wild type oyster in an attempt to quantify the effect of inbreeding on growth. The resulting out crossed progeny demonstrated significant superiority over the white inbred progeny for total weight and rate of growth. A higher rate of mortality in the inbred strain compared to the crossed strains was also observed. Beattie *et al.*, (1987) reported a reduction in shell size and wet and dry meat weight relative to control and out bred stocks in 2-year-old progenies of first generation brother-sister mating in *Crassostrea gigas*. Longwell and Stiles (1973) reported reduced viability of gametes in the American oyster, *Crassostrea virginica*, (reduced larval viability and moderate growth rate reduction) which appeared to be associated with inbreeding but which they contributed to gamete incompatibility. In experiments on the Pacific oyster, *Crassostrea gigas*, Lannan (1980) also noted a degree of gamete incompatibility, but ruled out inbreeding being the cause. The source of gamete incompatibility was rather thought to be unsynchronised gonadal development. Mallet and Haley (1983) proposed a revision of the results of work done by both Lannan (1980) and Longwell and Stiles (1973) after experimentation done on *Crassostrea virginica*. The results of their work indicated the positive effects due to inbreeding for larval survivorship but depression for spat size at an inbreeding level of 0.25.

Gjerde *et al.* (1983) observed an increased growth depression with increased inbreeding in populations of rainbow trout and concluded that significant non-additive genetic variation is present. Estimates of inbreeding depression were high within three populations of the oyster, *Ostrea edulis*, and ranged from 1, 0.44 and between 0.02 and 0.43 for the third population (Naciri-Graven *et al.*, 2000).

### **2.1.3.2 Hybridization**

The mating of members of different species is called hybridization. In nature, species that exist in close proximity to each other do not hybridise as a rule, but some evidence of hybridization between natural populations does exist. Trego (1997) reported on evidence of a natural occurrence of hybridization between the black abalone, *Haliotis cracherodii*, and the pink abalone, *Haliotis corrugate*. Protein electrophoresis investigations performed by Brown (1995) confirmed suspicions of the existence of a hybrid between the greenlip (*H. laevigata*) and the blacklip abalone (*H. rubra*) along the Southern Australian coastline.

Aquaculture species are readily hybridised in-vitro because of external fertilization, their high fecundity and the ease of handling the gametes. Artificial hybridization in aquaculture is often performed to combine the favourable characteristics of the two different parental species creating unique phenotypes with improved economic value in the hybridised progeny. Advantages associated with hybridization include improved growth, disease resistance, feed utilization (carp), adaptability to environmental conditions, sex ratio (tilapia) and meat quality, to name a few. Hybridization also often results in various forms of sterility among progeny groups (delayed or inhibited sexual maturation, monosex broods), which may have advantageous effects in commercial production systems. Advantages due to sterility include the controlled reproduction within production stocks as in the case of tilapia. Sterile hybrids of crosses between yellowtail flounder and winter flounder had the associated advantage that

potential escapees would not be capable of reproducing in the wild and contaminating natural stocks (Park *et al.*, 2003).

When hybridization is performed aimed at a progeny that is superior to both parents for a specific trait, the realised superiority of the progeny is termed hybrid vigour. Hybrid vigour is associated with hybridization and occurs when the performance of the hybrid progeny exceeds that of the average of the parental species. In an attempt to combine the favourable characteristics of two freshwater prawn species, *Macrobrachium malcolmsonii* and *M. rosenbergii*, Soundarapandian and Kannupandi (2000) succeeded in producing viable hybrid larvae through artificial insemination as well as through natural mating. Leighton and Lewis (1982) reported on artificial hybridization of abalone and the occurrence of hybrid vigour for growth rate between crosses of the Red, *H. rufescens*, and Green, *H. fulgens*, abalone as well as between the Red and White, *H. sorenseni*, abalone. Red and Pink, *H. corrugata*, abalone produced offspring with survival far superior to that of homologous Pink abalone in culture. The authors reported on the production of viable offspring from all crosses made between the four species. Hoshikawa *et al.* (1998) hybridised the pinto abalone (*H. kamtschatkana*) and ezo abalone (*H. discus hannai*) anticipating increased growth at low water temperatures. Vigour was observed in the hybrid at the top water temperature range, indicating superiority over pure strains. In spite of many reports of hybrid vigour for growth, however, hybrids usually perform intermediate between parental strains.

### **2.1.3.3 Crossbreeding (Intraspecific hybridization)**

Crossbreeding is the mating of genetically distinct individuals from different sub-populations or strains within the same species. Crossbreeding is used in commercial animal production as a means of exploiting heterosis (Sang, 1956; Bowman, 1959), when the desired phenotype is a combination of existing lines/breeds, or to improve the efficiency of an operation through the use of specialised sire and dam lines. Heterosis is the term used to describe the deviation of

the mean of the reciprocal offspring from the mean of the pure parental offspring for a specific trait and can be positive, negative or neutral. In principle, crossbreeding results in a reshuffling of alleles in the next generation bringing new alleles in contact with one another. This creates unique new combinations between alleles without changing the frequencies of genes. When distinct individuals are crossed, the interaction of these alleles at specific loci might give rise to dominance variance ( $V_D$ ), which is the major component of non-additive genetic variation. Dominance genetic variance ( $V_D$ ) is the variance at a single locus attributable to dominance of one allele over another. This could result in a shift in the mean value of a trait in the crossbred groups relative to that of the parental groups. This effect is not repeatable in successive generations but a once off occurrence in the first (F1) generation. Due to the reshuffling of the alleles, it is impossible to predict the effect of crossbreeding in the progeny. When the performance of the crossbred progeny exceeds that of both parental strains, positive heterosis is present. In contrast, negative heterosis exists when the crossed progeny performs inferior to the pure parental progeny.

Crossbreeding provides an alternative, short-term strategy to selection aimed at improving traits with low heritability (circumstances of high non-additive genetic variance and little additive genetic variance) or in species with very long generation intervals. The effect of crossbreeding is immediate and present in all F1 progeny of the crossbreeding parents. Crossbreeding is therefore useful to improve economic important traits such as growth rate, survival, disease resistance etc.

Although crossbreeding has been well documented in fish species, very little information is available on crossbreeding in shellfish. Dunham *et al.*, (2001) reviewed extensively on crossbreeding in fish species such as carp, catfish, rainbow trout and silver barb. Newkirk (1978) observed heterosis in the American oyster, *Crassostrea virginica*, for growth and survival although the expression depended on salinity, indicating a genotype by environment interaction. These findings correspond with earlier findings by Newkirk *et al.*

(1977) of the presence of heterosis for survival in crosses of two populations of *Crassostrea virginica*. On sub-species level, Hongen (2000) reported on a hybridization technique to increase resistance against disease in cultured abalone. *H. discus Reeve* was crossed with *H. discus Lannai* resulting in an increased time during which spat feed on diatoms as well as an improvement in growth rate (33 percent), growth of juveniles (1.5cm to 2.0cm), increased survival rates (20 percent to 80 percent), yield (15000m<sup>-2</sup> to 5000m<sup>-2</sup>). Crossbreeding would therefore be a useful genetic improvement strategy in *H. midae* due to its extremely long generation interval of five years. Wohlfarth (1993) observed positive heterosis for growth rate in crosses among different races of the common carp. Heterosis was limited, however, by genetic factors, genotype-environment interactions, and age and weight of fish. Mallet and Haley (1984) observed significant male and female mean square (suggesting non-additive genetic variance) for larval viability in diallele crosses produced from three geographically isolated natural populations.

Crossbreeding is also applied in inbred lines to restore heterozygosity. It has the opposite effect of inbreeding in that it results in an increase in heterozygous genotypes and a decrease in homozygous genotypes (Falconer, 1989). One generation of crossbreeding of highly inbred lines will restore those lines to a fully heterozygous state. These heterozygotes are almost always superior to their inbred homozygous parents (Hedgecock *et al.*, 1995).

Naciri-Graven *et al.* (2000) observed heterosis for growth in the crossbred progeny of three populations of the flat oyster, *Ostrea edulis* with high relative values of inbreeding depression. Bayne *et al.* (1999) reported the presence of heterosis for growth and superiority for physiological traits in the progeny of crosses between inbred lines of the Pacific oyster, *Crassostrea gigas*. Hedgecock *et al.* (1995) observed significant non-additive genetic components of variance for larval mortality, larval size, and juvenile size and concluded that heterosis for economically important traits can be observed and quantified effortlessly among inbred lines of the Pacific oyster.

Crossbreeding does not necessarily result in a heterosis effect in the progeny. This might be true in instances where considerable levels of heterozygosity already exist in parental lines, limiting the effective increase in heterozygosity among crossbred offspring. Tave *et al.* (1990) found that crosses between two strains of *Tilapia nilotica* produced results intermediate between the parental strains for survival under ambient temperatures. Growth of the crossed progeny of two diverse populations of the American oyster, *Crassostrea virginica*, was found to be intermediate between pure parental progeny groups (Hawes *et al.*, 1990). Longwell (1976) reported similar findings in crossed offspring generated from geographically separated populations of the same species. In a crossbreeding experiment between two geographically isolated populations of the catarina scallop, *Argopecten circularis*, Cruz and Ibarra (1997) determined that the crossbred offspring expressed no significant heterosis for larval growth despite the fact that significant differences were observed between the two populations.

## **2.2 Improvement through Genetic Manipulation**

Recent developments in biotechnology and molecular biology present an alternative method for genetic improvement in comparison to conventional crossbreeding and selection, particularly in view of a shortened time frame and cost. As mentioned before, long generation intervals associated with selection and breeding render these methods unattractive to the aquaculturist. Genetic manipulation also provides for genetic improvement in the absence of variation. The above-mentioned attributes make genetic manipulation an attractive alternative or complementary approach to genetic improvement.

### **2.2.1 Chromosome set manipulation**

Manipulation of the chromosome set is relevant for the production of individuals tailored for specific commercial requirements such as the production of mono-sex progeny groups, sterile individuals or highly inbred lines. The induction of polyploidy through chromosome set

manipulations has been achieved in a variety of aquaculture species including those belonging to freshwater and marine water fish, as well as shellfish. The main objective of the induction of polyploidy is to induce sterility, which is associated with polyploidy, and triploidy in particular. Sterility holds advantages with regard to improved growth performance and product quality. Triploid juveniles and adults of the Pearl oyster grew more rapid in size and weight than diploids (Jiang *et al.*, 1993). Inspection of the gonads of the triploids revealed that they were retarded in development.

Various methods are used for the induction of triploidy, gynogenesis, or androgenesis in aquatic species. These include radiation, chemical, temperature or pressure treatment during the first or second meiotic division in order to induce the retention of the polar body, or to neutralise sets of nuclear material. The application of these procedures are comparatively easier for aquatic species in comparison with mammals, due to the relative ease of handling gametes, induction of in vitro fertilization and the relatively high viability of gynogenetic and polyploid genotypes (Wolfarth and Hulata, 1989).

### **2.2.1.1 Gynogenesis and Androgenesis**

Gynogenesis refers to the production of offspring without any paternal genetic contribution. This procedure involves the fertilization of normal eggs by radiation-inactivated sperm followed by the induction of a physiological shock (heat, cold, or pressure). The paternal DNA is destroyed and the sperm therefore cannot contribute the paternal chromosome set to the zygote. The sperm provide the biological stimulus of fertilisation but without any genetic contribution.

In contrast, androgenesis refers to the production of offspring without any maternal contribution. Eggs in which the maternal DNA complement was inactivated by means of shock treatment are fertilized using normal sperm. Androgenesis is not as popular as gynogenesis and more difficult to perform, though, and will not be explained in detail.

The Early sperm inactivation was done through exposure to X-rays or gamma rays but lately by UV light irradiation. In some instances it is possible to use sperm from entirely different or incompatible species to activate the fertilization process. Normal eggs are exposed to inactivated sperm, which triggers mechanisms in the egg to expel the second polar body along with the maternal genetic material it contains as part of the fertilization process. At this stage the zygote contains a haploid maternal set of DNA that needs to be duplicated in order for the zygote to survive. This is done by subjecting the eggs to chemical, temperature or pressure shock treatment to impede the mechanisms of polar body expulsion resulting in the retention of the second polar body. The cell continues with its normal development making use of the two sets of maternal chromosomes it now contains. The end result is a diploid individual having double the maternal chromosome set. While the 2N “meiotic gynogens” produced in this way contain genetic material only from their maternal parents, they cannot be considered clones, since many loci may inherit distinct alleles from the respective chromosomal components of the second polar body and the egg cell (Lutz, 1997).

A second type of gynogenesis, mitotic or homozygous gynogenesis, is achieved when shock treatment of eggs treated with irradiated sperm is delayed until after extrusion of the second polar body. The eggs each contain a unique haploid chromosome complement of the maternal parent. Since the haploid zygotes are not viable, a diploid state needs to be restored. Shock treatment at the stage after duplication of the haploid chromosomal complement prior to the first mitotic division will restore the normal diploid chromosomal condition in the zygote that is homozygous at every locus. In androgenesis, shock treatment to restore the diploid chromosomal condition is also delayed until expulsion of the second polar body at first cell division - the only time that diploidy can be induced. Fairbrother (1994) reported the production of viable meiotic gynogens in the mussel *Mytilus edulis* by using irradiated sperm to activate the fertilization process and cytochalasin B to inhibit meiosis I or meiosis II. Gynogenetic offspring was also produced through the use of ultraviolet irradiated sperm in the



Pacific abalone (Arai *et al.*, 1984), Pacific oyster, *Crassostrea gigas*, (Guo *et al.*, 1993) and the mussel, *Mytilus galloprovincialis* (Scarpa *et al.*, 1994)

Gynogenesis and androgenesis are used to produce cloned populations as well as partial or completely inbred individuals rapidly. Inbred individuals may be useful in genome studies. Gynogenesis was used by Guo and Allen (1995) in mapping of allozyme loci in the dwarf surfclam, *Mulinia lateralis* Say. It can also be used to produce large numbers of clones for genetic studies. Inbred lines can also be used to realize a heterosis effect after crossbreeding. Gynogenetic individuals could be helpful in the establishment of populations of sex-reversed individuals to produce all-female or all-male livestock in that species. Quillet and Gaignon (1990) observed all-female progeny in Atlantic salmon, *Salmo salar*, and noted that it may be valuable in establishing all-female livestock.

#### **2.2.1.2 Polyploidy**

Polyploidy refers to the presence of multiple sets of chromosomes in an individual, as opposed to the normal diploid status of two sets. Poliploidy is induced through the fertilization of eggs with normal sperm followed by treatment similar to that as described for gynogenesis, which results in more than two chromosome sets in the progeny.

Shock treatment during the initial stages after fertilisation leads to triploid progeny due to retention of the second polar body. The zygote contains double the maternal chromosome set as well as a haploid paternal set. Shock treatment during the latter stages after fertilisation prevents the first mitotic cell division, resulting in the formation of tetraploid progeny with two chromosomal sets of both parents. Tetraploids in it self demonstrate no commercial benefits except when used in the production of triploids. A procedure for the mass production of triploids can be obtained by the crossing of tetraploid genotypes with normal diploid genotypes. Viable triploids have been produced in a few *Haliotis* species, which include the South African specie, *H. midae* (Stepto and Cook, 1998), the tropical

abalone, *H. asinina* (Norris and Preston, 2003), the Pacific red abalone *H. rufecence* (Maldonado *et al.*, 2001) and the Pacific abalone, *Haliotis discus hannai* Ino (Zhang *et al.*, 1998; Arai *et al.*, 1986).

The benefits to be derived from triploids are often associated with sterility, improved meat yields and quality, as well as an increase in growth rate. Stanley *et al.* (1984) reported on meiosis I triploids in the American oyster, *Crassostrea virginica*, which grew faster than diploids during the first three years of production as well as an increase in dry meat weight of 40%. Guo *et al.* (1996) reported successful mass production of triploid Pacific oysters, *Crassostrea gigas*, through the crossing of tetraploid and diploid genotypes. They concluded that individuals produced in this manner were better suited to aquaculture than individuals produced by altering meiosis. In the Pacific oyster, triploidy offers an improved quality product, particularly during summer months when normal diploids undergo sexual maturation that affects the product quality, taste and market acceptance (Chew, 1994). Furthermore, polyploidy can facilitate the production of otherwise unviable interspecific hybrids.

### **2.2.2 Manipulation of DNA**

The application of DNA manipulation technologies is a relatively new discipline in aquaculture compared to other improvement strategies i.e. selection, crossbreeding, etc. The field of DNA based biotechnology is however increasing rapidly within aquaculture, to the extent that current literature is dominated by topics on bioengineering.

The application of these technologies focuses mainly on the enhancement of growth through the regulation of metabolic growth hormone levels, whilst specific disease resistance is also receiving some attention. Biotechnology offers attractive alternatives to conventional genetic improvement strategies, with the ability to ensure significant improvements over the short term. Hormone enhancement of growth allows for improvement in growth rate of up to 300 percent (Dunham *et al.*, 2001). Agellon *et al.* (1988) reported weight gains twice that of

control groups in yearling rainbow trout treated with a biosynthetic growth hormone. An important technique included under bioengineering is transfer of genes from one species to another. Gene transfer is the introduction of foreign genes or DNA fragments into the host organism for incorporation into its own DNA. Tsai *et al.* (1997) used sperm as a vector to introduce a foreign DNA fragment into oocytes of the Japanese abalone, *Haliotis diversicolor suportexta*. The results demonstrated that sperm could potentially be used as a vector for the mass transfer of genes in species such as abalone. Successful gene transfer has also been reported by Smitherman *et al.* (1996) in the catfish species of *Ictalurus punctatus* and *Clarias gariepinus* through the use of microinjection and electroporation. The author has confirmed both the incorporation of the foreign genes into the host genome, as well as their expression and inheritance. Transgenic *Ictalurus punctatus* containing salmonid growth hormone genes grew 20-40 percent faster than controls. Maclean *et al.* (2002) has also reported on the development of growth-enhanced lines of tilapia through the use of transgenic technology. These fish have displayed no abnormalities and a considerable increase in growth rate of more than 200 percent against a control.

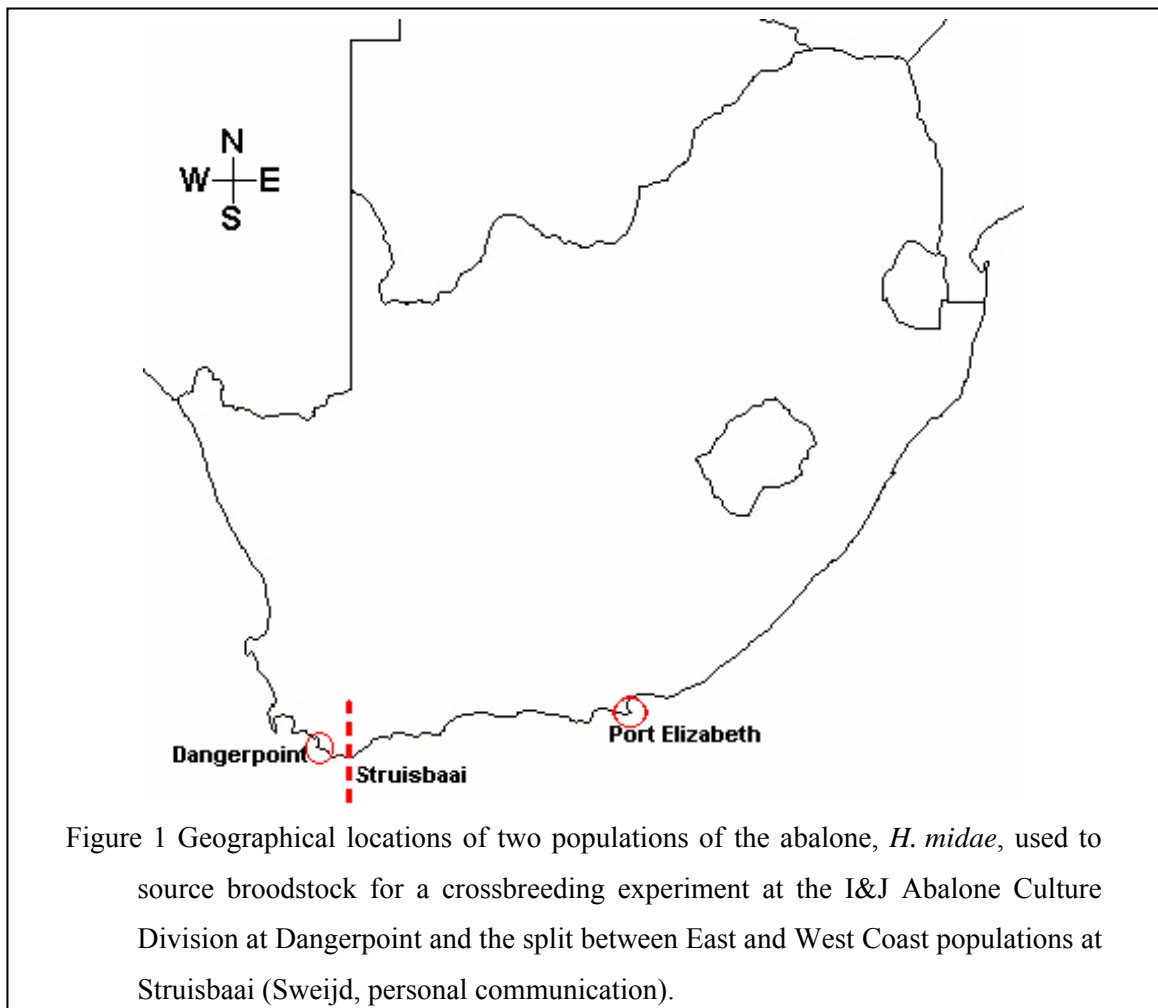
It is anticipated that DNA manipulation technologies will eventually be integrated with conventional genetic improvement practices, such as selection and breeding, to optimise the efficiency of such improvement programmes. It is believed that the negative public perception regarding transgenic technology will gradually dissipate as the technology becomes standard procedure in aquaculture production.

## CHAPTER THREE

### 3. MATERIALS AND METHODS

#### 3.1 Experimental Material

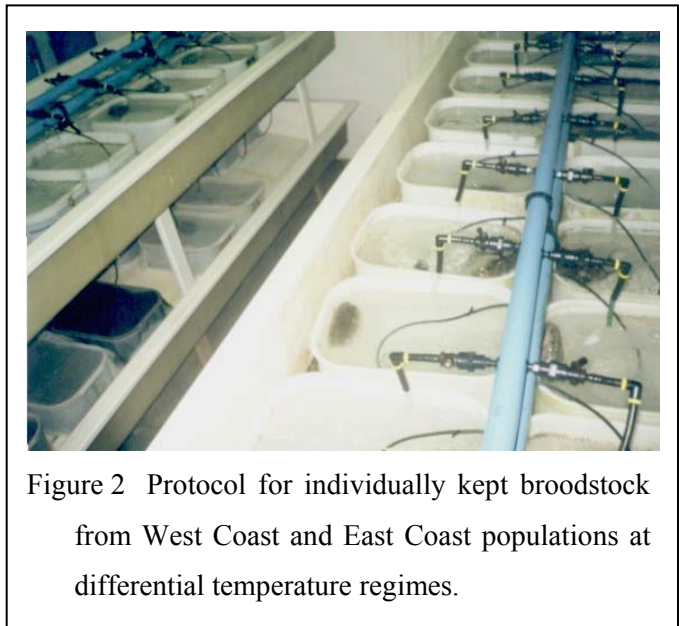
The experiment was conducted at the Abalone Culture Division of Irvine and Johnson (Pty. Ltd) situated at Dangerpoint, Gansbaai on the South-west Coast of South Africa (Figure 1). This site was chosen on the basis of the availability of adequate facilities and support systems for the execution of a complex and time consuming crossbreeding experiment. The two populations used in the crossbreeding experiment, one from the West and one from the East Coast region, were selected for the purpose of this experiment on the basis of their genetic distinction as reported by Sweijd (personal communication). The assessment by Sweijd was



done on the basis of differences in allele frequencies, using mitochondrial DNA markers. The expectation was that heterosis may be demonstrated in crossbreeding of these populations on the basis of the genetic distance between them.

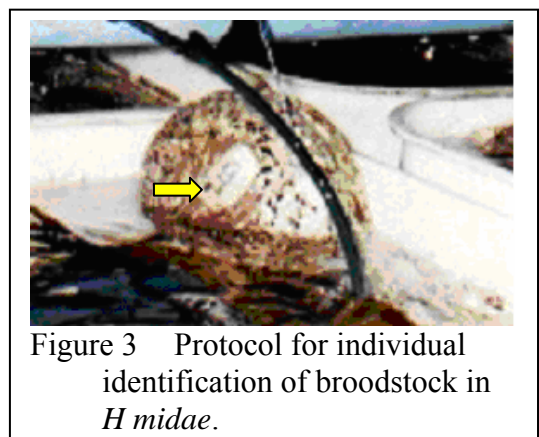
The West Coast Group (W) was obtained from the Dangerpoint hatchery (Figure 1) and an East Coast Group (E) obtained from the wild in the Cape Receive Nature Reserve near Port Elizabeth (Figure 1). Thirty West Coast animals were selected at random from a collection of broodstock that was originally

obtained through random sampling from the wild population in the Dangerpoint area. Fifty East Coast animals were collected from the wild population in the Cape Recife Nature Reserve during July of 1999. These animals were transported to the I&J Abalone Culture Division hatchery by road in cooled, oxygenated polystyrene boxes.



### 3.2 Conditioning and spawning of broodstock

Broodstock from the respective locations were kept in separate holding systems (Figure 2), to allow for differential temperature regimes required for conditioning and spawning. The West Coast animals were kept at a constant water temperature of 16.5 degrees Celsius and the East Coast animals were kept at 19.5 degrees Celsius.



The temperature profiles were chosen on the basis of optimal ambient temperature in the region of origin. All broodstock animals were fed on an *ad-lib* diet of the macro-algae, *Ecklonia maxima*. Photoperiod was regulated to simulate the natural spawning season. Broodstock were tagged on an individual basis through the application of quickset putty onto the shell of the animals, followed by the impression of individual identification numbers into the putty (Figure 3).

After six months of conditioning, ten males and ten females from each of the East and West populations were transferred to individual 20 litre containers. The same water temperatures were applied to the respective Groups, and each bin was supplied with aeration in the form of an air stone. Animals were fed on a twice-weekly basis on a diet of *Ecklonia maxima*, with *ad hoc* supplementation with *Gracilaria* and *Porphyra*. All bins were cleaned on a regular basis.

The selected animals were induced to spawn on a monthly cycle by a standard industrial method described by Morse et al. (1977). Problems were experienced from time to time with synchronisation of induction of spawning among East Coast broodstock. This complication caused a considerable delay in the preparation of experimental material and was attributed to a loss of condition, often associated with a reduction in gonadal activity. The loss of condition was believed to be a result of incomplete adaptation of East Coast animals to the new location over the preceding six-month period regarding water quality regime, nutrition, management, etc. Each parent that was successfully used in a complete diallele cross was substituted with unused broodstock from the reserve group.

Immediately after induced spawning, eggs of an East Coast female were siphoned into a measuring jug and transferred in equal volumes to two 20 litre plastic bins for the stage of incubation and hatching (Figure 4). Siphon tubes and measuring jugs were rinsed in warm fresh water in between transfers of eggs from different animals to prevent contamination of eggs. The procedure was then repeated for a West Coast female, to allow for the formation of four Groups of eggs, two from each female. Sperm from an East Coast male was then collected and used to fertilize one Group of the eggs from the East Coast and one from the West



Figure 4 20 Litre hatching bins wherein hatching took place with eggs (top) and sieves for washing of eggs (bottom).

Coast female. Sperm was collected from a West Coast male in a similar manner and used to fertilize the other half of the eggs from the respective East Coast and West Coast females. Care was taken to destroy any residual sperm in the transfer containers in order to prevent contamination of sperm from different males. Fertilized eggs were prepared according to a standard industrial method described by Hahn (1989). Care was taken at all times to prevent an accidental exchange of eggs between the different Groups during their return to the hatching bins.

### 3.3 Mating design

The mating design was based on a complete diallele cross between East and West Coast individuals with each set of four crosses constituting a block. Each block consists out of four Groups, comprising two pure (WW and EE) and two crossbred Groups (WE and EW) as

presented in Table 3. A total of 15 complete blocks, with of a minimum of 100 individuals per Group, were successfully prepared over a 20 month period through the use of this mating design. Each parent was used only once, except for two East Coast females that were used twice and one West Coast female that was used twice. This was the result of difficulties in spawning enough female broodstock.

Table 3 The experimental mating procedure according to a completed diallel block design, consisting out of crosses between East Coast and West Coast males and females, constitute four Groups of an experimental Block.

Experimental Block	West Coast Male	East Coast Male
West Coast Female	<b>West X West (WW)</b>	<b>East X West (EW)</b>
East Coast Female	<b>West X East (WE)</b>	<b>East X East (EE)</b>

### 3.4 Larval stage

After a hatching period of  $\pm 18$  hours, larvae from each cross or Group were collected through a siphoning method and transferred into a separate 70 litre rearing bin with suitable air and water supply. The incoming water was filtered with 20micron nominal wound filters and temperature was regulated at 16.5 degrees Celsius. All larvae underwent a larval developmental stage of approximately 4-5 days in the rearing bins. Standard husbandry procedures were followed with regard to the maintenance of water quality and hygiene during this developmental phase. Although broodstock were kept at differential temperature regimes, all progeny groups were kept at standard water conditions as it applied to the I&J Abalone Culture Division.



### 3.5 Settlement stage

Settlement bags were inoculated two weeks prior to settlement with diatoms favourable to settlement and feeding of the larvae. Settlement bags were clearly marked in accordance with the specific parental combination and Group identification. After a larval period of four to five days, larvae were ready for transfer to settlement bags prepared with diatoms. Before each transfer, larvae counts were done for every Group to determine the total number of larvae in each rearing bin. Volumes corresponding to the number of larvae required for each settlement bag



Figure 5 Examples of larval settlement bags with brown algal medium, air-inlet at the bottom and water inlet and outlet at the top.

were calculated for each Group. A total of between 700 and 800 spat was required from each bag and larval density was calculated working with a 1% survival rate. Rearing bins with larvae were drained into sieves with a mesh size of 100micron. Larvae were then washed out of the sieve into a measuring jug and a volume corresponding to the correct stocking density transferred to a settlement bag. Larvae were settled and reared in clear, plastic, cylindrical bags with a diameter of 300mm and length of 1.8m suspended from a metal structure (Figure 5). The ends of the bags were rolled onto a piece of timber that slotted into metal fittings on the metal structure enabling them to hang from the structure once filled with water. Larvae were simply poured into a bag using a funnel. The water supply in the settlement bags were turned off to prevent washing out of larvae and air supply was kept at a minimum. This procedure was repeated for all Groups in all of the blocks taking care to rinse all transfer

equipment in warm fresh water in between transfers of different Groups. Water conditions i.e. temperature, pH and dissolved oxygen (DO) were monitored throughout the settlement stage until water was turned on again. Larvae were closely monitored for signs of settlement and the water supply was turned on at the first sign of settlement of sufficient numbers of larvae. The air supply was kept at a minimum to prevent larvae from being washed off the sides of the bag. Air supply to the bags was increased after one to two weeks after settlement. The bags were constantly ventilated through an air supply in the bottom and a water supply at the top to allow for the circulation of fresh water and oxygen through the bag. A water outlet was created at the top of the bag to allow for drainage of excess water.

### **3.6 Weaning**

Juvenile abalone (referred to as spat) remained in the settlement bags for as long as sufficient diatoms were available, often not longer than two months. Weaning bins with dimensions of 480mm x 560mm x 140mm were prepared with suitable diatoms for feeding two weeks prior to moving spat from settlement bags. Each bin was clearly marked corresponding to the Group it would receive. After about two months, spat were moved from the settlement bags, each Group to its own bin. Bags were drained into a sieve to prevent loss of spat. Each bag was then cut into three parts in order to fit into the bins to be fully submerged while spat were gently brushed off the plastic sheet using a commercial paintbrush. Each bin was provided with sufficient water, aeration and shelter in the form of halves of 50cm long pipes, with diameter 11cm, split along the length. Individuals that ended upside-down were corrected upon completion of transfer. Outlet pipes were fitted with mesh of appropriate size to prevent juveniles from escaping during nightly excursions. It was later established that sufficient space should be left between the water level and the top of the bin since juveniles leave the water from time to time when water conditions become unfavourable. An increased distance to the edge of the bin could prevent the loss of animals since they would only reach half way

to the top before getting stranded. Bins were also placed far enough apart from each other to prevent individuals from moving between different bins. Spat were immediately introduced to commercial tropical fish flakes supplied by Amatikulu Pet Products in Durban, while still feeding on diatoms. After one week, fish flakes were substituted by a standard formulated abalone feed supplied by Sea Plant Products in Hermanus. The feed was crushed according to the size of the juveniles and as spat grew bigger, granule size was increased accordingly. An *ad-lib* feeding regime was followed throughout the weaning period.

### 3.7 Tagging

Cohabitation of experimental Groups in the same environment is necessary during the genetic evaluation of different Groups in order to minimise environmental differences between Groups. A suitable method for Group identification of abalone at an early stage of development (<6mm) was required to allow for the placing of different Groups in a common rearing environment. No standardised procedures or methods for the identification of abalone during the early developmental stages were available at the time. Several methods, including different dyes, glues and marker pens were evaluated without success. A method through which fish is marked with the use of a needleless



Figure 6 Group identification technique in juvenile abalone (+/- 10mm) using colour coded silicon strips.

Panjet ink injection apparatus loaded with Alcian Blue ink also failed to deliver satisfying results. High levels of mortalities were experienced due to excessive tissue damage.

An innovative tagging method was developed that involves the threading of a silicone cord through one of the respiratory pores (tremata) in the shell of an individual (Figure 6). Different colours were then allocated to each of the four Groups within a block. This method requires strips of silicone rubber of about 10mm in length to be prepared according to dimensions that correspond with the respiratory pore of the animals to be marked. Animals were anaesthetised with Magnesium Sulphate prior to handling and implant of the tags. Each individual was tagged by inserting a colour coded silicone strip from the inside of the shell through the third or fourth respiratory pore above the respiratory cavity using a pincer. The silicone strip was then pulled through the respiratory pore from the outside of the shell, far enough as to leave it just about

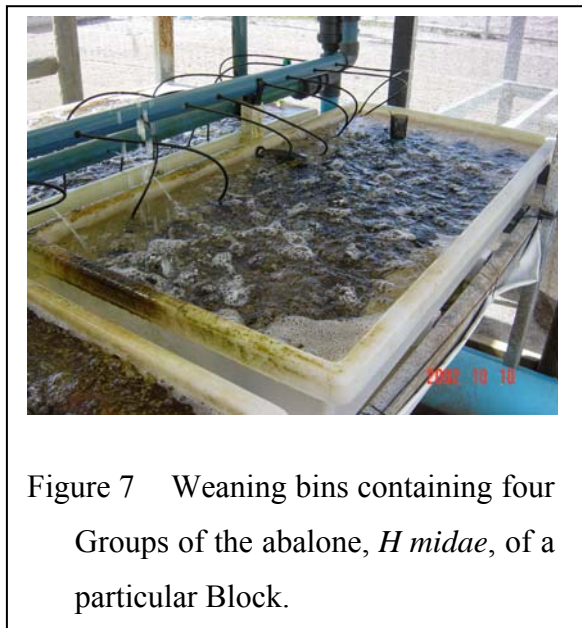


Figure 7 Weaning bins containing four Groups of the abalone, *H midae*, of a particular Block.

intact. Excessive protrusion on the inside of the shell should be avoided due to irritation of the skin on the back of the head causing fatality. The inserted silicone strips were detected as a foreign object in spite of every precaution taken to minimize discomfort and was covered with a shell layer within weeks of insertion.

Juveniles could be tagged through this method only from an age of six months ( $\pm$  10mm). All individuals belonging to a particular Group within a block were kept in separate rearing units until the colour tagging of all four Groups belonging to a Block were completed. On completion a random sample of three hundred individuals from each of the four Groups in a Block were transferred to a common weaning bin. The bins were approximately 135 litres in volume and of dimensions 600mm x 1500mm x 150mm (Figure 7). The animals,



representative of the four Groups in a particular Block, were kept together in these containers for the duration of the comparative growth stages. Each Block was thus assigned to a single rearing bin and contained the four Groups belonging to it. In instances where Groups consisted of less than 300 animals, untagged individuals of a similar size range from commercial stock were included to fill the bins to the required density of 300 individuals per Group. This was done in an effort to standardise stocking densities over the blocks, i.e.  $4 \times 300 = 1\ 200$  animals per block.

### 3.8 Sampling

A non-destructive sampling method was used to collect individuals for measurement. Habitats are cone-shaped structures serving as shelter for abalone in rearing bins (Fig 8). Four habitats connected by a stainless steel rod and supplied with an aeration hose make up a habitat unit. Rearing bins were supplied with eight detached habitat units resulting in

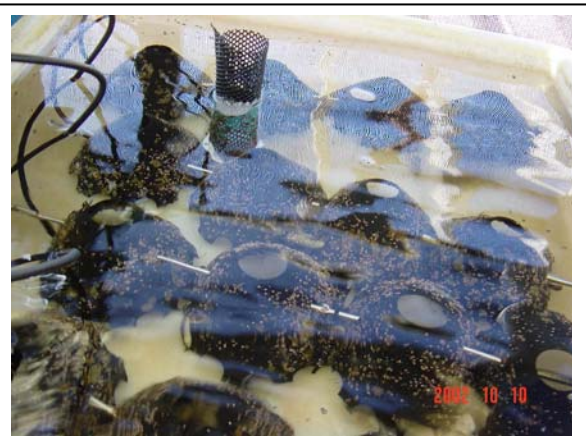


Figure 8 Weaning bins containing cone-shaped habitats serving as shelter and numbered units for random sampling.



Figure 9 Tagged abalone sheltering underneath habitats in a rearing bin.

32 habitats per bin. Habitats were assigned a number from 1 to 32 and random numbers between 1 and 32 corresponding to the relevant habitats were drawn for every Block during sampling.

A Block was sampled first by random selection of habitats. Individuals were then randomly collected from the habitats in the order in which they were selected until the

full complement of 16 animals per Group was obtained. If a particular habitat did not provide for the full complement of 16 animals of a particular Group, an additional habitat was selected to provide the remainder of the required animals.

### 3.9 Weighing and Measuring

The randomly sampled animals were kept in a net in a bucket of water. Individuals were taken out of the water in Groups of ten and placed on absorbent paper to standardise the removal of excess water before any measurements for weight or length were recorded. Individual weight ( $W$ ) was measured in grams to the second decimal of a gram on a Denver XS410 Scientific electronic balance (Figure 10). Individual length ( $L$ ) was measured on the shell in millimetres (mm) to the nearest two hundredth of a millimetre through the use of standard vernier callipers (Figure 10). The length measurement was taken across the longest section of the shell from front to back.

A random sample of 16 individuals from each of the four Groups in a Block were weighed and measured over 9 months at



Figure 10 Growth measurements in the abalone, *H. midae*. Weight (g) to the second decimal and length (mm) to the nearest two hundredth of a millimetre.

monthly intervals, as shown in Table 4. All Groups were measured at nine intervals over the growth period except for Groups 1 to 4, which were only measured eight times. The inconsistent sampling during the early stages in Groups 1-4 was due to the sampling protocol still being developed. Data obtained from Groups 1-4 were, however, included in the analysis

even though the Groups were raised during the developmental stages of the experiment. The interruption in sampling at the end of Groups 11-15 was due to a misunderstanding during absence of leave. The absence of the sampling points was accounted for during the statistical analysis of the data.

Table 4 A summary of the number and intervals of sampling of abalone from the different Blocks, over a period of 20 months, beginning August 2001 to March 2003.

Year	2001					2002												2003		
Group	08	09	10	11	12	01	02	03	04	05	06	07	08	09	10	11	12	01	02	03
1	•				•	•	•	•	•				•							
2	•				•	•	•	•	•				•							
3	•				•	•	•	•	•				•							
4			•		•	•	•	•	•	•			•							
5						•	•	•	•	•	•	•	•	•						
6						•	•	•	•	•	•	•	•	•						
7						•	•	•	•	•	•	•	•	•						
8							•	•	•	•	•	•	•	•	•					
9							•	•	•	•	•	•	•	•	•					
10									•	•	•	•	•	•	•	•	•			
11										•	•	•	•	•	•	•	•			•
12										•	•	•	•	•	•	•	•			•
13										•	•	•	•	•	•	•	•			•
14										•	•	•	•	•	•	•	•			•
15											•	•	•	•	•	•	•	•		•

### 3.10 Growth rate

Growth rate of the respective Groups is expressed in terms of average weight and length gain over time (months). Using SAS (2001), weight gain ( $b_w$ ) was calculated as the regression coefficient of the log increase in weight (g) over time (month), and length gain ( $b_L$ ) as the regression coefficient of the increase in length (mm) over time

## CHAPTER FOUR

### 4. RESULTS AND ANALYSIS

The results and analysis of the growth performance of the different Blocks and Groups of the abalone, *Haliotis midae*, originating from the reciprocal crossing of the two different stocks of origin (West and East Coast, Table 3), is presented in sections 4.1 to 4.3. Growth rate is expressed in terms of the regression of weight ( $b_w$ ) and length gain ( $b_L$ ) over time. Weight gain ( $b_w$ ) was calculated as the regression coefficient of the log increase in weight (g) over time (month), and length gain ( $b_L$ ) as the regression coefficient of the increase in length (mm) over time (see section 3.10). The data was analysed through the use of the statistical analyses programme, SAS (2001).

#### 4.1 Weight Gain ( $b_w$ )

The main objective of the study was to determine the presence and significance of heterosis for growth traits, i.e. weight and length gain respectively, between reciprocal crosses of different stocks of abalone (*H. midae*). The data for weight gain ( $b_w$ ) obtained from 15 Blocks, each Block consisting of four Groups, is presented in Table 5.

Table 5 Results of the growth rate of abalone (*H. midae*) expressed as a regression of weight gain ( $b_w$ ), measured in grams over a growth period of 20 months (Intercept = log of initial weight = log weight at time zero; slope =  $b_w$  = regression coefficient of the log of weight (g) over time (month))

Observation	Block	Group	RMSE	Intercept	Weight gain, $b_w$ , (log gram/month)	Standard error
1	1	WW	0.326	-2.577	0.257	0.035
2	1	EW	0.382	-2.980	0.282	0.041



3	1	WE	0.366	-2.947	0.278	0.039
4	1	EE	0.357	-2.757	0.264	0.038
5	2	WW	0.226	-1.669	0.220	0.024
6	2	EW	0.324	-2.573	0.238	0.035
7	2	WE	0.301	-2.114	0.254	0.032
8	2	EE	0.340	-2.639	0.253	0.037
9	3	WW	0.407	-2.983	0.286	0.044
10	3	EW	0.406	-2.847	0.274	0.044
11	3	WE	0.338	-2.477	0.251	0.036
12	3	EE	0.367	-2.501	0.249	0.040
13	4	WW	0.324	-2.019	0.267	0.038
14	4	EW	0.375	-2.710	0.285	0.044
15	4	WE	0.382	-2.245	0.285	0.045
16	4	EE	0.368	-2.425	0.268	0.043
17	5	WW	0.214	-1.616	0.205	0.028
18	5	EW	0.152	-1.905	0.215	0.020
19	5	WE	0.228	-2.535	0.260	0.029
20	5	EE	0.162	-1.984	0.236	0.021
21	6	WW	0.244	-1.939	0.233	0.032
22	6	EW	0.170	-1.682	0.193	0.022
23	6	WE	0.218	-1.621	0.211	0.028
24	6	EE	0.210	-1.985	0.228	0.027
25	7	WW	0.109	-1.570	0.227	0.014
26	7	EW	0.180	-1.964	0.237	0.023
27	7	WE	0.161	-1.662	0.221	0.021
28	7	EE	0.184	-1.615	0.210	0.024
29	8	WW	0.191	-1.667	0.260	0.025
30	8	EW	0.172	-2.083	0.274	0.022
31	8	WE	0.119	-1.321	0.203	0.015

32	8	EE	0.208	-1.451	0.192	0.027
33	9	WW	0.185	-1.839	0.226	0.024
34	9	EW	0.248	-2.113	0.269	0.032
35	9	WE	0.188	-2.207	0.236	0.024
36	9	EE	0.195	-1.854	0.234	0.025
37	10	WW	0.128	-2.818	0.342	0.018
38	10	EW	0.120	-1.732	0.289	0.017
39	10	WE	0.082	-2.183	0.315	0.012
40	10	EE	0.106	-2.495	0.346	0.015
41	11	WW	0.125	-1.222	0.217	0.014
42	11	EW	0.188	-1.650	0.234	0.021
43	11	WE	0.126	-1.762	0.224	0.014
44	11	EE	0.102	-1.602	0.221	0.011
45	12	WW	0.126	-1.260	0.209	0.014
46	12	EW	0.077	-0.928	0.175	0.009
47	12	WE	0.094	-1.663	0.225	0.010
48	12	EE	0.146	-1.811	0.225	0.016
49	13	WW	0.089	-1.636	0.216	0.010
50	13	EW	0.144	-1.478	0.234	0.016
51	13	WE	0.169	-1.997	0.264	0.019
52	13	EE	0.106	-1.630	0.234	0.012
53	14	WW	0.243	-2.699	0.313	0.027
54	14	EW	0.115	-1.950	0.249	0.013
55	14	WE	0.143	-1.619	0.240	0.016
56	14	EE	0.101	-2.035	0.242	0.011
57	15	WW	0.113	-1.847	0.260	0.013
58	15	EW	0.135	-2.194	0.270	0.015
59	15	WE	0.119	-1.745	0.236	0.014
60	15	EE	0.101	-1.618	0.216	0.012

RMSE = Root mean square error

The RMSE indicates the degree of fit of the regression model to the data. The RMSE values for the Groups of Blocks one to four are far higher than those of the rest of the Blocks and decreases gradually in Blocks produced towards the end of the experiment. This can be ascribed to the investigational methodology that was still being standardised at the early stages of the experiment and the resulting stress these Blocks were subjected to. Blocks one to four were much older than the rest of the Blocks at first measurement.

#### 4.1.1 Differences between Groups: Weight gain ( $b_w$ )

The significance of the observed variation in weight gain of the different Groups was analysed by means of an Analysis of Variation (ANOVA) for weight gain, the results of which is presented in Table 6.

Table 6 An Analysis of Variance of weight gain ( $b_w$ ) of four Groups (WW, WE, EW, EE) of the abalone, *H. midae*, over a series of 15 repeats (Blocks), generated from a reciprocal crossing of two populations from the East and West coast of South Africa.

Source	df	Mean Square	P
Blocks	14	0.003397	< 0.001
Groups	3	0.000187	0.780
Error	42	0.000516	

Significant differences were observed between Blocks ( $P < 0.001$ ). Efforts were made to standardise various managerial aspects of the experimental protocol related to the treatment of Blocks, such as stocking densities, food supply, water quality, etc. There were, however, some factors of environmental and managerial variation that could not be standardised for Blocks over the extended experimental period of 20 months. These include variation in ambient water temperature, daylight length, water quality, etc. The observed differences between the average growth rates of Blocks can therefore be explained by the relative

exposure of Blocks to these common environmental effects (Falconer, 1989). This aspect was taken into account during further analysis of the data in terms of the observed differences between Groups.

The ANOVA (Table 6) indicates that no significant statistical difference was observed between Groups over Blocks ( $P = 0.780$ ) in terms of weight gain ( $b_w$ ). The average weight gain of the respective Groups is presented in Table 7. The Group, WW, recorded an average weight gain ( $b_w$ ) of 0.249g per month over the 20-month growth period, compared to the 0.248g of EW, 0.247g of WE and 0.241g of Group EE. A t-test, as presented in Table 7, confirms the results with a similar clustering of the different Groups. The results indicate the absence of heterosis effects for weight gain ( $b_w$ ) between the different progeny Groups of the abalone, *Haliotis midae*, obtained from the reciprocal crossing of two populations from the East and West Coast of South Africa.

Table 7 A t-test of the average weight gain ( $b_w$ ) of four Groups (WW, WE, EW, EE) of the abalone, *H. midae*, over a series of 15 repeats (Blocks), generated from a reciprocal crossing of two populations from the East and West coast of South Africa.

Group	N	Mean $b_w$	t-Grouping
WW	15	0.249	A
EW	15	0.248	A
WE	15	0.247	A
EE	15	0.241	A

#### 4.1.2 Differences between male and female parental groups: Weight gain ( $b_w$ )

A secondary interest was to assess whether any significant differences existed between progeny assigned to the respective male and female parental groups, i.e. East Coast males *versus* West Coast males, and East Coast females *versus* West Coast females, as well as males

*versus* females overall. The growth data of the relevant progeny groups was therefore analysed to investigate the differences in weight gain between them.

The ANOVA (Table 8) again confirms significant differences between Blocks ( $P < 0.001$ ) in terms of weight gain as expected due to common environmental factors and explained in section 4.1.1.

Table 8 Analysis of Variance of weight gain ( $b_w$ ) of East Coast male and West Coast male parental groups, East Coast female and West Coast female parental groups and male and female parental groups overall, of the abalone, *H. midae*, over a series of 15 repeats (Blocks), generated from a reciprocal crossing of two populations from the East and West coast of South Africa.

Source	df	Mean Square	P
Blocks	14	0.003397	< 0.001
Male Parental Groups	1	0.000187	0.549
Female Parental Groups	1	0.000306	0.445
Combined Male x Combined Female	1	0.000068	0.718
Error	42	0.000515	

The observed average weight gain of progeny from the West Coast males ( $b_w = 0.248$ ) was better than that from the East Coast males ( $b_w = 0.245$ ). The differences between male groupings (East Coast *versus* West Coast) were however not statistically significant ( $P = 0.549$ ). The observed average weight gain of progeny from the West Coast females ( $b_w = 0.249$ ) was superior to that from the East Coast females ( $b_w = 0.244$ ). These differences between female groupings (East Coast *versus* West Coast) were also not statistically significant ( $P = 0.445$ ). An analysis of variance of the overall performance of males *versus* females also reveals no significant differences ( $P = 0.718$ ) in terms of weight gain. The

observed average weight gain of the respective progeny groups is presented in Table 9. A t-test (Table 9) confirms similar results with uniform clustering of the different progeny groups.

Table 9 A t-test of observed weight gain ( $b_w$ ) of 30 male and female parental groups of the abalone, *H. midae*, generated from a reciprocal crossing of two populations from the East and West coast of South Africa.

Male	N	LSM ( $b_w$ )	t-Grouping
W	30	0.248	A
E	30	0.245	A
Female	N	LSM ( $b_w$ )	t-Grouping
W	30	0.249	A
E	30	0.244	A

The lack of significant differences in weight gain of offspring assigned to the respective male and female parental groups indicated that no significant differences exist between parental groups of the two populations from the East and West coast of South Africa.

#### 4.1.3 Differences between Groups: Initial weight ( $W_0$ )

It was observed that Group means for weight at the initiation of the experiment (time = 0) differed between Groups. The variation in average weight at a standard age of six months at the start of the experiment can be attributed to differences in stocking densities between Groups during the period from spawning to the start of the experiment. These differences were caused by natural variation in fecundity and survival during the larval and post-larval stages. Groups with a smaller number of individuals had more favourable feeding conditions, i.e. less competition, leading to faster growth and higher average weight at the start of the comparative growth phase. There was a concern that the larger animals might have had an

advantage over smaller ones when competing for food, favouring specific Groups during the period of combined evaluation. No corrective measures could be taken to standardize the Group weight in terms of numbers due to the metamorphic complexities of early larval and post-larval stages. The Group age, numbers and overall densities were however standardised from the start of the comparative growth phase. No standardisation was done for weight at the beginning of the experiment. The relationship between initial weight ( $W_0$ ) and weight gain ( $b_w$ ) is presented in Section 4.1.3.2. The relationship between initial weights of the four different Groups was investigated through an Analysis of Variation, the result of which is presented in Table 10.

Table 10 An Analysis of Variance of initial weight ( $W_0$ ) of four Groups (WW, WE, EW, EE) of the abalone, *H. midae*, observed over a series of 15 repeats (Blocks), generated by a reciprocal crossing of two populations from the East and West coast of South Africa.

Source	df	Mean Square	P
Blocks	14	0.674	< 0.001
Groups	3	0.024	0.873
Error	42	0.105	

Significant differences ( $P < 0.001$ ) were observed in the initial weight ( $W_0$ ) of the Groups. This observation was attributed to the exposure of the Blocks to common environmental factors, as explained in section 4.1.1. Differences were observed in the averages of initial weights of the Groups. The Group, WW, had the highest average initial weight (0.141g) followed by the WE (0.134g) and EE (0.132g) Groups. The EW Group (0.128g) performed worst compared to the rest of the groups. The difference in initial weight between the Groups were, however, not statistically significant ( $P = 0.873$ ). The clustering of all Groups resulting from a t-test analysis (Table 11) confirmed this assessment.

Table 11 A t-test of the average initial weight ( $W_0$ ) of four Groups (WW, WE, EW, EE) of the abalone, *H. midae*, observed over a series of 15 repeats (Blocks) generated by a reciprocal crossing of two populations from the East and West coast of South Africa.

Group	N	Intercept	t-Grouping
WW	15	-1.957	A
WE	15	-2.007	A
EE	15	-2.027	A
EW	15	-2.053	A

(N = number of Blocks)

#### 4.1.3.1 Differences between male and female parental groups: Initial weight ( $W_0$ )

The difference between the offspring of specific male and female parental groups, regarding initial weight, was determined as for weight gain. The data was analysed to determine the difference in initial weight between progeny of specific parental groups, i.e. East Coast males *versus* West Coast males and East Coast females *versus* West Coast females, as well as overall male *versus* overall female, and is presented in Table 12.

Significant differences in initial weights ( $W_0$ ) were observed between Blocks ( $P < 0.001$ ) as was expected due to previous findings. The analysis indicates that no significant differences were observed in initial weight of male parental groups ( $P = 0.493$ ). Similarly, no significant difference in initial weight was observed between female parental groups ( $P = 0.891$ ). The ANOVA in Table 12 also revealed that overall male and overall female parental groups did not differ significantly ( $P = 0.655$ ) regarding initial weight.



Table 12 Analysis of Variance of initial weight ( $W_0$ ) of East Coast male and West Coast male parental groups, East Coast female and West Coast female parental groups and male and female parental groups overall, of the abalone, *H. midae*, over a series of 15 repeats (Blocks), generated from a reciprocal crossing of two populations from the East and West coast of South Africa.

Source	df	Mean Square	P
Blocks	14	0.67415	< 0.001
Male Parental Groups	1	0.04992	0.493
Female Parental Groups	1	0.00197	0.891
Combined Male x Combined Female	1	0.02118	0.655
Error	42	0.10459	

#### 4.1.3.2 Relationship between Weight gain ( $b_w$ ) and Initial weight ( $W_0$ )

The observed variation in initial weight ( $W_0$ ) between Groups at the start of the experiment (see 4.1.3) raised concern regarding the effect of initial weight on further growth performance. The relationship between weight gain ( $b_w$ ) and initial weight ( $W_0$ ) was therefore assessed through an Analysis of Covariance, the result of which is presented in Table 13.

Table 13 Analysis of Covariance of initial weight ( $W_0$ ) and weight gain ( $b_w$ ) in 60 progeny groups (four Groups over 15 repeats) of the abalone, *H. midae*, generated from a reciprocal crossing of two populations from the East and West coast of South Africa.

Variable	Parameter Estimate $\pm$ Standard error	P [ $H_0$ : parameter = 0]
Growth rate	0.141 $\pm$ 0.0129	< 0.001
Initial weight	-0.053 $\pm$ 0.00623	< 0.001

The Analysis of Covariance confirmed the presence of a significant relationship ( $P < 0.001$ ) between initial weight ( $W_0$ ) and weight gain ( $b_w$ ) and is described by Equations 7 and 8 and Figure 11:

Regression: 
$$\text{Weight gain} = a + b (\text{Initial weight}) \quad (7)$$

$$= 0.140 - 0.053 (W_0) \quad (b = -0.053)$$

Correlation: 
$$\text{Weight gain} = r (\text{Initial weight}) \quad (8)$$

$$= -0.743 (W_0) \quad (r = -0.743)$$

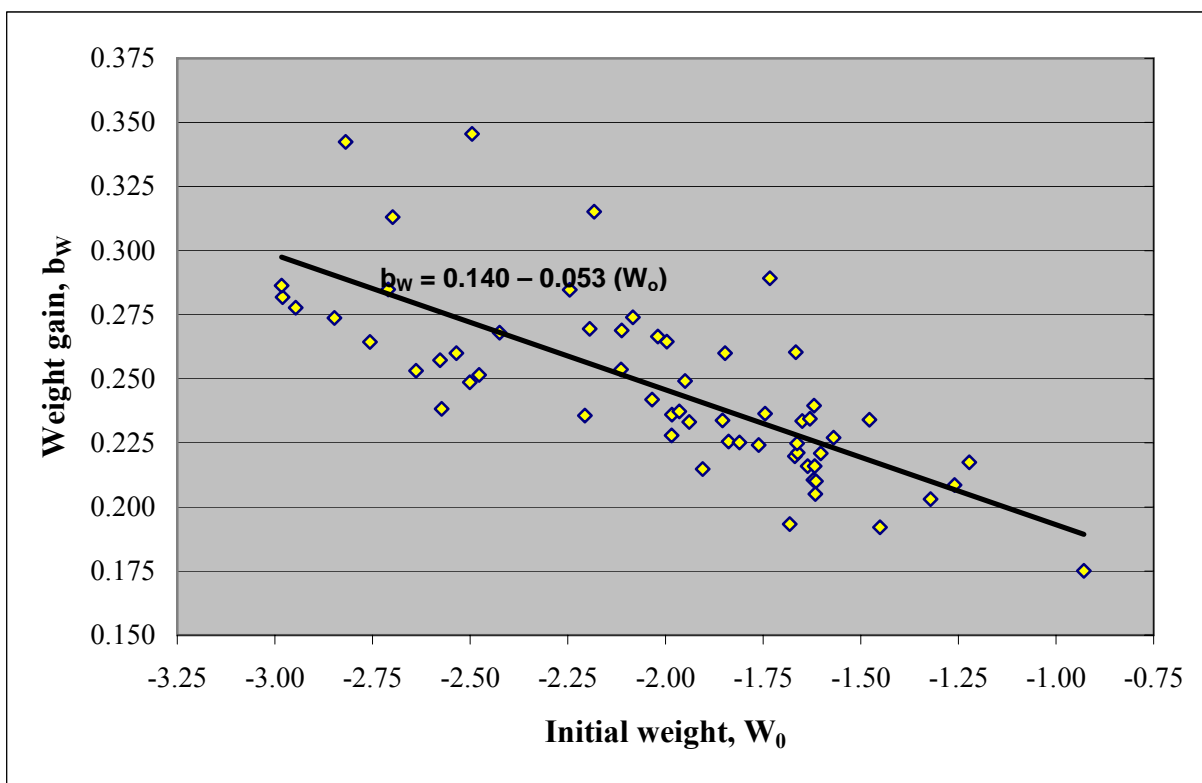


Figure 11 The relationship between Initial weight ( $W_0$ ) and Weight gain ( $b_w$ ) of 60 progeny Groups (4 Groups over 15 repeats) of the abalone, *Haliotis midae*.

This implies that Groups with a smaller initial weight tend to demonstrate higher weight gain during the comparative growth phase than Groups with a larger initial weight. On the basis of these results it was decided to correct the original data for differences in initial weight and run

a complete repeat analysis in order to establish whether any significant changes in the findings had occurred.

#### 4.1.4 Adjustment of data for differences in Initial weight ( $W_0$ )

A covariance analysis was conducted on weight gain ( $b_w$ ). The initial weight ( $W_0$ ) was used as covariate. The results of a repeat analysis on the adjusted means are presented in the sections 4.1.4.1 to 4.1.4.4.

##### 4.1.4.1 Differences between Groups based on Adjusted data: Weight gain ( $b_w$ )

The results of an ANOVA of the variation in weight gain ( $b_w$ ) between Groups, after correction on the basis of initial weight, are presented in Table 14.

Table 14 An Analysis of Variance of weight gain ( $b_w$ ) of four Groups (WW, WE, EW, EE) of the abalone, *H. midae*, based on adjusted data, observed over a series of 15 repeats (Blocks), generated by a reciprocal crossing of two populations from the East and West coast of South Africa.

Source	df	Mean Square	P
Intercept	1	0.038503	< 0.001
Blocks	14	0.001694	< 0.001
Groups	3	0.000372	0.085
Error	41	0.000157	

Significant differences in weight gain still exist between Blocks ( $P < 0.001$ ) after adjusting for initial weight ( $W_0$ ). Differences in weight gain between Groups were, as before, not statistically significant, although the P-value has declined from  $P = 0.780$  to  $P = 0.085$  and almost significant on the 5% significance level. It would therefore appear as though the correction has improved the merit of the data set. A comparison of the observed average

Group means for weight gain, after correction for initial weight, reveals that the WW Group ( $b_w = 0.252$ ) still performed better than the rest of the Groups. The WE Group ( $b_w = 0.247$ ), however, performed second best in the analysis based on adjusted data compared to results prior to correction for initial weight (see section 4.1.1). Next followed the EW Group ( $b_w = 0.245$ ) and then the EE Group ( $b_w = 0.240$ ).

A t-test (Table 15) confirms the change in the results indicating the divergent clustering of the Groups. In addition, the t-test analysis indicates that the WW, WE and EW Groups were more or less the same as well as the WE, EW and EE Groups regarding the mean weight gain. The WW and EE Groups, however, were significantly different according to the t-test analysis.

Table 15 A t-test of the adjusted LSMean weight gain ( $b_w$ ) of four Groups (WW, WE, EW, EE) of the abalone, *H. midae*, over a series of 15 repeats (Blocks), generated from a reciprocal crossing of two populations from the East (E) and West (W) coast of South Africa.

Group	Adjusted LSMean ( $b_w$ )	t-Grouping
WW	0.252	A
WE	0.247	AB
EW	0.245	AB
EE	0.240	B

#### 4.1.4.2 Differences between male and female parental groups based on Adjusted data: Weight gain ( $b_w$ )

The growth data of the progeny groups assigned to the respective male and female parental groups i.e. East Coast *versus* West Coast males, and East Coast *versus* West Coast females, as well as males *versus* females overall, was analysed after correction for initial weight. The result of the ANOVA is presented in Table 16 and is presented in comparison with Table 8, which is based on unadjusted data.

The ANOVA again confirms significant differences ( $P < 0.001$ ) between Blocks in terms of weight gain, as expected due to common environmental factors and explained in section 4.1.1.

Table 16 Analysis of Variance of weight gain ( $b_w$ ) of East Coast male and West Coast male parental groups, East Coast female and West Coast female parental groups and male and female parental groups overall based on adjusted data, of the abalone, *H. midae*, over a series of 15 repeats (Blocks), generated from a reciprocal crossing of two populations from the East and West coast of South Africa.

Source	df	Mean Square	P
Intercept	1	0.03850	< 0.001
Block	14	0.00169	< 0.001
Male Parental Groups	1	0.00071	0.040
Female Parental Groups	1	0.00040	0.117
Combined Male x Combined Female	1	< 0.00001	0.981
Error	41	0.00644	

The observed average weight gain of progeny from the West Coast males ( $b_w = 0.250$ ) was better than that of the East Coast males ( $b_w = 0.243$ ). The weight gain of progeny of male parental Groups, i.e. East Coast and West Coast, differed significantly ( $P = 0.040$ ) from each other on the 5% significance level. The difference was unlike that obtained from data prior to correction for initial weight (see Table 8), however, and is indicated by the t-test analysis in Table 17. The observed average weight gain of progeny from the West Coast females ( $b_w = 0.249$ ) was superior to that from the East Coast females ( $b_w = 0.244$ ). There was, however, still no significant difference in weight gain between female parental groups ( $P = 0.117$ ), i.e. East Coast and West Coast females. The ANOVA reveals that no significant difference in weight gain exists between the males overall and females overall ( $P = 0.981$ ).

Table 17 A t-test of Least Square Means for observed weight gain ( $b_w$ ), of male and female parental groups based on adjusted data, generated from a reciprocal crossing of two populations from the East and West coast of South Africa.

Male	Adjusted LSM ( $b_w$ )	Pr >   t	t-Grouping
W	0.250	0.039	A
E	0.243		B
Female	Adjusted LSM ( $b_w$ )	Pr >   t	t-Grouping
W	0.249	0.117	A
E	0.244		A

## 4.2 Length Gain ( $b_L$ )

The data for length gain ( $b_L$ ), obtained from 15 Blocks, each Block consisting of four Groups, is presented in Table 18.

Table 18 Results of the growth rate of abalone (*H. midae*) expressed as a regression of length gain ( $b_L$ ), measured in grams over a growth period of 20 months (Intercept = initial length = length at time zero, slope =  $b_L$  = regression coefficient length gain (mm) over time (month))

Observation	Block	Group	RMSE	Intercept	Length gain, $b_L$ , (mm/month)	Standard error
1	1	WW	1.515	-3.998	2.137	0.163
2	1	EW	1.659	-6.174	2.314	0.179
3	1	WE	1.807	-5.244	2.202	0.195
4	1	EE	1.454	-3.694	2.084	0.157
5	2	WW	1.252	-0.380	2.092	0.135
6	2	EW	1.685	-1.970	1.854	0.182
7	2	WE	1.571	-3.409	2.376	0.169
8	2	EE	1.619	-2.265	1.959	0.175
9	3	WW	2.259	-5.159	2.212	0.243
10	3	EW	2.252	-3.107	2.070	0.243
11	3	WE	2.103	-1.643	1.991	0.227
12	3	EE	2.254	-1.081	1.932	0.243
13	4	WW	1.855	0.332	2.206	0.219
14	4	EW	2.285	-1.997	2.077	0.270
15	4	WE	2.362	-0.609	2.316	0.279
16	4	EE	1.925	1.201	1.876	0.228
17	5	WW	1.287	7.100	1.375	0.166
18	5	EW	1.336	6.279	1.337	0.173

19	5	WE	1.123	0.939	1.772	0.145
20	5	EE	0.748	3.396	1.732	0.097
21	6	WW	1.406	5.283	1.564	0.182
22	6	EW	1.002	7.203	1.252	0.129
23	6	WE	1.288	6.588	1.483	0.166
24	6	EE	1.121	4.685	1.554	0.145
25	7	WW	0.494	4.596	1.847	0.064
26	7	EW	0.936	4.131	1.714	0.121
27	7	WE	0.830	5.275	1.679	0.107
28	7	EE	1.173	6.260	1.533	0.151
29	8	WW	0.906	4.960	2.073	0.117
30	8	EW	0.679	2.823	2.013	0.088
31	8	WE	0.749	8.613	1.486	0.097
32	8	EE	1.370	8.941	1.224	0.177
33	9	WW	0.765	6.354	1.446	0.099
34	9	EW	1.214	3.952	1.829	0.157
35	9	WE	0.864	4.922	1.436	0.112
36	9	EE	0.981	5.945	1.557	0.127
37	10	WW	0.977	1.073	2.067	0.138
38	10	EW	1.305	3.943	2.188	0.184
39	10	WE	0.799	2.290	2.248	0.113
40	10	EE	1.058	-0.054	2.493	0.149
41	11	WW	0.364	6.815	1.799	0.040
42	11	EW	0.570	5.009	1.826	0.063
43	11	WE	0.670	5.215	1.593	0.074
44	11	EE	0.584	5.538	1.659	0.064
45	12	WW	0.545	8.317	1.543	0.060
46	12	EW	1.091	9.315	1.402	0.120
47	12	WE	0.694	5.379	1.693	0.076



48	12	EE	0.525	5.400	1.560	0.058
49	13	WW	0.621	5.561	1.563	0.068
50	13	EW	0.523	6.086	1.773	0.058
51	13	WE	0.319	3.674	1.897	0.035
52	13	EE	0.697	5.545	1.751	0.077
53	14	WW	0.748	0.493	2.052	0.082
54	14	EW	1.012	3.457	1.803	0.111
55	14	WE	0.526	5.065	1.864	0.058
56	14	EE	0.596	4.357	1.634	0.066
57	15	WW	0.349	3.752	1.911	0.040
58	15	EW	0.520	2.925	1.829	0.060
59	15	WE	0.575	4.592	1.731	0.066
60	15	EE	0.415	5.957	1.548	0.048

\* RMSE = Root mean square

The RMSE indicates the degree of fit of the regression model to the data. A similar trend is observed in the RMSE values for length as in weight and can also be ascribed to the investigational methodology that was still being standardised at the early stages of the experiment.

#### 4.2.1 Differences between Groups: Length gain ( $b_L$ )

The main objective of the study was to determine the presence and significance of heterosis for growth traits, i.e. weight and length gain respectively, between reciprocal crosses of different stocks of abalone (*Haliotis midae*). The significance of the observed variation in length gain ( $b_L$ ), of the different Groups, was analysed by means of an Analysis of Variation (ANOVA) for length gain, the results of which is presented in Table 19.

Table 19 An Analysis of Variance of length gain ( $b_L$ ) of four Groups (WW, WE, EW, EE) of the abalone, *H. midae*, over a series of 15 repeats (Blocks), generated from a reciprocal crossing of two populations from the East and West coast of South Africa.

Source	df	Mean Square	P
Block	14	0.2595	<0.001
Groups	3	0.0445	0.300
Error	42	0.0352	

Significant differences were observed between Blocks for length gain ( $b_L$ ) ( $P < 0.001$ ), similar as for weight gain ( $b_W$ ). The experimental protocol relating to the treatment of Blocks, such as stocking densities, food supply, water quality, etc., was standardised as for weight gain. There were, however, some factors of environmental and managerial variation that could not be standardised for Blocks over the extended experimental period of 20 months. These include variation in ambient water temperature, daylight length, water quality, etc. The observed differences between the average length gain of Blocks can therefore be explained by the relative exposure of Blocks to these common environmental effects (Falconer, 1989). This aspect was taken into account during further analysis of the data in terms of the observed differences in length gain between Groups.

The ANOVA (Table 19) indicates that no significant statistical difference was observed between Groups over Blocks ( $P = 0.300$ ) in terms of length gain ( $b_L$ ). The average length gains of the respective Groups are presented in Table 20. The WW Group recorded an average length gain ( $b_L$ ) of 1.859mm per month over the 20-month growth period compared to the 1.851mm of WE, 1.819mm of EW and 1.740mm of the EE Group. A t-test (Table 20) confirms similar results with a comparable clustering of the different Groups.

The results indicate the absence of heterosis effects for length gain ( $b_L$ ) between the different progeny Groups of the abalone, *Haliotis midae*, obtained from the reciprocal crossing of two populations from the East and West Coast of South Africa.

Table 20 A t-test of the average length gain ( $b_L$ ) of four Groups (WW, WE, EW, EE) of the abalone, *H. midae*, over a series of 15 repeats (Blocks), generated from a reciprocal crossing of two populations from the East and West coast of South Africa.

Group	N	Mean	t-Grouping
WW	15	1.859	A
WE	15	1.851	A
EW	15	1.819	A
EE	15	1.740	A

#### 4.2.2 Differences between male and female parental groups: Length gain ( $b_L$ )

A secondary interest was to establish whether any significant differences in length gain existed between progeny assigned to the respective male and female parental groups (East Coast males *versus* West Coast males, East Coast females *versus* West Coast females and males *versus* females overall). The growth data of the relevant progeny groups was therefore analysed to investigate the differences between them regarding length gain. The ANOVA (Table 21) again confirms significant differences ( $P < 0.001$ ) between Blocks in terms of length gain as in the case of weight gain, due to common environmental factors as explained in section 4.2.1.

Table 21 Analysis of Variance of length gain ( $b_L$ ) of East Coast male and West Coast male parental groups, East Coast female and West Coast female parental groups and male and female parental groups overall, of the abalone, *H. midae*, over a series of 15 repeats (Blocks), generated from a reciprocal crossing of two populations from the East and West coast of South Africa.

Source	df	Mean Square	P
Blocks	14	0.2595	<0.001
Male Parental Groups	1	0.0863	0.125
Female Parental Groups	1	0.0282	0.376
Combined Male x Combined Female	1	0.0190	0.467
Error	42	0.0352	

As with weight gain, the observed average length gain of progeny from the West Coast males ( $b_L = 1.855$ ) was superior to that from the East Coast males ( $b_L = 1.779$ ). The differences between male groupings were however not statistically significant ( $P = 0.1250$ ). The observed average length gain of progeny from the West Coast females ( $b_L = 1.839$ ) was higher than that of progeny from the East Coast females ( $b_L = 1.796$ ). These differences between female groupings were also not statistically significant ( $P = 0.376$ ). An analysis of variance of the overall performance of males versus females also reveals no significant differences ( $P = 0.467$ ) in terms of length gain. These findings are similar to that obtained for weight gain. The observed average length gain of the respective progeny groups is presented in Table 22. A t-test (Table 22) confirms similar results with uniform clustering of the different progeny groups.

Table 22 A t-test of observed length gain ( $b_L$ ) of 30 male and female parental groups of the abalone, *H. midae*, generated from a reciprocal crossing of two populations from the East and West coast of South Africa.

Male	N	LSM ( $b_L$ )	t-Grouping
W	30	1.855	A
E	30	1.779	A
Female	N	LSM ( $b_L$ )	t-Grouping
W	30	1.839	A
E	30	1.796	A

The lack of significant differences in length gain of offspring assigned to the respective male and female parental groups indicated that no significant differences exist between parental groups of two populations of the abalone, *H. midae*, from the East and West coast of South Africa.

#### 4.2.3 Differences between Groups: Initial length ( $L_0$ )

Groups also differed in Group mean length at the initiation of the experiment (time = 0). The variation in average length was also attributed to differences in stocking densities between Groups during the period from spawning to the start of the experiment. These differences were caused by natural variation in fecundity and survival during the larval and post-larval stages. No corrective measures could be taken to standardize the Group length in terms of numbers due to the metamorphic complexities of early larval and post-larval stages. The Group age, numbers and overall densities were however standardised from the start of the comparative growth phase. No standardisation was done for length at the beginning of the experiment. The relationship between initial length ( $L_0$ ) and length gain ( $b_L$ ) is presented in Section 4.2.3.2. The relationship between initial lengths of the four different Groups (WW,

WE, EW, EE) was investigated through an Analysis of Variation, the result of which is presented in Table 23.

Table 23 An Analysis of Variance of initial length ( $L_0$ ) of four Groups (WW, WE, EW, EE) of the abalone, *H. midae*, observed over a series of 15 repeats (Blocks), generated by a reciprocal crossing of two populations from the East and West coast of South Africa.

Source	df	Mean Square	P
Blocks	14	55.015	< 0.001
Groups	3	1.043	0.788
Error	42	2.968	

A significant difference ( $P < 0.001$ ) was observed in the initial length ( $L_0$ ) of the Groups, as with initial weight ( $W_0$ ). This observation was attributed to the exposure of the Blocks to common environmental factors, as explained in section 4.2.1. The observed differences for initial length were different to that for initial weight, though. The WW parental Group did not have the greatest average initial length, as was the case with initial weight. The EE Group (3.34mm) outperformed the rest of the Groups followed by the WW (3.01mm) and EW (2.79mm) Groups. The WE Group (2.78mm) performed poorer than the rest of the Groups. The difference in initial length between the Groups were, however, not statistically significant ( $P = 0.788$ ). The clustering of all Groups resulting from a t-test analysis (Table 24) confirmed this assessment.

Table 24 A t-test of the average initial length ( $L_0$ ) of four Groups (WW, WE, EW, EE) of the abalone, *H. midae*, observed over a series of 15 repeats (Blocks) generated by a reciprocal crossing of two populations from the East and West coast of South Africa.

Group	N	Mean initial length (mm)	t-Grouping
EE	15	3.34	A
WW	15	3.00	A
EW	15	2.79	A
WE	15	2.78	A

#### 4.2.3.1 Differences between male and female parental groups: Initial length ( $L_0$ )

The difference in mean initial length of progeny groups from specific male and female parental groups was assessed as for initial weight. The data was analysed to determine the difference in initial length between progeny of specific parental groups, i.e. East Coast males *versus* West Coast males and East Coast females *versus* West Coast females, as well as overall male *versus* overall female, and is presented in Table 25.

Table 25 Analysis of Variance of initial length ( $L_0$ ) of East Coast male and West Coast male parental groups, East Coast female and West Coast female parental groups and male and female parental groups overall, of the abalone, *H. midae*, over a series of 15 repeats (Blocks), generated from a reciprocal crossing of two populations from the East and West coast of South Africa.

Source	df	Mean Square	P
Blocks	14	55.051	< 0.001
Male Parental Groups	1	0.461	0.696
Female Parental Groups	1	0.384	0.721
Combined Male x Combined Female	1	2.284	0.385
Error	42	2.968	

Again, significant statistical differences in initial lengths were observed between Blocks ( $P < 0.001$ ) but, as was expected, the difference in initial length between male parental groups ( $P = 0.696$ ) was not significant. Similarly, no significant difference was observed between female parental groups ( $P = 0.721$ ) either. Offspring of East Coast males (3.07mm) had a bigger average initial length than that of West Coast males (2.89mm). The average initial length of offspring of East Coast females (3.06mm), were bigger than that of West Coast females (2.90 mm). The ANOVA in Table 25 revealed that the overall male and overall female parental groups did not differ significantly ( $P = 0.385$ ) regarding initial length.

#### 4.2.3.2 Relationship between Length gain ( $b_L$ ) and Initial length ( $L_0$ )

Concern over the effect of initial length ( $L_0$ ) on length gain ( $b_L$ ) prompted an investigation into the relationship between these two parameters. The results of an Analysis of Covariance are as presented in Table 26.

Table 26 Analysis of Covariance of initial length ( $L_0$ ) and length gain ( $b_L$ ) in 60 progeny groups (four Groups over 15 repeats) of the abalone, *H. midae*, generated from a reciprocal crossing of two populations from the East and West coast of South Africa.

Variable	Parameter Estimate $\pm$ Standard error	P [ $H_0$ : parameter = 0]
Growth rate	2.001 $\pm$ 0.029	< 0.001
Initial length	-0.062 $\pm$ 0.006	< 0.001

The Analysis of Covariance confirmed the presence of a significant relationship ( $P < 0.001$ ) between length gain ( $b_L$ ) and initial length ( $L_0$ ) as well. The relationship is described by Equations 9 and 10 and Figure 12:

$$\begin{aligned} \text{Regression:} \quad \text{Length gain} &= a + b (\text{Initial length}) & (9) \\ &= 2 - 0.062 (L_0) & (b = -0.062) \end{aligned}$$



Correlation:  $\text{Length gain} = r (\text{Initial length})$  (10)  
 $= -0.8061 (L_0)$  ( $r = -0.8061$ )

This implies that Groups with a smaller initial length tend to demonstrate higher length gain during the comparative growth phase than Groups with a larger initial length.

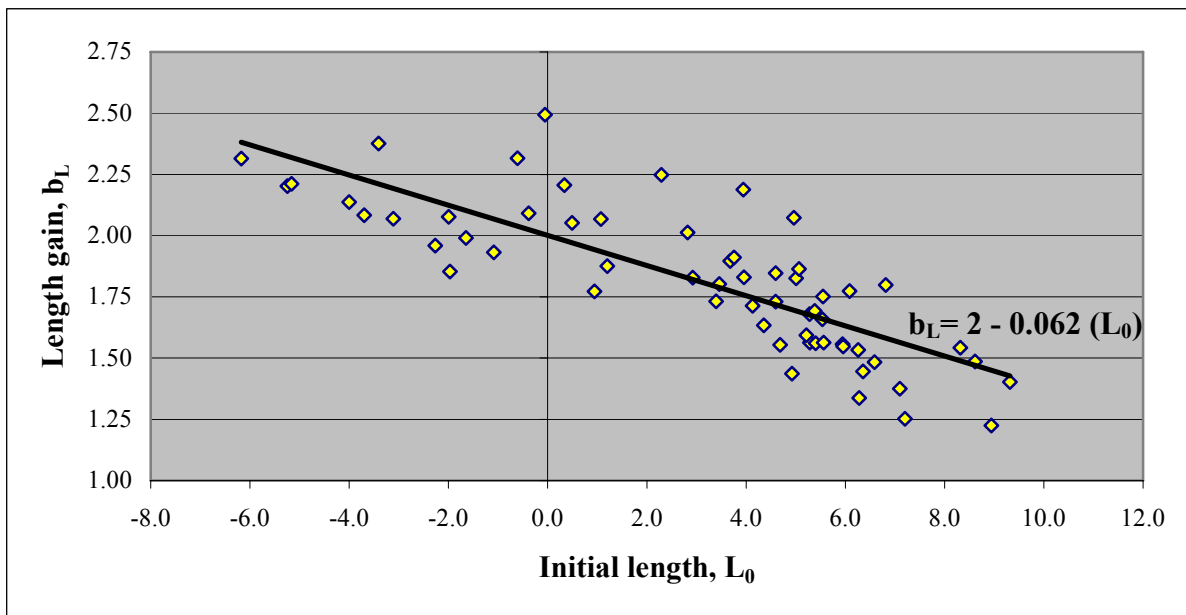


Figure 12 The relationship between Initial length ( $L_0$ ) and length gain ( $b_L$ ) of 60 progeny Groups (4 Groups over 15 repeats) of the abalone, *Haliotis midae*.

This is in agreement with results obtained from weight analysis. On the basis of these results it was decided to correct the original data for differences in initial length, as in the case of weight analysis, and run a complete repeat analysis in order to establish whether any significant changes in the findings had occurred.

#### 4.2.4 Adjustment of data for differences in Initial length ( $L_0$ )

A covariance analysis was conducted on weight gain ( $b_L$ ). The initial weight ( $W_0$ ) was used as covariate. The results of a repeat analysis on the adjusted means are presented in the sections 4.2.4.1 to 4.2.4.4.

#### 4.2.4.1 Differences between Groups based on Adjusted data: Length gain ( $b_L$ )

The results of an ANOVA of the variation in length gain ( $b_L$ ) between Groups, after correction on the basis of initial length, are presented in Table 27.

Table 27 An Analysis of Variance of length gain ( $b_L$ ) of four Groups (WW, WE, EW, EE) of the abalone, *H. midae*, based on Adjusted data, observed over a series of 15 repeats (Blocks), generated by a reciprocal crossing of two populations from the East and West coast of South Africa.

Source	df	Mean Square	P
Intercept	1	3.4082	< 0.001
Blocks	14	0.0782	< 0.001
Groups	3	0.0236	0.244
Error	41	0.0164	

Significant differences in length gain still exist between Blocks ( $P < 0.001$ ) after adjusting for initial length ( $W_0$ ). Differences in length gain between Groups are still not statistically significant and the P-value ( $P = 0.244$ ) has not changed especially (see Table 19). A comparison of the observed average Group means for length gain (Table 28), after correction for initial length, reveals that the WW Group ( $b_L = 1.861$ ) still performed better than the rest of the Groups followed by the WE Group ( $b_L = 1.835$ ), EW Group ( $b_L = 1.804$ ) and the EE Group ( $b_L = 1.769$ ), which performed worse of all the groups. From the results it can be seen that correction of the data for the effect of initial length had no significant effect on the results of the data set. A t-test (Table 28) reveals that the clustering of the different Groups remained unchanged after correction of the data (see Table 20).

Table 28 A t-test of the adjusted LSMeans length gain ( $b_L$ ) of four Groups (WW, WE, EW, EE) of the abalone, *H. midae*, over a series of 15 repeats (Blocks), generated from a reciprocal crossing of two populations from the East (E) and West (W) coast of South Africa.

Group	LSMean ( $b_L$ )	t-Grouping
WW	1.861	A
WE	1.835	AB
EW	1.804	AB
EE	1.769	B

#### 4.2.4.2 Differences between male and female parental groups based on Adjusted data:

##### Length gain ( $b_L$ )

The growth data of the progeny groups assigned to the respective male and female parental groups i.e. East Coast *versus* West Coast males, and East Coast *versus* West Coast females, as well as males *versus* females overall, was analysed after correction for initial length. The result of the ANOVA is presented in Table 29 and is presented in comparison with Table 21, which is based on unadjusted data. The ANOVA again confirms significant differences ( $P < 0.001$ ) between Blocks in terms of length gain, as expected due to common environmental factors and explained in section 4.2.1.

Table 29 Analysis of Variance of length gain ( $b_L$ ) of East Coast male and West Coast male parental groups, East Coast female and West Coast female parental groups and male and female parental groups overall based on adjusted data, of the abalone, *H. midae*, over a series of 15 repeats (Blocks), generated from a reciprocal crossing of two populations from the East and West coast of South Africa.

Source	df	Mean Square	P
Intercept	1	3.408241	< 0.001
Block	14	0.078218	< 0.001
Male Parental Groups	1	0.056690	0.070
Female Parental Groups	1	0.013875	0.363
Combined Male x Combined Female	1	0.000256	0.901
Error	41	0.016358	

Progeny from West Coast male parental groups ( $b_L = 1.848$ ) still performed better than that of East Coast male parental groups ( $b_L = 1.786$ ) concerning average length gain based on adjusted data (Table 30). Even though the progeny of male parental groups (East Coast and West Coast) did not differ significantly ( $P = 0.070$ ), the P-value is close to significance on the 5% level (compare  $P = 0.30$ , Table 19).

Progeny of West Coast female parental groups ( $b_L = 1.832$ ) also performed better than East Coast female parental Groups ( $b_L = 1.802$ ) regarding the average length gain based on adjusted data (Table 30). The difference between the progeny of female parental groups (East Coast and West Coast) remained insignificant ( $P = 0.363$ ) and almost unchanged from results prior to correction for initial length (see Table 19). The ANOVA reveals that there was no significant difference between overall male and overall female parental groups ( $P = 0.901$ ) based on adjusted data. Least Square Means of length gain of East Coast and West Coast male and female parental groups are presented in Table 30.

Table 30 A t-test of Least Square Means for observed length gain ( $b_L$ ), of male and female parental groups based on adjusted data, generated from a reciprocal crossing of two populations from the East and West coast of South Africa.

Male	Adjusted LSM ( $b_L$ )	Pr >   t	t-Grouping
W	1.848	0.069	A
E	1.786		B
Female	Adjusted LSM ( $b_L$ )	Pr >   t	t-Grouping
W	1.832	0.362	A
E	1.802		A

## CHAPTER FIVE

### 5. DISCUSSION OF RESULTS ON GROWTH PERFORMANCE OF THE ABALONE, *HALIOTIS MIDAE*

The aim of this study was to investigate the presence of heterosis for growth related traits, weight and length gain, among the crossbred offspring of two distinct populations of the South African abalone, *Haliotis midae*. The two populations used in the crossbreeding experiment, one from the West and one from the East Coast region, were selected for the purpose of this experiment on the basis of their genetic distinction as reported by Sweijd (personal communication). Using mitochondrial DNA markers, Sweijd indicated a clear distinction between populations of *H. midae* to the East and the West of Struisbaai, based on differences in allele frequencies (Figure 1). This genetic differentiation between populations of *Haliotis midae* formed the basis of a decision to investigate the incidence of heterosis among crossbred progeny groups from these populations. Crossbreeding was chosen as a strategy for genetic improvement of *H. midae* because of the long generation interval of the species of four to five years that render conventional selection a fairly unattractive option. Short-term benefits can be obtained through the exploitation of heterosis effects for growth rate through crossbreeding of natural populations. Hongen (2000) reported marked improvement in growth in crosses among abalone while Wohlfarth (1993) observed positive heterosis for growth rate in crosses among different races of the common carp.

#### 5.1 Adjustment for variation in initial weight ( $W_0$ ) and length ( $L_0$ ) between Groups

A trend in the experimental data of variation in initial weight ( $W_0$ ), and its potential effect on weight gain ( $b_w$ ), was a cause of concern. The relationship between initial weight ( $W_0$ ) and weight gain ( $b_w$ ) was therefore analysed in order to establish whether the observed differences in initial weight had any effect on the weight gain as determined during the course

of the experiment. Growth rate is dependent on initial size and Moav and Wohlfarth (1976) were obliged to make corrections to the incremental length gains to compensate for differences in the initial length of fish. An ANOVA was consequently performed (Table 10, Section 4.1.3), providing no indication of significant differences in initial weight between Groups (WW, WE, EW, EE). A further ANOVA of initial weight (Table 12) indicated no significant statistical difference existed between male parental groups from the East and West Coast population. Similarly, no significant difference existed between female parental Groups from the East and West Coast population (Table 12) either. These results indicate that variation in initial weight is randomly distributed over Groups.

A covariance analysis between initial weight ( $W_0$ ) and consequent weight gain ( $b_w$ ) provided evidence of a set relationship described by the regression coefficient of  $b = -0.053$  between these traits (Equation 7, Figure 11). This relationship was confirmed by the correlation coefficient of  $r = 0.743$  (Equation 8). A negative relationship exists between initial weight ( $W_0$ ) and weight gain ( $b_w$ ) in all progeny Groups as described by Figure 11 and the negative regression value ( $b_w = -0.053$ ) in Equation 7 (section 4.1.3.2). This implies that Groups with a smaller average initial weight ( $W_0$ ) demonstrated a higher weight gain ( $b_w$ ) during the subsequent comparative growth phase. The variation in initial weight was removed by assigning the mean initial weight over all Groups as the new initial weight for all Groups (section 4.1.4).

A similar variation was observed in the experimental data for initial length ( $L_0$ ). The analysis of the relationship between initial weight ( $W_0$ ) and weight gain ( $b_w$ ) was repeated for length in order to establish whether the observed differences in initial length had any effect on the consequent length gain. An ANOVA indicated that no significant differences existed in initial length between Groups (Table 23). A similar ANOVA indicated that no significant differences in initial length existed between male parental groups, female parental groups, and

overall male and female parental groups (Table 25). As with initial weight, the results indicated that variation in initial length is randomly distributed over Groups.

A covariance analysis between initial length ( $L_0$ ) and consequent length gain ( $b_L$ ) provided evidence of a similar set relationship described by the regression coefficient  $b = -0.062$  between these traits (Equation 9, Figure 12) as in the case of  $b_W$  and  $W_0$ . This relationship was confirmed by the correlation coefficient of  $r = -0.8061$  (Equation 10). A negative relationship exists between initial length ( $L_0$ ) and length gain ( $b_L$ ) in all progeny Groups as described by Figure 12 and the negative regression value ( $b_L = -0.062$ ) in Equation 9 (section 4.2.3.2). This implies that Groups with a smaller average initial length ( $L_0$ ) demonstrated a higher length gain ( $b_L$ ) during the subsequent comparative growth phase. Initial length was standardised as for weight (section 4.2.4).

Adjusting the data for initial weight brought about changes in the results of an ANOVA of differences in weight gain between Groups (Table 14). The P-value decreased from  $P = 0.780$  to  $P = 0.085$  and came near to significance on the 5% level. The two crossed Groups (WE and EW) switched places in the ranking of the Groups for average weight gain. A t-test analysis gave indication of the WW and EE Groups were significantly different (Table 15). Differences in length gain ( $b_L$ ) between Groups after adjustment for initial length remained insignificant ( $P = 0.244$ ), but a t-test analysis of the adjusted LS Mean length gain ( $b_L$ ) revealed that the WW and EE Groups differed significantly from each other (Table 28).

The results of an ANOVA of differences in weight gain between male and female parental groups also changed (Table 16). Differences between West Coast and East Coast males were significant on the 5% significance level and were confirmed by a t-test (Table 17). The difference in length gain between West Coast and East Coast male parental groups also came close to being significant on the 5% level ( $P = 0.070$ ) and is confirmed by a t-test analysis (Table 30). All further analyses and assessment of weight gain were performed on the



data adjusted for initial weight on the bases of differences in results after adjusting data for initial weight.

## **5.2 Assessment of Heterosis effect for weight gain ( $b_w$ ) and length gain ( $b_L$ )**

The results of an ANOVA of weight gain ( $b_w$ ) and length gain ( $b_L$ ) of the four genotypes (WW, WE, EW, EE) of a cross between two populations of the abalone, *Haliotis midae*, (Table 14, Table 27) revealed significant differences between the 15 Blocks evaluated during the experiment. These differences between Blocks occurred consistently throughout the experiment, for all traits that were evaluated, and are best explained by common environmental effects. In spite of efforts to standardise environmental factors over Blocks, such as density, handling, feeding, weaning, etc., differences in environmental conditions between Blocks could not be avoided. These differences include seasonally linked factors such as, photoperiod, water temperature, etc. due to a time span of almost a year in generating the first and last Blocks. Other factors include differences in the size of habitats and placement in the weaning section. The placement of weaning bins was in an arrangement of two bins in series. Both bins in series had its own water inlet, but one of the bins received the effluent water of the other. The experimental design and analysis of results accounted for these differences during further analysis of differences between Groups.

No statistically significant differences in weight gain ( $b_w$ ) or length gain ( $b_L$ ) were detected between the different experimental Groups (WW, WE, EW, EE) during the course of the experiment although the difference for weight came close to significance ( $P = 0.085$ ) on the 5% level (Table 14, Section 4.1.4.1). It is therefore concluded that no significant heterosis was detected for weight gain or length gain between the four genotypes (WW, WE, EW, EE) generated from a cross between the two specific populations of the abalone, *Haliotis midae*, as determined under the specific conditions of the experiment. Cruz and Ibarra (1997) observed no useful heterosis (better than mid-parent value) for larval length in crosses

between two geographically isolated populations of the Catarina scallop, *Argopecten circularis*, even though significant differences between the populations ( $P=0.001$ ) were observed. No explanation for the lack of heterosis is presented, however. Longwell (1976) reported on the intermediate performance of crossed offspring generated from geographically separated populations of the American oyster, *Crassostrea virginica*, but failed to state the reasons for this. Hawes *et al.*, (1990) reported similar results between two isolated populations in the same species. Reasons for their findings were not clear from the source.

A further analysis of specific factors related to weight and length gain, i.e. the relationship between origin, sex and weight and length gain, revealed a significant difference ( $P = 0.040$ ) in weight gain on the 5% level between male parental Groups from the East and West Coast populations (Table 16, Section 4.1.4.2) but no significant difference ( $P = 0.070$ ) in length gain (Table 29, Section 4.2.4.2). This result gives an indication of the presence of a male paternal effect on weight gain between East Coast and West Coast males. West Coast males are therefore expected to yield a significantly higher weight gain in comparison to the East Coast males. Differences in the weight gain ( $P = 0.117$ ) and length gain ( $P = 0.363$ ) of female parental groups were not statistically significant indicating the absence of a female parental effect on weight gain between East Coast and West Coast females (Table 16, Section 4.1.4.2 and Table 29, Section 4.2.4.2). An overall analysis of differences between male and female parental groups in terms of weight gain ( $P = 0.981$ ) and length gain ( $P = 0.901$ ), irrespective of origin, also gave no indication of statistically significant results.

A comprehensive analysis of the data for weight gain and length gain, therefore, gave no indication of significant heterosis effects between any of the progeny groups from reciprocal crosses between these populations. In the absence of any significant heterosis effects on weight gain and length gain among the progeny Groups, it is concluded that no significant economic benefits will be derived from a program of crossbreeding between these specific

East and West Coast populations of abalone, *Haliotis midae*, irrespective of the parental combination in terms of sex or origin. Growth rate ( $b_w + b_L$ ) was the only parameter tested for in this experiment, however. The possibility that heterosis might exist for other economically important traits such as disease resistance, age at maturity etc., cannot be excluded. It is recommended that further quantitative investigations into growth of *H. midae* only be undertaken in light of new molecular evidence of substantial genetic differentiation between populations.

### **5.3 Recommendations**

It is recommended that future populations for use in crossbreeding experiments be selected on the basis of genetic evidence, rather than operational convenience. A minimum of 16 individuals (irrespective of sex) is required as being representative of that population.

The mating design should provide for sufficient numbers of broodstock to be spawned at a single time ensuring the formation of the required number of complete diallele Blocks in an acceptable period of time. No less than nine Blocks should be produced over the shortest possible period of time within the limits of the facilities. This will decrease the age difference between Blocks and as a result the common environmental effects. Standardizing husbandry techniques and systems, i.e. feeds, densities, housing systems, handling, etc. is important in further reducing common environmental effects that tend to obscure genetic differences.

The importance of the method of group identification in comparative analyses should be stressed. Placement of progeny groups in a common environment limits the environmental effects that distort genetic differences, i.e. the accuracy of performance assessments. It is recommended that an alternative method of tagging be identified to the one used in this experiment. The current method is time as well as labour intensive. Furthermore, the initial material used to produce tags required re-tagging after four months, resulting in a high percentage of tag loss implying a loss of the individual representing that Group. A reliable tag

that could be applied practically from a very small size (< 6mm) would decrease the time of separate rearing and further reduce common environmental effects.

The presence of a possible Genotype x Environmental interaction cannot be excluded. A different result may be obtained from the same experiment performed in a different management system or under different environmental conditions, i.e. water temperature, feed, housing systems, etc. The results are therefore not directly applicable to another production system. It is also possible that a heterosis effect for growth rate may manifest in another production system.

Considerable effort went into the development of experimental material and it is therefore recommended that multiple traits of economic importance be evaluated simultaneously. This evaluation only made use of length and weight as criteria for growth assessment. The use of width as an additional indicator of growth should be considered. The data analysis has indicated that the log scale is the best-fit model for analysis of weight gain, whilst the linear model is acceptable for assessment of length gain.

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