MITIGATION METHODS FOR *TEREBRASABELLA HETEROUNCINATA*, A PROBLEMATIC SABELLID POLYCHAETE, POPULATIONS WITHIN AN ABALONE (*HALIOTIS MIDAE*) PRODUCTION SYSTEM

Assignment presented in partial fulfillment of the requirements for the degree Master of Philosophy in Livestock Industry Management: Aquaculture, at the University of Stellenbosch

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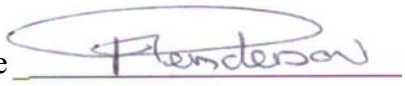
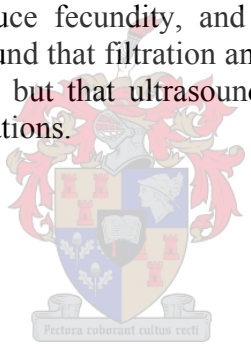
Declaration

I the undersigned hereby declare that the work contained in this assignment is my own work and has not previously in its entirety or in part been submitted at any University for a degree.

Signature  Date 10 March 2006

ABSTRACT

T. heterouncinata is a sabellid polychaete endemic to South Africa and found primarily in the shells of the abalone *Haliotis midae*. With the intensification of abalone aquaculture around the world, *T. heterouncinata* has become a problematic pest by causing shell deformities, reducing abalone growth rates and, in some instances, high abalone mortalities. The problem of this sabellid was first noticed in Californian in the early 1980's in Red abalone (*Haliotis rufescens*) production facilities. Many mitigation methods have been tested over the years and this paper investigates another two methods; a reduction in particulate load in the tank to reduce the food source of the sabellid which perhaps will reduce fecundity, and to use ultrasound as a possible mitigation method. This study found that filtration and reduction in suspended particles did not have a significant effect, but that ultrasound did have a significant effect in reducing *T. heterouncinata* populations.



OPSOMMING

T. heterouncinata is ´n sabellid polychaete endemies aan Suid Afrika en is hoofsaaklik te vinde in die skulp van die perlemoen *Haliotis midae*. Met die intensifikasie van perlemoen produksie in die wêreld, is *T. heterouncinata* problematies in die sin dat dit skulp abnormaliteite veroorsaak, groei van perlemoen verlaag en in sommige omstandigehede aanleiding gee verhoogte mortaliteit. Die probleem van hierdie sabellid was die eerste keer waargeneem in Kalifornieë in die vroeë 1980's in produksie stelsels van die Rooi perlemoen (*Haliotis rufescens*) . Baie metodes is al getoets om die organisme te beheer of van onslae te raak. Hierdie ondersoek behels die evaluasie van twee verdere metodes, naamlik; ´n verlaging van gesuspendeerde partikels om die voedselbron van die sabellid te verwyder wat miskien ook die fekunditeit van die sabellid sal verlaag, en die gebruik van ultraklank. Hierdie narvorsing het aangetoon dat filtrasie en ´n reduksie van gesuspendeerde partikels nie 'n betekenisvol effek op sabellid besmetting het nie, maar dat ultraklank wel ´n betekenisvol effek gehad het om die populasie van *T. Heteruncinata* te verlaag.

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The wet weight of the particulate was determined by: **WP = TW – SW**

Where: WP = Wet weight of the particulate (g)

TW = Total Weight of sieve and particulate (g)

SW = Predetermined wet weight of the sieve (g) (Constant)

LITERATURE REVIEW

1.1 HISTORY OF ABALONE AQUACULTURE

Abalone is one of the most prized sea delicacies worldwide. Entirely comprised in the genus *Haliotis*, these herbivorous marine gastropods have been exploited for hundreds of years for food and for making ornaments and the manufacture of jewelry (Bevelander, 1988; Leighton 1998, Stevens, 2003).

As a result of years of the intensification of fishing activities (both sport and commercial), poaching, predation, pollution of mainland habitat, disease, and inadequate wild stock management, there has been a collapse of wild fisheries of major abalone species in many parts of the world, especially over the past two to three decades (White, 1995; Stevens, 2003). In an effort to rebuild stocks, the commercial fishery for abalone in California was closed in 1997 (Stevens, 2003). Iin South Africa, the recreational fishery closed in the 1990's and it is believed that if the 2004/2005 commercial fishery is as bad as the previous year, the fishery will be unsustainable by 2006 (Steinberg, 2005).

The declining yields from wild fisheries have stimulated the development of intensive shore-based abalone aquaculture in a number of countries (Britz, 1996) as a means of enhancing over-exploited wild stock and to satisfy market demand (White, 1995). Abalone aquaculture was pioneered in Japan in the 1950's and 1960's. At present the main supply of abalone is Mexico, California, Australia, New Zealand, S. Africa, and Japan (Bevelander, 1988). The development of abalone culture technology in South Africa only began in earnest in 1989/1990 (White, 1995). Of the six species of *Haliotis* which occur in South Africa only *H. midae* occurs in sufficient quantities to warrant commercial exploitation (Newman, 1967; White, 1995) and supports a large abalone industry (Newman, 1969).

1.2 CLASSIFICATION OF THE ABALONE: *Haliotis midae*

A detailed classification of the species *Haliotis midae* can be presented as:

Kingdom	Animalia
Phylum	Mollusca
Class	Gastropoda
Subclass	Prosobranchia
Order	Archeogastropoda
Suborder	Zygobranchia
Superfamily	Pleurotomoniacea
Family	Haliotidae
Genus	Haliotis
Species	midae

Abalone belong to the Phylum MOLLUSCA which includes the chitons, snails, clams, tooth shells, squids, octopuses and others. These are soft-skinned, unsegmented animals possessing a head, muscular foot, a visceral hump usually covered by a calcareous shell and a *mantle fold* covering the gills. In all mollusks except the bivalve shellfish, the mouth contains a characteristic *radula* or ribbon of chitinous teeth for rasping the food (Day, 1974; Bevelander, 1988). The body cavity contains blood and is thus haemocoele and the coelome is reduced. The sexes are usually separate, though many hermaphrodite forms occur. Development of primitive aquatic forms is by means of a trochophore larva which later becomes a veliger before it settles down as an adult. There are five classes of mollusca of which the abalone is of the class Gastropoda (Day, 1974).

The Class GASTROPODA includes the snails, slugs and nudibranchs. These are mollusks with one-piece shells, or no shells at all, that move by means of a broad muscular foot and show some degree of torsion or asymmetry (Day, 1974; Bevelander, 1988), a distinct head with eyes and tentacles and mouth with a radula (Day, 1974). Formal classification is based on the character of the teeth on the radula and the internal anatomy (Day, 1974).

Abalones are members of the Subclass PROSOBRANCHIA which are the abalones, limpets, periwinkles, cowries, whelks and conchs'. They are classified as such because they are gastropods that undergo torsion during the veliger larval stage so that the mantle cavity and gill or gills come to lie at the front of the body, and the nervous system is twisted into a figure 8 (Crofts, 1929; Bevelander, 1988).

Abalone are classified into the order ARCHAEOGASTROPODA (limpets, abalones top shells). This order includes the Prosobranchs that have no siphon or proboscis and have bipectinate gills (with filaments on both sides of the gill axis) (Bevelander, 1988).

The abalone make up the Family Haliotidae. They are classified as such because the visceral mass and shell are markedly flattened, and the spire is greatly reduced. The shell has a row of holes through which the respiratory current exits, carrying also feces, urine, and sometimes gametes. (Crofts, 1929; Bevelander, 1988). The holes are successively obliterated as new holes form at the shell margin. Mantle tentacles clean the open shell holes. The ctenidia are placed symmetrically on either side of the anus and are washed with fresh water, when the shell lifts, along the whole free anterior and right border. The right ctenidium shows slight reduction. There is complete torsion of the mantle cavity and shell through 180^{o}. The nervous system is very primitive in having a long labial commissure, several anterior anastomoses and the pedal ganglia in the form of long anastomosing cords. The family is further characterized by the enormous development of the epipodium, which bears a profusion of sensory structures (Crofts, 1929).

The "sea-ear" is mentioned, in the fourth century B.C., by Aristotle in his 'Historian Animalism" but the generic name Haliotis, meaning "sea-ear", appears to have been first given by Linnaeus, in 1740 (Crofts 1929) or 1758 (Bevelander, 1988), in his "System nature," Ed. II to this Gastropod genus with ear-shaped shell (Crofts, 1929). Linnaeus distinguished nineteen species (Crofts, 1929), Crofts (1929) 75 species and Bevelander (1988) closer to 100 species. There are six species of *Haliotis* which occur in South Africa, but only *Haliotis midae* occurs in sufficient quantities to warrant commercial exploitation (Newman, 1967; White, 1995).

Abalone are found world wide in temperate waters ranging from the low tide line to depths of 30 meters (Newman, 1969) or even in excess of 400m (White, 1995). Their preferred habitat are crevices on rocky reefs and overhangs, which provide protection from light and predators (Crofts, 1929; White, 1995). *H. midae* occur sub-intertidally along the coast between St. Helena Bay and Qora River Mouth and are abundant in certain areas where mean annual temperatures vary between 15^{o}C and 17^{o}C (Newman, 1969). Britz, Hecht and Mangold (1997) concluded in their studies that temperatures between 12 and 20°C are physiologically optimal for *H. midae.* Macroalgae are the major source of food for abalone (Newman, 1969; Bevelander, 1988) with the large

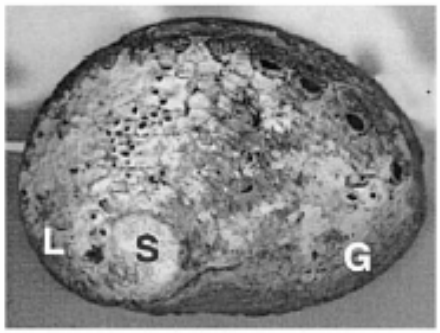
kelps *Ecklonia maxima* and *Laminaria pallida* being the main source of food for *H. midae* (Newman, 1969).

1.3 SHELL DEVELOMENT IN ABALONE

The most significant characteristic of abalone is the ear-shaped, flattened shell and for the purposes of this paper it is important for the writer to focus on this feature.

The abalone shell is ear-shaped and has a small spire located posteriorly. Most of the shell, however, consists of a large whorl (Barnes, 1980; Bevelander, 1988) which has an enormous aperture (Bevelander, 1988). The dorsal surface (Figure 1.1) is convex and exhibits a number of striations, the growth rings. These rings are an interruption of the orderly growth of the shell brought about by drastic changes in the water temperature and availability of food. They are also correlated with spawning periods and change in habitat (Bevelander, 1988). There are various methods of determining the growth rate of mollusks. Annual rings provide a convenient method of age determination and have been applied to *Haliotis discus hannai*, but in *Haliotis midae* these rings are not evident (Newman, 1968).

Another prominent feature of the external surface of the shell is the presence of respiratory pores (Figure 1.1). The first pore forms as the mantle separates at the anterior margin of the shell. This creates a slit giving rise to an opening or pore on the growing surface of the shell. Additional pores are formed as the shell increases in size. After four or five pores are formed, the first formed pore is closed. This process is repeated as the shell increases in size and accordingly there may be several closed pores but only four or five open pores present at any time (Bevelander, 1988).



G - Growth edge
S - Spire
L - Lip

Figure 1.1 Photograph of the abalone shell structure as viewed from the top (Bettiol; et al, 1999).

The first shell is laid down by the larva and is called the protoconch and it is represented by the smallest whirls (Barnes, 1980). The juvenile and adult shell is derived from cells of the outer fold and outer surface of the mantle. The first indication of mineralization appears as crystals of calcium carbonate within the confines of conchiolin (organic) envelopes (Newman, 1968; Bevelander, 1988). The tissue responsible is the mantle (Newman, 1968; Day, 1974; Lin & Meyers, 2004) and the rate of increase in shell area is therefore a function of the mantle area (Newman, 1968). The rate of increase in shell thickness and weight is, however, a function of the rate of secretion of the calcium carbonate and the organic matrix (Newman, 1968). Subsequent events give rise to a shell consisting of three layers (Figure 1.2): an outer horny periostracum, which is occasionally fibrous, a thick calcareous prismic layer and an inner pearly nacre (Day, 1974, Barnes, 1980; Bevelander, 1988). As it grows, more and more whorls are formed around the central axis or columella, each whorl being separated from the next by a spiral groove or suture (Day, 1974). The prismic layer is composed of calcite crystals (Bevelander, 1988) and is responsible for the shell's normal linear growth (Culver, Kuris & Beede, 1997). The nacreous layer is composed of aragonite, and nacre, the so called mother of pearl, giving the inner surface the characteristic iridescent appearance (Bevelander, 1988). Nacre can also be deposited when shell damage is being repeated or when a foreign object cannot be dislodged from beneath the mantle (Culver, *et al*, 1997).

Lin and Meyers (2004) conducted more detailed studies on the growth and self-assembly of aragonitic calcium carbonate found in the shell of abalone (*Haliotis*) and confirmed that the growth of the aragonite component of the composite occurs by the successive nucleation of aragonite crystals and their arrest by means of a protein-mediated mechanism. Their findings suggested a mechanism of *c*-axis aragonite growth arrest by the deposition of a protein layer of approximately 20–30 nm that is periodically activated and determines the thickness of the aragonite platelets, which are remarkably constant (0.5 μm).

The structure of nacre within the shells of abalone is composed of a "brick-like" tiled structure of crystalline aragonite (an orthorhombic polymorph of $CaCO_3$); moreover there is a very high degree of crystallographic texture characterized by a nearly perfect "*c*-axis" alignment normal to the plane of the tiles. Aragonite is metastable at low pressures (lower than 0.4 GPa) and forms orthorhombic crystals that are often twinned and pseudo-hexagonal in cross-section. Three morphologies of the

aragonite polymorph are observed in the abalone shell: tiles, block-like, and spherulitic. The two forms of $CaCO_3$, calcite (rhombohedral) and aragonite (orthorhombic), constitute the inorganic component of this ceramic/organic composite (95 wt.% ceramic, 5 wt.% organic material) (Lin & Meyers, 2004).

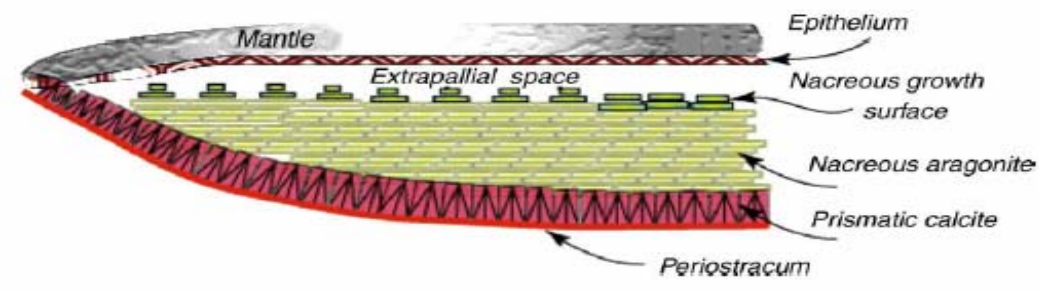


Figure 1.2 Structure of typical mollusk shell (Lin & Meyers, 2004).

The mantle epithelium of the abalone is responsible for secreting the chemicals that produce growth. It ejects them into the extrapallial space. Shell growth begins with the secretion of proteins that mediate the initial precipitation of calcite, followed by a phase transition from calcite to the aragonite. Prismatic calcite is composed as columnar, crystallographically textured, crystals of rhombohedral calcite. There are at least seven proteins involved in the process (Lin & Meyers, 2004).

First, a proteinaceous layer is deposited. Then, a calcite layer is formed. The aragonite crystals nucleate and grow, with a characteristic spacing. They have the orthorhombic structure and the *c* direction is perpendicular to the protein plane. In the absence of inhibiting proteins, this is the rapid growth direction. There is stereo selective adsorption of proteins in the growth of calcite crystals; this results in a slowing down of growth in the *c* direction and completely alters the final shape of the crystals. The time during which the protein is being deposited to arrest and reinitiate the process of bio mineralization is approximately equal to five times the growth time (Lin & Meyers, 2004).

Important to this study, they compared laboratory-raised and naturally-grown abalone and indicated that growth is regulated by the level of proteinaceous saturation. Naturally-grown abalone exhibits mesolayers (growth bands) 0.3mm apart and proposed that they result from seasonal interruptions in feeding patterns, creating thicker (10–20nm) layers of protein. These mesolayers play a critical role in the mechanical properties, and are powerful crack deflectors. The viscoplastic deformation of the

organic inter-tile layers is responsible for the significant improvement of tensile strength over the tensile strength of monolithic aragonite (Lin & Meyers, 2004). They noticed that after 6months there was a change in the case cultured abalone from tiled aragonite growth to a block-like structure due to environmental changes in the circulating seawater in the holding tanks. They deducted there was a switch from aragonite growth to calcite growth and noticed the shells to be brittle compared to samples not showing this calcitic mesolayer. Thus they concluded that the block-like or spherulitic growth takes place when arrestor proteins are not injected into the growth areas (Lin & Meyers, 2004). This would have a significant compounding affect with a sabellid infestation of the shell.

Increase in length is reduced or negligible after the shell attains a certain size, whereas thickening of the shell continues throughout the life of the individual (Bevelander, 1988).

1.4 THE ABALONE PARASITE: *Terebrasabella heterouncinata*

Organisms living in the same biosphere interact with individuals of the same species as well with individuals of other species. Many of these established interactions are known as direct relationships. These relationships usually convey a high degree of specificity, to the extent that at least one of the involved partners can no longer be considered a free-living organism. Among the polychaetous annelids, which for the most part are free-living, crawling, burrowing and tube-dwelling, the establishment of close associations with other marine invertebrates is a rather common phenomenon (Martin & Britayev, 1998). These relationships are often enhanced or accelerated in aquaculture facilities with dramatic and often devastating consequences in commercial culture systems, particularly so in relation to the previously unknown sabellid polychaete.

In the early 1980s the exotic sabellid worm *Terebrasabella heterouncinata*, then unknown to science, was accidentally introduced into California abalone farms with imported South African abalone, *Haliotis midae*. The worm established infestations in the shells of the California Red Abalone, *Haliotis rufescens* (Leighton, 1998; Culver; *et al*, 1997; Cohen & Webb, 2002; McEnnulty, Bax, Schaffelke & Campbell, n.d.) and wreaked havoc on nearly every abalone aquaculture facility in the state (Stevens, 2003). It was not until 1993 when Californian abalone production facilities recognised the problem (Ruck & Cook, 1998; Cohen & Webb, 2002) and by 1995, all California abalone farms were infested with the sabellid via stock transfers, bankrupting some

growers, and infested native snails in the ocean in at least one site (Cohen & Webb, 2002).

In 1993, Dr. Kirk Fitzhugh of the Los Angeles County Museum of Natural History recognized that this worm was actually an undescribed member of the family Sabellidae, whose members are collectively known as "fan worms" (Culver, *et al*, 1997). It was also discovered that it was a non-indigenous species to California, but accidentally introduced from South Africa (Culver, *et al*, 1997; Ruck & Cook, 1998; Cohen & Webb, 2002).

Prior to the introduction of the sabellid into California, this worm was unrecognized even in its native habitat (Cohen & Webb, 2002). Infestations of sabellid polychaetes were only found in South African-farmed abalone in 1994 (Culver, *et al*, 1997; Ruck & Cook, 1998) Surveys of populations of mollusks on the South African coastline revealed that the sabellid is endemic to South Africa (Ruck & Cook, 1998; Simon, Kaiser, Booth & Britz, 2002). This sabellid has a broad host specificity and able to infest many different gastropods, not just abalone (Culver *et al.* 1997; Ruck, 2000).

1.5 CLASSIFICATION OF: *Terebrasabella heterouncinata*

A detailed classification of the species *Terebrasabella heterouncinata* can be presented as:

Phylum	Annelida
Class	Polychaeta
Subclass	Sedentaria
Family	Sabellidae
Genus	Terebrasabella
Species	heterouncinata

Phylum ANNELIDA is characterized by metamerically segmented worms with a soft skin and elongate, bilaterally symmetrical bodies. Internally there is a simple alimentary canal running from the mouth to the anus, and between the alimentary canal and the body-wall there is a true coelomic body-cavity lined with an epithelium. The metameric segmentation affects all parts of the body except the alimentary canal, thus the whole length of the worm is divided externally into a series of rings or segments, each of which typically posses setae, sometimes borne on a pair of parapodia; internally the coelome is divided by traverse septa and the muscles, excretory organs, gonads and nerve-ganglia are also repeated in each segment (Day, 1974; Barnes, 1980).

The Class Polychaeta are Annelids with numerous setae and often well-developed parapodia and head-appendages. The sexes are usually unisexual. They are almost all free living and mainly found in marine environments (Day, 1974; Barnes, 1980).

Polychaetes may be broadly divided into active forms or *Polychaeta Errantia* and burrowing or tube-dwelling forms or *Polychaeta Sedentaria*. The SEDENTARIA are all particle feeders. Some construct tubes and collect suspended food particles from the water by means of a frilly membrane around the mouth, or by a number of buccal cirri or by a funnel shaped branchial crown which serves both for feeding and respiration. In all the sedentary forms the parapodia tend to be reduced, particularly in the posterior region, and the anterior part of the body then differs from the posterior part. Similarly the setae are usually small and often form a series of hooks (Day, 1974).

Polychaetes are one of the best represented groups in marine benthic communities showing a large variety of feeding types and life strategies. They are also one of the groups with the highest diversity of reproductive traits among marine invertebrates (Glangrande, 1997; Pernet, 2003). This is probably due to the relative simplicity of their reproductive system, and to their high plasticity and adaptability to different habitats (Glangrande, 1997). Known reproductive patterns in the polychaete family Sabellidae include: (i) broadcasting of gametes, (ii) depositing of benthic egg masses, (iii) brooding outside the lip of the tube, and (iv) brooding within the tube (McEuen; *et al*., 1983; Ruck 2000), with the only consistency being that all have lecithotrophic larvae (Ruck, 2000). Intratubular breeding is a widespread strategy among polychaetes due to the tube-life habitats of most of the forms, especially in the small-size forms. In addition, brooding in polychaetes is also frequently associated with hermaphroditism rather than with small size and can be a strategy against desiccation in intertidal habitats, or against the high variability of physical conditions. Those species with continuous reproduction must have well developed seminal receptacles for sperm storage by the female so that each batch of eggs may be fertilized without the simultaneous discharge of gametes by males (Glangrande, 1997).

The species name, *heterouncinata* is derived from the presence of different types of uncini (acicular anteriorly and avicular posteriorly) within the same body region (Oakes and Fields, 1996).

1.5.1 Life Cycle (Figure 1.3)

T. heterouncinata is a simultaneous hermaphrodite, producing both eggs and sperm at the same time (Oakes & Fields, 1996; Culver, *et al*, 1997; Ruck & Cook, 1998; Ruck, 2000). The structure of the sperm, as well as the presence of a spermatheca, suggests that these animals normally cross-fertilize (Simon in Britz, Chalmers, Gray, Henderson, Kaiser, Simon and Winter, 2005). It has been established that *T. heterouncinata* are able to self-fertilize and produce viable reproductive offspring (Ruck, 2000). Outcross fertilization is regarded as the rule for many simultaneously hermaphroditic animals, to prevent the phenomenon of inbreeding depression of which there are many examples. However, inbreeding depression is not always found and there have been arguments defending the advantages of self-fertilization under certain conditions (Hsieh, 1997; Ruck, 2000).

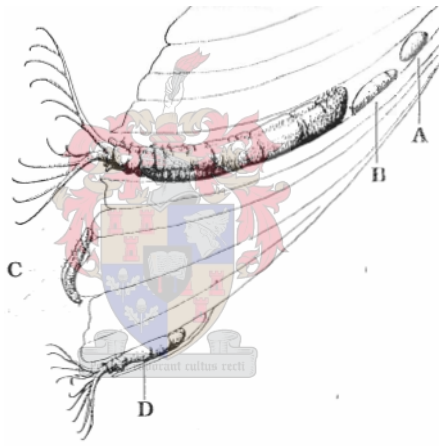


Figure 1.3 Life cycle of the sabellid worm. A. Eggs of the adult sabellid are laid and brooded within the tube. B. After fertilization, eggs develop into larvae within the tube. C. Fully developed larvae crawl out of the parental tube and eventually settle on the parental host or new host. Once the larva has settled, nacreous shell is deposited by the host, thus forming a new tube. D. Within the tube, the larva metamorphoses into a juvenile, which now has the characteristic crown and tentacles. (Culver, et al, 1997)

Eggs are laid, fertilized, and brooded within the posterior end of the tube in the host's shell (Culver; *et al*, 1997, Ruck 2000), while sperm are presumably broadcast from the tube's aperture (Culver; *et al*, 1997). This strategy is probably intended to ensure a high survival of offspring and would place the sabellid among *k*-selected organisms. This strategy may be related to small body size, since it is limited in the number of eggs it can produce and would thus prefer to ensure survival of the offspring

rather than release them into the plankton. Brooding is frequently associated with hermaphroditism and for this species, which already inhabits a protective tube; it is logically the favorable strategy. The egg production and development of larvae appear to be rapid (Ruck, 2000).

Larval development continues within the tube until segmentation is complete and bristles (required for locomotion) are visible. At this point, the larvae are able to actually crawl, not just flex from side to side, (Culver, *et al*, 1997) fairly rapidly over the substrate (Oakes & Fields, 1996; Ruck & Cook, 1998; Ruck, 2000), presumably as an adaptation to avoid being dislodged by strong water currents (Ruck & Cook, 1998; Ruck, 2000). They locomote in a gliding fashion using a band of cilia on the ventral side, that extends down their entire length (the neurotroch). This crawling larval stage is the infesting stage, and it leaves the parental tube in search of a host (Culver; *et al*, 1997). A likely cue for larval release appears to be the lunar cycle. Further studies observed that the most pronounced increase in total intensity and prevalence of larval infestation occurred in September suggesting that the spring season influences reproduction (Gray in Britz, *et al*, 2005). Most of the larvae appear to exit through the anterior opening of the worm's tube, but a few have been observed to emerge through an opening in the posterior end of the tube. This opening is much smaller than the diameter of the adult worm, and larvae have been observed exiting the tube through this opening, placing them between the abalone's shell and the mantle (Oakes & Fields, 1996). At this stage, its branchial crown has not formed, and the larva cannot feed and usually settle on the underside of the host's shell along the growing margin, or on the outer lip around the abalones respiratory pores (Culver; *et al*, 1997).

Once it has settled, the larval worm secretes a mucous sheath, which is rapidly covered by shell deposited by the host, thereby forming a tube for the worm (Oakes & Fields, 1996; Culver, *et al*, 1997; Ruck & Cook, 1998; Ruck 2000). Within the tube, the larva now metamorphoses into a juvenile (Culver, *et al*, 1997; Ruck 2000), easily identified by the crown of tentacles (Culver, *et al*, 1997; Ruck & Cook, 1998). They lose their eyespots and sensory tentacles and as they grow they develop more setigers and there is elongation of the body (Ruck, 2000). There is also evidence that they enlarge the size of the tubes they inhabit (Ruck, 2000). Maturation from juvenile to the adult stage occurs within the tube in about a month, but it is still unclear when the adult begins reproducing (Culver; *et al*, 1997).

1.5.2 Adult Morphology

Adult *T. heterouncinata* have only 11 segments (Culver; *et al*, 1997). The worm has a number of pairs of setae which are present on some of the segments of the body. The first 5 pairs are present from an early development stage as they can also be seen in the larvae. These are the largest setae and are probably used for rapid retraction into the burrow. Each setael group consists of a number of setae of various sizes. The shape of the setae on the dorsal side are elongated and tapered, whereas the setae on the ventral side are shorter and have a tooth-like projection at the end. These setae are termed uncini, defined as being sharp and claw-liked, often bearing teeth. Posterior are setigers bearing more uncini which have a different shape and have many teeth. The presence of different types of uncini (acicular anteriorly and avicular posteriorly) within the same body region, hence the species name – heterouncinata. As these teeth face forward, their function could be to prevent the worm being dislodged from its burrow (Ruck, 2000). The sabellid is not attached to the surface of its tube, and can move freely within the tube by means of setae positioned along its body. It maintains an opening at the anterior end of its tube which is equal to or greater than, the diameter of the worm and opens to the exterior of the abalone shell (Oakes and Fields, 1996).

At the basal region of the worm there are also a number of smaller setae which are perhaps used for grip at the base of the tube or for manipulation of the stored eggs. A faecal groove, which is lined with a large band of cilia, runs the length of the worm on the dorsal side. It is used to transport faeces away from the anus to the exterior of the burrow (Ruck, 2000).

The feeding crown consists of two branchial lobes, which have two palps in the center which are presumably involved in food selection. The feeding tentacles are covered by cilia, which work together in waves. These cilia create strong feeding currents, which draw the particle-laden water into the center of the crown (Ruck, 2000).

In live worms, the blood can be observed coursing through the major blood vessels, which are visible through the skin of the worm. A ring of blood is present around the base of the feeding crown, which appears to join the blood to the dorsal and ventral vessels. This makes sense since the branchiole is also the region for gaseous exchange and indeed the blood vessels can be seen in the branches of the radioles (Culver, *et al*, 1997; Ruck, 2000).

The adult worms are hermaphroditic, possessing both eggs and sperm simultaneously (Oakes & Fields, 1996; Culver, *et al*, 1997; Ruck & Cook, 1998; Ruck, 2000). The eggs are fairly large and each worm may harbor a number of eggs at various stages of development at the base of the tube (Oakes & Fields, 1996; Ruck, 2000). The eggs are orange and can sometimes be seen from the interior side of the abalone shell (Oakes & Fields, 1996).

The reproductive biology of *T. heterouncinata* suggests that some form of sperm transfer and storage must take place. Mature sperm are approximately 5µm long, excluding the tail. The head is elongate, with a modified acrosome and mid-piece. A single spermatheca is situated ventral to the mouth, and extends as far as the first segment, for more than 100µm. The total number of sperm stored has not been accurately determined, but appears to be less than 100. The sperm ducts that lead from the male segment open into the ciliated faecal groove that runs the length of the animal. This groove is analogous to the sperm duct that runs dorsally along the length of some other sabellids. The sperm are released into this groove and they move to the anterior of the animal where they are released into the water column. The sperm are then presumably picked up by other individuals (probably in their feeding current) and stored in the spermatheca, the mouth of which opens into the ventral part of the feeding crown (Simon in Britz, *et al*, 2005).

The ability to store sperm affords the worm with several advantages. The uptake of sperm can occur before the eggs are laid, and this eliminates the need to synchronize the spawning of sperm and the maturation of eggs. Several clutches of eggs can be fertilized after a single uptake of sperm. When the eggs are laid, they are fertilized by the sperm that are released from the spermatheca. This also increases the fertilization success (Simon in Britz, *et al*, 2005).

T. heterouncinata has only one female segment consisting of two ovaries. The ovaries are attached to the septum that separates the female and male reproductive segments, and lie on either side of the ventral blood vessel. Each ovary contains oogonia at different stages of development. The oogonia are released into the body cavity before vitellogenesis (i.e. the production of yolk) commences. At any one time, the body cavity may contain one or two late vitellogenic oocytes and 3 or 4 oocytes at earlier stages of development. The oocytes incorporate yolk precursors from outside the cell, probably from the extracellular fluid (Simon in Britz, *et al*, 2005).

The longevity is unknown at present. The age of reproductive maturity lies between 1 and 3 months (Ruck & Cook, 1998).

1.5.3 Larval Morphology

Most authors describe the eggs belonging to these sabellids as large and rich in yolk. Egg size has long been thought of as a rough indicator of reproductive pattern in polychaetes (McEuen; *et al*., 1983). The eggs and larvae are orange in color and are large relative to the adults (Ruck, 2000). A larval worm lacking a branchial crown develops directly from the eggs (Fields & Oakes, 1996). The final stage of larvae before emerging from the burrow are approximately 500µm in length with 5 pairs of setae on the sides of the body and two dark eyespots. A closer view of the head region shows the eyespots and also numerous "whiskers" in the front which are presumably important for sensory purposes and enables the larvae to target the correct area for settlement. The neurotroch is a broad cilial band with five pairs of setae on the ventral side of the body, which is used for locomotion and anchorage (Ruck & Cook, 1998; Ruck, 2000). Juveniles posses a functional feeding crown approximately seven days after settlement (Gray in Britz, *et al*, 2005).

1.6 SABELLID LARVAL SETTLEMENT, EFFECTS ON ABALONE SHELL GROWTH AND THE IMPLICATIONS FOR AQUACULTURE

T. heterouncinata larvae seem to have the ability to detect suitable areas to settle, which is quite consistent with larval behavior in other species (Ruck, 2000; Ruck & Cook, 1998). Studies conducted by Gray (in Britz, *et al*, 2005) indicated that sabellid larvae showed a preference to settle around the thinnest area of the shell edge. Thus, the heterogeneous distribution of sabellid larvae on the shell edge indicates the presence of settlement cues. Differences in shell mineralogy and composition have been shown for different types of abalone shell and it has been observed that larvae appear to settle most readily on prismatic shell. The differences in chemical or physical properties between prismatic shell and nacreous shell may be detected by sabellid larvae and may provide a cue to locate settlement sites. The respiratory pores are also sites of active nacre deposition (Leighton, 1998) and these sites may also be attractive to sabellids as the increased movement of water through the pores during respiration may lead to better food availability than other shell areas (Culver, *et al*, 1997). In response to the worm,

the abalone deposits repair nacreous shell over the mucous sheath secreted by the worm (Oakes & Fields, 1996; Culver, *et al*, 1997) (Figure 1.4).

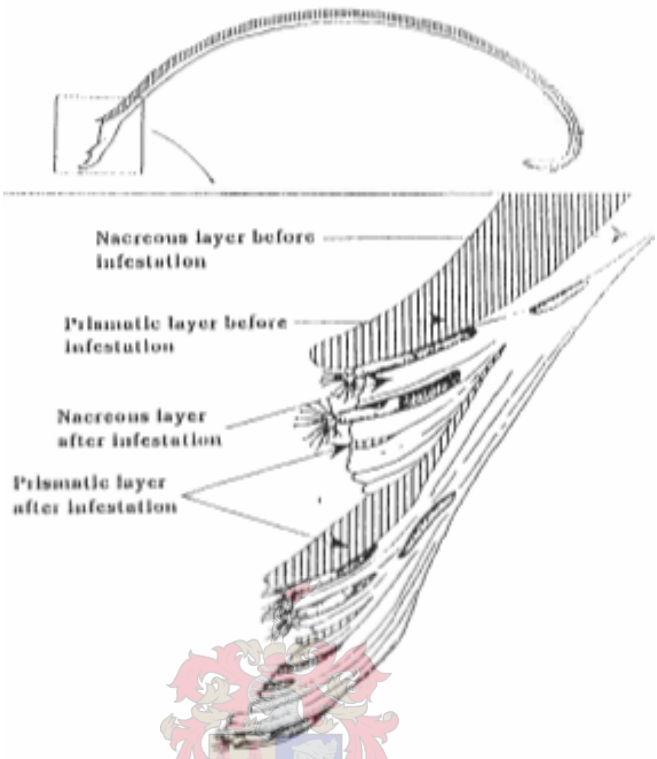


Figure 1.4 Cross section of sabellid-infested abalone shell. Prior to infestation, both prismic and nacreous shell are deposited. Upon settlement of the worm formation of the prismic layer is disrupted and only nacreous shell is deposited. Deposition of the prismic layer resumes once tube formation has been completed, but it can be disrupted again if additional worm settlement occurs

The larvae settle in areas of the shell with the greatest rate of deposition as it may provide an environment ensuring fast encapsulation (Culver, *et al*, 1997; Leighton, 1998). This response results in formation of a tube with one open end, which permits the worm to feed and release its young (Culver, *et al*, 1997). As the abalone tries to produce new shell, the sabellid worm keeps the area around its tube open. The result is the appearance of a worm that has burrowed into the shell, but the worm has actually been encapsulated by the abalone (Oakes & Fields, 1996). This process of establishment is unique among shell inhabiting parasites, which normally bore directly into the host shell using mechanical or chemical methods (Cohen & Webb, 2002; Simon, *et al*, 2002).

Nacreous layers continue to be deposited, creating a thickened shell, but marginal increment is curtailed (Leighton, 1998). The presence of the worm thus disrupts the normal linear extension of the host's shell, creating a vertical growth instead (Culver; *et al*, 1997). The resulting shell is also very weak and porous (Oakes & Fields, 1996) and prone to breakage, which presumably leads to mortalities (Ruck & Cook, 1998). Under these conditions, growth of body tissue appears also to be greatly decreased or stopped altogether (Leighton, 1998). Growth studies confirmed that sabellid infestations reduce growth rates of farmed abalone. Growth is influenced because of the interference caused by larvae at the mantle-shell interface, but low levels of infestation do not appear to slow growth rates. Presumably, this is because the shell deposition can continue relatively unhindered when there are only small isolated pockets of disturbance. On the other hand, when the entire shell margin is covered by larvae, the normal process of linear shell deposition is not possible (Ruck & Cook, 1998). A fast-growing abalone can encapsulate a small number of worms and extend its shell beyond them. When the encapsulated worms reproduce, their juveniles are in turn rapidly covered and passed by. On a slow growing abalone, the worms at the leading edge have time to reproduce and their larvae settle before any appreciable shell can be formed (Oakes & Fields, 1996).

The sabellids can infest a population of abalone quite rapidly. They spread from one abalone to another when the abalone come in contact with each other. Studies conducted reveal that uninfested abalone placed in a tank with infested abalone will become host to a population of sabellids within 60 days. Abalone downstream from an infested tank may not pick up any sabellids. This led researchers to believe that the sabellid does not have a planktonic stage, a useful fact when it comes to managing infested abalone. The infested abalone can be quarantined together with minimal risk of them spreading the infestation to abalone downstream via water flows (Oakes and Fields, 1996). To complicate matters, it has also been shown that live worms are unaffected by the absence of a live host (Simon, *et al*, 2002) the larvae will either die or they have to disperse to find a suitable living host. In the absence of a live host *T. heterouncinata* continued to grow and reproduce (Simon, et al, 2002; Culver et al., 1997). This indicates that once the worms have settled, they are not dependent on a live host for survival (Simon, Kaiser, Booth & Britz, 2002). It does, however, mean that infested shells can serve as a reservoir of worms that can infest other abalone (Chalmers in Britz, et al, 2005).

It has been shown that farmed abalone have a higher density of sabellids (Simon, *et al*, 2002) because of the high stocking densities, food availability and other factors. It has also been shown that this is compounded by the fact that a higher proportion of worms on farmed abalone are reproductively active than on wild abalone and a greater proportion of the offspring are likely to reach adulthood (Simon, *et al*, 2002). Abalone that are 3mm or smaller are significantly less susceptible to infestation than larger individuals (Culver; *et al*, 1997).

The implications for the abalone aquaculture industry are that although infestations by the sabellid do not affect the quality of the abalone's meat, they can deform the shell to the point where the animal's growth slows or virtually ceases (Oakes & Fields, 1996; Culver, *et al*, 1997; Leighton, 1998). The results is an increases the length of time it takes for growers to get a marketable product. In the worst cases, the abalone remain too small to be marketable, with shells that are brittle, unsightly, or grossly deformed and even increased mortalities (Oakes & Fields, 1996; Culver, *et al*, 1997), bringing enormous economic losses to the aquaculture facilities (Culver, *et al*, 1997).

T. heterouncinata can therefore be considered as pests that drain the energy by causing the host to increase metabolic requirements to keep the shell intact, though they are uninterested in the hosts as such. Though the host is not attacked directly it is affected negetively, by the worms that that use its shell as substrate (Martin & Britayev, 1998).

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Chapter 2:

METHODS OF MITIGATION OF SABELLID INFECTION IN ABALONE USING FILTRATION AND THE REDUCTION OF PARICULATE LOADING

2.1 INTRODUCTION

The problem of *T. heterouncinata* was first identified in California and culturists who had been badly affected by the sabellid had attempted to control or eradicate the worm by a variety of means, including exposing infested abalone to air or extreme temperatures, as well as treating them with fresh water, chlorine, or insecticides (Leighton, 1988; Culver, Kuris & Beede, 1997; McEnnulty, Bax, Schaffelke & Campbell). Ultimately, they found that applying wax and other nontoxic substances like shellac to the outside of the abalone's shell did effectively reduce infestations by smothering the worms, and that normal shell growth resumed after treatment. However, these applications did not completely eradicate the sabellids, and re-infestations on new shell growth occurred. Furthermore, coating shells was very labor intensive (Leighton, 1988; Culver; *et al*, 1997).

Infestations of sabellid polychaetes were only found in South African-farmed abalone in 1994 (Culver, *et al*, 1997; Ruck & Cook, 1998) and initial attempts to control or eradicate the problem was pretty much on an ad hoc basis. Management recommendations proved useful in containing infestations and allowing reasonable growth of abalone, but there was still a primary demand for a treatment to contain or eradicate the worm (Ruck, 2000). This prompted the Abalone Farmers Association of South Africa (AFASA) to concentrate their efforts by funding research in trying to understand the biology and then finding solutions to either eradicating or controlling the sabellid problem more effectively. The department of Ichthyology at Rhodes University and the department of Zoology at the University of Cape Town have assisted AFASA in this research.

So far, experiments looking for an effective chemical agent have not revealed any substance which will kill the worm without also killing the abalone. Owing to the nature of this polychaete, its ability to withdraw into a tube with a small opening, it is unlikely that any environmental agent will be found which will poison the sabellid without affecting the abalone (Oakes & Fields, 1996; Ruck, 2000).

The idea of a potential predator, such as an isopod to be used as a bio-control was considered. There is a possibility that predators could be found which would nip away the exposed branchial crown. This would act to reduce the time for feeding and thus decrease production. This would be a limited control since it would be non-lethal but inhibit growth and reproduction. But this was not likely to be effective, as sabellids show remarkable powers of regeneration (Ruck, 2000).

The possibility for control by non-invasive approaches may exist wherein an environmental factor is changed that exerts a more stressful action on the worm than on the abalone (Leighton, 1998). However, it is perceived that that since these two species have been associated in a symbiotic relationship for so long, their environmental tolerances would probably be the same. Therefore, manipulating an environmental factor to affect the sabellid will in all likelihood affect the abalone negatively as well.

A major difference that was apparent was that the worms are filter feeders whereas the abalone are grazers (Ruck, 2000). Therefore, Oakes & Fields (1996) suggested that there may be methods of poisoning the worm via its food supply or controlling it with a substance that interferes with its reproductive cycle. Ruck (2000) tried to introduce an encapsulated toxin in microparticle form, which could target the worm only, by virtue of the fact that it would be the only one able to ingest significant amounts, but with only limited success.

Among environmental factors, both the quality and quantity of food have been considered to be the most important factors responsible for variation in fecundity of marine invertebrates (Quian & Chia, 1991). Rhodes University has conducted extensive research on *T. heterouncinata* and have identified that diet has the most significant effect on the sabellid. It affects the time it takes to reach maximum size, growth rate (Simon, Kaiser, Booth & Britz, 2002; Gray in Britz, Chalmers, Gray, Henderson, Kaiser; Simon & Winter, 2005), time to reach sexual maturity (Simon in Britz, *et al*, 2005) and influences the reproductive output (Chalmers, in Britz, *et al*, 2005). Cultured abalone are fed on either Kelp (*Ecklonia maxima*) or a commercial pelleted feed, Abfeed® or a combination of the two. The high level of nutritionally rich suspended solids found in aquaculture facilities provides an ideal environment for filter-feeding organisms such as *T. heterouncinata* (Chalmers in Britz, *et al*, 2005). These suspended particles include fragmented abalone feed and feces suspended or stirred up by the host (Simon, *et al*, 2002) but mainly by the aeration in the abalone production tanks (Chalmers in Britz, *et al*, 2005).

It was observed by some farmers that sabellid populations were higher on abalone receiving the Abfeed® diet and it possible that the supplementation of Abfeed® with other organic matter results in a more nutritious food source (Chalmers in Britz, *et al*, 2005). Farmers also reported that high sabellid infestations were associated with high abalone stocking densities. With increased stocking density there is an increase in the amount of suspended solids within the system providing more food for the worm (Chalmers in Britz, et al, 2005). However, if the food quantity is limiting, the worms will reproduce at a smaller size and at a later age (Simon, et al, 2002). There is, however, conflicting evidence on the effect of different diets and the fecundity and offspring size (Qian & Chia, 1991; Simon, et al, 2002).

Chalmers (in Britz, et al, 2005) found that *T. Heterouncinata* is capable of ingesting particles up to 35µm in size whereas particulates above 50µm were responsible for disrupting the Sabellids' feeding pattern. This resulted in a reduction in the duration the sabellids spent feeding, thus decreasing the quantity of feed particles they were able to ingest, resulting in smaller sabellid sizes from kelp raceways in comparison to Abfeed® raceways. Therefore, a decrease in suspended particulate is thought to cause a decrease in sabellid population numbers and increase growth rate of the abalone and, or alternatively, a decrease in suspended particulates will not cause a decrease in sabellid population numbers and will have no effect on the abalone growth rate. Therefore, it was this hypothesis that was tested in these experiments.

2.2 FARM SURVEY

An initial survey on as many abalone production facilities as possible was undertaken to a) gain a better understanding of the different production systems and management practices, b) identify possible "problem" facilities by observing examples of infected abalone, c) to identify which feed type and feeding protocols individual facilities use and d) to gain input as to what filtration systems might be effective.

All the abalone production facilities visited where pump-ashore, flow-through systems, with one exception where grow-out abalone where kept in tanks using recirculation technology. The air lines in the tanks at all facilities ran along the bottom of the tank stirring up particulates from the bottom and keeping the majority of particulates in suspension.

Only one farm had the tanks in a raceway layout where outlet water from one tank flowed into another tank. It may be coincidence that this facility also experienced a higher infestation rate. This could be due to one of two reasons or a combination thereof. Firstly, the average water temperature at this facility is lower than other production facilities in South Africa and therefore the metabolic rate of the abalone is much lower. A reduced metabolic rate causes a slower growth rate allowing sufficient time for *T. heterouncinata* larvae to settle on the growing edge and insufficient time for the abalone to "outgrow" the larvae. Secondly, an increased particulate loading because of the raceway system could potentially provide a greater source of particulate of the correct size for the sabellid to ingest.

The abalone facility with the least or no *T. heterouncinata* infestations, have strict management practices, lower stocking densities per basket and have less baskets and higher water flow per tank than any other facility.

For the most part, all production facilities use various combinations of Abfeed®, Kelp (*Ekclonia maxima*) and/or other species of seaweeds, however managers were reluctant to provide specific feeding regimes.

All facilities were in agreement that *T. heterouncinata* was problematic and that particulate loading was the major contributing factor to sabellid infestations. All suggested that by removing a greater proportion of the particulate and thereby reducing the foodsource, would bring about a reduction in the sabellid infestations. Known methods of filtration or flocculation are either too expensive or detrimental to abalone health, therefore, practical and more cost-effective methods of reducing the particulate loading had to be developed.

2.3 EXPERIMENT 1: EFFECT OF WATER FILTRATION ON SABELLID INFECTION

From the information in the introduction to this section, it was decided to test the null hypothesis that filtration and hence a reduction in particulate loading would not bring about a reduction in *T. heterouncinata* populations in a production facility and subsequently improve the growth rate of abalone in an intensive rearing system. In order to do so, it was decided to utilize a known means of water filtration.

2.3.1 Materials and Methods

Two standard abalone grow-out tanks in a production facility were randomly selected as for a treatment and control group respective. Eight baskets, with abalone of 45 – 50mm size class distribution and stocking density of 300 abalone per basket, were randomly allocated to each of the tanks and their specific positions in the tank in relation to the inlet were recorded and maintained for the duration of the experiment. Both tanks received equal volumes of fresh incoming seawater and air was introduced along the bottom of the tanks. The abalone in both tanks received a ration of Kelp (*E. maxima*) and Abfeed® as per the usual production protocols. The tanks were drained and cleaned every second week.

A standard swimming pool 40µm sand filter was used to continuously filter the water of the experimental tank. The water was drawn from the tank along its length just below the one end of the baskets and then pumped through the 40µm sand filter from where it was reintroduced back into the tank along its length above the opposite end of the baskets (see Figure 2.1). The intake in the tank and the return over the baskets were

Figure 2.1 The layout of tanks and baskets in Experiment 1 to investigate the effect of water filtration on sabellid infection in abalone, *H. midae*.

identical with the same number and diameter holes. The filter was backwashed daily to remove filtered particles and preventing the filter from clogging.

A similar set of data was collected from each of the treatment and the control group in order to draw a comparison amongst them. At the start of the experiment, a non-destructive, random sample of abalone were measured and the number of sabellid larvae on the growing edge counted. Twenty (20) abalone were sampled from each of the baskets numbered 1 and 2 at the inlet side of the tank, 20 from each of the baskets numbered 4 and 5 in the middle of the tank and 20 from each of the baskets numbered 7 and 8 at the outlet side of the tank (see Table 2.1).

Table 2.1 Layout of tanks and baskets for treatment and control groups in Experiment 1. (n = sample size)

Treatment Tank	Control Tank
Basket 1 (n = 20)	Basket 1 (n = 20)
Basket 2 (n=20)	Basket 2 (n=20)
Basket 3 (n=0)	Basket 3 (n=0)
Basket 4 (n=20)	Basket 4 (n=20)
Basket 5 (n=20)	Basket 5 (n=20)
Basket 6 (n=0)	Basket 6 (n=0)
Basket 7 (n=20)	Basket 7 (n=20)
Basket 8 (n=20)	Basket 8 (n=20)

The sampled abalone were used to measure shell length and count the number of sabellid larvae on the growing edge of the shell. The length of the abalone was determined using a vernier caliper by measuring the abalone shell from the edge at the spiracle end to the edge between the eyes of the abalone.

To examine the growing edge and count the number of settled sabellid larvae, the abalone is turned upside down and the mantle has to be gently pushed back to expose the sabellid larvae on the growing edge (Culver; *et al*, 1997, Gray, *pers.comm*.). Using a magnifying glass, all the larvae were counted along the entire growing edge from the first respiratory pore to where the edge meets the spire. These measurements were repeated every two weeks for a period of eight weeks to determine abalone growth rate and the rate of sabellid larval settlement. An ANOVA statistical analysis was conducted in order to determine any significant relationships.

Water samples were taken every two weeks, the day before the tanks were cleaned, to determine the effect of water filtration on particulate loading within the tanks and the size distribution of the suspended solids. To determine the particulate loading, water was siphoned from a fixed depth of 400mm at the inlet and outlet sides of the tank via a siphon system into a 2L volumetric flask. A sample was also collected from the actual outlet by catching the water in a 2L volumetric flask. These samples were shaken vigorously to distribute the particles evenly through the water column and a 10mL sample collected. The 10mL sample was tested for turbidity in an Orbeco-Hellige spectrophotometer as per the instruction manual and the determined result recorded. An ANOVA statistical analysis was conducted in order to determine any significant relationships.

In order to determine the size distribution of the suspended solids, water was siphoned from a fixed depth in the center of the tanks through a series of sieves of differing micron (100µm, 50µm, 41µm, 30µm and 20µm) for thirty (30) minutes. The collected sample was washed from each sieve through a 20micron sieve with distilled water. The filtered material was spun through the air by means of the container attached to a piece of nylon string by the researcher for 5 minutes to remove excess water. The wet weight of the particulate was determined by: $WP = TW - SW$

Where: WP = Wet weight of the particulate (g)

TW = Total Weight of sieve and particulate (g)

SW = Predetermined wet weight of the sieve (g) (Constant)

A Friedman statistical analysis was conducted in order to determine any significant relationships. This test is non parametric and does not assume any normality in the data.

Also, just before the tanks were cleaned water samples were collected from the inlet side, the middle and outlet side of the tanks to test the toxic ammonia content. A

sample from the center of the tank was collected once the tanks had completely refilled with water after cleaning as it was assumed that ammonia toxicity would be uniform in the tank after cleaning. The samples were tested using a standard Palin Test method in a spectrophotometer from Orbeco-Hellige.

Water quality parameters (Dissolved Oxygen (DO), Temperature (T) and pH) were collected weekly in order to determine if filtration had any positive or detrimental effect00 on water quality. Measurements were taken at the inlet side of the tank, both at the surface and bottom of the water column, in the middle of the tank at the surface and at the outlet side, at the surface and bottom of the water column. All measurements were taken with water analysis instruments from Orbeco-Hellige. No statistical analysis was conducted on the ammonia or water quality parameter data sheets as the observed differences were neglible and it was assumed that there would be no significant statistical differences.

2.3.2 Results and Discussion

2.3.2.1 Abalone Shell Length and Sabellid Larval Counts

At the end of the trial, the only significant statistical difference observed was for that of change in length over time, i.e. the number of days ($P<0.0001$) and an interaction between the treatments and the number of days for the number of sabellid larvae on the growing edge of the abalone shells ($p<0.0001$).

Table 2.2 Descriptive statistics of the relationship between mean shell length (mm) and the number of days for the duration of the trial

No. of Days	Mean Length (mm) (+/- s.d.)
0	50.917^{d} +/- 7.544
15	53.775^{c} +/- 7.542
28	54.079^{c} +/- 7.318
43	55.583^{b} +/- 7.179
53	57.988^{a} +/- 8.470

The results described in table 2.2 are to be expected, as normal abalone shell growth should increase over time as the average sabellid infesations was considered low. For both the control and the experimental tank, abalone growth rate was comparative for the duration of the experiment and there was no improvement in the growth rate of the abalone in the treatment tank compared with the control (Figure 2.2).

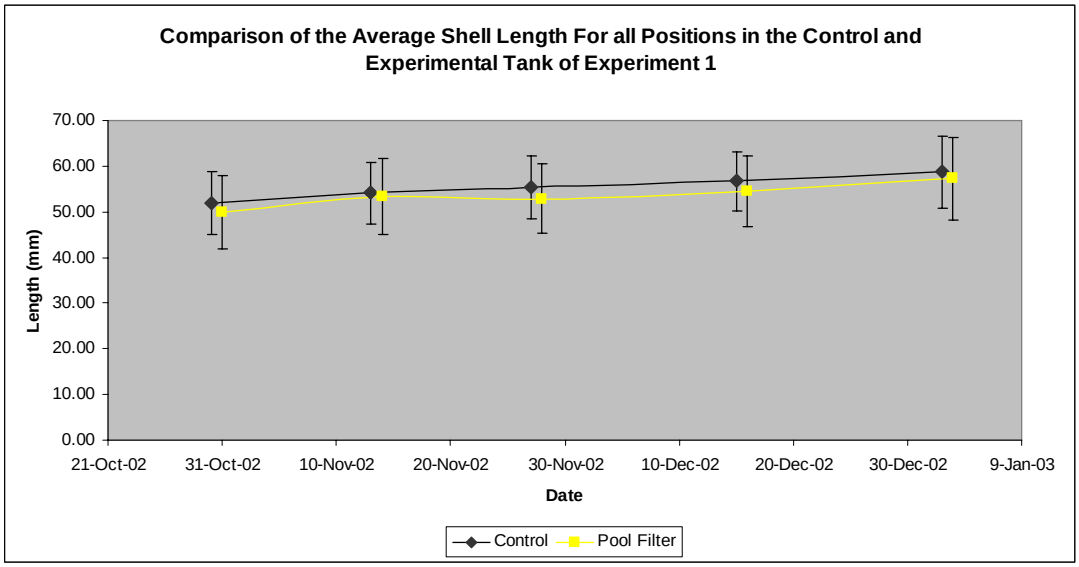

Figure 2.2 A comparison of the average growth rate (shell length (mm)) for the control and treatment tanks in Experiment 1.

Table 2.3 (Figure 2.3) show that for both the control and experiment tanks there was an increase in the sabellid larvae counts after 15 days and then a gradual decrease for the rest of the periods. However, there was a sudden increase in sabellid larvae found in both tanks from the second last to the final date, 43 to 50 days. This could probably be explained by the fact that for the first period, the tanks were not cleaned two weeks prior and two weeks after the commencement of the experiment. A managerial oversight by the farm management also caused the tanks not to cleaned and the filter not backwashed during the last 3 weeks of the experiment, which may have affected the water quality and hence the outcome of this experiment.

Table 2.3 Descriptive statistics of mean sabellid larvae counts on the growing edge of the abalone shells, between the different treatments and the number of days (+/- s.d.).		
Control	0	20.383^{a} +/- 14.60
Control	15	25.333^{ab} +/- 18.53
Control	28	22.100^{a} +/- 12.83
Control	43	23.825^{a} +/- 15.12
Control	53	36.433^{cd} +/- 16.77
Filtration	0	35.625^{c} +/- 14.61
Filtration	15	40.800^{d} +/- 15.23
Filtration	28	39.317^{cd} +/- 15.05
Filtration	43	22.492^{a} +/- 10.78
Filtration	53	30.308^{b} +/- 11.15

Although there were no other statistical significant relationships ($p>0.05$) there were some observed differences, such as differences in abalone shell length and sabellid larval counts on the growing edge of the abalone shell at the different positions in the tanks where data was recorded. Figure 2.3 and 2.4 depict the averages at the three sample positions in the abalone tanks of abalone shell length and the number of sabellid larvae counted on the growing edge. For both the control and the experiment, it can be observed that the average growth rate of abalone at the inlet side of the tanks were better than those in the middle and those at the outlet side of the tank at the end of the experiment.

For both the control and the treatment, it can be observed that the sabellid larval counts were higher on abalone at the outlet side of the tank where one would assume there would have been a higher concentration of suspended solids, and hence a food source for the sabellids, and water quality poorer which would influence the abalone metabolic rate. At the start of the experiment there was a wide distribution of sabellid larvae on the abalone shell edge at the three different positions. However, at the end of the end experiment there was still an observed wide distribution in the control tank whereas in the experimental tank, the sabellid larval counts were almost the same at all three positions. This would indicate that filtration might well have had some impact in reducing the particulate load, thereby causing a reduced sabellid population or the impact of filtration and the even distribution of 20micron suspended particles may have influenced this (Figure 2.3). Further experiments would have to be conducted in order to confirm this.

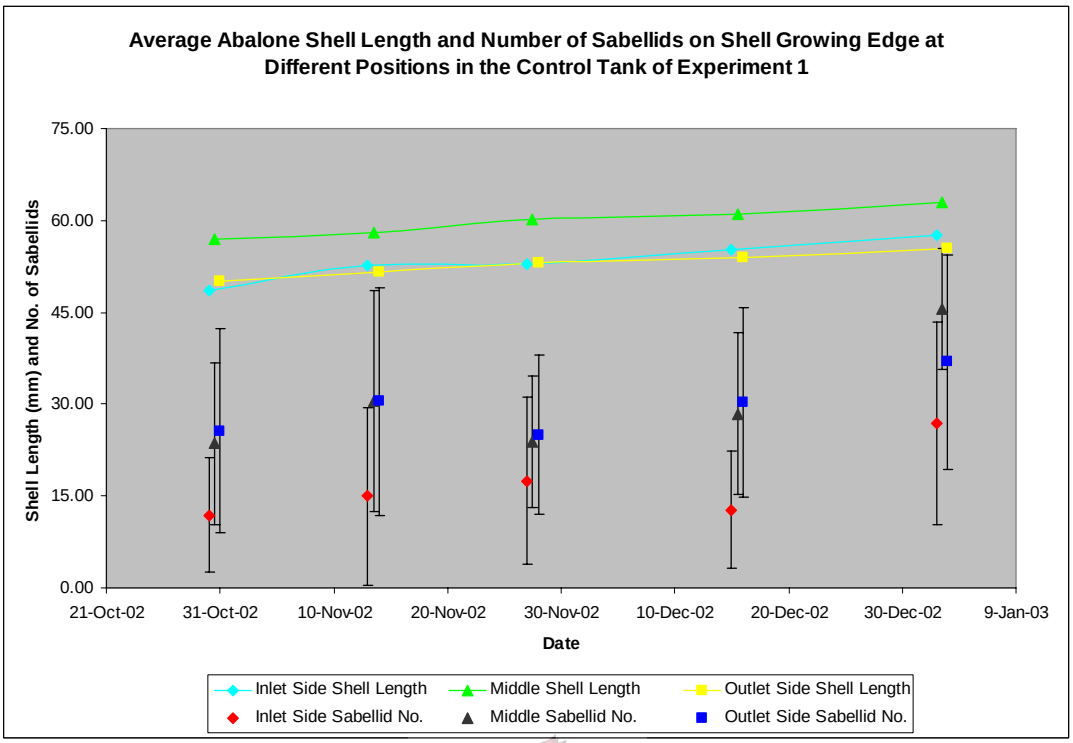

Figure 2.3 Average abalone shell length (mm) and the number of sabellids on the shell growing edge at the inlet, middle and outlet of the Control tank for Experiment 1.

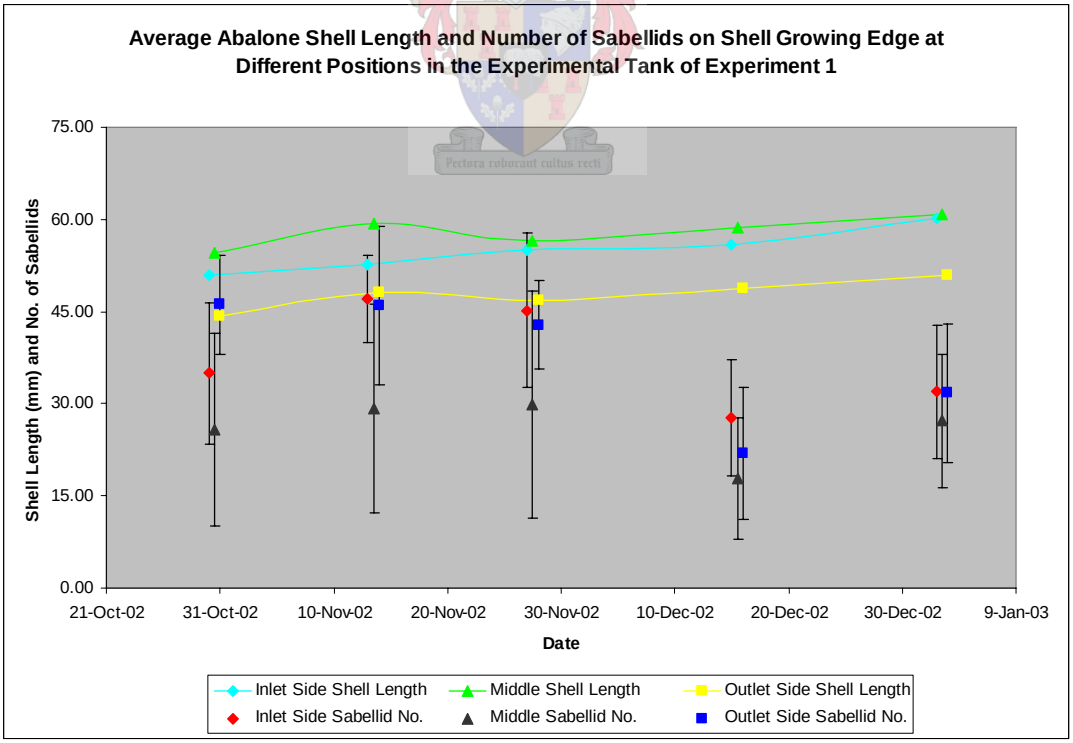

Figure 2.4 Average abalone shell length (mm) and the number of sabellids on the shell growing edge at the inlet, middle and outlet of the Treatment tank for Experiment 1.

2.3.2.2 Total Suspended Solids

The statistical analysis at the end of the experiment showed there was a significant statistical difference for location ($p=0.0208$) and between treatments ($p=0.0152$) for total suspended solids, but there was no significance in the treatment and location interaction ($p>0.05$).

Table 2.4 Descriptive statistics of mean turbidity (Formazin Turbidity Units) between location for the duration of the trial.

Location	Mean (+/- s.d.)
Outlet	0.814^{a} +/- 0.401
Bottom End	0.757^{a} +/- 0.398
Top End	0.588^{b} +/- 0.2509

For both the control and experimental tank it can be observed (Table 2.4) that the total suspended solids is greater at the outlet and outlet side of the tank compared with the inlet side of the tank which is to be expected as, as the water column moves toward the outlet side of the tank there is a gradual accumulation of suspended solids.

Table 2.5 Descriptive statistics of mean turbidity (Formazin Turbidity Units) between treatments for the duration of the trial.

Treatment	Mean (+/- s.d.)
Control	0.948^{a} +/- 0.261
Filtration	0.491^{b} +/- 0.297

Table 2.5 illustrates that filtration reduced the total suspended solids by about 50% compared to the control. It was also observed that filtration effectively reduced the amount of suspended particulate in the experimental tank at all the sample positions when compared the results of the control tank.

2.3.2.3 Particulate Size Distribution

At the end of the trial there were no significant statistical associations ($p=0.1797$) between the treatments or the different sieve sizes. However, it was observed that filtration reduced the particulate load in the experimental tank by 23.65% (Figure 2.5 and 2.6) compared to the control tank.

From Figure 2.5 and 2.6, interesting observations can be made. In both the control and treatment tank, it was observed that there was a higher particulate loading in the

optimum size range for sabellid feeding of 20-30 micron than that found in the 41 micron size range. In the experiment tank it was also observed that there was more particulate in the 20 micron size class than in each of all the other size ranges. It is deduced that the 40 micron sand filter is effectively filtering the larger particulates from the water, however it seems that within the sand filter, these particles are being reduced under pressure and reintroduced into the tank in excess quantities of the optimum size range for sabellid feeding. It is believed that problem was further compounded during periods when the filter was not backwashed at the scheduled times and could further explain the rapid increase in sabellid counts at the end of the experiment as explained in 2.3.2.1 above.

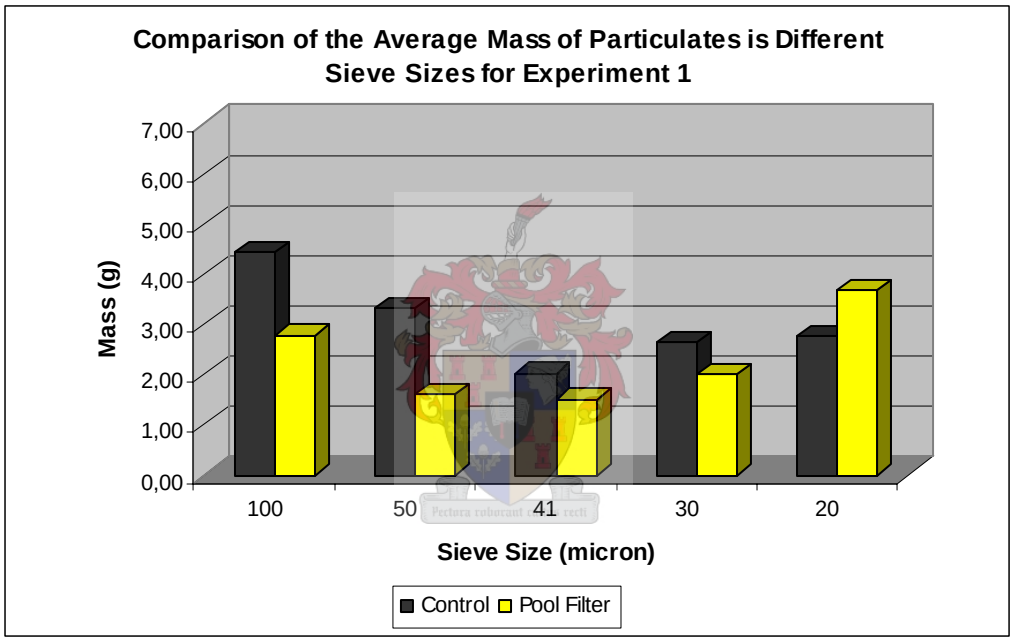

Figure 2.5 A comparison of the average mass of particulates per sieve size in Experiment 1.

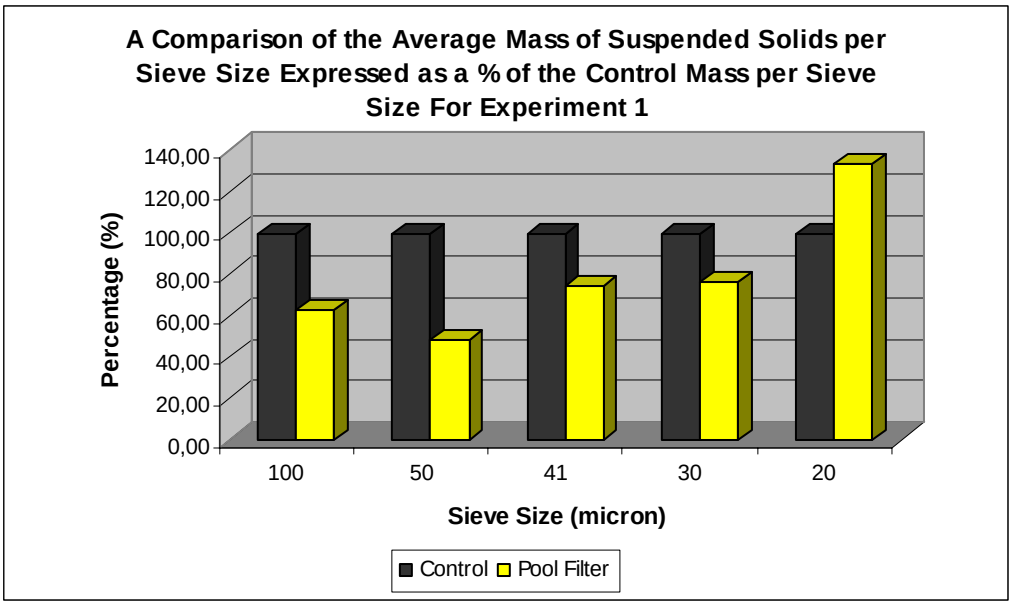

Figure 2.6. A Comparison of the average mass of particulates expressed as a percentage of the control mass per sieve

2.3.2.4 Toxic Ammonia

A toxic ammonia level of 0.018-0.020 is considered to be the maximum allowable level in an abalone production system before negative metabolic reactions begin (N. Dormehl, *pers. comm.*). pH levels are critical in determining the toxic ammonia levels. At a pH=8.28 non toxic NH_3 is rapidly converted to toxic NH_4^+ (Timmons; *et al*, 2002, Loubser, *pers. comm.*). The toxic ammonia levels recorded for the duration of the experiment were at tolerable levels and no cause for concern (Figure 2.7).

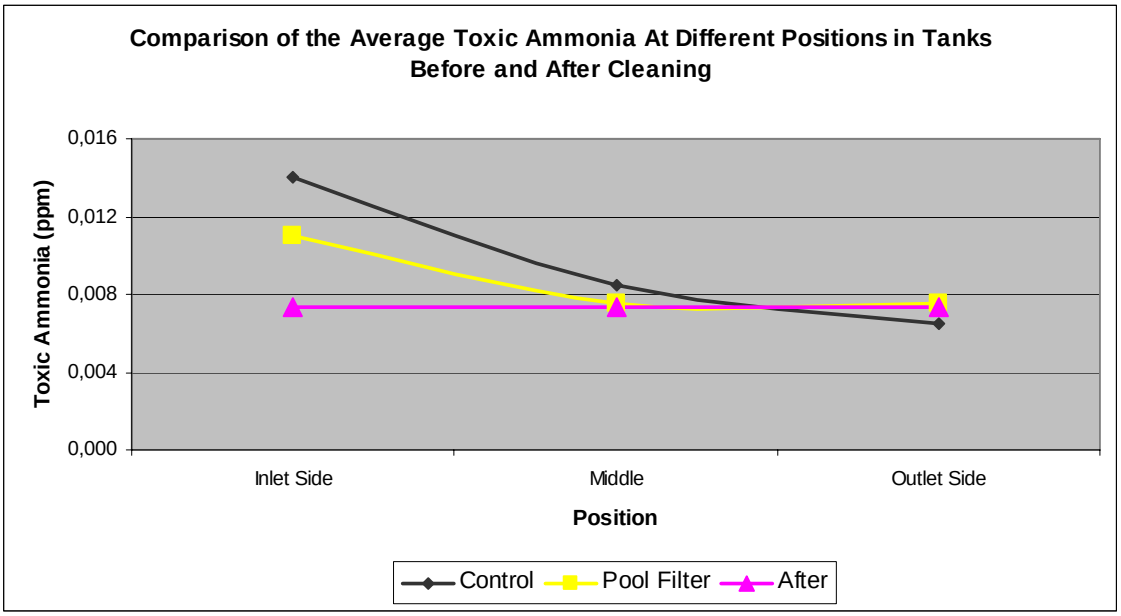

Figure 2.7 A comparison of the toxic ammonia levels at different positions in the control and experimental tank before and after cleaning.

It was further observed that the ammonia levels were higher at the inlet side of the tank before cleaning and gradually reduced along the length of the tank. This indicated that as the water from the inlet side of the tank moved toward the outlet side, the kelp fed to the abalone in the baskets was still metabolically active and actively absorbing the nitrates in the water and thereby reducing the toxic ammonia levels. This was confirmed by conducting separate trials comparing tanks with and without kelp for toxic ammonia levels (data not included).

2.3.2.5 Water Quality Parameters

The average temperature of the control and treatment tank was 16.2°C and 16.8°C respectively. The higher temperature of treatment tank could be atributed to friction of water movement through the filtration unit. An increase in temperature increases the metabolic rate of abalone (Hahn, 1989) and an average difference of 0.6°C, observed here, could potentially be beneficial to abalone production over an extended period of time, especially during the colder winter periods.

The dissolved oxygen content was only slightly higher in the experimental tank in most positions compared to the control. A more significant increase was observed at the outlet side of the tank (Figure 2.8) which was more comparable with the values

recorded in the middle of the tank. Just as temperature has an effect on the metabolic rate of abalone, so does available oxygen and the improved dissolved oxygen could have an effect on the growth rate of the abalone at the outlet side of the tank in this system over time. However, this was not observed in this experiment as growth rates were comaparable at the outlet side of the control and experimental tanks for the duration of this experiment.

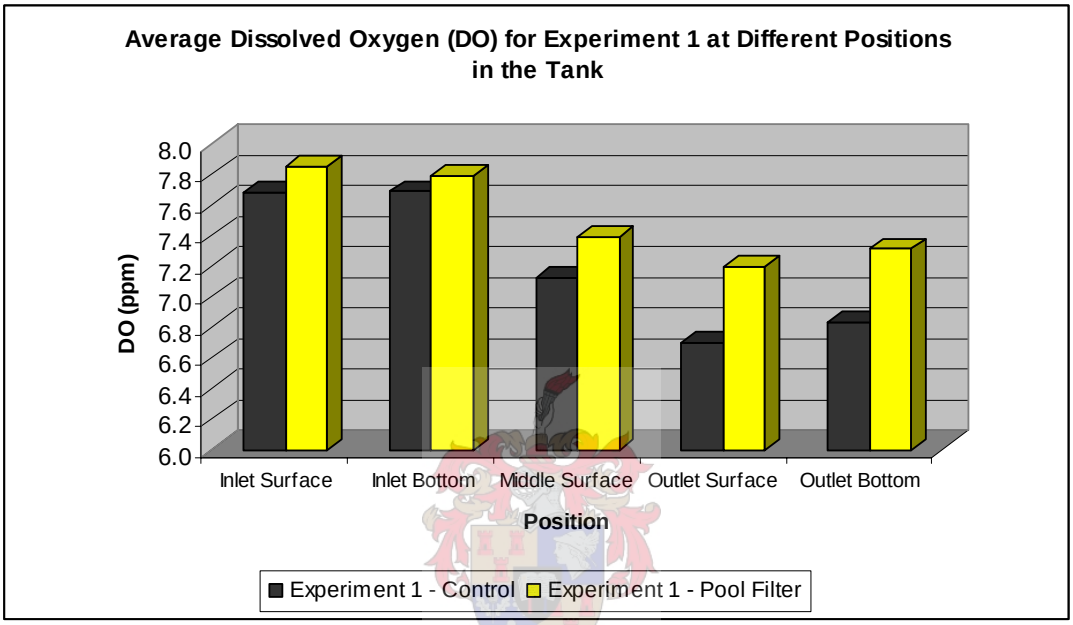

Figure 2.8 A comparison between the control and experimental tank of the average dissolved oxygen (ppm) recorded at different positions in Experiment 1.

2.3.3 Conclusion

Although filtration did reduce the amount of total suspended solids, it also seemed to increase the the amount of particulate in the 20 micron size class which is within the optimum size class for sabellid feeding. For this experiment, filtration alone had no significant effect on sabellid larval settlement or on abalone growth rate and therefore based on these results, we have to accept the null hypothesis that a reduction in particulate loading through filtration by means of a standard pool filter method will not reduce sabellid larval settlement and hence have no effect on abalone growth rate.

2.4 EXPERIMENT 2: EVALUATION OF METHODS OF ON-FARM REDUCTION OF PARTICULATE

In practice, even if it is proven that a reduction in particulate loading causes a reduction in sabellid infestations, it would not be feasible to fit a pool filter to every tank in an abalone production facility to reduce the particulate load in the tanks. Filtration methods had to be developed and tested which would prove cost effective, require the minimum amount of change to already existing abalone production tanks, and cause as little disruption to the production process. The only two forces available at each tank to drive filtration units are the respective air and water supply systems.

The primary purpose of the air supply to an abalone tank is to improve the water movement and mixing. It increases the surface area exposed to the air and mixes the fresh incoming water more efficiently and is therefore responsible for the majority of the water movement in the tanks. But, as discussed earlier, it is also responsible for causing faeces and other organic matter, the feed source of the sabellid, to be kept in suspension. In all production facilities visited during the cause of the investigation the air lines rest on the bottom of the tank, enhancing the suspension of particulate matter. It can also be responsible for the spread of the sabellid by dislodging the larvae from the host causing them to become free floating (Culver; *et al*, 1997) and "swim" at the surface attached to a mucous thread (Ruck & Cook, 1998) and possibly settle on other abalone. Therefore, the air supply and its dynamics in an abalone tank became the focus of attention in how it could be utilized to either drive filtration or settlement units, or reduced to reduce the amount of suspended solids.

Three methods of reducing the particulate loading were developed and tested during this experiment. The first two methods of reducing the particulate load in abalone production tanks was based on the concept of an under-gravel filter usually found in most common household fish tanks. This system of filtration functions by means of air lift pumps drawing water to the base of the tank through a grid under the gravel, and hence through the gravel. The airlift pump operates on the basis of a gas, usually air, that is injected at the base of a submerged riser tube. As a result of the gas bubbles suspended in the fluid, the average density of the two-phase mixture in the tube is less than that of the surrounding fluid. The resulting buoyant force causes a pumping action (Timmons, Ebeling, Wheaton, Summerfelt & Vinci, 2002). A grid was designed to fit in the bottom of the tank with six airlift risers which would draw water downward in the tanks and hence the suspended solids.

One of these two methods involved using airlift pumps to have a second function in driving hydrocyclone filter units built on site. Hydrocyclones employ the principle of centrifugal sedimentation, i.e., the suspended solid particles are subjected to centrifugal acceleration, which makes them separate from the liquid more rapidly by effectively increasing their density. The rotational flow of the inlet water imparts a centrifugal motion in the particles that causes the heavier particulate material to move to (or remain at) the outer portion of the vessel. Simultaneously, the particles are affected by gravity, which causes them to fall through the water, and move towards the bottom center drain (Timmons; *et al*., 2002).

For the third method, it was observed that the air in a tank causes distinct water flow patterns between the baskets housing the abalone and this aspect was utilized in an attempt to reduce the particulate loading. This method relied on settlement of suspended particulates in a gutter unit.

2.4.1 Materials and Methods

Four abalone tanks in a production facility (different to that of Experiment 1) were randomly selected, three as treatment tanks and the other as a control tank. Eighteen baskets, with abalone of 65 – 70mm size class distribution and stocking density of 250 abalone were placed in each of the tanks and their specific positions tagged and kept in those positions for the duration of the experiment (Table 2.6).

All the tanks received equal volumes of fresh incoming seawater and air was introduced into the tanks. The abalone received a ration of Kelp (*E. maxima*) and Abfeed® as per the usual production protocols. The tanks were drained and cleaned every week.

Table 2.6 Layout of tanks and baskets for treatment and control groups in Experiment 2. (n = sample size)

Treatment Tanks		Control Tank	
Basket 1 (n = 10)	Basket 2 (n=10)	Basket 1 (n = 10)	Basket 2 (n=10)
Basket 3 (n=10)	Basket 4 (n=10)	Basket 3 (n=10)	Basket 4 (n=10)
Basket 5 (n=0)	Basket 6 (n=0)	Basket 5 (n=0)	Basket 6 (n=0)
Basket 7 (n=10)	Basket 8 (n=0)	Basket 7 (n=10)	Basket 8 (n=0)
Basket 9 (n=10)	Basket 10 (n=10)	Basket 9 (n=10)	Basket 10 (n=10)
Basket 11 (n=0)	Basket 12 (n=10)	Basket 11 (n=0)	Basket 12 (n=10)
Basket 13 (n=0)	Basket 14 (n=0)	Basket 13 (n=0)	Basket 14 (n=0)
Basket 15 (n=10)	Basket 16 (n=10)	Basket 15 (n=10)	Basket 16 (n=10)
Basket 17 (n=10)	Basket 18 (n=10)	Basket 17 (n=10)	Basket 18 (n=10)

2.4.1.1 Bottom Filtration and Settlement Treatment

The first method involved constructing a grid of PVC pipe with six airlift risers and placing it in the base of an abalone tank (Figure 2.9). The grid and position of the airlifts had to be designed in such a manner that the risers were positioned between the baskets and sturdy enough to withstand being manhandled during the tank cleaning procedure, and that the grid did not disrupt cleaning procedures. The grid had holes drilled of equal size and distance apart as calculated for maximum efficiency of water movement (the sum of the hole diameters must not exceed that of the diameter of the main line) per airlift pump. Each airlift had one 8mm and one 6mm airline inserted into the base. The air lines in the tank were raised above the grid to just below the abalone baskets, effectively creating a "dead zone" of little or no water movement, allowing settled particulate matter to remain settled. The airlift pumps dumped the water into gutters running along the edge of the tank, particulates settled in the gutter and the water overflowed back into the tank effectively doubling the water turnover in the tank. Furthermore, an outlet line was added to the base of the tank allowing the outlet water, and thereby settled particulates, to be drawn from the length of the tank. Again, the number and diameter of holes was calculated for maximum efficiency.

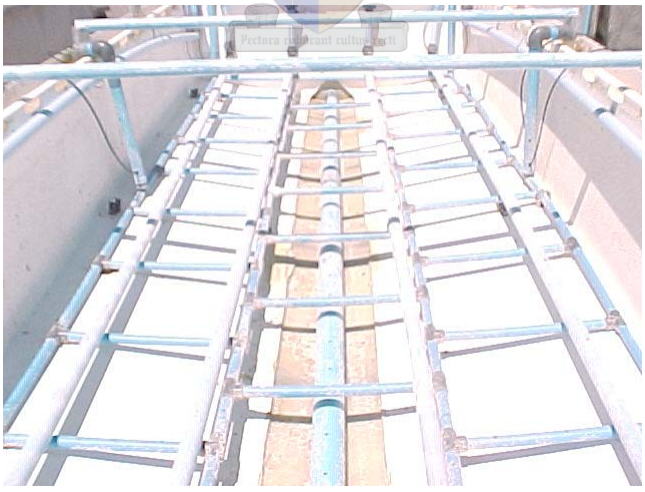

Figure 2.9 Pipe layout for the grid design and airlift pumps, drain and airlines.

2.4.1.2 Cyclone Filtration Treatment

An identical grid as the one described in section 2.4.1.1 was constructed for this treatment. The airlines were also lifted above the grid and just below the baskets, causing the "dead zone". The water pumped by the airlifts was collected into a single pipe running along the top edge of each side of the tank which then flowed into a hydrocyclone filter unit constructed on site (Figure 2.10). It was initially found that the unit was too small for the volume of water pumped by the airlifts and a second unit was added so that thee airlifts on one side served one cyclone and the three on the other served the second cyclone. The outflow water from the cyclone units was returned to the abalone tank. No alterations were made to the outflow of the abalone tank. The incoming water lines were raised from the bottom of the tank to half way up either side of the tank in order to prevent incoming water from stirring up settled particulates as well as to assist in forcing a downward water movement.

Figure 2.10 Pipe layout of grid and airlifts, the collection lines on the top of the tank and the two hydrocyclone filters at the top end.

2.4.1.3 Sedimentation Treatment

Based on observations mentioned above, this trial simply required standard rainwater guttering, usually found on houses, modified slightly, to be placed along the length of the tank between the baskets (Figure 2.11). Slots were cut into the side of the guttering in positions where the water flow was greatest between the baskets. The gutter was placed between the baskets at a slight gradient towards the one end to allow the collected water to flow out one end. PVC baffles were placed in the guttering, forcing

the water to follow a specific pattern and allowing the particulates to settle out (Figure 2.11). No other modifications were made to the tank pipe work.

Figure 2.11 Modified PVC guttering with baffles, in the centre of the tank between the abalone baskets.

All data collected from the experimental systems was collected from the control to try and gain a meaningful correlation between them. At the start of the experiment, a non-destructive, random sample of abalone were measured and the number of sabellid larvae on the growing edge counted. Ten (10) abalone were sampled from each of the first four (4) baskets (total of 40) at the inlet side of the tank, ten from each of four baskets in the middle of the tank and ten from each of the baskets at the outlet side of the tank. The same individual abalone were used to measure length and count the number of sabellid larvae on the growing edge of the shell. Every four weeks, the same sampling was conducted to determine growth rate of the abalone and sabellid larvae settlement. The same sampling methodology was used as described in Experiment 1. An ANOVA statistical analysis was conducted in order to determine any significant relationships

Every two weeks, the day before the tanks were cleaned, water samples were taken to determine the effect of water filtration on particulate loading within the tanks and the size distribution of the suspended solids. The same sampling methodology as described in Experiment 1 (Section 2.3.1) was used. An ANOVA statistical analysis was conducted on the suspended solids data sheet and a Friedman analysis on the particulate loading data sheet in order to determine any significant relationships.

Also, just before the tanks were cleaned water samples were collected from the inlet side, the middle and outlet side of the tanks to test the toxic ammonia content. A second sample from the center of the tank was collected once the tanks had completely refilled with water after cleaning as it was assumed that ammonia toxicity would be uniform in the tank immediately after cleaning. The samples were tested using a standard Palin Test method in a spectrophotometer from Orbeco-Hellige.

Water quality parameters (Dissolved Oxygen (DO), Temperature (T) and pH) were collected weekly in order to determine if filtration had any positive or detrimental effect on water quality. Measurements were taken at the inlet side of the tank, both at the surface and bottom of the water column, in the middle of the tank at the surface and at the outlet side, at the surface and bottom of the water column. All measurements were taken with water analysis instruments from Orbeco-Hellige. No statistical analysis was conducted on the ammonia or water quality parameter data sheets as the observed differences were neglible and it was assumed that there would be no significant statistical differences.

2.4.2 Results and Discussion

2.4.2.1 Abalone Shell Length and Sabellid Larval Counts

At the end of the experiment there was a statistical significance of abalone shell length for position ($p=0.0008$) and a significant difference for the number of days ($p<0.0001$). For sabellid larval settlement counts there was a significant interaction between the treatments and position ($p=0.0304$) and there was also a significance over the number of days ($p<0.0001$).

Table 2.7 and 2.8 confirms an assumption made at the start of the experiment that abalone growth rates (shell length) were lower and sabellid larval settlement higher at the outlet side of the tank, even though this was a very small difference. This could be due to two reasons, poorer water quality, affecting abalone metabolic rates, and higher suspended particulate loading, an increased feed source for the filter feeding sabellids.

Table 2.7 Descriptive statistics of mean shell length (mm) between different tank positions for the duration of the trial.	
Position	**Mean Length** (mm) (+/- s.d.)
Inlet Side	71.9895[a] +/- 4.265
Middle	71.292[b] +/- 4.043
Outlet Side	70.9645[b] +/- 4.012

It is assumed that because water quality is better at the inlet side of the tank and then gradually deteriorates along the length of the tank, that abalone in better water quality conditions have a better metabolic rate and hence improved growth rate. A slower abalone growth rate allows sabellid larvae enough time to settle and the abalone to produce a nacre tube and the increased food supply has obvious benefits to the sabellid, therefore an expected higher sabellid count on abalone shells at the outlet side of the tank, as was the case in this trial.

Table 2.8 Descriptive statistics of mean Sabellid larval counts and the interaction between treatments and tank position for the duration of the trial.		
Treatment	**Position**	**Mean** (Count)
Control	Inlet Side	39.281[b] +/- 13.630
Control	Middle	48.581[f] +/- 15.377
Control	Outlet Side	60.975[h] +/- 14.033
Bottom Filtration	Inlet Side	35.956[a] +/- 17.417
Bottom Filtration	Middle	43.062[c] +/- 14.349
Bottom Filtration	Outlet Side	44.500[cd] +/- 19.330
Cyclone Filtration	Inlet Side	43.519[c] +/- 16.699
Cyclone Filtration	Middle	47.269[ef] +/- 17.160
Cyclone Filtration	Outlet Side	46.406[def] +/- 18.607
Sedimentation	Inlet Side	48.363[f] +/- 15.088
Sedimentation	Middle	45.488[cde] +/- 20.750
Sedimentation	Outlet Side	55.331[g] +/- 16.018

An increase in abalone shell length for all the tanks in the experiment over time for the duration of the experiment as depicted in Table 2.9 is to be expected as normal abalone shell growth should increase over time. The growth rates of the abalone in the four tanks are comparative for the duration of the experiment (Figure 2.12).

Table 2.9 Descriptive statistics of mean shell length (mm) between days for the duration of the trial.

Days	**Mean Length (mm) (+/- s.d.)**
0	68.096[a] +/- 2.493
28	69.677[b] +/- 2.872
63	72.088[c] +/- 2.822
91	75.796[d] +/- 3.477

Table 2.10 Descriptive statistics of mean Sabellid larvae counts between days for the duration of Experiment 2.

Days	**Mean Count (+/- s.d.)**
0	54.7406[a] +/- 18.886
28	50.938[a] +/- 17.787
63	42.275[b] +/- 14.7782
91	38.292[b] +/- 14.312

There was, however, an observed slight improvement in the growth rates of the abalone in the experimental tanks with the airlifts and bottom sedimentation, and cyclone filtration when compared with the control. This may improve over time and could be confirmed if the experiment is repeated for a longer period of time.

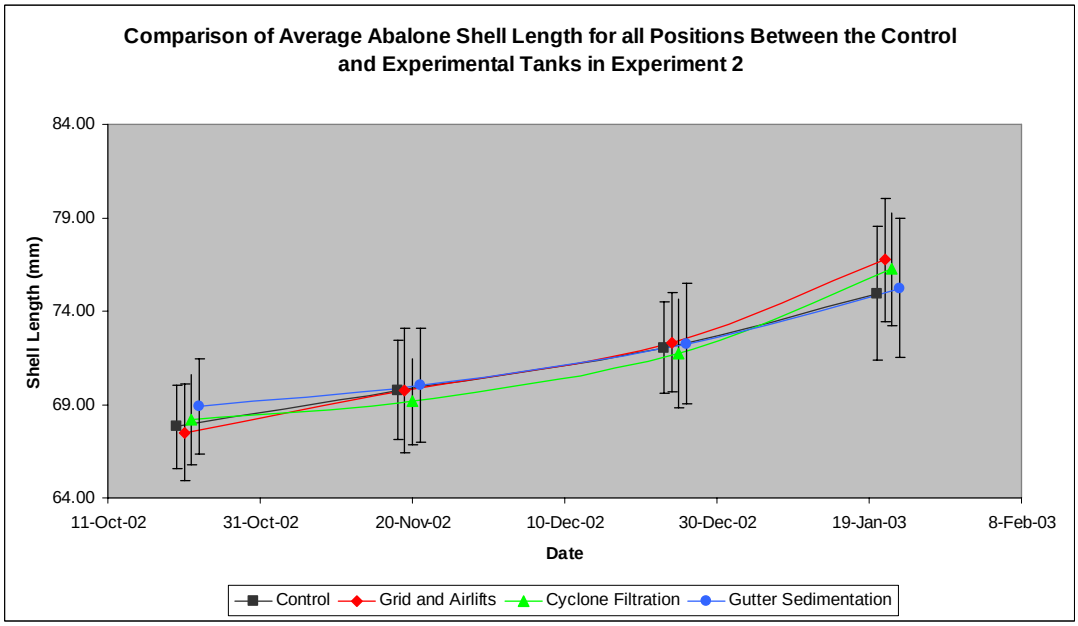

Figure 2.12 A comparison between the control and experimental tanks of the total tank averages of abalone shell length (mm) for the duration of Experiment 2.

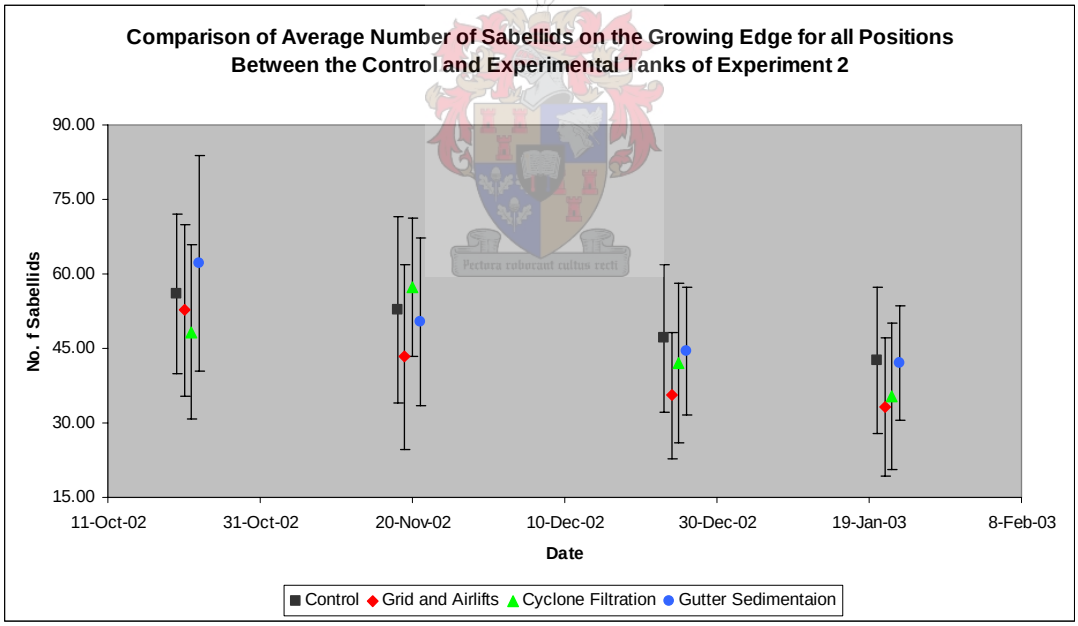

Figure 2.13 A comparison between the control and experimental tanks of the total tank average of Sabellid larval counts for the duration of Experiment 2.

A further observation which can be seen in Table 2.10 and figures 2.12 and 2.13 is that as abalone shell length increased, sabellid larval settlement decreased over time for the duration of the experiment. This seems to be more directly related to normal abalone growth rather than a result of the filtration mechanisms as the control tank had an comparative growth rate as well as a comparative decrease in sabellid larvae counts.

As discussed earlier, abalone of the size class used in this trial begin to outgrow sabellid infestations by depositing shell faster that what sabellid larvae are able to settle and this seems to be the case here.

2.4.2.2 Total Suspended Solids

There was a statistical significant difference of total suspended solids at the different locations ($p=0.0021$) and between treatments ($p<0.0001$), but there was no significant interaction between treatment and position ($p=0.5463$) as seen from the results in Table 2.11.

Table 2.11 Descriptive statistics of mean turbidity (Formazin Turbidity Units) between location for the duration of the trial.

Location	**Mean**
Top End	0.491^{a} +/- 0.176
Bottom End	0.627^{b} +/- 0.161
Outlet	0.633^{b} +/- 0.166

Total suspended solids is greater at the outlet side and outlet side of the tank compared with the inlet side of the tank which is expected as, as the water column moves toward the outlet side of the tank there is a gradual accumulation of suspended solids.

Table 2.12 Descriptive statistics of mean turbidity (Formazin Turbidity Units) between treatments for the duration of the trial.

Treatment	**Mean**
Control	0.696^{a} +/- 0.127
Bottom Filtration	0.403^{b} +/- 0.201
Cyclone Filtration	0.612^{a} +/- 0.126
Sedimentation	0.623^{a} +/- 0.141

According to Table 2.12 and Figure 2.14 the only significant reduction in total suspended solids compared to the control occured in the bottom filtration experiment as described in Section 2.4.1.1. This system of filtration effectively reduced the amount of suspended particulates in the tank at all sample positions when compared to the results of the control tank.

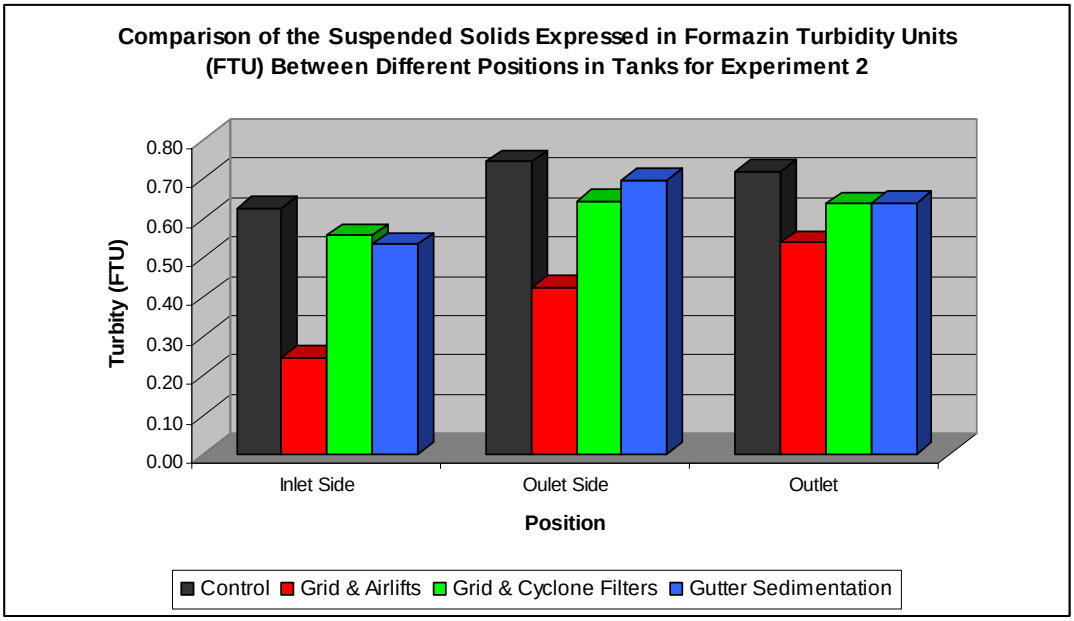

Figure 2.14 A comparison of the suspended solids at the inlet side, outlet side and outlet of the control and experimental tanks in Experiment 2

2.4.2.3 Particulate Size Distribution

No statistical interaction was observed, but an interesting observation in Figure 2.15 is that all three experimental filtration systems effectively reduced the particulate loading in the 100micron size range and only the bottom filtration mechanism had any significant difference in the optimum sabellid feeding range of 20-30 micron.

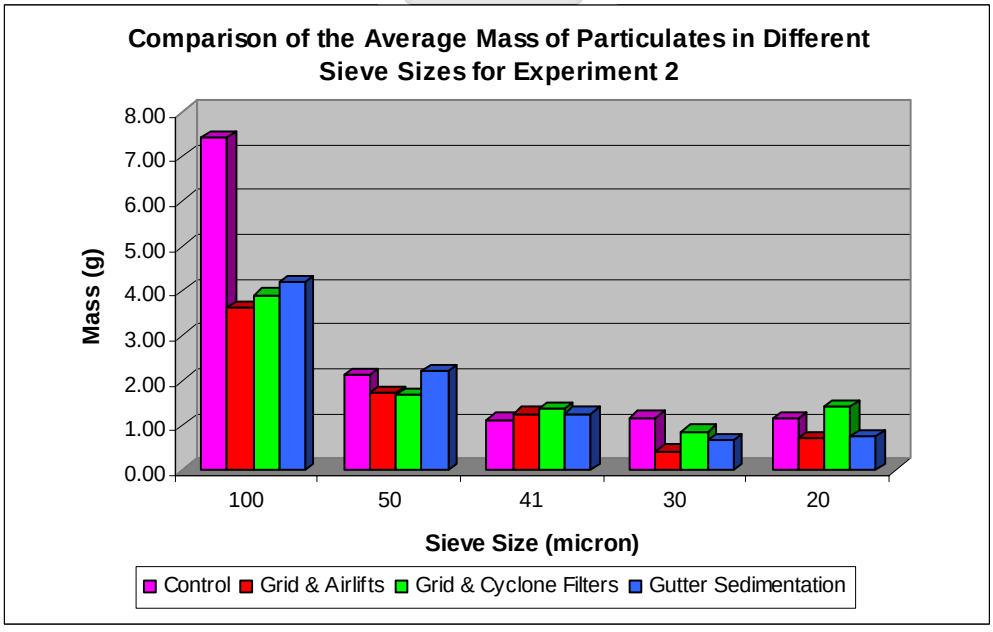

Figure 2.15 A comparison of the average mass of particulates collected in different micron sieves in Experiment 2

The total mass of suspended solids was reduced by 40%, 29% and 30% by the bottom filtration, cyclone filtration and gutter sedimentation respectively (Figure 2.16).

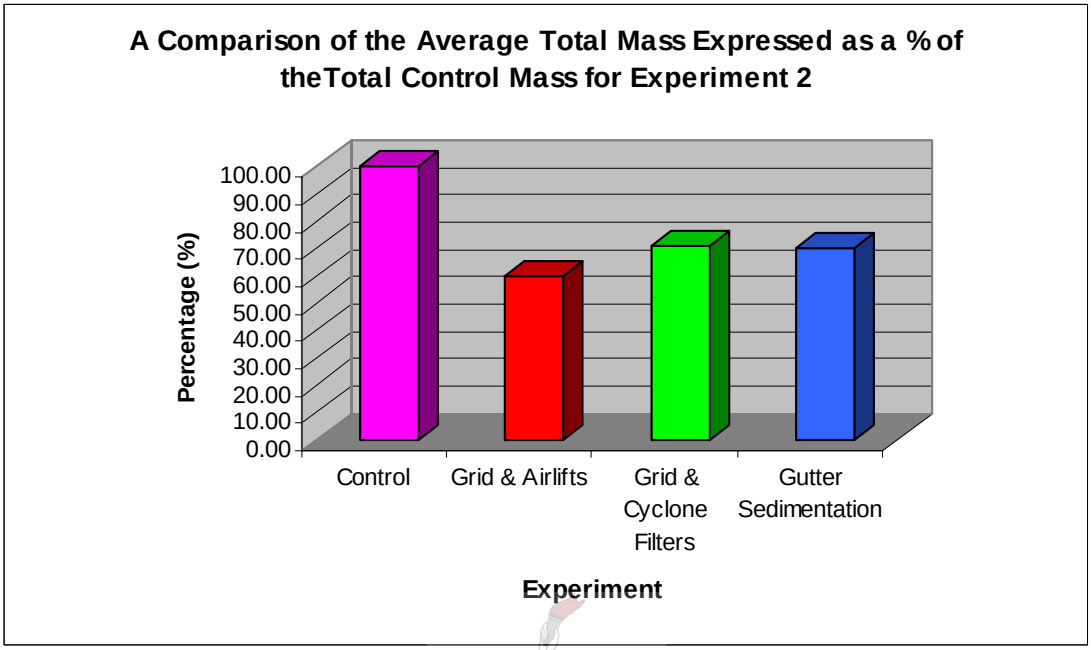

Figure 2.16 A comparison of the average total mass of particulates as a percentage of the total control mass.

2.4.2.4 Toxic Ammonia

As mentioned in section 2.3.2.4, toxic ammonia level of 0.018 to 0.020 is considered to be the maximum allowable level for an abalone production unit. The average toxic ammonia levels recorded for the duration of the experiment at the inlet side of all the tanks in the experiment were higher than this desired level before cleaning. These levels gradually reduced along the length of the tank to more acceptable levels (Figure 2.17), which indicates that as water from the inlet side of the tank moved toward the outlet side, the kelp fed to the abalone in the baskets was still metabolically active and actively absorbing the nitrates in the water and thereby reducing the toxic ammonia levels.

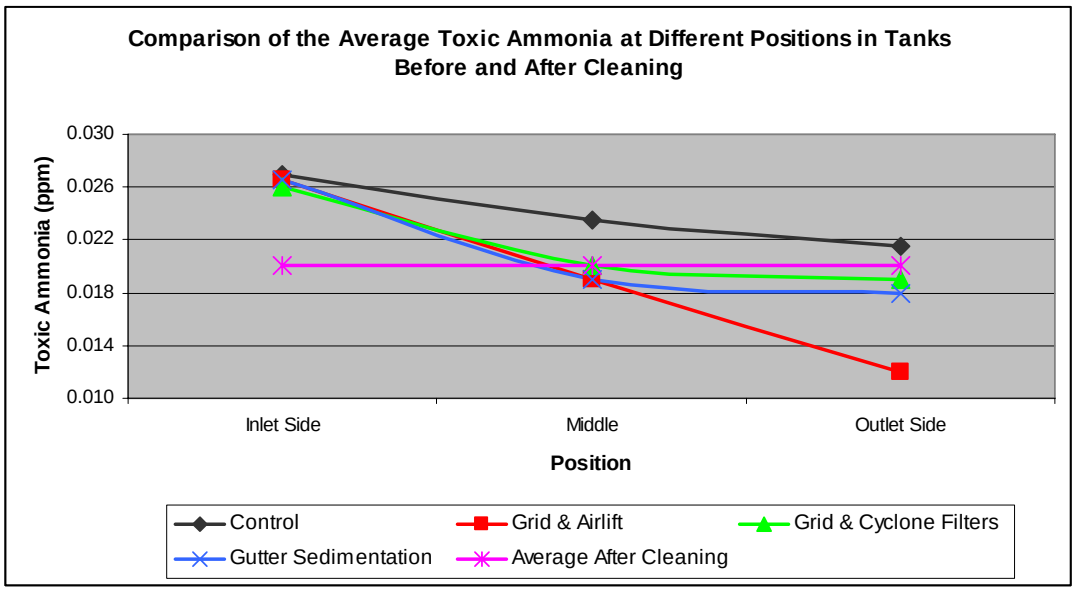

Figure 2.17 A comparison of the average toxic ammonia (NH_4) at different positions in the tanks used in Experiment 2.

However, it was also observed that the toxic ammonia levels in the bottom filtration tank reduced more rapidly to a significantly lower level at the outlet side of the tank.

This also has a definite relationship to the decreased amount of particulate loading in this tank. A reduced particulate loading would mean a reduced Nitrogen loading available for conversion to toxic ammonia. A second possible contributing factor is that because the water from the airlifts were been dumped into a gutter along the sides of the tank, more volatile nitrogenous gases were being vented off making less Nitrogen available.

2.4.2.5 Water Quality Parameters

The average temperature for the control, bottom filtration, cyclone filtration and gutter sedimentation units for the duration of the experiment were 16.90°C, 16.90°C, 16.97°C and 16.79°C respectively. Therefore temperature had no comparative effect for this experiment.

The dissolved oxygen content was only slightly higher in the experimental tanks in all positions compared to the control and therefore also had no significant comparative effect on this experiment (Graph B.1 in Appendix B).

2.4.3 Conclusion

The abalone in the bottom filtration treatment tank had a slight observed improvement in growth rate and there is the possibility that this could be as a result of the reduced suspended solid loading and the improved water quality in terms of ammonia content. However, there was no statistical difference in growth rates of the abalone or reduction of sabellid larvae on the growing edge of the shell between the control and the experimental tanks. Furthermore, it cannot be stated for certain whether the observed reduction in sabellid larvae counts over the duration of the experiment is as a result of a reduced particulate loading because there was a reduction in the control tank as well. It is known however that the size class abalone used in the experiment, are less prone to sabellid infestations as they begin to outgrow infestations which is the most likely scenario observed here. Therefore, we have to accept the null hypothesis to be true that a reduction in particulate will not cause a reduction in sabellid larval settlement and influence the abalone growth rates.

However, there is still evidence from the research conducted by Rhodes University (Britz, *et al*. 2005) that a reduction in the particulate load in an abalone production tank should have an effect in reducing a *T. heterouncinata* population and cause an improved growth rate of the abalone. The experiments in this paper should not be considered conclusive as they may just have been ineffective and alternate methods may be tested. The time period for the experiments may have been too short to observe any significant differences; therefore future experiments should be conducted for longer periods of time.

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CHAPTER 3

MITIGATION OF SABELLID INFECTION OF ABALONE BY USE OF ULTRASOUND

3.1 INTRODUCTION

In a previous study, ultrasound micro-cavitation treatment of infested abalone for 1-10 minutes killed all sabellid eggs and juveniles and destroyed feeding crowns of a high proportion of adult worms impairing feeding and reproductive activity (Loubser and Dormehl, 2000; McEnnulty, Bax, Schaffelke & Campbell). Simon (in Britz, Chalmers, Gray, Henderson, Kaiser, Simon & Winter, 2005) also suggested that the location of the spermatheca of *T. heterouncinata* makes it potentially vulnerable to treatment by ultrasound. The spermatheca are just over 100µm long and it might be in the tissue that is destroyed by ultrasound and thus, if the spermatheca are destroyed, the animal will not be able to fertilize its eggs until a new spermatheca is produced and more sperm collected. Therefore it was decided to investigate the potential of using ultrasound as a mitigation method.

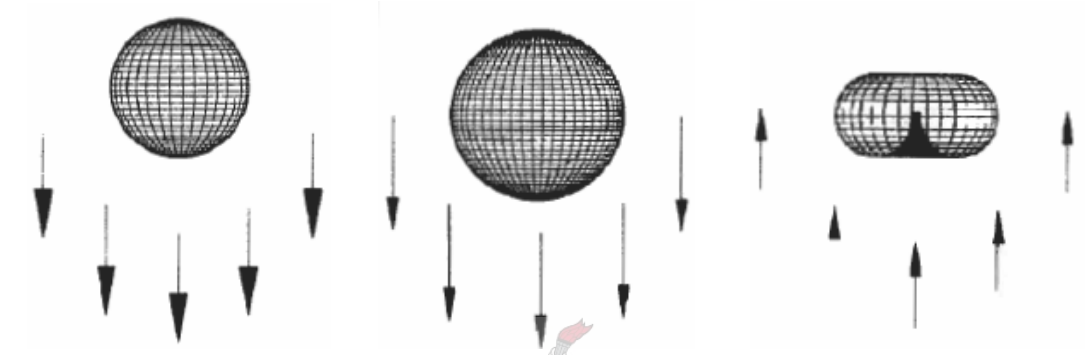
3.2 ULTRASOUND CAVITATIONAL PROCESS

Sound waves are composed of both expansion cycles, where molecules of liquid in the cleaning tank are pulled apart, and compression cycles, where the molecules of liquid are compressed together. If enough energy exists during the expansion cycle to overcome the binding forces which hold the liquid molecules together, the molecules are ripped apart, creating a gap in the fluid medium. This partial vacuum gap is known as a cavity. The cavity, after formation, continues to grow in size for the entire duration of the expansion cycle. The longer the expansion cycle in duration, the larger the cavity will grow.

Upon completion of the expansion cycle, the sound wave converts to the compression cycle, which causes a violent collapse of the cavity (Figure 3.1). This collapse creates a microscopic jet of liquid which travels at an estimated speed of 400+ kilometers per hour. If this weren't enough, the rapid compression of gasses contained within the cavity during implosion causes the area immediately surrounding the cavity to momentarily superheat to temperatures approaching that of the surface of the sun,

over 5000 degrees Celsius. However, due to the microscopic size of the cavities, and the enormous temperature difference between cavity and surrounding fluid, damage caused by heating never occurs, and fluid heats up only very slowly.

This combination of superheated cavities, and violent cavity implosions, creates a cleaning environment which cannot be equaled by any other mechanical means. Microscopic cavities continuously scour any objects submerged in the cleaning fluid.



A. During expansion, transducers pull downward, creating a rip in the fluid medium.

B. The cavity grows in size for duration of expansion cycle of sound wave.

C. When compression begins, cavity implodes, causing A jet of liquid responsible for cleaning

Figure 3.1 Phases of the Ultrasound Cavitational Process (www.zenith-ultrasonics.com)

3.3 THE ANAESTHESIA OF *H. midae* AND *T. heterouncinata*

The problem faced with using ultrasound, is how to get the ultrasound treatment to be effective on the sabellid while still in the shell of a live abalone, without causing too much stress on the abalone. Sabellids are able to detect any chemical or osmotic pressure changes in the water, withdraw into the burrow and "cap" it with a bubble, effectively isolating themselves in the burrows (Ruck, Hugo & Loubser, pers. comm.). This renders the ultrasound ineffective on the sabellid as ultrasound works directly on a surface. An effective anaesthesia had to be found that anaesthetises the abalone and causes the sabellids to be exposed out of their burrows.

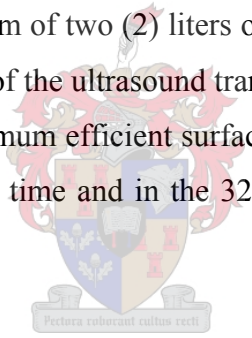
A prerequisite of the U.S.A. Food and Drug Administration (FDA) is that a drug used in aquaculture should be safe to the animals. Mortality-free anaesthesia is also an important financial consideration in aquaculture. It is therefore important to know how long animals can be exposed to an anaesthetic without any mortalities, and whether the animals can survive the anaesthetic exposure times required during commercial farming

practices. A "safe anaesthesia" implies that the anaesthetic has no immediate detrimental or long term sublethal effect on the abalone, that it is safe for the farmer, the consumer and the environment. Prolonged exposure of abalone to 14g.100ml-1 $MgSO_4$ revealed that the animals can be exposed for up to six hours to the anaesthetic without any mortalities (White, 1995; White, Hecht & Potgieter, 1996).

Day (1974) mentions that for observation/identification sabellids can be relaxed using magnesium sulphate ($MgSO_4$). Hugo (*pers. comm.*) suggested that abalone left in a $MgSO_4$ solution of 36ppm for exactly one hour would cause the sabellids to become anaesthetized and literally hang out of their burrows.

3.4 ULTRASOUND TREATMENT WITH LABORATORY AND INDUSTRIAL ULTRASOUND BATHS

This experiment was conducted using two different size and frequency ultrasound baths, a 40KHz laboratory ultrasound bath and an industrial 32KHz ultrasound bath. The 40KHz bath could hold a maximum of two (2) liters of water and the 32KHz bath, forty (40) liters. According to the size of the ultrasound transducers, a maximum efficiency is attained only according to a maximum efficient surface area. For the 40KHz bath, only two abalone could be treated at a time and in the 32KHz bath thirty abalone could be treated.



3.4.1 Materials and Methods

Fifty (50) abalone were randomly selected from a production tank and kept in the lab to purge for seven days to minimize stress during the treatment. A Magnesium Sulphate ($MgSO_4$) solution was prepared using distilled water and adding $MgSO_4$ crystals until a concentration of 36ppm was measured using an Orbeco-Hellige electronic hygrometer. Sixteen of the purged abalone were placed in the $MgSO_4$ solution and after one hour the sabellids were anaesthetized and "hanging" limp from the burrows totally exposed. These abalone were then treated, two at a time, with ultrasound in the 40KHz bath containing two litres of distilled water. After the treatment, they were immediately placed in fresh, aerated seawater and the bin marked. The water from the ultrasound bath was then drained through a sieve to collect any sabellids that might have been damaged by the ultrasound. If any, they were examined under a microscope and photographed.

Thirty of the purged abalone were then placed in a 36ppm $MgSO_4$ solution for one hour, after which they were placed in the industrial 32KHz bath containing forty (40) litres of fresh tap water, and treated for 1 minute. After the treatment, they were placed in fresh, aerated seawater and the bin marked. All the abalone were then monitored for recovery and the number of mortalities recorded after 48 hours.

Just prior to the experiment commencing, the abalone in each trial were measured with a vernier calliper and the number of sabellid larvae on the growing edge counted. Sixty days after the treatment, the abalone were measured and the number of sabellid larvae on the growing edge were counted again.

An ANOVA statistical analysis was conducted in order to determine any significant relationships.

3.4.2 Results and Discussion

When observed under a microscope it was noticed that the feeding crowns of the sabellids were destroyed or ripped off completely in most instances (Figure 3.2a, b). When one compares the feeding crown of a healthy specimen such as in Figure 3.3 with that of the one in Figure 3.2b, the effect of the ultrasound is quite evident. There was also evidence of sabellids being removed from their burrows and some were completely destroyed by the ultrasound.

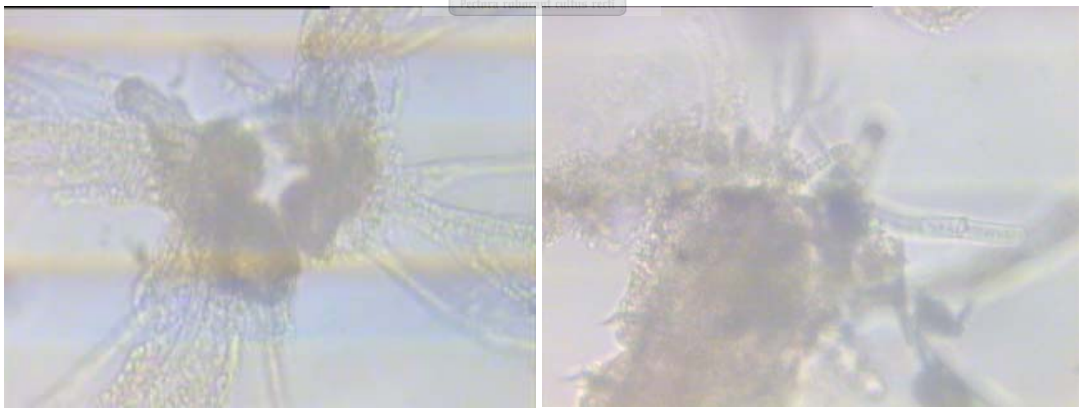


Figure 3.2 Feeding crown of *T. Heterouncinata* destroyed by ultrasound treatment.

The ideal is to have all the sabellids being removed from their burrows and destroyed in the ultrasound bath. However, this procedure did not affect 100% of the *T. heterouncinata* population and some did survive but the number of those which survived without any damage was extremely low. Sabellids which survived the ultrasound but had their feeding crowns damaged or removed would obviously not be able to feed.

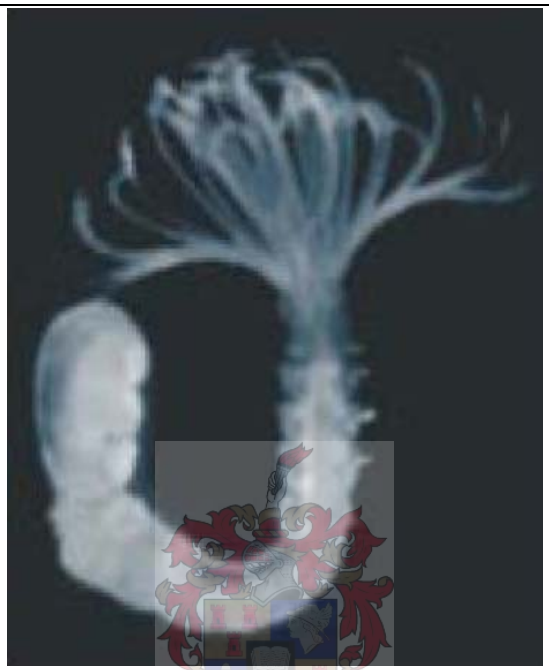


Figure 3.3 Adult sabellid displaying and intact feeding crown (picture by Carolynn Culver).

At the end of the experiment a statistical significant interaction between the treatments and time ($p<0.0001$) was detected (Table 3.1).

Table 3.1 Descriptive statistics of mean sabellid larval settlement between treatments and time for the duration of the experiment.

Treatment	**Time**	**Mean larval settlement (+/- s.d.)**
Large Bath (32KHz)	0	26.667^{b} +/- 8.751
Large Bath (32KHz)	60	15.583^{a} +/- 8.272
Small Bath (40KHz)	0	44.064^{c} +/- 11.096
Small Bath (40KHz)	60	13.250^{a} +/- 10.253

Figure 3.4 presents a graphic depiction of Table 3.1 excluding the standard deviation to show the dramatic decrease in sabellid larval settlement on the growing edge of the

abalone shells 60 days after treatment with two different frequencies of ultrasound. Abalone treated in the higher frequency laboratory ultrasound bath had a more significant decrease in larval sabellid settlement than the larger, lower frequency, ultrasound bath, therefore the higher frequency treatment was more effective.

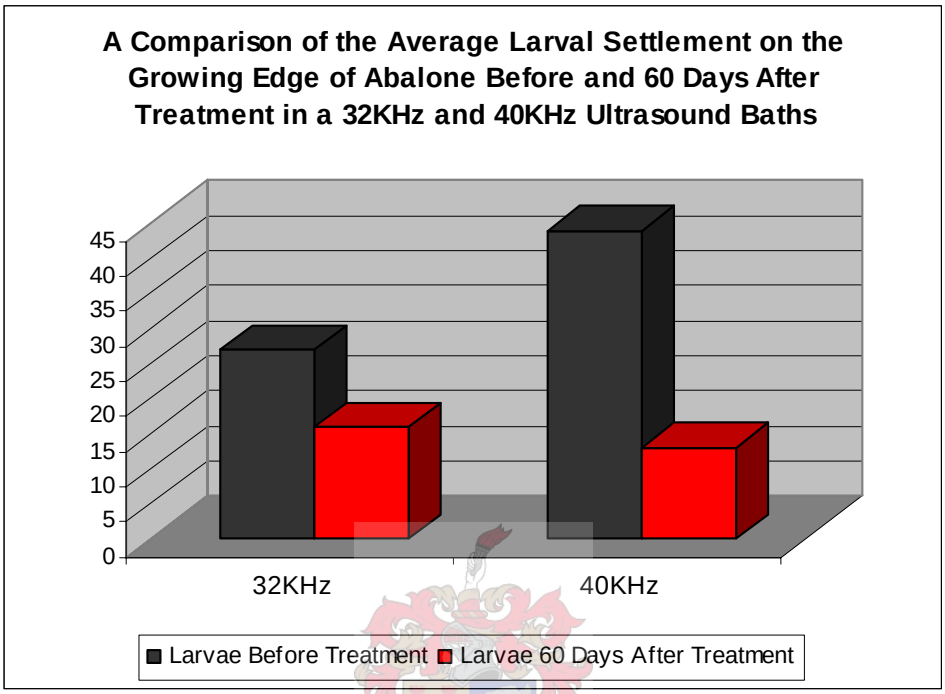


Figure 3.4 A comparison of the average sabellid larval settlement before and 60 days after treatment in 32KHz and 40Khz ultrasound baths

3.5 TREATMENT OF AN ENTIRE TANK OF ABALONE WITH ULTRASOUND.

After a number of trials, an entire tank of abalone were treated with ultrasound in the 32KHz industrial ultrasound bath. Because of the incredibly high value and in order that the abalone were not exposed to the anaesthetic or rising water temperatures for extended periods of time and risk mass mortalities, a careful plan had to be designed to choreograph the procedure.

3.5.1 Materials and Methods

An abalone tank containing 18 baskets of abalone, each stocked with a 150 abalone was selected for the experiment. A schedule was determined as to the procedure in order to conduct the treatment as quickly as possibly. The ultrasound bath could only effectively treat 30 abalone at a time which means one basket of abalone would require 5

treatments. It was determined with a team of people that we could load the bath with abalone in 1 minute, treat them for one minute and then "unload" the bath in one minute, therefore, taking fifteen minutes to complete one basket. Since the abalone had to be the anaesthetic for 1 hour, we could effectively place one abalone basket into the anaesthetic 15 minutes apart (see appendix C). A tank able to hold four abalone baskets at one time was made available next to the ultrasound bath. This tank was filled with fresh tap water (municipal drinking water) and $MgSO_4$ crystals added until a concentration of 36ppm was determined using an Orbeco-Hellige electronic hygrometer. The dissolved oxygen and temperature of the tank was monitored throughout the duration of the experiment with an Orbeco-Hellige water analysis probe. Forty liters of fresh tap water was added into the ultrasound bath. An abalone basket was placed in the anaesthesia tank and the next basket fifteen minutes later and another, fifteen minutes later and so on. A 300 liter bin was also placed near the ultrasound bath with fresh, aerated seawater and an empty abalone basket.

When the first basket had been in the anesthesia for one hour, thirty abalone were loaded into the ultrasound bath and treated for one minute with ultrasound. The abalone were then placed in the basket in the 300 liter bin and the next thirty abalone loaded. Once a basket was complete, and all the abalone were in the new basket, it was tagged and placed back into the production tank and monitored for 48 hours for mortalities. This process was repeated for all eighteen baskets.

After every two baskets, the water in the ultrasound bath was drained and replaced in order that the water temperature did not exceed $20^{o}C$ since ultrasound raises the water temperature which could cause mortalities in abalone.

If sabellid worms are reproductively active, eggs and larvae will be present. Reproductive status can be useful in projecting the likelihood of new infestations (Culver; Kuris & Beede, 1997). Before commencing with the experiment sixteen abalone from the experimental tank and sixteen from a control tank were sampled in order to determine a reproductive index. This is a destructive form of sampling and by breaking the shell apart the different stages of development are easily observed. Sixty days after the experimental treatment with ultrasound, another sixteen abalone were taken from each of the control and experimental tanks and sampled. On both occasions, the abalone were measured with a vernier caliper and the number of sabellid larvae on the growing edge counted by gently pushing back the mantle to reveal the growing edge of the shell. The abalone were shucked and the shells crushed with a pliers into a glass

Petri dish with sea water. The Petri dish was gently shaken to loosen the sabellids from the crushed shell and as many pieces of shell as possible were removed to reveal the eggs, larvae and adult sabellids. The Petri dish was placed under a microscope and the number of eggs, larvae and adults were counted. Counting ceased once 100 adults were counted. This was done for each of the sixty four samples.

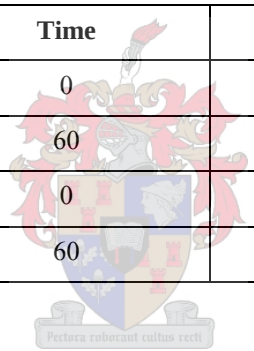
An ANOVA statistical analysis was conducted in order to determine any significant relationships.

3.5.2 Results and Discussion

At the end of the experiment, the only statistical significance for abalone shell length was for time ($p<0.0001$) which is to be expected if normal abalone growth continues (Table 3.2).

Table 3.2 Descriptive statistics of mean shell length between treatments and time for the duration of the experiment.

Treatment	Time	Mean shell length (mm) (+/- s.d.)
Control	0	68.5635[a] +/- 1.548
Control	60	73.6885[b] +/- 3.114
Ultrasound (32KHz)	0	67.9385[a] +/- 1.526
Ultrasound (32KHz)	60	73.6885[b] +/- 2.387



For the uncrushed shells there was a statistical significant interaction between treatments and time for both the number of burrows ($p<0.0001$) and number of larvae ($p<0.0001$) on the growing edge of the shells. For the crushed shells there was also an interaction between treatments and time for the number of larvae ($p<0.0001$) and the number of sabellid eggs counted in the crushed shell ($p<0.0001$).

Table 3.3 Descriptive statistics of mean number of sabellid burrows on the growing edge of uncrushed abalone shell between treatments and time for the duration of the experiment.

Treatment	Time	Mean No. Burrows (+/- s.d.)
Control	0	21.625[a] +/- 4.098
Control	60	22.063[a] +/- 5.234
Ultrasound (32KHz)	0	22.938[a] +/- 4.250
Ultrasound (32KHz)	60	14.438[b] +/- 3.265

Table 3.4 Descriptive statistics of mean number of sabellid larvae on the growing edge of uncrushed abalone shell between treatments and time for the duration of the experiment.

Treatment	Time	Mean No. of Larvae (+/-stdev)
Control	0	12.375[b] +/- 4.829
Control	60	15.375[a] +/- 4.717
Ultrasound (32KHz)	0	15.063[a] +/- 3.586
Ultrasound (32KHz)	60	8.188[c] +/- 2.455

It can be observed in the Table 3.3 and 3.4 that the number of sabellid larvae and burrows on the growing edge of abalone after treatment with ultrasound were significantly reduced by almost 50%, sixty days after treatment whereas the control abalone had a slight increase in the number of settled sabellid larvae and burrows on the growing edge.

Table 3.5 Descriptive statistics of mean number of sabellid larvae in crushed abalone shell between treatments and time for the duration of the experiment.

Treatment	Time	Mean No. of Larvae (+/-stdev)
Control	0	69.563[b] +/- 7.312
Control	60	61.000[c] +/- 9.859
Ultrasound (32KHz)	0	75.750[a] +/- 13.650
Ultrasound (32KHz)	60	36.625[d] +/- 8.725

Table 3.6 Descriptive statistics of mean number of sabellid eggs in crushed abalone shell between treatments and time for the duration of the experiment.

Treatment	Time	Mean No. of Eggs (+/-stdev)
Control	0	131.063[a] +/- 14.626
Control	60	130.125[a] +/- 17.408
Ultrasound (32KHz)	0	133.375[a] +/- 24.552
Ultrasound (32KHz)	60	65.938[b] +/- 16.377

The reason for crushing the shells and counting the eggs and larvae relative to the number of adults is to determine the reproductive index, and from the Tables 3.5 and 3.6 above and Figure 3.5. and 3.6 below, the reproductive index was reduced by more than 50% sixty days after the treatment with ultrasound.

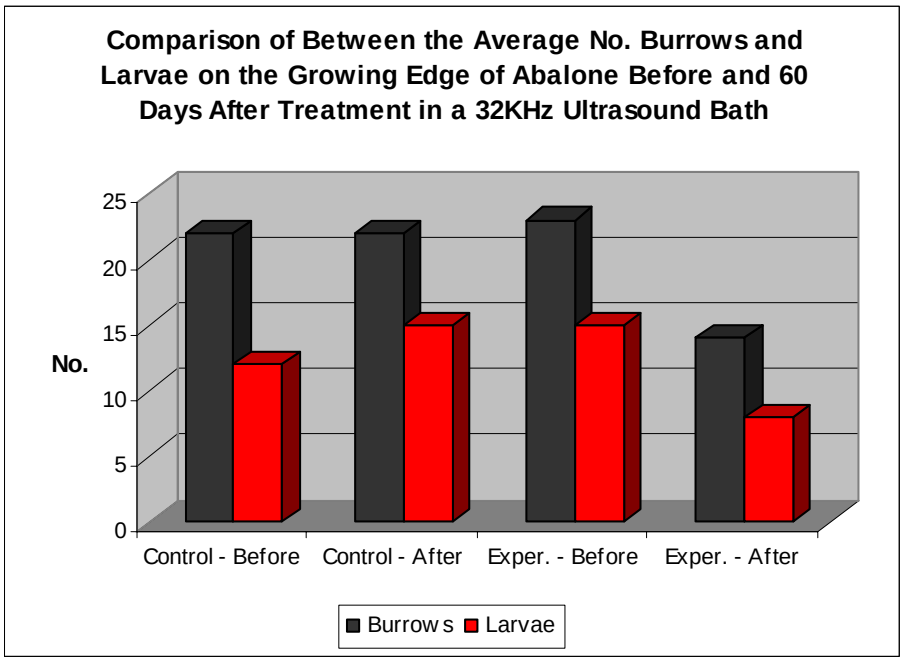


Figure 3.5 A comparison of the average number of sabellid burrows and larvae on the growing edge of uncrushed abalone shell before and 60 days after treatment in a 32KHz ultrasound bath.

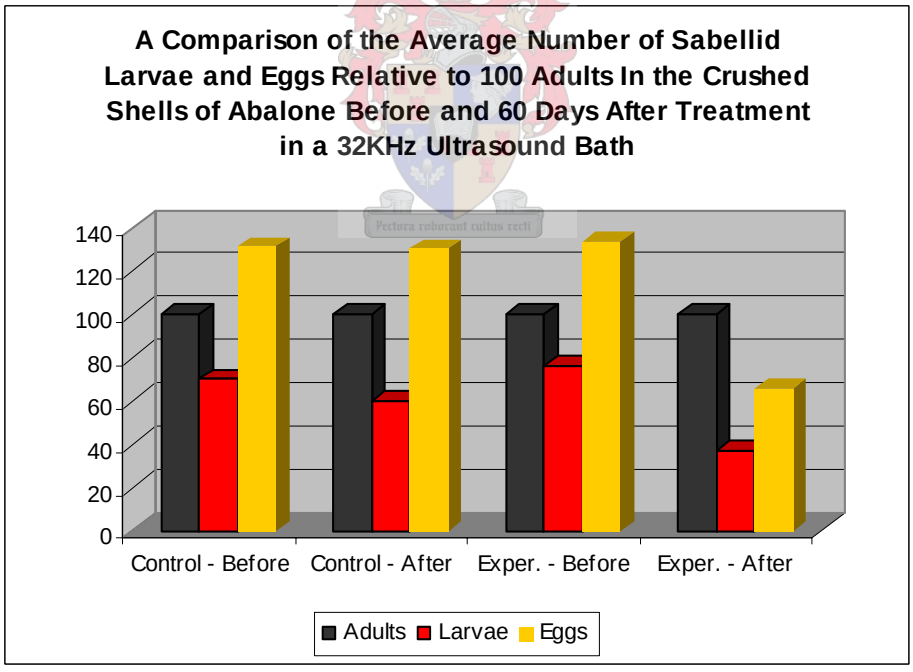


Figure 3.6 A comparison of the average number of sabellid larvae and eggs relative to 100 adults in crushed abalone shell before and 60 days after treatment in a 32KHz ultrasound bath.

Only three mortalities were recorded within forty eight hours after treatment and a further five mortalities recorded within sixty days after treatment.

3.6 CONCLUSION

Ultrasound treatment of abalone infested with *T. heterouncinata* did prove to be an effective method of reducing these populations by either, destroying the individuals completely, removing them from the burrows, or removing or damaging the feeding crowns. This had an effect in reducing the reproductive index by more than 50% sixty days after treatment and the inability to feed either led to mortality or a reduced fecundity as a result of not been able to feed (Quian & Chia, 1991).

However, polychaetes have relatively great powers of regeneration (Ruck, 2000). Tentacles, palps, and even heads ripped of by predators are soon replaced (Barnes, 1980). This means sabellids which had their feeding crowns damaged or ripped off during treatment with ultrasound had a good chance of survival by regenerating these damaged areas. It is uncertain of the time period for this regeneration to occur and for the sabellid to become fully fecund again. Therefore future trials need to take this into consideration and a longer time period after treatment to determine the reproductive index may be necessary to determine the time period for a sabellid population to recover.

Abalone are able to outgrow a low sabellid population on their shells from approximately 65-75mm shell length. If the infested abalone are treated within this size class, ultrasound treatment may have a more significant long term effect, by reducing the sabellid population in order that this size class animal can begin to outgrow them faster. There have been discussions with one abalone production manager to implement ultrasound in the grading process. Since the abalone have to be anaesthetized for this process, ultrasound could be added to treat sabellid populations on infested abalone.

Temperature is critical to the post treatment survival of the abalone. Water temperatures of the anaesthesia tank, ultrasound bath and recovery tank need to be maintained below a maximum of 20^{o}C. At temperatures above 25^{o}C abalone mortalities become problematic. Ultrasound is very stressful to the abalone and they will begin to twist in their shells, therefore the treatment procedure needs to be as fast as possible and abalone placed in cool, fresh seawater with aeration and Kelp as soon as possible.

3.7 REFERENCES

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APPENDIX A: ADDITIONAL INFORMATION REGARDING EXPERIMENT 1

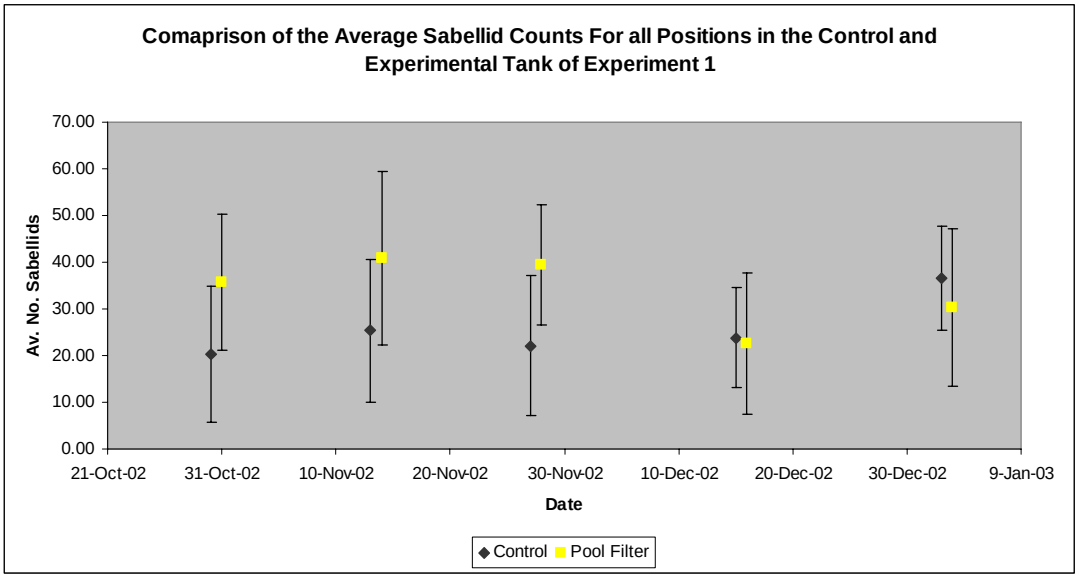

Figure A.1 A comparison of the average number of sabellids on the growing edge of abal all positions in the control and experimental tanks of experiment 1.

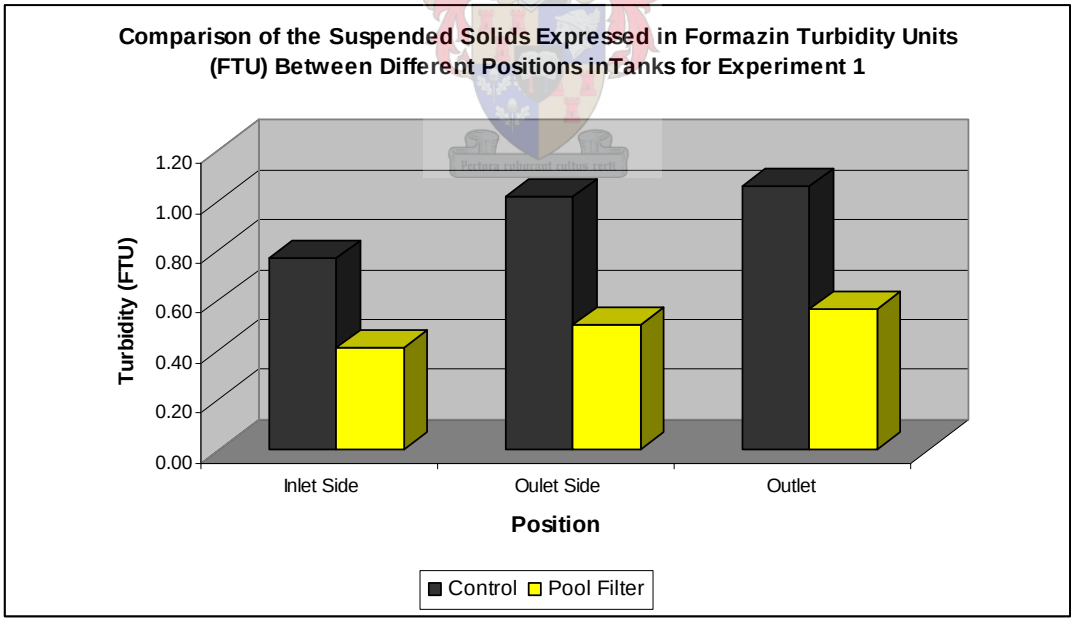

Figure A.2 A comparison of the suspended solids at the inlet side, outlet side andoutlet of both the control and experimental tank in Experiment 1.

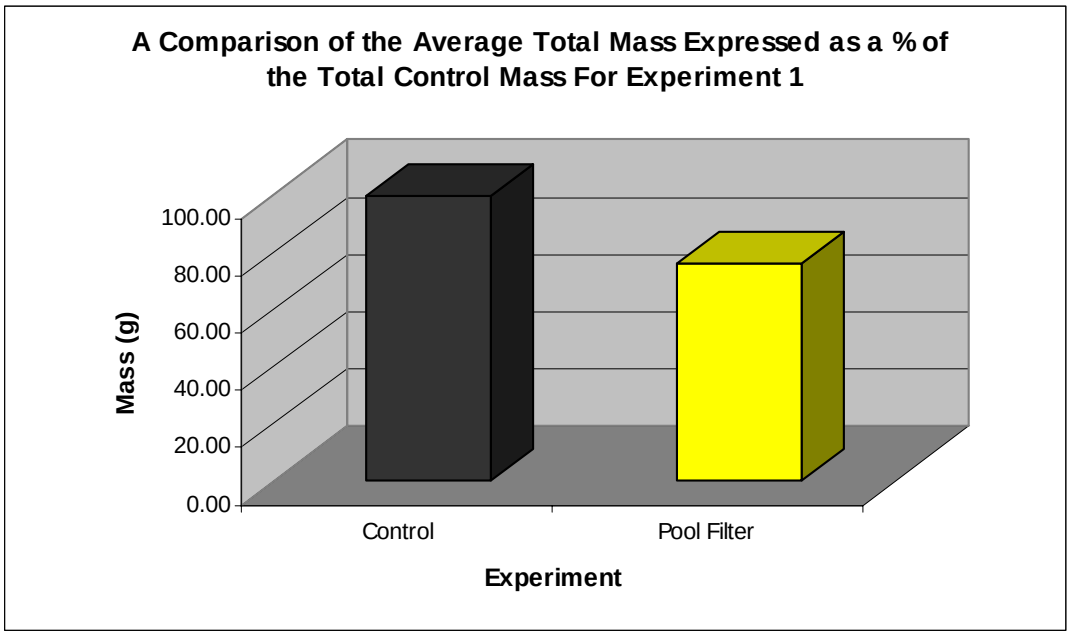

Figure A.3 A comparison of the average total mass expressed as a percentage of the control total mass for Experiment 1.

APPENDIX B: ADDITIONAL INFORMATION REGARDING EXPERIMENT 2

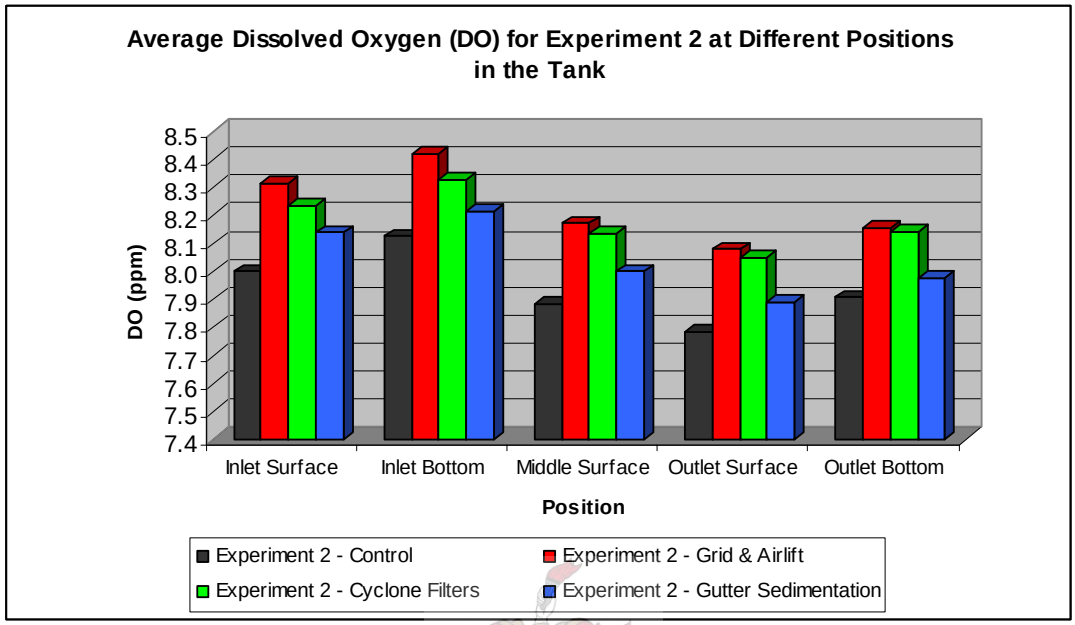

Figure B.1 A comparison between the control and experimental tanks of the average dissolved oxygen (ppm) recorded at different positions in Experiment 2.

APPENDIX C: TIME SCHEDULE USED DURING ULTRASOUND TREATMENT OF AN ENTIRE ABALONE PRODUCTION TANK