The effect of triploidy on the growth and survival of the indigenous abalone, *Haliotis midae*, over a 24 month period under commercial rearing conditions

by

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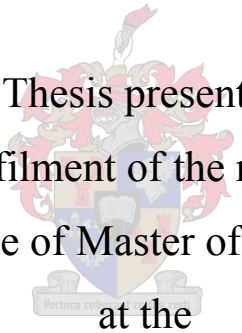

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DECLARATION

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

Triploidy is the genetic state of containing three sets of chromosomes per cell in stead of two as in diploid organisms. The South African abalone (*Haliotis midae*) is naturally a diploid organism that sexually matures between four to eight years of age. Early sexual maturity is a disadvantage in cultured abalone stock, as the process of gonad development and spawning is energy demanding, causing energy to be diverted away from somatic growth. This same problem has been extensively experienced in diploid bivalve molluscs, where triploidy has since been applied as a means to prevent sexual maturation from occurring, thereby speeding up the growth process and shortening the time to marketing.

Because triploidy was effective in bivalves, it was thought that it could contribute to faster growth in abalone as well. A procedure for the induction of triploidy in the abalone, *Haliotis midae*, was developed by De Beer (2004) and yielded up to 100 percent triploidy in treated abalone larvae. The next step was to compare the growth of the diploids and triploids to establish whether there was indeed a growth advantage on the part of the triploids, in view of commercial application.

By using the same techniques as described by De Beer (2004), three groups consisting of triploid and diploid siblings were produced and subscribed to a comparative growth trial. The groups were spawned in three different seasons. The main objective was to establish whether there was in fact a difference in growth between diploid and triploid siblings, and whether seasonal effects were associated with growth advantages for either triploids or diploids.

The two growth parameters measured were shell length and body weight. Measurements commenced at eight months of age, when the abalone could be individually tagged and continued up to the age of 24 months.

The over-all results provided no convincing evidence of statistically significant faster growth of triploid juveniles compared to that of diploids up to two years of age. Growth differences were detected between seasons, but could not confidently be ascribed to seasonal environmental effects. The regression of shell length to body weight was similar for diploids and triploids.

UITTREKSEL

Triploiede organismes bevat drie stelle chromosome per sel in plaas van twee soos dit normaalweg in diploiede diere voorkom. Die Suid Afrikaanse perlemoen (*Haliotis midae*) is van nature ‘n diploiede organisme wat tussen die ouderdom van vier tot agt jaar seksueel aktief word. Vroeë seksuele aktiwiteit is ongewens in kommersiële akwakultuur aangesien energie spandeer word aan gonade ontwikkeling in plaas van somatiese groei. Dieselfde probleem is vroeër in die oester bedryf ondervind waar dit deur middel van triploiede induksie aangespreek is. Triploiedie veroorsaak steriliteit en kan gebruik word as ’n metode om steriliteit op groot skaal te induseer. Steriliteit sou dan meebring dat meer energie beskikbaar is vir somatiese ontwikkeling, wat verhoogde groeitempo en n verkorte tyd tot bemarking beteken.

Op soortgelyke wyse is dus gepostuleer dat triploiedie in perlemoen ook tot steriliteit kon lei. ‘n Triploiede induksie metode was ontwikkel deur Mathilde de Beer (2004) wat ‘n hoë persentasie triploidie in geinduseerde perlemoen opgelewer het. Die volgende logiese stap was om die groei van diploiede diere met die van triploiede diere te vergelyk om te bepaal of triploiedie wel ’n groei voordeel tot gevolg het met die oog op kommersiële toepassing.

Deur van dieselfde tegnieke as De Beer (2004) gebruik te maak, is drie groepe, elk bestaande uit verwante diploiede en triploiede diere, geproduseer en ingeskryf aan n vergelykende groei proef. Die groepe was in drie verskillende seisoene geproduseer. Die hoof doelstelling van die proef was om groeitempo van diploiede en triploiede diere te vergelyk, asook om die invloed van seisoen op groei van diploide en triploide te bepaal.

Twee groei eienskappe naamlik skulp lengte en liggaamsmassa is gemeet vanaf ‘n ouderdom van agt maande (wanneer die diere individueel gemerk kon word) tot ‘n ouderdom van 24 maande.

Die algehele resultate het gedui op geen betekenisvolle verskil tussen die groei van triploiede en diploiede perlemoen tot op die ouderdom van twee jaar. Verskille het voorgekom in die groei tussen seisoene, maar daar kon nie bewys word dat die verskille die gevolg van seisoenale omgewingseffekte was nie. Diploiede en triploiede het dieselfde skulp lengte tot liggaamsmassa verhouding getoon tot op twee jaar ouderdom.

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He has made everything beautiful in its time. He also planted eternity in men's hearts and minds (a divinely implanted sense of a purpose working through the ages which nothing under the sun but God alone can satisfy), yet so that men cannot find out what God has done from the beginning to the end. I know that there is nothing better for them than to be glad and to receive and do good as long as they live; and also that every man should eat and drink and enjoy the good of all his labour – it is the gift of God. And God does it so that men will (reverently) fear Him (worship Him, knowing that He is.)

Ecclesiastes 3: 11 - 13

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1.1 Introduction

1.1.1 The status of the abalone fishery and farming sectors in South Africa

Haliotis midae is the only species among the six indigenous abalone species occurring in South African coastal water that is commercially exploited (Genade *et al.* 1988, Tarr, 1995). Although the abalone fishery in South Africa has existed since 1949 (Tarr, 1992), over-exploitation of the wild resource, together with illegal poaching over the past 20 years has led to the demise of the commercial abalone fisheries from a level of total allowable catches in excess of 2 700 tons in the 1970's to a level of 125 tons in 2006/2007.

The demise of the commercial fishery has shifted the emphasis to that of commercial abalone farming. The cultivation of *Haliotis midae* in South Africa was initiated with the first successful spawning of brood stock and rearing of offspring in captivity in 1981 by Genade *et al.* (1988). Since then twenty-two abalone farms came into existence on the South African coast, ranging from Port Nolloth on the West Coast, through the area of main concentration near Hermanus on the South Coast onwards to East London on the East coast (Troell *et al*., 2006). The on-shore rearing of juvenile abalone in tank-based culture systems remains the main abalone rearing method applied in South Africa.

Commercial production of farmed abalone has increased since its inception in the 1980's to approximately 500 tons in 2003 (FAO, 2004), with South Africa listed as the second-largest abalone producer after China. South African abalone production has since increased to over 900 tons in 2006 (AFASA, 2006) and is expected to continue increasing as the industry expands and more farming permits are being considered (Brink, 2003, Troell *et al*., 2006).

Ranching is not currently a commercial practice although a number of trials have been done in South African waters. De Waal *et al.* (2003) showed that shallow, sheltered bays are best suited to ranching for various reasons, but sites such as these are uncommon on South African coast lines, thus inhibiting the expansion of this industry. The current poaching status of *Haliotis midae* also inhibits investment into this sector, where security poses to be a limiting factor.

1.1.2 The application of triploidy in abalone farming in South Africa

The abalone farming sector in South Africa has developed over the last two decades mainly through the in-house development of appropriate technology (Sales & Britz, 2001), supplemented by technology transfer from foreign industries - mainly New Zealand and California (Troell *et al*., 2006). Most of the research and development was directed towards improvement of farming techniques and practices. This goal-orientated approach to research of the South African abalone industry, in relation to the commercial culture of *Haliotis midae,* has inevitably led to the introduction of genetic technologies directed towards improving the commercial productivity of the species.

The I&J Abalone Culture Division started in 1996 with initial investigations, in collaboration with the University of Cape Town (Stepto, 1997) and later the University of Stellenbosch (Vorster, 2003; De Beer, 2004) into the genetic enhancement of *Haliotis midae*, including the development of a technique for the induction of triploidy in an effort to secure the prospect of enhanced growth. This initiative by I&J has since lead to the formation of a consortium, consisting of industry partners, the University of Stellenbosch in collaboration with the South African Innovation Fund Trust, to oversee the genetic enhancement of the species for commercial application. Triploidy was one of the aspects incorporated into the genetic enhancement venture, especially for the benefits that could be derived in terms of improved rate of growth and sterility. Sterility in itself addressed two major concerns:

1. Biosecurity: Using triploids eliminate the potential risks of genetic contamination of natural populations by individuals escaping from genetically altered farmed populations. The use of triploids could also enable farming in conservation areas and even 'ranching' of exotic genotypes in non-indigenous habitats of particular species (Liu *et al.,* 2004).
2. Intellectual property: Triploidy provides a means of protecting expensive improved genetic strains form being propagated by abalone buyers (Dunstan *et al.,* 2007).

Triploidy has been successfully applied in the oyster industry as a means of inducing sterility with the associated benefits of improved quality as well as increased growth rates. Triploidy has been commercialised in oysters in North America as early as 1985 (Nell, 2002) and has since initiated investigations into triploidy in many other commercial molluscs

species, including abalone. Faster growth rates, however, have not been observed in all species, as studies on a variety of molluscs have demonstrated highly variable triploid growth responses.

The aim of this study was to investigate the effect of triploidy on the growth rate and survival of the South African abalone, *Haliotis midae*. This study followed on the work done by De Beer (2004), who successfully developed a protocol for the induction of triploidy in *Haliotis midae* through the use of hydrostatic pressure and the consequent retention of the second polar body at fertilization. The availability of a reliable technique for induction of triploidy in *Haliotis midae* necessitated the comparative evaluation of the growth rate and survival of triploid and diploid genotypes, in view of possible commercial application.

1.2 Biological aspects of the abalone, *Haliotis midae*

1.2.1 General classification

Abalone, or Haliotids, are classified as part of the phylum Mollusca, under the class *Gastropoda* (Table 1.1). Day (1974) described the Class *Gastropoda* as molluscs with a distinct head, eyes and tentacles: mouth with radula and the foot broad and flattened. The body is typically asymmetrical and covered by a single spiral shell. *Haliotids* (abalone) belong to the Order *Archaeogastropoda* which is the oldest and least specialized group of prosobranch gastropods (Muller, 1986).

Table 1.1 General classification system for molluscs, including the abalone, *Haliotis midae* (Branch *et al.,* 1994).

PHYLUM MOLLUSCA	
Class Polyplacophora	Chitons
Class Bivalvia	Clams, mussels, oysters
Class Scaphopoda	Tusk Shells
Class Gastropoda	
Subclass Prosobranchia	Snails, abalone, limpets
Subclass Opisthobranchia	Nudibranchs
Class Cephalopoda	Octopi, squid

South Africa is home to a total of six indigenous species of *Haliotis* that are distributed along its coastline, as summarised in Table 1.2. *Haliotis midae* is the largest of the South African species (230 mm) (Muller, 1986).

Table 1.2 *Haliotis* species and its occurrence in South Africa (Muller, 1986)

Species	Distribution
H. midae	Saldanha to Port St. Johns
H. parva	Cape Town to East London
H. spadicea	Cape Town to Sodwana
H. speciosa	Port Alfred to Port St. Johns
H. queketti	East London to Durban
H. pustulata	North of Sodwana

In the wild the shell is described as being reddish in colour, but this is normally obscured by marine growths (Muller, 1986). From the author's experience, marine growths do not occur readily on the shells of farmed abalone. On farms, shell colour in early life stages is heavily influenced by diet, so much so that abalone grown on different farms can be distinguished due to their differing diets. At I&J, where kelp is the main source of feed, the shells of the export size abalone are grey-white with an occasional greenish tinge.

The shell is identified by corrugations that run obliquely to the lines of growth (Muller, 1986). Juveniles show numerous fine spiral ridges and corrugations when the shell is about 3,75 mm in major diameter. The foot is pale cream to mottled light brown, and the tentacles and gills are yellow (Muller, 1986). On farms, foot colour is also influenced by the major feed component in the diet and can vary between dark grey, light grey, green, and yellow-green with dark grey mottles.

1.2.2 Physiological aspects related to growth and growth measurement

1.2.2.1 The shell

Haliotis larvae form initial shells (protoconchs) through shell glands present in the embryo prior to day five after fertilization. It is spiral shaped and serves as protection to the veliger larvae. The post-embryonic shell consists of three layers: the outer periostracum, a calcerous prismatic layer (composed of calcite crystals) and the inner nacre which is composed of aragonite (Bevelander, 1988).

The protoconch forms the apex of the adult shell, so that incremental growth is achieved by the deposition of new shell material by the mantle, on the growing edge (aperture) of the shell (Bevelander, 1988). Growth of the shell continues throughout the life of the abalone. Shell length tends to increase only up to a certain point; in *Haliotis midae* up to about 200mm (Newman, 1968), but thickening of the shell continues throughout its life (Bevelander, 1988).

In abalone aquaculture, shell quality and strength is of great importance for the overall presentation of a good quality live abalone product. Poor management that allows high stocking densities and inadequate shelter increases stacking of the abalone on top of one another which in turn increases competition for food (Huchette *et al.*, 2003). Competition for food affects shell quality by causing breakages in weak spots (Tarr, 1995). Juvenile abalone

reflect their life histories in their shells, in terms of what they ate, their growth rates as well as environmental conditions such as water quality, exposure to parasitic shell invaders and stocking densities. The average shell quality of a batch of abalone is thus a direct indicator of the general health and well-being of that batch.

Because shell quality is such an important indicator of the life history of animals the following aspects should be taken into account during the establishment of experimental groups:

1. No animals with damaged shells should be incorporated / included into growth trials because they demonstrated severe and permanent retardation in growth. Once a shell is damaged, energy spent in trying to correct the flaw or irritation results in less energy spent on overall growth. The environmental influence on their growth overshadows their genetic ability to grow in genetic comparisons, (or in feed trials where the feed conversion ratios of a feed is determined), thus making damaged abalone unsuitable as growth trial material.
2. Groups of animals with differential rates of damage / growth should not be compared to each other, either within replicates or between batches.

1.2.2.2 Tagging methods

The tagging of abalone poses quite a challenge during comparative experimental work during the juvenile life stages. A reliable cost efficient tagging method for large numbers of abalone in the size range of 5-10mm was difficult to find. The required tagging method should be durable; lasting for two years or longer and should not impose any stress on the animals.

The two main methods used in tagging of abalone are attaching a tag to the shell via an adhesive or by attaching a tag to a shell pore. A problem with juvenile abalone is that the shell is smooth and fragile, preventing the use of an adhesives such as Pratley Putty in combination with a coloured tags, which has become a proven method on larger animals (larger than 30mm shell length). Various studies have shown that the method of attaching a tag to a shell pore causes a significant amount of stress to the tagged abalone (Newman, 1968; McShane *et al*., 1988) leading to unrealistic growth patterns. The insertion of soft silicone tags into the first breathing pore of 15mm juveniles also proved to be unreliable due

to unacceptable levels of tag loss within four months (Vorster, 2003; pers. comm., Vlok, 2007).

The tagging method deemed most suitable for this study were similar to that used by the South African Innovation Fund Abalone Project (Pers. comm., Vlok, 2007) that is based on the use of a liquid quick-set adhesive (Superglue) in combination with a colour coded tag. Although tag loss was still experienced it lasted long enough to allow for retagging of the animals at a larger size when the shells were more ridged. The same tagging method was then applied, but the tags were placed in-between the shell ridges where they displayed very good adherence.

1.2.2.3 The foot or muscle

The foot of the abalone is made up of muscle cells. It represents the bulk of the soft tissue weight and total weight of the abalone. The foot is used for locomotion, adhesion to the surface it resides on and for feeding. It is also the only energy storage depot available to the animal.

It is reported that triploid individuals of certain abalone and oyster species cope better in stressful environments than their diploid siblings (Stanley *et al*., 1984, Tabarini, 1984, Allen & Downing, 1986, Maguire *et al*., 1995, Garnier-Gere *et al.,* 2002). Section 1.4.1.3 elaborates on these findings. If a similar trend could be established for *Haliotis midae*, triploid genotypes might display additional advantages in terms of reduced weight loss during stressful events such as adverse environmental conditions, dietary changes and handling during grading and live export.

1.2.2.4 The reproductive organs

Haliotids possess a single gonad, either male or female. The gonad develops on the right side of the body. In the adult it lies around the digestive gland and forms a large part of the superficial region of the visceral mass (Newman, 1967, Bevelander, 1988). A ripe male gonad appears cream coloured and the female gonad has a light greenish colour (Bevelander, 1988). This is due to the white sperm inside the male gonad and the green eggs inside the female gonad.

The lumen of a well-developed testis is vertically traversed by connective tissue tubes. The testis wall and outer surface of the connective tissue tubes are lined with germinal

epithelium (Newman, 1967). The germinal cells of the epithelium of the testis give rise to spermatocytes, which in turn develop into spermatids. The spermatids produce sperm which are about 6µm long, excluding the tail. When fully mature, the whole testis lumen is packed with sperm (Newman, 1967).

In a fully developed ovary the lumen is filled with mature eggs of up to 200µm in diameter. These eggs are embedded in a gelatinous matrix. Together with the fully developed eggs, smaller immature eggs are also present, attached to trabeculae. Evidence suggests that this group of smaller eggs form the basis of the next spawning (Newman, 1967).

Although triploid abalone is expected to be sterile, this does not mean that they will not develop gonads. Several spawning events have been observed in triploids of other shellfish species. Allan and Downing (1986) recorded spawning events in triploid oysters of the species *Crassostrea gigas* and Guo and Allen (1994) found that gametes produced by these triploids were fully capable of fertilization, but aneuploid progeny resulted which did not survive to metamorphosis and settlement. They concluded that although the triploids spawned, they were effectively sterile through the inability of their gametes to produce normal larvae.

Dunstan *et al*., 2007 found that in *Haliotis laevigata*, triploidy more adversely affected adult triploid females than males, in the sense that males were observed to develop small gonads where females displayed almost no gonad development.

If triploid abalone do develop gonads similar to diploids, no comparative growth advantage should theoretically be observed based on the principle that energy is diverted towards somatic muscle growth instead of gonadogenesis.

1.3 Life stages of *Haliotis midae* applicable to the experimental design

1.3.1 Larval development

The larval development stages of *Haliotis midae* was studied by Genade *et al*. (1988) and is presented in Table 1.3.

Table 1.3 The stages of larval developmental of *H. midae* at 20^{o}C (Genade *et al*., 1988).

Stage	Description	Time from fertilization	
		Hours	± Days
1	Hatching	14	
2	Free-swimming trochophore	22	
3	Cap-shell, early veliger	24	1
4	Inflate-shell veliger	31	
5	Early operculate veliger, pre-eyespots	46	2
6	Incipient cephalic tentacle, operculate veliger	51	
7	Mid-formed cephalic tentacle	86	3
8	Digitate (branched) cephalic tentacle	97	4
9	Crawling, settlement	118	5
10	Total metamorphosis (loss of cilia, but no mouthparts or feeding yet observed)	145	6
11	Peristomial growth	169	7

The times related to larval development stages are influenced to a large extent by water temperature. Larvae raised at lower temperatures (e.g. 17.5^{o}C) took two days longer to develop to Stage 11, the peristomial growth stage (Genade *et al.,* 1988). Larvae reared at the I&J Danger Point Abalone Hatchery generally followed the same developmental patterns as above. No comparison, however, was made between the developmental times of diploid and triploid larvae. It might be expected for the triploid larvae to develop a little slower than the diploids due to the stress imposed by the method of induction, though no evidence were observed in this regard.

1.3.2 Settlement

The most critical stage in the life history of benthic organisms is recognized to be settlement, where the availability of suitable substrates for settling larvae is of utmost importance (Genade *et al.*, 1988). It is generally accepted that both physical and chemical characteristics

of substrates play a role in settlement rates (Genade *et al.,* 1988), indicating differential settlement success on different substrates. This is commonly experienced in settlement systems, especially where settlement takes place on naturally grown substrates (chemical differences between substrates), but also within mono-culture diatom films, where diatom density causes the variance (physical differences of substrate). Daume *et al.* (2004) showed that different settlement substrates also supported different growth rates in settled larvae, and that this variance in growth persisted and was amplified with time after settlement.

It is clear that settlement represents the first obstacle in a comparative growth trial between different groups of abalone, such as diploids and triploids. Settlement rates will always be unpredictable and variable due to the nature of seawater and variability of natural algae present. The design of any settlement survival or growth comparison experiments should therefore try to minimise the variance caused by settlement in the following manners:

1. When different groups of larvae have to be settled in different containers, the presentation of a similar diatom composition on the respective settlement substrates is very important. This can be achieved by settling on mono-diatom settlement cultures, or at least settlement substrates that have been grown in the same conditions and which are similar in age.
2. A number of smaller containers should rather be settled in to create more repeats of settlement substrates, rather than using one or two larger containers for settlement. This may assist to dilute the effect of differences in settlement substrate between experimental groups.

1.3.2.1 Feeding and growth

According to Barkai and Griffiths (1988), about 63 percent of energy derived from the feed that an abalone consumes is excreted as faeces. A further 32 percent is used for respiration, leaving a mere 5 percent of consumed energy available for growth and reproduction. In juveniles this is mostly allocated towards somatic growth, while in adults, energy spent on reproduction increases relative to sexual maturity. This may account for the relatively slow growth of abalone as only a small portion of the total energy intake is used for growth.

In addition to this, abalone is known to be erratic feeders, sensitive to a number of environmental and physiological stimuli (Huchette *et al.,* 2003). These environmental stimuli

often causes the channelling of energy into physiological stress responses, resulting in fluctuating feed intake and growth rates (pers. comm., Vosloo, 2007). Growth in juvenile abalone is primarily dependant on the availability of and type of diet. Seasonal variation in algal diet quality and water quality parameters (Day & Fleming, 1992), together with other factors such as competition, parasite load and management affect growth rates in commercial systems.

From a commercial perspective, the most important economical goal is achieving constant, good growth rates among juvenile abalone, so that they reach the appropriate size in an economically viable time frame (Fleming, 1995). This objective can be achieved by keeping the environmental conditions inside the rearing units as stable and conducive to feeding as possible. Not only do good growth rates render animals ready to be sold at a younger age, thus lessening the time spent on the farm, it also promotes healthy shell growth and quality of the end product. Fast growth further minimizes the attack from parasitic organisms, thus promoting the general health status of the juveniles (pers. comm., Loubser, 2006).

Another important growth-determining factor is the extent of competition amongst abalone in the holding units, which is a direct result of stocking densities. Competition between juveniles is influenced by the availability of feed. The availability of feed is determined by the amount of feed provided per abalone in a holding unit as well as the ability of each abalone to reach the feed. Stocking densities thus play an important role where space for feed is limited and set feeding times are applied as in a commercial set-up (pers. comm., Loubser, 2006). During the execution of a comparative growth trial at a high stocking density, it is of utmost importance to monitor stocking densities on a regular basis in order to limit competition and to prevent unnecessary damage to the abalone. It is recommended that the factors inducing variance in growth, such as stocking densities, feeding regimes, water and air supplies and handling should be kept constant over treatments and groups.

1.3.2.2 Survival

Observations made by Genade *et al.* (1988), indicated the major factors that influence the mortality rate of juvenile *H. midae*. The first major factor was micro-predators that preyed on the settled larvae on the settlement plates, during the first two months, particularly when the

water chemistry around the settlement substrate was inadequate. The second was mortalities caused by deplating and handling of the juveniles and the third was predation by policlads. Most of these factors, they claimed, could be eliminated by proper management techniques (Genade *et al*. 1988). Similar observations were made during this growth trial in as much as that the highest mortality rates occurred within the first two weeks of settlement (about 97 percent), followed by only a few mortalities after deplating (about 5 percent of the remaining animals) with negligible levels thereafter.

1.3.2.3 Movement

Juvenile abalone are very active and photosensitive so they will move to more suitable residing places whenever the need arises, away from sunlight (Huchette *et al*., 2003). This makes them easily containable during the day by providing suitable habitats in their holding units. However, during the night they forage actively and will walk to wherever food is available (Huchette *et al*., 2003). When competition for food in a basket is too high, some of the abalone will crawl out of the baskets in search of food. These animals are referred to as "crawl-outs" and often die due to exposure when they are not discovered in time to be placed back into their baskets.

The crawl-out factor warrants serious consideration during a growth trial. When replicate groups are placed into the same housing system, ways of preventing them from crawling over into other baskets need to be found. The best means of preventing crawl-outs and consequent contamination of experimental groups is to physically cover all the containers so that crawl-outs cannot occur. This is not always possible in which case it is recommended to use different colour tags for each group of experimental animals in order to identify animals that have crossed over from one group to another. Staff should also be trained in how to deal with animals that have left their respective experimental group to prevent contamination.

1.3.3 Adult Stages

1.3.3.1 Spawning

In the wild, fifty percent of animals reach sexual maturity at a live weight of 140g, equivalent to a shell width of 8.0 cm, with 100 percent of individuals mature at a size of 275g or 10.5cm

shell width. Most wild populations spawn twice a year, once in spring or early summer and again in late summer or autumn (Newman, 1967).

Spawning can be artificially induced by changing certain water quality parameters and thus the environment the abalone resides in. A variety of chemical and physical treatments can be used to induce spawning, e.g. Ultra-violet light, pH alteration or water temperature manipulation (Fallu, 1991).

When collecting gametes for a comparative growth trial, one should ensure that the material used is not genetically biased. It is recommended that gametes are randomly sourced from as wide a pool of brood stock as possible, so that parental genetic influences in the offspring are minimised.

1.3.3.2 Feeding and growth

Adult abalone are usually retained for brood stock purposes, in which event growth is not a consideration. Energy and nutrients from the feed is required to sustain gonadogenesis; a well balanced diet that supplies the dietary requirements is therefore required to keep brood stock in good condition.

1.4 Triploidy in aquaculture

The occurrence of polyploidy in natural populations has been recorded; though uncommon and seen mainly as a result of a numerical mutation of chromosomes (Guo & Allen, 1994). Research on polyploidy in molluscs began in America in the early 1980's in response to a request by the aquaculture industry to produce sterile oysters (*Crassostrea virginica*) that could be marketed throughout the year (Utting, 1995). The need to farm commercial aquatic species in non-endemic waters and bays also contributed to research in mass sterility induction. Chromosome number alteration, especially triploidy induction was identified as a potential method of mass sterilization of commercial quantities of shellfish (Allen & Guo, 1996).

Commercial production of triploid Pacific oysters (*Crassostrea gigas*) on the West Coast of North America started in 1985. Since then triploidy of various other oyster species were investigated, but not yet commercialised (Nell, 2002).

However, triploidy has since been successfully induced in various species of molluscs, including oysters (Garnier–Gere, 2002, Davis, 2004), clams (Liang & Utting, 1994), mussels (Brake, 2004), scallops (Tabarini, 1984, Yang *et al.*, 2000) and abalone species (Zhang *et al.,* 1998, Elliot *et al.,* 2004, Liu & Heasman, 2004), as well as finfish species such as trout (Bonnet *et al.,* 1999), salmon (O'Flynn *et al*., 1997) and sunshine bass (Kerby *et al*., 1995). The effects of triploidy on the above mentioned species vary greatly and advantages in addition to sterility such as faster growth, improved yield and superior product quality that triploidy may offer is reviewed in the following section.

1.4.1 General effects of triploidy on shellfish

1.4.1.1 The effect of triploidy on growth

As research into the artificial induction of triploidy progressed, growth trials provided evidence of differential growth between diploids and triploids. Bonnet *et al.* (1999) and Kerby *et al*. (1995) reported that, in finfish species, the diploids seemed to perform better in terms of growth rate, but in molluscan species, the triploids seemed to have displayed a general superior growth rate (Tabarini, 1984, Zhang *et al.,* 1998).

Various theories have been presented as possible explanations for the faster growth observed in triploids of certain molluscan species compared to diploids. These theories can be summarized as:

1. Triploid genotypes are sterile and hence channel energy towards somatic growth that would otherwise have been used for gametogenesis (Tabarini, 1984, Allen & Downing, 1986, Barber & Mann, 1991, Ruiz-Verdugo *et al*., 2000). This theory is referred to as the "triploid advantage" theory.
2. Triploid populations display higher levels of heterosis due to an increase in heterozygosity because of a higher probability of possessing two or even three different alleles at each gene (Magoulas *et al.,* 2000, Hawkins *et al.,* 2000, Garnier-Gere *et al.,* 2002).
3. Even in the absence of heterosis, the higher probability that diploids might exhibit depressed growth due to the expression of deleterious mutations caused by the partial loss of chromosomes give triploids an advantage (Zouros *et al.,* 1996, as cited by Garnier-Gere *et al,*. 2002).
4. The "gene dose" hypothesis states that triploids have three homozygous alleles at each gene. This means that triple the amount of gene products is available, or at least that transcription of the genes which might affect and enhance the growth rate of the organism is facilitated faster (Magoulas *et al*., 2000).
5. Guo and Allen (1994) suggested that having three sets of chromosomes per cell may cause a marginally increased cell size.

Furthermore, a difference has also been observed in the growth rate of Meiosis I and Meiosis II triploids, where the type Meiosis I abalone grew faster (Hawkins *et al*., 1994, 2000). This is thought to be the combined effect of more than one of the above theories (see Section 3.1.4).

In researching the roles that the above theories played on the growth of triploid individuals of a variety of species, it was evident that the importance of each theory differed from species to species and was also dependant of environmental conditions. The relationships of the above mentioned theories with each other to produce the growth effects in the triploids was unique in almost each instance and this provided contradicting results in terms of the comparative growth rates between diploids and triploids. It could therefore not

be concluded from literature which hypotheses/theories or any combination of these provided the most probable explanation for the superior (or inferior) growth performances observed in triploids.

1.4.1.2 Yield and quality

Oysters are probably the most highly researched organisms that display economic benefit obtained from triploidy. Triploid oysters display superior growth in relation to their diploid siblings due to the mechanism of theory 1 (triploid advantage), thereby reducing the time taken to obtain market size by 6 to 18 months, whilst also maintaining better meat condition and therefore better yield and quality throughout all seasons (Allan & Downing, 1986; Nell *et al.,* 1995).

Utting *et al*. (1996) showed that triploid Manilla clams (*Tapes philippenarum*) were heavier and had a higher condition index than diploids of the same age. Contrary to the above, Mason *et al.* (1988) found that energy allocation between different tissues in triploid and diploid soft-shelled clams, *Mya arenaria* was not related to ploidy status at all.

An important factor when assessing the quality of the live abalone product is the quality of the shell. This is largely determined by on farm management practices, such as the regular grading of animals to limit size variation and to maintain standardized stocking densities. Abalone growth tends to be quite variable in nature, probably due to high levels of environmental and genetic variation. A decrease in the observed variation in growth would be of value in terms of general management as it would reduce competition between individuals and the need for grading that is the major cause of shell damage. A trial by Mason *et al.* (1988) on *Mya arenaria* indicated that the variance with regard to energy budget parameters of triploids was significantly reduced in comparison to diploids siblings. The increased heterozygosity measured in the triploid clams was correlated to a decrease in the variance of physiological and morphological parameters. These findings indicate the possibility that triploid populations of shellfish may display less variable growth which could have important benefits in terms of reduced managerial inputs (grading) and improved product (shell) quality. More uniform growth could have specific benefits in the nursery stage as the limitation of size variance would reduce competition among the ungraded juveniles which in turn would yield larger proportions of good quality seed stock.

1.4.1.3 The effect on survival and disease resistance

Garnier-Gere *et al.* (2002) reported that mortality rates for triploid oyster larvae were mostly higher than for diploids but after this stage, mortality rates were equal for diploids and triploids. The same pattern was recognized in this study, where triploid survival rates at settlement were much lower than for diploids, but about equal after these initial stages.

In later stages of life, where disease resistance is more important, reports indicated the probability that triploids may have improved resistance to diseases or other stress factors (Allen & Downing 1986). This was ascribed to the fact that both heterozygosity and sterility may lead to lower metabolic energy requirements, so that more energy is available to support the immune system under stressful conditions (Hawkins *et al.,* 2000).

Allen and Downing (1986) found a superior survival rate in triploid oysters, *Crassosstrea gigas*. Maguire *et al*. (1995) however, found triploid *Crassostrea gigas* to have a lower survival rate than the diploid siblings. Other studies found comparable survival of triploid and diploid genotypes such as for *C. gigas* (Garnier-Gere *et al.,* 2002), *C. virginica* (Stanley *et al*., 1984), *Saccrostrea commercialis* (Nell *et al.,* 1995) and *Argopecten irradians* (Tabarini,1984).

1.4.2 Effect of stage of induction (Meiosis I or II) on growth and survival

Examination of the differences that might exist between triploids created by retention of the first polar body (Meiosis I triploids) and triploids created by the retention of the second polar body (Meiosis II triploids) was done by Hawkins *et al*., (1994). They showed that Meiosis I triploids of *Ostrea edulis* grew about 60 percent faster than Meiosis II triploid and diploid groups, with no difference between Meiosis II triploids and their diploid siblings. They consequently tested 6 polymorphic enzyme loci and found that single-locus heterozygosity was the highest in the Meiosis I triploids and the average multiple locus heterozygosity in Meiosis I triploids were about 50.5 percent higher than in diploids and Meiosis II triploids.

These results were a confirmation of the work of Beaumont and Kelly (1989) done on *Mytilus edulis*, where Meiosis 1 triploid larvae outgrew their Meiosis II and diploid siblings. In a later study, Hawkins *et al.* (2000) showed that there was a maternal effect that interacted with genotype in *Crassostrea gigas*. Among full siblings, 42 percent of the variance in physiological performance was accounted for by allelic variation, measured as multi-locus

enzyme heterozygosity. Allelic variation was greater in both Meiosis I and Meiosis II triploids than in diploids, but was highest in Meiosis I triploids.

Stanley *et al.* (1984) also observed faster growth in *Crassostrea virginica* Meiosis I triploids. The Meiosis I triploids outgrew both the diploid controls and the Meiosis II triploids, both of which grew at the same rate. They also found the heterozygosity of Meiosis I triploids to be higher than the others and concluded that the faster growth must have been due to heterozygosity rather than triploid advantage.

Garnier-Gere *et al*. (2002) compared diploid *C. gigas* and Meiosis II triploids, in differing environments (sites), in an effort to establish the roles of both triploid advantage (theory 1) and heterozygosity (theory 2) in the faster growth of Meiosis II triploids (which grew faster at both sites). They found that the average heterozygosity was significantly higher in the meiosis II triploids, but the ranges of both diploid and triploid variations overlapped substantially. This indicated an advantage of the triploid state *per se,* whatever the diversity of the individual (Garnier-Gere *et al*., 2002). They attributed this triploid advantage to either the sterility of the oysters or the gene dosage theory (theory 4) or both, and were not able to quantify the role each may have played, if both were in fact involved. Their study also indicated that triploid advantage could exist in both favourable and unfavourable environments.

It is clear from the above that different species show differing heterozygosity patterns in their meiosis I triploid-, meiosis II triploid- and diploid siblings. The exact reasons for triploid advantage have thus not yet been accurately quantified, and the fact that different species react so differently to triploid induction reinforces the need for further study into the field. However, the above findings suggest that, should growth not be markedly different in Meiosis II triploid abalone compared to diploids, it might be worth the while following the triploid route via meiosis I as in oysters, unless sterility is the only objective.

In relation to abalone, Zhang *et al.* (1998) found no significant difference in growth between Meiosis I and Meiosis II triploids of the Pacific abalone *Haliotis discus hannai,* contrary to the results in oysters by Stanley *et al.* (1984). Stepto (1997) reported poorer survival rates and higher abnormality rates among Meiosis I triploids than among Meiosis II triploid *Haliotis midae,* as did Zhang *et al.* (1998) for Pacific abalone. Norris and Preston (2003) compared the induction methods for Meiosis I and II triploids and concluded that it

was physically much easier to block the release of polar body II to produce Meiosis II triploids for abalone. Since the survival of the Meiosis II larvae is higher and there is no difference between subsequent growth rates, Norris and Preston (2003) concluded that blocking of polar body II would be preferentially used in commercial scale triploid abalone production.

1.4.3 Specific effects of triploidy in shellfish and finfish

1.4.3.1 Specific effects of triploidy in abalone species

Zhang *et al.* (1998) found that triploid individuals of the Pacific abalone, *Haliotis discus hannai*, performed better than their diploid counterparts at 10mm shell length. They hypothesized that the size difference could be due to any of a number of reasons, including the relatively high heterozygosity of the triploids or selection effects from use of the chemical 6-DMAP or both. They reasoned that the triploid larvae that survived the initial induction treatment (chemical or shock treatment) may have had a greater vigour and thus withstood environmental challenges better during the juvenile stage.

Elliot *et al.* (2004) produced *Haliotis laevigata* Meiosis II triploids and compared their growth performance to that of their diploid siblings. They found that after 2.5 years, triploid groups grew at a lower absolute growth rate, but when expressed in terms of specific growth rate, i.e. relative to initial size (the triploids were smaller at the first measurement of 13 months), the values for diploids and triploids were similar.

Liu *et al*. (2004) induced triploidy in *Haliotis rubra* and reported that the growth of triploids and diploids where equal at 19 months of age, although at 22 months of age the triploids weighed significantly more. When sexual maturation stepped in, the triploids had more developed testes than those of their diploid counterparts.

Stepto (1997) reared diploid and triploid groups of *Haliotis midae* and reported trends showing the superior growth of that triploids up to 550 days, but these growth differences were not statistically significant.

1.4.3.2 Oysters, mussels and scallops

Garnier–Gere *et al*. (2002) found that triploid and diploid genotypes of the oyster *C. gigas* displayed different growth performances during specific times of year, related to

conditions of stress, where triploids tended to perform better. They concluded that this was due to triploid advantage, so that more energy was available to handle the stressful environments. Maguire *et al.* (1995) found that in *C. gigas,* triploid oysters grew 23.4 percent and 19.6 percent faster than their diploid siblings in both good and poor environments respectively. Their respective survival rates were similar as were their meat quality.

Barber and Mann (1991) found that triploid individuals of the Eastern oyster (*C. virginica*) were significantly heavier than their diploid counterparts and reached commercial size five months earlier than the diploids.

Both Nell *et al.* (1995) and Hand *et al*. (1998) found that triploid Sydney rock oysters, *Saccostrea commercialis,* grew significantly faster than their diploid siblings and also displayed significantly lower mortality rates. Hand *et al.* (1998) also found that triploids had a higher rate of resistance against "winter mortality", caused by infestation of the protistan parasite *Mikrocystos roughleyi* during winter months.

Brake *et al*. (2004) studied the performance of diploid and triploid mussels *Mytilus edulis* and found that the triploid mussels demonstrated a greater growth rate than the diploids. When compared to each other after a spawning event, the triploids weighed 62 percent heavier and had a shell length 10.9 percent greater than their diploid counterparts. An interesting observation was that a highly skewed sex ratio, in favour of the males, existed in the triploid population.

As for scallops, Tabarini (1984) reported a 73 percent heavier adductor muscle in triploids of the bay scallop, *Argopecten irradians.* For catarina scallop (*Argopecten ventricosus*), Ruiz-Verdugo *et al.* (2000) reported a difference of 167 percent and Ruiz-Verdigo *et al.* (2001), a difference of 121 percent in adductor muscle weight, in favour of triploids.

Yang *et al.* (2000) reported an average muscle increase of 44 percent for triploid *Clamys farreri* (zhikong scallop). Maldonado-Amparo *et al*. (2004), on the other hand, found no growth differences between diploid and triploid scallops *Nodipecten subnudosus*, even though triploids were unable to mature and form gametes, showing 95 to 99 percent sterility when compared to diploids. They attributed this lack of growth advantage to the productive strategy of the species, where energy for maturation of the gonads was not derived from stored resources but rather from newly ingested feed.

1.4.3.3 Specific effects of triploidy in finfish species

Bonnet *et al.* (1999) reported significant differences in body weight of both Rainbow trout and Brown trout, where diploids were heavier than triploids, though no significant differences in length were observed. O'Keefe and Benfey (1999), found no difference in growth rate or food consumption when comparing diploid and triploid brook trout, *Salvelinus fontinalis*.

Kerby *et al*. (1995) showed that triploid individuals of Sunshine bass grew significantly slower than their diploid siblings, though the triploids had a slightly higher survival rate. O'Flynn *et al.* (1997) compared the growth and survival of diploid and triploid Atlantic salmon. They had mixed results but concluded that the overall yield of triploids were lower than diploids under culture conditions.

It can thus be concluded from the researched material that finfish species do not react favourably to triploid induction in relation to growth.

1.5 The use of triploidy for biological containment

The need for biological containment of aquaculture organisms associated with commercial farming activities has increased in recent years due to the expansion of such industries into environments to which these species are not native or indigenous. The induction of reproductive sterility such as with triploidy, presents a feasible method of containment of aquatic species that are non-native or genetically differentiated from the natural stocks (Allen & Guo, 1996).

Triploid animals in many species are thought to be sterile. This does not mean, however that they do not develop gonads, though in most cases gonad development is retarded. Allan and Downing (1986) found that triploid Pacific oysters showed retarded gonadal development, but spawned never the less. They attributed the spawning to a behavioural response caused by sperm in the water column which acted as an environmental cue to trigger spawning, even in triploids. This suggested that spawning was not only regulated by the maturity of the gametes, as was first thought, but also by environmental triggers (Allen & Downing, 1986). Guo and Allen (1994) found that gametes produced by triploid oysters, *Crassostrea gigas,* were fully capable of fertilization, but resulted in aneuploid progeny which did not survive to metamorphosis and settlement. Therefore, although triploid *Crassostrea gigas* were not sterile with reference to gamete production, their reproduction potential remained virtually zero.

Allen and Guo (1996) found a high proportion of heteroploid mosaics among their triploid stock of *Crassostrea gigas*, suggesting that at least some of the triploids were unstable and could revert from triploidy to mosaics. These mosaics might have become sexually active, but this could not be proved. Their research into the subject continues, but on the basis of available results, triploidy can be assumed to be a safe mechanism of sterilization, providing that the treatment can be certified to be 100 percent effective, i.e. 100 percent triploidy resulting from the induction.

Dunstan *et al*. (2007) found that female triploid *Haliotis laevigata* did not develop sufficient gonads to be able to spawn by the age of four years. However, the males did show some gonadal maturation but not enough to respond to conditioning and repeated spawning queues.

1.6 Methods of induction of triploidy

In a review article Chao *et al.* (1993) described the different methods that can be used to induce triploidy in a variety of aquatic organisms. This included the use of thermal treatment, hydrostatic pressure treatment and chemical treatment to suppress polar body formation or to block mitosis. The efficiency of these treatments depends on three main parameters, namely:

1. Treatment conditions (cold or heat in thermal shock, pressure intensity in hydrostatic pressure shock, kind and concentration of chemicals in chemical shock).
2. The duration of the shock treatment.
3. The timing of treatment in terms of the cell/meiotic cycle.

Figure 1.1 illustrates the mechanisms of inducing triploidy through chemical, temperature or pressure shock. When a sperm cell fuses with an egg cell, the egg is "activated" and mechanisms are initiated that result in the expulsion of polar body II, which contain a single complete copy (1N) of the maternal DNA (Lutz, 2001). However, by applying a stressor at this specific time for a sufficient duration to disrupt these mechanisms, failure of the expulsion of the second polar body can be achieved, resulting in a triploid fertilized egg (Lutz, 2001).

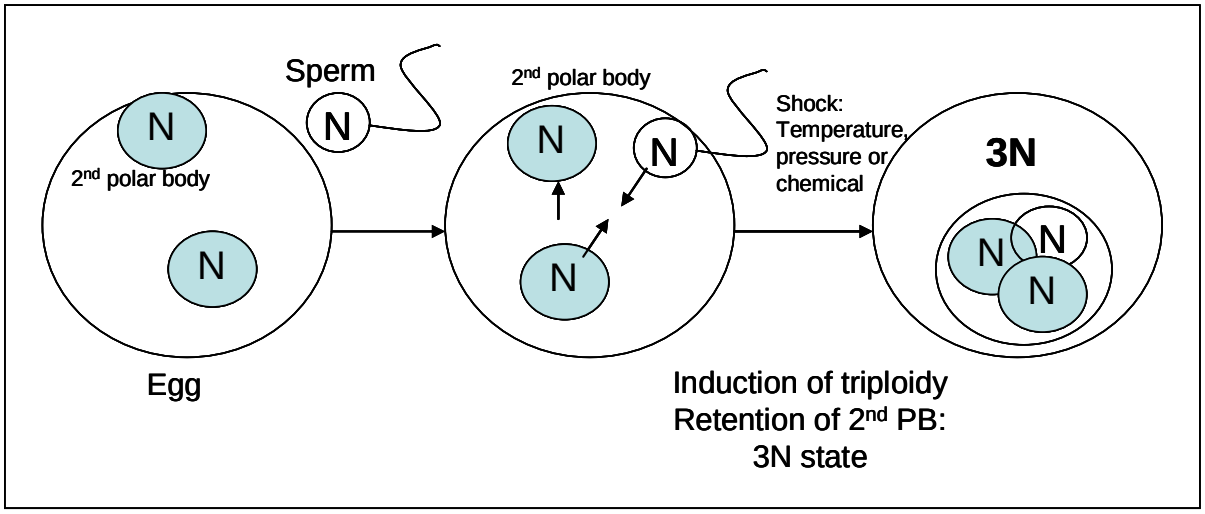

Figure 1.1 The induction of triploidy through the retention of the second polar body (Lutz, 2001).

1.6.1 Chemical treatment

The chemical Cytochalasin B (CB) is the most commonly used chemical shock treatment to induce triploidy in bivalves. It is thought to inhibit micro-filament formation during cell division. Cytochalasin B is hydrophobic and is, therefore, dissolved in DMSO (dimethyl suphoxide) as a carrier solution before being made up to the desired concentration (0.1 – 1.0 mg CB/liter) with filtered sea water. Fertilized eggs are held in this solution for the appropriate time (15 – 20 min). Eggs are then transferred to a 0.01 to 0.1 percent solution of DMSO in filtered sea water for 15 – 20 minutes to remove the remaining Cytochalasin B. Thereafter, the eggs are returned to filtered sea water and reared the normal way (Beaumont & Fairbrother, 1991).

Beaumont and Fairbrother (1991) were able to produce 83 percent triploid Pacific oysters, *Crassostrea gigas*, by using Cytochalasin B. Maguire *et al.* (1995) produced 76 percent triploids by using Cytochalasin B in the same species. Tabarini (1985) was able to produce 94 percent triploidy in the bay scallop, *Argopecten irradians,* by treating newly fertilised eggs with 0.1 mg/L Cytocalasin B. Maldano-Amparo *et al*. (2003) had a success of triploid induction in scallops, *Nodipecten subnodusus,* of 87 percent and 95 percent with Cytocalasin B concentrations of 0.75 and 1.0 mg/L respectively. Li and Li (2004) used Cytochalasin B to produce 61 percent triploidy in the abalone, *Haliotis laevigata* by treating the fertilised eggs with 0.5mg/L of CB for 15 minutes. Stepto and Cook (1998) were able to produce 70.9 percent triploidy by blocking polar body II and 48.4 percent triploidy by blocking polar body I in *Haliotis midae*, using Cytochalasin B at a concentration of 0.5 mg/l.

The chemical 6-dimethylaminopurine (6-DMAP) has also been used by Norris and Preston (2003) to induce triploidy in the abalone, *Haliotis asinina,* at 90 to 96 percent levels of triploidy. Vadopalas and Davis (2004) were able to induce 92 percent triploidy with survival rates of 30 percent in geoduck clams, *Panopea abrupta,* by using 6-DMAP.

In short, chemical induction of triploidy is successful, but not 100 percent successful.

1.6.2 Thermal treatment

Effective heat shock temperatures range from 25°C to 38°C and cold shocks from 0°C to 5°C, although the actual differential between normal and thermal shock temperature is probably of more importance than the temperature ranges (Beaumont & Fairbrother, 1991).

Johnson *et al.* (2004) used heat-shock to induce 94 percent triploidy in Chinook salmon. They submerged fertilised eggs at 25 minutes after fertilization in a uniformly heated and aerated water bath for 10 minutes at 29.0 ± 1.0^{0}C, followed by 30 minutes of air-cooling at a mean air temperatures of 8.4 ± 0.3°C.

This is not a widely used method in molluscs.

1.6.3 Hydrostatic pressure treatment

Hydrostatic pressure treatment has been successfully applied in finfish and abalone species. Johnson *et al.* (2004) used hydrostatic pressure shock to induce 96 percent triploidy in Chinook salmon. They applied hydrostatic pressure for 5.0 min at 6.89 x 10^4 kPa (10,000 psi) of pressure, at 30 minutes after fertilization. Kerby *et al.* (2002) induced triploidy in Sunshine bass by employing hydrostatic pressure shock treatments to ova at 4.0- to 5.0-min intervals after fertilization at a pressure of 8000 psi for 1.5 to 2.0 min.

The use of hydrostatic pressure treatment in molluscan species is limited and only a small number of references could be found. Chaiton and Allen (1985) and Allen *et al*. (1986) have produced triploid oysters, *Crassostrea gigas,* at a range of 10 to 60 percent using 6000 to 8000 psi pressure shock treatment of 10 minute duration, applied 10 minutes after fertilization. The best reported yields of up to 60 percent were achieved by applying a shock of 7200 psi at 29°C. Arai *et al*. (1986) ran experiments to find the optimum induction procedure for the abalone, *Haliotis discus hannai* using pressure treatment. They found that the inhibition of polar body I could be achieved by starting the pressure treatment 7 minutes after fertilization and the formation of polar body II could be inhibited when pressure was applied 22 minutes after fertilization. They used a pressure of 200kg/cm^2 and the duration of the treatment was 5 minutes. Triploidy up to levels of 60 percent was achieved.

De Beer (2004) applied hydrostatic pressure treatment to induce triploidy in the abalone, *Haliotis midae,* through the inhibition of polar body II. She recommended the application of

25 to 35 MPa for 7 to 10 minutes, roughly 23 minutes after fertilization. This protocol yielded 95 to 100 percent triploidy in *Haliotis midae.*

1.6.4 The use of tetraploid brood stock

In his review of triploidy in oyster farming, Nell (2002) confirmed that 100 percent triploid populations displaying uniformity in condition and complete sterility was only possible through the crossings of diploid and tetraploid brood stock. Neither physical nor chemical methods can guarantee 100 percent triploidy.

There are also the effects of the shock process to consider. For triploid yellow perch, Malison *et al*. (1992) examined the effects of heat and pressure shocks on their internal development and found, through a series of comparative growth experiments that the shock process itself had the effect of slowing down growth, whether triploidy was attained through the process or not. Diploids that received the same shock treatment as triploids, performed similarly to the triploids, relative to growth. Both of these groups subsequently performed worse than untreated diploids. In light of these findings, Lutz (2001) recommended the use of tetraploid brood stock in comparison to chemical, heat or pressure treatment to attain triploidy.

However, the only successful production of tetraploid brood stock oysters involves the blocking of polar body I in eggs from triploids, fertilized by sperm from diploids (Guo & Allan, 1994). The use of physical or chemical induction can thus not be discarded, especially in new species where tetraploids are not available. Also, tetraploidism is not viable in all species, e.g. Sydney rock oysters, where tetraploid larvae do not survive up to the settlement stage (Nell *et al*., 1998).

2 Materials and Methods

2.1 Spawning and Fertilization

Abalone brood stock of the species *Haliotis midae* were induced to spawn at the I&J Abalone hatchery at Danger Point, South Africa (Plate 2.1). Three progeny groups were produced from three spawning sessions, three to four months apart. For each experimental spawning, a unique group of brood stock were selected from the commercial brood stock population at the hatchery. The hatchery brood stock tanks contained five permanently residing brood stock each. Three tanks of females and two tanks of males – a total of 15 females and 10 males - were randomly selected during each spawning event. The respective groups of male and female abalone brood stock were then induced to spawn in mass according to standard hatchery procedures, in a manner similar to that described by Liu *et al*. (2004). Various other techniques for the artificial spawning of abalone are described by Fallu (1991). In all three induced spawnings, the abalone brood stock produced gametes in abundance.

After completion of spawning, the unfertilized eggs were collected from each of the three female groups by siphoning the eggs from the bottom of the spawning bins into a one litre glass container and then allowed to settle down. Two hundred ml of the concentrated eggs were then siphoned from the bottom of the container and filtered through a 400µm sieve to retain any debris whilst the eggs were captured in a 100µm mesh sieve which was partially immersed in 17.0^{o}C seawater. Twenty five ml of sperm was then added to the eggs contained in the 100µm sieve (Plate 2.2 and Plate 2.3). The eggs and sperm were kept together according to standard fertilization procedures to allow for fertilization to occur.

Due to the limited volumetric capacity of the pressure induction equipment of 150 ml, three to four fertilizations and subsequent triploidy treatments had to be conducted during each spawning event.

2.2 Triploid Induction

2.2.1 The Pressure Treatment Apparatus

The pressure apparatus consisted of a stainless steel cylinder with a volume of 150 ml. A headpiece with a valve and pressure gauge fitted tightly into the cylinder, with a rubber o-ring that sealed the junction. The cylinder with its headpiece, together with a hydraulic jack, was fitted into a steel frame so that the top of the headpiece and bottom of the jack were supported by the frame, clamping the cylinder between them (Plate 2.4 and Plate 2.5). Pressure in the cylinder was increased by increasing the hydraulic jack pressure, and could instantaneously be released by releasing the pressure valve of the jack.

2.2.2 Pressure Treatment

For each treatment a standardized random sample of unfertilized eggs were collected from each of the three female spawning bins and combined. Sperm was collected in a similar fashion from each of the two male spawning bins. This was done to ensure that all of the participating animals, males and females, contributed equally to all the fertilizations.

After fertilization the zygotes were collected and poured into the pressure apparatus. A nylon hammer was used to gently tap the sides of the cylinder to remove trapped air bubbles in order to prevent super-saturation and the consequent formation of intra-cellular air bubbles on termination of the pressure treatment. The headpiece was positioned onto the cylinder and placed into the frame. Pressure was applied to the zygotes according to the recommendations by De Beer (2004).

The fertilized eggs were then transferred to a hatching bin. The eggs hatched after approximately 14 hours and trochophore larvae emerged, swimming upwards in columns. Twenty hours after fertilization the trochophore larvae were transferred to rearing bins, with the water temperature remaining at 17.5^{0}C. The larvae stayed in the rearing bins for five more days at which point they were ready to settle onto a substrate.

2.3 Settlement, larval rearing and growth of juveniles

2.3.1 Preparation of settlement bags

Larvae were transferred to prepared settlement bags that provided the required substrate for settlement, five days after fertilization (Plate 2.6). They were kept in the settlement bags for a further two months. The settlement bags were prepared, starting two weeks earlier, by filling them with filtered, UV treated seawater, and seeding it with diatoms used for settlement induction whilst nutrients were added for diatom growth. The settlement bags were made of clear plastic with a volume of 35 litres per bag. The bags were suspended from a 1.8 meter high horizontal ladder and were supplied with air and water lines. Three to five days before settlement waterlines were opened, providing inflowing water which was not filtered or UV treated but was heated to 17.5^0C.

2.3.2 Settlement

At the age of 5 days abalone larvae normally showed signs of initiating settlement, at which stage they were transferred to the settlement bags to settle on diatoms as a food source (Plate 2.7). Before being transferred to the bags, five samples were taken to determine the larval concentration. The larvae were then poured into the settlement bags at appropriate larval densities, allowing them to settle. During their stay in the bags the water temperature was kept at 17.5^0C. The diatom source in the settlement bags tended to become depleted after about two months, at which time they were removed from the settlement bags and transferred to weaning trays where additional diatoms could be provided as well as an artificial diet (Plate 2.8).

2.3.3 Transfer to trays and weaning onto artificial diet

New weaning trays were prepared one week before transfer. They were filled with ambient water and diatoms were seeded into the trays. After transfer, juveniles consumed the diatoms and were weaned to a commercial abalone feed, i.e. Abfeed (supplied by Marifeed Pty Ltd), visible in Plate 2.8, over a couple of days in accordance with normal farm management procedures.

The triploid juveniles showed a higher mortality rate during the post-settlement stages. Their numbers were thus far less than that of the diploids, causing the effective stocking

densities of the triploids to be lower than the diploids, not only in the bags, but also in the initial weaning stages as it was difficult to standardize stocking density in the bins. This could have created an initial advantage for the surviving triploids in that they had better access to feed, causing them to grow faster on average than the diploids. They were therefore larger than the diploids when tagged at an age of seven to eight months. No quantitative data were collected on the post settlement survival of juveniles.

2.3.4 Tagging and transfer to bins

At about eight months of age the abalone were large enough to be tagged, with the smallest animals in the group at the size of 5.0 mm. The diploids were tagged with yellow and the triploids with blue tags (Plate 2.11).

Because of the general lower numbers of triploids in the spawned Blocks, they were tagged and counted first to determine the amount of triploid animals. They were subsequently divided into three bins. Equal numbers of diploids were then randomly selected and added to the triploid animals in each bin, to complete the 3 replicates per *Block*. A Block therefore consisted of 3 replicates of equal numbers of triploid and diploid animals that originated from the spawning of the same brood stock.

One of the mistakes made in this trial was using similar colours to tag all three spawnings (Blocks): blue for all of the triploids and yellow for all of the diploids. It would have been more practical to tag the diploids and triploids of each Block with unique colours – thus utilizing six different colours. The benefit of that would have been that where the juveniles escaped, there would have been no doubt as to which Block the animal belonged to, and all of the Blocks could have been kept in close proximity. As the case was, however, the three Blocks had to be kept separate in order to know where escapees belonged. This arrangement incurred practical difficulty and extra management efforts.

2.3.5 Tagging procedure

A solution of magnesium sulphate was used as an anaesthetic to relax the muscular feet of the juvenile abalone in order to handle them during tagging. The juveniles were tagged in batches of about 200 animals at a time to keep the period out of the water to less than one hour.

The magnesium sulphate solution was added to the bin in which the abalone was kept and after ten minutes the abalone could be removed with ease and placed on a wet sponge sheet with their shells facing upwards (Plate 2.11). A paper towel was used to dab excess water from the shells. The use of an air conditioner provided mild airflow that facilitated shell drying and helped to keep the animals cool. Elevated body temperature was established as the main cause of mortalities during the initial tagging attempts, rather than the time out of the water. Shepherd and Hearn (1983), as quoted by Day and Fleming, 1992, also found that stress due to elevated temperatures during tagging could have adverse affects on abalone growth if not lethal.

When the shells were dry, a small droplet of Super Glue^{TM} was placed on the abalone shell just underneath the whorl, away from the shell growth edge and breathing pores. A colour coded tag was then placed onto the drop of glue and left to dry for the remainder of the allowed one hour handling period. All the abalone were then carefully placed into their new bins.

2.4 Experimental Layout

2.4.1 Random Block Design

The experimental layout was based on a Random Block Design. The procedures of spawning and fertilization were explained in Section 2.1. Each spawning event was used to establish a Block. Three spawnings were conducted on three-monthly intervals through which a total of three Blocks (A, B and C) were generated to make up the experimental material. The three Blocks were generated three and four months apart to provide information on the comparative growth performance of triploids over different seasons and thus differing environmental stress regimes.

Each Block contained two treatment groups namely diploids and triploids. Before tagging, the triploids were raised separately from the diploids. At the age of approximately seven months the abalone were tagged and each Treatment group were randomly divided into three Repeats. A minimum of 300 animals per treatment were required for each repeat at the onset of the comparative growth phase, but there were more available in every case, so that 350 animals per repeat could be placed. A summary of the experimental design is presented in Table 2.1.

Table 2.1 The standard experimental layout (random Block design) that was used as a basis for the comparison of growth rates and survival of diploid and triploid abalone, *Haliotis midae* (n = number of animals/treatment at start of trial).

Blocks (A, B and C)	Repeat 1	Repeat 2	Repeat 3
Treatment 1: Diploid	n = 350	n = 350	n = 350
Treatment 2: Triploid	n = 350	n = 350	n = 350

2.4.2 Procedures for random sampling and growth measurements

Measurements for individual shell length (mm) and body weight (g) were taken on a total of 30 randomly sampled animals from each treatment in each repeat. The measurements were conducted in a non-destructive manner and were repeated on a monthly basis. A specific procedure was followed to ensure that random sampling was attained throughout the trial.

The assumption was made that animals were randomly distributed throughout the housing units containing the habitats provided for shelter. Sampling therefore started with a random selection of habitats from the respective housing units during initial parts of the trial and baskets during the latter growth stages (Plate 2.10). All of the animals obtained from a particular habitat or panel were anaesthetized and spread out evenly onto an oyster-net covered frame that allowed excess water and slime to drip off. The frame was divided into 12 numbered squares of 10cm x 10cm. The squares to be sampled were randomly selected. Starting with the first square, all the triploids and then all the diploids were measured. A second and third square was then selected until a total of 30 triploids and 30 diploids were recorded. Measurement of length was taken with a Vernier Calliper while weight measurements were taken on a 200g x 0.01g calibrated electronic scale.

2.4.3 Housing systems

Two types of housing systems were used during the two year trial period.

2.4.3.1 Trays

A tray system was used for the housing of the animals during the first four months of measuring. Each repeat was placed in a separate tray with its own water and air supply. Each tray contained six specific habitats, in the shape of cones, under which the abalone could find shelter. The habitats were labelled A to F. At each measurement a particular habitat was drawn at random, with the animals attached to it and underneath it on the tray surface included into the draw (Plate 2.9).

2.4.3.2 Baskets

After an initial period of four months in the trays the animals were transferred to a basket holding systems. This was done to improve the ease of maintenance and feeding and to reduce handling stress, stocking densities and competition amongst animals. At the stage of transfer from the trays to the baskets each repeat (tray) were split randomly into two baskets. This was done by anaesthetizing and weighing the entire group, after which the animals were randomly divided into two groups of equal weight and placed into separate baskets. This procedure implied that a particular repeat was split into two identifiable baskets of equal

biomass, though the number of diploids and triploids in a particular basket may not have been equal.

Each basket contained a corrugated sheet grid that provided four panels as substrates, with a cover sheet on top (Plate 2.10). At each measurement a particular panel was chosen at random, with all associated animals, from which a random sample of 30 animals was selected for measurements.

2.5 Analysis of Ploidy Status

It was considered important to test and verify the ploidy status of the treatment groups at various stages throughout the trial period, because some studies have shown the reversion of triploid tissue cells to the diploid state or various other aneuploid chromosome structures. Triploidy was first tested through the collection of larval samples at two days of age and tissue samples at a later stage in the trial period, when juveniles were about 20 mm in length.

2.5.1 Collection, preservation and preparation of tissue samples

2.5.1.1 Sampling of Larvae for initial triploidy testing

Initial samples of larvae to be tested for triploidy were taken 48 hours after spawning. A 100µm sieve was used to scoop abalone larvae from the water column of the larval rearing bin. The collected larvae were preserved according to the protocol specified by De Beer (2004). The larvae were washed off the sieve into a glass beaker with the use of UV sterilized water. Under a laminar flow, the larvae were poured from the glass beaker through a piece of 100µm sieve folded into a cone shape. The larvae concentrated in a small spot on the sieve, making it easier to wash into a Falcon tube. Ten to twenty ml of KCl was used to wash the larvae into the Falcon tube, where it was left for ten minutes. When the ten minutes expired, the larvae were again passed through a 100µm sieve, in the same fashion as before, to drain the KCl. A sterile cryovial was then opened and used to scoop up the larvae from the sieve. One and a half ml Vindelov citrate buffer and 150 µl DMSO were then added and the larvae sample frozen at -20^{o}C.

Before the samples could be run through a flow cytometer it had to be prepared in such a way that the nuclei were separated from one another and stained. This was done in accordance with the procedure described by De Beer (2004). Samples were taken from the freezer and thawed at room temperature. Fifty microlitres of concentrated larvae were extracted from the cryovial and put into an Eppendorf tube. Fifty microlitres Marine phosphate saline buffer (MPBS) and 500 µl Propidium Iodide (PI) staining solution were added to the larvae. The suspension was then aspirated through a 1 ml syringe fitted with a 26½ G needle until clogging at the tip of the needle stopped. The cell suspension was filtered twice through a 25 µm nytex screen and centrifuged for five minutes at 5000 rpm.

The supernatant could then be removed and the pellet was resuspended in 500 µl MPBS in a plastic Eppendorf tube. The tube was wrapped in foil and kept on ice until it was processed through the flow cytometer.

2.5.1.2 Sampling of epipodia tissue for verification of triploidy

Tissue samples were also taken from experimental animals at the age of two years in order to reconfirm their ploidy status. Tissue samples were obtained from 15 animals from the triploid group and two animals from the diploid group and processed in accordance with the method prescribed by Yang *et al.* (2000). Epipodial tissue was used instead of the prescribed gill tissue due to the fact that non-destructive sampling was required. The epipodia was cut using sterile equipment and placed in 0.075 M KCl for 10 minutes. It was then cut into smaller pieces, and placed in 75 percent ethanol. The ethanol was changed once.

The samples were also prepared for flow cytometry to ensure that the nuclei were stained and separated from one another, according to a procedure described by De Beer (2006)

Samples were washed in MPBS once and transferred to PI staining solution with 10 percent DMSO (MPBS + 0.1% Triton X100 + 0.2mg/ml RNAse A + 0.02 mg/ml PI). They were then frozen at -80^{o}C and thawed again, after which it was vortexed. The resulting fluid was then aspirated five times through a 25G needle and filtered through a 25 micron screen. The sample could now be processed through the flow cytometer.

2.5.2 Fluorescence microscopy

A fluorescence microscope was used to assess whether the samples were prepared properly. Each sample was viewed under a 20 x magnification and screened for clumps of nuclei. The presence of numerous clumps of nuclei was an indication that the sample was not prepared correctly and would not give adequate results when processed through the flow cytometer. The evaluation of the samples confirmed its suitability for processing through flow cytometry, with relatively few clumps of nuclei being observed.

2.5.3 Flow cytometry

The prepared samples were processed with a Becton Dickinson FACSCalibur flow cytometer with a He/Ne laser of wavelength 488nm. The instrument was a bench-top type with a cuvette flow-cell, where the cells were delivered singularly to a specific point at which the

laser was focused (De Beer, 2004). Samples were vortexed for 1 minute before it was transferred to sterile 5ml Becton Dickinson flow cytometry tubes. For each sample 50 000 nuclei were acquired at a rate of 100 – 250 nuclei per second to generate a histogram. The instrument settings used were those specified by de Beer (2004) and is presented in Table 2.2.

Table 2.2 Instrument settings for the Becton Dickinson Flow Cytometer, for the use of the CellQuest ProTM programme to analyze the levels of ploidy in larval cells of the abalone, *H. midae* (De Beer, 2004). P = Photomultiplier detectors (P1: Forward angle light scatter; P2: Right angle light scatter; P4: Fluorescent label 2 PI-DNA; P6: Fluorescent label 2 Area; P7: Fluorescent label 2 Width).

	Volt	**Amp**	**Mode**
P1	E00	3.00	lin
P2	500	1.00	lin
P3	320	1.00	log
P4	490	1.00	lin
P5	380	1.00	log
P6	-	1.00	lin
P7	-	3.94	lin
Threshold		20	
Compensation		0	

These settings gave adequate histograms in as far as that triploid and diploid samples could be easily distinguished. The thresholds were set to ignore debris and minimize electronic noise, where DNA fluorescence was used as the threshold parameter (De Beer, 2004).

Modfit LT (Verity Software House, Topsham, ME, USA) and WinMDI Version 2.1.3 (Copyright 1993 – 1996, Joseph Trotter) software programs were used to analyze the raw data generated by the flow cytometer and to compile histograms. The histograms showed peaks where most of the nuclei fluoresced, this being the point used to determine whether the samples were diploid or triploid. The peak position was measured by channel numbers on the horizontal axis and reflected the relative DNA content per nucleus, while the number of nuclei recorded was shown on the vertical axis (Yang *et al.,* 2000). The area under the curve represented the relative contribution of a certain ploidy (Allen, 1983).

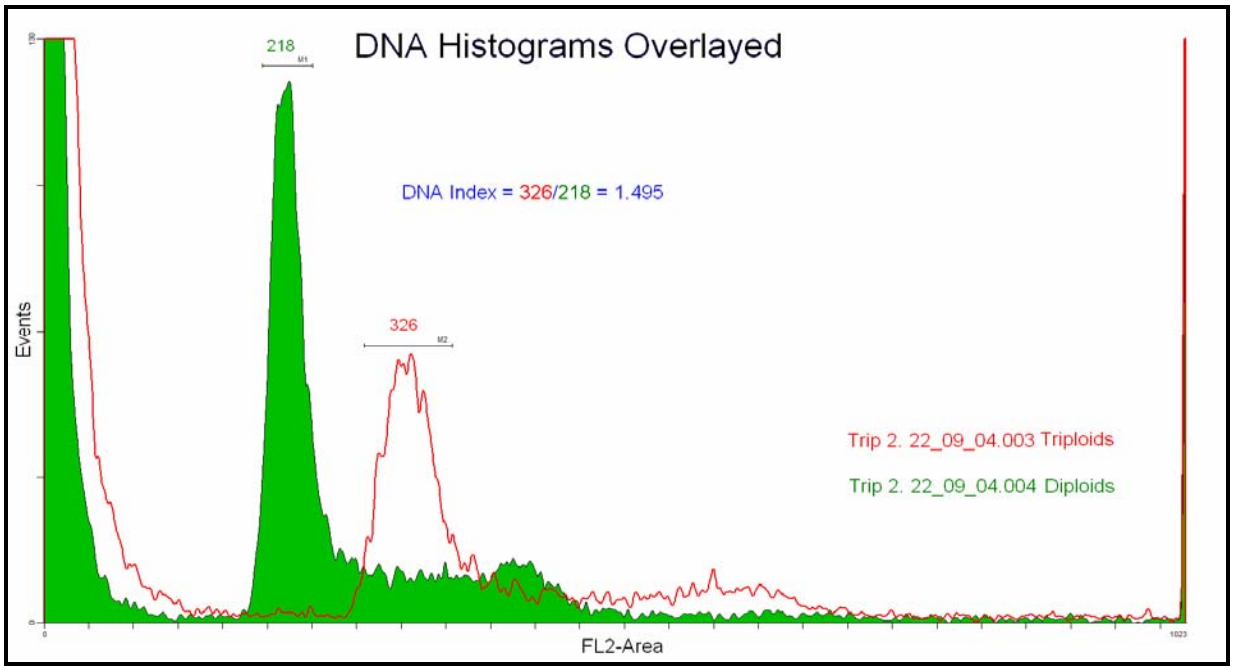

Figure 2.1 Flow cytometry histograms comparing a Triploid and Diploid population of nuclei detected by the FL2-A fluorescent density detector.

2.6 Validation of triploidy status in latter growth stages

In a quest to test triploidy as a means of reliable mass sterilization for the purpose of biological containment of *Crassostrea gigas* (Pacific oysters), Allen and Guo (1996) unexpectedly generated data indicating heteroploid mosaics i.e. individuals containing both diploid and triploid cells, in two populations of "certified triploids". The triploid populations were created using tetraploid and diploid brood stock, which was believed to generate 100% triploid offspring. The authors attributed the occurrence of heteroploid mosaics to the reversion of the triploids to a mosaic state, through chromosome set loss. Contrary to these findings, Elliot *et al.* (2004) found no reversion from triploid to diploid status when re-sampling triploid *Haliotis laevigata* at 32 months of age. It was thus decided to test whether an indication of reversion to the heteroploid state existed amongst the 100% certified triploids used in this trial and to what extent pure triploidy was still present in the experimental triploids at an older age.

In addition to the larval testing, flow cytometry could also be used as a quick and reliable method to determine and validate the ploidy levels of older abalone in a non-destructive manner through tissue samples (De Beer, 2004). As long as the samples were prepared in the

correct manner and the nuclei containing the DNA were intact, though separated from one another, the readings of the flow cytometer provided reliable indications of the ploidy status of either larvae populations or individual animals. Sample preparation techniques that were followed are presented in Section 2.5.1.

Fifteen individuals were sampled from the triploid group and tested for ploidy at 26 months of age, as presented in Figure 2.2. Animals from the diploid group were used as a control, i.e. to calibrate the readings. De Beer *et al*. (2006) reported a 93 percent level of aneuploidy amongst animals from the triploid group, i.e. DNA indices significantly above that of diploid. A total of 67 percent displayed DNA indices as for triploid or higher and a further 26.3 percent displayed DNA indices between that of diploid and triploids. A total of 7 percent (one individual) of the sampled animals displayed a loss of aneuploidy that is an indication of a reversal to the diploid state, in comparison to the 100 percent triploidy assessment during the larval stage (De Beer *et al*., 2006). The animals classified as aneuploids, i.e. 93 percent of the triploid groups, are therefore expected to be functionally sterile.

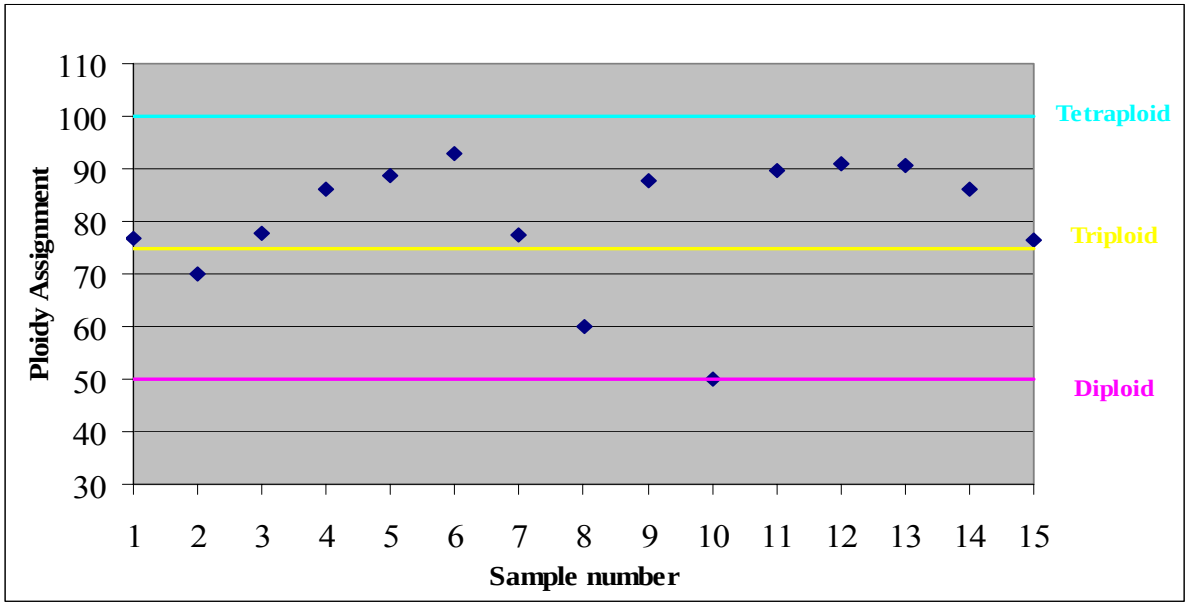

Figure 2.2 **Comparison of the ploidy levels (DNA indices) of "triploid" and diploid abalone from Block A of growth trials (De Beer *et al*., 2006).**

2.7 Plates: Illustration of triploidy induction procedures

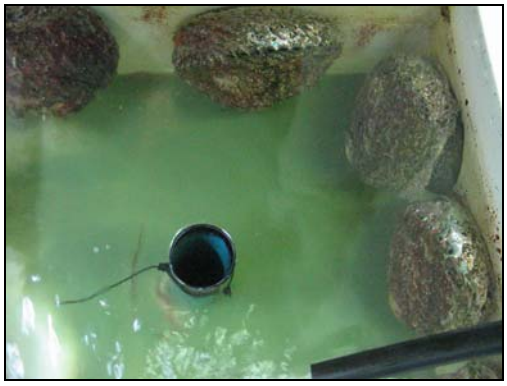

Plate 2.1 Spawning of female abalone in holding tank.

Plate 2.2 Preparation of eggs (left) and sperm (right) for fertilization and induction of triploidy.

Plate 2.3 Fertilized eggs in a water bath prior to induction.

Plate 2.4 Hydrostatic pressure induction apparatus.

Plate 2.5 Close-up view of head piece inserted into steel cylinder that contains the larvae

2.8 Plates of abalone development and tagging

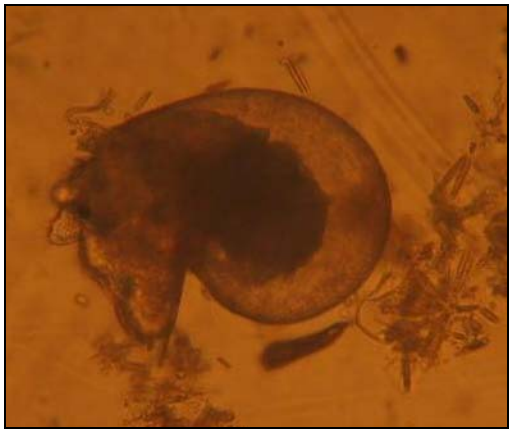

Plate 2.6 a 6 day old larva**.**

Plate 2.9 Five month old juveniles (±10mm shell length) underneath a habitat unit in a weaning tray.

Plate 2.7 Settlement bags containing settled juveniles.

Plate 2.10 Oyster net baskets in which the juvenile abalone were housed after being tagged.

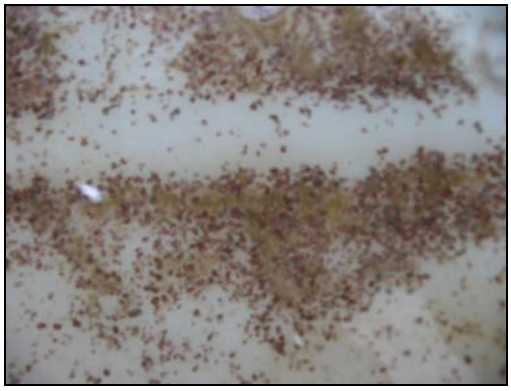

Plate 2.8 2 month old juveniles moved from the settlement bags into weaning trays.

Plate 2.11: Tagged 8 month-old juveniles, with a population average of about 16mm in shell length. Note the size variation of the siblings.

3 Results and Discussion

The main objective of the study was to assess whether there was a difference in the growth rate of diploid and triploid genotypes of the abalone, *Haliotis midae*, over an initial growth period of 24 months, prior to the onset of maturation. Secondary objectives were to investigate the effect of seasons on the growth rate of the species and the two genotypes in particular, and to establish the relationships between growth traits over time.

3.1 Data analysis

Growth data, in the form of individual body weight (weight) and shell length (length) was collected on a monthly basis, over a period of 15 months, starting at approximately eight months of age. The experimental layout was that of a Random Block Design, consisting of three Blocks, each Block containing three Repeats of two Treatments, namely diploid and triploid (see Chapter 2 for further detail). The Blocks, each related to a Season, were based on separate spawnings in three consecutive seasons, namely summer, winter and spring.

The data was then analyzed in a progressive manner to:

a. Assess the effect of season on the average growth performance, ignoring ploidy, by means of a covariate analysis (Section 3.2).
b. Assess the effect of ploidy on the average growth performance, ignoring seasons, by means of a covariate analysis (Section 3.3).
c. Compare the growth performance of diploid and triploid groups, over Blocks (Section 3.3.2.1 and 3.3.3.1) and between Blocks (Sections 3.3.2.2 and 3.3.3.2), by means of regression analyses for length and weight on age.
d. Describe the weight-length relationship for triploid and diploid groups (Section 3.4).

3.2 Assessment of the effect of Season on Average Growth Performance

Growth rates of abalone during the winter months are known to be slower due to the lower, but relatively constant water temperatures. During the summer months, water temperatures fluctuate more, but are higher on average. Early summer is normally also the natural spawning season for the abalone, and all of this results in more sporadic, but higher average growth rates during summer months.

The effect of season on the average growth performance of diploid and triploid abalone was assessed by means of a covariance analysis with age as covariate. Blocks A, B and C were spawned in late summer, winter and spring respectively. Because of the delay between spawnings, there may also have been a managerial influence on the respective Blocks. As an objective was to assess the effects that spawning season may have had on the growth performance of the abalone in general and in particular on that of diploids or triploids comparatively, two covariance analyses with age as covariate were conducted to assess the differences in the growth rate (length and weight) of triploid and diploid abalone; first between Blocks (seasons) adjusted for age, and then over Blocks adjusted for age.

For the first assessment (Section 3.2.1) i.e. the general effects of spawning season on growth rates, the data of the triploids and diploids were pooled for each Block and the length and weight were corrected for age. This resulted in a mean for length and a mean for weight for each of the Blocks, as presented in Tables 3.2 and 3.4. These means reflect the general growth performance of the abalone in each Block, regardless of the genetic status, allowing a broad assessment of growth differences between Blocks or seasons.

3.2.1 Assessment of the effect of Season on the Adjusted Mean Length of Blocks, ignoring ploidy

A covariate analysis with age as covariate was performed on the data to compare the mean length of the Blocks over seasons, ignoring ploidy. A difference between these age adjusted means would indicate a seasonal (or Block management) effect on the length gain of abalone.

Table 3.1 Results of the covariance analysis with age as a covariate for length-wise seasonal growth differences where triploid and diploid abalone were pooled within Blocks.

Source (Model)	df	Mean square	P
Age (Regression)	1	7251.11	<0.0001
Block	2	65.32	<0.0001
Ploidy	1	93.99	<0.0001
Error	198	1.65	
Uncorrected total	202		

Table 3.2 The adjusted mean length of abalone (*H. midae)* in Blocks A, B and C with the results of a pair-wise t-test to indicate the similarity of the mean length (mm). (For p-values > 0.05, the means were considered as similar and for p < 0.05 the means were considered as statistically different from each other.)

Group	Adjusted Mean Length	T-tests
Block A	21.27	a
Block B	22.38	b
Block C	22.47	b

	Block B	Block C	
Block A	-5.133	-5.323	t-value
	<0.0001	<0.0001	p-value
Block B		-0.362	t-value
		0.718	p-value

The results of the pair-wise T-test and the related p-values as presented in Table 3.2 indicate a similarity in the adjusted mean length of abalone in Blocks B and C, but a significant difference from the adjusted mean length of Block A. It can therefore be concluded that seasons do have a significant effect on the consequent growth of abalone in the form of average length.

3.2.2 Assessment of the effect of Season on the Adjusted Mean Weight of Blocks, ignoring ploidy

The age adjusted mean weight of all three Blocks was compared by means of a covariate analysis with age as covariate. Differences between the age adjusted means would be an indication of seasonal effects.

Table 3.3 Results of the covariance analysis with age as a covariate for weight-wise seasonal growth differences where triploid and diploid abalone were pooled within Blocks.

Source (Model)	df	Mean square	P
Age	1	726.08	<0.0001
Block	2	4.79	0.0073
Ploidy	1	6.68	0.0006
Error	198	0.46	
Uncorrected total	202		

LSD values were consequently calculated as an indicator of the statistical significance of the observed differences between adjusted diploid and triploid mean Block weights.

Table 3.4 The adjusted mean weight of abalone (*H. midae)* in Blocks A, B and C with the results of a pair-wise t-test to indicate the similarity of the mean weight (g).

Group	Adjusted Mean Weight	T-tests
Block A	2.63	a
Block B	2.89	b
Block C	2.98	b

	Block B	Block C	
Block A	-2.305	-2.984	t-value
	0.0222	0.0032	p-value
Block B		-0.730	t-value
		0.4662	p-value

The results of the pair-wise T-test and the related p-values as presented in Table 3.4 also indicate a similarity in the adjusted mean weight of abalone in Blocks B and C, but a significant difference from the adjusted mean weight of Block A. Similarly, it be concluded that seasons do have a significant effect on the consequent growth of abalone in the form of average weight.

The results from Section 3.2.1 and Section 3.2.2 provide evidence of a significant difference in the adjusted average length and weight of Block A compared to that of Blocks B and C, with Block A performing significantly worse than Blocks B and C. In order to explain these observed differences one has to consider all factors that could have contributed, which include:

- The spawning and settlement of abalone of Block A in March 2004 was adversely affected by extraordinary cold sea water conditions. Further to this the hatchery water could not be heated during the consequent weaning process that resulted in slow growth during the first months, although no abnormal mortalities occurred. This delay in growth during the initial stages, prior to the start of the experiment at the age of 8 months, may have had an effect on the group averages towards the end of the experiment.
- Block A was also the first batch to be spawned and reared by a new and inexperienced group of hatchery staff. The lack of experience could also have contributed to a reduced growth performance by this Block due to managerial effects.
- In comparison to Block A, Blocks B and C were spawned under controlled hatchery water temperature conditions. Their respective initial survival and growth rates were consequently superior to that of Block A.

The difference in starting weight of the Blocks was therefore influenced by factors such as a lack of control of hatchery water temperatures and managerial experience that had little to do with the typical seasonal differences between the groups. The starting weight in turn could have had an influence on the subsequent growth rate of the groups during the subsequent experimental periods.

Although significant differences in the growth rate was detected between Blocks, these differences could not be ascribed with confidence to seasonal environmental effects, *visa ve* low though stable ambient water temperatures during winter and high though variable water temperatures during summer.

The “seasonal effects” as observed, although statistically significant, could therefore not necessarily be ascribed to environmental effects such as water temperature, because of the nature and influence of other managerial effects that differed between Blocks and could not be separated from the observed seasonal effects.

3.3 Assessment of the effect of Ploidy on Average Growth Performance

3.3.1 Assessment of the effect of Ploidy on the basis of Adjusted Mean Length and Weight

The age adjusted means, as presented in Table 3.5, were calculated over Blocks, thereby ignoring seasonal effects. This allows for the observed differences in length and weight to be ascribed to the effect of the treatments, i.e. the difference between diploids and triploids.

Table 3.5 The age adjusted mean length and weight of diploid and triploid abalone (*H. midae*), over Blocks.

Parameter	*Treatment*	*Adjusted Mean*	*T Value*	*P Value*
Length	Diploid	21.36 mm	-7.54	<0.001
	Triploid	22.72 mm		
Weight	Diploid	2.65 g	-3.83	0.0002
	Triploid	3.01 g		

The results gave an indication of significant differences between the lengths and weights of the diploid and triploid abalone, when assessed over Blocks. The nature of the differences (1.36 mm in terms of length and 0.37 g in terms of weight), although statistically significant, are considered of lesser significance in economic terms.

3.3.2 Assessment of the effect of Ploidy on the basis of the regressions of Length on Age

3.3.2.1 Regression of Length on Age, for diploids and triploids, over Blocks

The shell length growth of the diploids and triploids over Blocks were assessed by means of a length on age regression analysis. Both linear and quadratic equations were developed to describe the respective relationship between the length of diploids and triploids and age. The best fitting equation was then used in further comparisons between the growth of diploids and triploids.

A linear regression analysis was done first. Figure 3.1 displays the linear regression for diploid and triploid abalone length gain over time.

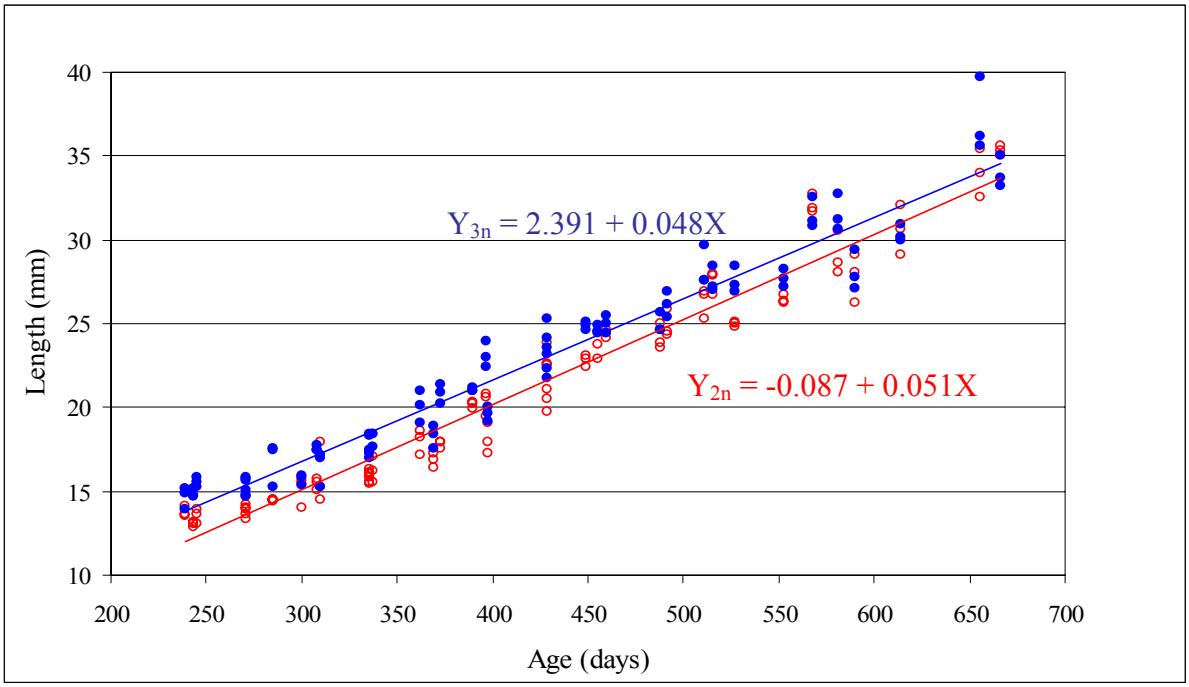

Figure 3.1 The linear regression of shell length on age of diploid and triploid abalone, *H. midae* (Solid dots = triploid, open dots = diploid).

The respective linear regression equations between length (Y) and age (X) were calculated as:

- Diploid: $Y = -0.087 + 0.051(X)$
- Triploid: $Y = 2.391 + 0.048(X)$

with an R^2-value of 0.9963 as indication of a very good fit to the data.

Application of these equations to predict the respective length of abalone at 200 and 650 days provided the following results which reaffirmed the economic insignificance of the difference in length gain between diploids and triploids:

Average length at age 200 days:	Diploid	= 10.11mm
	Triploid	= 11.99mm
Average length at age 650 days:	Diploid	= 33.06mm
	Triploid	= 33.59mm

The quadratic regression for diploid and triploid abalone length gain over time is presented in Figure 3.2.

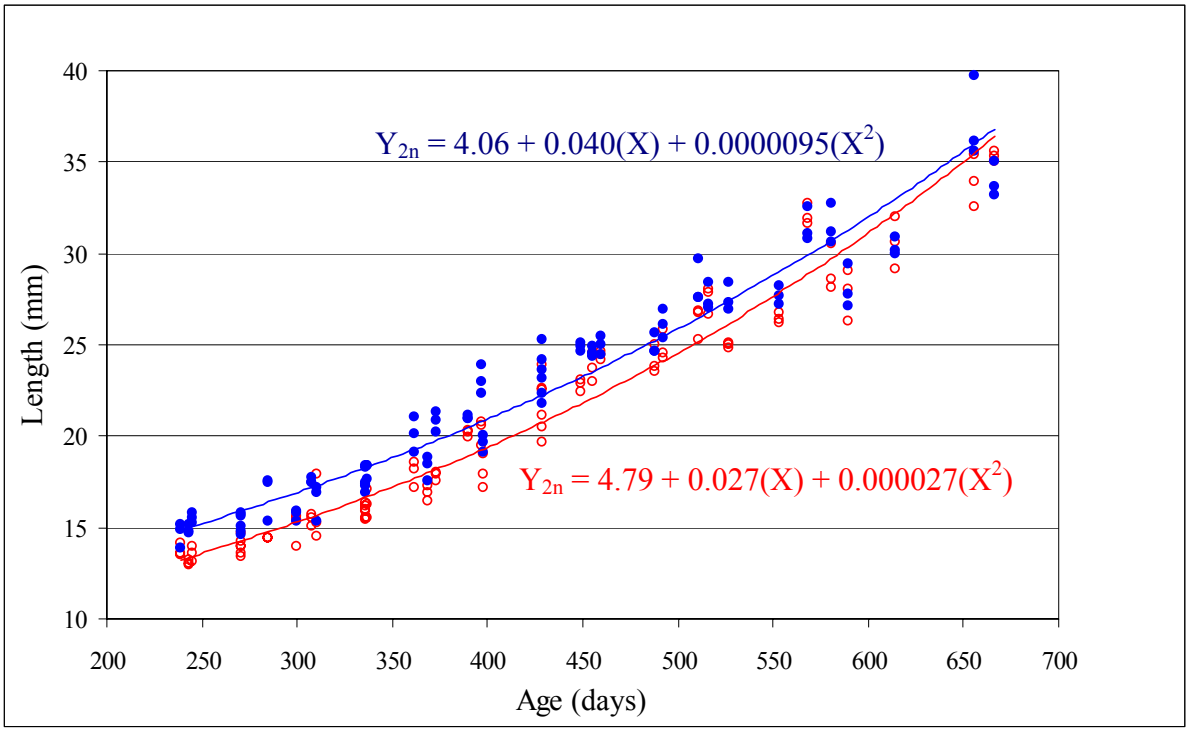

Figure 3.2 The relationship between shell length and age of diploid and triploid abalone, *H. midae,* plotted as a quadratic regression (Solid dots = triploid, open dots = diploid).

The quadratic regression equations between length (Y) and age (X) were calculated as:

- Diploid: $Y = 4.79 + 0.027(X) + 0.000027(X^2)$
- Triploid: $Y = 4.06 + 0.040(X) + 0.0000095(X^2)$

with an R^2-value of 0.9965, also as indication of a very good fit to the data.

Predictions of the length of abalone at 200 and 650 days respectively deliver the following results:

Average length at age 200 days:	Diploid	= 11.27mm
	Triploid	= 12.44mm
Average length at age 650 days:	Diploid	= 33.75mm
	Triploid	= 34.07mm

The respective R^2-values of 0.9963 and 0.9965 indicates that both the linear and quadratic regression provide an acceptable and equally good fit, hence it was decided to use the linear regression for length in all of the length analyses as it is considered easier to use in practice.

Table 3.6 illustrates the analysis of variance for the linear length regressions, followed by an LSD test which indicates differences between diploid and triploid length regressions. The intercepts indicates starting lengths and the slopes, length gain in mm/day.

Table 3.6 Results of the linear ANOVA for the mean length increase of diploid and triploid abalone, *H. midae,* over Blocks.

Source	*df*	*Mean square*	*P*
Regression	4	26 306.6	<0.0001
Diploid Intercept	1	47 251.0	<0.0001
Triploid Intercept	1	50 683.4	<0.0001
Diploid Slope	1	3 976.1	<0.0001
Triploid Slope	1	3 315.6	<0.0001
Error	199	1.93	
Uncorrected total	203		

LSD values were consequently calculated as an indicator of the statistical significance of the observed differences between intercept and slope of diploids and triploids.

Table 3.7 Results of the linear regression for length (mm) on age (day) in diploid and triploid *H. midae*. Estimates and Standard errors of the Regression Coefficients are displayed, as well as the LSD results for differences between diploid and triploid groups. LSD (P≤0.05) for intercept = 1.4; LSD (P≤0.05) for slope = 0.0032

Parameter	*Estimate*	*Standard Error*	*T Value* (H_0:θ=0)	*P Value*	*LSD tests for intercept and slope*
Diploid Intercept	-0.087	0.493	-0.18	0.8601	a
Triploid Intercept	2.40	0.505	4.73	<0.0001	b
Diploid Slope	0.051	0.00112	45.35	<0.0001	a
Triploid Slope	0.048	0.00116	41.41	<0.0001	a

The slope of the diploid group (0.051±0.00112) was higher than that of the triploids (0.048±0.00116), but insignificantly so, as indicated by the LSD (P>0.05) value of 0.0032. It is therefore concluded that there is no significant difference in length gain of diploid and triploid groups over the initial period of 24 months.

However, a significant difference (2.487) was detected between the Y-axis intercepts of the diploid (-0.87±0.493) and triploid groups (2.4±0.505). This is an indication of a better growth rate obtained by the triploids prior to tagging and the onset of the growth trial. A more detailed discussion of these results are presented in Section 3.3.4

3.3.2.2 Regression of Length on Age, for diploids and triploids, within Blocks

Separate analyses were conducted for growth in terms of length gain for each of the three Blocks. Each Block was spawned in a different season that may have had an effect on the subsequent growth rates of the treatment groups.

3.3.2.2.1 Regression of length on age for Block A (summer season)

Figure 3.3 displays the averaged length gain curves of the three pooled repeats of diploids and three pooled repeats of triploids within Block A.

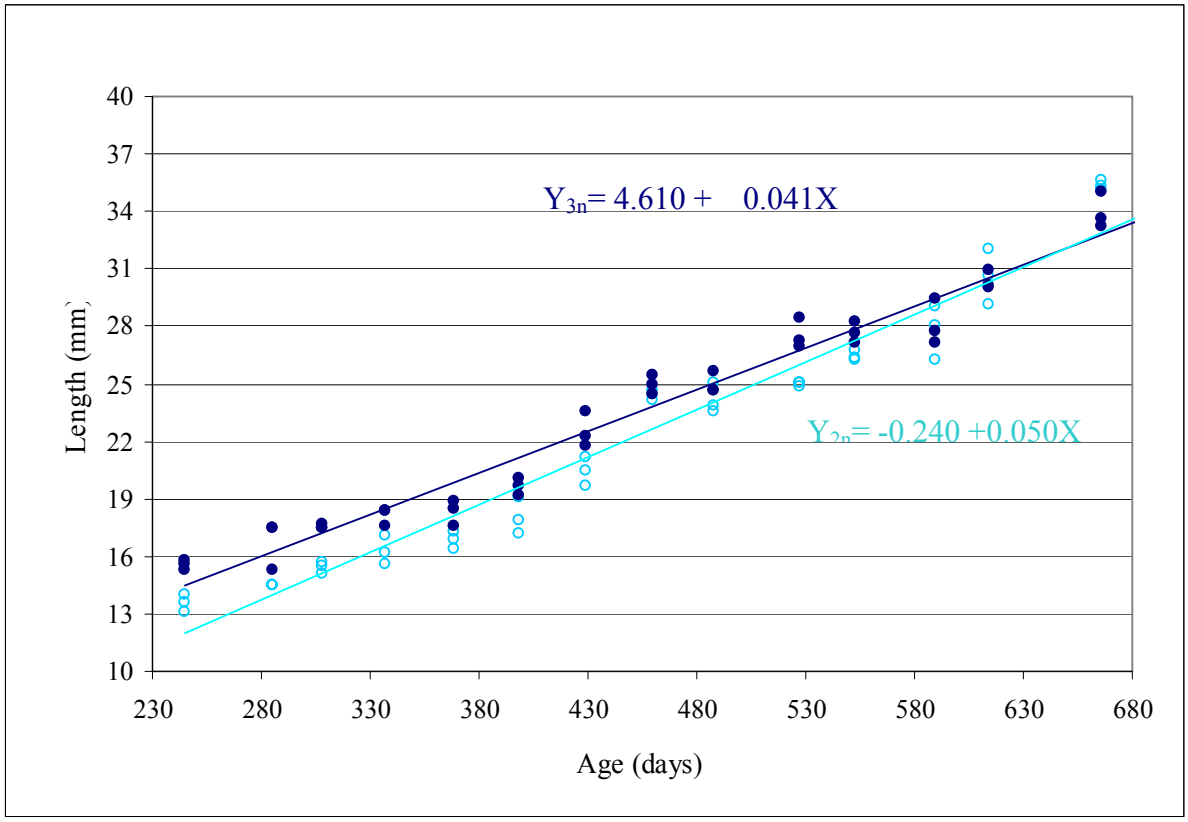

Figure 3.3 The length-wise growth, recorded as mm per day, of the diploid and triploid abalone over two years in Block A (Solid dots = triploid, open dots = diploid).

The following straight line equations were extracted (Y = weight; X = age):

Block A Diploid Y = -0.240 + 0.050X

Block A Triploids Y = 4.610 + 0.041X

where $R^2 = 0.997$ indicated a good fit of the regression equations to the data.

Table 3.8 Results of the ANOVA of the length (mm) over age (days) of diploid and triploid abalone in Block A.

Source	*df*	*Mean square*	*P*
Regression	4	10 752.37	<0.0001
Diploid Intercept	1	20 429.50	<0.0001
Triploid Intercept	1	19 928.70	<0.0001
Diploid Slope	1	1 652.40	<0.0001
Triploid Slope	1	991.86	<0.0001
Error	77	1.52	
Uncorrected total	81		

LSD values were consequently calculated as an indicator of the statistical significance of the observed differences between intercept and slope of diploids and triploids.

Table 3.9 Results of the linear regression for length (mm) in Block A for diploid and triploid abalone. Estimates and Standard Errors of the Regression Coefficients are displayed, as well as the LSD results for differences between diploid and triploid groups. LSD (P≤0.05) for intercept = 2.0; LSD (P≤0.05) for slope = 0.0044

Parameter	*Estimate*	*Standard Error*	*T Value* (H_0:θ=0)	*P Value*	*LSD tests for intercept and slope*
Diploid Intercept	-0.24	0.702	-0.35	0.7286	a
Triploid Intercept	4.61	0.732	6.31	<0.0001	b
Diploid Slope	0.0500	0.00151	33.02	<0.0001	a
Triploid Slope	0.0414	0.00161	25.53	<0.0001	b

The results as presented in Figure 3.3 and Table 3.9 indicate that there were significant differences between the intercepts of diploid and triploid abalone in Block A as well as between the slopes of the regression curves. The differences in slope, i.e. length gain, may be attributed to the significance of the different starting lengths; while the differences in starting lengths could be attributed to stocking density differences before the commencement of measurements (see Section 3.3.4).

3.3.2.2.2 Regression of length on age for Block B (winter season)

Figure 3.4 displays the averaged length wise growth curves of the three pooled repeats of diploids and three pooled repeats of triploids within Block B.

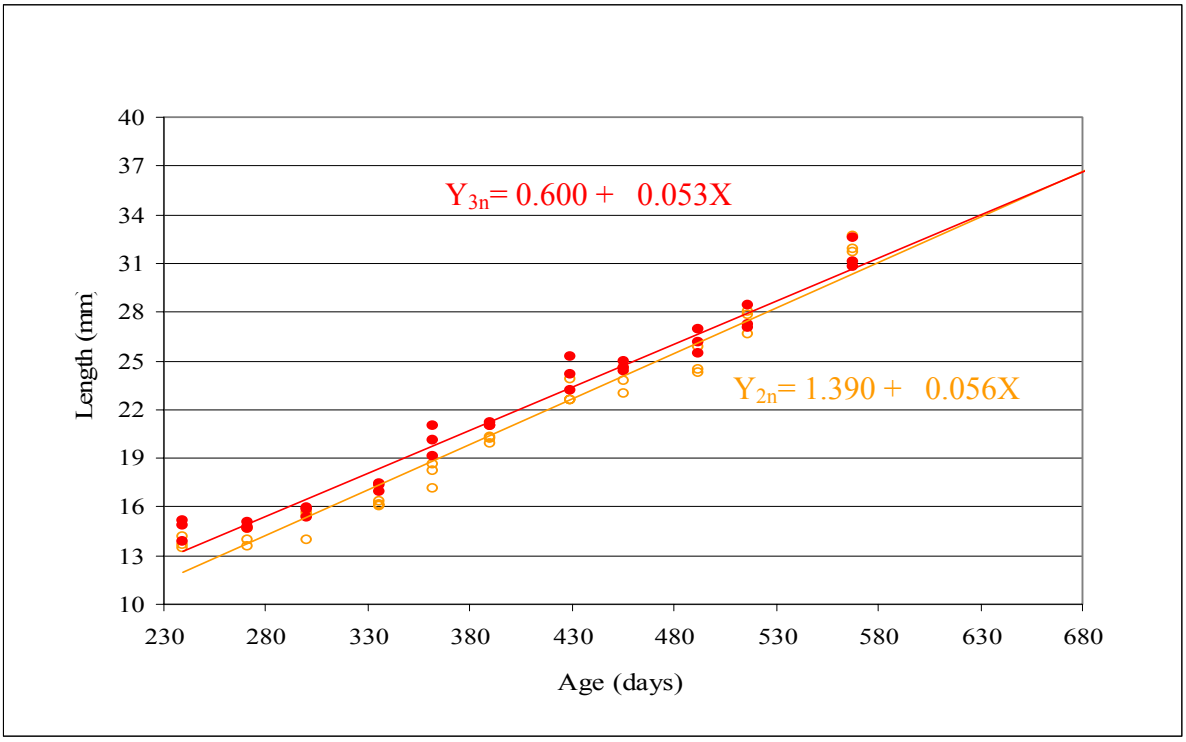

Figure 3.4 The length-wise growth, recorded as mm per day, of the diploid and triploid abalone over two years in Block B (Solid dots = triploid, open dots = diploid).

The following straight line equations were extracted (Y = weight; X = age):

Block B Diploids Y = -1.390 + 0.056X

Block B Triploids Y = 0.600 + 0.053X

where R^2 = 0.998 indicated a good fit of the regression lines to the data.

Table 3.10 Results of the ANOVA of the mean length (mm) increase over age of diploids and triploids of the abalone, *H. midae,* in Block B.

Source		*df*	*Mean square*	*P*
Regression		4	7 896.80	<0.0001
	Diploid Intercept	1	14 213.80	<0.0001
	Triploid Intercept	1	15 380.70	<0.0001
	Diploid Slope	1	1 046.80	<0.0001
	Triploid Slope	1	943.17	<0.0001
Error		62	1.08	
Uncorrected total		66		

LSD values were consequently calculated as an indicator of the statistical significance of the observed differences between intercept and slope of diploids and triploids.

Table 3.11 Results of the linear regression for length (mm) in Block B for diploid and triploid abalone *H. midae*. Estimates and Standard Errors of the Regression Coefficients are displayed, as well as the LSD results for differences between diploid and triploid groups. LSD ($P \leq 0.05$) for intercept = 2.07; LSD ($P \leq 0.05$) for slope = 0.0051.

Parameter	*Estimate*	*Standard Error*	*T Value* (H_0:θ=0)	*P Value*	*LSD tests for intercept and slope*
Diploid Intercept	-1.39	0.733	-1.89	0.0633	a
Triploid Intercept	0.60	0.733	0.82	0.4137	a
Diploid Slope	0.056	0.00179	31.16	<0.0001	a
Triploid Slope	0.053	0.00179	29.54	<0.0001	a

As is reflected in Table 3.11, no significant differences were observed between the intercepts or slopes of the diploid and triploid regression curves in Block B.

3.3.2.2.3 Regression of length on age for Block C (spring season)

Figure 3.5 displays the averaged length wise growth curves of the three pooled repeats of diploid and three pooled repeats of triploid abalone within Block C.

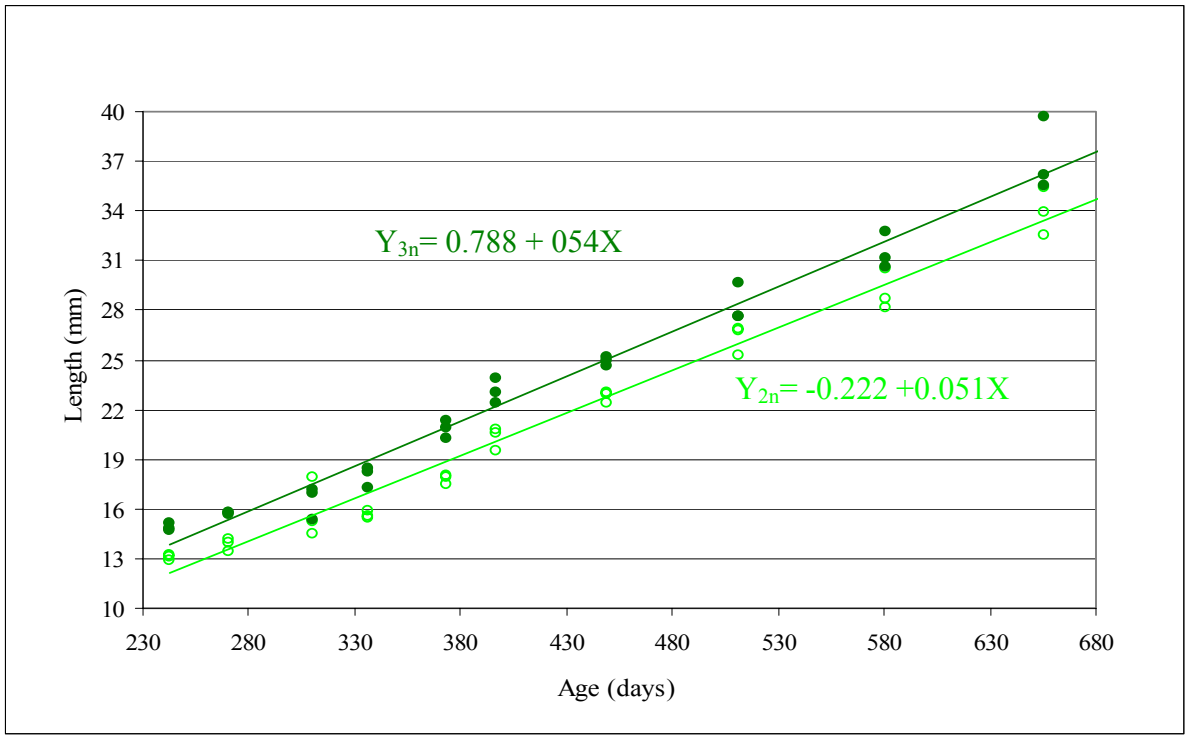

Figure 3.5 The length-wise growth, recorded as mm per day, of the diploid and triploid abalone over two years in Block C (Solid dots = triploid, open dots = diploid).

The following straight line equations were extracted (Y = weight; X = age):

Block C Diploids Y = -0.222 + 0.051X

Block C Triploids Y = 0.788 + 0.054X

Where R^2 = 0.998 indicated a very good fit of the trend lines to the data.

Table 3.12 Results of the ANOVA of the mean length (mm) increase over age of diploid and triploid abalone, *H. midae,* in Block C.

Source	*df*	*Mean square*	*P*
Regression	4	7 692.55	<0.0001
Diploid Intercept	1	12 640.12	<0.0001
Triploid Intercept	1	15 428.44	<0.0001
Diploid Slope	1	1 276.43	<0.0001
Triploid Slope	1	1 425.21	<0.0001
Error	52	1.10	
Uncorrected total	56		

LSD values were consequently calculated as an indicator of the statistical significance of the observed differences between intercept and slope of diploids and triploids.

Table 3.13 Results of the linear regression for length (mm) in Block C for diploid and triploid abalone, *H. midae*. Estimates and Standard Errors of the Regression Coefficients are displayed, as well as the LSD results for differences between diploid and triploid groups. LSD ($P \leq 0.05$) for intercept = 1.91; LSD ($P \leq 0.05$) for slope = 0.0044.

Parameter	*Estimate*	*Standard Error*	*T Value* (H_0:θ=0)	*P Value*	*LSD tests for intercept and slope*
Diploid Intercept	-0.222	0.674	-0.33	0.74	a
Triploid Intercept	0.788	0.674	1.17	0.24	a
Diploid Slope	0.051	0.00154	33.37	<0.0001	a
Triploid Slope	0.054	0.00154	35.26	<0.0001	a

As is reflected in Table 3.13 by the LSD values, no significant differences were observed between the intercepts or slopes of the diploid and triploid regression curves in Block C.

3.3.3 Assessment of the effect of Ploidy on the basis of the regressions of Weight on Age

3.3.3.1 Regression of Weight on Age, for diploids and triploids, over Blocks

A weight on age regression analysis was used to compare the growth rates of diploids and triploids over Blocks. Both linear and quadratic equations that describe the weight gain of triploids and diploids over time were developed to identify the equation that best fit the data. Firstly, as displayed in Figure 3.6 a linear regression analysis was done to render linear equations of diploid and triploid weight gain.

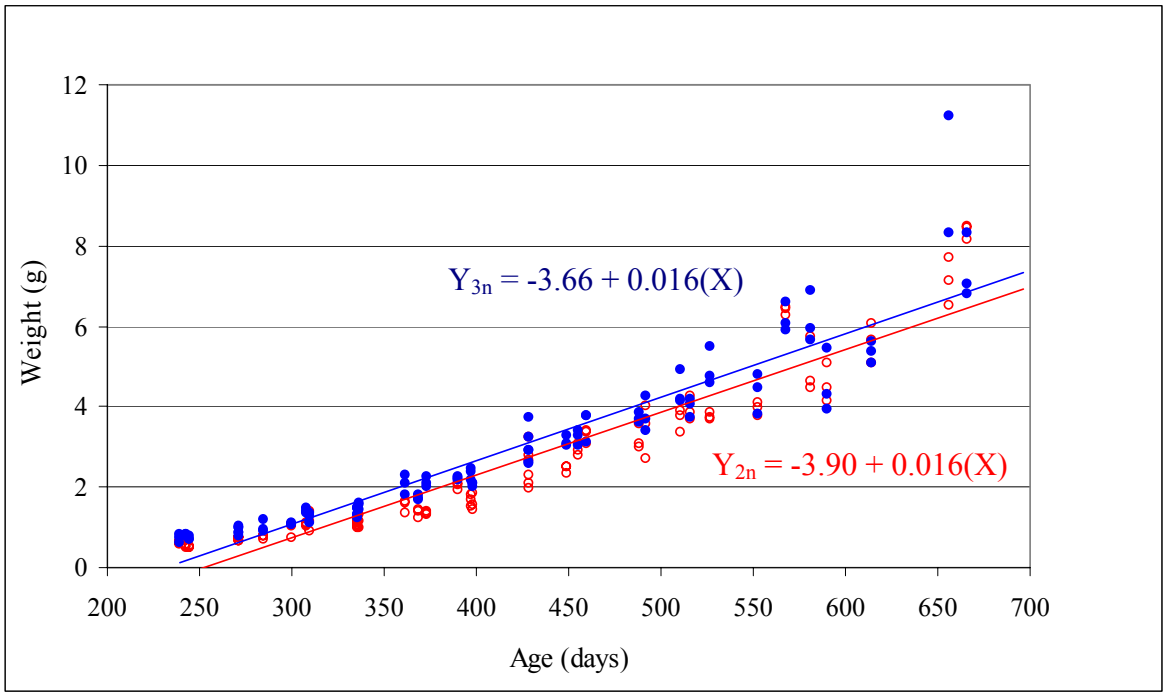

Figure 3.6 The relationship between weight and age of diploid and triploid abalone, *H. midae,* plotted as a linear regression (Solid dots = triploid, open dots = diploid).

The respective linear equations between weight (Y) and age (X) were calculated as:

- Diploid: Y = -3.90 + 0.016(X)
- Triploid: Y = -3.66 + 0.016(X)

with an R^2-value of 0.961 that gives a good fit to the data.

Applying these equations to predict the respective weights of abalone at 200 and 650 days provided the following results and put the difference in weight gain between diploids and triploids into perspective:

Average weight at age 200 days: Diploid = -0.70g
Triploid = -0.46g
Average weight at age 650 days: Diploid = 6.50g
Triploid = 6.74g

Figure 3.7 presents the quadratic regressions for weight gain in triploids and diploids over time.

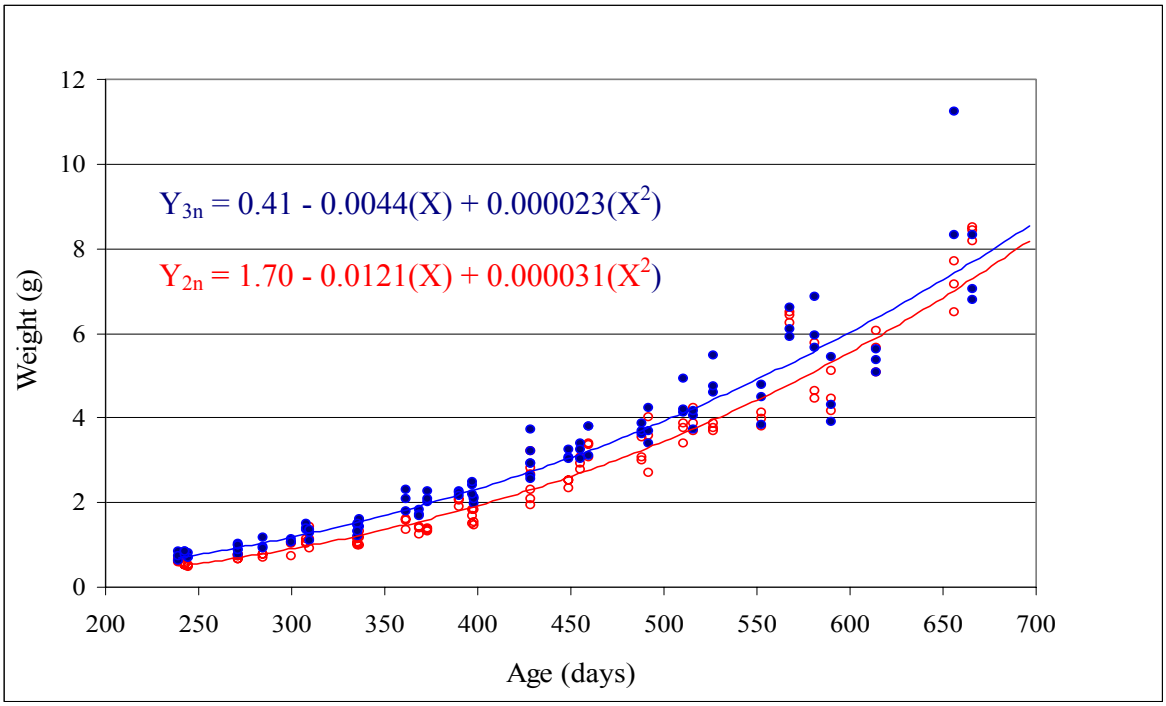

Figure 3.7 Mean Weights of diploid and triploid *H. midae* plotted as a quadratic regression. (Solid dots = triploid, open dots = diploid).

Quadratic equations were calculated where (X = age; Y = weight):

- Diploid: $Y = 1.70 - 0.0121(X) + 0.000031(X^2)$
- Triploid: $Y = 0.41 - 0.0044(X) + 0.000023(X^2)$

with an R^2-value of 0.975 that gives a good fit to the data.

Similar predictions of the weight of abalone at 200 and 650 days respectively delivered the following results:

Average weight at age 200 days: Diploid = 0.489g

Triploid = 0.618g

Average weight at age 650 days: Diploid = 7.856g

Triploid = 8.055g

For the weight regressions there was a larger difference between the R^2 values (0.961 for the linear and 0.975 for the quadratic equations), where the quadratic equation gave a slightly better fit to the data. This difference, however, was not considered large enough to outweigh the advantage of simplicity that the linear regression offered. The linear regressions were therefore applied during further analyses of weight up to the age of 650 days.

Table 3.14 Results of the ANOVA of the mean weight gain over age of diploid and triploid abalone, *H. midae,* over Blocks.

Source		*df*	*Mean square*	*P*
Regression		4	583.970	<0.0001
	Diploid Intercept	1	735.980	<0.0001
	Triploid Intercept	1	870.830	<0.0001
	Diploid slope	1	371.500	<0.0001
	Triploid slope	1	357.580	<0.0001
Error		199	0.476	
Uncorrected total		203		

LSD values were consequently calculated as an indicator of the statistical significance of the observed differences between intercept and slope of diploids and triploids.

Table 3.15 Results of the linear regression for weight gain (g) in diploid and triploid *H. midae*. Estimates and Standard errors of the Regression Coefficients are displayed, as well as the LSD results for differences between diploid and triploid groups. LSD (P≤0.05) for intercept = 0.69; LSD (P≤0.05) for slope = 0.0016.

Parameter	*Estimate*	*Standard Error*	*T Value* (H_0:θ=0)	*P Value*	*LSD tests for intercept and slope*
Diploid Intercept	-3.900	0.245	-15.93	<0.0001	a
Triploid Intercept	-3.657	0.251	-14.59	<0.0001	a
Diploid slope	0.015	0.00056	27.94	<0.0001	a
Triploid slope	0.016	0.00058	27.41	<0.0001	a

The LSD values in

Table 3.15 indicated that the diploid and triploid groups were statistically similar with regard to the intercepts and slopes for weight gain.

It can thus be concluded that, over blocks, no significant differences were detected between diploid and triploid weight gain, neither for the intercept (average starting weight) nor for the slope (average weight gain).

3.3.3.2 Regression of weight on age between Blocks (seasons)

Weight gain for each Block was analysed separately in this section. Due to the fact that each Block was spawned in a different season there may have been effects on the subsequent growth rates of the treatment groups within the blocks.

3.3.3.2.1 Regression of weight on age for Block A (summer seasons)

Figure 3.8 displays the averaged weight gain curves of the three pooled repeats of diploids and three pooled repeats of triploids within Block A.

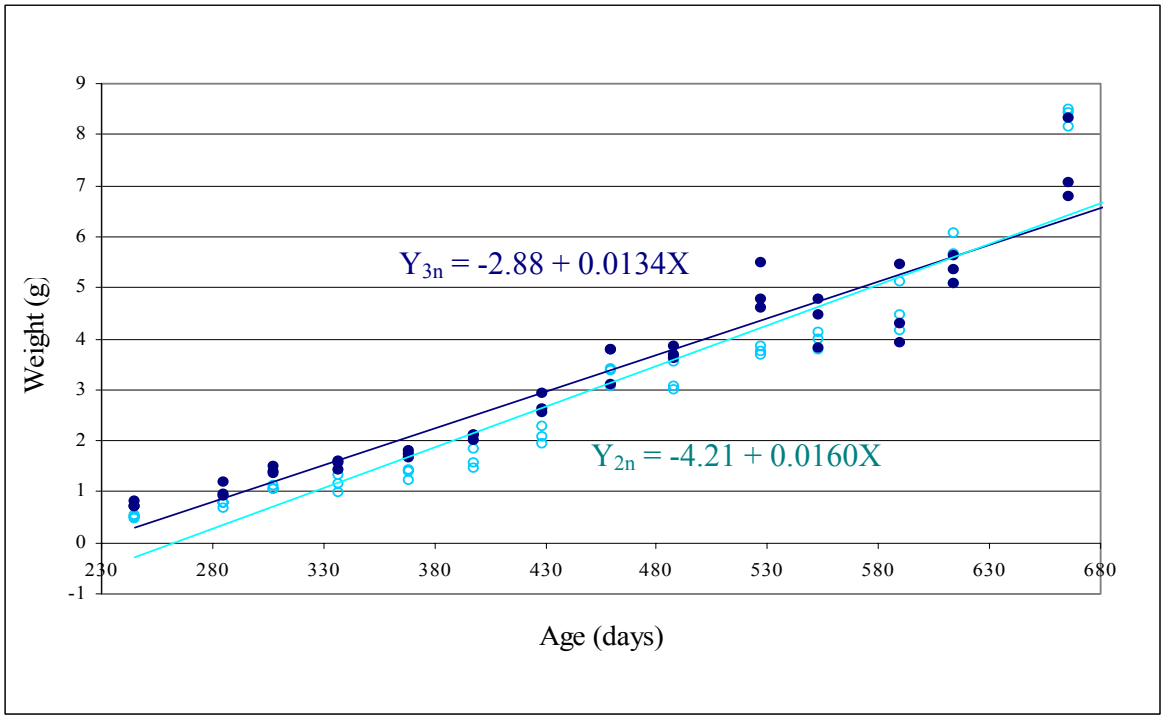

Figure 3.8 Weight gain, recorded as gram per day, of the diploid and triploid abalone over two years in Block A (Solid dots = triploid, open dots = diploid).

The following straight line equations were extracted (Y = weight; X = age):

- Block A Diploids Y = -4.21 + 0.0160X
- Block A Triploids Y = -2.88 + 0.0134X

with an R^2-value of 0.972 that gives a good fit to the data.

Table 3.16 Results of the ANOVA of the mean weight increase of diploid and triploid abalone of *H. midae,* in Block A.

Source	*df*	*Mean square*	*P*
Regression	4	246.51	<0.0001
Diploid Intercept	1	364.89	<0.0001
Triploid Intercept	1	345.03	<0.0001
Diploid slope	1	170.93	<0.0001
Triploid slope	1	105.19	<0.0001
Error	77	0.37	
Uncorrected total	81		

LSD values were consequently calculated as an indicator of the statistical significance of the observed differences between intercept and slope of diploids and triploids.

Table 3.17 Results of the linear regression for weight gain (g) in Block A diploid and triploid *H. midae*. Estimates and Standard Errors of the Regression Coefficients are displayed, as well as the LSD results for differences between diploid and triploid groups. LSD (P≤0.05) for intercept = 1.00; LSD (P≤0.05) for X = 0.002.

Parameter	*Estimate*	*Standard Error*	*T Value* (H_0:θ=0)	*P Value*	*LSD tests for intercept and slope*
Diploid Intercept	-4.209	0.348	-12.08	<0.0001	a
Triploid Intercept	-2.884	0.363	-7.94	<0.0001	b
Diploid linear coefficient	0.016	0.00075	21.34	<0.0001	a
Triploid linear coefficient	0.013	0.00080	16.76	<0.0001	b

The LSD values in Table 3.17 mirrors the results of the diploid and triploid length regression differences of Block A in Section 3.3.2.2.1. Once again, the differences in slope, i.e. weight gain, may be attributed to the significance of the different starting weights; while the differences in starting weights could be attributed to stocking density differences before the commencement of measurements (see Section 3.3.4).

3.3.3.2.2 Regression of weight on age for Block B (winter season)

Figure 3.9 displays the averaged weight gain curves of the three pooled repeats of diploids and three pooled repeats of triploids within Block B.

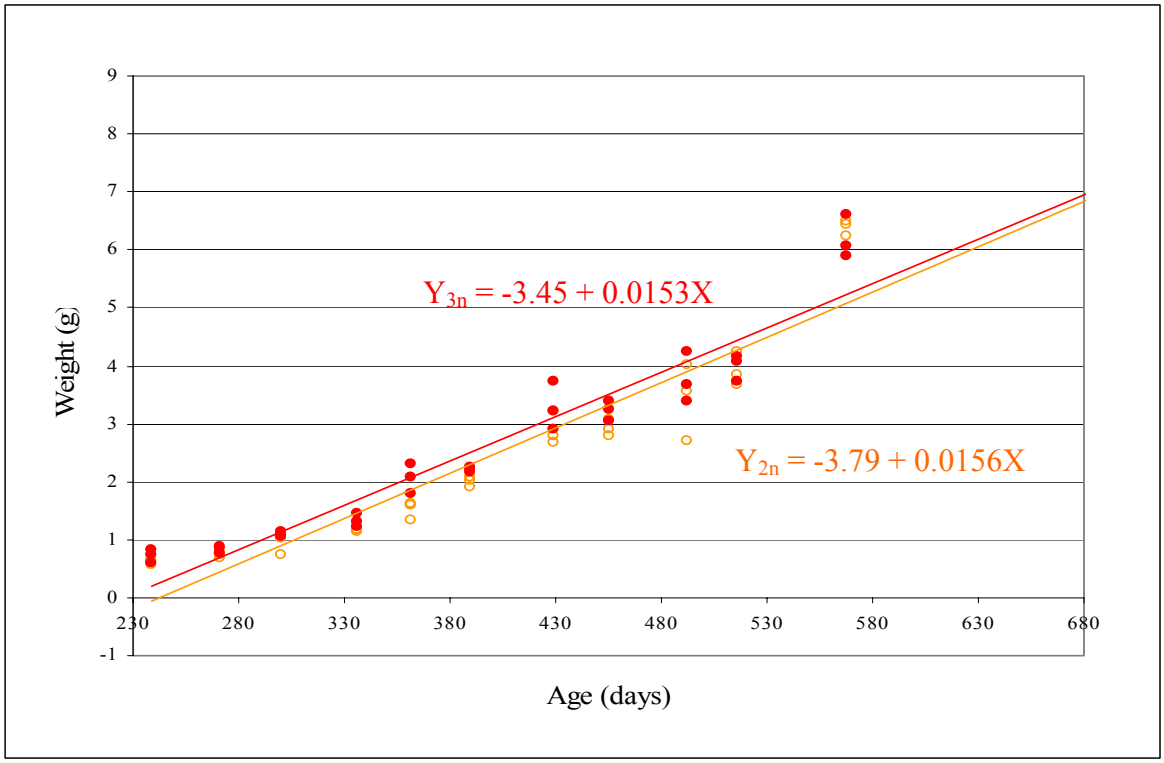

Figure 3.9 Weight gain, recorded as grams per day, of the diploid and triploid abalone over two years in Block B (Solid dots = triploid, open dots = diploid).

The following straight line equations were extracted (Y = weight; X = age):

- Block B Diploids $Y = -3.79 + 0.0156X$
- Block B Triploids $Y = -3.45 + 0.0153X$

with an R^2-value of 0.971 that gives a very good fit to the data.

Table 3.18 Results of the ANOVA of the mean weight increase of Block B diploid and triploid groups of *H. midae* over age.

Source	*df*	*Mean square*	*P*
Regression	4	144.28	<0.0001
Diploid Intercept	1	191.71	<0.0001
Triploid Intercept	1	224.43	<0.0001
Diploid slope	1	82.27	<0.0001
Triploid slope	1	78.71	<0.0001
Error	62	0.28	
Uncorrected total	66		

LSD values were consequently calculated as an indicator of the statistical significance of the observed differences between intercept and slope of diploids and triploids.

Table 3.19 Results of the linear regression for weight gain (g) in Block B diploid and triploid *H. midae*. Estimates and Standard Errors of the Regression Coefficients are displayed, as well as the LSD results for differences between diploid and triploid groups. LSD ($P \leq 0.05$) for intercept = 1.06; LSD ($P \leq 0.05$) for X = 0.0026.

Parameter	Estimate	Standard Error	T Value (H_0:θ=0)	P Value	LSD tests for intercept and slope
Diploid Intercept	-3.788	0.373	-10.15	<0.0001	a
Triploid Intercept	-3.454	0.373	-9.25	<0.0001	a
Diploid slope	0.0156	0.00091	17.13	<0.0001	a
Triploid slope	0.0153	0.00091	16.76	<0.0001	a

No significant differences were observed in either intercept or slope between diploid and triploid regression equations in Block B.

3.3.3.2.3 Regression of weight on age for Block C (spring seasons)

Figure 3.10 displays the averaged weight gain curves of the three pooled repeats of diploids and three pooled repeats of triploids within Block C.

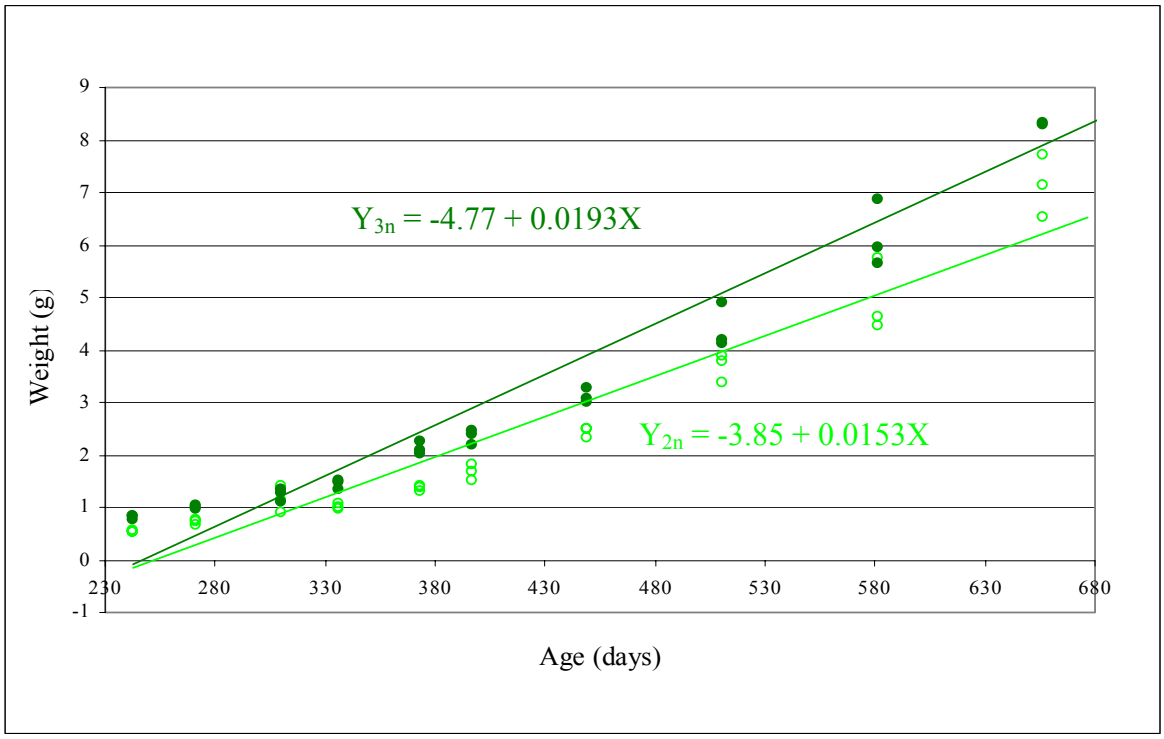

Figure 3.10 Weight gain, recorded as grams per day, of the diploid and triploid abalone over two years in Block C (Solid dots = triploid, open dots = diploid).

The following straight line equations were extracted (Y = weight; X = age):

- Block C Diploids Y = -3.85 + 0.0153X
- Block C Triploids Y = -4.77 + 0.0193X

Where an R^2 value of 0.962 indicated good fit of the lines to the datasets.

Table 3.20 Results of the ANOVA of the mean weight increase of Block C diploid and triploid groups of *H. midae* over age.

Source	*df*	*Mean square*	*P*
Regression	4	197.42	<0.0001
Diploid Intercept	1	185.10	<0.0001
Triploid Intercept	1	309.15	<0.0001
Diploid linear coefficient	1	114.03	<0.0001
Triploid linear coefficient	1	181.40	<0.0001
Error	52	0.60	
Uncorrected total	56		

LSD values were consequently calculated as an indicator of the statistical significance of the observed differences between diploid and triploid intercepts and slopes.

Table 3.21 Results of the linear regression for weight gain (g) in Block C diploid and triploid *H. midae*. Estimates and Standard Errors of the Regression Coefficients are displayed, as well as the LSD results for differences between diploid and triploid groups. LSD ($P \leq 0.05$) for intercept = 1.42; LSD ($P \leq 0.05$) for X = 0.0032.

Parameter	*Estimate*	*Standard Error*	*T Value (H_0:θ=0)*	*P Value*	*LSD tests for intercept and slope*
Diploid Intercept	-3.845	0.490	-7.85	<0.0001	a
Triploid Intercept	-4.771	0.490	-9.74	<0.0001	a
Diploid slope	0.015	0.0011	13.73	<0.0001	a
Triploid slope	0.019	0.0011	17.32	<0.0001	a

There were once again no significant differences in either the intercepts or slopes of the diploid and triploid weight gain equations in group C.

3.3.4 Discussion of the effects of Ploidy on the Length and Weight Regressions on Age, Over and Between Blocks

This discussion looks at the overall outcomes of the data displayed in the previous sections so that practical conclusions can be drawn from it.

1. The Regressions of length and weigh on age Over Blocks

For these analyses (Section 3.3.2.1 and Section 3.3.3.1) the diploid abalone from all three Blocks were pooled and the triploids from all three Blocks were pooled. Length- and weight gain regressions for both these triploid and diploid groups were then calculated and compared.

The results from these comparisons were:

For length gain, there were no significant differences between the slopes, but there was a significant difference between the Y-axis intercepts, where triploids had a longer average shell length than diploids. This is an indication of a better growth rate obtained by the triploids prior to tagging and the onset of the growth measurements.

For weight gain however, no significant differences were detected between diploids and triploids, neither for the regressions' Y-axis intercepts (average starting weight) nor for the slopes (average weight gain).

The fact that there existed significant intercept differences for length, but not for weight, meant that triploids weighed the same as diploids at the start of the trial, but had longer average shell lengths. The triploids and diploids thus exhibited a differential length to weight ratio at the start of the measurements which could not be explained within the scope of this trial.

2. The Regressions of length and weight on age Between Blocks

For these analyses diploid and triploid length and weight regressions were compared within each Block. For Blocks B and C, no differences between diploid and triploid length or weight regressions (slopes) or Y-intercepts (starting weights) were observed.

For Block A (see Section 3.3.2.2.1 and Section 3.3.3.2.1), however, there were significant differences in Y-intercepts or starting lengths and weights where the triploids were superior to the diploids. This can partially be explained by the differences in stocking densities of diploids and triploids that occurred during the period prior to the start of the

growth trials, i.e. during settlement and weaning. These differences could have been due to the following reasons:

1. A differential survival rate of diploids and triploids during the early life stages i.e. the hatching and settlement phases. The induction of triploidy through the application of hydrostatic pressure on fertilized gametes had a predictably negative impact on larval hatching and settlement survival which was observed, but not quantified during the trial. This could have caused a lower stocking density in the triploid settlement bags in comparison to the diploid bags, which in turn could have had a positive effect on growth for the Triploids during the first two months of life.
2. Extraordinarily cold water conditions during the first two months of life due to a lack of a warm water source for the settlement bags at the time. A heater was installed for Groups B and C.
3. Stocking densities were not standardized between two and eight months of age. This stocking difference was highest in Block A due to ignorance, after which stocking densities were more evenly spread out in Blocks B and C. The lower stocking densities amongst triploids lead to lower levels of competition and better access to food, causing faster growth and higher average starting lengths and weights at the commencement of measurements.

As for weight gain, diploids in Block A fared significantly better than the triploids over the measurement period, both in weight gain and in length gain. This could indicate that as soon as stocking densities were standardised when the measurements started, the diploids showed compensatory growth.

Overall, one can conclude that Block A showed the largest differences between diploid and triploid weight gain while Blocks B and C showed similar trends in both diploid and triploid weight gain.

3.4 Regression of length on weight

The relationship between shell length and body weight of abalone is an important indicator of proper yield and condition in the culture of abalone. It also allows for the prediction of one parameter such as weight on the basis of sampling and measuring another such as length, a procedure that is used with regular effect on abalone farms.

The relationship between shell length and body weight of abalone, based on the regression of the combined lengths and weights of diploid and triploid abalone is presented in Figure 3.11.

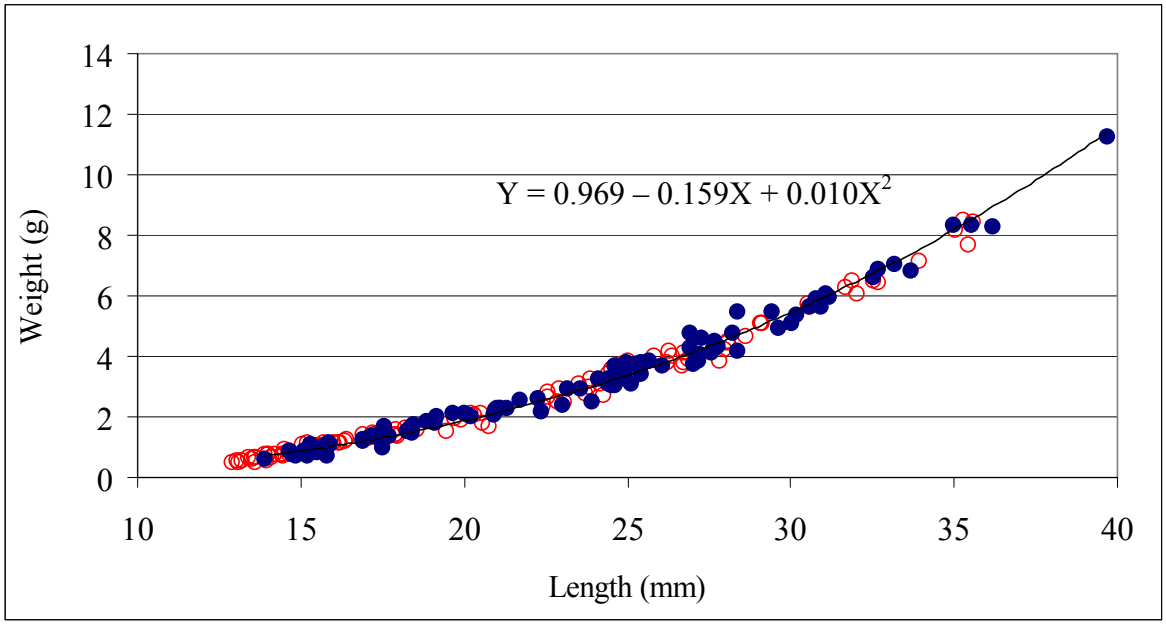

Figure 3.11 The relationship between the mean length and weight of *H. midae* over treatments (diploid and triploid) and over Blocks (different seasons). Solid dots = triploid, open dots = diploid.

Separate quadratic regressions were calculated for Diploid and Triploid groups, but revealed no significant differences. The data was thus pooled for a combined analysis. The overall relationship between length and weight of both triploid and diploid abalone over time is described by the following equations:

Quadratic equation:	$Y = 0.969 – 0.159X + 0.010X^2$	$R^2 = 0.990$
Linear equation:	$Y = -4.9 + 0.319X$	$R^2 = 0.950$

where Y = weight in grams, X = length in mm and N = 197.

The R^2 value indicated a good fit for both the quadratic and linear equations to the data. The quadratic equation is displayed in Figure 3.11.

The above regression equation may only be applicable for juvenile abalone prior to an age where sexual maturity is reached or at least for the duration of this trial, where the abalone reached 24 months of age. As the abalone become older, the relative energy expended in shell deposition verses soft tissue may change and one may even see a difference in ratios between diploids and triploids as sexual maturity sets in.

This assumption is based on a study done by Kesarcodi-Watson *et al.* (2001), where they concluded that triploid oysters (*Saccrostrea commercialis*) soft tissue versus shell content was relatively higher than in diploids, indicating a difference in the resulting weight-length equations between diploid and triploid oysters that are sexually mature. Utting *et al*. (1996) described heavier triploid Manila clams (*Tapes philippinarum*) in comparison to diploids of the same age, with a higher condition index, indicating a difference in yield and thus in weight-length regression equations between diploids and triploids.

However, Dunstan *et al*. (2007) measured the lengths and weights of diploid and triploid *Haliotis laevigata* up to 42 months of age and found no differences in the length-weight relationship at any age.

4 Conclusion and Recommendations

This project was aimed at comparing the growth rates of diploid and triploid abalone. A Random Balanced Block design was used and a common environment was ensured for the rearing of experimental animals during the comparative growth stage from eight to 24 months. Although significant seasonal effects were observed, no conclusion could be made relative to the influence of environmental/seasonal effects on the growth rate due to the possible influence of managerial factors on the performance of the respective Blocks. Possible advantages by spawning in a particular season to obtain improved overall growth could therefore not be confirmed by this investigation.

Statistically significant differences were observed in relation to the growth rates of diploids and triploids on the basis of overall adjusted mean length and weight, as well as on the basis of length and weight gain for Block A. No such differences could however be detected for Block B and Block C, nor for the combined Blocks. The observed differences in length and weight between diploids and triploids were also of such a limited physical nature that it was considered as insignificant in economic terms. It is therefore concluded that the induction of triploidy had no significant effect on the growth rate of abalone over the initial period of 24 months of age. Although no growth benefits are to be derived during this stage, the importance of this finding in terms of commercial application lays in the fact that the induction of triploidy had no adverse effect on initial growth stages, compared to that of diploids. The application of triploidy for commercial benefits related to inhibition of reproductive development, biosecurity, improved growth, yield and product quality at later growth stages can therefore still be considered.

Recommendations as outcomes from the study include:

1. Differences were observed in the survival of diploid and triploids larvae at the settlement stage (day five to six). The triploids in general displayed a lower survival rate than the diploids, which could be ascribed to the aggressive and robust nature of the hydrostatic shock treatment. It is therefore recommended that the larval survival rates of triploids and diploids be determined to assess the economic feasibility of triploidy induction.

2. It is virtually impossible to control the settlement rates and hence the stocking densities on settlement surfaces during the first two months of life. Stocking densities however have a significant influence on initial growth rate. It therefore recommended that, once the animals are moved from the plates, every effort be employed to standardise the stocking densities up to the point at which the animals could be tagged, i.e. eight months of age. This approach will reduce the size variation and uneven competition between groups, at the stage were they are to be placed into a common experimental environment.
3. Although no meaningful differences were detected in the growth rate of diploids and triploids from the age of eight to 24 months, it is recommended that the growth comparisons be continued up to at least 4 years of age. Gonadal development and sexual maturation is expected to set in during this subsequent growth period that may lead to a differential growth rate between diploids and triploids. The measurement of traits during this period should also be expanded to include yield and gonadal index.

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