# AN INVESTIGATION INTO THE FEEDING BIOLOGY AND FACTORS INFLUENCING THE POPULATION DYNAMICS OF *Terebrasabella heterouncinata* (POLYCHAETA:SABELLIDAE), A PROBLEMATIC TUBE-DWELLING POLYCHAETE IN FARMED ABALONE IN SOUTH AFRICA

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# ABSTRACT

Since the discovery of a new species of sabellid worm in 1993, the Californian and South African abalone industries have experienced increasing shell breakage and reduced growth rates of abalone, resulting in reduced market value of product. This polychaete worm can reach very high infestation levels. It was described as *Terebrasabella heterouncinata* in 1999, having originated from the South African coastline where it had been observed on several wild mollusc species. Although not a shell borer, it occupies tubes on the growing edge which are formed by deposition of nacreous shell over sabellid larvae by the host abalone. Heavy sabellid infestation levels have placed the economic viability of several South African farms under threat. Past research methods have not been successful in establishing effective means of eradication or control, partly due to the lack of knowledge of basic biological information on this species. This study forms the first part of an ongoing research project into the biology of *Terebrasabella heterouncinata*. Abalone farms in South Africa utilise two main diets in abalone culture. An artificial formulated feed, Abfeed, is produced in South Africa and is used by the majority of farms as it increases abalone growth rate. Kelp, a natural diet of abalone, is harvested and also used as a feed. Farmers have observed higher sabellid infestation levels on Abfeed-fed abalone and suspect that diet may be an important factor influencing sabellid infestation. Abalone stocking density has also been identified as a potential factor influencing sabellid population growth on commercial farms. In order to better understand sabellid nutrition, the feeding behaviour of the sabellid in response to suspended solids of various particle size classes from both abalone diets was investigated. Both the effect of abalone diet and stocking density and their effects on sabellid morphometrics was quantified. The proximate composition and particle size distribution of suspended-solids present within kelp and Abfeed abalone raceways were also quantified. Results indicate that sabellid feeding follows no structured behavioural pattern for both abalone diets. The quantity of suspended solids in kelp raceways was greater than in Abfeed raceways ($p<0.02$) while there was no difference in particulate level between three abalone stocking densities ($p>0.05$). Protein ($p<0.0001$) and energy ($p<0.01$) levels of suspended solids were significantly greater in raceways dedicated to feeding Abfeed. The infestation level (number of tubes/centimetre on the growing edge) was significantly higher ($p<0.001$) in Abfeed-fed abalone. The morphometrics of the sabellids indicated that sabellids from Abfeed-fed abalone were larger in various body measurements: length ($p<0.00001$); neck width ($p<0.001$); base width ($p<0.001$); and surface area ($p<0.001$). This study suggests that abalone diet has an important influence on the infestation level and size of sabellids and that this is likely to be due to the fragmentation and leaching of nutrients from the artificially enriched commercial abalone diet.

# CHAPTER 1

# GENERAL INTRODUCTION

The South African abalone industry has developed and expanded rapidly in the past ten years in order to meet increasing demands for abalone products. This was a result of the declining harvest from abalone fisheries due to excessive poaching, which has decimated natural abalone stocks. Abalone farming is like any intensive animal husbandry and requires a large capital investment and has high running costs. Animals are stocked at high densities in shore-based facilities where they are reared on seaweeds and/or artificial diets (Sales, 2001). In 1996 a problematic sabellid worm was identified in Californian abalone facilities, which was subsequently found in South African abalone farms. It is indigenous to South Africa and is found in a wide range of intertidal molluscs where it occupies burrows on the external surface of the shell. The sabellid is capable of reaching epidemic levels within abalone facilities due to the favourable conditions of intensive monoculture. This results in reduced abalone growth rates, due to interference of larvae at the mantle-shell interface (Ruck and Cook, 1998), and a reduction in the market value of live abalone for the Far East market due to loss of aesthetic appeal. The sabellid infestations associated with abalone farming are thought to be due to the combined effects of intensive farming techniques and the use of enriched artificial diets. This study aims to identify the relationships between abalone diet and culture conditions and sabellid biology.

## *Development of South African abalone aquaculture industry*

In response to the decline of the natural stocks in South Africa due to fishing pressure, the then Division of Sea Fisheries launched an abalone research programme in 1962 (Newman, 1966). The development of intensive abalone farming techniques in South Africa began in 1989/1990 (Cook, 1998). Research into the spawning, larval settling, nutrition and environmental requirements of *Haliotis midae* have allowed pilot scale facilities to be up-scaled to farms for commercial production (White, 1995). Since 1990 in excess of R80 million has been invested to build a South African abalone farming industry (Cook, 1998) comprising 12 commercial farms situated between Port Nolloth on the west coast and East London on the east coast (Sales, 2001). Although there is a high initial capital investment, land-based abalone farming is a feasible business with the prospect of high returns due to the high economic value of abalone products coupled with the exchange rate and devaluation of the South African Rand. Abalone farming is intensive and the combined annual projected production of the 12 farms is 500-800 tonnes (Sales and Britz, 2001). All farm-produced abalone in South Africa are intended for the export market as current permits prevent the sale of farmed abalone in South Africa. This is because farm-produced abalone are grown to a size of 80-100mm, smaller than the minimum harvestable size for wild abalone (11.43cm), and the prohibition of selling abalone of this size range in South Africa is aimed at preventing the illegal harvesting of undersized animals (Cook, 1998). Intensive aquaculture strives to maximise production while reducing capital and operating costs (Lyon, 1995). A high level of control is maintained over intensive production processes, temperature and photoperiod may be regulated and the inflowing water is filtered and sterilised, in order to minimise the risks associated with these operations and to ensure that the market can be guaranteed a regular supply of abalone of uniform size and quality (Britz, 1995). The existing abalone aquaculture industry contributed less than 10% to the global abalone yield in 1994 (FAO, 1994). Farm production has been on the rise, with many of the South African farms attaining their maximum production levels in recent years, which is aiding in alleviating the shortfall in supply to the global market.

Abalone are well suited to aquaculture due to several favourable attributes; they have a high stress threshold and adapt well to captive conditions; they can be cultured at high densities due to their non-aggressive and sedentary nature; they are resistant to bacterial infection and there are no known viral infections; captive breeding techniques are known and possible; and they accept artificially enriched and formulated diets (Britz, 1995). The only negative attribute of abalone is their slow growth (15-40 mm/year) and therefore long grow-out periods (Britz, 1995).

The South African abalone industry is primarily a land-based industry where, besides the high start-up costs, space is the main limiting factor. The sedentary and non-aggressive nature of abalone makes them ideal candidates for intensive monoculture. The slow growth of the abalone requires farmers to grow abalone for 4-5 years before they attain market size. The stocking density is calculated as the percentage cover of the available surface area by the abalone. Abalone farmers often face the problem of having too little space to stock the abalone at the accepted stocking densities towards the end of grow-out periods and when the overseas market demand drops. As a result, stocking densities often exceed the levels recommended and recognised by the farmers as the optimal stocking densities for best abalone growth. Abalone farmed at very high densities show reduced growth rates and farmers need to find a compromise between the costs of additional grow-out facilities and reduced growth at high stocking densities (Fallu, 1991). The Abalone Farmers Association of South Africa has recommended a maximum stocking density of 35% of the available solid surface area, as densities above this appear to stifle abalone growth rate (Anonymous (a)). Although poor water quality can be avoided at excessively high densities by increasing the flow rate and aeration, high stocking densities, poor hygiene and high nutrient loading have been suspected by the South African abalone farmers as the primary causes of problematic sabellid infestations. The sabellid, *Terebrasabella heterouncinata*, is compromising the South African abalone industry due to its implication on abalone shell deposition. Sabellid larvae settle on the growing edge, where the abalone deposits a nacreous layer over them, thereby creating a tube for them to metamorphosise and mature in. This results in a cessation of the normal growth of the abalone as more effort is invested in shell deposition over the larvae rather than in shell extension. Consequently growth rate declines and an irregular domed shell is formed, which decreases the market value of live abalone. The formation of tubes within the abalone shell results in a porous and weaker shell, which is prone to breakage during the rigorous hand-sorting techniques common in the abalone industry.

## *Introduction to the Family Sabellidae*

Sabellids are among the most beautiful of the sedentary polychaetes (Barnes, 1987). They are commonly referred to as fan worms or feather dusters and are often admired by aquarists and divers for their bright coloration and feathery appearance. Sabellids are suspension feeders and live in strong sandy tubes attached to various substrates. The tube serves as a protective retreat from predators as well as a lair from which to catch passing prey and food items. The tubes of polychaetes are composed of secreted material, sand particles cemented together or a combination of both. Most species live on rocks but the smaller species may be found on the shells of living molluscs or on the fronds of algae (Day, 1967). Although single individuals are found, most species are gregarious and form dense aggregations (Day, 1967). The majority of tubicolous polychaetes are highly modified for a tube-dwelling existence and are unable to leave their tubes. The feathery branchial crown is used for respiration and feeding. Particles suspended in the water column are filtered from the water and sorted according to size and composition (Fitzsimons, 1965; Bock and Miller, 1997). Larger sand particles are used for tube construction while the smaller organic particles are channelled, by the cilia lining the radioles, to the mouth where they are ingested. The mechanism of particle selection is not well understood (Barnes, 1987). This lack of understanding is due to the fact that most studies on filter feeders have concentrated on describing the feeding structures. The cilliary selectivity and fluid mechanical aspects of particle capture have been neglected (Jørgensen, 1981).

A problematic epibiont commonly referred to as the sabellid by abalone farmers, was identified as a new genus and species endemic to South Africa (Fitzhugh and Rouse, 1999). It was first noticed in Californian abalone facilities where it is thought to have been introduced in the late 1980s on the shells of South African abalone, *H. midae*, imported for commercial research (Leighton, 1998). In any abalone aquaculture facility there is a small percentage of slow growers that never attain premium market size and in 1990 a population of slow growing abalone introduced from South Africa into a Californian facility was noticed to have a characteristically deformed shell, with the growing edge thickening and growing downwards instead of outwards creating a domed shell (Oakes and Field, 1996). Closer examination of the growing edge revealed that there was a small worm living in the shell. The sabellid, *Terebrasabella heterouncinata*, was discovered in 1993 and later described (Fitzhugh and Rouse, 1999). Infestations of sabellid polychaetes were found in South African abalone in 1994 when it was also noted that one group of abalone exhibited virtually zero growth and experienced higher mortalities than other groups (Ruck and Cook, 1998). The shells of these abalone were porous, exceptionally weak and prone to damage, and had slightly abnormal shapes (Ruck and Cook, 1998). The porous nature of the shell was due to the numerous tiny holes occupied by a polychaete worm which was originally thought to be of the *Polydora* genus, a documented shell borer in abalone (Blake and Evans, 1973). Closer examination of the worm suggested that it was the same polychaete that had been discovered in Californian abalone in the previous year (Ruck and Cook, 1998).

*T. heterouncinata* has caused severe damage to the Californian and South African abalone industries. Studies in both countries have been designed to determine the effects of the sabellid on both the abalone farms and the natural environment (Culver *et al.*, 1997). Although sabellid infestations do not affect the quality of the abalone meat, they deform the shell, reducing the value of live abalone by up to 50% for the Asian market and they reduce abalone growth, increasing the duration required to reach market size (Culver *et al.*, 1997). Heavily infested live abalone are generally in poor condition, do not eat well and slight fluctuations in external parameters increase the stress levels of the animals, resulting in increased mortality (P. Pesch, *pers. comm.*).

### *Interaction between sabellids and host gastropods and implications for abalone aquaculture*

In the natural environment the sabellid is found on a number of mollusc species (Ruck, 2000). Snails, limpets and other marine gastropods are susceptible to infestation and may act as vectors for transporting and introducing sabellids into abalone facilities which were previously uninfected (Anonymous (a)). Limpets are commonly introduced into raceways with kelp fed to the abalone. Wild individuals of *Haliotis midae* are infested to a moderate degree without any noticeable effect on the host (Ruck, 2000). On the abalone farms, under the intensive monoculture conditions, the sabellid infestation can reach epidemic proportions which are clearly harmful to the host as is indicated by reduced growth rates, weakened shells and possible mortalities (Leighton, 1998; Ruck and Cook, 1998). The symbiotic classification of *T. heterouncinata* is unclear due to the lack of knowledge of its relationship and dependence on the host for nutrition. Abercrombie *et al.* (1951) defined parasitism as the relationship between an organism (parasite) living in or on another organism (host) and deriving food from it. Campbell (1993) provides a more general definition of parasitism; A symbiotic relationship in which the symbiont (parasite) benefits at the expense of the host by either living within or outside the host. In both cases the sabellid fits the definition as it lives on its host and although the sabellid does not actively feed on and harm its host, it may derive food from it indirectly in the form of particulates from faecal matter. The abalone also suffers due to brittle and deformed shell growth when infested by sabellids, which can ultimately cause mortality of the abalone.

The parasitic nature of the sabellid is further emphasised by the fact that it does not have a free-living form (Ruck, 2000) and cannot survive extended periods of time without a living gastropod host. Emerging larvae do not settle in abandoned burrows on gastropod shells and if the host gastropod is dead, the emerging larvae will either die or they must disperse to find a suitable living host (Simon *et al.*, 2002). The sabellid is clearly dependent on the abalone for shelter, protection and possibly food. The abalone, however, is not dependent on the sabellid, but is harmed, and the relationship between the two organisms cannot be considered a symbiotic one. The feeding relationship between the host gastropod and the sabellid is yet to be determined but it is possible that they feed on particulates originating from host faecal material. Investigating this feeding relationship forms part of this study. A true parasite does not usually kill its host (Opperdoes, 2000). It is not individual sabellids but the combined effects of several hundreds of sabellids on each abalone shell which are responsible for increased mortalities in the abalone industry. At this stage the sabellid has not been clearly classified as to its relationship with the abalone but is commonly referred to as a parasite within the abalone farming industry (Anonymous (a)). This creates a need to further our knowledge on its relationship with the host, its life history and its basic biology.

Although not much is known about the life history of the sabellid, its basic mode of reproduction is known. *T. heterouncinata* is a simultaneous hermaphrodite (Oakes and Fields, 1996; Fitzhugh, 1996; Ruck, 2000). It has recently been established that the sabellid is able to self-fertilise and produce viable offspring, but it is uncertain whether this mode of reproduction occurs in the wild (Finley *et al.*, 2000). The eggs are laid, fertilised and brooded in the posterior end of the adult tube where they hatch into crawling benthic larvae. Adult burrows commonly contain 1-3 eggs as well as 1 or 2 larvae at various stages of development (Fitzhugh and Rouse, 1999). Present work on South African abalone farms has shown that there are 3.2 ± 2.2 eggs and 1.8 ± 1.3 larvae within the adult sabellid tube (M. Gray, *pers. comm.*). The small brood size and parental protection and care allows for a greater survival rate and is characteristic of k-selected organisms. Brooding is frequently associated with hermaphroditism and for this species, which already inhabits a protective tube, this appears to be the preferred strategy (Ruck, 2000). Larval development continues within the tube until segmentation is complete and bristles are visible (Culver *et al.*, 1997). Larvae have eyespots and exit the parental tube via the opening and crawl in search of a suitable settlement site. At this stage the branchial crown is not developed and there is no evidence of gut formation (Fitzhugh and Rouse, 1999). The larvae cannot feed and still have yolk granules in the body (Culver *et al.*, 1997). The larvae crawl to the growing edge of a gastropod host where they settle on the underside of the shell between the mantle and the shell edge. They settled with the anterior end on the shell edge, each larva secretes a mucus sheath around itself and maintains an opening to the outside. They may also settle on the outer lip or around the respiratory pores of gastropods (Culver *et al.*, 1997). The host gastropod deposits a calcified layer over the mucus-coated larvae, forming a calcareous tube for metamorphosis and the adult worm to reside in. Metamorphosis takes place approximately one week after settlement after which the adult is easily identifiable by the branchial crown (Culver *et al.*, 1997). Maturation from juvenile to adult occurs with the development of setigers 7-11 (sperm in setiger 8 and oocytes in setigers 9-10) at approximately one month (Fitzhugh and Rouse, 1999). Adults are sexually mature and capable of reproduction after the 11th setiger has completely developed, approximately 50 days after hatching (Fitzhugh and Rouse, 1999; Finley *et al.*, 2000).

Adult sabellids are sessile and incapable of infesting new hosts themselves. The brooding of the eggs and crawling larvae stage eliminates the possibility of dispersal and infestation by eggs and pelagic larvae. Wild gastropods are usually sessile and dispersed, rarely aggregating, reducing the possibility of sabellid larval transmission from one host to another. Fluctuating environmental conditions and food availability commonly experienced in the wild create harsh conditions for reproduction and reduce the possibility of high infestations of sabellids occurring in wild gastropods. In abalone aquaculture facilities abalone are held at extremely high stocking densities and during the day it is common for abalone to sit with their shells touching and rubbing on each other, and at night, while actively feeding, they crawl over each other, increasing the possibility of transfer and infestation of previously uninfested individuals. Aeration in tanks and increased water flow in intensive aquaculture may cause larvae to be suspended in the water column and transported from one basket to another. Reducing the density of abalone in tanks and keeping abalone in suspended baskets off the bottom of the tanks has been effective in reducing the spread of sabellids within tanks (Culver *et al.*, 1997) but is not a sustainable method of control in the abalone industry, as decreasing the stocking density increases the production costs and space required, which is already restricting. Further research is required to determine the overriding factors which influence sabellid growth and infestation levels. At this stage there is no information on the feeding biology and behaviour of *T. heterouncinata*. This information would increase our understanding of the relationship between the sabellid and the abalone and help identify those factors associated with increased levels of infestation and sabellid growth. The information will also help to identify management techniques for controlling infestations under intensive culture conditions. This study aims to address these issues. It is part of a programme of research into the life history and reproduction and brooding capabilities of the sabellid.

## *Potential control, management and eradication of the sabellid in the abalone industry*

At present there is no method for eradicating or even controlling sabellid infestations under culture conditions. Past research by researches and farmers into possible control mechanisms has been unsystematic, often ending with poor results. This and accompanying studies are structured to obtain basic biological information on the sabellid in order to identify potential areas and potential methods which would be applicable to the problem.

The past control approaches by farmers have achieved poor levels of success due to several traits which the sabellid uses to its advantage. The sabellid is capable of withdrawing into its tube, providing an excellent retreat when unfavourable conditions are detected in the external environment. For this reason the sabellid is very resilient to changes in the environment, vermicides, dehydration and anoxic conditions (Ruck, 2000). Trevelyan *et al.* (1994) conducted several trials with different chemical agents but found nothing that killed the sabellid without harming the abalone. In several instances the abalone has been more sensitive to treatments such as salinity changes, dehydration and chemical agents than the sabellid, possibly due to the fact that the sabellid can withdraw into a safe microhabitat.

Leighton (1998) performed studies to evaluate the effect of raised water temperature on sabellid survival. *Haliotis fulgens* and *H. corrugata* have a higher thermal tolerance than other species of abalone of up to 30°C. Results indicate that all life stages of the sabellid can be eradicated if exposed to water temperatures of 28-29°C for a period of 48 hours. Unfortunately this temperature lies outside the temperature range for *H. midae* which ranges from 11-19°C. Temperatures above this range stress the abalone and result in growth reduction (Ruck, 2000). The use of temperature as a control mechanism for sabellids in the South African abalone is therefore not possible.

Control of pests and parasites using biological methods is preferred over the use of chemical agents. This is particularly true in the food industry where chemical contamination must be avoided. Biological control involves using one organism to suppress another (Hoffmann and Frodsham, 1993). Natural enemies of the problematic organism can be used for control, as has been demonstrated in the control of several invasive species such as the European corn borer in North America (Hoffmann and Frodsham, 1993). The use of biological agents to control sabellids is of limited use as they show exceptional powers of regeneration (Ruck, 2000). Redevelopment of the branchial crown has been observed to occur after ultrasonic treatment (Abalone Farmers Association of South Africa (AFASA), *pers. comm.*). Destruction of the branchial crown would, however, reduce the ability of the sabellid to feed and may therefore limit the energy available for the sabellid to invest in reproduction. Kuris and Culver (1999) experimented with possible biological agents for control of the sabellid. They tried fish, crabs, shrimps, isopods, flatworms and starfish, all known to feed on encrusting organisms, to control sabellid infestations under experimental conditions, but they had no success.

The use of ultrasound technology in South Africa has had very little success (AFASA, *pers. comm.*). The treatment is effective in destroying the fragile branchial crown of the sabellid but does not kill the adult sabellid, allowing it to regenerate the feeding crown.

Although very labour intensive, physical control methods have had the greatest success. Trevelyan *et al.* (1994) had some success in reducing sabellid infestations by coating the abalone shell with a low melting point wax. The wax smothered the sabellid tubes and killed all stages of the sabellid life cycle, but this method caused unacceptably high mortalities in the abalone (Ruck, 2000).

Considerable research into the use of liposomes as a vector for presenting toxins to the sabellid has been carried out. Liposomes are artificial spheres consisting of a lipid bilayer encapsulating a toxin (Ruck and Sales, 1999). It is a highly effective method for delivering toxins (Ruck, 2000). Since the worms would concentrate the liposomes in their gut, the toxins would accumulate in a localised area, making them more efficient while overall doses are kept low (Ruck, 2000). The use of liposomes takes advantage of the fact that sabellids are suspension feeders and filter particles from the water column, while abalone are grazers and feed off the substrate. Encapsulated toxins suspended in the water column would therefore not be available for consumption by the abalone, preventing abalone mortality. Although the liposomes proved effective as a delivery method of the toxin, the sabellids seemed incapable of digesting the oil beads (Ruck and Sales, 1999; Ruck, 2000). Worms could also detect the toxins in beads that were not dialysed properly and would not feed on them. In addition sabellids were able to void their gut contents very rapidly (Ruck, 2000) possibly due to the detection of unfavourable substances. Further refinement of this technology and a better understanding of the sabellids' feeding and digestion capabilities may provide an effective treatment. Ruck (2000) suggests that the use of hydrogels could also be used to control sabellid infestations. Hydrogels are carefully engineered gels which expand or contract by many orders of magnitude depending on the external conditions (Zhang *et al.*, 1992; Annaka and Tanaka, 1992). Factors such as temperature, pH or salinity can produce these changes (Ruck, 2000). These polymers can potentially be used to rupture or block the gut. The advantage of this method over liposomes encapsulating toxins is that no toxins would be released into the environment and the worms would be unable to develop a resistance to this treatment as it is mechanical (Ruck, 2000). This technique, however, is relatively new and untested.

The need for work on factors affecting worm nutrition is essential for understanding its versatility and factors influencing growth and reproduction, which may play an important role in identifying potential control options. Sabellids are subject to a wide range of fluctuating conditions on abalone farms on which they are reliant for nutrition. Environmental conditions within raceways on abalone farms are highly variable depending on location and management strategies. Stocking density, cleaning frequency, splitting frequency, feeding frequency and diet can all modify the conditions within a raceway making them more or less suitable for the sabellid. The quantity of waste material within the water column increases as production and stocking density increases (Cripps, 1995). The abalone farming industry in South Africa has two diet options and each farm has its own preferred feeding routines and preferred diets. South African abalone are reared on a formulated diet, harvested kelp (*Ecklonia maxima*), cultured *Gracilaria* spp., or a combination of the above (Cook, 1998). Formulated diets can minimise costs while increasing growth of the abalone. Since the discovery of sabellid infestations in South African abalone farms, it has been speculated that the artificially formulated diet used as a grow-out feed causes greater infestation levels (Anonymous (a)). This speculation appears plausible as formulated diets can maximise the nutritional value for the target organism while also increasing the nutrient loading within the water column, as leaching of nutrients from unstable pellets is known to occur. The main nutrient components in aquacultural water are phosphorus and nitrogen compounds and suspended solids (Cripps, 1995). Elevated levels of these substances result in a nutrient rich environment, creating the ideal conditions for filter feeding organisms to thrive. *T. heterouncinata* appears to thrive under the conditions in abalone raceways, leading to high infestation levels. The effect of abalone diet on infestation levels and sabellid condition, has, however, not been shown or quantified conclusively.

Some sabellids are known to feed selectively, ingesting certain organic particles while rejecting others of lower nutritional value. The selective process is not well understood and it was previously thought that sabellid species select particles on size (Fauchald and Jumars, 1979). Bock and Miller (1997), however, showed that organic coating of particulates was the prime determinant in particle selection in polychaetes. The contradictory evidence on polychaete feeding calls for a better understanding of the sabellid feeding biology. The aim of this study was to document and quantify the feeding biology, and to determine the effect of physical parameters on population morphometrics of *T. heterouncinata*.

The main objectives of the study were:

a) To develop an observational technique to quantify the feeding behaviour of the sabellid under laboratory conditions,
b) To quantify the feeding behaviour of the sabellid in response to different feed particle sizes and origins,
c) To compare proximate composition of particulates originating from kelp and Abfeed raceways,
d) To compare particulate abundance between different abalone stocking densities,
e) To investigate the effect of stocking density on sabellid infestation levels and population morphometrics, and
f) To investigate the effect of diet on sabellid infestation levels and population morphometrics.

# CHAPTER 2

# DEVELOPMENT OF THE METHODS FOR OBSERVATIONAL STUDIES

## INTRODUCTION

Suspension feeding is widespread in benthic marine habitats (Turner and Miller, 1991) with many species of polychaetes feeding in this manner. The understanding of the feeding methods and strategies of these organisms is, however, poorly understood. Behaviour of an organism is linked to its natural physical environment (Vogel, 1981) and the complex forces experienced by suspension feeders under oscillatory and steady flows has led to speculation that organisms behave differently under static, oscillatory and steady flow conditions (Turner and Miller, 1991).

Suspension feeders feed by capturing particles from the water column. Active suspension feeders create a feeding current of their own in order to draw particles towards their mouth parts, whereas passive suspension feeders protrude a feeding organ into a current to capture passing particles (Levinton, 1995). Passive suspension feeding is therefore highly dependent on local environmental conditions and water movement patterns. Hydrodynamic forces experienced at the burrow entrance often induce switches in feeding from suspension to deposit feeding in many species (Levinton, 1995). Suspension feeders must be able to avoid clogging of the feeding apparatus during periods of high particulate loading and survive periods when food items are scarce.

Oscillatory flow in natural conditions is favourable for many species of suspension feeders as it re-suspends particulate matter, increasing the total amount of carbon and nitrogen available for consumption (Wainright, 1990). Most feeding studies have concentrated on bivalve molluscs (Turner and Miller, 1991) which are active suspension feeders creating water currents through their bodies to filter suspended particulates with their gills. Perhaps because of this active mode of suspension feeding in many filter feeders, previous studies have concentrated on the levels of particulates filtered and not the types of flow and particle size and distribution which resulted in the suspension of the particulates (Turner and Miller, 1991). Under natural conditions these factors are unpredictable and highly variable (Navarro and Iglesias, 1993). Many natural processes contribute to fluctuations in the concentration and quality of suspended particles (Grant and Thorpe, 1991) which suspension feeders are subjected to. Changes in the flow characteristics around sessile organisms are very important, not only for supplying food but also for the behaviour of an organism. Sessile suspension feeders may experience pressure drag in the presence of strong currents and may respond by changing the orientation of their feeding apparatus away from the flow, creating a sheltered area behind the tubes in which to collect particles (Levinton, 1995), or by withdrawing into the safety of their burrows. Although stronger currents may result in re-suspension of more particles, providing the ideal feeding opportunity for filter feeders, they may spend a shorter duration feeding during times of high particle density.

Most studies on the feeding behaviour of deposit and suspension feeders have been carried out in uni-directional flow chambers, with observations being taken at certain time intervals (eg. Dauer, 1983; Bock and Miller, 1996; Bock and Miller, 1997). Many species of marine polychaetes utilise multiple feeding modes. Therefore, some authors looked into the effect of current velocity on the feeding mode of filter feeders capable of switching between suspension and deposit feeding (Dauer, 1985; Bock and Miller, 1996). Recent studies have examined the effect of oscillatory flow on the feeding rate and behaviour of filter feeders (Turner and Miller, 1991; Bock and Miller, 1996). An important factor to take into consideration when designing feeding experiments is to simulate the natural conditions as closely as possible while controlling potentially confounding factors. Sabellids in the wild tolerate a wide range of environmental conditions to which they have adapted (Levinton, 1995). The abalone aquaculture industry, like any intensive farming practice, controls the environment in the culture tanks as much as possible to create constant conditions favourable for abalone growth. In doing so, abalone farmers have created an ideal habitat for other marine invertebrates to colonise. *T. heterouncinata* thrives in the farm environment, causing infestation of farmed abalone to epidemic proportions.

An observational system for observing the feeding biology of the sabellid was designed to represent the flow regimes most likely experienced within raceways on abalone farms as closely as possible. Circulation was created by introducing water into a cone-shaped observational chamber and thereby maintaining particulates suspended in the water column.

The objectives of this series of pilot studies were to design and test a recirculating system for feeding observations; to observe and quantify the feeding behaviour in order to get reference feeding behaviour data; and to test various methods which could be used to quantify feeding observations.

# MATERIALS AND METHODS

## *Experimental animals*

Heavily infested abalone were obtained from Sea Plant Products Ltd. in Gansbaai, South Africa. The abalone were held in a constant environment room in 106-litre glass aquaria, fitted with a 36-litre biological filter. An airlift pump maintained water flow through the filter. The water temperature in the aquaria was maintained between 15-18$^{o}$C and the salinity at 35 ± 2 ppt. The abalone were previously fed an exclusively artificial diet and were maintained on artificial pellet feed while being held for the observational studies. They were fed once daily and the tanks siphoned once per week, with one third of the water in each aquarium being replaced with filtered seawater. The photoperiod was set at 12L:12D, with the dark period starting at 17:00.

## *Reference feeding behaviour*

All reference feeding behaviour observations were done using tanks with static water maintained at 17 ± 1.4$^{o}$C for the duration of the recording. A Panasonic surveillance camera linked to a video monitor and a videocassette recorder were adapted to fit onto the C-mount of a dissecting microscope. A freshly shucked abalone shell was placed into the 500-ml observational vessel and the microscope positioned over it. During the recordings seawater filtered to 30 µm was used. The microscope magnification and focus was adjusted to obtain a clear image of a single sabellid’s tube entrance and branchial crown when open. Once set up for recording, the sabellids were allowed a 5-minute acclimation period to recover from the disturbances of setting up the equipment. Ten-minute recordings were taken of individual sabellids. At the end of each recording the water was replaced and another sabellid was identified and used for the following recording. A set of 30 ten-minute recordings was taken to establish the reference feeding conditions of the sabellid.

The video recordings were replayed and the activities of the sabellid were entered into observational event recording software. The duration (sec.) of time spent with the branchial crown exposed and open was assumed to be the time the sabellid spent feeding. This was quantified along with the frequency of withdrawal. Duration events were averaged and results given as mean ± standard deviation.

### *Reference feeding behaviour results*

Sabellids exposed to static water conditions and in the presence of no particulates spent 51.2 ± 15.8 percent of the observational duration open for feeding. The average frequency of withdrawal was 8.1 ± 2.1 times per 10-minute observation period.

Figure 2-1 is a frequency histogram for the times the sabellids spent feeding with their branchial crowns fully open before withdrawing into their burrows. The sabellids most often spent between 30-50 seconds feeding before withdrawing into their burrows. No clear reason could be identified for withdrawal and/or cessation of feeding.

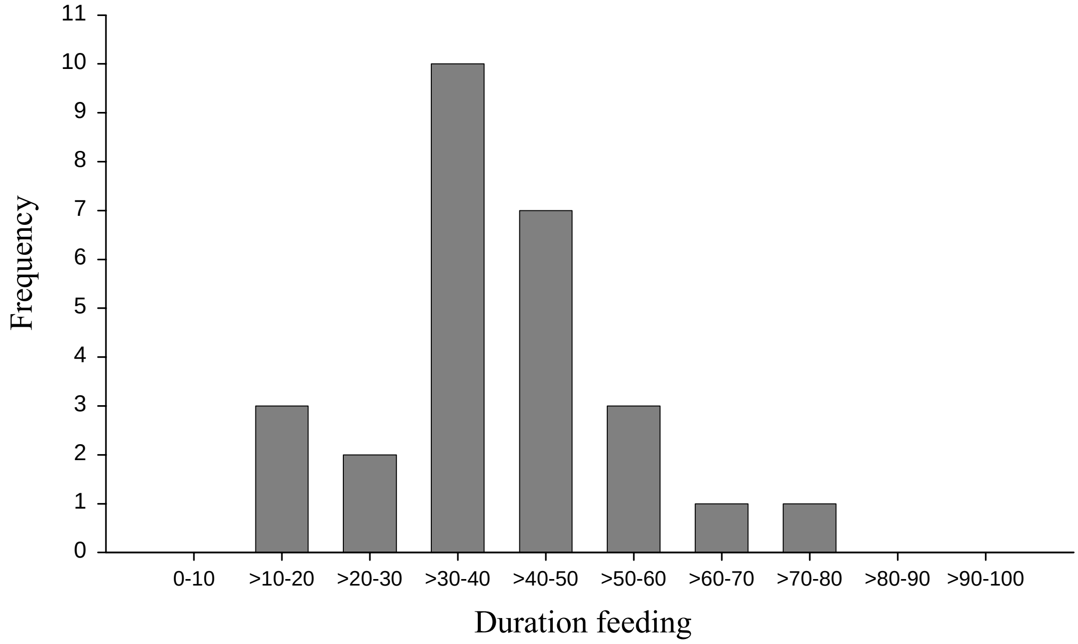


**Figure 2-1: Frequency histogram for the duration (sec.) sabellids spent feeding under static water conditions.**

Figure 2-2 is a frequency histogram of durations the sabellids spent in their burrows after withdrawing. On average sabellids spent 48.8 ± 15.8 percent of the time withdrawn in their burrows. Most sabellids spent between 20-40 seconds in their burrows between feeding sessions.

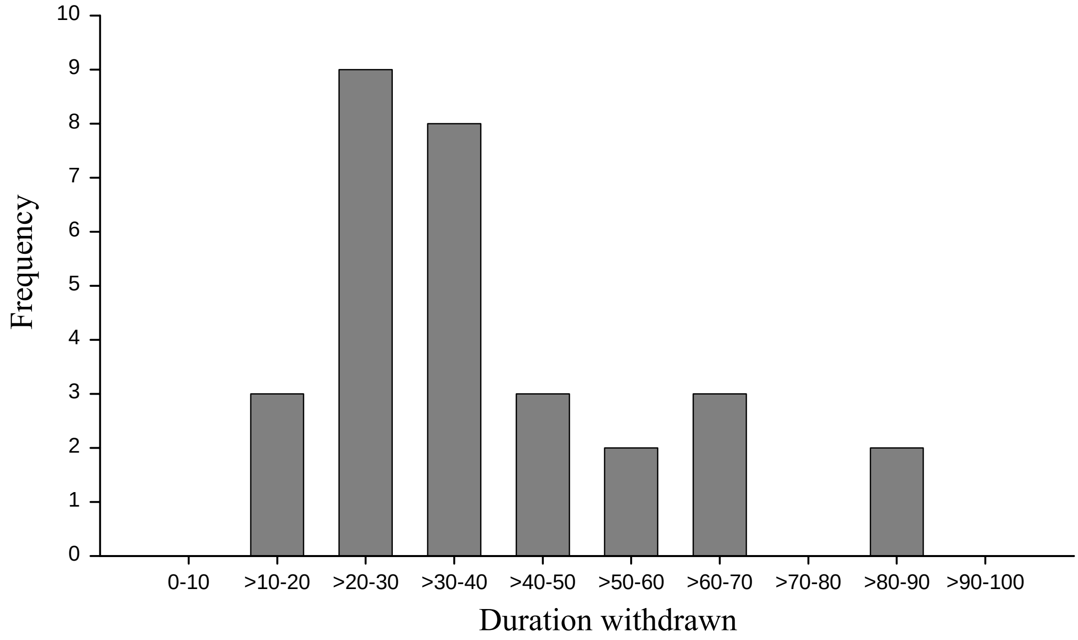


**Figure 2-2: Frequencies for different durations (sec.) sabellids spent withdrawn within burrows under static water conditions.**

## *Description of feeding behaviour*

*T. heterouncinata* is a passive suspension feeding polychaete. As seen from the results of the reference feeding conditions, it spent approximately half of the time with its branchial crown open. During this period the sabellid exhibited limited movement of the branchial crown, only changing its orientation slightly, presumably in relation to water movement. Although there was no observed movement of the radioles for particle capture, particles were clearly seen passing beyond the reach of the branchial crown and changing their direction of movement rapidly while moving towards the radioles of the branchial crown. This was presumably due to the movements and water currents created by the cilia lining the length of each radiole. Under the observation conditions the magnification and focusing capabilities of the equipment used were not high enough to observe the movement of the cilia. Particles which were observed to come into contact with the outer tip of the branchial crown were either rejected and expelled immediately or they were moved down the length of the radioles to the mouth of the sabellid. At this point two behaviours were noted. The particle was either rapidly ejected from the mouth region, passing out of the branchial crown without being ingested, or it disappeared from view, being ingested. At this stage the branchial crown was fully open, apparently catching and sorting particles simultaneously. Occasionally ingested particles were expelled from the oral cavity. When this occurred the particle was propelled well beyond the reaches of the branchial crown, preventing it from being re-captured. Particles which were not expelled from the oral cavity after being ingested were presumed to be consumed and digested as food particles. Some particles caused the branchial crown to close and withdraw with the particle enclosed in it. This was usually with larger particles which were expelled on reopening of the branchial crown. Faecal pellets are clearly distinguishable from other particles due to their uniform elongated shape. They can easily be observed being expelled from the opening of the faecal groove in the mouth region of the sabellid. Like other expelled particles, they were propelled well beyond the reach of the branchial crown.

Withdrawal of the branchial crown and sabellid into its burrow occurred regularly and usually for no obvious reason. On these occasions the branchial crown was closed and withdrawn slowly, sometimes halfway. When an obvious disturbance occurred, the sabellids withdrew rapidly into their burrows and took a longer period to reopen. The sabellid could remain withdrawn in its burrow for extended periods of time, as is shown in Figure 2-2, indicating that some respiration might occur within the burrow. Particle selection definitely occurred. Particles were first sorted on the outer regions of the branchial crown and if selected were moved down to the oral cavity where the selection process may have reoccurred. In this way, the sabellid may be able to select and ingest only preferred particles.

### *Observational system design and suspension of particulates*

Feeding behaviour experiments required that particles remained in suspension or were periodically resuspended for the duration of the observation period. The initial idea was to use a magnetic stirrer to resuspend the particles (Figure 2-3). The abalone shell was placed on a grid above the magnetic stirrer bead. The observation chamber was placed onto a magnetic stirrer and a circular water current could be created by adjusting the rotation speed of the stirrer bead. Using this method a current could be created continuously or periodically as required to keep the particles suspended. The effect of this method on the sabellid behaviour was tested prior to use in feeding experiments as it appeared to cause vibrations which could possibly alter the natural feeding behaviour. No particles were used and recordings were made in the same manner as the reference observations. The results from these trials indicated that there was no significant difference ($p>0.05$) to the reference conditions but the vibration caused a ripple effect on the surface of the water, causing the image to lose focus, thus making it difficult to observe the sabellid.

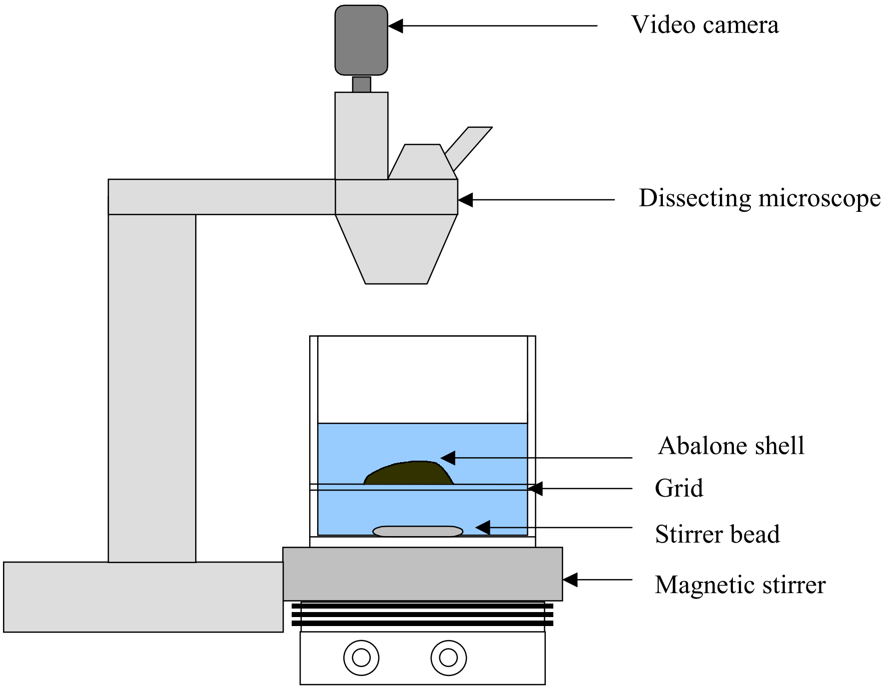


**Figure 2-3: Observation setup using a magnetic stirrer to create currents.**

It was thus decided to construct a small recirculating system to maintain the particles in suspension (Figure 2-4). The system consisted of an observation chamber, over which a dissecting microscope and video camera were mounted, and a sump having a volume of 8 litres. Trials with this system proved to be more efficient as the flow rate could be set and the sabellids did not experience the effects of the currents directly on their branchial crowns, while the particles were effectively maintained in suspension. Once the flow speed had been set the surface of the water remained flat, resulting in the sabellid image being clearer. This recirculating system was used for all following observation experiments involving particulates.

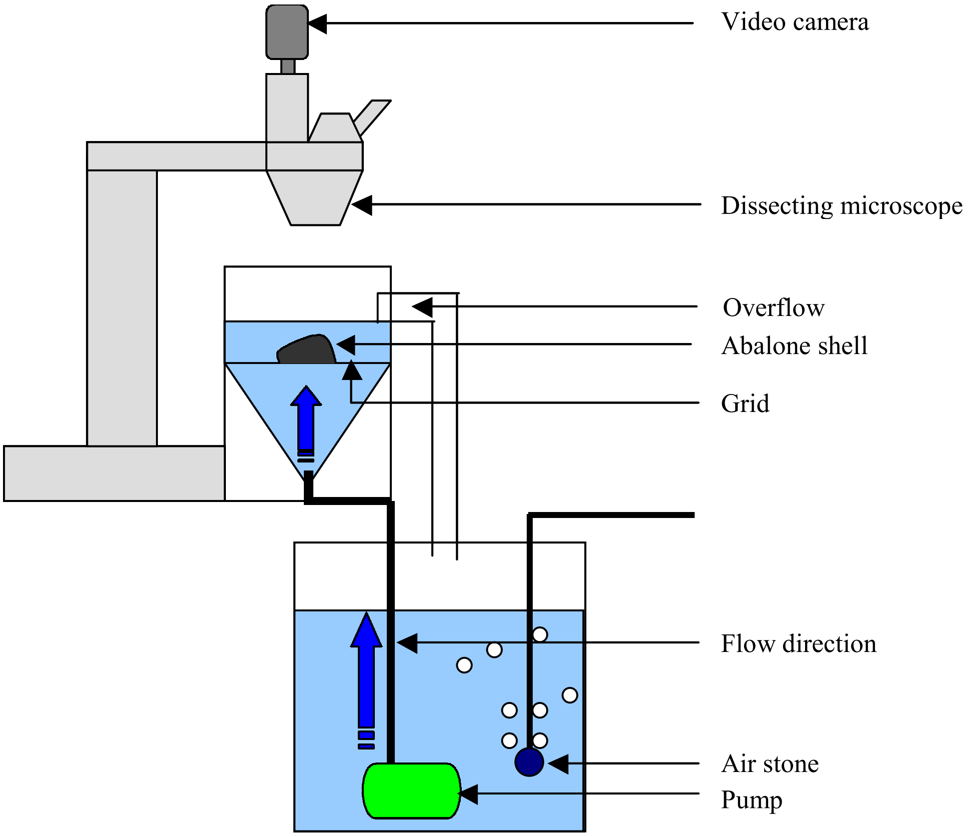


**Figure 2-4: Recirculating system used for feeding and behaviour observations.**

## *Particle size selectivity*

Particle size selectivity trials were performed using Abfeed, milled and sieved into size classes. The size classes were 10-20 µm, >20-30 µm, >30-40 µm, >40-50 µm and >50 µm. Initial trials were conducted for each size class separately to determine the accuracy of measuring particle size off the monitor. The aim was to measure the diameter of the particles ingested by rewinding and pausing the cassette when a particle which was about to be ingested was visible on the branchial crown. The overall aim of using this method was to combine all the particle size classes into one size selectivity experiment and determine the size of the ingested particles. This method proved to be unreliable and inaccurate for measuring the size class to which the particle belonged.

Since particle size could not be determined, the possibility of colouring different size particles was investigated. In order to determine if the food dye affected the feeding behaviour, a solution of food dye in filtered seawater (3:10) was prepared and a freshly shucked infested abalone shell was placed into the observational vessel. The behavioural responses of the sabellids to the dye were recorded as for the reference behaviour.

Commercially formulated Abfeed was repeatedly milled in a hammer mill using a 500µm screen until a fine powder was formed. The Abfeed particles were sieved through a series of filters to a final size of 30 µm. A solution of commercially available food colourant was formulated using 30 ml of colourant in 100 ml of distilled water. The Abfeed powder was added to the colourant and stirred for a period of 1 hour using a magnetic stirrer. The suspension was filtered and the food particles were dried at 35°C overnight. The dyed particles were re-milled and sieved according to their colour so that each colour represented a particle size class. The size classes were 10-20 µm, >20-30 µm, >30-40 µm, >40-50 µm and >50 µm. The particles were sieved through the respective meshes three times to ensure an accurate size range.

The system was filled with water filtered to 20 µm and run for 20 minutes with a 20 µm filter between the overflow and sump, removing residual particles prior to the experiments. An abalone shell was placed into an observational chamber of the recirculating system and an individual sabellid was focused on, as in the reference behaviour studies. The same mass (1 g) of the different coloured size classes was introduced into the system and was allowed to run for 5 minutes to distribute the particles before commencement of video recording. The experiment was run for 45 minutes and the water temperature was maintained between 16-18°C. Behavioural observations were made from the video recordings.

At the end of the feeding duration the shell was removed and preserved in 2.5% gluteraldehyde for 24 hours. The sabellids were removed by digesting the abalone shell with 6.5% nitric acid in 70% ethanol. The abalone shell was placed in a large beaker with nitric acid and stirred with a magnetic stirrer. As the shell softened, pieces were broken off and the sabellid tubes opened, releasing adult and larvae sabellids as well as some eggs. All adult sabellids were collected and held in 2.5% gluteraldehyde until analysed for gut content analysis.

The 60 intact adult sabellids were prepared on wet mounts and examined under a compound microscope. The length of the digestive tract was traced and examined for the presence of dyed food particles. A sample of the particles was prepared on a wet mount for comparison of size and colour.

# RESULTS AND DISCUSSION

## *Behavioural observation*

Results from the behaviour studies were unclear as too few particles were observed to be ingested and the colouration of the particles was not clearly distinguishable. Thus, the behaviour data were not analysed further.

## *Examination of digestive tract*

The digestive tract and faecal groove of the sabellid could be traced fairly easily using good lighting and a compound microscope under high magnification. Most of the digestive systems of the sabellids appeared empty, with only a few sabellids showing signs of having ingested the dyed food particles or having particles within the gut. Sixty sabellids were prepared and analysed and out of these only 11 worms showed signs of ingested particles.

The duration of the feeding period could have been too short. Ruck and Sales (1999) found that sabellids had ingested liposomes after a 20-minute period. In their experiments they supplied the sabellids with particles for periods of up to 2.5 hours, at the end of which the sabellids were observed to have liposomes within their hind gut. The feeding period in these trials could therefore have been too short to allow sabellids to ingest a sufficient number of particles. However, the concentration of particles within the water appeared to be sufficiently high to allow particles to be available to the sabellids. Since sabellids were observed to ingest liposomes after 20 minutes by Ruck and Cook (1999), a feeding duration of 45 minutes was decided upon as it would be of sufficient duration to allow particles to be detected and ingested without any particles passing through the digestive tract and being eliminated via the faecal groove. Size, type and shape of the food particles may have been inappropriate. When observed under a microscope, the particles appeared to have an irregular shape with sharp and protruding corners. It may be possible that sabellids did not ingest many particles due to the irregular shape.

A final explanation for the low ingestion rates was that the sabellids may have been able to detect the food dye incorporated into the feed particles. This was not expected as non-toxic food dyes were used. The dye may, however, have reduced the feeding response. To test this hypothesis, an experiment was carried out to test the effect of the food dye on sabellid behaviour.

The average time the sabellid spent open and feeding was 154.3 ± 85.7 sec., which is significantly lower than the reference feeding behaviour (307.1 ± 94.9 sec.) ($p < 0.0001$). The frequency of withdrawal was 6.4 ± 3.3, which is significantly lower ($p<0.001$) than the frequency of withdrawal of the reference behaviour. Figure 2-5 shows the average duration the sabellids spent exposed between withdrawal events. Ninety percent of the sabellids remained open for less than 10 seconds (mean 5.0 ± 3.6 sec.) per feeding event. This is significantly different ($p<0.0001$) from the reference data where the mean average time feeding was 39.5 ± 14.8 sec. The majority of the sabellids remained in their burrows for 20-100 sec. at a time (Figure 2-6). This produces a significantly higher average of 86.4 ± 58.7 sec. in comparison to the 39.1 ± 19.0 sec. found in the reference behaviour study.

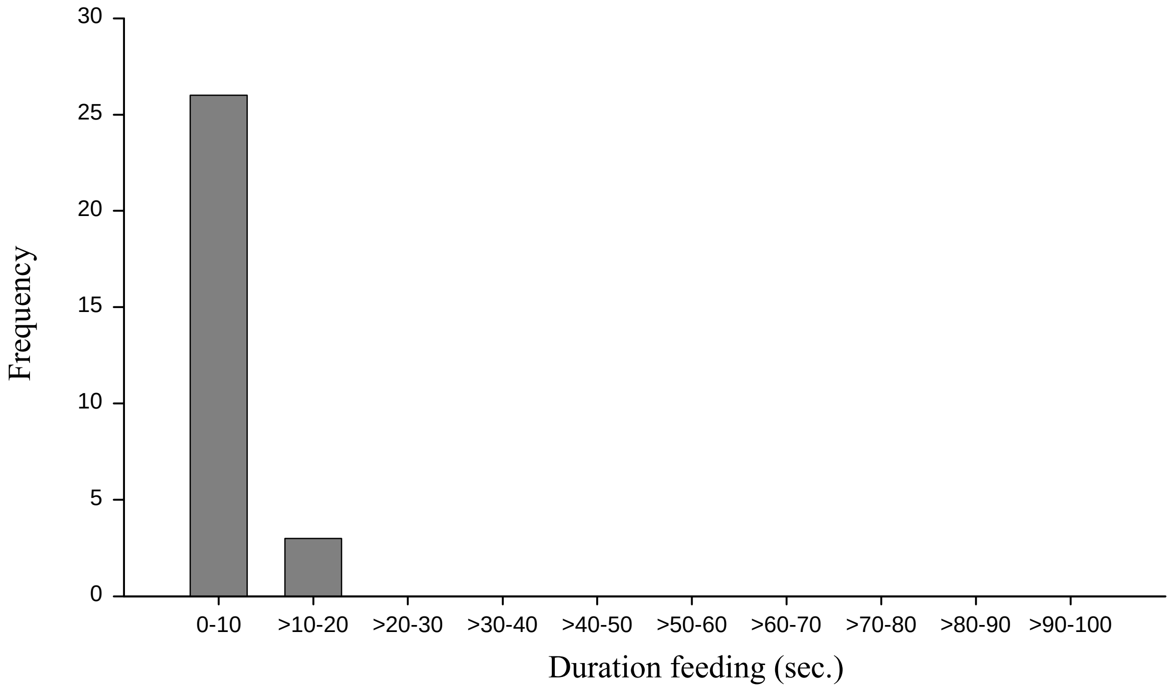


**Figure 2-5: Frequencies for different durations (sec.) sabellids spent feeding in the presence of food dye.**

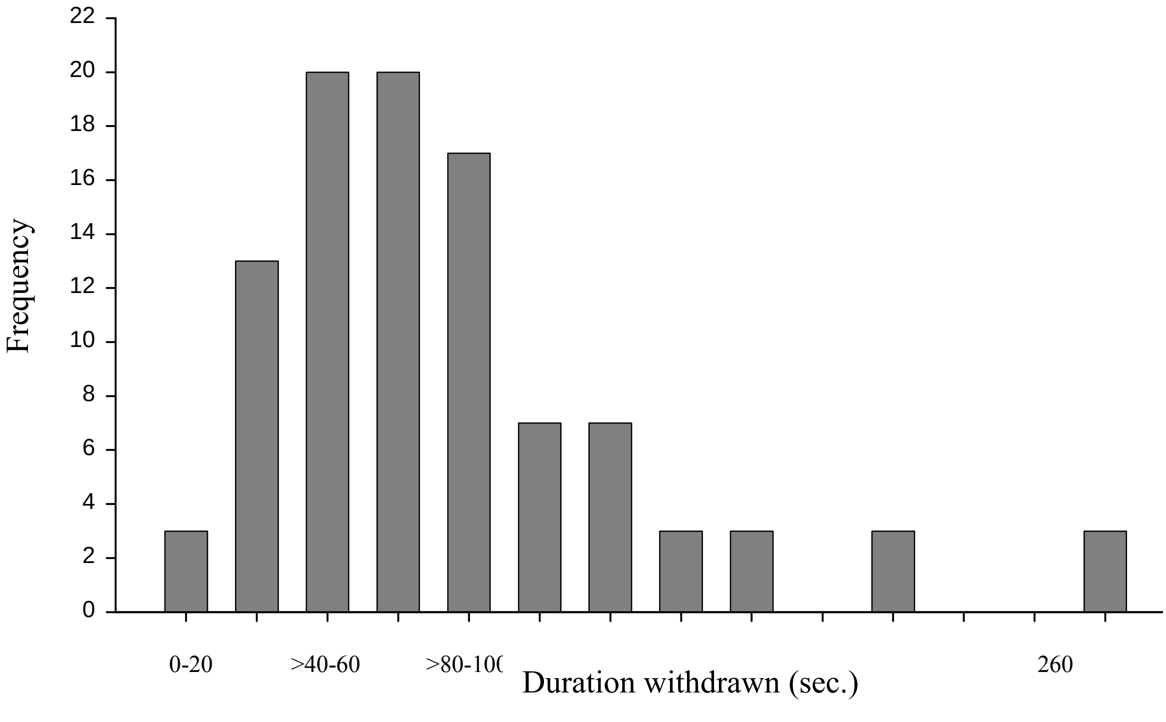


**Figure 2-6: Frequencies for different durations (sec.) sabellids spent withdrawn in burrows in presence of food dye.**

Thus, the food dye significantly altered the behaviour of the sabellids and further experiments using this method were abandoned. It was decided to pursue the feeding behaviour observational studies under the actual farm conditions rather than trying to simulate farm conditions in the laboratory. Further behavioural observations were carried out using the natural particulates present in the abalone raceways and available to the sabellids. These data are presented in Chapter 3.

# CHAPTER 3

# AN INVESTIGATION INTO THE INFLUENCE OF PARTICLE SIZE AND TYPE ON THE FEEDING BEHAVIOUR OF *Terebrasabella heterouncinata*

## INTRODUCTION

### *Polychaete feeding modes*

Polychaetes are known to feed on a wide size range of particles, live or dead material from plant or animal origin, and some species are capable of absorbing dissolved organic matter directly from the environment (Fauchald and Jumars, 1979). Detritus is the major food source for most polychaetes (Fauchald and Jumars, 1979) and the anatomical specialisation of a species determines its feeding method. For example species which have feathery branchial crowns are unable to actively scrounge for food and rely on water currents to bring suspended food particulates to them (e.g. *Sabellastarte longa*), while *Scolelepis squamata* has buccal tentacles and can gather detritus actively from the surface of the surrounding substrate (Day, 1969).

The polychaete class can be classed into errant and sedentary groups, which differ in their methods of food acquisition. Errant species are able to move about and actively search for food while sedentary forms are found in all particle feeders that are either collecting particles from the surface of the substrate (deposit feeding), or filtering suspended particles from the water column (suspension feeding) (Day, 1969). Suspension feeding is widespread in benthic marine habitats and suspension feeders are often the most conspicuous members of the benthic community (Hunter, 1989; Turner and Miller, 1991). Although they are often abundant and may dominate certain communities, there is little information on the quantitative (Shumway *et al.*, 1988) and behavioural aspects of filter-feeding in polychaete worms. Further research into the behaviour associated with feeding in sedentary polychaetes is required in order to better understand the processes behind particle uptake and selection. Dales (1957) performed studies on two species of sabellids, *Myxicola infundibulum* and *Sabella pavnina*, and concluded that fan worms are less efficient than other suspension feeders in both the volume of water they are capable of processing and the kind of particles they can retain. Only non-living and non-motile particles were retained, implying that sabellids are dependent on detritus for a food source. Shumway *et al.* (1988), however, present contradicting data from more recent experiments showing that *M. infundibulum* is capable of filtering algae from the water and at a faster rate than that of similar sized bivalves. This contradictory information highlights the need for further species specific research in order to understand the feeding modes and behaviour of particular organisms.

Deposit feeding tentaculate polychaetes have been more thoroughly studied than suspension feeders (Shimenta, 1996) and past research has concentrated on physical variables, such as current speed and particle concentration, which cause polychaetes to switch from suspension feeding to deposit feeding or vice versa, and not the quantitative assessment of suspension feeding. Benthic suspension feeders have developed the necessary specialisations to survive in an environment with typically low concentrations of particulates (Riisgård and Ivarsson, 1990) and the degree of anatomical specialisation determines the flexibility of the feeding behaviour and capabilities of each species. *T. heterouncinata* has a well developed branchial crown typical of the Sabellidae family but nothing is known about its feeding behaviour and feeding capabilities.

There are numerous anatomical and behavioural adaptations for food acquisition within the polychaete class (Dauer, 1983) and many species of marine polychaetes have multiple feeding modes (Bock and Miller, 1997). There are several instances where polychaetes have been observed switching between surface deposit feeding, where the tentacles scour the sediment surface around the burrow entrance for particulates, and suspension feeding, where particulates are filtered from the water column. *Spiochaetopterus oculatus* and *Spio setosa* are examples of polychaetes which switch between deposit and suspension feeding under conditions of different current velocity (Turner and Miller, 1991; Bock and Miller, 1997). The method of particle retention by suspension feeders is poorly understood. This is primarily due to the fact that most studies on filter feeders have concentrated on describing the feeding structures and not the cilliary selectivity and fluid mechanical aspects (Jørgensen, 1981). For a better understanding of particle capture it is necessary to understand the systems of water currents created by the cilliary tracts (Jørgensen, 1981) and how an organism's behaviour influences the flow dynamics during feeding. It is important to learn more about the feeding mechanism of the sabellid, *T. heterouncinata*, as behaviour could play an important role in feeding and may have resulted in the successful infestation by the sabellid within the abalone industry.

## *Feeding strategies*

There are several factors which influence the feeding strategy of polychaete worms. It is important to be able to identify those factors which influence the sabellid's feeding mode in order to understand their behavioural responses to changes in environmental conditions. Fauchald and Jumars (1979) studied and categorised the feeding guilds of the polychaete class according to feeding mode, behaviour and motility. There are two modes of feeding behaviour in the polychaetes, depending on both the particle sizes involved and the handling and transport method. Polychaetes that handle large particles one at a time, using most of the feeding appendages to transport the particle to the oral cavity, are termed macrophages, while microphages handle smaller food particles in bulk, usually transporting several particles to the oral cavity simultaneously. Feeding behaviour is further influenced by an organism's motility. Polychaetes can be divided into errant and sedentary forms, although the distinction is not always clear (Barnes, 1987). The structure and function of the feeding apparatus is also directly related to the motility of the organism. Polychaetes having developed jaws are generally carnivorous or omnivorous burrowers and crawlers and would be unable to feed on particulates suspended in the water column. Worms with highly developed elaborate branchial crowns for filter feeding on the other hand are unable to move and are usually sessile tube dwellers.

### *Sabellid feeding biology and anatomy*

Nicol (1930) described the structure and function of the tentacular crown, the feeding paths and the rejection paths in *Sabella penicillus*. Since this early work there has been very little research into the Sabellidae family and its feeding behaviour and strategies. *Terebrasabella heterouncinata* is a member of the Sabellidae family and Sabellinae subfamily, which are characteristically sessile suspension feeders inhabiting tubes of some kind. The prostomium and peristomium of sabellids is highly modified into a tentacular crown (Fauchald and Jumars, 1979) and it is used for gaseous exchange and to filter particles for both feeding and tube construction. *Terebrasabella heterouncinata,* however, does not construct its own tube from sand grains but is dependent on a gastropod host for its tube formation during its larval life phase. The branchial crown of *Terebrasabella heterouncinata* consists of two pairs of radioles each with 4-6 pairs of ciliated pinnules used for feeding (Fitzhugh and Rouse, 1999). Members of the subfamily Sabellinae remain in their tubes for life and usually only the head of the worm emerges from the opening of the burrow (Barnes, 1987). Some species within the family are capable of turning over their feeding crowns and touching the substratum to perform selective deposit feeding (Fauchald and Jumars, 1979). In the sabellid family there are differences in the activity levels within their tubes and this is an indication of their energy requirements; some species are highly active while others appear quiescent (Fauchald and Jumars, 1979). This behaviour implies differences in energy requirements. Unfortunately, no studies on quantifying the energetic requirements have been done on any species (Fauchald and Jumars, 1979). Sedentary species of polychaetes have a respiratory rate approximately 2.4 times lower than errant species (Shumway *et al.*, 1988) indicating the difference in activity levels between the two polychaete feeding modes.

The potential diet of a species includes all food items it is behaviourally and enzymatically capable of using in its diet (Fauchald and Jumars, 1979). The diets as well as the feeding behaviours vary considerably across the diverse class of the polychaetes and many motile species are capable of changing diets and locations according to fluctuations in local environmental conditions. Sessile filter-feeders either have to wait out unfavourable conditions or possibly modify their feeding mode according to the local conditions. Sabellids fall into this category, in particular the subfamily Sabellinae as they remain in their tubes for life (Fauchald and Jumars, 1979). According to Fauchald and Jumars (1979), sabellids apparently select particles exclusively on size and no evidence has been presented for selection based on other characteristics. Previous evidence has also been presented for particle selection in sabellids by Fitzsimons (1965), who showed that *Sabellastarte magnifica* could separate particles into three size classes, the smallest being food particles, and the two larger groups being used for tube construction or discarded.

Bock and Miller (1997), while working on *Spiochaetopterus oculatus* and *Spio setosa*, discovered that particulate organic coating functions as a cue that moderates feeding mode and behaviour, with nitrogenous compounds having an important role. Unlike *T. heterouncinata* which has a well developed branchial crown, both these polychaete species are bitentaculate interface feeders capable of switching between the deposit and suspension modes of feeding under differing particulate and flow regimes. The results also indicated that organic matter composition influenced feeding mode selection (deposit or suspension feeding) more significantly than the concentration of particles. Shimenta (1996) worked on *Pseudopolydora paucibranchiata*, a polychaete which is capable of both deposit and suspension feeding. He determined that when deposit feeding, *P. paucibranchiata* selects for smaller particles relative to abundance in comparison to suspension feeding, where there was apparent selection for larger particles. Bock and Miller (1997) observed that flow rate within the experimental system was a contributing factor in determining the feeding preference, with an increase of time spent suspension feeding with increasing flow speed. *T. heterouncinata* has the typical development of the branchial crown suited to suspension feeding and has therefore been postulated to be a facultative suspension feeder.

### *Flow speed and dynamics as determinants of feeding mode*

Fluid movements determine to a large degree the mechanical forces impinging on an organism, the rates of respiration and excretion, and the feeding mode employed by some organisms (Merz, 1984). Many species of Sabellidae form congregations of densely packed mounds (Hartman, 1969). These mounds have been shown to affect the flow patterns of water passing over aggregations of tube-dwellers (Eckman, 1983). The cluster of tubes acts as a breakwater causing the water to flow at a higher speed over the surface of the mound and hence pass through the area where the branchial crowns are situated (Merz, 1984). The current speed is lowest below the entrance to the tubes. Sabellids are therefore likely to experience higher flows in the branchial region when aggregated into clusters while experiencing less stress on the actual tube (Merz, 1984). It has been observed in the South African abalone industry that *T. heterouncinata* forms dense colonies on the abalone shells.

The behaviour of these sabellids in response to flow conditions could therefore be an important aspect of their feeding biology and requires further research.

## Aims and objectives

The aim of this chapter was to observe and document behavioural feeding patterns and feeding strategy of *Terebrasabella heterouncinata* in the presence of particulates from different on-farm conditions. To accomplish this, the feeding behaviour of sabellids exposed to different sized particulates originating from kelp and Abfeed raceways was quantified.

## MATERIALS AND METHODS

Infested abalone, 60-75 mm in size, were used as sabellid hosts for feeding behaviour observations. The abalone were removed from an on-farm grow-out flow-through system. The shells were well washed to remove particulates and the animals were placed into a 60-litre aquarium with aeration and flow-through water supply. The water temperature was 13.4 ± 2.6°C for the duration of the holding period. The aquarium glass was painted black to simulate the dark conditions of the farm raceways. On the farm the abalone had been fed on a predominantly kelp diet but they were purged for two days prior to, and for the duration of the behaviour observations.

The observational recordings were designed to quantify the feeding behaviour in response to particulates of different sizes and origins. During the observation the sabellids were kept in a small recirculating system. The system consisted of an observational chamber, over which a dissecting microscope and video camera were mounted, and a sump having a total volume of 8 litres (Figure 2-4). Seawater used for the observations was passed through 18 µm and 10 µm filters before it entered the system. Once filled to the required volume, the pump was turned on and the system was left to run for 20 minutes with the water flowing through a 20 µm filter placed between the observational chamber and the sump to remove particulates remaining within the piping of the system and the impeller of the submersible pump.

All infested abalone were housed in a dark tank prior to the sabellid behaviour recordings. Observations were carried out in a dark room with the only light coming from an optic fibre light source emitting a maximum light intensity of $0.34 \times 10^{17}$ quanta.sec$^{-1}$.cm$^{-2}$. Temperature for all recordings was 12.7 ± 0.6°C and the flow rate was maintained between 200-250 ml.min$^{-1}$.

Prior to the commencement of each observation, an infested abalone was shucked and the shell was rinsed with seawater and placed onto the false bottom of the observation chamber. The shell was positioned over the inflowing current to create currents along the edges and over the top of the shell. An external optic fibre light source was used to illuminate the shell sufficiently to see the behaviour of the sabellid. After the shell was placed into the system, the sabellids were allowed a five-minute period to acclimate and recover from the disturbance of being moved. The microscope was moved and adjusted accordingly until an individual sabellid, which was unobstructed and could be clearly seen, was in focus on the monitor. If possible a sabellid on the back edge of the abalone shell was used for the observation as they were situated directly in a current. For all observations involving particulates from kelp and Abfeed raceways, 2 g wet weight of particulates of the selected size class were introduced into the sump and stirred up to form a suspension. High particle concentrations were used in these experiments to increase the particle encounter rate during the observational recordings. During earlier experiments at lower particle concentrations the particle encounter rate was very low, and often no particles came in contact with the sabellid feeding crown during the observational period. Higher concentrations of particulates were therefore used to facilitate the observation process and did not appear to cause disturbance or fouling of the sabellid feeding apparatus.

The system was left to run for five minutes before the commencement of the recording session. Recordings were made on a minimum of three sabellids for a total of 45 minutes for each particle category. Recordings were also made using unfiltered water taken directly from kelp and Abfeed raceways to simulate the natural conditions found in raceways. In each case a 10-litre bucket was used to transport water from the raceways to the observation system. The water was removed from raceways with the same stocking densities and abalone sizes for each diet.

The particulates required for the treatments were collected by means of a siphon and series of polyester plankton mesh sieves from 30 cm below the surface of the raceways. The water was sieved through a series of sieves of mesh sizes 500 µm, 100 µm, 50 µm, 40 µm, and 20 µm. The particulates were stored on ice until used in the experiments.

The recordings were played using a video machine and the behavioural responses to the different conditions were entered into a computer programme. Behaviours identified are shown in Figure 3-1.

When the branchial crown was open, it was assumed that the sabellid was feeding. Particles which came in contact with the branchial crown were categorised as either being rejected, when they were expelled from the feeding apparatus by a water current, or accepted, when the particle was seen to be moved down the feeding apparatus towards the mouth opening where it was ingested. Once a particle was taken into the mouth, a waiting period of 10 seconds was allowed to observe if the particle would be rejected. If it was not ejected within this period, the sabellid was assumed to have retained the particle. When the feeding crown was retracted it was noted whether this was due to a disturbance by an external source (e.g. another polychaete or copepod species occurring on the shell), due to a particle coming into contact with the feeding crown, or if the crown was retracted for no obvious reason. If the crown was retracted after a particle came in contact with it, on re-opening it was recorded whether or not the particle was rejected or if it was retained. A waiting period of 10 seconds after opening was allowed to observe if the sabellid rejected the particle or not. The frequency of faecal pellet ejection was also recorded. The faecal pellets could be recognised by their large size and elongated shape.

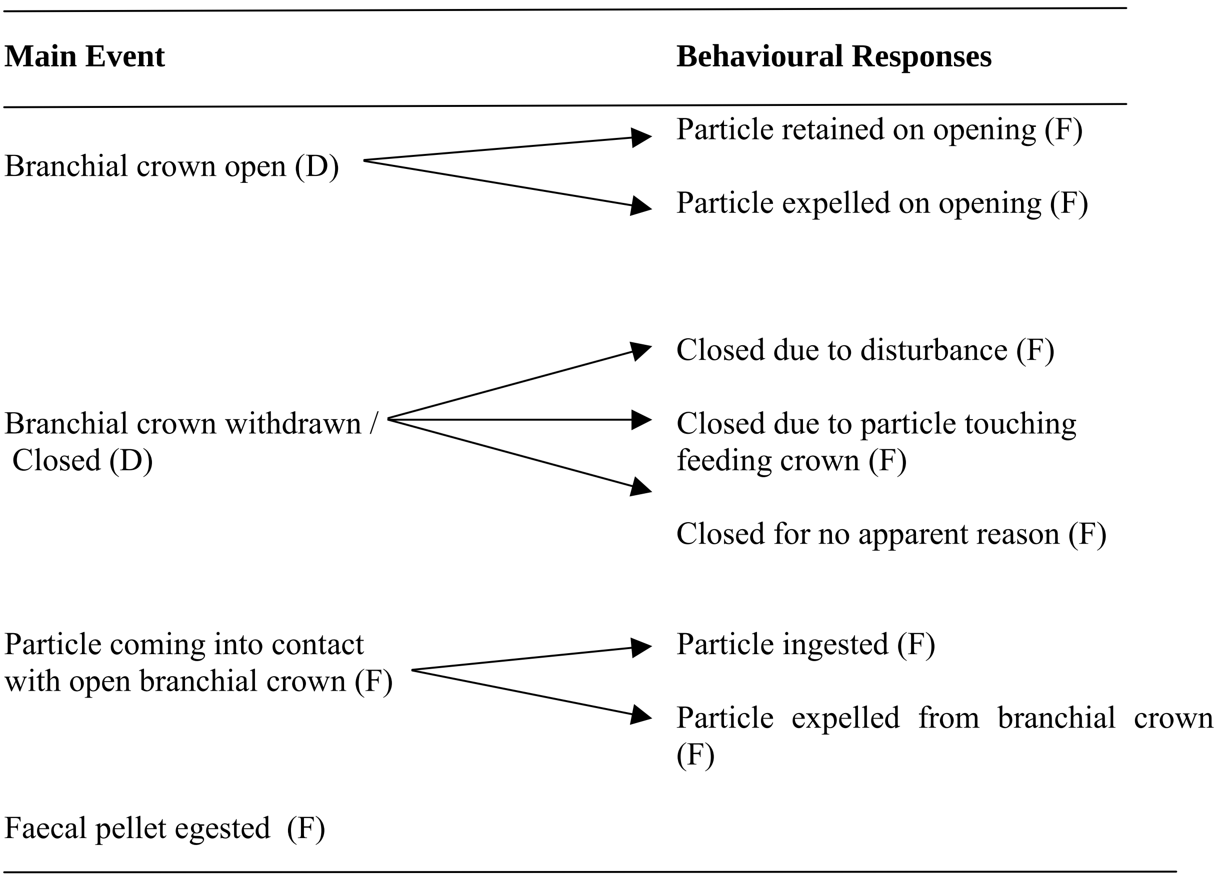


**Figure 3-1: Events recorded during the behavioural studies. D and F represent where duration or frequency was recorded, respectively.**

The events listed in Figure 3-1 were recorded from 15 two-minute observations on 5 individual sabellids for each treatment using computer software for behaviour observations (Programme: Observer).

## *Statistical analysis*

Where there were multiple observational recordings on an individual sabellid, the data were averaged. All data were tested for normality using the Lilliefors test, and Levene's test was used to test the homogeneity of variance assumption to meet the requirements for analysis of variance testing (ANOVA). Where required, data were log- and square root-transformed to meet the requirements for ANOVA testing. ANOVA and Tukey's multiple range tests were used to test for significant differences in the main effects, diet and particle size class, for all observations (Rosner, 1995). An alpha value of $p \leq 0.05$ was used for all tests. Initially the behavioural data were analysed as percentage particles accepted or rejected. This, however, resulted in a false representation as the n values for all replicates were very low and should therefore not be expressed as percentages. For this reason the sequence of behavioural events was tested for randomness using a Runs Test to determine if feeding behaviour occurred in a random or non-random fashion under different particle fluxes (Wackerly *et al.*, 1996).

# RESULTS

Because of the high frequency of disturbance in the >100 µm particle size treatments, these data were omitted from further analysis along with the "water only" treatment as this contained a mixture of particle sizes with very few contacts.

Percent of time spent feeding differed between particle size classes depending on diet ($p<0.0001$; $F_{2,24}=15.5$) (Figure 3-2). In Abfeed-fed sabellids, percent of time spent feeding increased from 40% to 80%, only as particle size increased from 40-50 to 50-100 µm. It decreased from 85% to 35% in sabellids fed kelp particulates of sizes 40-50 and 50-100 µm respectively.

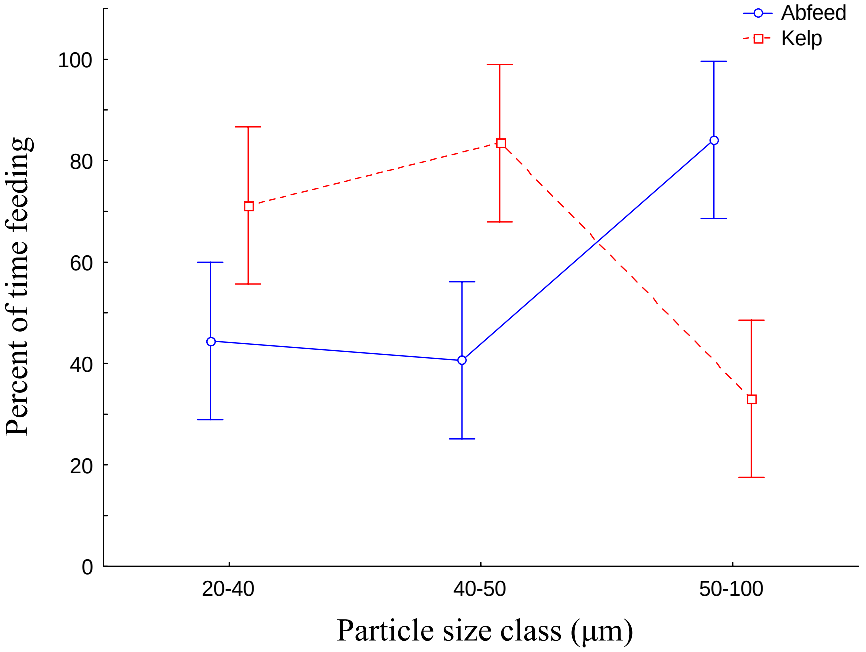


**Figure 3-2: Two way interaction of diet versus particle size showing average percent of time sabellid spent feeding. Error bars indicate 95% confidence limits.**

Diet and particle size had a significant interaction on the frequency of withdrawal ($p<0.00005$; $F_{2,24}=15.3$) (Figure 3-3). Frequency of withdrawal decreased with increasing particle size for Abfeed-fed sabellids and increased for kelp-fed sabellids.

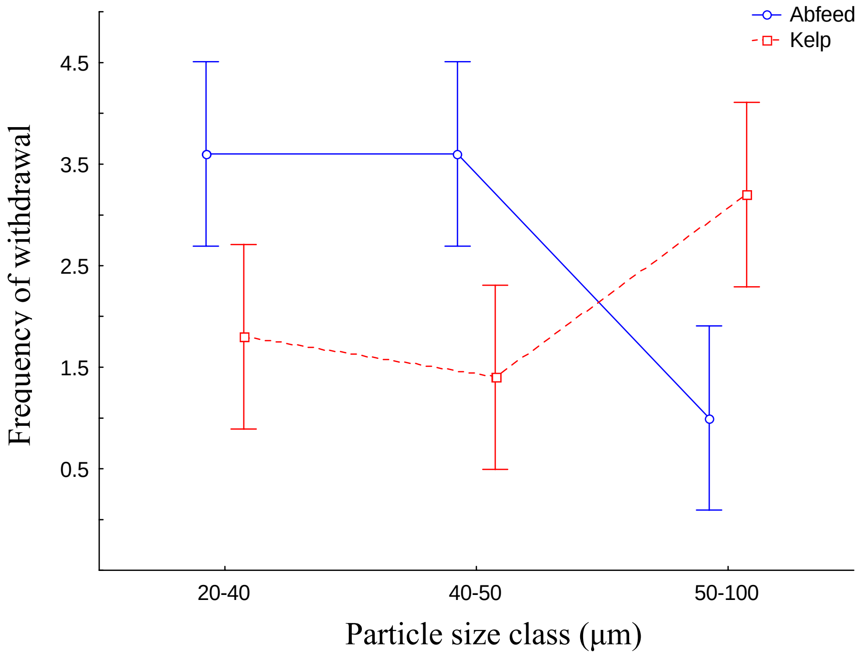


**Figure 3-3: Two way interaction of diet versus particle size showing frequency of withdrawal. Error bars indicate 95% confidence limits.**

There was a significant difference ($p<0.003$; $F_{1,24}=10.6$) and no interaction ($p<0.96$; $F_{2,24}=0.04$) in the time period the sabellids remained open and feeding after ingesting a particle between the two diets. The sabellids exposed to kelp particulates remained open for a longer period than those exposed to Abfeed particulates. (Figure 3-4). There was no significant difference between the particle size classes within the Abfeed diet ($F_{2,16}=2.98$; $p<0.079$) or the kelp diet ($p<0.84$; $F_{2,8}=0.16$).

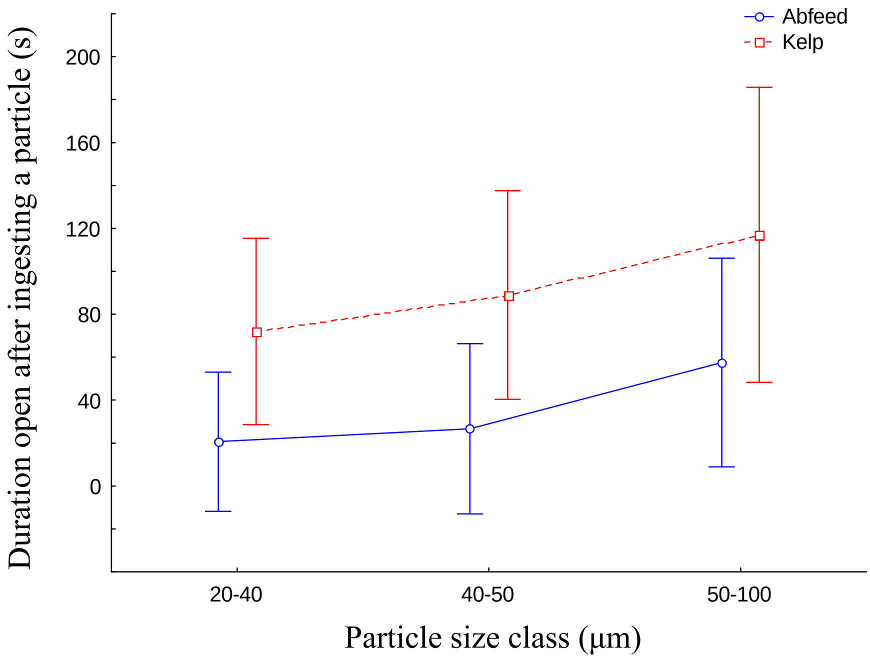


**Figure 3-4: Average duration (s) sabellid remained feeding after ingesting a particulate for three particle size classes for kelp and Abfeed. Error bars indicate 95% confidence bands.**

**Table 3-1: Particle contacts and particle rejection or acceptance for both abalone diets and all particle size classes. Data were pooled for all behavioural observations. Letters indicate where significant differences occur.**

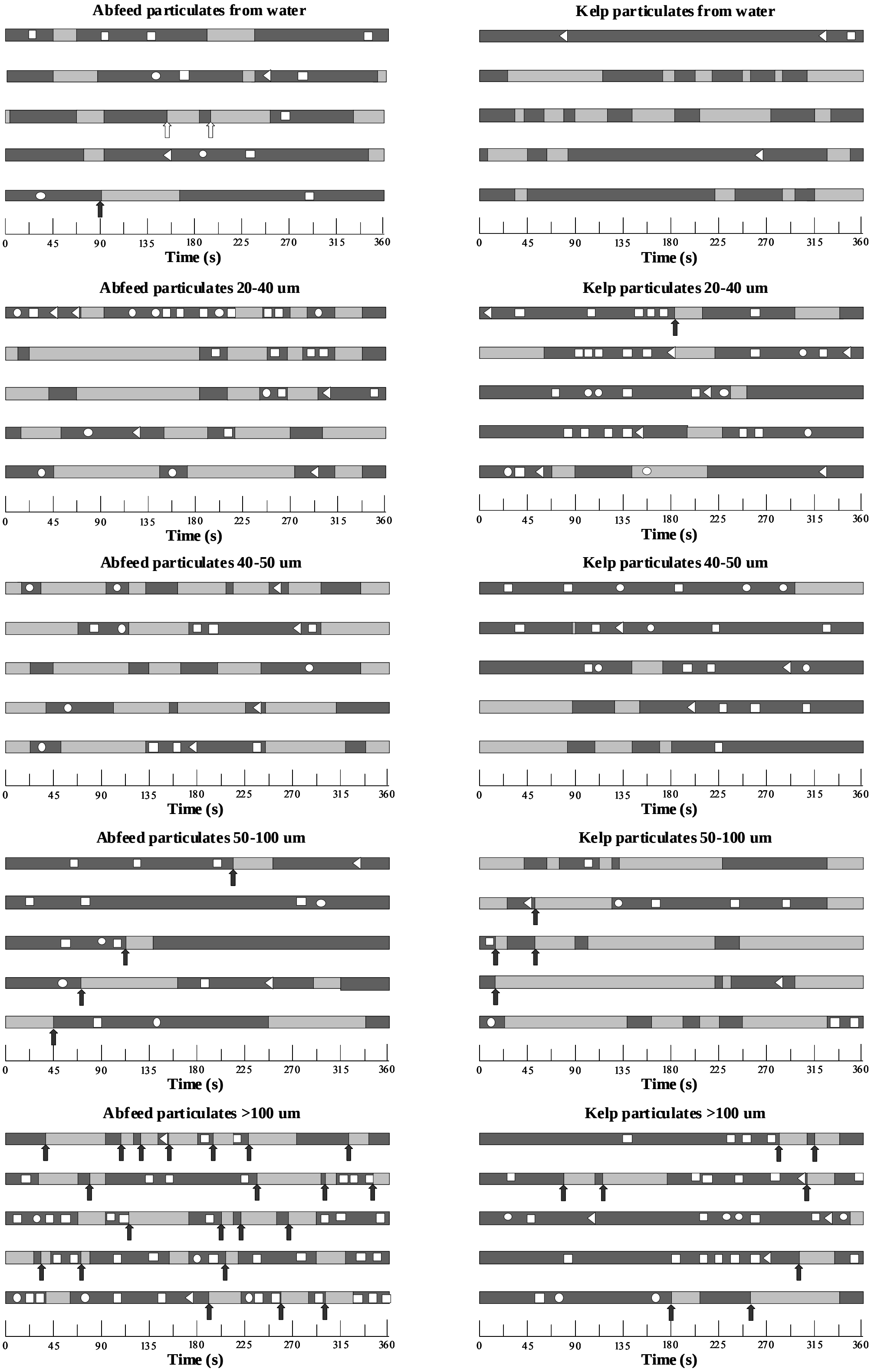
| Abalone diet | Particle size class | Number of particles | Number rejected | Number accepted | Number of faecal pellets egested |
|---|---|---|---|---|---|
| Abfeed | Water | $13^a$ | 10 | 3 | 2 |
| Abfeed | 20-40 | $24^a$ | 14 | 9 | 5 |
| Abfeed | 40-50 | $13^a$ | 7 | 6 | 4 |
| Abfeed | 50-100 | $18^a$ | 14 | 4 | 2 |
| Abfeed | >100 | $63^b$ | 58 | 5 | 2 |
| Kelp | Water | $1^x$ | 1 | 0 | 3 |
| Kelp | 20-40 | $30^y$ | 23 | 7 | 7 |
| Kelp | 40-50 | $20^y$ | 14 | 6 | 3 |
| Kelp | 50-100 | $13^x$ | 11 | 2 | 2 |
| Kelp | >100 | $30^y$ | 30 | 0 | 4 |

There was no significant difference for the number of particles coming into contact with the feeding crown between the two diet treatments ($p>0.05$; $F_{1,48}=5.2$). There was a significant difference in number of contacts between the Abfeed particle size treatment, with the >100µm particle treatment having significantly more contacts ($p<0.05$; $F_{4,20}=3.9$) than the other size classes. There were also significant differences between the kelp treatment ($p<0.0001$; $F_{4,20}=10.4$), with the 20-40, 40-50 and >100 µm particle size classes having more contacts than the kelp water and 50-100 µm particle size treatments, which were similar.

Tests for randomness were performed on the sequential feeding behaviour data to determine if there was an indication of a structured feeding response in terms of the frequencies for particle acceptance and rejection, and for durations between particle acceptance and rejection events. ANOVA testing has already indicated that there were significant differences between the treatments for particle ingestion, and the Runs test was used to determine if the sabellid fed in a structured manner within each treatment or whether particle ingestion was irregular and random in terms of number of particle contacts. The results of these tests indicated that all the behavioural responses were random and that there were no predictable feeding responses by the sabellids to any of the treatments (Table 3-2).

**Table 3-2: Results from tests for randomness on sabellid particle selection and rejection and frequency of withdrawal for particle size and diet treatments. * indicates the treatment where the n value was too small to perform a test for randomness.**

| Diet | Particle size class | p-value |
|---|---|---|
| Abfeed | Unfiltered water | p<0.23 |
| Abfeed | 20-40 | p<0.26 |
| Abfeed | 40-50 | p<0.29 |
| Abfeed | 50-100 | p<0.42 |
| Abfeed | >100 | p<0.61 |
| Kelp | Unfiltered water | * |
| Kelp | 20-40 | p<0.55 |
| Kelp | 40-50 | p<0.21 |
| Kelp | 50-100 | p<0.56 |
| Kelp | >100 | p<0.38 |



**Duration**

- ▬ Feeding crown open
- ▭ Feeding crown withdrawn

**Event**

- □ Particulate rejected from feeding crown
- ○ Particulate accepted and ingested
- ◁ Faecal pellet egested
- ⬆ Feeding crown withdrawn due to contact with particle
- ⇧ Feeding crown withdrawn due external disturbance

**Figure 3-5: Representation of sequential data for sabellid feeding behaviour studies. Bars represent replicate treatments and indicate duration the feeding crown is open or closed. The timescale (s) is situated on the x-axis of each treatment. Duration and occurrence of events are shown by symbols as described in the legend.**

# DISCUSSION

## *Effect of abalone diet on sabellid feeding*

The feeding behaviour and associated frequency of withdrawal is linked to the availability of particulates in combination with the diet. All sabellids are mucus feeders collecting particulates from the water column and transferring them to the mouth by way of grooves on the branchial crown (Bonar, 1972). Cilia are the primary mechanism of food collection in the sabellids and their primary roles are to produce circulating currents in the water, collect and transfer suspended particulates to the mouth and aid in the rejection of particulates from the branchial crown (Bonar, 1972). The selectivity of *T. heterouncinata* appears to be controlled by the cilia lining the radioles of the branchial crown, which appear to allow selection of particulates before reaching the pharynx. Particle selection was further observed to occur on reaching the pharynx where particles were either ingested or rejected. The majority of feeding studies have been performed on spionid polychaetes where it has been shown that particle selection occurs at the point of contact of the palp with the sediment surface (Dauer *et al.*, 1981; Levin, 1981; Taghorn, 1982), during transport along the palp (Fauchald and Jumars, 1979) and at the site of the pharynx (Levin, 1981; Taghorn, 1982). Selection methods along the palps, however, are passive and have been based on the adhesive strength of the tentacular mucus. Active particle selection at the pharynx has, however, been observed (Dauer *et al.*, 1981; Dauer, 1985). While working on *Spiochaetopterus oculatus* and *Spio setosa,* Bock and Miller (1996) discovered that organic coating was an important factor in particle selection. Selective rejection of nutritionally poor particles to increase the quality of ingested particles has also been observed in bivalves (Kiørboe *et al.*, 1980; Bacon *et al.*, 1998). These studies suggest that particle selection is based on organic quality, with inorganic particles having a low ingestion rate while particles of increasing organic content have an increasing ingestion rate. The protein and energy levels of the particulates from this study differed between kelp and Abfeed raceways (Chapter 4), which may have resulted in the observed difference in feeding selection of the sabellid between the two diets. Although certain species have been shown to be selective in their particle ingestion, others, such as *Scolelepis squamata*, show little or no selection at either the palp or pharyngeal level (Dauer, 1983). This indicates the diverse nature of feeding behaviour within the polychaetes and suggests that *T. heterouncinata* may be capable of changing its feeding pattern in response to changes in particulate composition and size.

Sorokin (1973) suggested that some sabellids may be concentrating on filtering the smaller size range of particulates composed of bacterio-, myco-, and small phytoplankton which are readily assimilated and constitute the greatest portion of the planktonic biomass (Sieburth *et al.*, 1978; Merz, 1984). In future studies it could be tested if bacteria colonise more rapidly on Abfeed particulates and if they constitute an alternative or additional food source for the suspension feeding sabellids. This could be tested by examining the particulates for the presence of bacteria and, if present, culturing these bacteria and using them for fluorescent or radio labelled feeding experiments. Additional food sources as a result of nutrient loading within Abfeed raceways could account for the higher infestation levels and population sizes observed in these raceways (Chapter 5). This is in part supported by the fact that Abfeed-fed sabellids are larger in size than kelp-fed sabellids (Chapter 5). Management procedures such as tank cleaning, stocking density and feeding protocols could therefore be a major factor influencing differences in sabellid infestations between kelp and Abfeed-fed abalone. Although not visually as dirty as kelp raceways, Abfeed raceways may require more regular cleaning and a modified feeding protocol in order to reduce the nutrient loading within the water column.

## *Effect of particle size on sabellid feeding*

Particle size influenced sabellid feeding behaviour depending on the abalone diet. The percent of time spent feeding increased with increasing particle size class for the Abfeed particulates and decreased for kelp particulates. Particle size selection is known to occur along the basal grooves of the Sabellid, *Chone mollis*, with small particles being carried along the groove to the mouth for ingestion, while larger particles are removed from the feeding crown (Bonar, 1972). Particle sorting according to size is also known to occur in *Sabella pavonina* (Nicol, 1930) and other fan worms but is crude compared with the sorting mechanisms in some other suspension feeding invertebrates (Dales, 1957). The most likely cause of particle size selectivity between the two diets requires further study. Statistical analysis indicated that the feeding behaviour for both diets was random. It is thus possible that the selectivity observed was due to satiation of the sabellids after ingesting several particles. The assimilation time of particles from kelp and Abfeed raceways may have differed due to the differences in organic quality (Chapter 4), which could help explain the difference in ingestion rates and feeding durations between the two diets.

Spionid polychaetes reduce the effects of browsing by epifauna by remaining withdrawn in their burrows (Woodin, 1981; Wilson, 1983; Dauer, 1985). Similarly, *T. heterouncinata* may withdraw into its burrow to assimilate particulates once it has reached satiation. More Abfeed particles from the smaller particle size classes were ingested than particles in the 50-100 µm class. The shorter feeding duration in the smaller particle size classes suggests that the sabellids may have become satiated more quickly under these conditions and withdrew into the safety of their burrows. The greater percent of time feeding at the larger Abfeed particle size class (50-100 µm) was possibly due to the smaller number of particles ingested as a result of the higher rejection rate observed. The feeding duration of kelp-fed sabellids was lowest at the larger particle size class (50-100 µm) and greatest at the two smaller particle size classes opposite to the behaviour of the Abfeed-fed sabellids. This difference in feeding behaviour may be related to the proximate composition of the particulates from each diet (Chapter 4). The gut evacuation time for kelp particulates may be quicker due to the lower protein composition of these particles (Chapter 4). A shorter gut evacuation time would allow the sabellids to ingest particles more frequently before they become satiated and stop feeding. This could account for the greater feeding duration of the sabellids in the presence of kelp particulates. The sabellids supplied with kelp particulates may also require a larger volume of food to obtain the same nutritional value as the Abfeed-fed sabellids and would therefore require a longer feeding duration. The short feeding duration of the sabellids exposed to 50-100 µm kelp particulates may have been due to unfavourable particulate size and disturbance from the large particles causing feeding to become unprofitable.

Sabellids provided with 50-100 µm Abfeed particulates had a lower frequency of withdrawal corresponding with their longer feeding duration. Together these factors suggest that these sabellids remain open in an attempt to select particles for greater nutritional gain. The majority of particulates in this size class were rejected, with only a small percentage being ingested, further indicating the selective rejection for this size class of Abfeed particulates. For all particle size classes sabellids exposed to kelp particulates remained open for a longer duration after ingesting a particle. This could be related to the quality and nutritional value of the particulates ingested (Chapter 4). Kelp particulates are lower in protein and energy level than Abfeed particulates and it is possible that sabellids feeding on kelp particulates require a greater quantity of food particulates in order to obtain sufficient nutritional value and therefore require longer feeding periods.

In summary, the suggestions explaining the causal factors for the feeding behaviour of *T. heterouncinata* are derived from both the information about the diets obtained in the other chapters of this thesis, and various hypotheses presented in the literature. At this stage they remain working hypotheses for future studies. This study was, however, able to provide directions and useful guidelines for future research. In particular, the combination of particle size and composition appears to be worth investigating. The organic composition of small Abfeed particles that cannot be revealed by proximate analysis (Chapter 4) may provide a clue for sabellid behaviour. In addition, the study showed that methods for quantifying satiation appear worth developing, as this information may help understand possible causes for the frequency of withdrawal.

## *Discussion of the data analysis*

There was a large variation in the number of particle contacts for each treatment and the related behavioural response. In addition sample sizes were small. Thus, less conventional methods of data analysis needed to be applied for this study. For example, analysis of percentage of particles ingested and rejected was not appropriate for the type of data. This was due to the low and variable n-values. During these experiments there could be no control over the number of particles passing the sabellid or coming into contact with the feeding crown. This resulted in varying n-values for particle contact, particle ingestion and particle rejection. When analysing small data sets, percentages do not give a true or accurate description of the data, as small changes in frequencies can produce very large effects. For these reasons data representation using percentages was avoided. Problems associated with small and variable sample sizes are rarely addressed in the literature and no standard statistical methods for analysing data of this type were available. Here, the data were tested for randomness of behavioural events. Tests of randomness use the number of events over a given time period to test if the occurrence of events occurs in a regular or random order (Wackerly *et al*., 1996). It was hypothesised that non-random behaviour of sabellids would indicate optimal feeding conditions in which sabellids control their feeding behaviour in order to maximise their net energetic gain according to the optimal foraging theory (Linton and Taghorn, 2000).

The results from these tests indicated that the feeding behaviour of the sabellid was random for all particle size classes and both diets. Particle size is thought to be the most important cue for suspension feeders and it was thought that the sabellid would feed more actively in the presence of one of the particle sizes tested. The statistical tests used, however, did not pick up any significant differences in the sequence of events between particle size classes. Many feeding studies on invertebrates have focused on testing predictions of optimal foraging theory. This theory focuses on processes such as feed particle selection and feeding rates which organisms might use in order to maximise energy intake while minimising energetic costs (Whitlatch and Weinberg, 1982). Several studies have found that certain aspects of invertebrate feeding behaviour are described by optimal foraging as opposed to random or non-optimal foraging (Doyle, 1979; Taghorn, 1982). These studies, however, have involved deposit feeders which expend energy foraging for food particles. Under conditions of poor or low food particle availability, expending energy foraging would be costly with little or no return. It is understandable therefore that these species should stop feeding under these conditions, and that they would therefore display non-random feeding behaviour. Suspension feeders on the other hand expose their branchial crowns from their burrows and rely primarily on the water currents to supply them with food particulates. Under conditions of high food availability, suspension feeders utilise their cilia to create local currents to increase the capture rate. For these reasons suspension feeders may not have an energy driven strategy in optimising feeding behaviour as has been observed in deposit feeders. Here, the prolonged feeding periods constitute a vulnerability to epifaunal feeders which may damage their branchial crowns. Thus, behaviour may be risk-driven rather than determined by energetic costs. This theory deserves further study.

Other suspension feeders such as bivalves are known to alter their feeding and digestive behaviour in response to fluctuations in the concentration and quality of suspended particles (Bacon *et al.*, 1998). They possess mechanisms to control ingestion of particulate organic matter, including regulating duration and rate of feeding, producing pseudofaeces, selecting more nutritious particles, and altering digestive efficiency (Hawkins *et al.*, 1990; Bayne *et al.*, 1993; Bacon *et al.*, 1998). *Placopecten magellanicus* and *Mya arenaria* have the ability to improve the quality of particulates ingested by the production of pseudofaeces and the preferential rejection of inorganic particles. The ability of these species to preferentially reject inorganic over organic particles decreased as the quality of the particulates decreased. Sabellids are exposed to continuously fluctuating conditions, ranging from extreme particulate loading to periods of very little nutrient availability, to which they have adapted. It is possible that their feeding behaviour patterns are continuously modified to conform to the environmental conditions and particulate abundance surrounding them. This is in part supported by the findings from this study, which showed that sabellid feeding behaviour was highly variable, and it should be tested if this is in response to changing conditions.

On a study of the sabellid, *Eudistylia vancouveri*, Merz (1984) discovered that the position of the branchial crown played an important role in particle capture. The branchial crown was positioned symmetrically and vertically over the tube in still water whereas in flowing water the crown was angled downstream. This is thought to aid in capturing particles from the water current. In this way behaviour of the organism was used to increase the particle capture. Warner (1977) suggests that this may be due to reduced flow speed and re-suspension and re-circulation of particulates in eddies increasing the chance of particle capture. However, flow rates are not the sole criterion by which filtration efficiency should be evaluated (Merz, 1984). If a suspension feeder can acquire all the food it needs by generating low filtration velocities, it may well be more efficient than suspension feeders that generate high flow rates at a greater energetic cost (LaBarbera, 1984). In this study *T. heterouncinata* was observed to rotate and reposition its branchial crown periodically. Although this was not quantified, it is suggested that this may have been a behavioural response in order to increase particle contact and capture after long unsuccessful feeding periods. Currents were created by the cilia on the radioles, which drew particles in proximity nearer for capture, where they were either rejected or transported along to the pharynx.

From this study it appears that the *T. heterouncinata* may modify its feeding behaviour in terms of crown positioning and feeding duration in order to maximise food intake, similar to *Eudistylia vancouveri*. The feeding duration and frequency of withdrawal appears to be diet dependent and requires further study in order to clarify the exact relationships. There was no structured feeding pattern for either diet or particle size treatments but selective particle rejection, particularly in the larger particle size classes, was observed to occur for both diets. This suggests that food quality and particle size play an important role in the particle selection process.

# CHAPTER 4

# INVESTIGATION INTO THE SIZE AND COMPOSITION OF PARTICULATES IN COMMERCIAL ABALONE RACEWAYS

## INTRODUCTION

One major and most commonly identified problem of aquacultural activities is feed-derived wastes and their effect on the environment (Cripps, 1995; Tovar *et al.*, 2000). Both the intensity and production of aquaculture operations is increasing throughout the world and the quantity of waste material within the effluent will increase as the production and intensity increases. The main components of the effluent from flow-through aquacultural systems are nutrients such as phosphorus and nitrogen compounds, suspended solids and pathogens (Cripps, 1995). Phosphorus in freshwater and nitrogen in seawater are usually the growth-limiting nutrients in natural ecosystems (Welch and Lindell, 1992). These nutrients are important feed components for culture organisms to increase growth rates and are therefore often incorporated into the feed in excess of requirements (Wiesmann *et al.*, 1988). Unused, regurgitated, or unmetabolised nutrients and organics are discharged into the water, creating a highly eutrophic environment which can lead to ecological changes (Anonymous (c), 1990).

Aquaculture effluent properties have been quantified to assess the environmental effects (Enell and Ackefors, 1991) and the efficiency of treatment devices (Bergheim *et al.*, 1993). Little work has been conducted on quantifying the physical properties, such as particle characteristics, with the aim of using the results to more effectively design treatment effort and improve treatment efficiency (Cripps, 1995). Diurnal and seasonal changes in water quality in aquaculture systems have been shown to occur (Poxton and Allouse, 1987; Poxton and Lloyd, 1989). Aquaculture wastewater typically has a low suspended solids concentration compared with various industrial and municipal wastewaters (Cripps, 1995).

Abalone farming is an intensive industry in South Africa with space often being a limiting factor. This is due to the slow growth of abalone and the long grow-out periods required for them to reach marketable size. Abalone are herbivorous gastropods which feed on marine seaweeds (Hahn, 1989). In the South African abalone industry a formulated pellet feed has been produced as a substitute for seaweeds, such as *Ecklonia maxima*, which is commonly fed. Formulated diets guarantee a constant and reliable supply of food, as seaweeds are difficult to obtain during certain seasons and heavy seas. These diets have their protein and energy ratios optimised to achieve best growth. They can be more cost effective than natural diets by improving abalone growth and thus farm production (Sales and Britz, 2001). A negative aspect of artificially formulated diets is that they may release more nutrients and particulates into the water column than seaweeds. In doing so they can create a nutrient rich environment which has negative implications for aquaculture. Oxygen deficiency and poor water quality, typical of a eutrophic environment, has been implicated with bacterial, protozoan and fungal diseases in fish (Ching *et al.*, 1993) and a high level of suspended solid may affect fish health (Ceronio *et al.*, 1995) and can be lethal (Alabaster and Lloyd, 1980). This stresses the importance of water quality on the health of aquatic organisms.

The high level of nutritionally rich suspended solids found in aquacultural waters provides an ideal environment for filter-feeding organisms. For example, bivalves have been shown to be effective in removing small suspended solids from shrimp pond water thereby improving the water quality for shrimp production (Wang, 1990). Bivalves can feed selectively, so that certain particles are ingested and digested while others are rejected as pseudofaeces (Clark and Wikfors, 1998). Suspension feeders thrive in the presence of a high load of suspended particles typical in intensive aquaculture facilities. In a study performed to determine the effectiveness of oysters in removing particulate organic matter from aquacultural effluent water, oysters were capable of reducing the total organic load by 50% (Jones and Preston, 1999). Most of the inorganic matter was in the form of very fine suspended particles which the oysters removed effectively. These fine particles were filtered and ingested or incorporated into larger pseudofaecal pellets which require a shorter time to settle out of suspension (Jones and Preston, 1999). During filtration, oysters sort particles by size and weight and accumulated rejected material (pseudofaeces) is expelled through the inhalant opening (Barnes, 1987). High sediment loads can reduce or even arrest oyster filtration (Loosanoff and Tommers, 1948) and this may also occur in other selective suspension feeders. High particle loading is known to cause a marked increase in particle rejection and decrease in clearance rates and ingestion rates in bivalve larvae (Gallager, 1988) and adults (Bricelj and Lonsdale, 1997). Thus, further studies are required to determine the effect of high organic and inorganic sediment load on suspension feeders growth and survival (Jones and Preston, 1999).

Compared to research efforts made on aspects such as nitrification and aeration in intensive aquacultural systems, the characterisation of solids remains scarce (Chen *et al.*, 1993). Particulate matter, consisting mainly of faeces and unconsumed feed, sinks to the bottom where it accumulates with serious environmental effects (Silvert, 1995). Therefore, suspended solids management is one of the key factors determining the success of recirculating systems because of their potential environmental impact (Chen *et al.*, 1993). Suspended solids promote gill damage in fish, reduce resistance to disease (Chen *et al.*, 1993), and can ultimately result in fish mortality (Chapman *et al.*, 1987). As a result of the problems associated with removal of fine particles from aquacultural water, the suspended solids concentration often exceeds the recommended limit of 15 $mgl^{-1}$ for recirculating systems (Chen *et al.*, 1993). From feeding studies on *Clarias gariepinus,* Hogendoorn *et al.* (1983) and Bovendeur *et al.* (1987) concluded that 79% of the dry matter and 74% of the nitrogen that was ingested was lost as faecal and non-faecal waste. Ceronio *et al.* (1995) discovered that approximately 80-85% of the dry material fed to the fish was sampled as suspended solids.

Thus the main waste products from aquaculture are from uneaten food, faeces and excreted dissolved inorganic nutrients which are transported in the water (Ceronio *et al.*, 1995; Pagand *et al.*, 2000). The most effective way to decrease the nutrient loading is to improve feeds and feeding (Mäkinen *et al.*, 1988). In systems with high water exchange, such as cage culture in the open sea, Norwegian fjords or in raceways, waste output is distributed at a low concentration in a large volume (Aure and Stigebrandt, 1990). Land-based flow-through systems result in point source sites of pollution, which have severe impacts on the local environment.

Air is introduced into abalone raceways for several reasons, the most important being to maintain dissolved oxygen levels at a sufficient level to support living organisms and to displace waste gases such as carbon dioxide and reduce their concentrations to an acceptable level (Backhurst and Harker, 1988). Air circulation also provides adequate mixing in the raceways to maintain a homogenous environment with regards to temperature and water quality for the successful rearing of the organisms (Backhurst and Harker, 1988). Unfortunately, the heavy aeration typical of the abalone culture industry causes constant re-suspension of particulate matter, resulting in a high level of particulate loading within the raceways. The high particulate loading of potentially nutritious particles appears to provide a good environment for suspension feeding organisms such as *T. heterouncinata*.

The aim of this study was to investigate and quantify the characteristics and levels of suspended solids within abalone raceways. Raceways dedicated to feeding either artificial feed or kelp were sampled to compare size distribution of particles and proximate composition of the waste related to the two abalone diets.

# MATERIALS AND METHODS

Suspended solids were sampled from kelp and artificial feed raceways to determine the size distribution and proximate composition from certain on-farm conditions. Particulates were collected from five separate raceways for each stocking density and diet to test the hypotheses. A siphon system was constructed to filter water from abalone raceways. Twenty-millimetre PVC piping was used to sample water 30 cm below the water surface. The flow rate of the siphon was regulated by a valve and the flow determined by timing the water flow into a 2 litre volumetric flask. This was repeated 10 times for each sample, and an average value of these ten samples was taken for further calculations. The abalone were 55.5 ± 2.2 (standard deviation) mm in size and they were fed between 17:00 and 17:30 in the evening. The water temperature for the duration of the sampling was 13.4 ± 0.79 (standard deviation) $^{o}$C.

The flow rate and total volume of water filtered was calculated using the following formulae:

**FR = L / d**

Where:

FR = flow rate (L/min)

L = the volume of the flask used to quantify the flow rate (L)

d = the time taken to fill the two-litre volumetric flask (min)

**T = FR * D**

Where:

T = total volume of water filtered (L)

FR = flow rate (L/min)

D = duration for which water was siphoned (min)

The following sieve sizes (μm) were used: 500, 100, 50, 40, 30, 20. Sampling took place from 8:00 to 17:00 with sediments on each filter being collected every 20 minutes (27 sediment collections in total) and immediately placed on ice. The sediments from each diet size class were spun at 10 000 rpm for 15 minutes in a Beckman centrifuge to remove excess water. The wet weight of the particles in each size class was determined by weighing the mass of the sediments remaining in the centrifuge tube after the water was poured out.

The wet weight of particles per litre of sea water for each size class was calculated using the following formula:

**WL= TW / T**

Where:

WL= wet weight of particles per litre (g/L)

TW= total weight of particles per size class (g)

T = total volume of water filtered (L)

Samples were freeze-dried and sent to the Department of Animal and Poultry Science, University of Natal for proximate analysis.

The hypotheses tested and the farms and conditions sampled are shown in table 4-1.

**Table 4-1: Hypotheses tested and details of the conditions from which particulates were collected.**

| | Farms sampled | Abalone diets | Stocking density | Dates sampled |
|---|---|---|---|---|
| **Ho: The wet weight and particle size distribution from kelp and artificial feed raceways are similar** | Aquafarm | Kelp<br>Abfeed | 24% | 20/3/01 |
| **Ho: The protein and energy content of particulates of kelp and Abfeed origin are the same** | HIK<br>Sea Plant Products Ltd. | HIK – Kelp<br>Sea Plant – Abfeed | 22% | 24/3/01 |
| **Ho: The protein and energy content of particulates originating from 40, 60 and 70 mm abalone are the same** | HIK<br>Sea Plant Products Ltd. | HIK – Kelp<br>Sea Plant – Abfeed | 22% | 25/3/01 |
| **Ho: The wet mass of particulates is the same for all stocking densities** | Aquafarm | Kelp | 20%<br>24%<br>32% | 22/3/01 |
| **Ho: The amino acid profiles from particulates from kelp and Abfeed raceways is similar** | HIK<br>Sea Plant Products Ltd. | HIK – Kelp<br>Sea Plant – Abfeed | 22% | 24-25/3/01 |

### *Statistical analysis*

Analysis of variance testing requires homogeneity of variance and that the data are normally distributed. Levene's test was used to test the homogeneity of variance assumption, and the Chi-square and Kolmogorov-Smirnow tests were used to check the normality assumption (Zar, 1999). Null hypotheses were rejected at a p-value $\leq 0.05$. If the data variance was not homogenous or the distribution was skewed, the data were log or square-root transformed and then retested for homogeneity and normality. Once the data satisfied the assumptions for ANOVA, one-way analyses of variance were run to determine whether there were any significant differences in average wet weight of particulates between diet, stocking density and between particle size classes, respectively. In the same way ANOVA was also used to compare proximate composition between diet, particle size and abalone size, respectively. Where appropriate, Tukey's multiple range test was used post-hoc to identify where significant differences between treatments occurred (Harraway, 1997).

# RESULTS

## *Wet weight and size distribution of particulates*

The average wet weight of particulates sampled from kelp and Abfeed raceways were significantly different from each other across the particle range tested ($F_{1, 18}$=7.5; p<0.02). There was a significant interaction between the two diets (p<0.0001; $F_{4,10}$=31.5). The majority of particles from the kelp raceways fell into the largest particle size class (>100 µm), which had a significantly higher wet weight per volume than all other size classes of kelp and Abfeed (p<0.001) (Figure 4-1). The wet weight of Abfeed particles per unit volume from the >100 and 50-100 µm and the wet weight per unit volume of the particulates from the kelp 50-100 and 40-50 µm were similar (p>0.05). The wet weight per unit volume of the particulates from the Abfeed and kelp 50-100; 40-50; 30-40 and 20-30 µm size classes were not significantly different from each other (p>0.05).

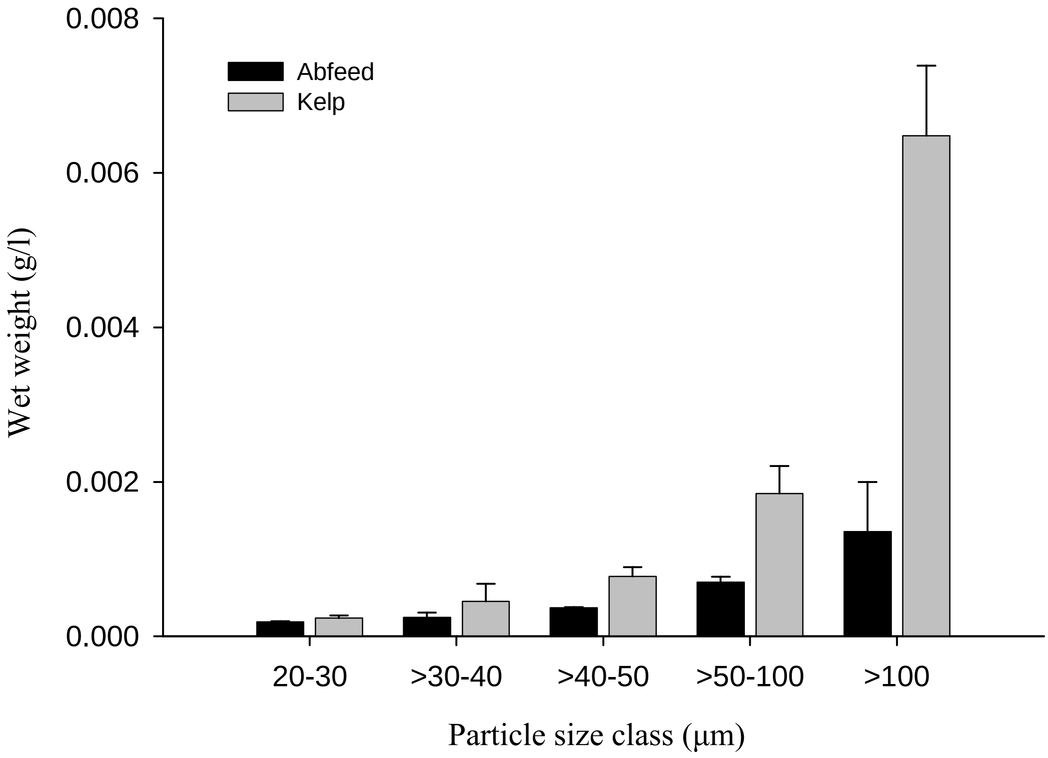


**Figure 4-1: Histogram of the wet weight of particles collected from each particle size class from kelp and Abfeed raceways.**

The filters were checked every 20 minutes during sampling and the finest filter (20µm) blocked most frequently, followed by the 30 and 40µm filters when filtering water from Abfeed raceways (Figure 4-2). The 50 and 100µm filters were not blocked by particulates from Abfeed raceways indicating the small particle size range. The kelp particulates blocked the fine filters (20 and 30µm) as well as the 50 and 100µm filters. This conforms with the finding that larger particle sizes were present in the kelp raceways than in the Abfeed raceways.

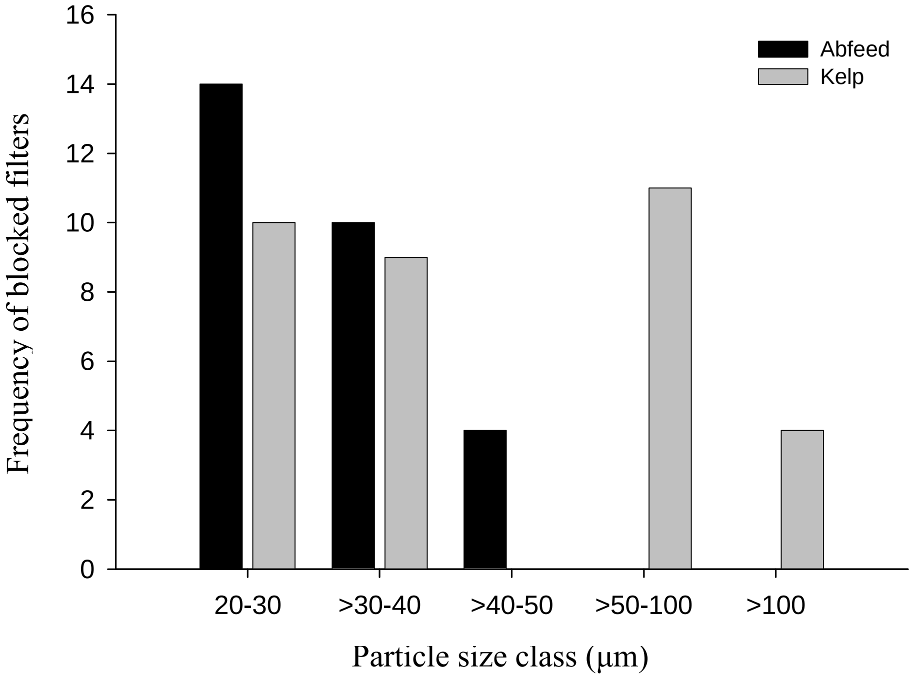


**Figure 4-2: Frequency histogram of the occurrence of the different filter sizes blocking from kelp and Abfeed raceways. Filters were cleaned every 20 minutes for the duration of the sampling period, with each filter size being cleaned a total of 27 times.**

## *Protein and energy composition of particulates sampled from kelp and Abfeed raceways*

Due to the small quantities of particulates filtered and the large dry weights required for proximate analysis, the samples were combined into two size classes of >100 and <100 µm for each diet. There was a significant difference in the average protein level ($p<0.0001$) and the energy level ($p<0.01$) between the particulates from each diet. Abfeed resulted in higher protein and energy values. The protein and energy contents of particles greater than 100 μm and less than 100 μm did not differ significantly for both kelp and artificial feed diets ($p>0.05$) (Table 4-2). There was no interaction between abalone diet and particle size class ($p>0.1$) for protein content, while there was a very weak interaction for energy content between the two factors ($p<0.04$).

**Table 4-2: Proximate composition of two size classes of particulates sampled from kelp and artificial feed raceways ± standard deviation. Letters indicate significant differences.**

| Diet | Particle size class | Protein (%) | Energy (%) |
|---|---|---|---|
| Kelp | >100 μm | 15.37±0.47 $^{a}$ | 8.34±0.04 $^{a}$ |
| Kelp | <100 μm | 16.12±0.03 $^{a}$ | 8.32±0.07 $^{a}$ |
| Abfeed | >100 μm | 30.24±0.15 $^{b}$ | 11.51±0.54 $^{b}$ |
| Abfeed | <100 μm | 28.57±1.73 $^{b}$ | 10.12±0.30 $^{b}$ |

### *Protein and energy composition of kelp and Abfeed particulates from abalone of different size classes*

The particulates from kelp raceways had significantly lower protein ($p<0.001$) and energy content than those from Abfeed raceways (Table 4-3). The particulates collected from kelp-fed abalone of different sizes had similar protein ($p>0.3$) and energy contents ($p>0.3$), and there were no significant differences in the protein ($p>0.09$) and energy ($p>0.13$) content of particulates from different size abalone from Abfeed raceways.

**Table 4-3: Protein and energy content (%) of particulates sampled from kelp and Abfeed raceways ± standard deviation. There was a significant difference between diets, but not between abalone sizes within diets.**

| Diet | Abalone size | Protein (%) | Energy (%) |
|---|---|---|---|
| Kelp | 40 | 16.07±1.23 | 8.31±0.07 |
| Kelp | 60 | 17.76±0.64 | 8.79±0.23 |
| Kelp | 70 | 16.65±1.13 | 8.32±0.45 |
| Abfeed | 40 | 30.47±2.15 | 11.02±0.11 |
| Abfeed | 60 | 26.15±1.12 | 10.28±0.31 |
| Abfeed | 70 | 34.05±3.04 | 11.97±0.94 |

## *The effect of abalone stocking density on wet mass of particulates*

Abalone stocking density did not influence wet weight of particles ($p > 0.05$) (Figure 4-3).

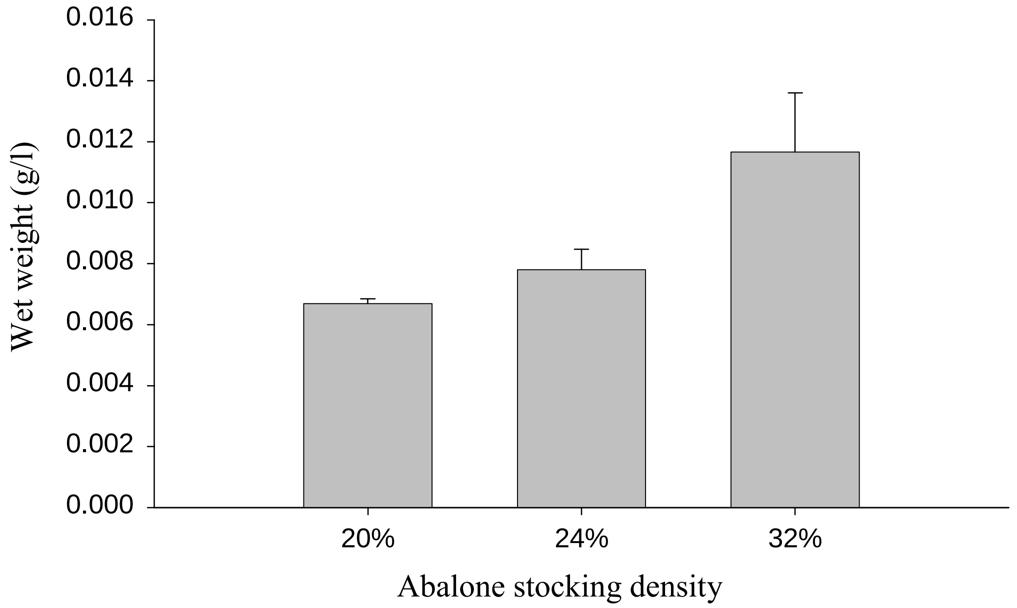


**Figure 4-3: Wet weight (g/L) of kelp particles present in the water column from raceways of different abalone stocking densities.**

## *Amino acid profiles of particulates sampled from kelp and Abfeed raceways*

The amino acid profiles for particulates collected from kelp and Abfeed raceways are very similar (Spearman-Rank correlation: $r^2$=0.98) (Table 4-4).

**Table 4-4: Amino acid profiles for particulates sampled from kelp and Abfeed raceways. Shown as % of sample weight.**

| Amino Acid | Abfeed | Kelp |
|---|---|---|
| Aspartic Acid | 4.36 | 3.76 |
| Threonine | 1.50 | 1.42 |
| Serine | 1.19 | 1.02 |
| Glutamine | 3.60 | 3.12 |
| Proline | 1.42 | 1.41 |
| Glycine | 1.79 | 1.45 |
| Alanine | 1.50 | 1.30 |
| Valine | 1.63 | 1.46 |
| Methionine | 0.45 | 0.36 |
| Isoleucine | 1.22 | 1.07 |
| Leucine | 1.85 | 1.55 |
| Tyrosine | 0.81 | 0.85 |
| Phenylalanine | 1.20 | 1.16 |
| Histidine | 0.51 | 0.53 |
| Lysine | 1.92 | 1.86 |
| Arginine | 1.23 | 1.20 |

## DISCUSSION

There has been considerable work on feed formulation and digestibility in abalone in order to derive a cost efficient feed (Britz, 1995; Shipton, 1999; and Sales, 2001). No work, however, has been carried out on the particulate loading within commercial abalone raceways resulting from feed and feed-derived wastes. This study indicates that the average wet weight of particulates collected from kelp and artificial feed raceways was significantly different and that the average weight and its particle size composition differed between the two diets. The distribution of wet weight of kelp particulates was highly skewed, with the largest proportion (64%) being greater than 100 µm in size. It has been suggested that the sabellid feeds primarily on protists and diatoms (Simon *et al.*, 2002), but the differences in infestation levels of farmed abalone on the kelp and artificial diet suggest that the worms also feed on fragmented abalone food and possibly faeces (Simon, *et al.*, 2002). The worms appear to ingest particles of different contents unselectively from the water column depending on their size (Ruck, 2000). Shields *et al.* (1998) and Ruck (2000) observed ingested microcapsules ranging in size from 3-30 µm and particles up to 35 µm within the gut of the sabellid, respectively. During the observational studies of Chapter 3, large particles were observed to disrupt the feeding of the sabellid when coming into contact with the feeding crown. In an attempt to escape the large particles, the sabellid would withdraw into its burrow often with the particle settling on the entrance. This suggests that the feeding size range of the sabellid is less than 40 µm, although they may be able to ingest larger particles. In abalone raceways uneaten food debris and abalone faeces would comprise particles in this size range (Ruck, 2000). This study indicated that the wet weight of the artificial feed is more suitable for sabellid consumption due to its smaller particle size. Kelp raceways had a larger wet weight of organic particulates but most of these nutrients are unavailable to the sabellid due to their large particle size.

Besides a large percentage of kelp particles being too large to ingest and unavailable to the sabellid, these particles may further disturb the feeding patterns of the sabellid by fouling the feeding crown and disturbing the sabellid, causing it to withdraw into its burrow. Thus kelp particulates may further reduce their availability by reducing the feeding duration of the sabellid. The lower abundance of large particles in artificial feed raceways may not impact as much on the feeding duration of the sabellid.

The frequency of blocked filters indicates that not only did the kelp particulates have a larger wet weight but also a greater particle frequency in the larger size classes as the two largest filters blocked in kelp raceways but did not in the artificial diet raceways. The two smallest filters blocked frequently for both the diets, indicating a similar particle frequency in these size classes. This suggests that there may be similar numbers of particulates within these size classes for each diet which are available to the sabellid to feed on. Differences in sabellid growth between the diets are not likely to be due to particle availability as there are similar numbers of small particles available to the sabellids for each diet. The difference in particle composition is likely to be more influential, as the Abfeed particulates have a higher protein and energy content than the kelp particulates.

In order to analyse the proximate composition of the particle sizes, they could be combined into two size categories, greater and less than 100 µm. According to this size categorisation there was no difference in proximate composition between the two size classes for each diet for both protein and energy content. There were, however, significant differences between the particulates from different diets, with the artificial feed raceways having particulates of greater protein and energy content than the kelp particulates. The protein content of fresh kelp is 8.1% (Britz, 1995) and that of the artificial diet is 34.6% (Anonymous (b)). This would suggest that the artificial diet increases the protein level of particulates within the water column either directly by pellet fragmentation or indirectly via abalone faeces production through undigested protein. Regardless of their origin, particles from the artificial diet abalone raceways appear to have higher nutritional value to the sabellid. The increased protein and energy levels in the artificial diet have been shown to increase the growth rate of abalone and they are a cost efficient feed for farmers. From these results it would appear that this diet may be producing beneficial conditions for growth and infestation of the problematic sabellid populations. This is shown in Chapter 5, where analysis of the sabellid morphometrics and infestation levels clearly shows that Abfeed causes higher infestation levels and larger body sizes of sabellids.

### *Effect of abalone size on proximate composition of particulates*

Undigested nutrients excreted by *H. midae* fed on artificial diets in comparison to animals fed on seaweed have been linked to a higher rate of infection by the sabellid (AFASA, *pers. comm.*). It has also been noted that abalone are more susceptible to infestations at certain sizes and it is uncertain if this is related to growth rate (AFASA, *pers. comm.*). It is widely known that organisms have different energetic and nutritional requirements at different life stages (Van der Wind, 1979; Charlon and Bergot, 1984; Dabrowski and Poczyński, 1988). It is therefore possible that abalone of different sizes and ages utilise different components of the artificial feed thereby producing a different waste composition. This study indicated that the particulates related to three abalone sizes were not significantly different in their protein and energy composition.

### *Effect of stocking density on particulate loading*

High sabellid infestations were thought to be the result of high abalone stocking densities which increased the particulate loading within the system (AFASA, *pers. comm.*). This study showed that particulate loading was not statistically different for the three stocking densities tested. There was, however, a slight increase in wet weight of particulates with increasing stocking density, which may be significantly different at higher densities or under different farm conditions. Commercial abalone farms stock abalone between 20-32% during the grow-out period as abalone growth is best between these ranges and water quality deteriorates at higher stocking densities. It was therefore not possible to quantify the particulate loading within commercial farm raceways at stocking densities greater than 32%. It is possible that at stocking densities above 32% the particulate mass within the water column may be statistically higher. This, however, requires further testing with raceways dedicated to abalone stocking densities higher than are usually found within the abalone industry.

Farm management appears to play a major role in particulate loading of the water and varies from farm to farm depending on their cleaning routines and feeding schedules. Although stocking density does have the potential to cause significant increases in particulates under extreme stocking conditions, the main cause for high levels of suspensoids seems to be management, and the current stocking densities employed by the South African farmers appear not to be a determining factor in particulate loading within abalone raceways.

### *Amino acid profiles of particulates from kelp and Abfeed diets*

The amino acid profiles of particulates sampled from kelp and artificial feed raceways are similar, suggesting that similar quantities of amino acids are available to the sabellid independent of abalone diet. This suggests that any differences in sabellid morphometrics (Chapter 5) cannot be due to an absence of essential amino acids in the waste particulates from abalone from either diet but may be due to differences in the quantity of protein available.

This study helped understand the differences in nutrient abundance and availability to the sabellid subjected to different commercial conditions. It has been suggested that sabellid infestation and size in the South African abalone industry may be influenced by nutrient availability (AFASA, *pers. comm.*). Particulate abundance in incoming water is likely to fluctuate according to local currents and upwelling events and thus would differ from farm to farm depending on well point location. More research should be focused at determining seasonal changes in the particulate components of water entering the farms before and after filtration to determine the quantity and composition of organic matter presently removed by drum filters. Improved filtration of incoming water may remove particulates important for sabellid nutrition.

Although it is possible that nutrients occurring naturally in the water may influence sabellid growth and infestation, this study indicates that abalone diet modifies the water within abalone raceways and significantly increases the nutritional value of particulates between natural and artificial feed raceways. This is supported by the findings in Chapter 5, which show that diet is a major factor influencing sabellid infestation and size. Abfeed, which was shown to have a higher protein and energy content in this study, resulted in both higher sabellid infestation levels and larger body sizes than kelp.

# CHAPTER 5

# INVESTIGATION INTO THE EFFECT OF DIET, ABALONE SIZE AND ABALONE STOCKING DENSITY ON SABELLID POPULATION SIZE AND STRUCTURE, AND MORPHOMETRICS

## INTRODUCTION

Worldwide, many economically important mollusc species are plagued by infestations of polychaetes (Handley and Bergquist, 1997). For example, the quality of oysters is markedly lowered by infestation with *Polydora* (Nel *et al.*, 1996) and they become unsuitable for the lucrative half shell market due to their undesirable appearance (Handley and Bergquist, 1997). Infestations of *Polydora* also cause the shells of oysters to be brittle and break during shucking, packaging and transport (Handley and Bergquist, 1997).

Boring organisms usually do not seriously affect the growth of abalone because they inhabit the outer portions of the shell (Oakes and Fields, 1996). However, sabellid infestations have become a concern among abalone culturists as they reduce growth, survival and marketability of the abalone (Leighton, 1998). Sabellid infestations were first found in South Africa on farmed abalone in 1994, when it was noted that infested abalone had reduced growth (Ruck and Cook, 1998). Abalone with high sabellid infestation levels exhibited slow growth and had deformed shells that were susceptible to breakage, causing mortalities (Ruck and Cook, 1998). Fluctuating environmental factors, such as food availability and water temperature, may play a role in controlling natural levels of infestation in wild gastropods (Ruck and Cook, 1998), resulting in lower infestation levels than those found in abalone cultured in monoculture farm conditions. Methods used to control or eradicate the sabellids include exposing infested abalone to air or extreme temperatures, as well as treating them with fresh water, chlorine or vermicides (Culver *et al.*, 1997; Ruck, 2000). The most effective treatment to reduce infestations was coating the outer abalone shell with wax, smothering the worms and allowing the abalone to assume normal growth for a limited time. This treatment was not completely successful and reinfestation occurred (Culver *et al.*, 1997). *T. heterouncinata* is a simultaneous hermaphrodite which broods its eggs to a larval stage (Culver *et al.*, 1997), thus a single sabellid can infest an entire stock of abalone. The abalone shell consists of two layers, the outer prismatic layer responsible for linear growth of the shell and the inner nacreous or pearly layer (Nakahara *et al.*, 1982). Nacreous shell is also deposited when shell damage is being repaired or when a foreign object cannot be dislodged from beneath the mantle (Barnes, 1987). High infestation levels on the growing edge lead to deposition of nacreous shell, resulting in a disruption of shell growth, giving the infested abalone their characteristic deformed shell pattern. The infestation level determines the degree of damage to the host's shell (Kuris and Culver, 1999). The transmission is affected by the proximity of infested animals, aeration and water flow, host size and specificity, and temperature and salinity tolerance of the sabellids (Culver *et al.*, 1997). However, the degree to which these factors influence transmission and infestation has not been quantified.

Older or slower growing abalone appear to be more susceptible to infestations and often have higher infestation levels than younger, faster growing abalone (AFASA, *pers. comm.*). Infestations of the sabellid can be reduced by maintaining optimal growing conditions for the abalone and low stocking densities in the grow-out tanks (Oakes and Fields, 1996). In all abalone aquaculture operations there is a small portion of the population that does not grow well and never attains a marketable size (Oakes and Fields, 1996). It is often these animals that are heavily infested by the sabellid and they can be a source of infestation for the rest of the farm (AFASA, *pers. comm.*).

Infestation levels between and within South African abalone farms are highly variable. System design, stocking densities, diet composition and the management strategies on the individual farms may play a role in controlling the level of infestation. Each farm has its own feeding schedule, diet and management strategy. In the Western Cape the two main diets are an artificially formulated pellet diet high in protein, Abfeed, and wild collected kelp. Feeding consists of either one or the other, or a combination of both of these alternated at some stage during the grow-out period. The farms feed their abalone either an artificial diet, fresh kelp or a combination of both. The artificial diet, Abfeed, is produced by Sea Plant Products Ltd. in Hermanus. The protein content of the diet is considerably higher than that of naturally occurring abalone foods (Table 5-1). Kelp is harvested locally by a private contractor and is delivered to the abalone farms fresh every day. During periods of heavy seas when the contractor is unable to harvest kelp, freshly washed up kelp is collected from the beaches. During these times there is often a shortage of kelp for the farmers. Feeding frequency is farm and water temperature-dependent but usually occurs daily for artificial feed and every 3-7 days for kelp. The artificial pellets are placed on a feeding plate (Figure 5-1) below which the abalone sit during the day. The abalone move onto the feeding plate at night to feed on the pellets and then return to the dark refuge below the plate during the day. This feeding method prevents overfeeding, as uneaten pellets remain on the feeding plate and can be recorded, and the amount of feed reduced the following evening. Kelp is placed under the feeding plate in amongst the plates where the abalone are. The kelp is therefore more readily available to the abalone during the day and they can feed on it without having to move to the surface of the feeding plate. The kelp remains in the baskets until completely eaten or until the baskets are cleaned once a week. Fresh kelp is introduced every 2-3 days to maintain a constant food supply.

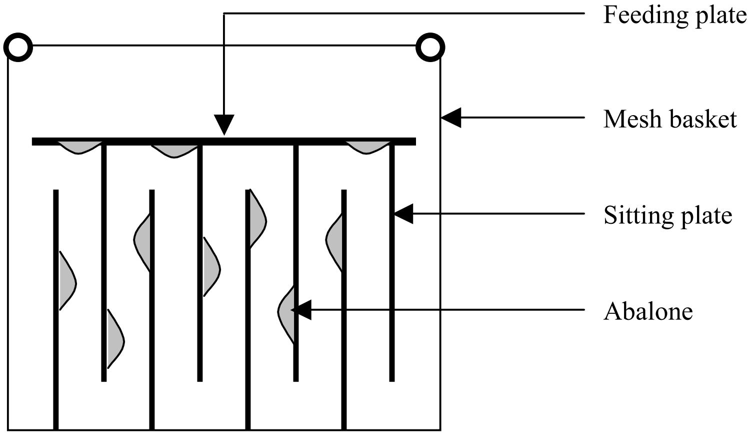


**Figure 5-1: End view of an abalone holding basket**

The farmers suggested that the sabellid infestations are directly related to the diets and tank cleaning routines. There has, however, been no direct comparison of sabellid infestation levels between feeding, cleaning and management procedures on different South African abalone farms due to the secretive nature of management on individual farms. The observed effect of abalone diet on sabellid infestation by certain farmers may be supported by the fact that the artificial feed and kelp differ significantly in their composition (Britz, 1995; Anonymous (b)), which results in variable growth rates of abalone of different sizes. The proximate composition of particulates collected from kelp and artificial feed raceways differed significantly in their protein and energy content and is likely to influence sabellid infestation and growth (Chapter 4). This study aims to compare and contrast sabellid infestations in relation to abalone stocking densities, abalone sizes / growth rates and abalone diets between farms with differing management practices.

Fishmeal is incorporated into the artificial diet to raise the protein level of the pellets above that of kelp, *Ecklonia maxima* (Table 5-1). The carbohydrate content of the artificial diet and kelp does not differ, while the fat content is lower and the ash content higher in kelp than in the formulated diet.

**Table 5-1: Proximate composition (%) of dry matter of an artificially formulated abalone diet[a] and kelp[b], a naturally occurring abalone food source.**

| Nutrient | Artificial pellet diet | Kelp (*E. maxima)* |
|---|---|---|
| Protein | 34.6 | 8.1 |
| Fat | 5.3 | 0.5 |
| Carbohydrate | 43.3 | 45.2 |
| Ash | 5.7 | 25.3 |

**a. Anonymous (b), Sea Plant Products Ltd.**

**b. Kelp Products Pty. Ltd, unpublished data in Britz, 1995**

Diet contributes to the condition of an organism. In aquaculture high protein feeds are commonly used to improve growth and overall condition of organisms to increase production. Britz and Hecht (1997) showed that protein levels in formulated diets for *Haliotis midae* significantly affected growth in abalone of different sizes. The abalone aquaculture industry incorporates fishmeal into the feed to increase growth and production. Artificial pellet diets break up and nutrients leach into the surrounding water, creating a nutrient-rich environment favourable for filter-feeding organisms. Increasing feeding frequency of artificial food can reduce nutrient leaching (Velasco *et al.*, 1999). In the abalone industry feeding takes place once a day in the evening and the feed is present in the water for several hours, providing plenty of time for nutrient leaching to occur. There is a need to test to what extent nutrient leaching and the availability of high quality nutrients in abalone facilities could be a cause of high sabellid infestations. Condition factor is a widely used term in aquaculture to describe the quality and overall health of fish in relation to the quality of the food they receive and their holding conditions (Bolger and Connolly, 1989). No work has been carried out on invertebrate morphometrics in relation to diet availability. The occurrence of two diets in the South African abalone industry provides an opportunity to investigate the possibility of using morphometrics of this polychaete species to determine how it is affected by diet.

Abalone are sedentary gastropods which attach to rocky substrates in the natural environment. In aquaculture facilities the rocky substrate is replaced by several plastic sitting plates (Figure 5-1, page 85) which are placed into the holding baskets in raceways. These plates are used to increase the available surface area for the abalone to attach to. Stocking density in the commercial industry is calculated as percentage cover of the available surface area on the sitting plates. As stocking density within a basket increases so does the percentage cover of the available surface area. Increases in stocking density, as in all aquaculture activities, increases the waste output into the water. High stocking densities above the recommended maximum (35%) (Anonymous (a)) are thought to increase the particulate loading within the water, thereby increasing the food availability for the sabellid. In doing so, stocking density is thought to influence sabellid infestation and growth.

Condition factor is widely used to compare the health of organisms from different aquatic systems and culture conditions. This study was designed to compare the condition of sabellids by using the length, width and surface areas from different farm conditions. The study of condition in fisheries ecology is based on length-weight data and assumes that a heavier fish of a given length is in better condition (Bolger and Connolly, 1989). This is believed to be a good indicator of general fitness of a population and is widely used in fisheries ecology studies (Bolger and Connolly, 1989). Condition has been most effectively used in three kinds of population analysis (Weatherly, 1972; Bolger and Connolly, 1989): (1) in comparing populations living under either different or similar conditions such as food density, climates etc.; (2) in determining timing and duration of gonad maturation; (3) as an indication of changing nutritional balance during alterations in feeding activity or food supply. Thus far there have been no such analyses on the condition of polychaete populations. This study investigated the possibility of using morphometric indices to describe the condition of sabellid populations exposed to different conditions.

Due to the small size of sabellids it was impractical and inaccurate to weigh individuals for use in the condition indices used by fisheries ecologists. The paucity of literature on length-weight relationships for polychaetes further prevented the use of these models. Length, width and area on the other hand could be measured accurately using magnified digital images and computer software. The measurements taken were designed to describe the size structure as well as the reproductive state of the population. By looking at each type of measurement for a population of sabellids, the size distribution could be obtained but this did not reflect on the health or condition of the population as different sizes could indicate different age classes within the population. To overcome this and to describe the health of the adult population, the measurements were looked at as ratios of each other, thereby providing a relationship of different morphometrics for sabellids of different ages.

Body length may be a general indicator of the size and age of an individual but it does not provide any information about the condition, as a worm may be long and thin but in poor health while a healthy worm may be shorter but wider. To analyse the condition of the sabellid populations it was assumed that neck width was the most constant growth parameter while growth in length could be highly variable. A ratio of the two values indicates the growth pattern and the health of the worms, providing a condition factor that can be used for comparisons of these data with that of other populations of sabellids. It is thus assumed that a high neck width for a given body length is an indicator of good condition.

Surface area of the sabellids could also be used to get an estimate of the size and condition of a population but when analysed on its own, it does not take the length of the sabellid into account. A long thin worm may have a similar surface area to a short wide worm and therefore a similar condition index, according to this interpretation. In order to overcome this problem the length of the sabellid needs to be taken into account. For this reason the surface area and length were combined to formulate a ratio.

The measurement of base width of the sabellids was used to get an indication of the sexual maturity and reproductive condition. As the sabellid matures and develops eggs, the abdomen region containing the eggs becomes wider. The base width was measured across the 9-10th abdominal setigers, which is the region for oocyte production (Fitzhugh and Rouse, 1999). Theoretically, as an adult worm matures sexually this region of the abdomen swells as oocytes develop. The measurement above cannot be used to indicate the reproductive state of the sabellid because the size of the sabellid needs to be taken into consideration. Thus, base width was investigated in conjunction with the surface area and neck width in order to obtain a better estimate of the reproductive state in relation to worm size. Also, the base to area ratio was used to estimate the reproductive state in relation to body size, with greater values possibly indicating sexual maturity of individuals. Unlike the neck width, which has a regular growth pattern, the length (Simon *et al.*, 2002) and possibly the base width may fluctuate on a seasonal basis according to reproductive activity. A ratio comparing these two factors may provide an indication of reproductive development and activity.

These reproductive indices make use of the female reproductive development, and the development of testis is not taken into account. *T. heterouncinata*, however, is a simultaneous hermaphrodite and produces sperm and oocytes at the same time (Fitzhugh and Rouse, 1999). Even though it is still unclear whether the sabellid receives spermatocytes from other individuals and cross-fertilises, or whether self-fertilisation occurs, both oocytes and spermatocytes are developed at the same time and the indices may give some reflection of its reproductive state.

The aim of the study was to investigate the effect of diet, stocking density of abalone and abalone size on the infestation level and morphometrics of sabellids. To achieve this aim, the infestation levels and sabellid morphometrics of abalone from three stocking densities, three size classes, and two abalone diets were quantified.

# MATERIALS AND METHODS

## *Farm conditions and sampling procedures*

Abalone were sampled from 3 different farms along the south coast of South Africa in the Hermanus area (34$^o$42'S; 10$^o$23'E) (Table 5-2) between 18 and 25 March 2001.

Abalone stocking density is calculated as a percentage cover of the surface area available to the abalone. For each farm the methods of calculating density vary considerably. Abalone surface area can be calculated as a triangular or circular surface area based on shell size. The available surface area includes both sides of the sitting plates and may include the bottom of the feeding plate but usually does not take into account the surface area of the sides of the mesh basket. The recommended maximum stocking density by the abalone management work group is 35% (Anonymous (a)) but often farmers maintain their abalone at a lower stocking density. Table 5-2 shows the management strategies, diet, tank sizes and mean temperatures for each of the farms sampled.

The experimental hypotheses, commercial farms sampled, abalone sizes, abalone stocking densities and abalone diets for the infested abalone sampled are shown in table 5-3.

## *Abalone selection and sampling*

A total of 15 abalone were sampled from five raceways for each stocking density and diet in order to test each hypothesis. (3 abalone x 5 raceways = 15 abalone for each treatment). The level of sabellid infestation was determined subjectively by the appearance of the abalone shell and examination of the growing edge for burrows by pushing the abalone's mantle back and visually assessing the number of burrows or shell damage. Heavily infested abalone have a deformed and slightly irregularly shaped shell, often with the growing edge being broken.

The abalone were shucked and the shells stored in 2.5% gluteraldehyde until the sabellids were extracted.

### *Quantification of sabellid infestation and occupation levels*

In the laboratory the shells were examined under a dissecting microscope. The number of tubes along the growing edge was counted. The tubes which appeared opaque white were counted as un-infested and those which were orange / yellow in colour were counted as infested tubes, as the larvae and adults appear orange in colour. The second and third centimetres of shells which had a regular (homogenous) distribution of tubes were marked and the number of infested and un-infested tubes within these marks were counted. All tubes were counted on shells which had an irregular (heterogeneous) distribution of tubes. A homogenous distribution of tubes shows a regular pattern along the growing edge of the abalone shell whereas in a heterogeneous distribution tubes are irregularly formed, often bunched into tight clusters with large gaps between them. The partial circumference (Figure 5-2) of the growing edge was measured (mm) and the infestation level of each shell was quantified as the average number of infested and un-infested tubes per centimetre of growing edge (tubes/cm G.E.).

This was done using the following equations:

Homogenous shells: $$\frac{\text{Infested or un-infested tubes}}{2}$$

Heterogeneous shells $$\frac{\text{Infested or un-infested tubes}}{\text{Length of growing edge (cm)}}$$

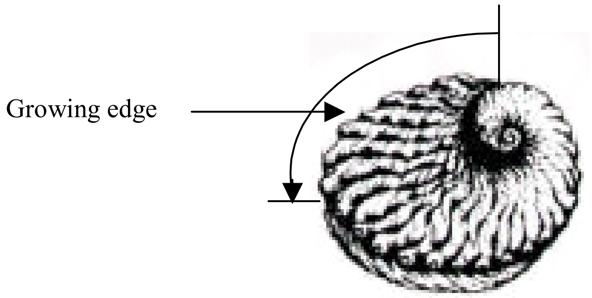


**Figure 5-2: Length of the partial circumference of the growing edge of the abalone shells**

**Table 5-2: Summary of the management procedures of the farms from which abalone were sampled.**

| | **Aquafarm** | **HIK** | **Sea Plant Products** |
|---|---|---|---|
| Location | Hermanus 34°42'S; 10°23'E | Hermanus 34°42'S; 10°23'E | Gansbaai 34°58'S; 19°36'E |
| Mean temp.-Summer<br>-Winter | 13.3°C<br>14.1°C | 13.3°C<br>14.1°C | 17.5°C<br>14.8°C |
| Abalone diets | Kelp and Abfeed | Kelp and Abfeed | Abfeed |
| Feeding schedule | Kelp – once every 3 days<br>Abfeed – once every day | Kelp – once every 7 days<br>Abfeed – once every day | Abfeed – every second day |
| Diet / abalone size | Kelp – abalone greater than 50-60mm<br>Abfeed – abalone smaller than 50- 60mm | Abfeed <50mm; >70mm<br>Kelp 50-60mm | Abfeed all size classes |
| Tank cleaning schedule | Kelp – every 1 or 2 weeks<br>Abfeed – every week | Once every 10 days | Once every 7 days |
| Abalone grading frequency | Once every 3-5 months if space permits, often only every 5 months | Once every 6 months | Once every 4 months |
| Tank -volume<br>-dimensions<br>-construction | 2.5 cubic metres<br>4.2 x 0.75 x 0.8m<br>Fibre glass, plastic (HDPE) and canvas | 4.5 cubic metres<br>3.8 x 1.8 x 0.7m<br>Canvas | 4 cubic metres<br>7 x 0.8 x 0.7m<br>Concrete |
| Water exchange rate | Kelp – 1 exchange every 2 hours<br>Abfeed – 1 exchange every 2.5-3 hours | 1 exchange every 4 hours | 1 exchange every 2.2 hours |
| Preferred stocking density | 18-24% | 16-20% | 20-35% |

**Table 5-3: Experimental hypotheses and sampling information of the abalone sampled for extraction of sabellids.**

| HYPOTHESES | | | |
|---|---|---|---|
| | **$H_o$: Abalone stocking density does not influence the sabellid infestation level and condition factor** | **$H_o$: Abalone diet does not influence the sabellid infestation level and condition factor** | **$H_o$: Abalone size does not influence the sabellid infestation level and condition factor** |
| Farm(s) sampled | Aquafarm | Sea Plant Products & HIK | Sea Plant Products & HIK |
| Abalone size class | 50-60mm | 70-75mm; 55-60mm; 40-45mm | 70-75mm; 55-60mm; 40-45mm |
| Average abalone size ± standard deviation | 55.5 ± 2.2 mm | 67.3±0.31mm; 55.7±1.1mm; 41.7±0.55mm | 67.3±0.31mm; 55.7±1.1mm; 41.7±0.55mm |
| Abalone size and/or age | 3.5 years | **Size** **Kelp** **Abfeed**<br>70-75mm 5 years 4.5 years<br>55-60mm 4 years 3.75 years<br>40-45mm 3.5 years 3.25 years | **Size** **Kelp** **Abfeed**<br>70-75mm 5 years 4.5 years<br>55-60mm 4 years 3.75 years<br>40-45mm 3.5 years 3.25 years |
| Monthly growth rate for different stocking densities and abalone sizes | 20% 1.5mm<br>24% 1.25mm<br>32% 1.2mm | **Size** **Kelp** **Abfeed**<br>70-75mm 1.2mm 1.4mm<br>55-60mm 1.1mm 1.45mm<br>40-45mm 1.2mm 1.1mm | **Size** **Kelp** **Abfeed**<br>70-75mm 1.2mm 1.4mm<br>55-60mm 1.1mm 1.45mm<br>40-45mm 1.2mm 1.1mm |
| Stocking density | 20%, 24%, 32% | 22% | 22% |
| Diet | Kelp | Kelp and Abfeed | Kelp and Abfeed |
| Date sampled | 22/3/01 | 24-25/3/01 | 20/3/01 |

## *Sabellid sampling*

Sabellids were extracted from the shells by dissolving the calcareous component of the shell using nitric acid. Individual shells were cleaned and placed into 6.5% nitric acid ($HNO_3$) in 70% ethanol. The nitric acid reacts with the calcareous component and softens the shell. Periodically the softened shell or shell fragments were gently broken until tiny fragments were left. One hundred of the most undamaged adult sabellids were removed from the debris of each shell. Sabellids were removed from ten shells from each of the conditions sampled.

Approximately 100 sabellids from each treatment were mounted onto slides and digital images were taken of them using a dissecting microscope and video camera at the Electron Microscope Unit of Rhodes University. All images were taken at the same magnification and a graduated 1 mm slide was used to calibrate the computer software used to analyse the images. All images were labelled and numbered before being measured using Sigma Scan computer software. The measurements are shown in Figure 5-3.

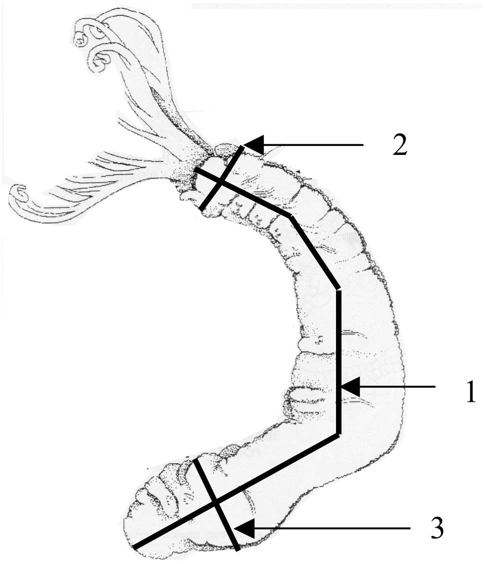


**Figure 5-3: Morphometric measurements**
**1 = body length; 2 = neck width; 3 = base width**

All measurements were taken in millimetres. The body length (1) was measured from the base of the branchial crown down the centre of the thorax and abdomen. Width (2,3) was measured as the perpendicular distance across the body at two locations. The first was below the basal flange (2) of the branchial crown at the collar, and the second was at the widest part in the abdomen region (3) of the worm. These measurements will be referred to as neck width and base width, respectively. The surface area ($mm^2$) of the thorax and abdomen was measured by outlining the perimeter of the thorax and abdomen and using the calibrated computer software to calculate the surface area outlined.

## *Statistical analysis*

Analysis of variance testing requires that the variance of data shows homogeneity of variance and that the data are normally distributed. Levene's test was used to test the homogeneity of variance assumption (Milton, 1992) and the Chi-square, Kolmogorov-Smirnov and Lilliefors tests were used to check the normality assumption (Milton, 1992; Zar, 1999). Null hypotheses were rejected at a p-value $\leq 0.05$. If the data variance was not homogenous or the distribution was skewed, the data were log or square-root transformed and then retested for homogeneity and normality. Once the data satisfied the assumptions for ANOVA testing, a one-way analysis of variance was run to determine whether there were any significant differences in infestation levels between the stocking densities, abalone diets and abalone sizes. Analysis of variance testing was also used to test for differences in sabellid lengths, widths and surface areas between the populations from abalone of different diets, sizes and stocking densities. A two-way ANOVA was run to determine if significant differences existed between the combined effect of diet and abalone size on the sabellid lengths, widths and surface areas. Tukey's multiple range test was used to identify where significant differences between treatments occurred (Snedecor and Cochran, 1991).

# RESULTS

## *Stocking density*

There was no significant difference in mean shell length ($p>0.05$; $F_{2,35}=1.98$), mean number of burrows per centimetre ($p>0.05$; $F_{2,35}=1.51$) and percentage of infested burrows ($p>0.05$; $F_{2,35}=1.03$) between abalone from the three stocking densities (Table 5-4). The mean ± standard deviation of shell length, number of tubes per centimetre on the growing edge and percent of tubes infested for all three treatments was 55.5 ± 5.3 mm; 6.1 ± 2.7 tubes/cm and 22.0 ± 14.5%, respectively.

**Table 5-4: Mean shell length, number of tubes per centimetre on the growing edge and percent of tubes infested ± standard deviation for abalone from stocking densities of 20, 24 and 32%. n is the sample size.**

| Stocking density | Mean shell length | Mean number of burrows/cm | % of tubes infested | n |
|---|---|---|---|---|
| 20% | 55.0 ± 5.4 | 6.6 ± 2.2 | 19.2 ± 11.9 | 15 |
| 24% | 57.9 ± 5.9 | 6.7 ± 3.5 | 20.1 ± 14.6 | 10 |
| 32% | 53.5 ± 4.5 | 5.0 ± 2.5 | 26.7± 16.9 | 13 |

Stocking density influenced all sabellid body measurements and their morphometric ratios (Figure 5-4), i.e. for each variable at least one variable was different to one of the others.

The sabellids from the abalone held at the highest stocking density (32%) had a significantly smaller body length (mean ± standard deviation) (2.77 ± 0.66 mm) than those at the lowest stocking density of 20% (2.98 ± 0.67 mm) (Figure 5-4). The length of the sabellids from the stocking density of 24% was not significantly different from either of the other two stocking densities. Sabellids from the 24% stocking density had a significantly greater neck width (0.21 ± 0.04 mm) than those from the 20% and 32% densities, which had similar average neck widths (0.16 ± 0.04 mm and 0.17 ± 0.03 mm, respectively). The base width of sabellids from the 24% density was significantly greater (0.37 ± 0.09 mm) than that of the 20% and 32% densities (0.32 ± 0.09 mm and 0.32 ± 0.07 mm, respectively). The surface area of the sabellids from the 24% abalone stocking density was significantly greater (0.7 ± 0.24 $mm^2$) than that of the 20% and 32% densities, which were similar (0.61 ± 0.19 $mm^2$ and 0.57 ± 0.18 $mm^2$, respectively).

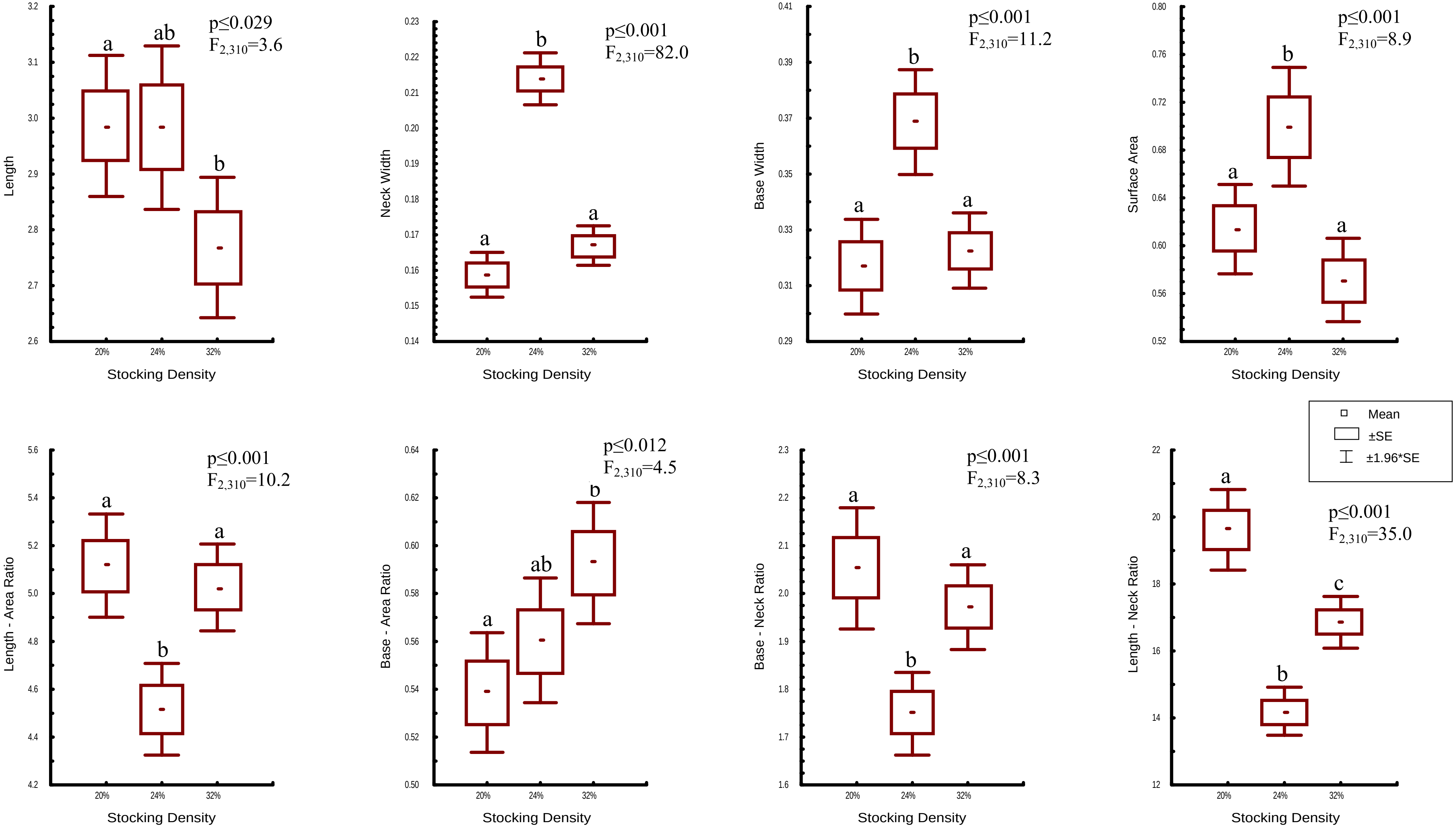


**Figure 5-4: Size measurements and morphometric ratios of sabellids extracted from abalone held at three stocking densities. Sabellids were sampled from abalone held at stocking densities of 20, 24 and 32% of available solid surface area. Length measurements were taken in mm and area in mm$^2$. Letters indicate significant differences between stocking densities.**

The length to area ratio was significantly lower in sabellids from the 24% abalone stocking density than in those from the 20% and 32% densities (Figure 5-4). The base to area ratio showed an increasing trend, with an increase in abalone stocking density with the 20% density being significantly different from the 32% stocking density. The base to neck ratio was significantly smaller for the 24% density than the 20% and 32% densities. The length to neck ratio for sabellids from all abalone stocking densities was significantly different, with the ratio for the 24% abalone stocking density being lowest followed by the 32% and 20% abalone stocking densities, respectively.

The frequency distributions for sabellid length, neck width, base width and surface area size for abalone stocking densities of 20% of surface area are shown along the left-hand side of Figure 5-5. The sabellid length ranged from 1.7-4.8 mm in length with the majority (89%) of the sabellids being in the 2.0-4.1 mm size range. The neck width had a skewness coefficient of 0.45 indicating a distribution skewed towards the smaller widths, with 81% of the sabellids having a neck width of 0.12-0.20 mm and only a small percentage (9%) having widths greater than 0.20 mm. Base widths ranged in size from 0.1-0.58 mm with relatively few sabellids being in the larger width classes. The majority (78%) of base widths fell between 0.21-0.42 mm. The sabellid surface areas were normally distributed ($p \geq 0.05$) with very few sabellids having areas greater than 1 mm$^2$ (3%) or smaller than 0.3 mm$^2$ (5%).

Frequency histograms of the infestation and occupation levels of abalone held at 20% stocking density are shown on the top axis of Figure 5-5. The number of tubes per centimetre of growing edge (infestation level) ranged from 4-11. Infestation levels between 5 and 6 tubes/centimetre occurred most frequently having 29 occurrences (27%). The occupation level of the tubes had a large variation and ranged from 4-45%. 62% of tubes had occupation levels between 13% and 23%. Significant correlations between the sabellid sizes and infestation and occupation levels are indicated by fitted lines and the regression statistics are given above each graph. The p-value refers to the significance of the slope of the model. No significant correlation existed between sabellid length and infestation and occupation level, and between sabellid surface area and occupation level ($p>0.05$). Sabellid neck width had a weak positive correlation with infestation level ($p<0.02$) and a negative correlation with occupation level ($p<0.0001$). The sabellid base width had a weak positive correlation with infestation level ($p<0.04$) and a weak negative correlation with occupation level ($p<0.02$). The sabellid surface area was positively correlated to infestation level ($p<0.002$). The $r^2$ value indicates the degree to which the variation in sabellid size can be explained by the variation in infestation and occupation levels, and is generally low indicating weak correlations.

Figure 5-6 shows the frequency histograms of sabellid size and infestation and occupation levels and any correlations which existed between these factors in sabellids from a 24% abalone stocking density. The skewness coefficient for sabellid length was 0.73 with the majority (81%) of the lengths values being between 1.8-3.6 mm but some attaining a maximum size of 5.1 mm. The neck width was normally distributed ($p\geq0.05$) with the values ranging from 0.13-0.32 mm. Most of the sabellid base widths were between 0.2-0.49 mm with very few (8%) individuals being larger than this size range. The surface area was distributed between 0.3-1.5 $mm^2$. The majority (76%) of abalone from the 24% stocking density had between 3 and 8 tubes per centimetre of growing edge (infestation level) with only a few having between 9-11 and 13-15 tubes per centimetre. The occupation level of the tubes was distributed between 6 and 40%. The most frequent occupation levels were between 14-22% and 32-40% of tubes, respectively. No correlations existed between any of the morphometric values or surface areas and infestation or occupation levels ($p>0.05$).

Sabellid length was left-skewed with the majority (94%) of sabellids measuring under 4 mm for abalone held at the 32% stocking density (Figure 5-7). The neck width was normally distributed ($p>0.05$) with an average of 0.17 mm and a range from 0.1 to 2.5 mm, with most widths (65%) being between 0.14-0.19 mm. Sabellid base width was skewed towards the smaller widths with the majority (76%) of sabellids having base widths below 0.36 mm but some attaining widths of up to 0.57 mm. Sabellid surface area was skewed towards the left-hand side with most sabellids (91%) having a surface area below 0.8 $mm^2$. The infestation level was concentrated between 2-8 tubes per centimetre of growing edge with a few outliers falling between 10-11 tubes per centimetre. The occupation level was distributed over a wide range and values were predominantly between 9% and 39 % but reached as high as 62%. No significant correlation existed between sabellid length, neck width and surface area and infestation and occupation levels. Sabellid base width showed a weak positive correlation with infestation level ($p<0.04$) and a weak negative correlation with occupation level ($p<0.04$).

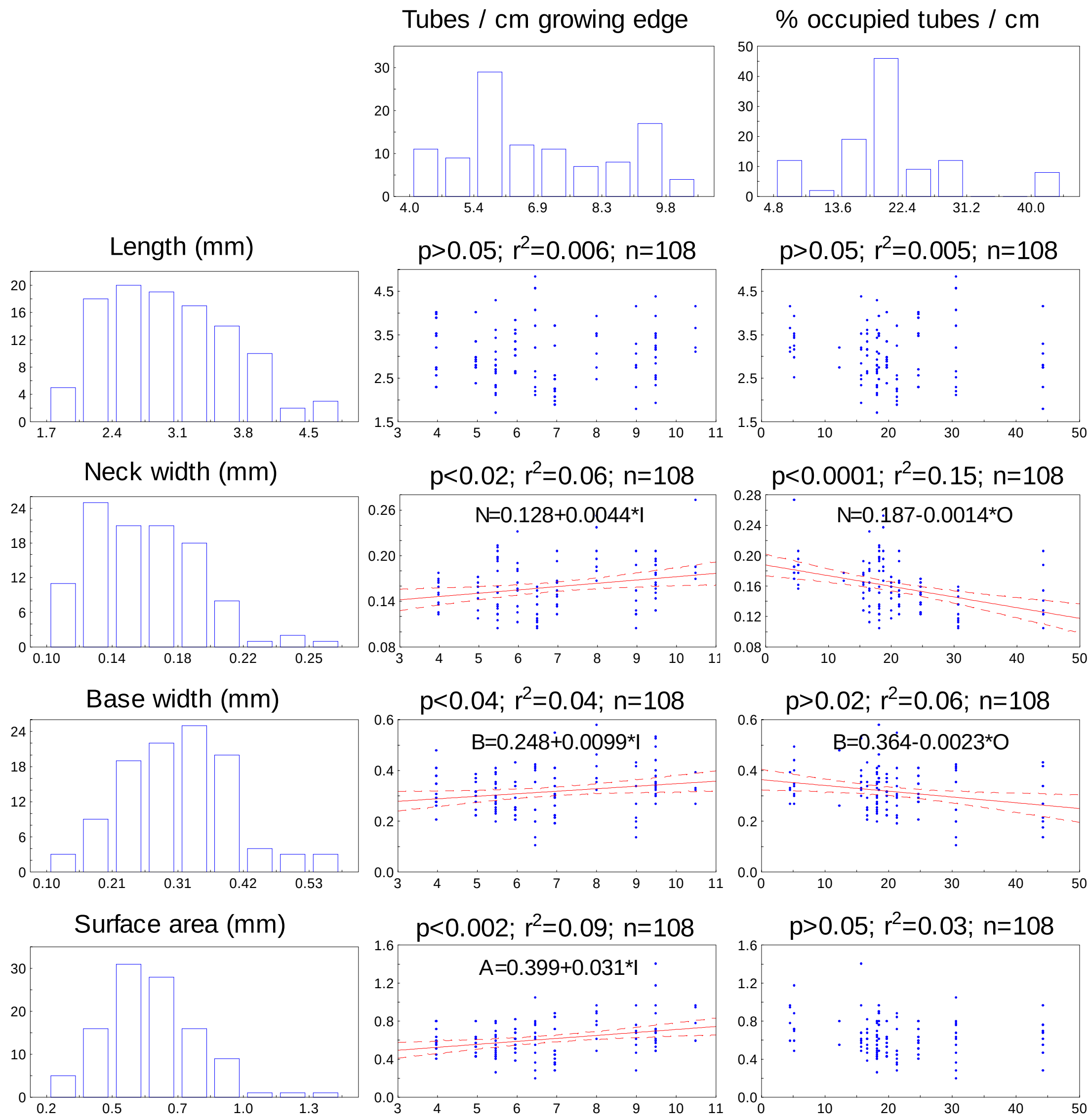


**Figure 5-5: Histograms of sabellid sizes and infestation and occupation levels from abalone stocking densities of 20% of available surface area. Scatter plots of the sabellid sizes versus infestation and occupation levels are shown. Where correlations exist a line is fitted and the equation displayed (N = neck width; O = percentage occupation; I = tubes per centimetre of growing edge). The histograms along the left-hand side of the page represent the y-axis variable in the scatter plots and the x-axis of the scatter plots is represented by the histograms on top of the page. Bars of all histograms are frequencies.**

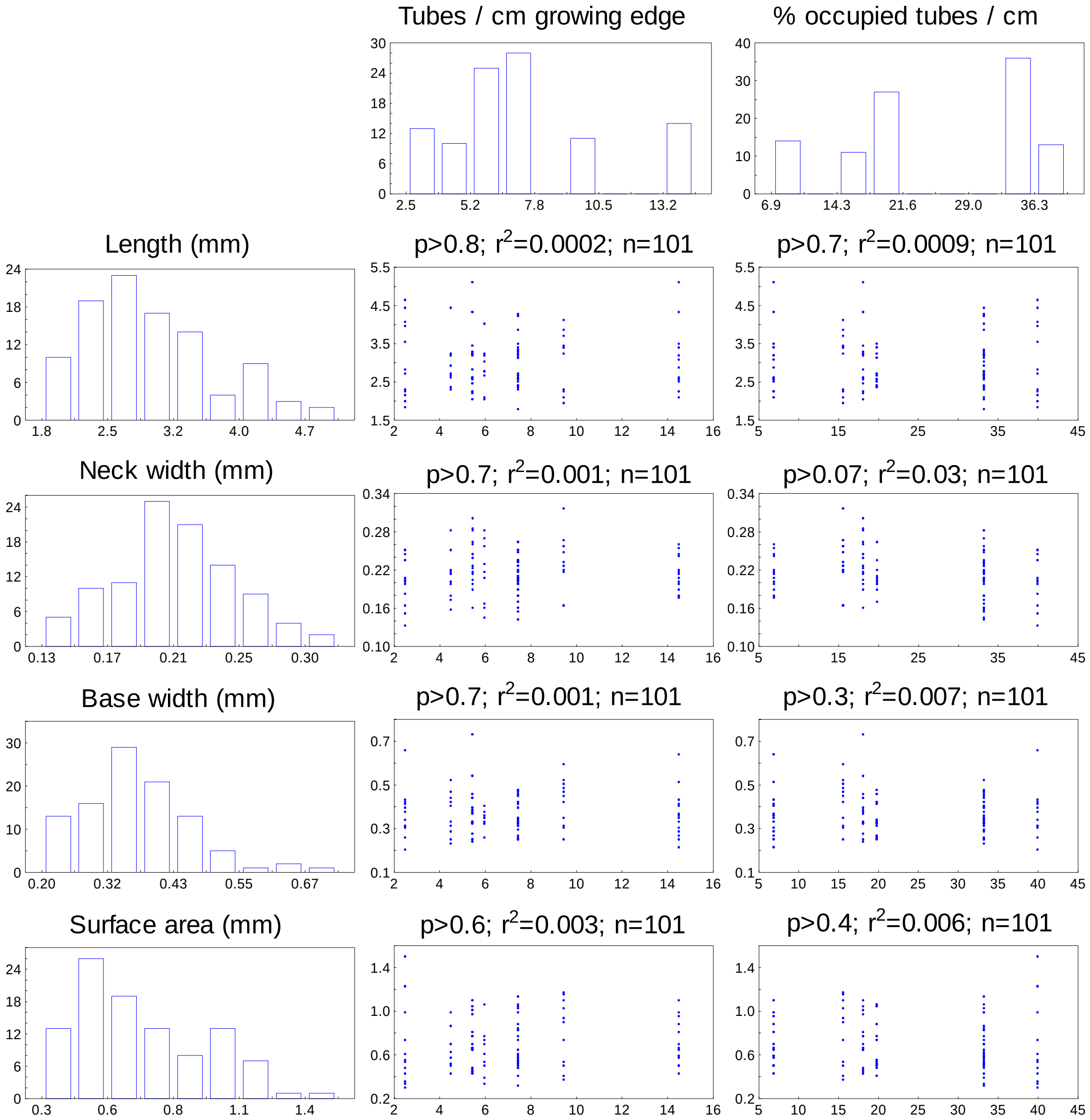


**Figure 5-6: Histograms of sabellid sizes and infestation and occupation levels from abalone stocking densities of 24% of available surface area. Scatter plots of the sabellid sizes versus infestation and occupation levels are shown. Where correlations exist a line is fitted and the equation displayed (N = neck width; O = percentage occupation; I = tubes per centimetre of growing edge). The histograms along the left-hand side of the page represent the y-axis variable in the scatter plots and the x-axis of the scatter plot is represented by the histograms on top of the page. Bars of all histograms are frequencies.**

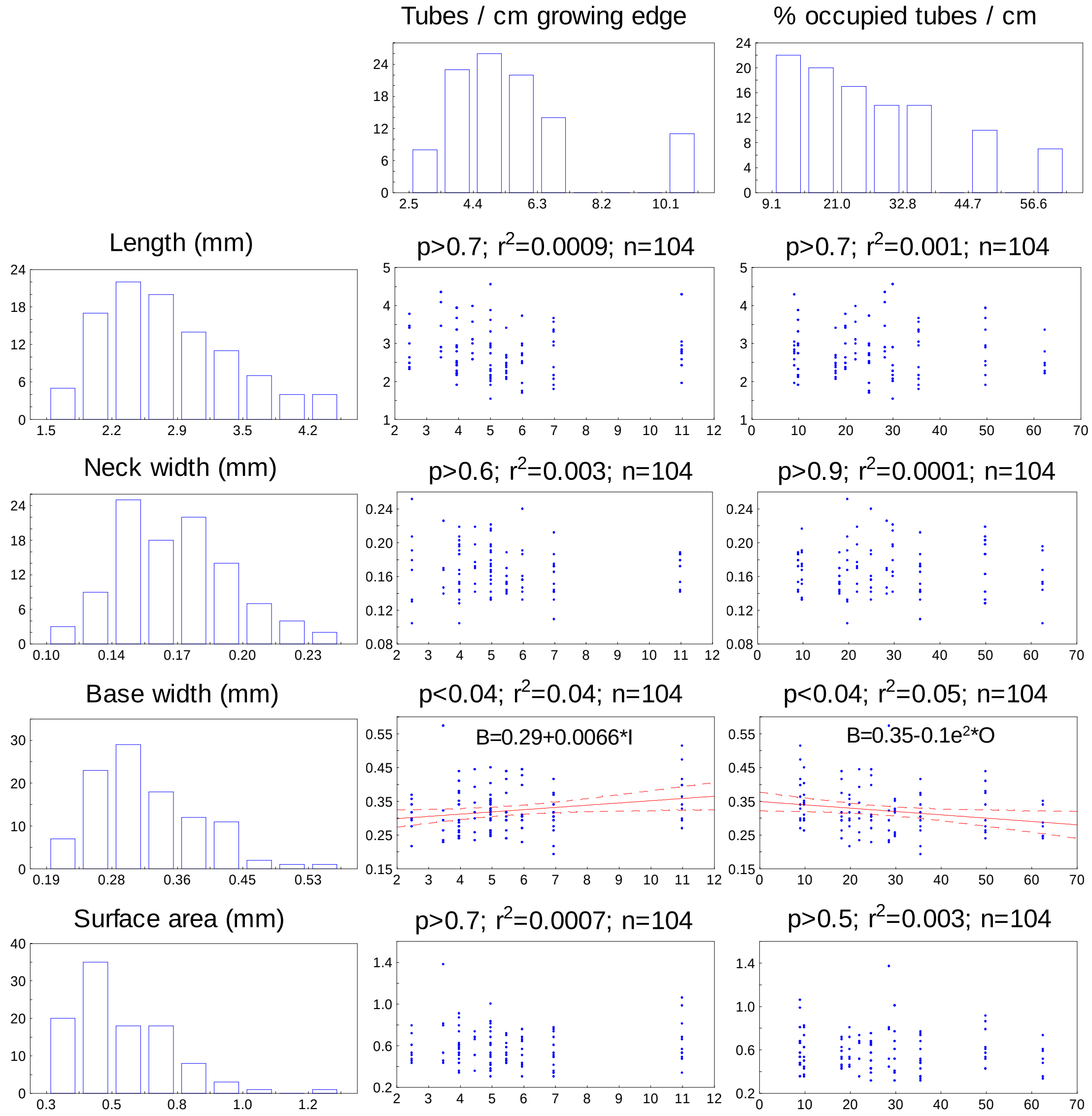


**Figure 5-7: Histograms of sabellid sizes and infestation and occupation levels from abalone stocking densities of 32% of available surface area. Scatter plots of the sabellid sizes versus infestation and occupation levels are shown. Where correlations exist a line is fitted and the equation displayed (N = neck width; O = percentage occupation; I = tubes per centimetre of growing edge). The histograms along the left-hand side of the page represent the y-axis variable in the scatter plots and the x-axis of the scatter plots is represented by the histograms on top of the page. Bars of all histograms are frequencies.**

## *Abalone size and diet*

The effect of diet on the number of tubes per centimetre (t/cm) of growing edge was significant, with the Abfeed abalone having a higher mean infestation level (5.9 ± 2.6 t/cm) than kelp-fed abalone (3.9 ± 1.6 t/cm) ($p \leq 0.001$; $F_{1,75}=13.3$) (Figure 5-8).

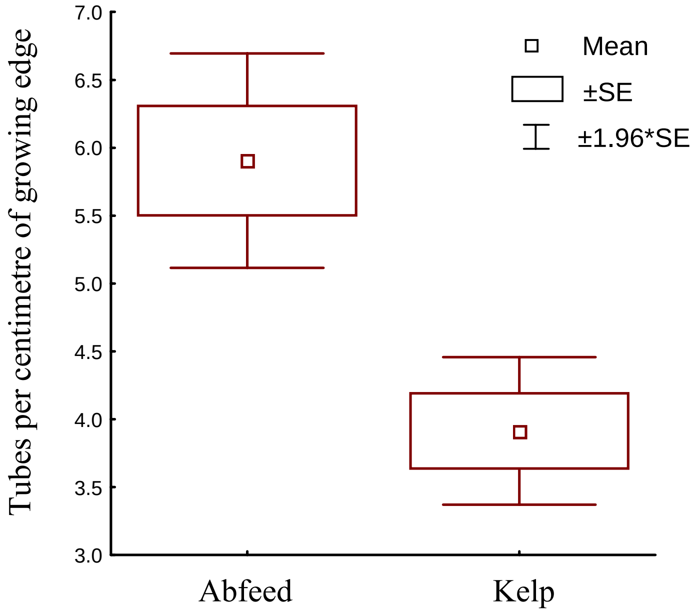


**Figure 5-8: Number of tubes per centimetre for abalone fed two diets.**

There was no significant interaction between abalone diet and abalone size on infestation level (tubes/cm) ($p>0.229$; $F_{2,71}=1.5$) (Table 5-5). There were also no significant differences in infestation level between abalone size classes within the Abfeed treatment. The mean number of burrows (infestation level) per centimetre of growing edge for kelp-fed abalone in the 40 mm size class was significantly greater than in the 60 mm abalone size class while similar to the 70 mm abalone size class (Table 5-5).

**Table 5-5: Mean shell length and number of tubes per growing edge ± standard deviation for abalone of different size classes on Abfeed and kelp diets. Superscripts indicate significant differences. n is the sample size.**

| Diet | Abalone size class (mm) | Mean shell length | Mean number of burrows/cm | n |
|---|---|---|---|---|
| Abfeed | 40 | 42.1 ± 3.8 | $6.4 \pm 2.8^{a}$ | 13 |
| Abfeed | 60 | 56.5 ± 4.5 | $4.6 \pm 1.6^{a}$ | 14 |
| Abfeed | 70 | 67.1 ± 6.1 | $6.7 \pm 2.9^{a}$ | 15 |
| Kelp | 40 | 41.3 ± 3.3 | $5.0 \pm 1.6^{x}$ | 10 |
| Kelp | 60 | 55.6 ± 4.9 | $3.3 \pm 1.7^{y}$ | 13 |
| Kelp | 70 | 67.7 ± 6.5 | $3.6 \pm 1.2^{xy}$ | 12 |

There was a significant interaction between abalone diet and abalone size on percentage of tubes occupied ($p<0.03$; $F_{2,71}=6.4$). The occupation level of sabellid tubes increased with increasing abalone size for kelp-fed abalone; the 70 mm abalone size class had a significantly higher occupation rate than the 40 mm abalone size class (Figure 5-9).

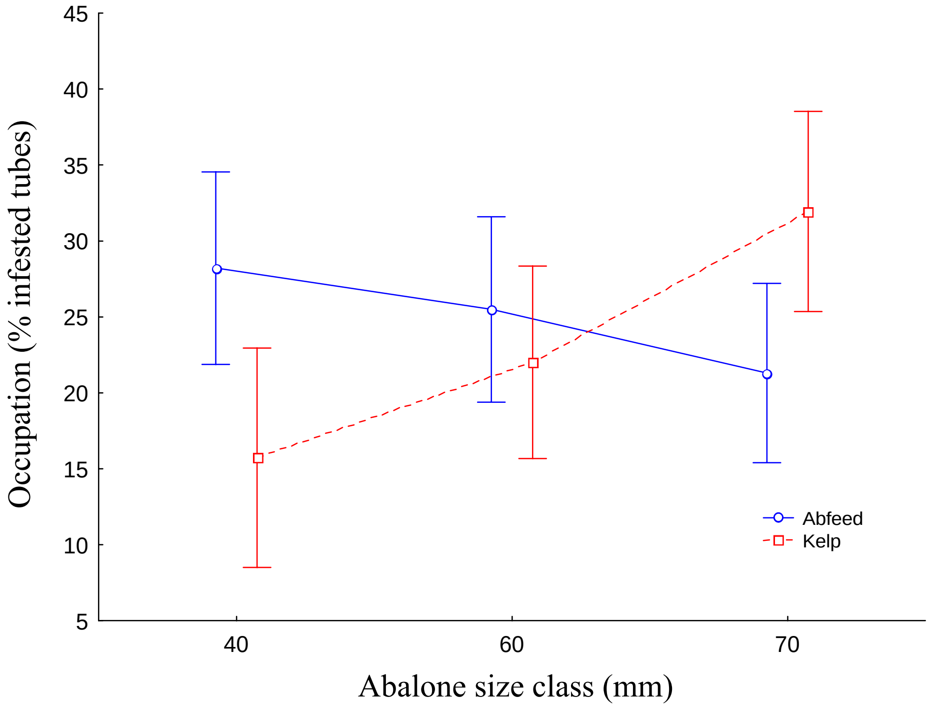


**Figure 5-9: Mean percentage occupation of tubes for abalone from kelp and artificial feed diets. Error bars indicate 95% confidence intervals.**

Figure 5-10 shows where significant two-way interactions exist between abalone diet and abalone size on the sabellid body sizes and morphometric ratios. Sabellids from Abfeed-fed abalone were generally larger, wider and had a greater surface area for all abalone size classes. There was no interaction between abalone diet and abalone size on sabellid length ($p<0.232$; $F_{2,612}=1.46$). The sabellids from abalone fed Abfeed had similar body lengths for all abalone size classes. The 70 mm abalone size class of kelp-fed abalone had sabellids of significantly greater length than the 40 and 60 mm abalone size classes.

There was an interaction between abalone diet and size for sabellid neck width, base width and surface area ($p<0.0001$; $F_{2,612}=16.48$; $p<0.0001$; $F_{2,612}=18.28$; $p<0.0001$; $F_{2,612}=15.06$, respectively) with a similar pattern for all variables. The Abfeed sabellids had similar widths or areas for abalone sizes of 40 and 60 mm, and these values decreased in the 70 mm abalone size class. Sabellids from kelp-fed abalone showed an opposite trend with the 70 mm abalone size classes having a greater value than the 40 and 60 mm abalone size classes for the widths and area values.

The length-area, base-neck and length-neck ratios had significant interactions between abalone diet and abalone size ($p<0.0001$, $F_{2,612}=26.2$; $p<0.0125$, $F_{2,612}=4.4$; $p<0.0025$, $F_{2,612}=6.2$, respectively). The length-area ratio of sabellids decreased with increasing abalone size for kelp-fed abalone and increased with increasing abalone size for Abfeed-fed abalone. The base-neck ratio was similar for all abalone sizes fed Abfeed while it increased from the 40 to 60 mm abalone size for kelp-fed abalone. Sabellids from Abfeed-fed abalone showed an increase in length-neck ratio with an increase in abalone size above 60 mm. Kelp-fed abalone had sabellids with a greater length-neck ratio. The sabellids from kelp-fed abalone showed an increase in length-neck ratio from 40 to 60 mm above which size it was fairly constant. There was no interaction between the base-area ratios ($p>0.05$, $F_{2,612}=2.87$), which were similar between the two diets with only the 60 mm kelp-fed abalone having a significantly higher value.

The number of tubes per centimetre of the growing edge for abalone on a kelp diet was not normally distibuted ($p<0.01$) and ranged from 1 to 7 tubes/cm with a median of 4.5 (Figure 5-11). The distribution of the percentage of tubes occupied ranged from 6.6 to 37.5% but was skewed (skewness coefficient of 1) with a large percentage (70%) of the total number of observations in the lower range of occupation below 17%. The frequency histograms of the sabellid measurements are shown on the left-hand side of Figure 5-11. The sabellid lengths from kelp-fed abalone were normally distributed with a mean of 2.9 ± 0.65 mm and a range of 1.3-4.8 mm. The neck width was slightly skewed (skewness coefficient of 0.38) towards the lower sizes with most observations (74%) falling in the 0.13-0.21 mm size range. The base width distribution was also skewed (skewness coefficient of 1.1) with most sabellids (76%) being between 0.20-0.40 mm in width at their base. The sabellid surface area histogram indicates a left-skewed distribution with a skewness coefficient of 0.89.

There was no significant correlation between sabellid length and tubes/cm, base width and tubes/cm and surface area and tubes/cm ($p>0.05$). There was a significant correlation between neck width and number of tubes/cm of growing edge ($p<0.05$) but with a low $r^2$-value of 0.015 indicating a very low predictive power of the model. Thus, only 1.5% of the variation in neck width could be explained by the variation in infestation. The sabellid length, neck width and surface area are correlated with percentage of tubes occupied ($p<0.05$) but all have similarly low $r^2$-values. There was no significant correlation between base width and occupation level ($p>0.05$).

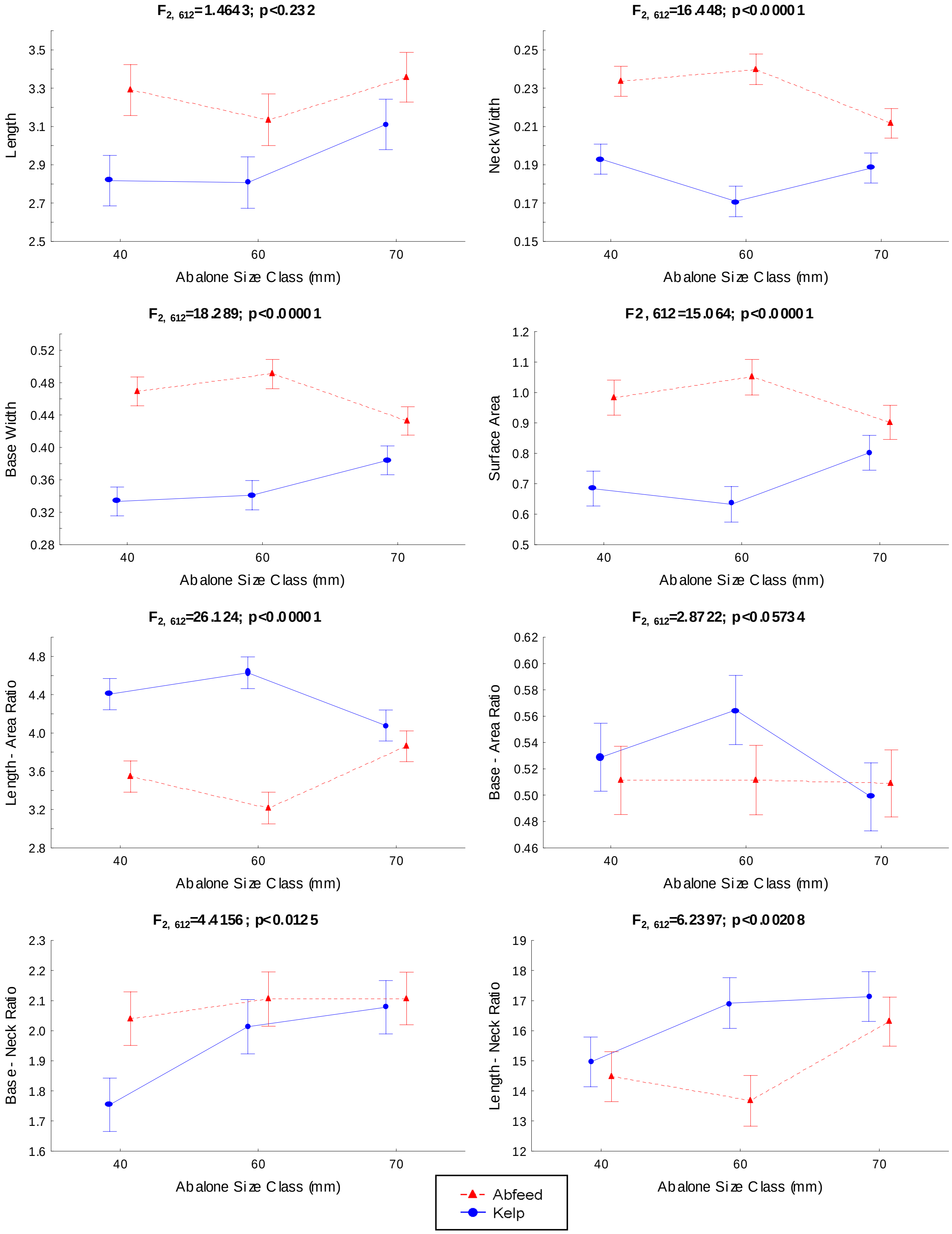


**Figure 5-10: Two way interaction of abalone size and diet and their effects on sabellid body proportion and the associated morphometric ratios. Error bars indicate 95% confidence bands.**

Both the number of tubes per centimetre of growing edge and the percentage occupation of the tubes was skewed towards the lower side of the distribution in Abfeed-fed abalone (skewness coefficient of 0.88 and 1.0, respectively) (Figure 5-12). The number of tubes per centimetre of growing edge attained maximum values of 12 in the Abfeed-fed abalone and was significantly higher than that of the kelp-fed abalone, which attained a maximum value of 7.5 tubes/cm ($p<0.0001$; $F_{2,612}=59.9$). The percentage of tubes occupied for Abfeed-fed abalone reached a maximum of 63% in comparison to a maximum of 37% in kelp-fed abalone. The distribution of the length of sabellids from the Abfeed diet was slightly skewed with a skewness coefficient of 0.32 and they had a greater average length than sabellids from the kelp diet treatment ($p<0.0001$; $F_{2,612}=38.5$). The neck width was slightly skewed (skewness coefficient 0.69) with the majority (80%) of the observations lying in the range between 0.17-0.29 mm. The base width of sabellids from Abfeed-fed abalone was normally distributed ($p<0.05$) and the sabellids attained larger mean widths (0.46 ± 0.10) than kelp-fed sabellids (0.35 ± 0.08). The surface area was skewed (skewness coefficient 1.0) towards the left with most (77%) of the sabellids having a surface area of between 0.6 and 1.4 $mm^2$ and a greater mean (0.98 ± 0.34) than the sabellids from the kelp diet (0.71 ± 0.25) ($p<0.001$; $F_{2,612}=119$). The only low significant correlation in Abfeed sabellids was that between length and infestation level (number of tubes/cm)($p<0.05$; $r^2=0.014$). There was no significant correlation between the other sabellid size parameters (neck width, base width and surface area) and the infestation and occupation levels ($p>0.05$). Sabellid length and occupation level were not correlated ($p>0.05$).

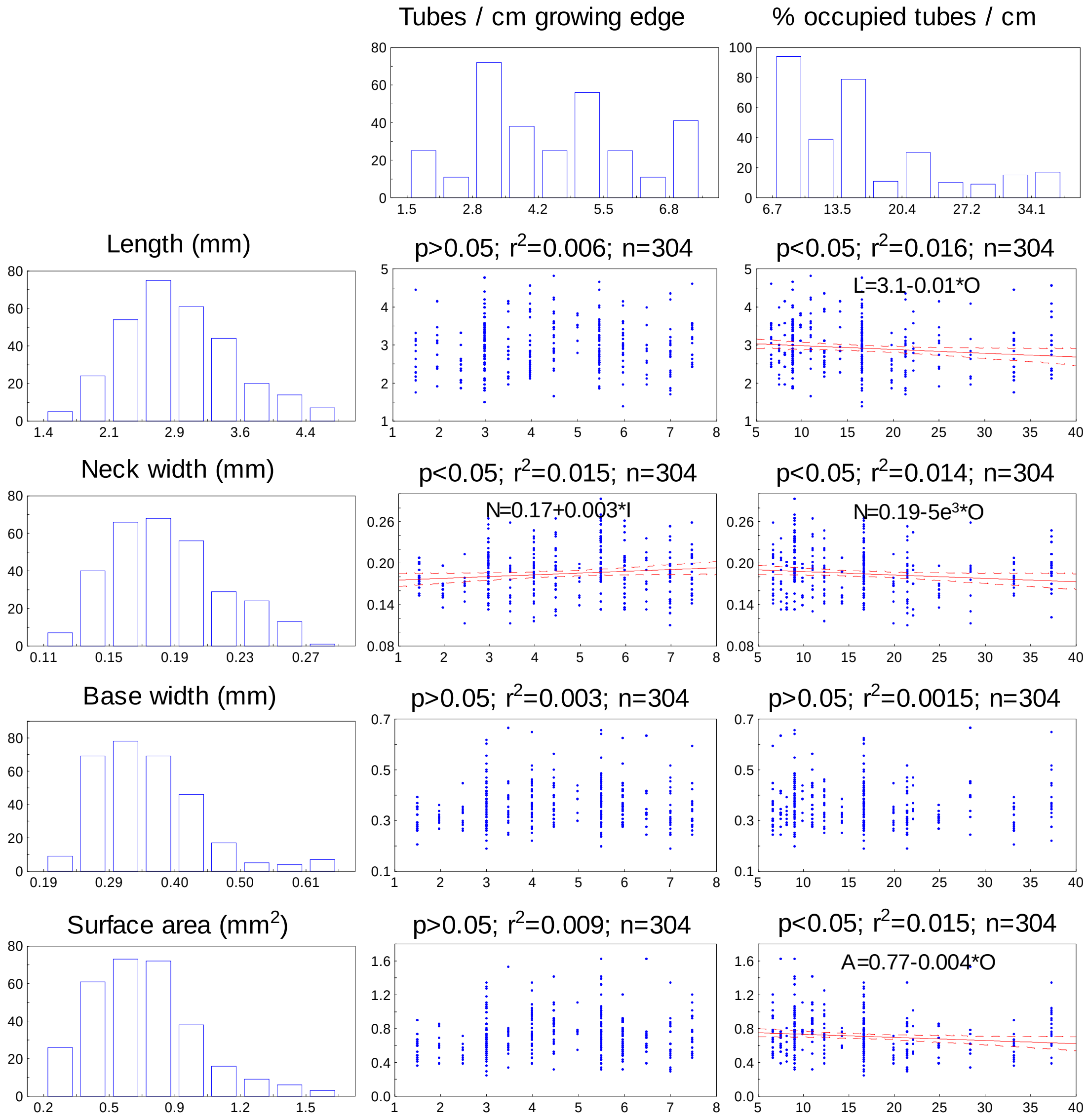


**Figure 5-11: Histograms of sabellid sizes and infestation and occupation levels from kelp-fed abalone and scatter plots of the sabellid sizes and infestation levels. Where correlations exist a line is fitted and the equation displayed (N = neck width; O = percentage occupation; I = tubes per centimetre of growing edge). The histograms along the left-hand side of the page represent the y-axis variable in the scatter plots and the x-axis is represented by the histogram on top of the page. Bars of all histograms indicate frequencies.**

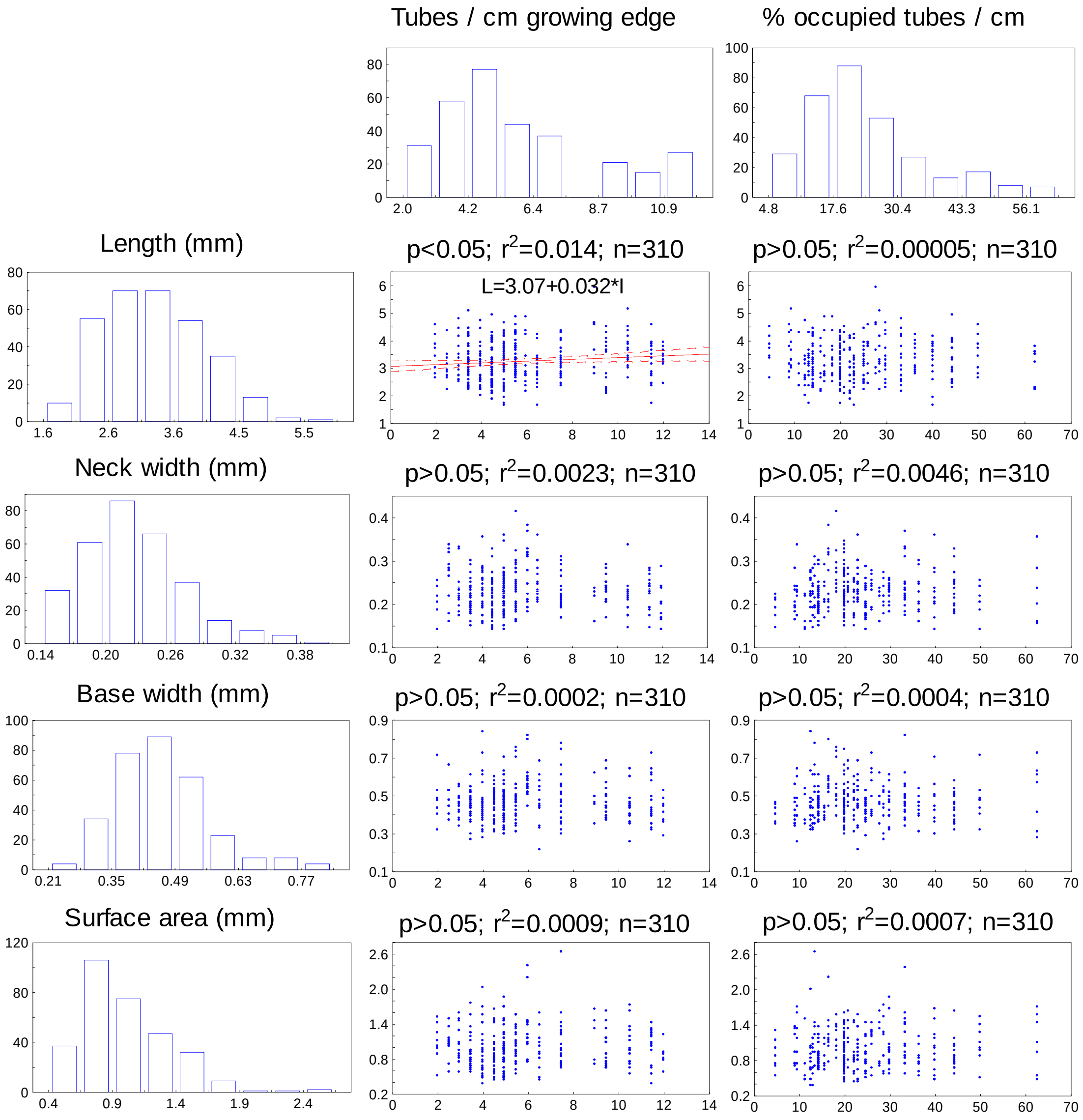


**Figure 5-12: Histograms of sabellid sizes and infestation and occupation levels from Abfeed-fed abalone and scatter plots of the sabellid sizes and infestation levels. Where correlations exist a line is fitted and the equation displayed (L = length; I = tubes per centimetre of growing edge). The histograms along the left-hand side of the page represent the y-axis variable in the scatter plots and the x-axis is represented by the histograms on top of the page. Bars of all histograms indicate frequencies.**

# DISCUSSION

## *Effect of abalone stocking density on sabellid infestation levels and morphometrics*

The stocking densities used in this study did not affect the infestation level (tubes/cm) or the percent of tubes occupied by the sabellid (occupation level). In the past abalone farmers have observed increased infestation levels at very high abalone stocking densities but there has been no documentation or experimentation to determine at what density the sabellid becomes problematic. Based on the farmers' observations it was predicted that sabellid infestation would be greater at higher abalone stocking density. This prediction was based on two potential factors related to stocking density in the culture environment: 1) Particulate loading within the water column at high stocking densities is expected to be greater than at low stocking densities. This is due to greater quantities of feed being used to feed a greater abalone biomass and due to increased abalone faecal production. This could increase food availability for the sabellid; 2) Physical contact between abalone at high stocking densities is more frequent than at low stocking densities. This increases the potential for transmission of sabellid larvae between hosts. Together these two factors would increase the sabellids' potential to infest abalone more successfully at higher stocking densities. In this study, however, these factors did not appear to affect the sabellid infestation and percentage occupation of tubes along the growing edge. This may have been because the particulate level for the three stocking densities did not differ significantly (Chapter 4) and they originated from the same abalone diet and therefore had similar nutritional values.

The morphometric values and ratios appear to be both variable and sensitive to abalone stocking density. This study has provided baseline data of sabellid population size and size variation of individuals within populations. This information is useful for future comparisons of sabellid populations between farms or between environmental conditions and for designing future experiments to test new hypotheses. The majority (67%) of the sabellid lengths were within a range of 1.4 mm, being between 2.2 - 3.6 mm. The minimum and maximum lengths, however, were 1.5 mm and 5.1 mm respectively, indicating the percentage of the population comprising outliers while most of the sizes occurred within a small size range around the mean. Although there were significant differences in sabellid lengths between abalone stocking densities, the coefficient of variation within each stocking density was high (20% density = 22%; 24% density = 25%; 32% density = 23%). This suggests that abalone stocking density within the range tested does not influence sabellid growth sufficiently to create populations with distinctly different length characteristics.

Neck width is thought to be the most constant morphometric variable of the sabellid as it is not known to fluctuate seasonally in response to reproductive activity. Base width and length fluctuate in response to reproductive activity (Simon *et al.*, 2002). The coefficient of variation of neck width across the stocking densities tested was similar to that of sabellid length suggesting that variability was proportionally similar between the two parameters (20% density = 22%; 24% density = 19%; 32% density = 19%).

The coefficient of variation indicated slightly greater variability in base width within populations (20% density = 29%; 24% density = 28%; 32% density = 22%). Base widths ranged from 0.10 and 0.73 mm, respectively, three times the range of the neck width. This indicates a wider size distribution in base width, which may vary seasonally and fluctuate according to reproductive activity. Simon *et al.* (2002) noticed that oocyte development resulted in a corresponding swelling of the abdominal region. This emphasises the potential importance of this variable in estimating sexual maturity and reproductive activity of sabellid populations in future research.

Sabellid surface area was the most variable of all morphometric values measured, having the highest coefficient of variation (20% density = 33%; 24% density = 38%; 32% density = 32%). The large variability in surface area is a function of both length and width. This variability indicates the potential sensitivity and adaptability of the sabellid to changes in microclimate and ultimately their biology.

Although there was large size variation across the stocking densities, the morphometric measurements indicate a population of larger sabellids in the 24% abalone stocking density. The variability in these parameters, however, suggests that stocking density within the range tested does not modify sabellid size and growth. It is possible that environmental factors played a stronger role than stocking density in influencing sabellid growth. Future experiments should be more sensitive to fluctuations in environmental conditions between treatments and aim to test hypotheses relating directly to the microhabitat experienced by sabellids as this may have a significant impact on morphometrics, influencing flow dynamics and therefore particle abundance and availability. Experiments within this study were run under commercial farm conditions in order to learn more about factors affecting sabellid populations in commercial systems. Unfortunately, unlike controlled laboratory experiments, where temperature, photoperiod and water quality can be finely tuned, environmental parameters in abalone systems vary greatly within and between farms. It is possible that combinations of these and other factors may have influenced the results, masking the potential effect of stocking density thereby suggesting sabellids from a 24% density are significantly larger. Such potentially confounding factors must either be isolated or investigated in future experiments. Ideally the effect of abalone stocking densities on sabellid population dynamics should be investigated across a wider range of densities at higher stocking levels than those currently found within the South African abalone industry.

The morphometric ratios appear to be sensitive to environmental conditions. Indices relating lengths and weights are widely used by aquaculturists and scientists to compare condition of aquatic organisms from different conditions or fed on different diets (Dadzie and Wangila, 1980; Bolger and Connolly, 1989). This is most common in commercially and economically important species where optimising the growth rate increases the production and yield. Condition indices have not been used on polychaete species before. Past research on problematic species, such as *Polydora*, has focused primarily on eradication and control methods rather than condition aspects (Nel *et al.*, 1996). Due to the economic impact of the sabellid to the abalone industry, basic information of sabellid population condition has become of utmost importance. These ratios were tested in an attempt to identify an index suitable for describing the condition of a population of sabellids from a range of conditions.

Stocking density appears to have a clear effect on the base-area ratio. The other morphometric ratios have irregular patterns, indicating that stocking density either has no clear effect on these ratios and that these ratios are poor indicators of sabellid condition or that other factors override the density effect. The base-area ratio appears to be a reliable index related to stocking density. The large variability in the base width and surface area values, however, implies that such an index may not be accurate. As previously mentioned, base width is variable in relation to reproductive activity and maturity. A ratio combining the area (which takes into account both the length and width) and base width would therefore appear to be a good estimator for sexual maturity. Further work is required to determine the accuracy of this index before it can be applied practically as a rapid method for assessing the condition and reproductive state of populations of sabellids.

It is important to understand the relationship between organisms and their environment (Begon *et al.*, 1996). Organisms are not distributed randomly through different kinds of environments but occur in specific areas where conditions favour their mode of life (Begon *et al.*, 1996). Flow conditions and water quality, in particularly suspended material, determines the suitability of a habitat for sessile filter feeding organisms (Levinton, 1995). Stocking density of aquatic organisms within culture systems can have a significant effect on the level of suspended and dissolved solids within the water column (Beveridge *et al.*, 1994). Feeding frequency (Velasco *et al.*, 1999; Smith *et al.*, 2002), water circulation and aeration (Hussenot *et al.*, 1998), and farm management (Beveridge *et al.*, 1994) have been identified as potential factors influencing the nutrient levels and water quality within culture systems. Space limitations within abalone farms often results in inadequate cleaning routines, which can account for variations in water quality conditions. Furthermore, size-grading and sorting frequency of abalone often depends on space availability, which, when limited, results in excessive abalone stocking densities for short periods of time. These factors contribute to the variability in water quality conditions and therefore nutrient availability to the sabellid. Combinations of these factors result in conditions unfavourable for abalone culture but suitable for filter feeding organisms such as the sabellid. On abalone farms under intensive commercial production these factors cannot be controlled efficiently and there is great variability both within and between farms. Continued variation in these environmental parameters within different raceways can influence the condition of the sabellid on a day to day basis. This could possibly explain the high variation in the variables measured and account for the small percentage of outliers which could be experiencing more extreme variations due to their location on the shell or in the raceway.

There was no clear correlation between sabellid morphometrics and infestation and occupation levels. A correlation indicates the extent to which two random variables either increase together or one increases while the other decreases in constant proportions (Harraway, 1997). In general an r-value of 0.7 or greater indicates a strong linear relationship between two variables while an r-value of below 0.3 implies a negligible linear association between two variables. According to this judgement, correlations between the sabellid morphometrics and infestation and occupation level for the three densities sampled were negligible. This means that there is no meaningful linear relationship between sabellid size and infestation level, indicating that the one does not influence or affect the other in any reliable manner. The lack of clear correlations for sabellid morphometrics and infestation and occupation levels for any of the stocking densities implies that the morphometrics for a population of sabellids could not predict its population size or survival after settling. The variability of the measurements both within and across the stocking densities implies that stocking density does not moderate population growth.

It is possible that the range of stocking densities sampled for this study may not have been wide enough to pick up clear differences in sabellid morphometrics. Abalone stocking density was identified by the Abalone Farmers Association of South Africa (AFASA) as one of the main causes of the sabellid problem. In an attempt to manage the sabellid problem, a maximum stocking density of 35% was recommended by AFASA (Anonymous (a)). As most farmers were having problems at the time, they modified their farming practices and reduced their stocking densities in an attempt to alleviate the problem. Currently no farms maintain abalone at densities above 40% in the conventional basket culture method. The aim of this study was to get an idea of what is currently occurring in the industry and the stocking densities sampled were therefore representative of farming practices presently found in the South African abalone industry. It could therefore not be tested if a wider range of stocking densities affected sabellid morphometrics. Future research should investigate the effects of significantly higher stocking densities on sabellid morphometrics in order to determine if above a certain level, it is a factor influencing sabellid growth and infestation.

The percentage of tubes occupied along the growing edge of the abalone shell was similar for all stocking densities sampled. In aquaculture an increase in stocking density above a threshold level is associated with a corresponding decrease in water quality (Beveridge *et al.*, 1994). Poor water quality is related to an increase in ammonia, nitrite, nitrate and suspended solids levels (Noble and Summerfelt, 1996). High particulate loading within the water column provides a nutritious environment for filter feeding organisms and one would therefore expect higher levels of sabellid infestations at higher abalone stocking densities. Contrarily to what was predicted for this trial, there was no significant difference in the quantity of particulates at each of the three stocking densities (Chapter 4) and similar particulate abundance was experienced by sabellids at all densities sampled. It is thus unclear as to what regulates the occupation level of the sabellid tubes. It has not been tested if competition for both space and food are controlling factors of post-larval sabellids. More research is required on larval settlement and metamorphosis to determine if competition for space becomes a controlling factor, regulating the occupation level of tubes.

From this study it would appear that the stocking densities currently in practice in the South African abalone industry do not promote excessive and problematic sabellid infestations. In addition, analysis of the quantity of particulates present within raceways indicated no significant difference in particulate abundance for the range of stocking densities tested (Chapter 4). Abalone stocking density is therefore not a major factor influencing sabellid infestation, occupation and morphometrics under the current practices. Thus, to prevent sabellid outbreaks, stocking densities should not exceed those tested in this study.

## *Effect of diet and abalone size on sabellid infestation and morphometrics*

The abalone industry has initiated considerable research on the nutritional requirements of abalone and feed formulation in order to maximise abalone growth by using artificial diets (Barkai and Griffiths, 1986; Knauer, 1994; Britz, 1995; Britz, 1996; Shipton, 1999; Sales, 2001). The artificial diet commonly used in South Africa, Abfeed, has improved the growth rates of abalone in comparison to those fed natural diets. It has, however, been suggested that the problematic sabellid infestations occurring in the abalone industry are linked to the use of this high protein diet but hitherto there has been no conclusive evidence for this. The aim of this study was to determine the effect of Abfeed on sabellid infestation and morphometrics. Abfeed has a significantly higher protein content than naturally occurring seaweeds (Britz, 1995) and it has been shown that there is a certain amount of nutrient leaching and particle fragmentation into the surrounding water column of commercial abalone raceways (Chapter 4). It was therefore hypothesised that the greater abundance of high protein particulates of preferable size (Chapter 3 and 4) observed in Abfeed raceways could potentially lead to an increase in sabellid infestation.

Abalone diet significantly affected the infestation level by sabellids. Abfeed is more nutritious and more available to the sabellids as it is more abundant than kelp in smaller particle size classes (Chapter 4) and resulted in a significantly greater number of tubes per centimetre of the growing edge. This indicates that abalone diet could be a factor influencing the reproductive output of the sabellid, with kelp sabellids producing fewer eggs, and therefore larvae, than Abfeed sabellids, resulting in a lower infestation level along the growing edge. The quality of the food particulates ingested by the sabellid could well affect the quality of the eggs produced and therefore their hatching success and post-hatching survival of larvae. Besides not hatching, poor quality eggs may not provide the emerging larva with sufficient energy to sustain itself until it is able to find the growing edge of the abalone shell. It is not known what type of cues attract the sabellid larvae to the growing edge and it could take the larvae a long time to discover the right location on the shell for tube formation. Food quality may also affect the formation of spermatids and their ability to fertilise the egg. Although it is currently not known what nutritional qualities affect reproductive success, abalone diet has a significant effect on the infestation level of cultured abalone. Presently work on egg size in relation to diet, and sabellid reproduction are being investigated.

Abalone size does not appear to have a strong effect on sabellid infestation levels, which were similar within each diet, except for the smallest kelp-fed abalone size class, which had a higher infestation level than the two larger kelp size classes. The growth rates of the kelp-fed abalone were similar and it is not clear as to what influenced the difference in infestation level between the abalone size classes. As mentioned earlier, it could have resulted from differences in the conditions between raceways.

Abalone size influences the occupation levels of tubes within both diets. Occupation level increases with abalone size in kelp-fed abalone and decreases with Abfeed-fed abalone. Again there is no clear reason for this. On smaller abalone the distance and surface area the sabellid larvae need to cover in order to find the growing edge is less, and the length of the growing edge is also smaller. These factors could result in higher infestation levels as was found in the kelp diet (Table 5-5). The interaction between sabellids at high infestation levels (tubes/centimetre) once they have metamorphosised may regulate the occupation level along the newly formed shell to a sustainable level for adults' survival. Intra-specific competition may play an important role in moderating the occupation level of tubes and should be investigated in the future. There is no clear explanation for the decrease in occupation level for the Abfeed-fed sabellids as they had similar infestation levels across abalone size. Again, this could have something to do with interactions within sabellid populations and should be investigated further.

There was no interaction between the diets for sabellid length, which increased from the 60 mm abalone to the 70 mm abalone in each diet. There were, however, interactions for the neck width, base width and surface area values across abalone size. In all these variables it appears that Abfeed sabellids begin decreasing in size from the 60 to 70 mm abalone size while kelp sabellids show an increase in size. This further illustrates the sensitivity of the methods used for measurement. Diet and abalone size have a combined effect on sabellid morphometrics although the reason for this cannot be established from this study. The quality of nutrients available to sabellids does not change across abalone size classes within each diet (Chapter 4) and the differences in sabellid morphometrics across abalone size cannot be attributed to nutritional quality of particulates. The size distribution of particulates was not tested for different abalone size classes in this study and could be a contributing factor to the observed differences in sabellid sizes. Different feeding rates or strategies of abalone could result in differences in particulate abundance and size distribution between small and large size classes, which could affect the availability of particulates to the sabellids differently in each diet thereby affecting their growth and size. Interactions between diet and abalone size also occurred for the length-area, base-neck and length-neck ratios indicating that both these factors influence the condition ratios of the sabellid. A low length-area ratio indicates a worm of better condition, as a short fat worm would presumably be in better condition than a long thin worm. This suggests that Abfeed sabellids are in better overall condition than kelp sabellids and that they begin to increase in condition with increasing abalone size. The kelp sabellids begin to decrease in size with a corresponding increase in abalone size. The Abfeed sabellids show a similar trend in the length-neck ratio, again indicating better condition than kelp sabellids and increasing condition with increasing abalone size. These ratios reflect the similar trends of the individual sabellid parameters measured, indicating that the Abfeed sabellids are in better condition while the actual measurements show the size difference between the two diets. The base-area ratio remains constant for Abfeed sabellids across abalone size class while being variable for kelp sabellids. The base-neck ratio appears to be the most reliable indicator of sabellid condition and possibly fecundity as it relates the width of the reproductive segments of the sabellid to the neck width, which increases proportionally as the sabellid grows. This ratio increases for both diets with increasing abalone size. Indices of condition can provide insight into the factors affecting isolated populations of organisms. The morphometrics from this study indicate that diet and abalone size interact to have an effect on sabellid size and condition, and should not be discussed in isolation of each other. The observed effects can have important implications for farm management, which could be modified to aid in alleviating the sabellid problems at different abalone stages.

Nutrient requirements of organisms may be dependent on the age of the organism and its digestibility capabilities (D'Abramo, 1998). Presently there is only one Abfeed formulation used as feed for all abalone size classes. It has been noticed by farmers that Abfeed results in different growth rates of abalone of different size classes. Abalone have also previously been observed to be most susceptible to sabellid infestation at a size of 35-45 mm shell length (AFASA, *pers. comm.*). For this reason the study tested three abalone size classes for both diets. There were no significant differences in infestation level across Abfeed size classes, while the smallest kelp group was significantly different to the other two kelp treatments. There was a significant interaction between sabellid occupation level and abalone size class for the two abalone diets. Kelp-fed abalone had an increasing occupation level with increasing abalone size class while Abfeed-fed abalone showed a decreasing occupation level with increasing abalone size (Figure 5-9). The average morphometric sizes of sabellids from Abfeed raceways were significantly greater than those from the kelp raceways. This highlights the importance of diet as a major factor influencing sabellid size and infestation.

Only very weak correlations existed between sabellid morphometrics and infestation/occupation levels for both abalone diets. The length (Abfeed=22%; kelp=22%), neck width (Abfeed=22%; kelp=19%) and base width (Abfeed=22%; kelp=24%) all had similar coefficient of variations for both diets. The surface area, however, had a larger but similar coefficient of variation for both diets (Abfeed=36%; kelp=35%), which can be attributed to this parameter being a function of both length and width. For all parameters Abfeed resulted in larger sabellids, which suggests that the sabellid is deriving greater nutritional value from the formulated feed raceways which can be invested into growth, as shown by this study, and possibly reproduction, which needs to be researched further. Abfeed had a higher maximum infestation level (12 tubes/centimetre opposed to 7 for kelp) of sabellids, supporting the theory that the difference in diet composition can lead to increased reproductive output of the sabellid, which is reflected in the degree of re-infestation of the growing edge. The occupation level (% tubes occupied/centimetre) for Abfeed also had a higher maximum value than kelp abalone shells. This indicates the potential difference in egg quality and larval survival between the two abalone diets. More research, however, needs to be conducted in this area in order to validate these assumptions.

From this study it appears that the stocking densities sampled and currently used in the South African abalone aquaculture industry do not have a significant effect on sabellid populations and infestation levels. Diet, however, appears to have a significant influence on both sabellid infestation and population size structure, with the artificial diet, Abfeed, resulting in greater infestation and sabellid size. This is likely to be due to the greater nutritional value of Abfeed particulates, as was documented in Chapter 4.

# CHAPTER 6

# GENERAL DISCUSSION

## OBJECTIVES OF THE STUDY

The main objectives of this study were to investigate the nutritional requirements of the sabellid, *T. heterouncinata*, using morphometrics and behavioural feeding studies with commercial farm diets under conditions present in the South African abalone industry. The individual objectives of this study complemented each other and aided in understanding the feeding biology of the sabellid, which provides a basis for future studies (Figure 6-1).

Due to its recent economic impact on the viability of abalone farming, *T. heterouncinata* has become the focus of attention by the farmers. Research into its biology has become necessary to identify key factors that may influence its prolific infestation in farmed abalone. Past investigations into the feeding biology of suspension feeding polychaetes have resulted in species-specific results. It has been suggested that suspension feeding is induced by the presence of suspended particles (Dauer *et al.*, 1981; Taghorn, *et al.*, 1982). However, particle size (Nicol, 1930; LaBarbera, 1984; Shimenta, 1993, 1996; Quian and Chia, 1997), organic quality (Taghorn, 1982; Shimenta, 1996; Bock and Miller, 1997), flow characteristics (Dauer *et al.*, 1981; Shimenta and Jumars, 1991; Shimenta, 1993; Shimenta, 1996; Quian and Chia, 1997) and particle concentration (Kiørboe *et al.*, 1981; Bricelj and Malouf, 1984) have all been identified as factors influencing the feeding behaviour of suspension feeding organisms.

Diet, comprising particle size and composition, stocking density, flow rate and farm management have been identified by the abalone farmers as factors that may result in conditions suitable for this suspension feeding species. From its prolific infestation of farmed abalone, *T. heterouncinata* appears to have found a niche in which it benefits from a combination of these factors.

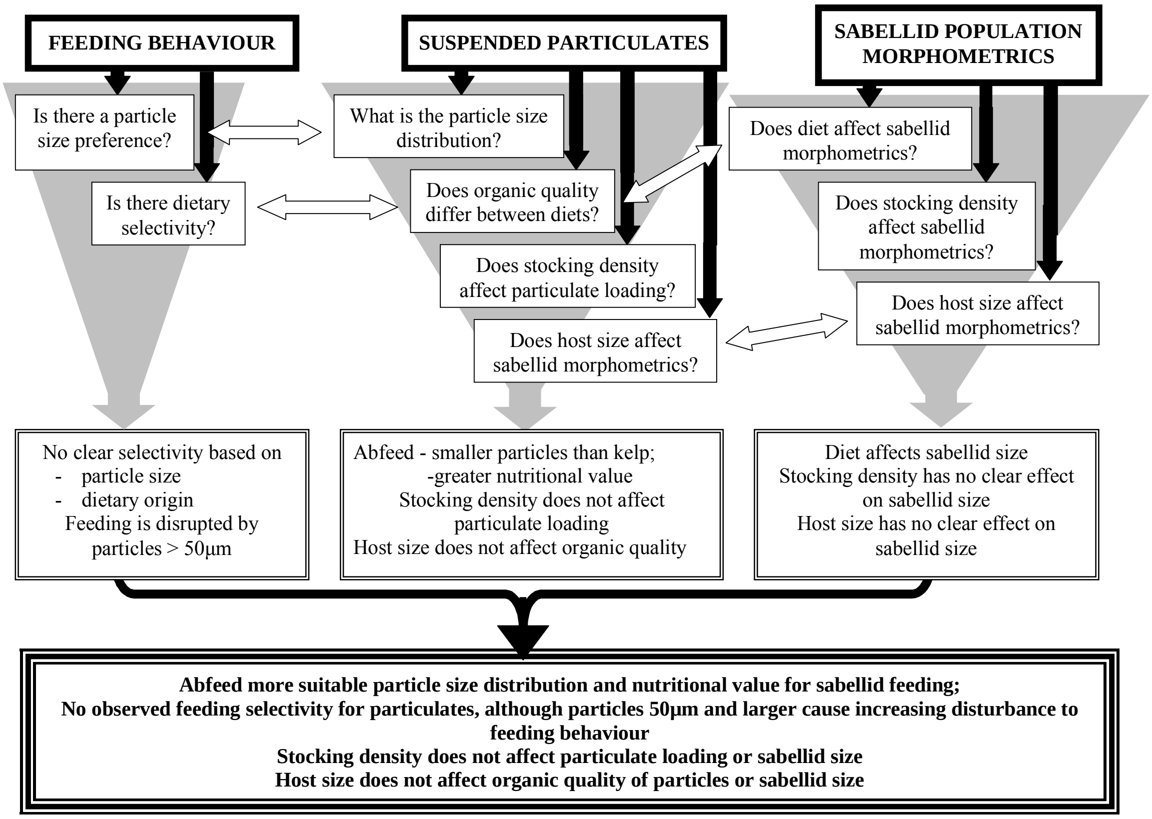


**Figure 6-1: Flow diagram of the project layout, highlighting main objectives and results for each component and overall conclusions.**

## *Particle size*

The particulate abundance and size distribution from kelp and Abfeed raceways differed significantly. *T. heterouncinata* is capable of ingesting particles up to 35 μm in size (Shields *et al.*, 1998; Ruck, 2000). This study revealed that particulates within Abfeed raceways were more evenly distributed across the preferred size range, whereas a large proportion of the kelp particulates occurred in the particle size class of >100 μm, which is out of the feeding size range of this sabellid. Abfeed raceways therefore have a higher percentage of particulates within the size range the sabellids are capable of ingesting and although kelp raceways had a greater total wet weight of particulates, the majority of these were too large for ingestion and therefore less available to the sabellid. Further evidence for the predominantly small particle size range in Abfeed raceways is indicated by the frequency of blocked filters while collecting particulates. This also gives an indication as to the number of particulates within each size class, which shows a definite decrease in abundance with increasing particle size for Abfeed particulates but not for kelp particulates. Although the filters also blocked in the small size classes for kelp particulates, the two largest filters (50 and 100 μm) blocked regularly, further indicating the wider size range and greater abundance of large kelp particulates.

The results from the analysis of particle size distribution helped in interpreting the findings from the behavioural observation and potentially the requirement of the sabellids for particle size and quality. Behavioural observations indicated that particulates above 50 μm were responsible for disrupting the sabellids' feeding pattern, causing them to withdraw into their burrows more often. During periods of exposure to large particulates (>50 μm), the sabellids spent more time within their burrows and generally had a higher frequency of withdrawal. This may be because the particulates were too large for ingestion and may have been potentially damaging to the feeding crown of the sabellid. Only particulates smaller than 50 μm caused no disruption to the feeding pattern of the sabellid for both diets. This suggests that particulates below 50 μm in diameter are within the feeding size range of the sabellid, while particles larger than this cause disturbance and are more often selectively rejected from the branchial crown.

The large quantity of particulates greater than 50 μm within the kelp raceways were likely to reduce the energetic benefits for sabellids in these raceways, as they resulted in increased frequency of withdrawal into the burrow by the sabellid. This reduced the duration the sabellids spent feeding, thus decreasing the quantity of feed particles they were able to ingest. This may ultimately decrease the net energetic gain of the sabellids' feeding effort. This is supported by the smaller sabellid sizes from the kelp raceways in comparison to the Abfeed raceways.

## *Particle composition*

Protein and energy content differed clearly between particulates from the two abalone diets. Particulates originating from Abfeed raceways had both higher protein and energy levels than particulates from kelp raceways. Sabellids feeding on Abfeed particulates may have derived greater nutritional value than those feeding on kelp particulates. It could therefore be hypothesised that sabellids from abalone fed Abfeed would grow faster and attain a larger size than those sabellids present on abalone fed a predominantly kelp diet, due to the difference in proximate composition of particulates available to them. Although no work was done on sabellid growth, this hypothesis can be supported by the results from the sabellid population morphometrics for each abalone diet (Chapter 5). The populations of sabellids sampled from both kelp and Abfeed raceways differed significantly in their morphometric values, with sabellids from Abfeed raceways being larger in all respects. This difference in size may have been due to the difference in proximate composition of the respective particulates available to the sabellids within each raceway, as all other environmental conditions were similar between treatments. Growth and reproductive output have been shown to respond to changes in quality and quantity of food (Meidel and Scheibling, 1999; Jones *et al.*, 2002; Weiss *et al.*, 2002; Vos *et al.*, 2002) and it appears that abalone diet in the South African abalone industry has an important impact on the size of the sabellid, *T. heterouncinata*.

There was no difference in feeding behaviour of *T. heterouncinata* when subjected to particulates originating from Abfeed and kelp raceways (Chapter 3). Sabellid feeding appeared random in both diets with no indication of selectivity. An absence in dietary preference may suggest that there is little or no selectivity by *T. heterouncinata* based on organic quality of the food particles. From a study on sabellids and serpulids, Dales (1957) concluded that only inert particles are retained and that free swimming algae escape through the feeding crown, implying that fan worms are dependent solely on suspended detritus for food. Dales, however, did not investigate the selectivity of the sabellids based on organic quality of the detritus particles. Most studies on tentaculate suspension feeders have addressed particle selection based on categorisation of organic versus mineral constituency (Muschenheim, 1987; Shimenta, 1996) and particle organic coating has been shown to be an important cue in suspension feeding behaviour, with nitrogenous compounds being a driving factor (Bock and Miller, 1997). This allows organisms to react to variability in food concentration and composition to increase acquisition of organically important compounds (Bock and Miller, 1997). Suspension feeding behaviour is partly controlled by chemical cues, with chemoreception playing an important role in food particle detection and selection (Bock and Miller, 1997; Qian and Chia, 1997; Vannier *et al.*, 1998). The absence of an obvious ability for particle selection by the sabellids may have been a result of sabellids only being subjected to one type of particle at a time. Under these conditions the sabellids may resort to feeding on what is available under the current conditions rather than rejecting particles of lower nutritional value. From this study it cannot be predicted if, in the presence of particulates of differing organic quality, selection will take place. Yet, the combined discussion of the results from the proximate analysis of the diet and the morphometrics suggest that diet itself must have an important role.

## *Abalone stocking density*

The quantity of particulates present in raceways was similar for all abalone stocking densities tested. In aquaculture, stocking density has been associated with a corresponding increase in particulate loading. It appears, however, that the stocking densities investigated in this study do not have a significant influence on the abundance of particulates within the water column. This is thought to be due to the range of stocking densities currently used on the abalone farms. Previous water quality problems, poor abalone growth rates and sabellid infestations have been linked to excessive abalone stocking densities in the past (AFASA, *pers. comm.*), and this has led to a reduction in farm stocking densities. It is likely that before this change a difference in particulate abundance may have been detectable between higher and lower stocking densities and that this reduction in stocking density has resulted in the desired effect of lowering the particulate abundance within the raceways to an acceptable level. In doing so, this may have reduced the degree to which the sabellid colonised and infested abalone.

This is supported by the finding that there was no apparent effect of stocking density on sabellid morphometrics. Although differences did exist between stocking densities for some parameters they do not appear to be a direct result of abalone stocking density. Criteria such as a water quality (pH, ammonia, nitrate and nitrite levels) and temperature appear to be most suitable variables for further studies, as they may provide an insight into sabellid condition. Future studies can use these results as reference points to evaluate the effect of different environmental conditions on sabellid development. As this is the first study to provide baseline data on sabellid morphometrics, comparisons with results from other such studies are not possible, yet there was sufficient variability in the data to assume a certain sensitivity to changes in conditions. This could be explored further using the current data set as a guideline.

## *Sabellid infestation and occupation level*

Abalone stocking density did not affect the infestation level (number of burrows/cm) or occupation level (percentage of tubes occupied) of the sabellids. Due to the sessile nature of the sabellids and the benthic crawling larval stage, transmission from one host to another can only occur via direct contact between abalone or possibly via suspension and transmission of the larvae in the water column. Thus, stocking density could have had a noticeable effect on infestation level, as at high densities physical contact between abalone is more frequent. The absence of a stocking density effect can be attributed to the narrow and low stocking densities currently in practice in the abalone industry. It needs to be tested if stocking densities above 40% of the available surface area will lead to differences in infestation level.

The occupation level of the burrows was approximately 20-25% for all stocking densities. Similar levels of occupation have been recorded in several farms from the South African abalone industry (Dr A. Mouton, Onderstepoort Veterinary Institute; C.A. Simon, Department of Ichthyology and Fisheries Science, Rhodes University). It therefore appears that only approximately a quarter of the larvae that settle on the growing edge of abalone shells survive, and metamorphosise into adults. Currently, there is no explanation for this mortality. To evaluate if competition for either food or space regulated the occupation level, the behaviour study provided some suggestions. From these observations space did not appear to moderate behaviour. On heavily infested abalone shells, where sabellids were situated closely to one another, they were observed to be feeding with their branchial crowns continuously touching without disturbing each other. Disturbances from neighbouring sabellids did not have a noticeable effect on feeding duration. It could be investigated if the mortalities occur at the larval phase before metamorphosis and development of the branchial crown. On hatching the larvae have an energy reserve to sustain themselves until the branchial crown is fully developed when they are capable of feeding. This energy reserve may be dependent on the size and quality of the eggs. Small eggs, or eggs of poor quality may only have a small energy reserve capable of sustaining the larva for a short period of time. If the larva spends time searching for the growing edge, it may utilise essential energy required for metamorphosis and development of the branchial crown before the abalone has completed shell deposition over the larva forming a burrow. If this occurs, the larva may not survive to become an adult and die during the formation of its burrow, or die after metamorphosis during development of the branchial crown. These are possible factors which could account for the low occupation rate of the burrows formed. Currently there is no evidence to support this hypothesis but further research into factors affecting egg and brood size is presently being conducted.

### *Abalone size*

The effect of abalone size on sabellid infestation and population morphometrics was investigated in order to determine whether particulate organic quality was related to abalone size, thus influencing sabellid growth. The protein and energy levels of the suspended particles did not vary across the abalone size classes for each diet. This suggests that abalone of the sizes tested did not appear to have different nutritional requirements, assimilating their feed in a similar manner, releasing particulates of similar quality into the water. This could have supplied the sabellids with a uniform food quality across abalone sizes within each diet and it was therefore hypothesised that sabellid populations would have similar morphometrics across abalone size. In Chapter 5, however, abalone size was shown to have an effect on sabellid morphometrics, with width and surface area decreasing with increasing abalone size for Abfeed while increasing for kelp. The decrease in width and surface area, along with a corresponding increase in length for Abfeed sabellids from increasing abalone size, indicates a possible drop in sabellid condition. Since there is no difference in the particulate composition available to the sabellids from each abalone size class, the drop in condition may not have been due to quality of the feed particles. It can, however, be due to the availability of food particles.

While this study could not answer all questions, a few hypotheses can be proposed for further research. For example, at smaller abalone sizes, more abalone are placed into grow-out baskets than large abalone in order to obtain the same final stocking density. In doing so each basket may have a similar biomass but the smaller abalone consume more food per kilogram of biomass thus releasing more small feed particulates and faecal matter into the water. Unfortunately the particulate loading within the water columns for each abalone size class was not quantified in this study.

## DISCUSSION OF THE METHODS USED IN THE STUDY

### *Behaviour observations*

Previous feeding studies have monitored particle concentration in order to quantify the selectivity and feeding rates of polychaetes (Dales, 1957; Shumway *et al.*, 1988; Riisgård and Ivarson, 1990; Nielsen *et al.*, 1995). In some behavioural studies observations were done at regular time intervals over a given feeding period (Dauer, 1983; Bock and Miller, 1996, 1997), while other authors have made continuous observations either by observing the worms directly or by using a video camera to record the behaviour for later analysis (Turner and Miller, 1991; Quian and Chia, 1997). The majority of these studies, however, have involved species which are capable of switching between suspension and deposit feeding and have been performed to determine factors that affect feeding behaviour. The system and methods developed to observe *T. heterouncinata* feeding behaviour were designed to document continuous feeding behaviour and involved using a dissecting microscope and video recorder similar to the equipment used by Quian and Chia (1997). The method was successful for recording feeding durations and frequency of withdrawals as, if set up correctly, the branchial crown could be clearly observed. Unfortunately, methods to measure particle size from the monitor using a pre-recorded cassette proved to be inaccurate, giving an unreliable interpretation of the particle sizes ingested and rejected. Movement of particles along the branchial crown could generally be clearly observed. Problems did, however, arise on focusing on the three-dimensional feeding crown due to the magnification and when the branchial crown was repositioned during the recording period. Focus adjustment resulted in some disturbance to the observational chamber and, at times, caused the sabellid to withdraw. Improvements to this technique could improve the quality and reliability of observational results.

Observational experiments using artificially coated beads as a food source, as done by Bock and Miller (1997), rather than utilising naturally occurring particulates, could improve our understanding of particle selectivity in this species. The aim of this study, however, was to determine the selectivity of the sabellid in response to naturally occurring particulates within farm raceways and therefore particulates were used originating from raceways of the two major abalone diets found in the farming industry. The use of inert particulates could be used to quantify particle size and particle type selectivity more accurately as they can be collected from the digestive tract of the sabellids at the end of a feeding cycle. This type of study would provide additional results about feeding selectivity compared to behavioural selectivity observations only.

In conclusion, this study was successful in developing a technique for quantifying and observing sabellid feeding behaviour when subjected to particulate matter. Furthermore, the proximate composition, abundance and size distribution ranges of particulates from kelp and Abfeed were investigated and significant differences were shown to exist between the waste generated by these abalone feeds. A useful and suitable technique was developed for morphometric analysis of sabellid populations. This technique was used to investigate the effect of abalone stocking density, abalone diet and abalone size on the size structure of sabellid populations, and significant differences between populations were identified. Sabellid infestation levels were also investigated from abalone from different stocking densities and abalone diets. All variables investigated in this study were compiled in a diagram to show relationships between environmental factors and sabellid growth, development and reproduction. Figure 6-2 provides a set of hypotheses that link these factors to the various stages of the life cycle.

Abalone diet, resulting in differences in particulate proximate composition and size range, was identified as a key factor influencing sabellid condition and level of infestation success. Past research methods for controlling sabellid infestations have focused on eradication of the sabellid by means of liposome and chemical treatments. This study has helped to identify a possible area where sabellid infestations could potentially be curbed and to some degree controlled. Development of an effective method for controlling the particulate loading within abalone raceways could potentially restrict the food available to the sabellid and therefore reduce its potential to reproduce and re-infest cultured abalone. Possible methods for reducing the particulate loading could involve improved feeding techniques in order to minimise feed wastage and input into the raceways, and cleaning routines to remove unwanted debris more regularly. Polyculture, involving the combined culture of abalone and another filter feeding organism, such as oysters or mussels, could also aid in alleviating the problem of excess particulates while providing an alternative crop to complement the existing one.

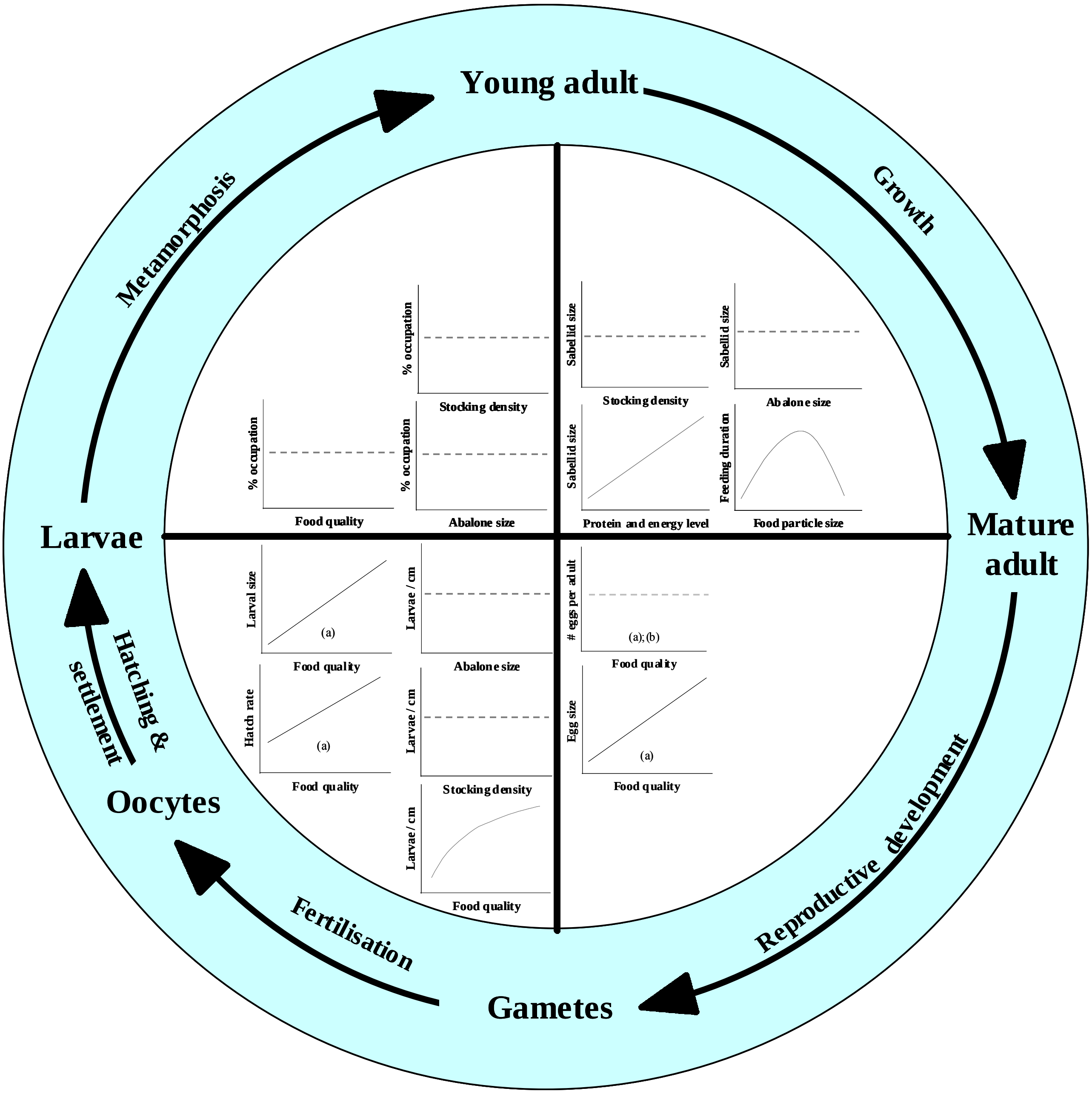


**Figure 6-2: Representation of factors that influence the various stages of the sabellid life cycle. Solid lines indicate clear relationships; dashed lines weak or no clear effect; and letters indicate results from other studies (a) – M. Gray, *pers. comm.*; (b) - M. Winter, *pers. comm.*. GE = growing edge.**

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