MORPHOMETRICS AND REPRODUCTION OF *Terebrasabella heterouncinata* (POLYCHAETA: SABELLIDAE), INFESTING ABALONE (*Haliotis midae*) FROM DIFFERENT CULTURE ENVIRONMENTS.

Submitted in Fulfilment of the
Requirements for the Degree of
MASTER OF SCIENCE
Rhodes University

by
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February 2003

DEDICATION

This thesis is dedicated to my parents for their genuine support and encouragement throughout my career.

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ACKNOWLEDGEMENTS

My sincere gratitude to Dr. Horst Kaiser for his patience, persistence, support and encouragement during the many ups and downs of this study, especially during the write-up stage. He recognised the potential of the data where I did not and I am truly grateful for that. Also to Professor Pete Britz for his assistance in the organisational and administrative aspects of the project.

I would like to thank the member of the Rhodes "worm clan", Carol Simon and Russ Chalmers, for the many hours spent discussing and debating. The contributions from which proved invaluable.

This project would not have been possible without the co-operation of the abalone farmers and I would like to thank the following farms for providing abalone for the study: Aquafarm Development, Sea Plant Products, Hermanus Abalone and HIK. Special thanks must go to Andre Du Plessis and the staff of Aquafarm Development for the use of their facilities and for their support during the experimental phase of the project. Particularly I would like to thank Gavin Johnston, Peter Pesch, Pieter Truter, Otto, Dolf, Dennis and Bryan for assistance and guidance while working on the farms. Special thanks must go to the Hermanus crowd for the 'social support' during my stay in Hermanus. The financial support of the Abalone Farmers Association of South Africa (AFASA) is gratefully appreciated and acknowledged.

Thanks to the staff and students of DIFS who provided assistance and moral support throughout the project.

Thanks to my parents for their continued motivation and support throughout my varsity career. Finally, to Bernice, thanks for the endless encouragement and patience during the years spent apart.

ABSTRACT

In the late 1980's abalone culturalists noticed reduced growth rate and shell deformities in some abalone stocks. These problems were the result of infestations by a shell boring polychaete, *Terebrasabella heterouncinata.* Under intensive abalone culture conditions the level of infestation can reach epidemic proportions and there are often severe consequences for the host abalone. Heavy sabellid infestation levels have placed the economic viability of several South African farms under threat. This study formed part of an ongoing project that is aimed at investigating the basic biology of *Terebrasabella heterouncinata.* The majority of abalone farmers in South Africa feed their abalone either naturally occurring kelp (*Ecklonia maxima)* or the formulated abalone feed, Abfeed. Farmers have suggested that the use of Abfeed is associated with higher sabellid infestation levels and changing the abalone diet from Abfeed to kelp helps reduce sabellid infestation. Speculation has arisen indicating that older, slower growing abalone are more susceptible to sabellid infestation. The effect of host abalone diet history and their growth on sabellid settlement success, morphometrics and reproduction was quantified. To better understand the plasticity of the expression of life history traits the variability of morphometric and reproductive characteristics was compared between different farm environments.

A change in abalone diet from kelp to Abfeed resulted in smaller adult sabellids ($p \leq 0.001$), larvae ($p \leq 0.001$) and eggs ($p \leq 0.0001$), compared to sabellids from abalone that experienced a diet change from Abfeed to kelp. Abalone diet history and growth rate did not influence the occupation level of tubes ($p > 0.05$) or the sabellid intensity (i.e. number of tubes per centimeter of the shell edge) ($p > 0.05$). Sabellids from slower growing abalone were larger in various body measurements and other characteristics;

(length ($p<0.0001$); base width ($p<0.0001$); stage 2 larvae length ($p<0.001$); egg volume ($p<0.001$); number of stage 1 and stage 2 larvae per adult ($p\leq0.0004$); and number of eggs per brood ($p\leq0.0001$). The combined effect of slow abalone growth and the feeding of Abfeed resulted in increased number and size of the sabellids, indicating a confounding effect of these two conditions.

This study suggests that sabellids are essentially *K*- selected, exhibiting variation in reproductive and morphometric characteristics under different conditions. The number of larvae per adult (CV= 113- 79%), number of eggs per brood (CV= 86- 58%), sabellid intensity (CV= 79- 39%) and number of larvae per egg (CV= 126- 84%) were the life-history- related variables that exhibited the greatest variation for all studies. The smallest variation in sabellid characteristics included the larval length (CV= 11- 17%), base width (CV= 12- 31%) and occupation level (CV= 19- 27%). Thus, in all studies the numbers of individuals of the life-cycle stages were more variable than the sizes. The greatest variation occurred between the farms with the least variation occurring between abalone of different growth rate. Larval settlement was greatest on the thinnest and fastest growing region of the shell edge. Larvae settled most successfully at high tides. This study suggests that sabellid larval settlement is principally determined by abalone shell region, then by a change in diet, and least by abalone growth.

CHAPTER 1

General Introduction

The South African Abalone Industry

The South African abalone fishery was initiated in 1949 and is reliant on one species, *Haliotis midae* (Tarr, 1992). At the start of the fishery there were no controls on total effort by fishers, but by 1968 a quota system had been imposed due to increasing concern over declining catches (Tarr, 1992). By 1971, the quota system limited the total commercial catches to just over 600 tonnes, a level that appeared to be sustainable (Cook, 1998). Recently however, increased exploitation by recreational divers and illegal poachers placed increasing demand on the resource.

In response to the decline in natural *H. midae* stocks in South African waters, the division of Sea Fisheries launched an abalone research program (Newman, 1966). Intensive research and development effort was undertaken by the University of Cape Town, the Council for Scientific and Industrial Research and Rhodes University to develop a commercial abalone farming industry (Sales, 2001). Since then, over R80 million has been invested into commercial abalone farming in South Africa (Cook, 1998), resulting in the establishment of 12 commercial farms situated from Port Nolloth on the Atlantic coast to East London on the Indian Ocean (Sales, 2001). The combined output of the farms has been estimated at between 500 and 800 tonnes per annum (Sales & Britz, 2001). All commercially produced abalone are exported. Most producers aim to sell their abalone between 80 to 100 mm shell length, and as this is smaller than the minimum legal

harvestable size for natural abalone, the prohibition of the sale of farmed abalone within South Africa is aimed at preventing the harvesting of undersized abalone (Cook, 1998).

Other than an abalone ranching operation at Port Nolloth, all South African abalone farms use pump-ashore, land-based on-growing systems (Cook, 1998). In these systems the abalone are kept in man-made holding tanks, over which a high degree of control can be ensured. This control over the production process ensures a regular supply of abalone of relatively constant size and quality (Britz, 1995). Abalone are well suited to aquacultural production due to several characteristics; they have a high stress threshold and adapt well to the captive environment; there are no known abalone viral infections and they are resistant to bacterial infection; they are not territorial or aggressive and can be reared at high density; the captive breeding cycle has been closed and they reproduce year round; and they readily eat artificial pelleted feeds (Britz, 1995). The only major characteristic of the abalone unsuited to aquaculture is the slow growth rate. It takes 3 to 4 years to reach a marketable size of 100 g live weight in shell (Britz, 1995).

As growth of the abalone is slow, it is essential that the growth rate is maximised to ensure a reduction in the time it takes to get the product to a marketable size. Stocking density, temperature, diet and abalone size are considered to be the most important factors influencing the growth rate of abalone under culture conditions (Oakes & Fields, 1996; Culver *et al.,* 1997; Clayden, 2000). Space available for grow-out is one of the most important limiting factors of abalone production (Chalmers, 2002). The Abalone Farmers Association of South Africa (AFASA) recommends a maximum stocking density of 35% of the solid surface area available for abalone attachment (AFASA, *pers. comm.*). Often, during the end of the grow-out period or during periods of decreased market demands,

space becomes limited and the stocking density is increased above that recommended by AFASA. Abalone kept at high stocking densities show reduced growth (Pesch, *pers. comm.*).

Feed constitutes a major proportion of the costs of abalone production (Sales, 2001). In the past, because the majority of the abalone farms are situated on the South-western part of the country close to adequate supplies of kelp, the majority of farms fed their abalone kelp. However, the harvesting of kelp is dependent on sea conditions, complicating farm management and increasing the financial risk of such ventures (Britz, 1995). An artificial pelleted feed, Abfeed, has since been formulated by Sea Plant Products. Although more costly, the advantage of pelleted feed is in its reliability of supply and convenience from a management perspective (Britz, 1995). Farmers feed their abalone kelp, Abfeed or a combination of the two. Young, newly metamorphosised abalone are reared on diatoms cultured on plates or in bags and then weaned on to seaweed or formulated feed at approximately 4 to 6 mm shell length (Sales, 2001). Several farms feed the smaller abalone the formulated feed and change to kelp once the abalone reach 40 to 60 mm shell length. They report a small reduction in growth rate if the abalone continue to be fed Abfeed beyond this size (Pesch, *pers. comm.*). Abfeed and kelp differ in both the nutrient content and size and abundance of particulates produced during breakdown (Chalmers, 2002). Abalone farmers speculated that there may be a link between the use of the artificially formulated diet and high levels of infestation by the problematic sabellid worm, *Terebrasabella heterouncinata*, a recently described pest in abalone culture. *T. heterouncinata* has been identified as being responsible for reduced growth and deformation shells of some stocks of cultured abalone in California and South Africa. The speculation about the link between sabellid infestation and artificial feeds appears

plausible as formulated diets can potentially maximize the nutritional value for the abalone while also increasing the nutrient loading in the water column as leaching of nutrients from pelletised diets is known to occur (Chalmers, 2002). Chapter 4 of this study investigates the influence of a change in host abalone diet on the reproductive and morphometric characteristics of the sabellid worm.

High stocking densities, high particulate loads and poor tank hygiene have been suspected of producing higher infestation of the abalone by the sabellid. The sabellid has caused economic losses to the abalone industry both in South Africa and California (Sales, 2001). The infestation by the sabellid results in reduced abalone growth because of the interference caused by larval settlement at the shell-mantle interface (Ruck and Cook, 1998). The larvae of the sabellid settle on the growing edge of the abalone shell and the abalone responds by depositing a layer of nacre over the larvae, forming the tube in which the larva metamorphose into the adult worm. The number of larvae settling on an individual shell may become so high that the continual deposition of nacre by the abalone can reduce the growth rate as the abalone is investing more effort into covering the larvae and less into shell extension. Normal linear shell extension is reduced and the abalone takes on a characteristic domed-shaped appearance at high infestation levels. Thus, sabellid infestation results in reduced abalone growth rate, unsightly shells which have a reduced market value and shells which are more prone to breakage during handling.

The family Sabellidae

Sabellids are among the most easily recognisable of the polychaete groups due to their colourful branchial crown and by the sediment tubes that they inhabit (Rouse and Pleijel, 2001). They are commonly referred to as fan worms and all are suspension or deposit-

feeders. The crown is used in both feeding and respiration (Rouse, 2000). Particle selection during feeding is relatively advanced and particles that are too large for ingestion or tube building are rejected (Rouse and Pleijel, 2001). The tube they inhabit is constructed using the crown appendages, which actively sort particles and combine these with a complex mixture of organic compounds, usually referred to as mucus (Rouse, 2000). The tube serves to protect the sabellid from predators and provides a lair from which passing prey can be captured (Chalmers, 2002). Sabellids are distributed worldwide and are associated with hard surfaces or soft sediments at all latitudes from intertidal areas to different shelf depths. Most species live on rocky substrates but some species are known to live on the shells of molluscs or on the fronds of algae (Day, 1967).

An introduction to Terebrasabella heterouncinata

Whilst conducting growth trials on abalone on an Abalone farm in the Western Cape, South Africa, Ruck and Cook (1998) noticed that one group of animals exhibited a low growth rate and had a higher mortality than other groups. On examination of these animals, it was observed that the shells were brittle and abnormally shaped. It was initially suspected that the abnormal shell growth was associated with a polychaete worm, *Polydora* spp., a known shell borer in abalone (Ruck, 2000). Previously, in 1990, an abalone farm in Cayucos, California, had noticed that one of the culture tanks had what appeared to be a stock of slow growing abalone (Oakes & Fields, 1996). Upon closer inspection of the abalone, it was noticed that the entire population had deformed shells. The leading edge of the shell had begun to grow downwards instead of outwards, giving the shells a domed-shaped appearance. Initially, it was thought that the problem animal was a spionid polychaete but it was soon concluded that this was not the animal responsible for the deformation of the abalone shells (Culver *et al.*, 1997). Fitzhugh at the

Natural History Museum of Los Angeles identified the worm as a previously undescribed member of the family Sabellidae, which was later named *Terebrasabella heterouncinata* (Fitzhugh & Rouse, 1999). Studies were initiated to determine the origin of the sabellid worm and how it came to infest the abalone facilities (Culver *et al.*, 1997). It was concluded that the sabellid must have been introduced from elsewhere as it had not been found in wild abalone stocks in California (Lafferty & Kuris, 1996; Kuris & Culver, 1999). The worm was subsequently found to be endemic to South Africa, where it had not been previously recognised, and had been accidentally imported to California with abalone intended for commercial research (Culver *et al.*, 1997; Ruck & Cook, 1998). *T. heterouncinata* has caused considerable damage to the Californian abalone industry and has been released into the natural environment from farm effluents (Culver & Kuris, 1999). Studies, both in California and South Africa, have been conducted to determine the effects of the sabellid to both the abalone farms and the natural environment (Culver *et al.*, 1997; Ruck, 2000; Chalmers, 2002; Simon *et al.*, 2002). Subsequent to its discovery as an endemic species to South Africa it has been found from Port Nolloth on the West coast to Port Elizabeth on the East Coast (Ruck & Cook, 1998). Although infestations by the worm do not affect the quality of the abalone meat, they can grossly deform the shell, reducing its value for overseas markets, and increase the time it takes for an individual abalone to get to market size. Some farmers have reported high mortality and low meat yield in infested abalone (Culver *et al.*, 1997).

The sabellid life history

T. heterouncinata is a simultaneous hermaphrodite, producing both eggs and sperm (Fitzhugh, 1996; Oakes & Fields, 1996; Ruck 2000). It has been shown to self-fertilize and produce viable offspring (Ruck, 2000). The eggs are laid, fertilized and brooded

within the posterior end of the tube in the abalone shell. A burrow commonly contains 1to 3 eggs with one or two larvae at various stages of development (Ruck, 2000). The eggs are about 240 μm long and about 130 μm wide and are usually located at the proximal end of the burrow (Fitzhugh & Rouse, 1999). The fertilized eggs remain in the adult's burrow where they develop into larvae, which do not feed but instead continue to obtain nutrients from their egg yolk (Fitzhugh, 1996). Most brooding sabellids have young that leave the burrow as pre-adults with the branchial crown already formed so they can feed and commence building their own tube (Fitzhugh, 1996). *T. heterouncinata* is unique in that the young leaves the burrow whilst it is still a larva, without a developed branchial crown, and hence has no ability to feed until it becomes established. At this stage the larvae still has yolk granules present in the body and there is no evidence of formation of a gut (Fitzhugh and Rouse, 1999). The larvae can only leave the burrow once segmentation is complete and the "bristles" or setae are visible (Culver *et al.*, 1997). At this stage the larvae, complete with two eyespots and sensory tentacles at the anterior end, are able to crawl out of the burrow using the setae. This motile, crawling stage is the infesting stage and the larva exits the burrow and goes in search of a new host or area to settle on the same host. The released larvae normally crawl or "glide", using a band of cilia on the ventral surface, over the host's shell and settle under the lip of the shell, in contact with the mantle tissue (Fitzhugh & Rouse, 1999). Consistent with larval behavior in other species, the sabellid larvae seems to have the ability to locate suitable areas on the shell to settle, but this is an area in which more research needs to be conducted (Ruck, 2000). A study investigating factors influencing larval settlement may lead to the development of a management protocol aimed at reducing the survival of larvae settling on the abalone shell. The influence of host abalone diet on the success of larval settlement was addressed in Chapter 6. Furthermore, if the sabellid larvae settle preferentially on

certain areas of the shell edge they may be using settlement cues. The distribution of larvae settled on the abalone shell edge was investigated in Chapter 6. Within a few hours of leaving the burrow, the larvae usually settle on the underside of the abalone shell along the growing edge margin, although they may also settle on the outer lip or around the respiratory pores (Culver *et al.*, 1997). Within the first day of exiting the adult burrow, the larvae secrete a thin mucous sheath with the open anterior end at the shell margin. The abalone respond by depositing a thin transparent calcified layer over the ensheathed worm, forming a calcareous tube around the worm (Kuris & Culver, 1999). After settlement the larva metamorphoses into a juvenile after about one week at 18° C (Culver *et al.*, 1997). The formation of a functional feeding crown, together with the loss of the eyespots and sensory tentacles, about 7 days after settlement indicates the transformation from larva to juvenile (Fitzhugh & Rouse, 1999). Maturation from juvenile to adult occurs within the tube in about one month and the age of reproductive maturity occurs within about 3 months at 18° C (Simon *et al.*, 2002).

The adult worms are 1 to 4 mm in length and are more elongate with more setigers than the juveniles (11 setigers at maturity). The feeding crown of the adult consists of two branchial lobes, with two palps in the center, which are most likely used in food selection (Ruck, 2000). The feeding tentacles are covered with cilia, which are presumably used to direct food towards the mouth. Feeding is selective as behaviour studies showed that some particles taken in were rejected (Ruck, 2000). Other particles were deposited around the entrance to the burrow where they were combined with mucous to form an extension to the tube (Ruck, 2000). Five pairs of setae are present in the larval stage. These become the largest setae in the adult and are most probably used for retraction into the burrow (Ruck, 2000). Setae on the ventral surface are shorter than those on the dorsal side and

have tooth-like projections at the end. These setae, termed uncuni, are possibly used for preventing the worm from being dislodged from its burrow. There are different types of uncuni on the same body region, which is unique to this species hence, the name *T. heterouncinata* (Fitzhugh & Rouse, 1999). The adult worm possesses a faecal groove, lined by cilia, which runs the down the dorsal length of the body and is used to transport faeces from the anus to the entrance of the burrow (Ruck, 2000).

Interaction between the sabellid and the host

An abalone shell consists of two calcareous layers, the "prismatic" layer on the outside of the shell which is responsible for the shell's normal linear growth, and the pearly "nacreous" layer inside. Nacre is also deposited when shell damage is being repaired or when a foreign object cannot be dislodged from beneath the mantle (Culver *et al.*, 1997). At first it was thought that the sabellid bored into the abalone shell, as is the case with many other organisms that encrust on or bore into mollusc shells (Thomas & Day, 1995; Culver *et al.*, 1997; Kuris & Culver, 1999). However, it has been shown that in response to the presence of the larvae the deposition of prismatic shell is temporarily interrupted. The abalone deposits a thin layer of nacre over the larval sheath and the secretion of prismatic shell does not resume until there has been sufficient deposition of nacre to smooth over the irregularity associated with the worm tube (Kuris & Culver, 1999). Thus, while nacre is normally deposited over the prismatic front at the growing edge, the presence of the worm causes the premature deposition of nacre which disrupts the normal growth of the shell (Ruck, 2000). Therefore, the greater the number of larvae settling at any particular time, the more visible the growth disruption on the outer layer of the shell (Kuris, 1997). If larvae continue to settle over a longer period of time, the shell takes on a characteristic domed-shaped appearance (Culver *et al.*, 1997; Kuris & Culver, 1999). As

the abalone deposits more shell material, the worm keeps the entrance to its burrow open which results in the appearance of the sabellid having burrowed into the shell when in fact it has been encapsulated by the abalone (Oakes & Fields, 1996).

The intensity of infestation determines the extent of damage to the abalone shell. Light infestations (less than 20 newly settled worms on the shell edge at any time) result in little disruption to normal shell growth, thus the growth of the abalone appears unaffected (Culver *et al.*, 1997). Moderate to heavy infestation results in more nacre being deposited hence linear deposition is disrupted and the shell develops a thick outer lip. The formation of respiratory pores on the growing edge is impaired on heavily infested abalone. Existing respiratory pores may become settling sites for worms and may become blocked due to the presence of the worms or excessive nacre deposition (Culver *et al.*, 1997). Although the blockage of the respiratory pores does not cause instant mortality for the abalone, it has been shown that these abalone exhibit greater mortality (Oakes & Fields, 1996). If the rate of sabellid settlement is reduced or ceases, normal shell growth is resumed (Kuris & Culver, 1999). It is unclear whether slow growth of the abalone makes it more susceptible to sabellid infestations or whether the increased infestation reduces the growth rate of the abalone. Chapter 5 investigates the potential influence of abalone growth on the reproductive and morphometric characteristics of the sabellid.

Dispersal

The study of the means of dispersal of sabellid larvae is important to abalone farmers as it determines the spread of infestations within and between farms and to the natural environment. As was stated earlier, the adults are sessile and rely on motile larvae for the distribution of their offspring (Finley *et al.*, 2000). Ruck and Cook (1998) showed that

larvae placed in petri dishes were active, moving in a crawling motion at about 1.5 body lengths per second. The easiest place for larvae to settle would be on the same host as the parent but there may be increased competition between recruits (Ruck, 2000). There is, however, no experimental data to support this suggestion and an investigation into the possibility of intraspecific competition between sabellids on the shell edge may provide some insight into potential factors influencing post-settlement survival of the larvae. Experiments have shown that larvae are able to pass through the water column to infest new hosts (Culver *et al.*, 1997; Ruck & Cook, 1998; Ruck 2000). Aeration in tanks or increased water flow, for example during flushing of tanks, is most probably responsible for dislodging the larvae and allowing them to enter the water column (Culver *et al.*, 1997). Reducing the density of abalone in tanks and keeping abalone in baskets suspended off the bottom of the tanks are effective methods for reducing the spread of the sabellids within tanks (Culver *et al.*, 1997; Ruck, 2000)

Host specificity

Sabellids are able to infest abalone as small as 2 mm, but abalone 3 mm or smaller are much less susceptible to infestation than larger individuals (Culver *et al.*, 1997). The sabellid worm is native to South Africa and many other marine gastropods, snails and limpets are susceptible to infestation (Culver *et al.*, 1997; Kuris, 1997; Ruck, 2000). Considering the vast array of species found off South Africa's coast, only a few are commonly infested in the wild. If species are motile and gregarious, the spread of *T. heterouncinata* amongst those species is benefited (Ruck, 2000). Some species, such as the bivalves *Aulacomya ater* and *Mytilus galloprovincialis* and the limpet *Crepidula porcellana*, appear to be resistant to sabellid infestation (Ruck, 2000). This aspect needs to be studied in more detail to investigate whether these species possess a degree of

resistance to infestation. The range of species infested by *T. heterouncinata* is important in that abalone are not the only source for the transmission of the sabellid. Thus, any infested animals could spread the sabellid to other areas. The host does not even have to be alive to be a vehicle for the sabellid worm to survive, further increasing the risk of spreading the parasite to new areas through the distribution of shells (Culver *et al.*, 1997; Simon *et al.*, 2002).

Methods to control sabellid infestation

Although there are numerous methods of controlling on-farm sabellid infestation rates, there has been a need to completely eradicate this pest. Several attempts to eradicate the sabellid are discussed below.

Thermal tolerance

Leighton (1998) carried out a study to determine the effect of elevated seawater temperatures on both sabellids and abalone. The study aimed to ascertain if there was a temperature that was sub-lethal to the abalone but was lethal to all life stages of the sabellid (adults, juveniles, larvae and eggs). Other studies had shown green abalone, *Haliotis fulgens,* to be tolerant to temperatures of approximately 28°C with an LD_{50} (48 hours) for juveniles of 31.5°C (Leighton, 1998). Observations on the thermal tolerance of adult sabellids suggested temperatures above 29°C to be lethal if applied over a 1 to 2 day period (Leighton, 1998). Pink abalone, *H. corrugata*, were also subjected to the heat treatment. This abalone is also tolerant to temperatures in the range of 28 to 30°C (Leighton, 1998). It was concluded that temperatures of 28°C for 24 hours are sufficient to kill all stages of the worm (Leighton, 1998). Although this temperature range is below the upper thermal physiological limit for *H. fulgens* and *H. corrugata*, it is intolerable to

H. midae. Temperatures between 11 to 19°C are ideal for *H. midae* and temperatures above or below this range stresses the abalone and growth rate decreases (Ruck, 2000). Therefore, temperature as a potential method for the eradication of the sabellid worm in the South African abalone species is not applicable.

Liposomes as a drug vector

A major difference between abalone and the sabellid is the mode of feeding. Abalone are grazers and the sabellid is a filter feeder. To take advantage of this fact could provide a method for destroying the worm without harming the abalone. There have been several attempts to microencapsulate toxins which would be taken in by the sabellid (Shields *et al.*, 1998; Ruck & Sales, 1999; Ruck, 2000). Ruck (2000) used three methods of delivery for toxins. These were liposomes, oil in gelatin and oil emulsions. Both the oil in gelatin and oil emulsion treatments proved to be successful in carrying oil soluble drugs, but there was an apparent inability of the worms to digest the products (Ruck, 2000). The failure of the sabellid to digest these products is due to the fact that not enough information is available on the digestive enzymes present in the gut and since the worm is so small, it is difficult to identify enzymes using traditional methods. Liposomes were shown to have the most potential as a delivery mechanism, although these too did not prove to be successful in killing any of the sabellids (Ruck & Sales, 1999). Despite the liposomes being ingested, they were shown only to be partly digested in a few of the animals (Ruck, 2000). Shields *et al.* (1998) showed that in trials with encapsulated copper sulphate, mortality was not induced in the sabellids. It was suspected that there was significant leakage of the toxin from the microcapsule. Further refinement of methods discussed above is required to obtain a delivery technique that is effective in delivering a lethal dose of toxin to the sabellid worm. A potential method, which is so far untested, is

the use of synthetic polymer gels or hydrogels (Ruck, 2000). These polymers can be designed in such a way that they can shrink or expand by several orders of magnitude depending on environmental factors such as temperature, pH or salinity (Zhang *et al.*, 1992). These polymers may be used to rupture or block the gut of the sabellid after ingestion by manipulating the internal and external environment of the sabellid. The advantage of this technique is that no toxins are used and the worms would not be able to develop a resistance to the treatment as the mode of action is mechanical (Ruck, 2000).

Biological control

The problem with using potential sabellid predators, such as isopods, is that sabellids show exceptional powers of regeneration (Ruck, 2000). A study examining the regenerative powers of sabellid fanworms showed that after an anterior cut, the replacement of the entire head with branchial crown, mouth and neural ganglia was possible (Hill *et al.*, 1994). A potential use of these predators is that they may bite of the branchial crown of the sabellid, thereby reducing the time it has for feeding and hence its productivity (Ruck, 2000). Kuris and Culver (1999) collected a number of potential predators of the sabellid, including six fish species (*Hypsoblennis gilberti, Clinocottus analis, Girella nigricans, Citharichthys stigmataeus, Cymatogaster aggregata, Micrometrus minimus*); one crab (*Herbstia parvifrons*); two hermit crabs (*Pagarus samuelis, Pagarus hirsutiusculus*); three shrimps (*Lysmata californica, Heptacarpus paludicola, Pandalus gurneyi*); an isopod (*Cirolana harfordi*); two flatworms (*Notoplana articola, Stylochus tripartitus*) and a starfish (*Patiria miniata*). The sabellids were exposed to these predators, but none caused an increase in mortality (Kuris & Culver, 1999).

Chemical agents

The fact that the sabellid can retreat into its burrow when threatened means that it creates a microenvironment which gives it some protection against changes in the environment. This means that the worm has proved to be resilient to changes in the environment, such as salinity changes, dehydration, anoxia or the addition of vermicides (Ruck, 2000). Trevelyan *et al.* (1994) conducted several trials with different chemical agents, but found nothing that killed the worm without harming the abalone. This method for the eradication of the sabellid has proved unsuccessful, as the abalone is often found to be more sensitive than the sabellid.

Physical methods

Trevelyan *et al.* (1994) had some success in destroying sabellid infestations when they developed a method of using a low-melting point wax to cover the sabellid tubes. The dorsal surface of the abalone shell was dipped in the melted wax, smothering the tubes and killing all stages of the sabellid life cycle. This method is, however, labour intensive and caused unacceptable mortality in the abalone (Ruck, 2000). Another problem with this method is that once the abalone begins to grow again, it is re-infested along the growing edge by new tubes. The use of ultra-sound to destroy the feeding crowns of the adults has been investigated, however information regarding this aspect has not been published.

Management options for reducing the impact of infestations

Once abalone have been identified as being infested and have been properly isolated, it is necessary to control the growth of the sabellid population. One option is to provide the best possible conditions for maximizing abalone growth (Ruck, 2000). The faster an

abalone is growing, the better it is potentially able to cope with the interference caused by the infestation. The second task is to limit those conditions that favour the worm's productivity (Ruck, 2000). Below are some recommendations for managing existing sabellid infestations on farms (After Culver *et al.*, 1997; Clayden, 2000; Ruck, 2000).

1. Stocking density should be reduced. The lower the stocking density of the abalone the greater their growth rate and the less physical contact between individuals. There is, however, the problem of farmers having to increase the stocking density to recover capital investment (Ruck, 2000).
2. Sabellid larval development is faster at higher temperatures (Finley *et al.*, 2000). As was stated earlier, a temperature of 28°C for 24 hours is sufficient to kill all stages of the worm (Leighton, 1998). This treatment is of no use to *H. midae* farmers, as this abalone's temperature range lies between 11 to 19°C (Ruck, 2000). In this situation, it has been shown that the best results have been obtained at the optimum temperatures for abalone growth, even though this may increase sabellid production (Ruck, 2000).
3. Minimizing physical contact between abalone may reduce the rate of transmission (Ruck, 2000). Baskets, suspended off the base of the tank, are useful in curtailing the spread of sabellids within a tank.
4. Cleanliness of the tanks reduces the productivity of the sabellid. Although it is not known what the sabellid feeds on, the accumulation of waste, particulate organic matter and uneaten food, appears to contribute to the productivity of the worm (Clayden, 2000). Good hygiene is one of the characteristics of good aquacultural practice worldwide. Until more is known about the effect of biological waste on sabellid productivity, the tanks should be kept as clean as possible.

5. Although it is only a theory at present, it appears that the abalone become accustomed to a particular routine, which appears to favour growth (Clayden, 2000). Therefore, changing the routine or disrupting the abalone too often may reduce the growth rate, leading to increased susceptibility to infestation.
6. Abalone fed artificial feeds grow better when the diet is switched to kelp. The reason for this is not clear. Regarding the sabellid, it is possible that the kelp imparts a degree of resistance to the worm or perhaps particulates originating from the artificial feed supply it with nutrition, increasing its productivity (Clayden, 2000; Chalmers, 2002).

Gaining a sound understanding of the factors influencing the reproductive biology of the worm plays an essential part in developing a comprehensive database on all aspects of the worm's biology from which abalone farmers can access information. This information may be used by the abalone industry to develop new methods for on-farm treatment or even a means of eradicating this problematic animal.

The main objectives of this study were:

1. To develop, test and critically evaluate methods of removing and counting eggs and larvae of individual sabellids.
2. To examine the variation in sabellid morphometrics and offspring numbers between the different abalone culture facilities to provide an insight into the level of phenotypic and reproductive plasticity displayed by populations under differing environmental characteristics.
3. To investigate the potential relationship between host abalone diet history and the reproductive and morphometric characteristics of the sabellid.

4. To examine the potential influence of abalone growth rate on the reproduction, development and morphometrics of adult sabellids and their offspring under intensive abalone culture conditions.

Chapter 3 examined the variation in sabellid reproductive and morphometric characteristics in response to the variation in environmental conditions found on four abalone farms. This information was used to determine the variability of the sabellid life history characteristics and to place the individual life- history- related variables along the *r-K* continuum to provide an indication of the biology of the sabellid under farm conditions.

The influence of diet history of the host abalone on reproduction and growth of *T. heterouncinata* was investigated in Chapter 4. Infested abalone experienced a change in diet and were left for 185 days to test if the sabellid alters the expression of reproductive and morphometric characteristics in response to a change in host diet. Sabellids from abalone that were exposed to a change in diet were compared to those that did not experience a change in diet to determine the extent at which this may cause a shift in the position on the *r- K* continuum.

Chapter 5 investigated the effect of abalone growth rate on sabellid reproduction and growth. Fast and slow growing infested abalone were sampled from two farms. These comparisons of the reproductive and growth characteristics of sabellids from abalone with different growth rates were made separately for each farm.

The effect of abalone diet on sabellid larval settlement was examined in Chapter 6. The number of larvae settled per 96 hours was quantified for 36 days to determine if host abalone diet influenced the success of larval settlement on the shell edge. The distribution of larvae on the abalone shell was recorded.

CHAPTER 2

GENERAL MATERIALS AND METHODS

Farm conditions

All abalone used in this study were obtained from four abalone farms in the Hermanus (34°42'S; 19°23'E) and Gansbaai (34°58'S; 19°36'E) areas, on the South Coast of South Africa (Figure 2.1). Differences in farm management are listed in Table 2.1. Farms feed their abalone fresh kelp (*Ecklonia maxima*), an artificial diet, or a combination of the two (Figure 2.2). Kelp is commercially harvested and delivered to the farms. However, due to irregular supply as a result of rough seas, Sea Plant Products developed the pelletized formulated feed, Abfeed® (Figure 2.2). The proximate composition of the naturally occurring kelp and Abfeed is shown in table 2.2.

The abalone were held in baskets suspended off the bottom of the tank (Figure 2.3). A series of plastic sheets were placed into the baskets, which provided the surface area for the abalone (sitting plates) (Figure 2.3). A feeding plate was placed on top of the sitting plates, and was used for the abalone to feed on during the night while providing shade for the photophobic abalone during the day. Kelp was placed under the feeding plate, between the sitting plates. This allowed the abalone to feed on the kelp during the day without having to move up on to the feeding plate. Kelp was left in the baskets until it was eaten or removed when the baskets were cleaned once a week. Fresh kelp was introduced to the tanks once a week, depending on the farm (Table 2.1). Abfeed was placed on top of the feeding plates, and the abalone emerged during the night to feed (Figure 2.2). This feeding method reduced the problem of overfeeding as it allowed the

farmer to reduce or increase the ration per basket depending on the quantity of food left over from the previous night. Abalone were fed Abfeed either once a day or every second day, depending on the management strategy adopted by the farm (Table 2.1). Feeding is temperature-dependent and hence the frequency of feeding varied with water temperature. At low water temperatures the feeding rate of the abalone is decreased and hence less food is fed to the abalone. At higher temperatures, feeding rate increases and more food is required. An example of a typical commercial abalone raceway is shown in Figure 2.4.

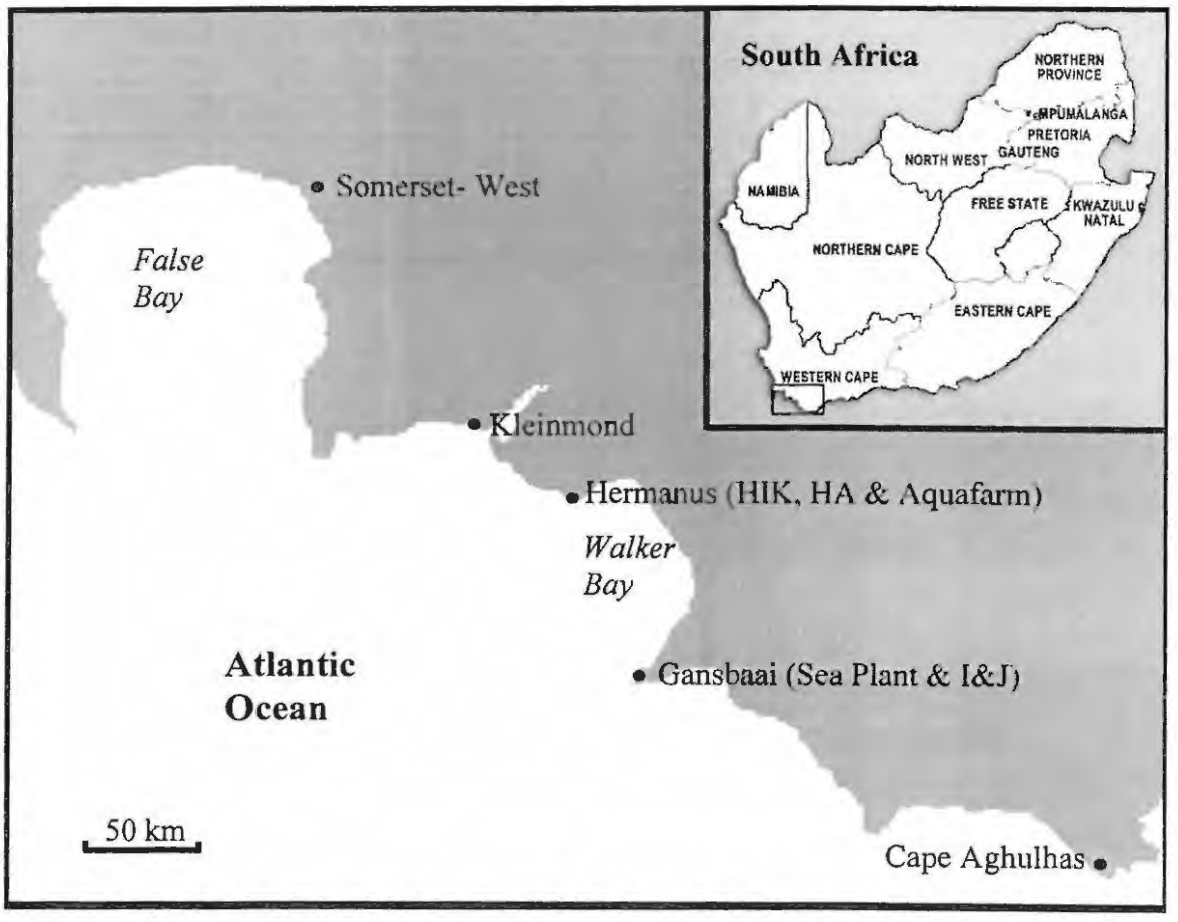

Figure 2.1: Location map indicating the position of Hermanus and Gansbaai and the location of farms from which abalone were sampled. (HIK= HIK Abalone; HA= Hermanus Abalone; Aquafarm = Aquafarm Development; Sea Plant= Sea Plant Products and I&J= Irvin & Johnson Abalone Culture Division).

	I & J	Sea Plant Products	HIK	Hermanus Abalone
Location	Gansbaai 34°58'S; 19°36'E	Gansbaai 34°58'S; 19°36'E	Hermanus 34°42'S; 19°23'E	Hermanus 34°42'S; 19°23'E
Abalone Diet	Kelp	Abfeed	Kelp and Abfeed	Kelp and Abfeed
Feeding regime	Every three days	Every second day	Kelp weekly Abfeed daily	Kelp and Abfeed daily
Size sorting	Every 6 months	Every 4 months	Every 6 months	Every 5 months
Stocking density (% Surface area)	30%	20-35%	16-20%	18-20%
Flow rate (exchanges per hour)	1.5	2.2	3.4	3.3
Mean Temperature (°C)	Summer- 18 Winter -16.2	Summer- 17.7 Winter- 15.8	Summer 15.2 Winter 13.4	Summer 15.2 Winter 13.4
Tank cleaning routine	Information withheld	Once every 7 days	Once every 10 days	Abfeed- every 7 days Kelp- every 10-14 days
Tank volume and construction	7.5 m^3 Fibre re-enforced concrete	4 m^3 Concrete	4.5 m^3 Canvas	3.5 m^3 Concrete

Table 2.1: A summary of the management procedures employed by the abalone farms from which abalone were sampled.

Table 2.2: The proximate composition (%) of dry matter of kelp (*Ecklonia maxima*) and Abfeed.

	Kelp	*Abfeed*
Protein	8.1	34.6
Carbohydrate	45.2	43.3
Fat	0.5	5.3
Ash	25.3	5.7

From Chalmers 2002, with permission.

Transport of abalone

Abalone sampled from the various farms were transported in polyurethane boxes containing a layer of wet sponge at the base of the box. These boxes are used for export of live abalone to overseas markets to reduce abalone mortality. As the distances between farms were small (< 50 km), no mortalities occurred in abalone transported during this study. All experiments and laboratory work carried out during this study were undertaken at Aquafarm Development, Hermanus.

Experimental conditions

Abalone that were not immediately shucked on arrival were kept in abalone baskets in 1.2 x 0.9 x 0.6 m (660 litre) white plastic (HDPE) tanks at Aquafarm Development in Hermanus. The tanks received 60 µm filtered seawater pumped ashore from Walker Bay. Aeration was provided by two pipes running along the base of the tank. Water was exchanged once every 2-2.5 hours and temperature fluctuated with the sea temperature. During the experimental period the temperature fluctuated between 11 and 21°C, with a mean of 14.4°C. The tanks were cleaned every 7 days. Abfeed was fed once a day and kelp every four days. Stocking density is calculated as the percentage of the available

surface area covered by the abalone. The available surface area includes both sides of the sitting plates and the underside of the feeding plate. The methods for calculating stocking density varied between farms and the abalone surface area can be calculated on a circular or triangular surface area based on the shell size. The Abalone Farmers Association workgroup recommends that abalone be stocked up to a maximum of 35%, but most farms maintained a lower stocking density. Abalone were size-sorted every few months to maintain the recommended stocking density.

Anaesthetising abalone

Before handling, abalone were anaesthetised in a solution of 7% magnesium sulphate in seawater. Anaesthetic was used to allow for easier removal of abalone from the sitting plates and the sides of the baskets if measurements, such as those of infestation level, were taken on live abalone. Approximately 30 kilograms of magnesium sulphate powder was added to a fibreglass tank filled with 400 litres of seawater (Pesch, *pers comm.*) The powder dissolved easily and aeration was introduced from a pipe at the bottom of the tank. The abalone basket was then placed for approximately 20 minutes into the tank containing the magnesium sulphate. After this time, the abalone were carefully removed by hand and placed in a polyurethane box containing a wet layer of sponge on the base. If some of the abalone in a basket were still able to adhere to the plates or basket, the basket was re-immersed in the magnesium sulphate solution for a further 10-15 minutes. Once all abalone from a basket had been removed, the lid was placed on the box to control the temperature and the abalone were moved to the laboratory.

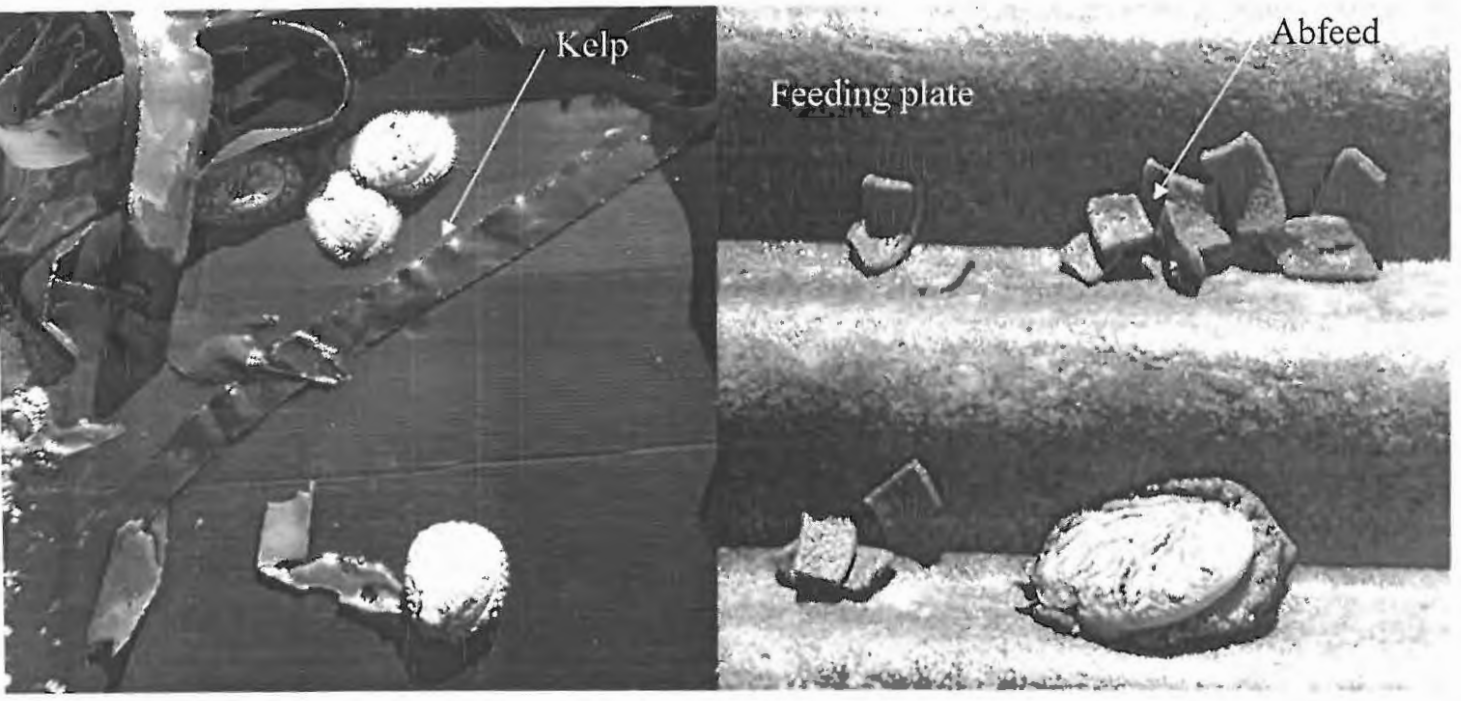

Figure 2.2: Abalone feeding on kelp (left) and Abfeed (right) on feeding plates

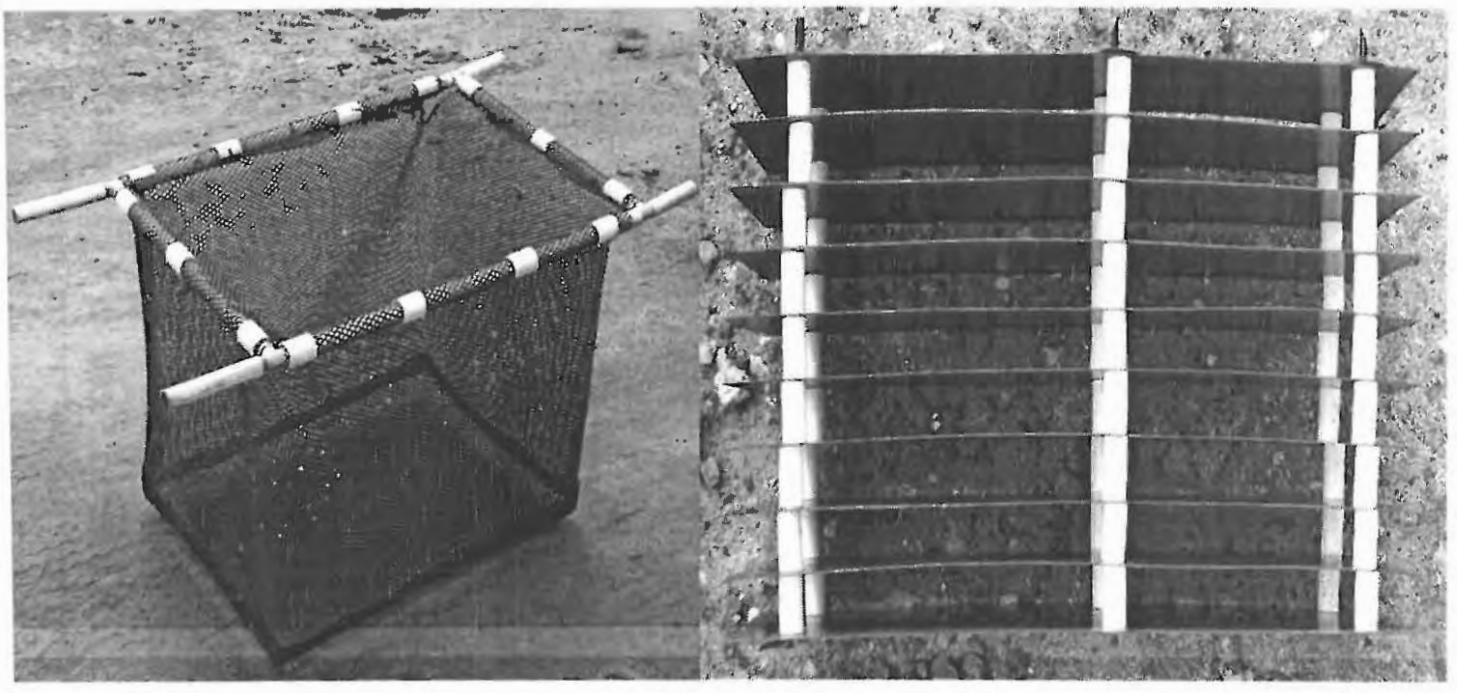

Figure 2. 3: A commercial abalone basket (left) and the sitting plates placed in the baskets to provide surface area for abalone attachment (right)

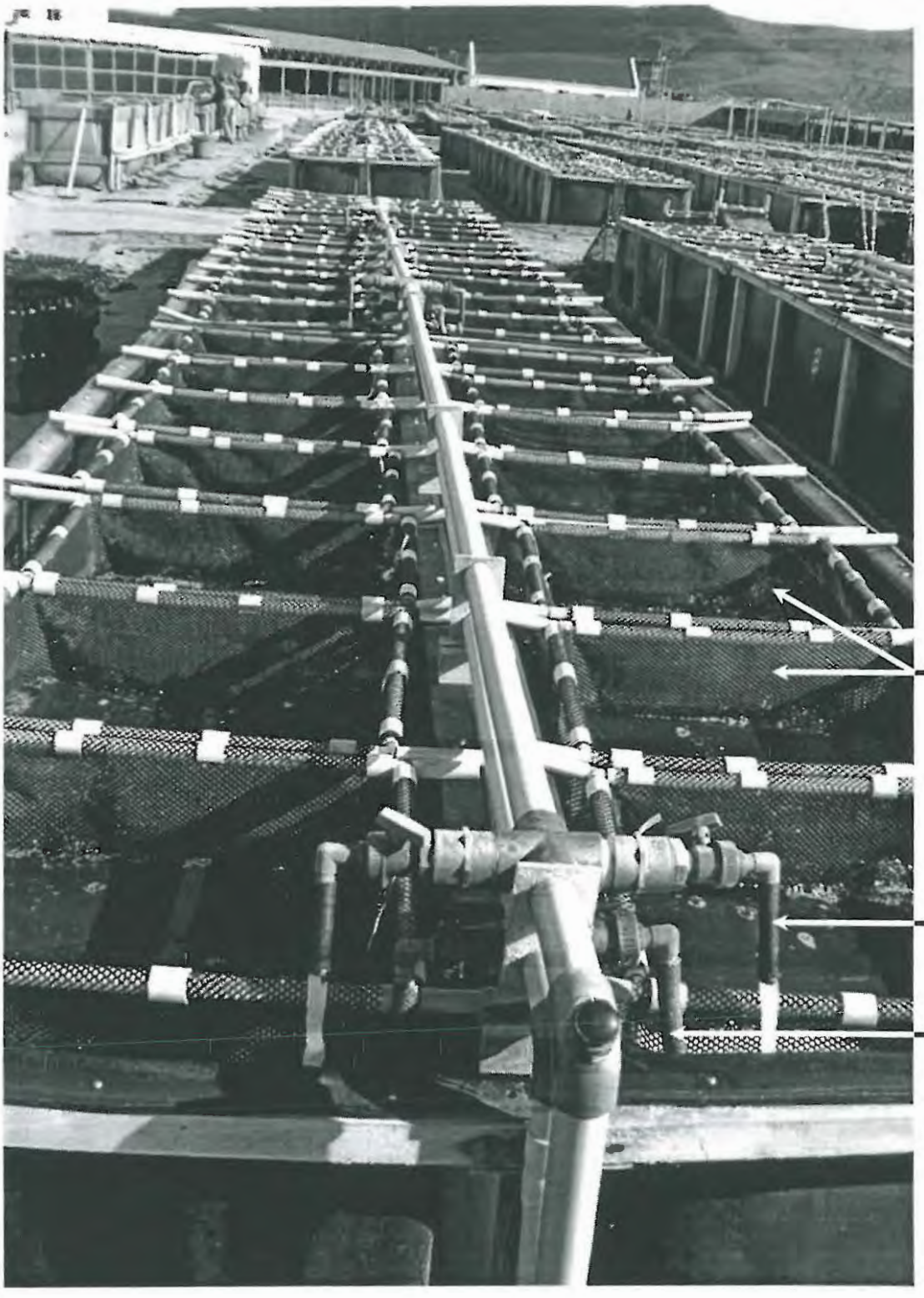

Figure 2.4: An example of a commercial abalone raceway containing abalone baskets. The aeration supply runs to airlines under the abalone baskets.

Quantification of intensity of infestation

Intensity is defined as the number of newly settled larvae on the growing edge of the abalone shell. The intensity gives an indication of the sabellid infestation level. Larvae less than one month old were recognized by pulling the mantle back and observing the inner margin of the shell edge. The larval tubes were visible as white tick marks about 2 mm long. Less than a week after settlement larvae could be seen in their tubes as at this stage they are translucent orange with two black eye spots. More than one week subsequent to settlement enough nacre had been deposited by the host to conceal the larvae in its tube. After about four weeks the tubes were no longer visible due to deposition of nacre and hence this method was only useful to identify new infestations. Therefore, a lack of new tubes along the shell margin does not indicate that the abalone is free of sabellids as settled sabellids may be present. Thus, only larvae less than one week old that had settled on the growing edge of the abalone shell were used to determine the infestation level.

The growing edge was taken as the inner shell edge margin between the point of the shell edge perpendicular to the shell apex and the shell edge region perpendicular to the most recently formed respiratory pore (Figure 2.5). A line was taken from the spire to the respiratory pore. The number of occupied and unoccupied larval tubes was counted on the growing edge 10 mm either side of the mid-point of this line (Figure 2.6). Infested tubes could be distinguished from uninfested tubes as the orange larvae could be seen through the thin nacreous layer deposited by the abalone. If a tube was regarded as uninfested, the tube was broken with a scalpel blade to ensure the larva was not present. The length of the shell margin of the growing edge was measured (mm) and intensity for each shell was determined as the mean number of tubes, both infested and uninfested, per centimeter of

the growing edge. This method has been used by other authors (Chalmers, 2002) and was used in this study so comparisons could be made with previous studies. The occupation rate (%) was calculated as the number of occupied tubes as a percent of the intensity. Abalone that were not required for continued studies on the intensity and occupation level were shucked to remove the adult sabellids and their offspring.

Removal of worms

After shucking the abalone the shells were labelled and preserved in a solution of 2.5% gluteraldhyde in filtered seawater buffer. Upon removal from the gluteraldhyde solution, the shells were rinsed with seawater and batches of five shells were immersed in a 500 ml solution of 6.5% nitric acid in 70% ethanol for approximately seven hours. This procedure was adapted from a technique first carried out by Brock and Brock (1977). It removes the calcium carbonate from the shell layers without damaging the protein matrix or the sabellids embedded in it (Brock & Brock, 1977; Culver *et al.*, 1997). The nitric acid reacts with the calcareous component of the abalone shell, softening it and reducing the time and effort needed to remove the worms. If the shell is left in the solution for more than seven hours the shell is further softened but the structural integrity of the worms and their offspring is compromised.

Shells were then rinsed with seawater to remove any traces of the acid, and were placed into 2.5% gluteraldehyde in filtered seawater. Sabellids kept in the 2.5% gluteraldehyde solution for longer than one month tended to have reduced structural integrity and were more easily damaged on removal from the abalone shell. Each shell was placed, ventral surface up, in a petri dish of filtered seawater under a Nikon SMZ-660 binocular dissecting microscope. Peeling back the protein matrix of the decalcified shell exposed

the sabellids. Thin glass rods were used to hook the sabellids and pull them out of their burrows. The glass rods were made by placing sections of a Pasteur pipette over a Bunsen burner and pulling them to draw the glass into long thin rods. The glass rods had to be thin enough to fit into the burrow and pull the worm out, without damaging the adult worm or its eggs and larvae. If there was an insufficient amount of the adult sabellid exposed, grooves were made down each side of the burrow with a scalpel. Once the grooves were deep enough (depending on the burrow), the scalpel blade was inserted into one of the grooves and turned, cracking the layer of shell and exposing a greater proportion of the worm. If it was not certain if the adult worm and all its eggs and larvae had been removed without damage, the worm was left and another was sampled. Approximately one out of three worms was successfully removed. Ten worms were removed from each abalone with confidence that all offspring specific to an adult sabellid had been obtained.

Once the adult and its offspring had been pulled from the burrow, they were moved using a dropper and placed in a labelled eppendorf tube, containing 2.5% gluteraldehyde in filtered seawater. For measurements each worm was placed in a drop of seawater on a petri dish under a binocular dissecting microscope. The eggs and larvae were removed from the adult by gently scraping them off the abdominal region of the worm using a fine glass rod.

All measurements (in micrometers) were taken with a graduated eyepiece using a compound microscope. The body length was measured from the base of the branchial crown to the tip of the abdomen (Figure 2.7). The neck width was taken as the perpendicular distance across the body below the basal flange of the branchial crown, at

the collar (Chalmers, 2002) (Figure 2.7). The base width was taken as the distance across the widest part of the abdomen region, across the 9th and 10th abdominal setigers (Figure 2.7). Two larval stages were identified for the purpose of this study. Stage 1 larvae are referred to as sabellid larvae that have less than 5 setigers, no cilia or setae and eyespots are absent (Fitzhugh & Rouse, 1999; Ruck, 2000). A stage 2 larvae is the final larval stage before emergence from the burrow. They are approximately 500 μm long, have five thoracic setigers, two dark eyespots and cilia and setae are present. Total body length and width at the widest part of the larvae were measured for both stages of larval development (Figure 2.7). The length and width at the widest part of the sabellid eggs were also measured (Figure 2.7). The number of eggs, young larvae and old larvae specific to each adult worm was counted. Egg volume was calculated according to the following formula:

$$V = {}^{4}/_{3}\,\pi \cdot W^{2} \cdot L$$

Where: V= egg volume in mm^3; W= egg width in mm and L= egg length in mm.

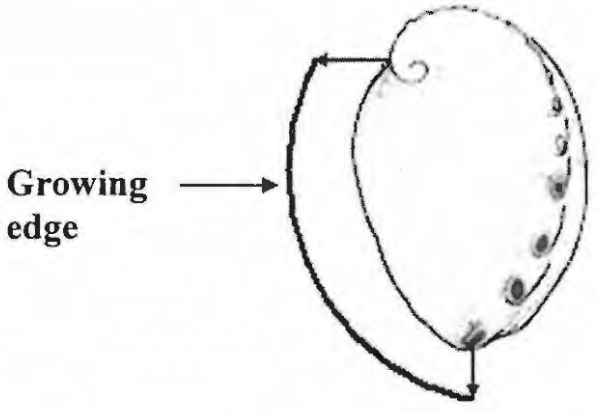

Figure 2.5: The growing edge of the abalone shell

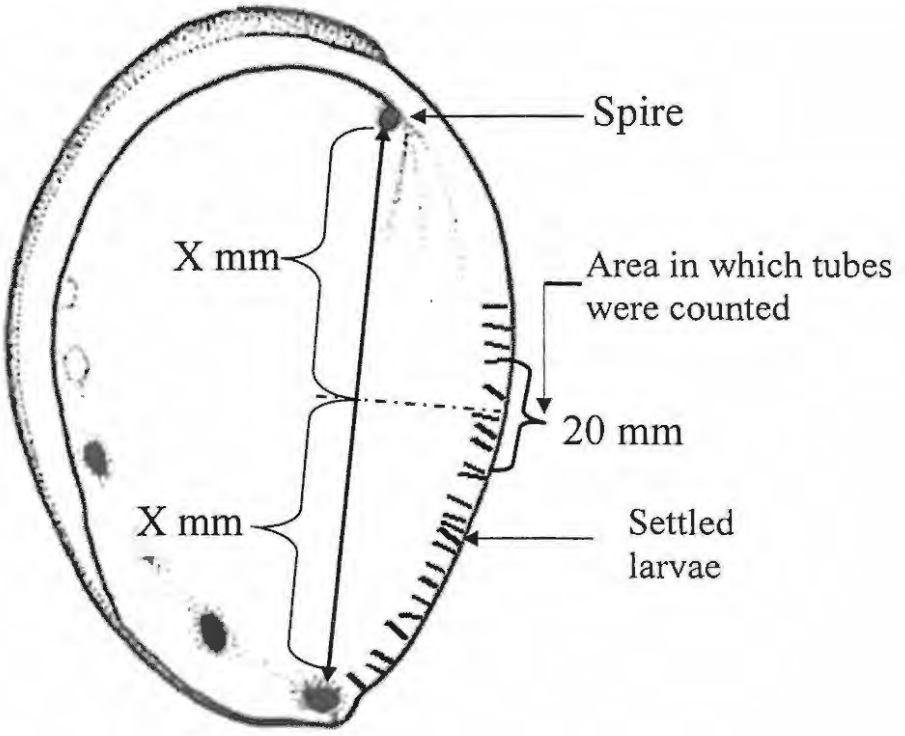

Figure 2.6: The section growing edge region from which the number of tubes was counted to determine the intensity. The area from which tubes were counted was 10 mm either side of the mid-way point between the line taken from the spire to the most recently formed respiratory pore of the abalone shell.

Statistical Analysis

The criteria for using either Student's t-test or an Analysis of Variance (ANOVA) is that the data are normally distributed and variance is homogeneous. The Levene's test was used to test for homogeneity of variance and Shapiro-Wilk's W test was used to determine if data were normally distributed. The null hypothesis was rejected at an α-error value of $p \leq 0.05$ for both tests. If the data were non-normally distributed or variances were unequal, they were log, square-root or arc-sin transformed. If the transformed data were not fulfilling the criteria for parametric testing, non-parametric tests were employed.

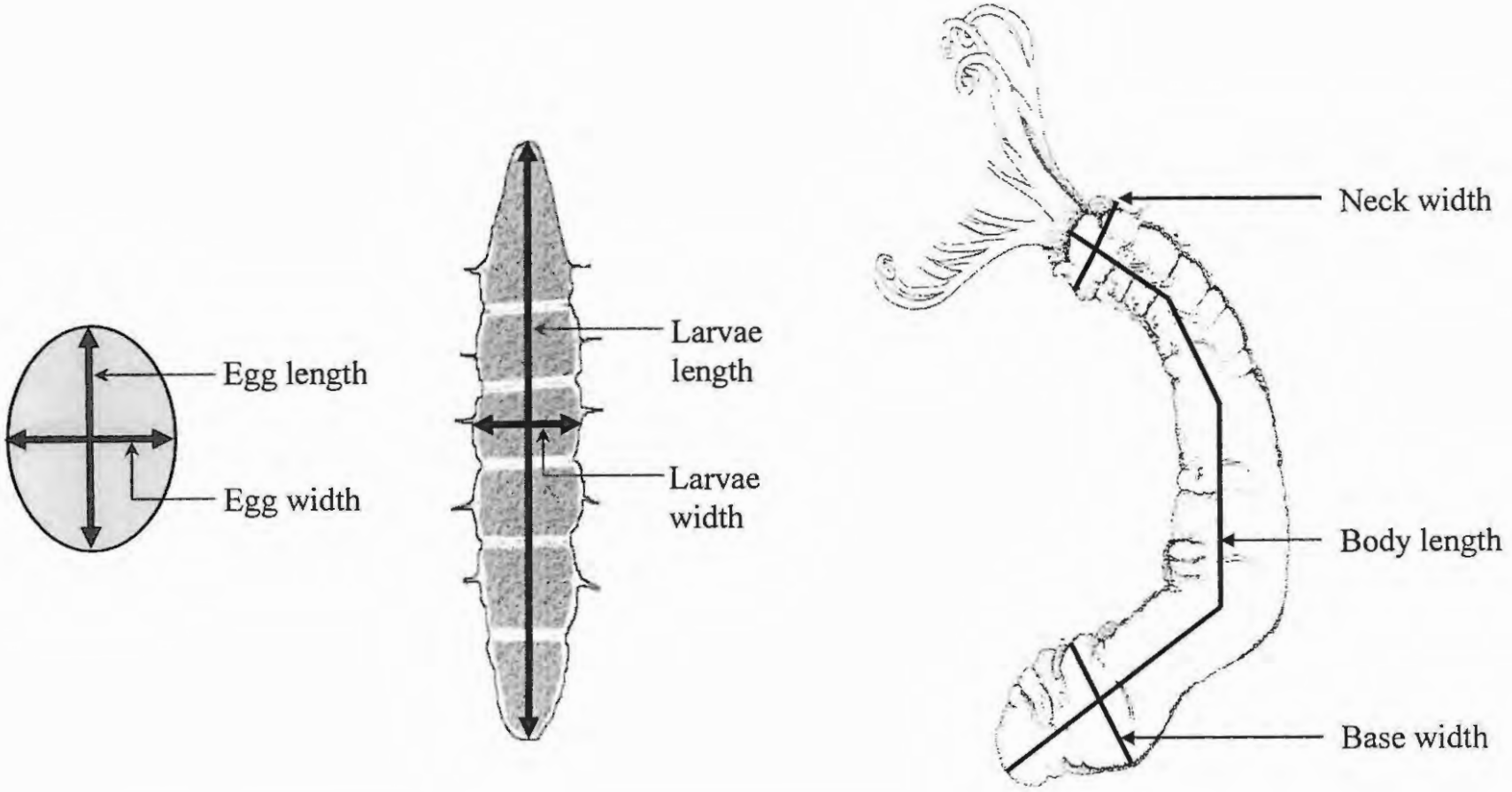

Figure 2.7: Morphometric measurements of the egg, larvae and adult of *Terebrasabella heterouncinata*

The Kruskall-Wallis test is a non-parametric alternative to one-way (between-groups) ANOVA. It is used to compare three or more samples, and it tests the null hypothesis that the different samples in the comparison were drawn from the same distribution or from distributions with the same median. As data could not be transformed to achieve normality or equality of variance the non-parametric test was used throughout this thesis. The alternative approach would have been the use of nested Analysis of Variance. However, this would also have required normally distributed data or equal variances. Thus, the Kruskall-Wallis ANOVA by ranks was used to determine differences in intensity and occupation level, body measurements, morphometric ratios and offspring counts of sabellids between different abalone diets, farms and abalone size classes. After testing data with the Kruskall-Wallis ANOVA by ranks, the Bonferroni correction was applied and data were then tested pair-wise with the Mann-Whitney U-test. The α- error of 5 % was divided by the number of planned comparisons (n) and pair-wise tests were considered significant at the new p-value of 0.05/ n.

CHAPTER 3

An investigation into the variability of reproductive and morphometric characteristics of *Terebrasabella heterouncinata* under intensive abalone culture conditions

INTRODUCTION

Natural populations of marine organisms are rarely exposed to constant environments for long periods. Environmental conditions may vary within an individual's lifetime, or over a longer period covering many generation intervals, and change may be slow or rapid. In the face of environmental change, differing reproductive success of genotypes may lead to inherited adaptive change in populations (Hoffmann *et al.*, 1995). If the genetic variation of a population is high, then adaptive differentiation may occur rapidly (Reznick *et al.*, 1997). Selection, may, however favour the evolution of phenotypic plasticity that allows for appropriate responses to a change in the environment to which individuals in a population are exposed (Baker & Foster, 2002). Phenotypic plasticity refers to the ability of the genotype to produce an array of phenotypes when exposed to differing environmental influences (Reznick & Yang, 1993). Because so many characters have shown to exhibit high levels of plasticity, including those closely linked to fitness, it is important to explore phenotypic plasticity in order to gain a better understanding of adaptive mechanisms. Knowledge of the extent of phenotypic plasticity of certain traits within a population, particularly those associated with reproduction, may yield important information for understanding the biology of the species. For example, if there is a high degree of variation in the size or number of eggs this knowledge may lead to the discovery of factors that may be influencing the adult into adjusting the numbers or size

of eggs depending on the environmental conditions. For the sabellid such an environment may be the abalone farm with its relatively stable environmental conditions and easy availability of food and hosts.

There are an estimated 9000 species of polychaetes occurring worldwide (Rouse & Pleijel, 2001), making it one of the most diverse invertebrate groups. Polychaetes exhibit a large diversity of reproductive strategies and life history traits. It has been hypothesised that this diversity may be due to the relatively simple reproductive systems and to their high degree of plasticity and adaptability to a changing environment (Giangrande, 1997).

Many references are available on polychaete life history styles (Thorson, 1950; Olive, 1984; Grahame & Branch, 1985; M^{c}euen *et al.*, 1993; Giangrande *et al.*, 1994; Qian, 1994). Despite this, less than 0.5 % of polychaete life cycles are well documented (Giangrande, 1997). Polychaetes exhibit a great variety of reproductive strategies including both sexual and asexual. Within a single family there may be species that brood a few large eggs that develop directly into juveniles whilst others may spawn many small, nutrient-poor eggs that are fertilised in the water column. The life cycle of polychaetes has been divided into two broad categories (Olive, 1984): semelparous and iteroparous. Semelparity refers to only one reproductive event in an organism's lifetime and iteroparity is the condition where breeding occurs several times in a lifetime (Rouse, 2000). Gonochorism is the most common mode of reproduction found in polychaetes, although, hermaphroditism is widespread. Hermaphroditism can be divided into two forms: simultaneous and sequential (Giangrande, 1997). Simultaneous hermaphroditism is characterised by the presence of both eggs and sperm at the same time, whilst

sequential hermaphroditism is the condition of containing both eggs and sperm but at different times (Rouse, 2000).

The embryology, type of development and larval forms are also highly variable amongst the polychaetes and are important indicators of life history strategies. Egg sizes range between 50 μm and 1 mm (Rouse, 2000). The energy content of the eggs is variable and has been shown to be influenced by factors such as the quantity and quality of food ingested by the adult. Both the number and size of polychaete eggs has been shown to vary with environmental conditions (Qian & Chia, 1991; Qian, 1994). Qian (1994) indicated that *Capitella* sp. respond to a change in the quantity of food available by altering both the size and number of eggs produced per spawning event. The energy content of the eggs influences the mode of larval development. For example, plasticity in polychaete larvae is demonstrated by 18 different developmental strategies (Giangrande *et al.*, 1994). Three basic categories are recognised by most authors and these include planktotrophic, lecithotrophic and direct development (Giangrande 1994; Giangrande *et al.*, 1994). Within some species, it has been recorded that populations may shift the type of larval development depending on environmental conditions (Giangrande *et al.*, 1994).

The diversity of polychaete life history traits and the high degree of plasticity of these traits displayed formed the basis for this study. The primary aim of this study was to determine the level of phenotypic plasticity displayed by the sabellid worm under different environmental conditions and to place the various life history traits of the worm along the continuum according to the *r*- and *K*-concept.

The central aspect in the study of life history strategies has been the inclination to divide life history traits, and the environments with which they are associated, into two contrasting types: '*r*' and '*K*'. (MacArthur and Wilson, 1967; Pianka, 1970). These letters refer to coefficients of the logistic equation, where *r* is the intrinsic rate of increase and *K* is the equilibrium population size. These terms indicate that *r*- selected individuals tend to maximize fitness by reproducing rapidly in an unpredictable environment (i.e. they have a high value for *r*), whilst *K*-selected individuals maximize fitness by making a large proportional contribution to a population that remains close to its carrying capacity.

The typical *r*-selected population lives in an environment that is unpredictable and it therefore experiences considerable environmental fluctuations. As a consequence, the population itself fluctuates widely in size and juvenile and adult mortality rates are highly variable (Bruton, 1989). A density-independent juvenile mortality rate tends to favour the production of many small young. If the fraction surviving remains constant, the number of surviving offspring increases with the number produced. Density- independent mortality amongst adults tends to support early and explosive reproduction (high reproductive effort), since effort towards maintenance and growth are worthless in the face of disaster. This explosive reproductive effort can be found in species with short life spans and generation times. The pattern of influence has, therefore, moved full circle: the selective pressures favouring *r*-type individuals- with earlier maturity; more, smaller young; smaller size; larger reproductive effort per reproductive event and shorter life- have been reinforced (Begon & Mortimer, 1986).

A *K*-selected species, on the other hand, lives in an environment that is either stable or predictably seasonal, and it therefore encounters very small environmental fluctuations.

As a result, a crowded population of relatively constant size is established in which there is strong, density- dependent competition between adults and little habitat available for young to become established (Bruton, 1989). Intense competition amongst adults favours successful competitors, and these will tend to be individuals allocating substantial effort to maintenance and growth, and therefore making a small reproductive effort per reproductive event and delaying maturity. The density- dependent complications experienced by small, young individuals will, similarly, guarantee that only the most successful competitors will survive. Thus, the production of few large young will be preferred (leading to large adults) as will dedication of considerable parental care to the young. This necessity for parental care, along with the need to reduce the density-dependent effects on the young, will lead to reproduction being extended over time (Begon & Mortimer, 1986). *K*-selected individuals- with later (more delayed) maturity; fewer, larger young; larger size; smaller reproductive effort; longer life; iterated breeding and parental care- are the result (Begon & Mortimer, 1986).

The objective of this study was to examine the variation in sabellid morphometrics and offspring numbers between four abalone culture facilities to provide an insight into the level of phenotypic and reproductive plasticity displayed by populations under different environmental characteristics. The data gathered on the life history traits of the sabellid during this study will be compared with data from other polychaete species in order to place the sabellid within the continuum of the *r-K* concept. They will also be used as reference data for the experimental chapters of this thesis. Thus, this chapter provides basic information to allow an evaluation of studies of the effect of diet, abalone growth and settlement.

MATERIALS AND METHODS

Infested abalone were sampled from four different abalone farms. Sabellids were extracted from the shells and the morphometric and reproductive characteristics of the adults and their offspring were examined to determine the level of variation between the different conditions found on the four farms.

Farms sampled

Abalone were sampled from four culture facilities in the Hermanus area (34°42'S; 19°23'E) on the South Coast of South Africa between the 26th June and 5th July 2001 (Figure 2.1). The management procedures and system design characteristics of these farms are provided in Chapter 2. The level of infestation was determined by visually assessing the extent of infestation by the appearance of the shell shape and examining the inner edge of the growing edge of the shell. Heavily infested abalone have a slightly deformed shell and the growing edge is often brittle due to the high number of sabellid tubes. Ten heavily infested abalone of the same cohort with a mean shell length ± standard deviation of 68.2 ±10.1 mm were randomly sampled from each of the four farms. The abalone were immediately shucked and the shells were preserved in a 2.5% solution of gluteraldehyde in filtered seawater until the sabellids were removed.

Quantification of intensity of infestation

In the laboratory each shell was placed under a binocular dissecting microscope. The tubes along the growing edge were counted to determine intensity (see Chapter 2). Both the tubes occupied and unoccupied by sabellids on the growing edge were counted to calculate the percent occupation.

Removal of sabellids

The shells were placed in a 6.5% nitric acid in 70% ethanol solution for seven hours. After this the shells were removed from the nitric acid solution, rinsed with seawater, and placed back into a 2.5% gluteraldehyde solution. Once all shells from a particular farm had been processed in this way, each individual shell was placed, ventral surface upwards, in a petri dish filled with seawater. Individual adult sabellids with their larvae and eggs were removed and placed individually in a 2.5% gluteraldehyde solution in seawater in labelled containers. Ten sabellids were removed from each of ten shells from each of the four farms. Each sabellid, together with the larvae and eggs specific to that adult, were placed onto a petri dish under the microscope. The length, width at the basal flange of the feeding crown and width of the widest part of the abdomen of the adult worm were measured with a graduated eyepiece using a dissecting microscope. Two larval stages were identified for the purpose of this study. Stage 1 larvae are referred to as sabellid larvae that have less than 5 setigers, no cilia or setae and eyespots are absent (Fitzhugh & Rouse, 1999; Ruck, 2000). The term stage 2 larva refers to the final larval stage before emergence from the burrow. They are approximately 500 μm long, have five thoracic setigers, two dark eyespots and cilia and setae are present. The length and width (at the widest part) of both the larvae and eggs was measured according to details provided in Chapter 2.

Statistical analysis

The Kruskall- Wallis ANOVA by ranks was used to determine differences in sabellid body measurements, offspring counts and infestation indices between the different farms. The Bonferroni correction for post-hoc planned pairwise comparisons was applied to arrive at the α- error level to be used for the Mann-Whitney U-test. The level of

significance was determined by dividing the error level by the number of planned pairwise comparisons. For all data tested with the Mann-Whitney U-test, the null hypotheses were rejected at $p \leq 0.0083$. Least- square regression analysis was used to determine the relationship between the adult sabellid length and the number of offspring and eggs per adult.

Determination of the r-K *position of T. heterouncinata*

For the determination of the position of the various life history traits of the sabellid on the *r-K* continuum, the measurements taken during this study were compared with the minimum and maximum values for that particular trait for species reviewed by Giangrande (1997) and Rouse and Fitzhugh (1994) (Table 3.3). For example, the range of eggs produced per brood of polychaetes reviewed by the above authors ranged between 1 and 1 million, the number of eggs per adult brood measured during this study ranged between 1 and ten, with a mean of 4 eggs per brood. Assuming that the polychaetes producing the least number of eggs (1 egg) are the 'absolute' *K*-strategists and those producing the most (1 million) are the *r*-strategists, then the sabellid will essentially be placed 4 units away from an "absolute" *K*-strategists on the *r-K* continuum for this trait. The solid blocks in Table 3.4 are an estimate of the position of a particular trait of the sabellid life history style when compared to the range of traits exhibited by other polychaete species (Table 3.3). Where categorical traits were used, for example the mode of larval development, it was not possible to place the sabellid on the continuum for that particular trait. Three modes of larval development have been identified, planktotrophic, lecithotrophic and direct development. Lecithotrophic development is more *K*-selected than planktotrophic development but more r-selected than direct development. Therefore,

in terms of "*r*- and *K*-ness", lecithotrophic development, as employed by the sabellid, is between planktotrophic and direct development and it is only possible to say it is more *r* or *K*-selected with respect to these other modes of development (Table 3.4).

RESULTS

All sabellid morphometric measurements and offspring counts were significantly different between two or more of the four culture facilities.

Sabellid intensity and occupation level

The mean sabellid intensity (tubes per centimeter) ranged from 0.25 to 18 tubes across all farms. Hermanus Abalone had abalone with significantly higher intensity than abalone from Sea Plant and I&J ($Z= 17.3$; $p \leq 0.0001$ and $Z= 7.6$; $p \leq 0.0001$, respectively) (Figure 3.1). The intensity for I&J, Sea Plant and HIK abalone was not significantly different ($H=1.07$; $p= 0.29$) between them.

The occupation level of sabellid tubes was significantly different between some of the farms ($H= 61.1$; $p \leq 0.0001$) (Figure 3.1). Abalone from I&J had a significantly higher sabellid occupation level (mean ± standard deviation) (78 ±5%) than abalone from Sea Plant Products and HIK (51± 12%; 54 ±12%, respectively). The occupation level of abalone from Hermanus Abalone and I&J was similar ($Z= 2.5$; $p= 0.02$).

Morphometrics

The average length of adult sabellids was different between some farms ($H= 154.1$; $p \leq 0.0001$) (Figure 3.1). I&J and Sea Plant Products had significantly longer sabellids (2.76 ±0.6 mm and 2.9 ±0.7 mm, respectively) than HIK and Hermanus Abalone sabellids (1.97 ±0.4 and 2.1 ±0.4 mm, respectively) ($p \leq 0.0083$). The length of sabellids from I&J and Sea Plant Products was not significantly different from each other ($Z=2.4$; $p= 0.016$), as was the case with sabellids from HIK and Hermanus Abalone ($Z= 1.85$; $p= 0.06$) (Figure 3.1).The length of the sabellids from the four farms was ranked as follows: I&J=SP>HIK=HA.

The base width was highest (0.4 ± 0.06mm) in sabellids from Sea Plant Products compared to the other three farms (H= 252.6; p= 0.001) (Figure 3.1). Sabellid base width for abalone sampled from HIK (0.3 ± 0.05mm) and Hermanus Abalone (0.3 ± 0.07mm) was not significantly different between farms (Z=0.69; p=0.49), and was significantly greater than in the sabellids from I&J, which had the smallest base width of all farms (0.2 ±0.06mm). The base width of sabellids from the different farms was ranked as follows: SP>HIK=HA>I&J.

The body length for stage 1 larvae was influenced by the origin of the sabellid population (H=10.8; p= 0.013). Stage 1 larvae from I&J sabellids had a significantly greater body length than sabellids from Sea Plant Products (Z=2.8; p= 0.0004). The Stage 1 larvae body length was not different between any of the other farms (H=1.26; p= 0.53) (Figure 3.2). The stage 2 larvae length was not significantly different between Sea Plant Products, HIK and Hermanus Abalone (0.70 ±0.08; 0.66 ±0.08 mm and 0.66 ±0.09 mm, respectively) (H=1.15; p= 0.56). I&J had significantly smaller stage 2 larvae than the other three farms (0.63 ±0.16) (Figure 3.2). The stage 2 larvae length from the four farms ranked as follows: SP=HIK=HA>I&J.

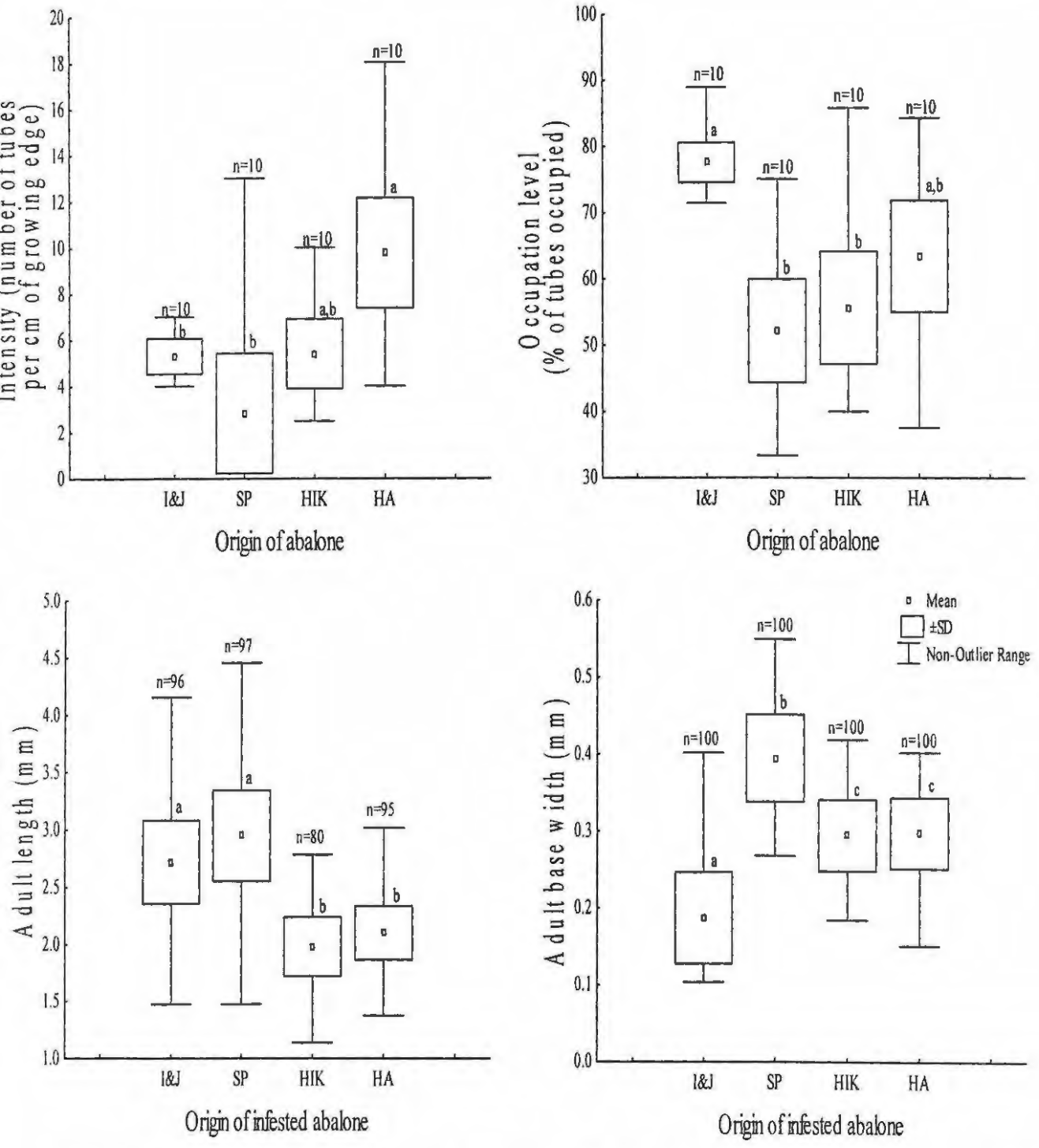

Figure 3.1: The intensity, occupation level, adult length and base width of sabellids extracted from abalone originating from four different abalone culture facilities. The p-value at which pairwise comparisons were considered significant was set at $p \leq 0.0083$ due to the Bonferroni- correction; different letters indicate significant differences. (Where: I&J = Irvin & Johnson abalone culture division; SP= Sea Plant Products; HIK= HIK abalone farm and HA= Hermanus Abalone).

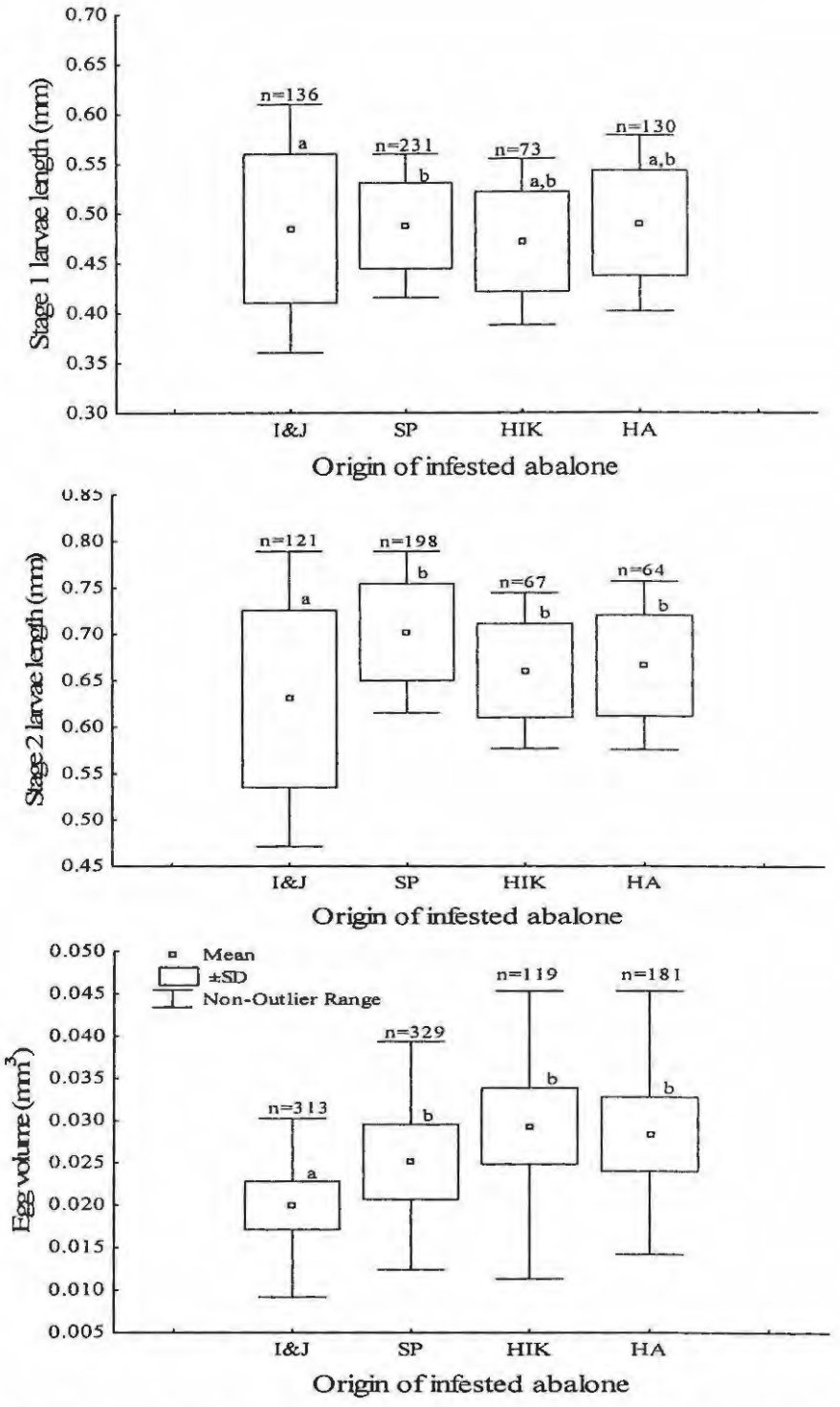

Figure 3.2: Larval length and egg volume for sabellids of abalone sampled from four farms. The p-value at which pairwise comparisons were considered significant was set at p≤0.0083 due to the Bonferroni- correction; significant differences are indicated by different letters. (Where: I&J = Irvin & Johnson abalone culture division; SP= Sea Plant Products; HIK= HIK abalone farm and HA= Hermanus Abalone).

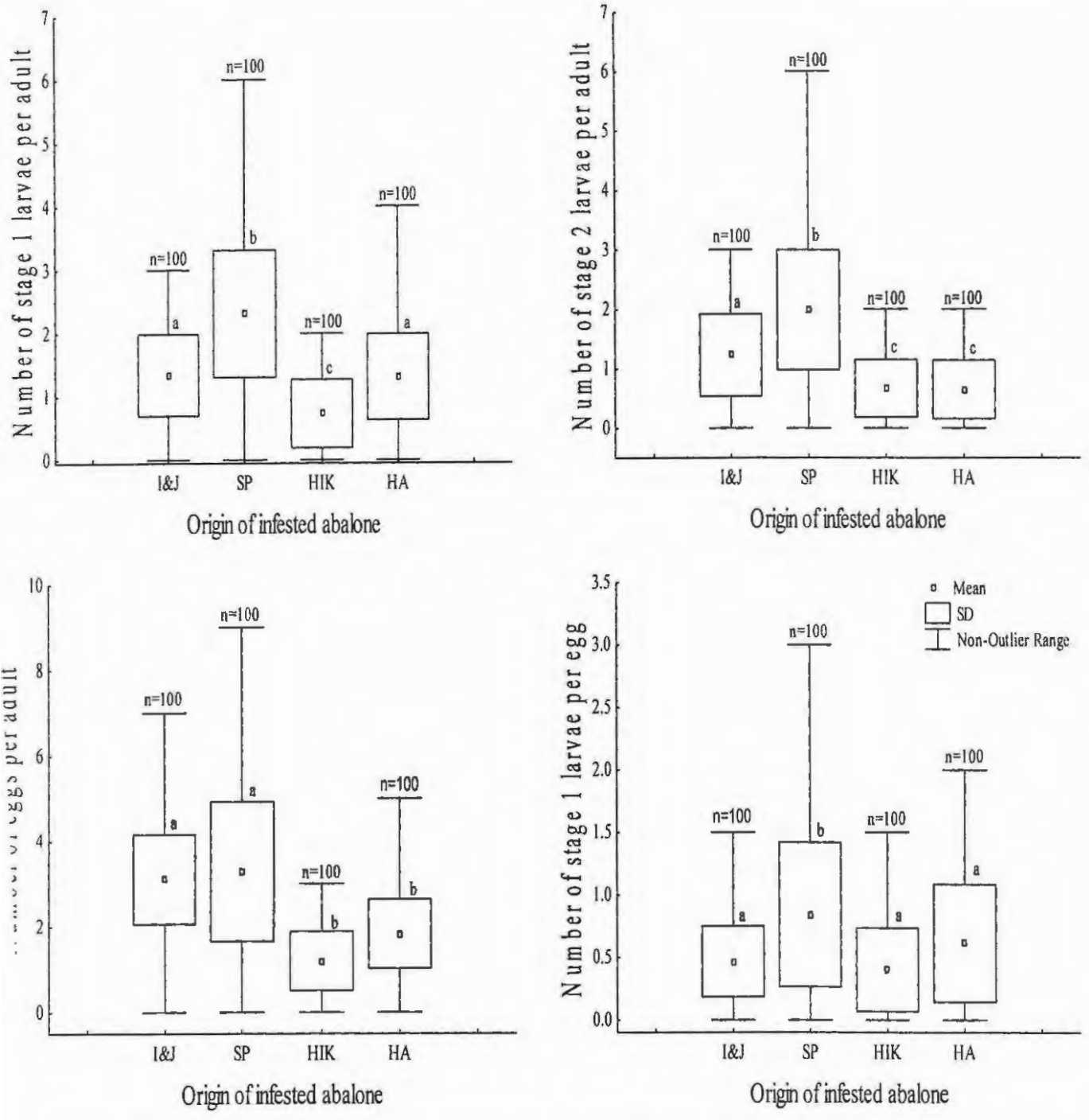

Figure 3.3: The number of larvae and eggs produced per adult for sabellids from four abalone farms. The p-value at which pairwise comparisons were considered significant was set at $p \leq 0.0083$ due to the Bonferroni- correction; different letters indicates significant differences. (Where: I&J = Irvin & Johnson abalone culture division; SP= Sea Plant Products; HIK= HIK abalone farm and HA= Hermanus Abalone).

Egg volume and number of eggs per brood

The volume of eggs produced by sabellids obtained from I&J was significantly lower than that from the other farms (H=203,6; $p \leq 0.0001$). The average egg volume of sabellids from Sea Plant Products, HIK and Hermanus Abalone was not significantly different between these farms (H= 2.89; p= 0.24), but all were significantly greater than for those eggs produced by sabellids from I&J (Figure 3.2). The mean egg volume recorded from each farm was ranked as follows; HIK=SP=HA>I&J. An increase in adult length was significantly correlated with an increase in the number of eggs per brood for sabellids for the four farms (Table 3.1). The number of eggs produced per adult was influenced by the farm from which the abalone were sampled (H= 20.9; $p \leq 0.0001$) (Figure 3.3). The number of eggs per sabellid varied up to a maximum of ten eggs per adult. I&J and Sea Plant Products sabellids produced significantly more eggs per adult (Up to a maximum of 7 and 10 eggs per adult, respectively) than HIK and Hermanus Abalone sabellids (up to 5 and 6 eggs per adult, respectively). The number of eggs per adult brood for each farm ranked as follows: I&J=SP>HIK=HA.

Number of larvae per adult and larvae morphometrics

Sea Plant Products sabellids produced the greatest average number of stage 1 larvae per adult (2.3 larvae) (Figure 3.3). The number of stage 1 larvae per adult was lowest in sabellids from HIK (0.7 larvae). Both I&J and Hermanus Abalone sabellids had a similar number of stage 1 larvae per adult (1.3 larvae and 1.3 larvae) (Z= 0.46; p= 0.65). A significant, but low correlation existed between the number of stage 1 larvae and the adult length for all farms (Table 3.1). Thus, only a low percent of the variation in the number of stage 1 larvae per adult could be explained by the variation in adult length. The number of stage 1 larvae per adult for the four farms ranked as follows: SP>HA=I&J>HIK.

The number of stage 1 larvae per egg gives an indication of the proportion of eggs that hatch viable offspring. Sea Plant Products sabellids had a significantly higher number of stage 1 larvae per egg than all other farms (0.9 larvae) (Figure 3.3). The number of stage 1 larvae produced per egg was not significantly different between HIK (0.4 larvae), Hermanus Abalone (0.6 larvae) and I&J sabellids (0.5 larvae) ($H=2.6$; $p= 0.063$). The number of stage 1 larvae per egg ranked as follows: SP>IJ=HA=HIK.

Sea Plant Products produced a greater number of stage 2 larvae per adult than any of the other three farms ($H= 55.3$; $p\leq0.0001$). HIK and Hermanus Abalone sabellids did not produce significantly different numbers of stage 2 larvae per adult ($Z= 0.22$; $p= 0.82$), but both produced a significantly lower number of stage 2 larvae per adult than Sea Plant Products and I&J (Figure 3.3). The number of stage 2 larvae produced per adult for each of the different farms ranked as follows: SP>I&J>HA=HIK.

The coefficient of variation (CV) ranged between 120% and 16% across the life history traits measured (Table 3.2). Offspring count and intensity showed the highest variation (Average CV =88%), whilst morphometric data and occupation level showed lower variation (Average CV= 21%).

Table 3.1: Coefficients of determination for the relationships between adult length and the number of eggs and larvae per adult for sabellids extracted from abalone obtained from the four farms. (Where I&J = Irvin & Johnson abalone culture division; SP= Sea Plant Products; HIK= HIK abalone farm and HA= Hermanus Abalone).

Independent variable	Dependent variable	Origin of sabellids	r^2(%)	p-value
Adult length	Number of eggs per adult	I&J	26	p≤0.0001
		SP	32	p≤0.0001
		HIK	16	p=0.0002
		HA	4	p=0.0027
Adult length	Number of stage 1 larvae per adult	I&J	29	p≤0.0001
		SP	19	p≤0.0001
		HIK	14	p≤0.0001
		HA	9	p=0.041

Table 3.2: The coefficient of variation of life history traits measured for sabellids extracted from abalone from all farms combined.

Variable	Coefficient of variation (%)
Number of larvae per egg	120
Number of larvae per adult	90
Number of eggs per adult	77
Intensity (tubes per cm)	64
Egg volume (mm^3)	33
Adult Length (mm)	21
Occupation level (% of tubes occupied)	20
Stage 1 larvae length (mm)	20
Base width (mm)	20
Stage 2 larvae length (mm)	16

DISCUSSION

Plasticity of life history traits

There were significant differences in the morphometric characteristics and offspring counts of sabellids between the four farms. However, it is problematic to isolate any single factor as the cause for these differences. Within each farm, and within each raceway, there is a multitude of different environmental and management-related influences that shape the conditions the sabellid populations are subjected to (Chapter 2). Individual factors may influence the sabellids more than others, but under culture conditions several factors may interact with one another resulting in the environment the sabellids are exposed to. The sabellids from Hermanus Abalone and HIK were not significantly different from each other in any of the morphometric features. These farms are located next to each other and share similar management procedures and system design characteristics (See Chapter 2). The differences in life history traits of sabellids between Hermanus abalone and HIK and the other two farms were more marked and there were significant differences in both the number of offspring produced and their morphometric characteristics. All offspring counts were significantly greater in Sea Plant sabellids than in those from both Hermanus Abalone and HIK. The reason for conducting this study was to gain insight into how the sabellid responds to the different conditions found on each of the farms, and to determine which of the sabellid's morphological and reproductive characteristics are most variable. In other chapters of this thesis potentially important factors will be investigated in isolation. These factors are diet and diet history of the abalone and abalone growth.

Plasticity of reproduction and development has been documented for many species (Grahame & Branch, 1985; Qian & Chia, 1991; Reznick & Yang, 1993; Giangrande *et al.*, 1994; Qian, 1994; Giangrande, 1997). It is defined as changes in an organism's phenotype in response to a change in some aspect of the environment (Reznick & Yang, 1993). Coefficient of variation was used to estimate which morphometric features and reproduction-related variables showed the greatest degree of plasticity. It was assumed that the higher the coefficient of variation, the greater the plasticity. The number of offspring and the shell edge tube count showed greater variation than the morphometric measurements (Table 3.2). Qian and Chia (1991) indicated that egg size is relatively constant within a species and that the number of eggs varies depending on environmental conditions. By comparing the range of the coefficient of variation for the egg and larvae counts (33- 106%) to the morphometric measurements of the eggs and larvae (16- 33%), it appears that the sabellid has the ability to respond to different environmental conditions by changing the number of offspring rather than the size of the various life stages. This is further supported by the fact that the size of the larvae and eggs was similar between farms, by contrast the number of eggs and larvae showed greater variability between the farms. This observation supports the hypothesis that the number of offspring is altered in the event of environmental change rather than the size of the offspring. Such flexibility is the means by which the sabellid appears to alter the expression of its life history traits in order to maximise its fitness in a changing environment. This forms a fundamental aspect of the theory of *r*- and -*K* selection (MacArthur & Wilson, 1967; Pianka, 1970).

Placement of Terebrasabella heterouncinata *into the r- and K- concept*

Polychaetes exhibit a great variation in form and adult size. They range from less than 1 mm to well over three meters (Rouse, 2000). According to the concept of *r*-and *K*-selection, a *K*-selected species would have a faster growth rate and a greater final adult size than *r*-selected species (MacArthur & Wilson, 1967; Pianka, 1970). Sabellids sampled in this study ranged between 0.9 mm to 4.5 mm body length. (Table 3.3). Within the family Sabellidae, *Fabriciola minuta* is the smallest species, attaining a maximum size of 0.85 mm (Rouse, 2000). *Schizobranchia insignis* is the largest recorded species in the family, reaching a maximum body length of 260 mm (Rouse, 2000). It is, however, not correct to place *T. heterouncinata* into the *r-K* continuum based on the absolute length of the adult. It is only possible to place different populations of the same species on the continuum as different species have different adult lengths. Only worms with offspring in their burrow were used in the analysis to avoid taking sabellids which were not fully grown. If comparisons are made between the four farms, the adult lengths of the sabellids were affected by the farm environment and are ranked as follows: Sea Plant=I&J>HA=HIK.

The timing of the onset of maturity is an important indication as to the life-history strategy employed by a species. Animals living in unpredictable environments, characterised by high adult mortality, tend to reproduce as soon as possible and maximise the reproductive effort in each reproductive event to produce as many progeny in as short a time as possible (Begon & Mortimer, 1986). A high intrinsic rate of increase (*r*) will be the result of producing offspring early in life (Giangrande *et al.*, 1994). Semelparity is the reproductive strategy where an individual breeds only once in its life. Breeding once or

more in an organism's lifetime is referred to as iteroparity (Grahame & Branch, 1985; Giangrande *et al.*, 1994; Giangrande, 1997). A high reproductive effort, early first reproduction and semelparity typify *r*- selected species. *K*-strategists are characterised by a delayed onset of maturity, low reproductive effort per event and continuous breeding (Grahame & Branch, 1985). *T. heterouncinata* matures after approximately four months (Ruck, 2000; Simon *et al.*, 2002), has a low number of offspring per reproductive episode and exhibits iteroparity, suggesting it is a *K*-selected species (Table 3.3 and 3.4).

Within the Polychaeta there is a vast diversity of reproductive and developmental modes. At least one-quarter of polychaete families are known to have more than one mode of fertilization and development (Rouse, 2000). Wilson (1991) reviewed sexual reproduction in polychaetes and identified 17 modes based on the larvae and site of development. He identified three broad categories of development;

1) Free spawning with no larval care; 2) brooding of larvae; 3) using gelatinous encapsulation of larvae (Wilson, 1991). *T. heterouncinata* broods its eggs in its burrow, exhibiting a high degree of parental care, placing it amongst the *K*-selected species. *r*-selected species show little or no parental involvement once fertilization has occurred. Of the 342 species of polychaetes reviewed by Giangrande (1997), over 50% of the species were brooding forms. The relationship between brooding and small adult size has been frequently shown in marine invertebrates (Strathmann and Strathmann, 1982; Giangrande , 1997). One hypothesis is that species with small adults are not able to produce enough young to risk an unprotected embryonic or larval phase (Strathmann and Strathmann, 1982). Another possible explanation is that for larger organisms, fecundity increases disproportionately with the area available for brooding and they are therefore less able to retain and ventilate the eggs (Strathmann and Strathmann, 1982).

Egg size is an important life history trait, being an indicator of energy investment per offspring. Of the sabellid polychaetes reviewed by Rouse and Fitzhugh (1994), egg volume ranged between 0.0007 mm^3 and 0.065 mm^3 (mean =0.0086 mm^3). *K*-strategists tend to produce larger eggs that are rich in yolk; *r*-strategists produced small eggs with a low energy content (Grahame & Branch, 1985; Balon, 1989; Giangrande *et al.*, 1994; Giangrande, 1997). Although the energy content of the eggs was not quantified for this study, egg size is considered to be related to the energy stored for later development (Giangrande *et al.*, 1994). Hence, a larger egg is assumed to have a higher energy content. The volume of eggs obtained from sabellids in this study ranged between 0.008 mm^3 and 0.06 mm^3, thereby placing the sabellid amongst the *K*-strategists. In future studies, egg size should be expressed in relation to adult size. While large animals may have a wide range of egg sizes, small animals are limited to producing small eggs. Data available from other studies do not allow for comparison of egg size in relation to adult size.

The number of eggs produced per adult is dependent on both the adult size and the size of the eggs, with smaller species tending to produce fewer large eggs than large species (Giangrande, 1997). The number of eggs produced per adult sabellid ranged up to ten eggs, with an average of 4 per adult. Giangrande (1997) summarised information on the reproduction of Polychaetes for over 500 taxa and found that the number of eggs produced per adult per reproductive event ranged between 1 and one million. Within the Sabellidae, the number of eggs produced per reproductive event ranged between one and 658 000, indicating that, when comparing the number of eggs, *T. heterouncinata* is strongly *K*-selected (Table 3.3 and 3.4). Although no information was available to compare larval size and number, several studies have indicated a close correlation between egg size and offspring size at hatching (Bagenal, 1965; Crump, 1984; Qian &

Chia, 1991). The larval size was relatively large as they hatched from large eggs and were non-feeding, indicating large amounts of yolk present in the body cavity for sustaining the larvae until settlement. This would place the sabellid amongst the *K*-strategists in terms of larval size.

The mode of larval development may also be used to classify a species according to the concept of *r*- and *K*- selection. Giangrande (1997) showed that amongst brooding Polychaetes, direct development occurred most frequently (59% of species), followed by planktonic development (25%), and the least common mode was lecithotrophic development (Giangrande, 1997). Planktonic development is characterised by the release of many small embryos into the water column that have to utilise a different resource to that used by the adult (Grahame & Branch, 1985). This mode allows for maximum dispersal of the progeny, but there is high mortality and very little parental investment per egg, placing these species amongst the *r*-strategists. At the other end of the *r*-*K* continuum for larval development modes is direct development. During direct development the embryonic stage is followed by morphogenesis of the juvenile, without an intermittent larval phase (Giangrande *et al.*, 1994). This mode is characterised by the production of a few large, energy-rich eggs that are most often brooded by the adult. Mortality is low but the parental investment per offspring is high, placing it at the *K*- selected end of the continuum. The sabellid larvae are derived from a few large eggs that are brooded within the adults burrow, but are different in that they have a larval phase. The larval phase is non-feeding and is termed lecithotrophic. As there are three categories of larval development mode, it is not possible to position the worm on the r- K continuum with respect to this trait and it is only suitable to indicate that lecithotrophy is less *K*- selected

when compared to direct development but more *K*- selected with respect to planktonic larval development.(Table 3.3 and 3.4).

This study has shown that *T. heterouncinata* is essentially a *K*-selected species (Table 3.4), characterised by few relatively large young, iterated breeding and a high degree of parental care. If it is assumed that a natural population of sabellids is living under optimum conditions, data collected from a wild population would provide baseline data with which a comparison could be made with sabellid populations from abalone farms. This would provide a better indication as to the extent of flexibility shown by life history traits of the sabellid under culture conditions. No data were collected from wild caught populations for this study, but future research into this area should take this into account.

Table 3.3: A summary of the life history traits of polychaetes reviewed by Giangrande (1997) and the comparison with traits measured for *Terebrasabella heterouncinata* during this study. Adult sizes, egg sizes and egg numbers for the *r*- and *K*- strategists represent the minimum and maximum values from the available literature (Giangrande, 1997; Rouse and Fitzhugh, 1994).

Life History Trait	***r*-strategists**	***K*-strategists**	*T. heterouncinata*
Adult Size	Small (0.85mm)	Large (260mm)	0.9- 4.5 mm
Reproductive effort per spawning	Large	Small	Small
Reproductive strategy	Semelparity	Iteroparity	Iteroparity
Parental care	Absent	Present	Present
Egg Size	Small (50 μm)	Large (1000 μm)	100 – 240 μm
Egg number per brood	Few (1)	Many (1,000,000)	0-10
Larval size	Small	Large	Large
Larval development mode	Planktonic→ Lecithotrophic→ Direct		Lecithotrophic

Table 3.4: A schematic representation of the position of various life history traits on the *r-K* continuum relative to polychaete species reviewed by Giangrande (1997). The blocks represent the range of traits measured for *Terebrasabella heterouncinata* relative to the minimum and maximum observed values for other polychaete species.

		r- strategist		*K- strategist*
Parental care	Other polychaetes	Absent	Gelatinous mass	Brooding
	T. heterouncinata			Brooding
Reproductive effort per brood	Other polychaetes	Large		Small
	T. heterouncinata			Small
Egg volume (mm^3)	Other polychaetes	0.0007		0.065
	T. heterouncinata			***0.027***
Egg number per brood	Other polychaetes	1000000		1
	T. heterouncinata			***4***
Larval length (mm)	Other polychaetes	Small		Large
	T. heterouncinata			Large
Reproductive strategy	Other polychaetes	Semelparity		Iteroparity
	T. heterouncinata			Iteroparity
Larval development mode	Other polychaetes	Planktotrophic	Lecithotrophic	Direct Development
	T. heterouncinata		Lecithotrophic	

Discussion of the sampling method

Two methods have been used for removal of sabellids from abalone shells (Culver *et al.*, 1997). Sabellids can be obtained by crushing the shell, rinsing the debris, and collecting the worms and offspring that have been dislodged from their burrows. This method is destructive and can damage the various life stages of the sabellid, rendering them useless for measurements. The second method involves partially dissolving the abalone shell with an acidic solution, softening the shell, allowing for easier removal of individual worms (see Chapter 2). For this study, the shells were dissolved in the acid solution and the adult sabellids and the offspring specific to those adults were removed from individual burrows with thin glass rods (see Chapter 2). The advantage of sampling the adult sabellids together with the eggs and larvae specific to that adult was that it was possible to relate morphometric measurements of the adults to the size range and number of its offspring. When crushing the shells, only a representative sample of eggs and larvae can be collected from a shell and it is not possible to relate offspring number or size to individual adults. It is also not possible to ensure that only adults, brooding sabellids are being sampled. An additional advantage to the method used in this study was that the offspring were not damaged during extraction. The only disadvantage to the method used for extraction of the worms was the time taken to sample and measure each individual adult and its offspring. Thus, sample sizes were small.

When counting the number of tubes on the growing edge of an abalone shell (Chapter 2) it is imperative that only the larvae of the same age or shell layer are counted. The greater the amount of time since a larvae has settled on the shell edge, the more obscured it becomes due to the deposition of nacre over the larvae by the abalone. The abalone shell edge is translucent and this makes it difficult to judge the depth of a larval tube within the

shell. Thus, a major problem with counting of tubes is that it has to be subjectively determined which larvae are considered "newly settled" and which are not. A potential solution for this problem is to fill in the intended area of the shell edge from which the larvae counts will be made with a pencil. Once the area has been covered with pencil marking, the abalone will be placed back into the system and left for a minimum of 48 hours to allow for new settlement to occur. After that time, the abalone will be removed and the shell edge can be examined for the presence of larvae over the pencil markings. The pencil markings contrast with the white tubes of the settled larvae allowing for easier counting. As the time since the abalone was placed back into the system was known, it is possible to determine the number of larvae settling in a known time period, reducing inconsistencies created by counting larvae from different shell layers. This research approach was addressed in Chapter 6.

CHAPTER 4

The effect of abalone diet history on the morphometric and reproductive characteristics of *Terebrasabella heterouncinata*

INTRODUCTION

Under natural conditions the sabellid polychaete, *Terebrasabella heterouncinata*, infests various mollusc species (Ruck, 2000). Sabellid infestations of the abalone, *Haliotis midae,* in the natural environment appear to be minimal and the abalone is not severely affected by their presence (Culver *et al.*, 1997). Under farm conditions, however, the sabellid population growth can reach epidemic proportions and there are often severe consequences for the host abalone, including reduced growth rate, a weakened and deformed shell and mortality. These problems associated with a high infestation level result in increased production costs and reduced profits for the abalone farmers and there is a need for the development of a protocol to control sabellid infestations.

Attempts to control *T. heterouncinata* under commercial settings have included the manipulation of water temperature, coating of the abalone shells with wax, quarantining of infested stocks, the use of novel therapeutic delivery systems using micro-encapsulation, ultrasound and improved sanitary practises (Oakes and Fields, 1996; Leighton, 1998; Ruck & Cook, 1998; Finley *et al.*, 2000; Loubser, *unpublished*). Unfortunately, most of these techniques have had limited success as sabellid infestations re-occur. Therefore, there is a need to develop a sound understanding of the sabellid's life history characteristics and the factors that influence them. This will help to establish ways for minimising the detrimental effects of infestations.

Diet is considered to be one of the most important ecological factors influencing reproduction and growth of marine invertebrates (Qian, 1994) and is considered to have a strong influence on the sabellid under farm conditions (Chalmers, 2002; Simon *et al.*, 2002). Abalone farmers in South Africa suggest that infestation by the sabellid is related to the diet fed to the abalone and the tank cleaning regime employed on the farm. However, a comparison between the management procedures used by the different farms and sabellid reproduction and growth is difficult due to the competitive and secretive nature of the South African abalone industry (Chalmers, 2002).

The majority of abalone farmers in South Africa feed their abalone either naturally occurring kelp (*Ecklonia maxima)* or the formulated abalone feed, Abfeed. These diets differ in both their nutrient composition and physical properties. Kelp is commercially harvested from the sea, and collection is therefore subject to suitable harvesting conditions. Abfeed is more convenient and cost-effective as it is readily available year round and the nutrient and energy levels have been tailored to ensure optimum abalone growth and hence farm production (Chalmers, 2002). Farmers speculate that the use of Abfeed is associated with higher sabellid infestation levels (Abalone Farmers Association of South Africa, *pers. comm.*). Formulated diets are known to leach nutrients into the water and increase the amount of particulate matter in the raceways (Chalmers, 2002). This results in a nutrient-enriched environment that may be favourable to the sabellid. Studies have shown that the diet fed to the abalone influenced reproduction and growth of the sabellid under culture conditions (Chalmers, 2002; Simon *et al.*, 2002). However, in these studies the effect of a change in diet on sabellid growth and reproduction was not investigated. For example, the study by Simon *et al.* (2002) was a laboratory study in which the two diets, kelp and Abfeed, were compared. Chalmers (2002) worked on host

abalone held under farm conditions but could not quantify the effect of the diet history as the abalone had been fed on their respective diets for various durations. Although diet has been suspected as an important factor controlling sabellid population growth, it has not been established if and how a sabellid population would respond to a change in diet and how quickly such a response would take place. Since some abalone farmers change the diets from Abfeed to kelp as part of their management practises the results from a study on diet history have potential applications for the control of sabellids.

The aim of this study was to show if or how the reproductive characteristics and growth of the sabellid changed in response to the diet history of their host abalone. Reproduction and growth of sabellids that had experienced a dietary change was compared to those that were not exposed to a change in diet to determine which characteristics became more *r*- or *K*-selected with the change in diet. To achieve this aim, infested abalone were held under different diet histories to determine the response of the sabellid worm's reproductive characteristics and growth to a change in diet. The suitability of Chalmers's (2002) indices of adult sabellid condition and reproduction was evaluated using regression analyses to relate adult morphometrics and the number of eggs per brood.

From the information provided the following hypotheses were set up:

H_0: A change in host abalone diet from kelp to Abfeed and Abfeed to kelp does not influence the sabellid intensity or occupation level.

H_a: A change in abalone diet from Abfeed to kelp affects the number of tubes on the abalone shell edge and the number of tubes occupied by larvae.

H_0: A change in diet history of the host abalone does not affect the morphometric or reproductive characteristics of *T. heterouncinata*.

H_a: Sabellids from abalone that experienced a diet change from kelp to Abfeed will be of different size and produce a different number of offspring than sabellids from abalone fed kelp followed by Abfeed.

MATERIALS AND METHODS

Experimental design

To test the effect of the abalone diet on the growth and reproduction of the sabellid, 400 uninfested abalone were experimentally infested by bringing them into contact with abalone that carried the worm.

The uninfested abalone were randomly selected from Aquafarm development on the 5th October 2001. All uninfested abalone had been fed kelp before they were brought into contact with the infested abalone. These animals were divided into four treatments of 100 abalone each. Each treatment was replicated twice, thus containing 50 abalone per basket (Figure 4.1). To each experimental basket ten heavily infested abalone were added for 185 days.

The infested abalone, sampled on the 28th September 2001, originated from two farms, I&J and Sea Plant Products. The mean shell length ± standard deviation of abalone sampled was 60.5 ± 1.56 cm and 56.9 ± 1.05 cm for I&J and Sea Plant products, respectively. Abalone from I&J were fed Kelp and those from Sea Plant were fed Abfeed. These abalone were labelled and the intensity of sabellid infestation and occupation level (Chapter 2) were recorded before the abalone were assigned to the experimental baskets (Figure 4.1).

Thus, the independent variable tested was the abalone diet fed to the mixed population of infested and uninfested abalone. The dependent variables were growth and reproduction of the worm on newly infested abalone as well as growth performance of their hosts. The

400 uninfested abalone were divided into four treatments: In two treatments abalone that had been fed kelp or Abfeed before the study were kept on their respective diets, and in two further treatments the diet was changed at the start of the study and the new diet was fed throughout the 185 days of the experiment.

The treatment codes used in this chapter are:

AA: Abfeed fed before and during the study

KK: Kelp fed before and during the study

AK: Abfeed fed before the study and then kelp for the 185 days of the experimental period

KA: Kelp fed before the study and then Abfeed for the 185 days of the experimental period.

It was thus possible to a) evaluate the growth and reproduction of sabellids on previously uninfested abalone in response to the diet fed to their hosts over the experimental period of 185 days; b) to test the effect of a change in diet from kelp to Abfeed on the growth of the abalone and their susceptibility to new infestations; and c) to describe any changes in sabellid intensity and tube occupation level on abalone that had experienced a diet change versus those that had been fed on the same diet before and during the study.

The experimental tanks were cleaned once every two weeks for the duration of the experiment. The stocking density was maintained at 20% of the total surface area available on the plates and the abalone were not size-sorted during the 185 days of the study.

Infesting abalone

After 185 days each basket was removed from the holding tank and the abalone were anaesthetised in a 7% solution of magnesium sulphate in seawater. The infesting abalone from each basket were shucked and their shells preserved in a 2.5% solution of gluteraldehyde in seawater buffer. The intensity and occupation level was determined by counting the number of larvae settled on the growing edge (Chapter 2). This allowed for the evaluation of the change in the occupation rate and intensity of sabellid infestation over the 185 days. The length and width of the shell of all infesting abalone was recorded to determine the growth of the abalone over the 185 days of the study. These abalone will be referred to as the "infesting abalone" throughout this chapter.

Removal of worms

A sample of twenty previously uninfested abalone was randomly taken from each basket and each animal was shucked and the shells preserved in 2.5% gluteraldehyde in filtered seawater. The number of sabellids settled on the growing edge was counted for each abalone to determine intensity and occupation level. The length and width of the shells was measured. Each shell was then placed in a 6.5% solution of nitric acid in 70% ethanol. The nitric acid reacts with the calcareous component of the shell and softens it. This allows for easier extraction of the worms from the burrows. Thin glass rods were used (see Chapter 2) to pull the adult sabellids, and offspring specific to that adult, from the burrow. Worms were extracted from the inner shell surface, mostly from around the area where the abalone foot attaches to the shell. The adult worm and its offspring and eggs were then placed in individually labelled containers containing 2.5% gluteraldehyde. Five worms were removed from each shell.

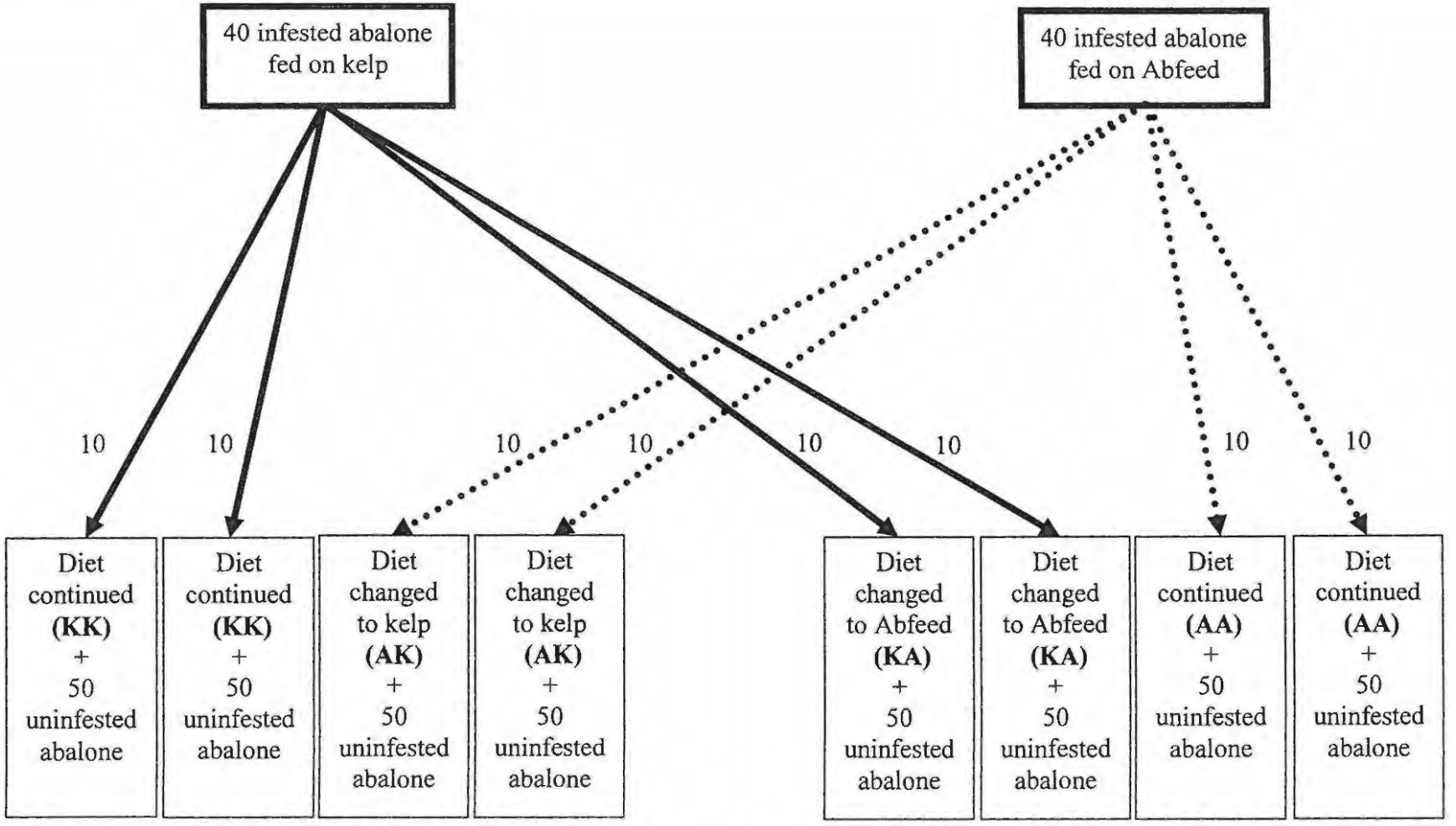

Figure 4.1: A schematic diagram of the experimental design used for testing the influence of a change of abalone diet on sabellid body measurements and reproductive characteristics. Numbers refer to the number of abalone in each of the respective treatments.

Once a worm had been removed and preserved, it was placed under a binocular dissecting microscope on a petri dish. The length, width at the basal flange of the feeding crown and width of the widest part of the abdomen of the adult worm was measured (Chapter 2). The length and width (at the widest part) of the eggs and larvae was also measured. Counts were done to determine the number of eggs and larvae per adult.

Statistical analysis

The non-parametric Kruskall- Wallis ANOVA by ranks test was used to determine differences in sabellid body measurements, offspring counts, morphometric ratios, intensity and occupation level between worms from abalone with different diet histories. The Bonferroni correction of the p-value was applied to pair-wise comparisons of treatments to arrive at the p-value used for the Mann-Whitney U-test. For this, the level of significance was determined by dividing 0.05 by the number of pre-planned pairwise comparisons. For all data tested with the Mann-Whitney U-test, the null hypotheses were rejected at $p \leq 0.05 / 6 = 0.0083$. The H-statistic refers to the results obtained for comparisons between variables using the Kruskall- Wallis ANOVA by ranks test and the Z-statistic refers to the Mann-Whitney U-test results.

Regression analysis was used to determine the relationship between number of eggs per adult and egg volume. The relationship between the number of eggs per brood and neck width: base ratio, base width and adult length was also evaluated using regression analysis.

For the infesting abalone the sabellid intensity and the occupation level from the beginning of the study was compared with that measured at the end of the study to

determine the changes in those variables that had occurred during the study. For both variables, the measurements taken at the end of the study were subtracted from those observed at the start. This provided the change that had taken place in both variables during the 185 days. The intensity refers to the number of newly settled worms on the growing edge of the shell. One week after larval settlement the tubes become obscured by the deposition of nacreous material by the abalone. Therefore, only larvae less than one week subsequent to settlement on the shell edge were used to determine the intensity. Thus, where the number of tubes decreases with time, it implies that the number of newly settled larvae had decreased, not the total number of larvae on the growing edge.

Figure 4.5 provides a comparison of the changes in reproductive and morphometric traits that have occurred after a change in diet. The Abfeed-only and kelp-only groups were taken as the control groups to which the changes in the expression of a particular sabellid life history trait for the kelp- Abfeed and Abfeed- kelp treatments were compared. The change in life history traits in the Abfeed- kelp and kelp-Abfeed treatments were expressed as a percentage of these reference values. Only those variables that were significantly different from the kelp-only and Abfeed-only diets were used for this comparison.

RESULTS

Abalone diet history did not significantly influence the number of larvae per adult (H=2.29; p= 0.51) and the number of larvae per egg (H=2.87; p= 0.41). For all treatments the mean number of larvae per adult and larvae per egg for the four treatments was 0.6 and 0.4, respectively. Diet history had a significant effect on all other sabellid morphometric measurements and offspring counts.

Morphometric measurements

The sabellids from abalone of the kelp-Abfeed treatment had a significantly smaller adult length (H= 83.5; p≤0.0001) than sabellids from all other treatments (Figure 4.2). The adult length of the sabellids from the other three treatments was not significantly different from each other (H = 1.4; p= 0.13). The Abfeed-only group produced sabellids with the greatest base widths of all treatments (0.30 ±0.04mm) (Figure 4.2). No significant difference in base-width was observed between the kelp-Abfeed, kelp only and Abfeed-kelp treatments (0.28 ±0.03 mm, 0.28 ±0.03 mm and 0.27 ±0.04 mm, respectively) (H= 1.3; p= 0.28).

The larvae from the kelp-Abfeed treatment were significantly smaller than those from any of the other groups (H= 45.2; p≤0.001). The larvae length for the other three treatments was not significantly different between treatments (H= 3.9; p= 0.13) (Figure 4.2).

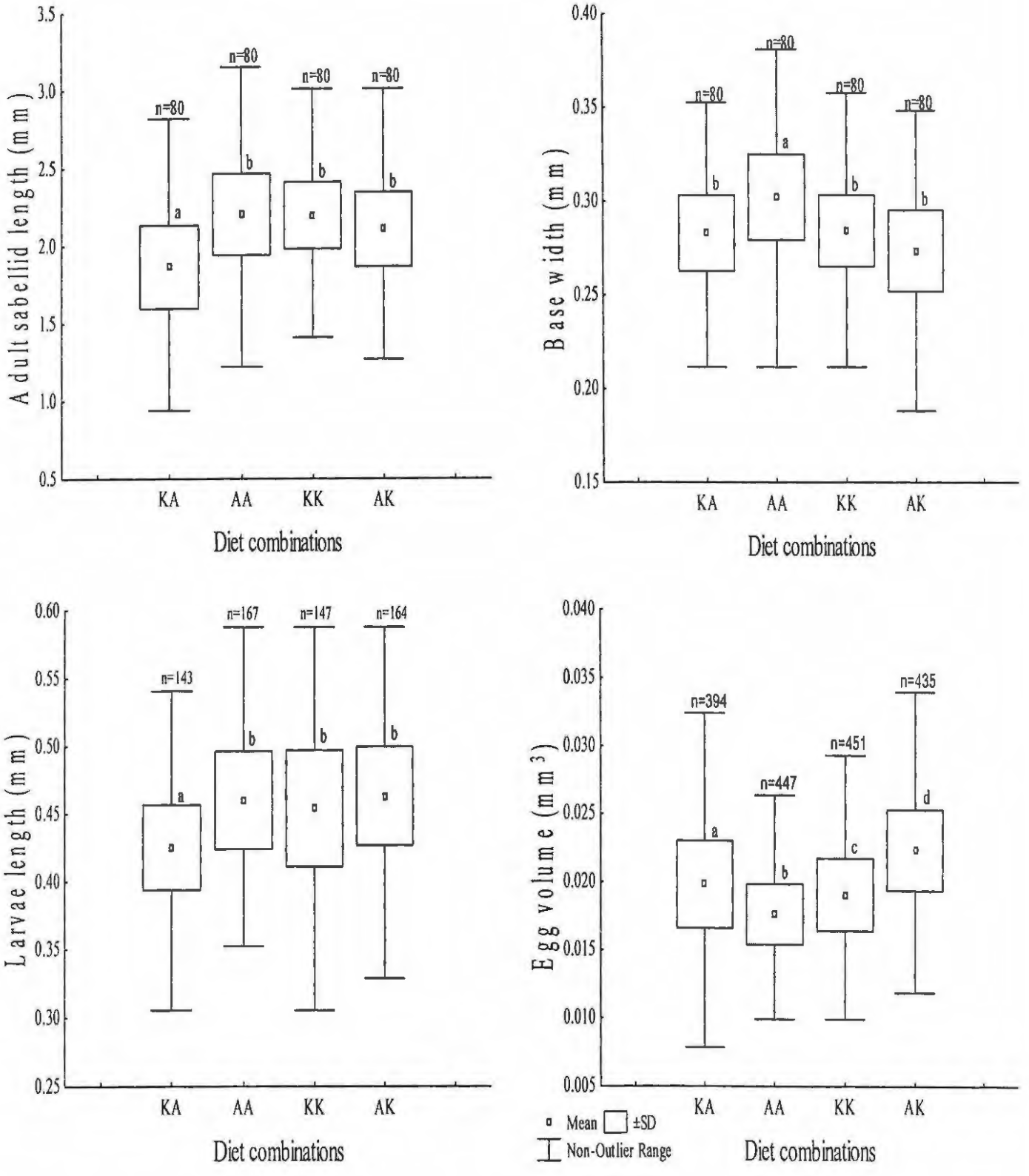

Figure 4.2: Adult length, base width, larvae length and egg volume for sabellids extracted from abalone fed different diet combinations. Where KA=kelp-Abfeed, AA=Abfeed only, KK=kelp only & AK=Abfeed-kelp. Different letters indicate significant differences ($p \leq 0.0083$).

Egg volume was significantly different between all treatments (H= 175.5; p≤ 0.0001). Egg volume was greatest in sabellids removed from abalone of the Abfeed-kelp group (0.021 ±0.007 mm^3). Sabellids from the kelp-Abfeed, kelp only and Abfeed-only groups produced progressively smaller eggs (0.020±0.005 mm^3, 0.019 ±0.005 mm^3 and 0.017 ±0.004 mm^3, respectively) (Figure 4.2). There was a significant but low correlation between the number of eggs per adult and egg volume in sabellids from the Abfeed-kelp and kelp only treatments (r^2= 23%; p= 0.0027 and r^2=15%; p= 0.044, respectively). Eggs from these sabellids decreased in volume as the number of eggs per adult increased. No such correlation could be obtained for the kelp-Abfeed and Abfeed-only treatments (p= 0.27 and p= 0.67, respectively).

Egg number per brood

Abalone diet history had a significant effect on the number of eggs per brood (H= 17.3; p≤0.0001) (Figure 4.3). Sabellids from abalone belonging to the kelp-Abfeed treatment had significantly less eggs per brood (1.9 ±1.1) than those from the kelp-only and Abfeed- only groups (Figure 4.3). All other treatments were not significantly different with respect to the number of eggs per brood (H= 2.9; p= 0.27). The results for the linear regression analyses for the number of eggs per brood and the neck width: base ratio, base width and adult length for all treatments are given in Table 4.1. The number of eggs decreased linearly with an increase in neck width: base ratio in the Abfeed- kelp treatment. No other significant relationships between the neck width: base ratio were found for any other treatments. An increase in the base width and adult length was positively correlated with an increase in the number of eggs per brood for all treatments (Table 4.1).

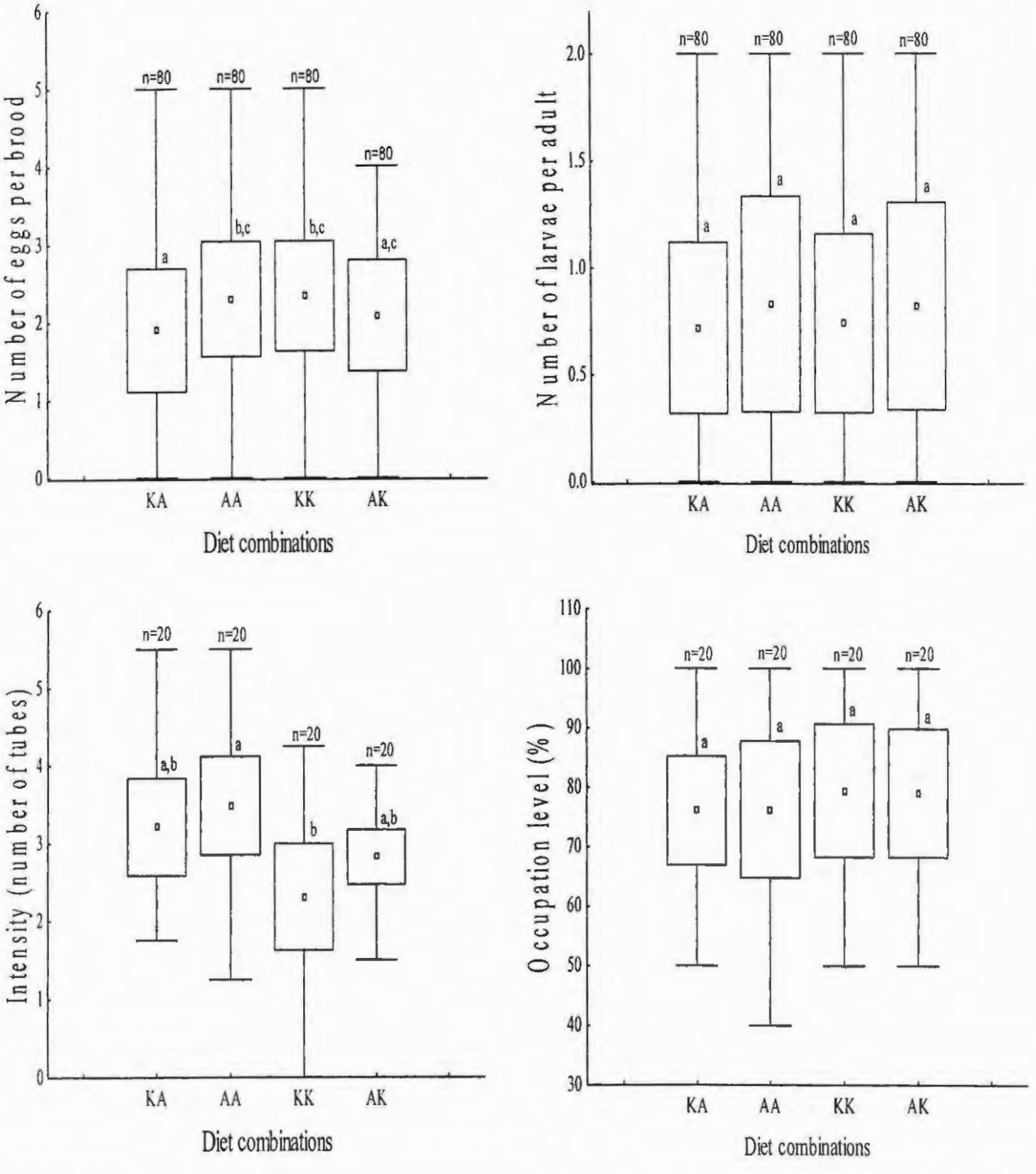

Figure 4.3: The number of eggs per brood, number of larvae per adult, intensity of infestation and occupation level of sabellids from abalone kept under different dietary conditions. Different letters indicate significant differences ($p \leq 0.0083$). Where KA=kelp-Abfeed, AA=Abfeed only, KK=kelp only & AK=Abfeed-kelp.

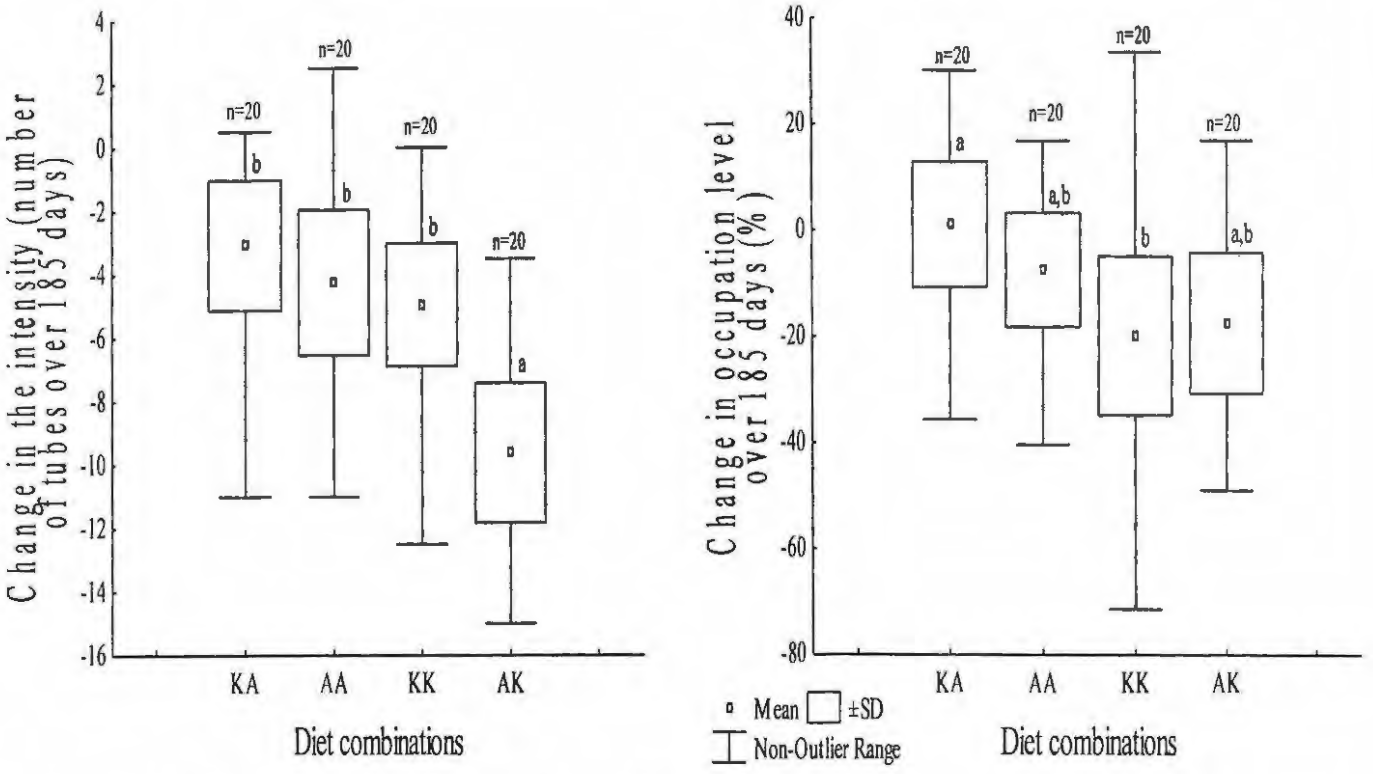

Figure 4.4: The change in the intensity of infestation and occupation rate of tubes for sabellids from the infesting abalone kept under different dietary conditions. Different letters indicate significant differences (p≤0.0083). Where KA=kelp-Abfeed, AA=Abfeed only, KK=kelp only & AK=Abfeed-kelp.

Intensity

The sabellid intensity was influenced by abalone diet history (H= 12.6; p= 0.006) (Figure 4.3). The Abfeed-only and kelp- only groups had significantly different sabellid intensities (Z= 3.1; p= 0.002). All other treatments were not significantly different with respect to intensity.

Change in intensity of infesting abalone

The decrease in the number of tubes on the shell edge of the infesting abalone during the study was greatest in the Abfeed-kelp treatment (9.6 tubes) and was significantly greater than in all other groups (Figure 4.4). The mean decrease in the intensity of infestation for

infesting abalone during the study for the kelp-only, kelp-Abfeed and Abfeed-only groups (4.9± 3.3, 3.1± 3.4 and 4.3± 3.8 tubes, respectively) were not significantly different between these treatments (H= 2.1; p= 0.59).

Occupation level

The occupation level (i.e. the percentage of the tubes that contained larvae) was not significantly different between treatments (H= 0.46; p= 0.93) (Figure 4.3). The mean occupation level ranged between 76.6% and 81.6% for all treatments.

Change in occupation level of infesting abalone

The occupation level decreased during the 185 day study for all but the kelp-Abfeed treatment (Figure 4.4). The decrease in the occupation level was highest in the kelp-only (19.7%) and Abfeed-kelp treatments (17.5%) followed by the Abfeed-only treatment (7%). The kelp- Abfeed group showed a small increase in occupation level during the study period (1%). The only significant difference in the decrease in occupation level occurred between the kelp-Abfeed and kelp-only treatments (Z =2.7; p= 0.006).

Growth of infesting abalone during the study

The increase in shell length of the infesting abalone during the study period was significantly influenced by the diet history (H= 30.6; p≤ 0.0001) (Figure 4.5). The mean ± standard deviation increase in shell length during the 185 days for the kelp- only (13.5 ± 2.2 mm) and Abfeed- kelp (13.9 ±2.6 mm) treatments was similar (Z= 2.0; p= 0.43) and was significantly greater than the kelp- Abfeed (9.8 ±2.6 mm) and Abfeed- only groups (10.8 ±2.6 mm)

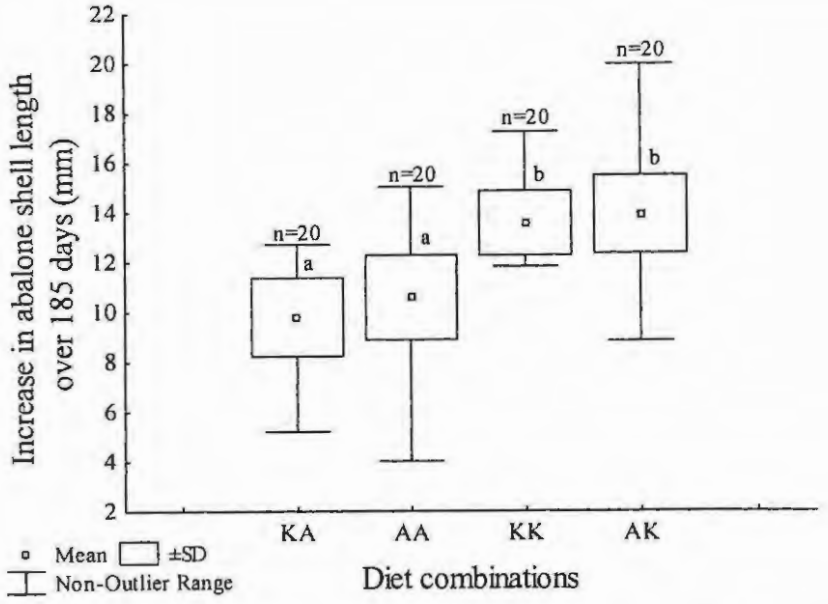

Figure 4.5: The increase in the shell length of abalone from different diet histories during the 185 study. Different letters indicate significant differences (p≤0.0083). Where KA=kelp-Abfeed, AA=Abfeed only, KK=kelp only & AK=Abfeed-kelp

Table 4.1: Coefficients of determination and p-values for the linear regression analyses of the number of eggs per brood and the neck width: base ratio, base width and adult length for all treatments.

	Number of eggs per brood vs:					
	Neck: base ratio		Base width (mm)		Adult length (mm)	
Treatment	r^2 (%)	p-value	r^2 (%)	p-value	r^2 (%)	p-value
Kelp-Abfeed	0.5	0.32	14.6	≤0.0001	38.1	≤0.0001
Kelp-only	1.5	0.09	13.6	≤0.0001	25.3	≤0.0001
Abfeed-only	0.18	0.55	12.2	≤0.0001	16.1	≤0.0001
Abfeed-kelp	9.6	≤0.0001	33.7	≤0.0001	25.2	≤0.0001

DISCUSSION

A change in diet history did not influence the sabellid intensity (tubes/ cm) or occupation level of tubes on the shell edge. With the exception of the number of larvae per adult, all other morphometric and reproductive characteristics of the sabellid were influenced by the diet history of the abalone. A comparison of the expression of life history traits of the kelp-Abfeed and Abfeed-kelp sabellids with those from abalone from the Abfeed- and kelp-only groups provides an estimate of the effect of a change in diet history on the sabellid population. The analysis of the expression of life history traits for sabellids from the mixed diets compared to those measured for either the Abfeed or kelp-only control groups showed a response to a change in diet (Figure 4.5). These changes occurred in a relatively short period. For example, the generation time, or time from larval settlement to the production of offspring for this sabellid species had been estimated at approximately four months (Ruck, 2000; Simon *et al.*, 2002). This study lasted 185 days, the equivalent of approximately 1.5 generations intervals of the sabellid. Therefore, changes occurring in either reproduction or morphometrics have occurred in a maximum of 1.5 generation intervals. No data could be found for other polychaete species for comparison of the rate at which changes in the expression of life-history- related variables takes place. Thus, it is unclear whether this change in the expression of life-history- related traits is slow or rapid relative to other species. Abfeed has a higher protein content than kelp (Britz, 1995) and speculation has arisen that the increase in the problems associated with the sabellid worm on South African abalone farms may be linked to their diet on the farms (AFASA, *pers. comm.*, Chalmers, 2002). Chalmers (2002) showed that Abfeed particulates had a higher protein and energy content and the size range of particulates was more suitable to the filter- feeding sabellid when compared to kelp. He suggested that the differences in the

nutritional and physical properties of the two diets may be responsible for potential differences observed in the intensity of sabellid infestation on the farms. The data from this study can make a contribution to this topic.

Intensity

A change in the diet from kelp to Abfeed or from Abfeed to kelp did not influence the intensity i.e., number of tubes on the growing edge. This is supported by a study conducted by Simon *et al.* (2003) who observed no dietary influence on sabellid intensity. Chalmers (2002) observed differences in intensity and occupation level with diet and indicated that Abfeed is a more suitable food source for the sabellid as it is more nutritious resulting in a higher sabellid intensity on Abfeed- fed abalone. However, Chalmers sampled abalone that had been exposed to the different diets for a longer time throughout the grow-out period suggesting that the 185 days of this study may not have been long enough to allow sufficient time for changes in the intensity to occur between the treatments. Although the occupation level was not different between any of the treatments, the intensity was significantly different between the kelp-only and Abfeed-only groups. This observation supports Chalmers's hypothesis that the nutritional value and suitability of Abfeed as a food source for the sabellid may potentially lead to a greater number of tubes on the shell edge (Chalmers, 2002). This observation also supports the suggestion that abalone have to be on the diet for more than 185 days to produce an effect. Therefore, larval settlement success appears to be affected by diet but at this stage, it is not clear what happens during the initial stages of settlement. Therefore, the influence of abalone diet on larval settlement success is addressed in Chapter 6. The mean number of tubes per centimeter of the growing edge in Chalmers's study was 5.5 tubes per centimeter. For this study the mean number of tubes for all treatments combined was 3

tubes per centimeter. A possible reason for the relatively greater number of tubes on the growing edge in Chalmers's study may potentially be due to a greater number of infested abalone in the raceways from which he sampled. Commercial abalone raceways contain up to 16 baskets of abalone. The experimental tanks used in this study contained one basket of abalone. Only ten infested abalone were used per basket in this study, therefore the number of larvae able to infest the abalone at any one time may have been lower than that in the raceways from which Chalmers sampled abalone. In addition, abalone from the Abfeed-only and kelp- only treatments had significantly different growth during the study. It has been suggested that slower growing abalone are more susceptible to sabellid infestations than fast growing abalone (Oakes & Fields, 1996; Clayden, 2000). Abfeed-fed abalone grew more slowly and had a significantly greater number of tubes on the growing edge compared to abalone fed kelp, indicating that abalone growth may possibly influence the intensity of sabellid infestation. Thus, it appears that both diet history and abalone growth are resulting in a response from the sabellid. A confounding factor creates a situation in which a measure of the effect of one factor is distorted because of the association with another factor that influences the variable under examination. Therefore, the difference in intensity can not only be attributed to diet due to the confounding effects of diet and abalone growth. Thus, to isolate the effect of abalone growth and diet, the influence of abalone growth rate on sabellid infestation was investigated in Chapter 5.

Tube occupation level

The occupation level of sabellid tubes on the growing edge was not different between any of the treatments. This implies that post-settlement mortality of larvae is not influenced by the diet fed to the host abalone within the first week after settlement. Chalmers (2002) observed differences in mean occupation level between diets and

showed that Abfeed-fed abalone had a higher sabellid occupation level than abalone fed kelp. He hypothesised that this may be due to differences in egg quality and larval survival between diets (Chalmers, 2002). Ruck (2000) suggested that fast growing abalone may be able to deposit enough nacre to smother the larvae soon after they settle, potentially reducing the occupation level of the tubes. The growth rate of the abalone during this study was different between treatments but occupation level was similar, suggesting that fast growing abalone are not able to cover the larvae with nacre at a rate which influences the occupation of tubes on the shell edge. Since there was no difference in occupation level between treatments the potential minimum abalone growth rate at which a reduction in larval occupation of tubes could not be determined. An investigation into the minimum growth required for a decrease in infestation may have considerable commercial application. Farmers with high levels of sabellid infestation may be able to curb the number of larvae surviving on the shell edge by ensuring abalone growth is maintained above a certain rate.

Morphometric measurements

Differences in adult length were observed between treatments. Diet had an effect on the size of adult sabellids in other studies (Chalmers, 2002; Simon *et al.*, 2002). In the abalone raceway Abfeed produces particulates of a higher nutritional content and of a more suitable size range for the sabellids than kelp (Chalmers, 2002). Both Chalmers (2002) and Simon *et al.* (2002) indicated that sabellids from abalone fed Abfeed were larger than those fed kelp. However, it had not been tested if sabellids on abalone that had experienced a diet change from kelp-Abfeed would be larger than those from the kelp-only and Abfeed-kelp groups. In this study, the kelp- Abfeed treatment sabellids were smaller than in all other treatments. The similarity in adult length between the Abfeed-

only and kelp- only groups suggests that other factors besides diet are influencing sabellid size within this initial period of settlement. Simon *et al.* (2002) using the Von Bertalanffy growth equation calculated that the worm reaches a theoretical maximum length after approximately four months. The study was therefore run for six months and only mature adults with offspring in their burrows were used for analysis. Thus, it appears that the study period was sufficient for adult worms to reach their maximum size and sampling of immature individuals was avoided.

The measurement of the base width of mature sabellids was taken to provide an indication of the reproductive condition of the worm. Chalmers (2002) used ratios of the different morphometric measurements for the first time to produce a condition index for the sabellid. Condition factor is commonly used in fisheries ecology and is based on length: weight data. However, it was impractical to weigh the sabellid due to their small size. To overcome this, Chalmers (2002) proposed the ratio of adult sabellid neck width: base width to provide an indication of the reproductive activity of mature sabellids. The base width was taken as the width of the abdomen across the 9^{th} and 10^{th} setigers, the site of oocyte production (Simon *et al.*, 2002). It was assumed that the base width increases in response to an increased number of eggs in these setigers (Chalmers, 2002). However, Chalmers did not count the number of eggs specific to individual adults and worked on the assumption that the width of the base was correlated with egg production. The results from this study could make a contribution for the first time. There was no significant correlation between the neck width: base width ratio and the number of eggs. The relationship between the adult length and the number of eggs per brood was the strongest of all the morphometric characteristics indicating that the adult length provides a good indication of the reproductive activity of the sabellid. This could be confirmed in future studies.

Egg number and volume

A change in abalone diet had a negligible effect on the number of eggs per brood for *T. heterouncinata*. Qian and Chia (1991) concluded that *Capitella* sp. modify their fecundity depending on the amount and quality of food available. Although there were differences in the number of eggs per adult they cannot be related to diet as both the Abfeed-only and kelp- only groups were similar and the difference between treatments where diet was changed was inconsistent. Winter (2001) showed differences in the number of eggs per brood between abalone fed different diets. Thus, sabellids exposed to the different diets for longer than 185 days may have produced different numbers of eggs per adult. However, as there was no difference in the number of eggs per brood between the kelp- and Abfeed- only groups, fed the same diet for longer than 185 days, diet did not influence the number of eggs per brood.

However, diet significantly influenced the egg volume. Qian and Chia (1991) showed that *Capitella* sp. varied its volume of eggs in response to different diets. In this study an increase in the number of eggs per brood was associated with a decrease in egg volume for the Abfeed- kelp and kelp- only treatments. This implies a potential trade-off between the number of eggs produced per brood and the size of eggs in sabellids from these groups. An increase in the amount of resources devoted to individual offspring is suggested to decrease the number of offspring that an adult polychaete is able to produce (Giangrande, 1997). With an increase in the number of eggs, less energy can be invested in each egg. This was found to be probable for the sabellid due to the drop in egg volume with increasing egg production for the Abfeed- kelp and kelp- only treatments. However, although significant, there was considerable variation between individuals. The lack of relationship between the egg volume and number in the kelp-Abfeed and Abfeed- only

treatments may indicate that due to the higher nutritional value of Abfeed, a trade- off may not have been necessary as there may be enough energy available to produce a higher number of eggs whilst maintaining the same volume. A study investigating the relationship between the egg volume and the energy content of the eggs may provide evidence to support this hypothesis.

Influence of diet history on larval size and number

The number of larvae per adult was not different between the treatments indicating that diet had a negligible effect on this variable. The larval length was significantly lower in the kelp- Abfeed group than in all other treatments. However, the influence of diet history appears to be negligible as the larval length was not different between the kelp- only and Abfeed-only groups and the nominal difference in larval length between some treatments was small. Simon *et al.* (2002) is the only other study that has examined larval length in sabellids. The length of the larvae was similar between their study and this study (0.49 mm and 0.47 mm, respectively). No significant difference was reported in the length of larvae between sabellids from Abfeed and kelp raceways (Simon *et al.*, 2002), supporting the suggestion that diet does not influence larval length.

Change in intensity and occupation level of infesting abalone

The abalone from I&J and Sea Plant that were used to infest the uninfested abalone from Aquafarm could be compared to test differences in intensity over time. In all treatments, the sabellid intensity on the infesting abalone decreased during the 185 day study. In a situation of even infestation it may be expected that the infested abalone would be continually infested by the other infested abalone. In this study there was little infestation to counter mortality, and thus a decline in intensity could be expected amongst the

originally infested abalone. The occupation of tubes on the shell edge for the infesting abalone decreased in all treatments during the study except in the kelp- Abfeed group which showed no significant change in occupation level. Thus there was a relationship between the diet history and the occupation level as the decrease in occupation level during the 185 days was different between treatments. The management practises employed on Aquafarm compared with those used on the farms from which abalone were sampled from may have potentially influenced intensity and occupation level. However, there is no information to support this hypothesis and due to the secretive nature of the South African abalone industry, the acquisition of the required data may prove to be problematic.

Change in life-history related traits as a result of diet change

Diet history appears to influence the expression of life history traits in the sabellid as the response to a change in diet was different between treatments. The change in the expression of traits occurred within approximately one and a half generation intervals of the sabellid. This provides some indication as to how rapidly the sabellid is able to modify the expression of various life-history- related characters in response to a change in environmental conditions. Farmers size-sort their abalone at approximately 6 month intervals (AFASA, *pers. comm.*). Thus, the sabellid is capable of modifying the expression of its life-history traits between each size-sorting event, potentially maximizing its productivity brought about a change in conditions resulting from the sorting and handling. Therefore, if a particular set of conditions is beneficial to a sabellid population, there may be an increase in sabellid productivity which may have a follow-on effect throughout the life-time of that cohort of abalone.

Farmers feeding their abalone formulated feeds report that if the sabellid populations are becoming a problem on the farms, a method often employed is to switch the diet from Abfeed to kelp and this seems to reduce the infestations by the sabellids (AFASA, *pers. comm.*). The results from this study suggest that a change in diet from Abfeed to kelp does not appear to influence the intensity of infestation or the occupation level of tubes on the shell edge. However, a change in diet from Abfeed to kelp resulted in an increased abalone growth rate. The reported reduction in infestation may be related to the improved growth rate of the abalone when the diet is switched from the formulated feed to kelp. It has been suggested that fast growing abalone reduce the survival of the larvae (Ruck, 2000). Abalone growth and diet appeared to potentially influence several of the variables measured during this study. Therefore, the influence of abalone growth rate on sabellid infestation, reproduction and morphometrics will be investigated in Chapter 5.

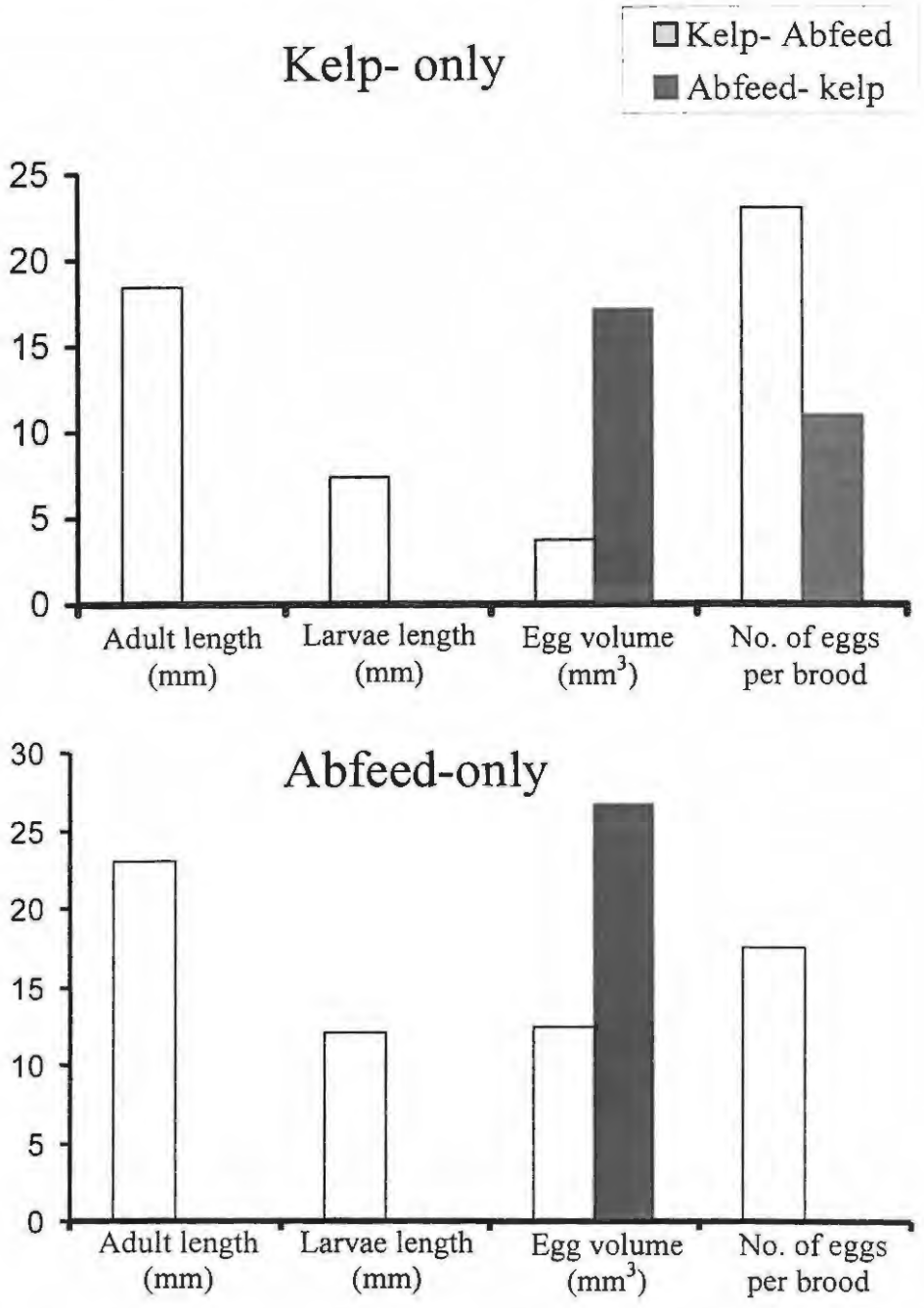

Figure 4.6: A comparison of the life history traits of both the mixed diets with those measured for both the Abfeed-only and kelp-only diets. The bars indicate the differences in traits measured for each mixed diet compared to those of the Abfeed- and kelp-only diets (% change). The Y-axis represents the percentage change of a particular trait affected by a change in diet. Only particular traits for the mixed diets that were significantly different the corresponding traits from either the Abfeed- or kelp-only diets are shown.

CHAPTER 5

The effect of abalone growth on the reproduction and morphometrics of *Terebrasabella heterouncinata*

INTRODUCTION

The sabellid, *T. heterouncinata*, was discovered in the early 1990's in California where it was found to be infesting the shells of commercially cultured abalone (Culver *et al.*, 1997). It is endemic to South Africa and has been responsible for economic losses in both the South African and Californian abalone industry (Ruck & Cook, 1998). Under culture conditions, infestations become heavy enough to result in deformation of the shell and reduced growth in the abalone, reducing the market value of the abalone and increasing the time it takes to produce a marketable product (Culver *et al.*, 1997). Older, slower growing abalone have been identified as having a greater susceptibility to infestation than younger, faster growing abalone (Oakes & Fields, 1996; Clayden, 2000). At this stage it is not certain if abalone growth is reduced by increased infestations by the sabellid or whether the slow growing abalone are more susceptible to infestations. Stocking density, temperature, diet and abalone size and age have been identified as important factors influencing the growth rate of abalone under commercial culture conditions (Oakes & Fields, 1996; Culver *et al.*, 1997; Clayden, 2000).

The Abalone Farmers Association of South Africa (AFASA) recommends a maximum stocking density of 35% of the available surface area (Clayden, 2000). Abalone maintained at a higher stocking density had slow growth and appeared to be more susceptible to sabellid infestation (Clayden, 2000). A low stocking density resulted in an

improved growth rate and a lower infestation level and it was suggested that this was a result of a lower incidence of physical contact between the abalone, resulting in a lower transmission of larvae between abalone (Ruck, 2000). The influence of temperature on sabellid larval success has not been investigated to date. However, an increase in water temperature is regarded as being the most important factor influencing the growth rate of abalone (Clayden, 2000). A high water temperature increased abalone growth up to an optimum from where a further raise leads to a decline in growth rate. Trends in growth rate, feed consumption, mortality, protein efficiency ratio and feed conversion ratio data indicate that a temperature range of 12-20°C is physiologically optimal for *H. midae* (Britz & Hecht, 1997). Temperatures higher than this reduce abalone growth and may result in an increase in sabellid infestation. Studies have suggested that sabellid reproduction is temperature dependent, with larval development being more rapid as temperature increases (Finley *et al.*, 2000). However, 28°C for 24 hours is sufficient to kill all life stages of the worm (Leighton, 1998), but this temperature is above the upper thermal limit of *H. midae* and is therefore an impractical method for the eradication of the sabellid. Ruck (2000) indicated that the best abalone growth rate is obtained in abalone kept at their optimum growth temperature range, regardless of the potential increase in sabellid production.

Two diets are primarily used in South African abalone culture; naturally occurring kelp and an artificial pelleted feed, Abfeed (Britz, 1995). Abfeed has a higher protein, lipid and energy level than kelp (Chalmers, 2002; Simon *et al.*, 2002). Chalmers (2002) reported that due to this high nutritional value and the size of the particulates produced upon breakdown, Abfeed particles are a more suitable food for sabellids than kelp. This has been supported by findings from the AFASA workgroup who suggested that abalone

fed Abfeed appear to be more susceptible to sabellid infestation (Clayden, 2000). This may be the consequence of a greater number of eggs and larvae produced by sabellids from abalone fed Abfeed (Chalmers, 2002; Simon *et al.*, 2002). Varied results have been reported for growth trials of abalone conducted by farmers testing the two diets (Pesch, *pers. comm.*). Different size animals respond differently to kelp and Abfeed, and several of the farms change the diet from Abfeed to kelp once the abalone reach a size of 45-65 mm shell length (Pesch, *pers. comm.*).

Thus, of the factors affecting sabellid infestations, the growth of the abalone has been the least well understood. Diet and stocking density, and in particular temperature appear to have an effect on abalone growth and sabellid infestation yet, as abalone growth is affected by any of these environmental variables, the effect of abalone growth on the sabellid is confounded by a combination of different variables. No study has yet been conducted in an attempt to isolate the potential effect of abalone growth on the success of sabellid settlement and population growth.

The aim of this study was to examine how abalone growth influenced the reproduction and morphometrics of adult sabellids and their offspring under intensive abalone culture conditions. Abalone of two different size classes from the same cohort were sampled from two farms. Depending on the farm, the abalone had been fed either kelp or Abfeed. The objective was to use the morphometric measurements and offspring counts of sabellids from different abalone size classes and diets to determine if abalone growth rate influenced these variables. This was done separately for the two different farm environments, i.e., comparisons between groups were only done within one farm environment. The approach of only comparing morphometric measurements and

offspring counts within farms was used to avoid having the farm environment as a confounding factor. This approach provided information on the differences between farms and their influence on the abalone growth effect.

From the above information the following hypotheses were formulated:

H_0: Abalone growth does not influence the sabellid intensity or occupation level.

H_a: Abalone growth affects sabellid intensity and occupation level.

H_0: Sabellids from abalone of different growth rate have similar reproductive and morphometric characteristics.

H_a: Abalone growth rate affects the morphometrics and reproduction of *T. heterouncinata*.

MATERIALS AND METHODS

Sampling of infested abalone

Infested abalone of two size classes were sampled from the same cohort from each of two abalone farms, Sea Plant and I&J. Ten of the largest and smallest infested abalone of the same cohort were sampled from raceways at Sea Plant products on the 30th November 2001 and I&J Abalone Culture Division on the 4th December 2001 (Chapter 2; Figure 2.1). The size range of the smaller animals was between 45-50 mm shell length. The larger abalone had a size range of 65-70 mm shell length. The abalone sampled from Sea Plant products had been fed on Abfeed, and those sampled from I&J had been fed on kelp throughout the grow-out period prior to the experiment. Sabellids and their offspring were removed from the abalone shell to determine if the abalone growth rate had an influence on the worm's morphometric measurements and offspring counts. A statistical comparison of sabellid growth and reproduction was carried out between abalone size classes of abalone within each farm.

The abalone were immediately shucked and the shells were preserved in 2.5% gluteraldehyde in filtered seawater until the sabellids were extracted. In the laboratory each shell was removed and placed under a binocular dissecting microscope where the inner margin of the shell edge was examined and the number of larvae that had settled on the growing edge was counted (Chapter 2). This gives a measure of the level of infestation by the sabellids and is referred to as the sabellid intensity. The number of tubes per centimeter represents the number of newly settled larvae. Only tubes less than one week old were considered. Older larvae become covered with a nacreous layer and it becomes difficult to determine the time since larval settlement. Larvae less than one week

after settlement are visible as orange larvae in the tubes. After this time the colour of the larvae becomes obscured by the nacre and it becomes difficult to identify if a larva is present in the tube. The number of empty and occupied tubes was counted on the growing edge. The occupation level was determined by dividing the number of occupied tubes by the total number of tubes, and the result expressed as a percentage value.

Removal of worms

Each shell was placed in a 6.5% nitric acid in 70% ethanol solution for 7 hours. This solution dissolves the calcareous component of the abalone shell, softens it and makes it easier to remove the sabellids. Once the shell was sufficiently softened, it was placed under a dissecting microscope and ten adult sabellids, together with eggs and larvae specific to those adults, were removed from the inner surface of the shell of each abalone (Chapter 2). The adult sabellids, their eggs and larvae were stored in labelled containers containing 2.5% gluteraldehyde in filtered seawater. Each adult and its offspring was placed on a petri dish under a dissecting microscope. The length, neck width and base width of the adult and the length and width of its larvae and eggs were measured with a graduated eyepiece using a dissecting microscope (Chapter 2). The number of stage1 and stage 2 larvae per adult and eggs per brood was counted for each adult sabellid. Stage 1 larvae are referred to as sabellid larvae with less than 5 setigers, no cilia or setae and eyespots are absent (Fitzhugh & Rouse, 1999; Ruck, 2000). The term stage 2 larvae refers to the final larval stage before emergence from the burrow. They possess five thoracic setigers, two dark eyespots and cilia and setae are present.

Statistical analysis

Assumptions of analysis of variance testing requires that the data are normally distributed and have equality of variance. Data were tested for normality using the Shapiro-Wilks test and for homogeneity of variance applying Levene's test. The null-hypotheses were rejected at the 5% error level. If data did not satisfy the assumptions for analysis of variance, they were logged and square-root-transformed and then retested. Data that were not normally distributed following log- or square- root transformation or exhibited significant heterogeneity of variance were analysed using non-parametric methods. The Mann-Whitney U-test was used to test the effect of abalone size class on sabellid body measurements and offspring counts within each farm. The null hypothesis was rejected at 5% α- error level.

RESULTS

Sea Plant Products sabellids

Sabellids on abalone from Sea Plant Products showed differences in a wide range of variables. There was, however, no difference in occupation level or sabellid intensity (number of tubes per centimeter of the growing edge) with different abalone growth rates, indicating that within the range of abalone growth rates tested, larval settlement was unaffected (Table 5.1). The adults were significantly longer on the slower growing abalone and had larger base widths than sabellids from faster growing abalone. The length of stage 1 larvae was not different on different size abalone from Sea Plant products, but the stage 2 larvae length was significantly greater on the slower growing abalone. Sabellids from slower growing abalone had more larvae, both stage 1 and stage 2, than abalone with a faster growth rate (Table 5.1). The sabellids from the slower growing abalone had significantly more eggs per brood, having almost double the number of eggs than sabellids from the faster growing abalone. The egg volume was not influenced by abalone growth rate. The most evidently influenced variables were the number of eggs and larvae per adult, with each being greater in the slower growing abalone (Table 5.1). Of the 10 variables considered; six were significantly different between the two abalone size classes i.e. fast and slow growing abalone. For the reproductive and morphometric characteristics that were different between the abalone size classes, all were significantly greater in the slower growing abalone (Table 5.1).

I&J sabellids

In sabellids from I&J the effect of abalone growth was less influential on the reproduction- related factors and morphometrics than in those from Sea Plant Products. For example, nine out of the ten variables did not differ between abalone from the two

size classes (Table 5.2). Only the egg volume was significantly different between slow and fast growing abalone. Sabellids on the slower growing abalone produced marginally, but significantly larger eggs.

The trends for changes in the reproductive characteristics and morphometrics were not similar between the two farms. Egg volume was the only variable influenced by abalone growth in I&J sabellids and this was one of only four variables not affected by abalone growth rate in sabellids from Sea Plant Products. It is clear from these data that under conditions at I&J the sabellids were not strongly influenced by abalone growth rate. In contrast, the sabellids responded more closely to abalone growth on abalone from Sea Plant Products.

Table 5.1: The number of observations, mean, median and range of measurements and counts taken for variables under consideration for both abalone size classes sampled from Sea Plant Products. The *Z*-statistic and *p*-value for comparisons between the abalone size classes for each variable are also given.

Variable	45-50 mm abalone size class				65-70 mm abalone size class				Comparisons between abalone size classes	
	n	*Mean*	*Median*	*Range*	*n*	*Mean*	*Median*	*Range*	*Z-statistic*	*p-value*
Shell length (mm)	10	47.43	47.65	44.9-50.1	10	69.28	69.85	65.4-71.5	3.77	≤0.0001
Growth rate (mm/ month)	10	1.19	1.19	0.9-1.3	10	1.54	1.54	1.5-1.6	3.77	≤0.0001
Occupation level (% of tubes occupied)	10	70.80	75.00	58.3-80.0	10	65.16	65	57.1-76.9	1.77	0.76
Number of tubes per cm of growing edge	10	7.91	8.25	2.5-12.0	10	6.00	5.73	3.5-9.5	1.73	0.82
Adult Length (mm)	100	2.84	2.81	1.8-4.8	100	2.42	2.33	1.2-4.3	4.72	≤0.0001
Base width (mm)	100	0.39	0.40	0.27-0.54	100	0.34	0.34	0.13-0.49	6.36	≤0.0001
Stage 1 larvae length (mm)	180	0.49	0.47	0.34-0.67	93	0.48	0.47	0.34-0.67	0.5	0.63
Stage 2 larvae length (mm)	171	0.67	0.67	0.47-1.04	121	0.64	0.63	0.34-0.94	2.66	0.007
Egg volume (mm^3)	348	0.025	0.025	0.011-0.048	195	0.026	0.025	0.011-0.051	0.05	0.96
Number of stage 1 larvae per adult	100	1.77	2	0-6	100	0.93	1	0-3	4.1	≤0.0001
Number of stage 2 larvae per adult	100	1.71	2	0-5	100	1.20	1	0-6	3.5	0.0004
Number of eggs per adult	100	3.45	3	0-8	100	1.96	2	0-9	5.8	≤0.0001

Table 5.2: A The number of observations, mean, median and range of measurements and counts taken for variables under consideration for both abalone size classes sampled from I&J. The *Z*-statistic and *p*-value for comparisons between the abalone size classes for each variable are also given.

Variable	45-50 mm abalone size class				65-70 mm abalone size class				Comparisons between abalone size classes	
	n	*Mean*	*Median*	*Range*	*n*	*Mean*	*Median*	*Range*	*Z-statistic*	*p-value*
Shell length (mm)	10	48.68	48.81	44.4-52	10	68.70	69.30	65.4-71.1	3.84	0.0002
Growth Rate (mm/month)	10	1.40	1.40	1.3-1.4	10	1.62	1.63	1.5-1.6	3.84	0.0002
Occupation level (% of tubes occupied)	10	64.3	68.20	20-80	10	56.98	64.20	28.5-73.9	1.28	0.20
Number of tubes per cm of growing edge	10	4.9	4.75	2.5-9.5	10	6.1	5.5	3.5-11.5	1.05	0.29
Adult length (mm)	100	2.98	2.93	1.3-5.7	100	3.05	3.01	1.2-5.5	0.52	0.59
Base width (mm)	100	0.35	0.35	0.21-0.48	100	0.36	0.36	0.21-0.47	0.45	0.65
Stage 1 larvae length (mm)	215	0.46	0.47	0.34-0.70	252	0.46	0.47	0.30-0.67	0.61	0.54
Stage 2 larvae length (mm)	209	0.63	0.62	0.44-0.85	197	0.62	0.60	0.47-0.87	0.67	0.49
Egg volume (mm^3)	440	0.027	0.027	0.011-0.052	437	0.026	0.025	0.010-0.049	3.22	0.0013
Number of stage 1 larvae per adult	100	2.16	2	0-7	100	2.52	2.5000	0-7	1.74	0.08
Number of stage 2 larvae per adult	100	2.10	2	0-6	100	1.97	2.0000	0-7	7.7	0.44
Number of eggs per adult	100	4.44	4	0-11	100	4.36	3.5000	0-13	0.92	0.35

DISCUSSION

There was no significant relationship between abalone growth and sabellid intensity or occupation level for either farm. All morphometric and reproductive variables except the stage 1 larvae length and egg volume were related to abalone growth for sabellids from Sea Plant. In contrast, the only significant effect on sabellids from I&J was that of growth on egg volume. It has been suggested that older, slower growing abalone are more susceptible to sabellid infestations than younger abalone with a higher growth rate (Oakes & Fields, 1996; Clayden, 2000). It is unclear whether slow growing abalone are more susceptible to sabellid infestation or whether the presence of sabellids on the shell causes a reduction in abalone growth. In Chapter 4 it was shown that abalone growth rate was significantly influenced by diet history and differences were observed in infestation, morphometrics and reproduction of the sabellid with different diets. It was thus hypothesised that the growth rate of the host abalone may be potentially related to the intensity, occupation level, development and reproduction of the sabellid.

To isolate the effect of growth the size classes of abalone sampled within each farm were from the same cohort, and hence it was possible to calculate growth rate as the age of the abalone was known.

Sabellid intensity

The intensity (number of tubes per centimeter of the growing edge) was not related to abalone growth rate for abalone from either of the two farms. This observation is supported by studies carried out by Chalmers (2002) who indicated that abalone size did not have a significant effect on sabellid infestations. Thus, abalone growth rate does not seem to play a role in regulating the number of tubes on the shell edge. The difference in abalone growth rate between the size classes

may not have been large enough to influence the number of larvae settled. Data from Chapter 4 can be used for further comparisons. The slow-growing abalone fed Abfeed in Chapter 4 had greater sabellid intensity than the fast growing kelp- fed abalone. The difference in abalone growth rate between this study and Chapter 4 may support the suggestion that the difference in growth rate between the abalone size classes may not have been great enough to influence sabellid intensity. The mean growth rate of the fast and slow growing abalone for both farms combined in this study was 1.6 mm per month and 1.3 mm per month, respectively. The mean growth rate of the treatments from Chapter 4 with the highest and lowest growth rate was 3.2 mm per month and 2.2 mm per month. The sabellid intensity for the fast and slow growing abalone in Chapter 4 was 2.5 and 3.4 tubes per centimeter. The intensity of fast and slow growing abalone for this study was 5.9 and 6.4 tubes per centimeter, respectively. The reason for the higher growth rates in the previous study is not clear as the stocking density was maintained at 20%, a level recommended by AFASA. The stocking density at which the abalone sampled for this study were kept prior to sampling was not known, but I&J normally keep their abalone at approximately 30% and Sea Plant at between 20 and 35% (Chapter 2). Depending on the time since these abalone were last size-sorted, the stocking densities may have been higher than the 20% used in this study, potentially reducing the abalone growth rates. A minimum growth rate may be required to influence the intensity of sabellid infestation. A study investigating the influence of a wide range of abalone growth rates on the settlement success of larvae may have potential applications in reducing the intensity of sabellid infestation on farms. Simon *et al.* (2002) observed that the intensity of sabellid infestation did not influence the growth rate of the abalone. This indicates that slow growing abalone are not more susceptible to sabellid infestation and that a high number of tubes on the shell edge does not reduce the growth rate of the abalone. Ruck (2000) and abalone farmers (*pers. comm.*) indicated that the growth rate of the abalone drops in response to high levels of sabellid infestation. Simon *et al.* (2002) suggested that the lack of influence of sabellid infestation on the abalone growth rate may

have been due to the lower intensity of sabellids on the shell edge in their study when compared to Ruck's experiment (Ruck, 2000). They suggested there may be a critical growth-reducing sabellid intensity (Simon *et al.*, 2002). Unfortunately comparison of the number of larvae between these studies is difficult as Ruck (2000) counted the number of tubes per centimeter of the shell edge and Simon *et al.* (2002) counted the total number of larvae settled on the growing edge of the shell. These authors recorded a maximum of 48 worms on the shell edge and Ruck observed a maximum of 25 tubes per centimeter. Therefore, from this information it is not possible to compare the two but the sabellid intensity observed by Simon *et al.* (2002) appears to be appreciably lower than that given by Ruck (2000). The determination of the minimum number of tubes on the shell edge necessary to elicit a reduction in abalone growth may provide farmers with an early warning of a potentially detrimental reduction in abalone growth rate. To contribute to this aspect the relationship between host diet and the success of sabellid larval settlement is addressed in Chapter 6.

Occupation level

Despite the wide range of conditions from which infested abalone were sampled, there appeared to be a similar relationship between abalone size and the occupation level for both farms. The occupation level was not affected by abalone growth rate for either farm. Chalmers (2002) showed that the occupation level decreases with increasing abalone size for abalone fed Abfeed, but increases with increasing abalone size for kelp- fed abalone. Ruck (2000) suggested that a high abalone growth rate may reduce the number of larvae occupying the tubes. Once the larvae leave the parent's burrow, they crawl over the shell and settle on the underside of the host's shell, usually along the growing edge (Culver *et al.*, 1997). The abalone responds by depositing nacreous shell over the larvae, thereby forming the tube for the worm. The worm is usually able to keep one end of the tube open (Finley *et al.*, 2000). However, if the rate of deposition of shell is high, the

sabellid may not be able to keep its burrow open, thereby smothering the worm causing mortality and decreasing the occupation level (Ruck, 2000). The growth rate of abalone from the previous study (Chapter 4) was up to 3.2 mm per month. The average growth rate of farmed abalone is approximately 2 mm per month (Pesch, *pers. comm.*). There was no difference in occupation level between treatments in Chapter 4, indicating that even under conditions of high abalone growth the occupation level is not affected. Thus, Ruck's (2000) hypothesis of abalone with a high growth rate smothering the settled larvae and reducing the occupation level could not be supported by data from this study.

Morphometric measurements

The adult length, base width and stage 2 larvae length were influenced by abalone growth in sabellids from Sea Plant. For sabellids from I&J, only the egg volume was significantly different between abalone with different growth rates. This suggests that the different conditions on the farms are resulting in a different response by the sabellid. Chalmers (2002) hypothesised that different feeding strategies of different size abalone may result in differences in particulate size and abundance. Chalmers (2002) provided no evidence to support his suggestion and it was not possible to corroborate his hypothesis using data collected during this study. An investigation into the potential difference in the number and size range of particulates produced by abalone of different size may yield information which may validate Chalmers's hypothesis.

Number of larvae per adult and eggs per brood

The number of stage 1 and stage 2 larvae per adult and eggs per brood was influenced by abalone growth in Sea Plant sabellids. Abalone size did not affect the number of larvae and eggs per adult for sabellids from I&J. This is the first study to investigate the influence of abalone growth on sabellid offspring number, therefore no comparison with data from other studies is possible. Simon

et al. (2002) counted the number of offspring per brood of sabellids from different diet histories but made no distinction between eggs and larvae. They found a maximum of 3 eggs or larvae per brood (Simon *et al.*, 2002). Ruck (2000) showed that there was a lower abundance of particulates in abalone kept in aquaria under laboratory conditions. The study carried out by Simon *et al.* (2002) was conducted in experimental aquaria in a laboratory. The maximum number of eggs and larvae per adult for this study was 13 and 7, respectively. The difference in the number of eggs and larvae between this study and that of Simon *et al.* (2002) may have been influenced by the lower abundance of particulates in the experimental aquaria used in their study. A maximum of ten eggs per brood and seven larvae per adult were observed in the study in Chapter 4, supporting the possibility that particulate abundance may have potentially influenced sabellid reproduction.

There was a decrease in both the morphometric characteristics and offspring number with increasing abalone growth for all variables that were different between abalone size classes. This indicates that the sabellids from both farms were influenced in a similar way by abalone growth rate. Six of the ten variables considered during this study were significantly different between abalone size classes for Sea Plant sabellids compared to only one for sabellids from I&J. Thus, the farm from which infested abalone where sampled appears to influence the reproduction and morphometrics of sabellids.

This study aimed to investigate cause and effect of the relationship between abalone growth and sabellid reproduction. As abalone growth and settlement were not related it could be concluded that the effect of shell growth is likely to shape the environment in which the sabellid matures and how fast and successfully it reproduces. Thus, the most likely explanation for changes in reproduction (i.e. numbers and sizes of offspring) is the growth of the abalone; it appears less likely from these data that the sabellid intensity reduces abalone growth. Yet, once the sabellids

have settled, their development depends on shell growth within the range tested here. They depend on the abalone growth as they were not able to choose favourable slow-growing abalone during the settlement phase.

In summary the results show a farm- specific effect of abalone growth on sabellids, except for intensity and occupation level which was not influenced by growth on either of the two farms.

CHAPTER 6

The influence of host abalone diet on the settlement success and distribution of *Terebrasabella heterouncinata* larvae on different areas of the shell

INTRODUCTION

World wide, many economically important mollusc species are plagued by polychaete infestations (Handley & Bergquist, 1997). In oysters, infestations by *Polydora* sp. form 'blisters' which affect the lucrative half-shell market, as these blisters can be punctured, releasing anaerobic metabolites, including hydrogen sulphide (Caceres-Martinez *et al.*, 1999). These infestations can also render the oyster shell brittle and easily broken during shucking, packaging and transport (Handley & Bergquist, 1997). Boring organisms do not seriously affect the growth of abalone as they normally inhabit the outer portions of the shell (Oakes & Fields, 1996). The sabellid worm, *Terebrasabella heterouncinata*, has however become a major concern for abalone farmers. Although infestations by the sabellid do not affect the quality of the abalone meat, they can cause severe deformities in the shell and reduce abalone growth (Culver *et al.*, 1997). This increases the time it takes to get the abalone to a marketable size and the appearance of the shell reduces the market value of the product.

The larval phase is the only motile stage of the sabellid life cycle and it is the only means by which progeny can be distributed to a new host. The level of sabellid infestation on abalone shells is highly variable. Possible factors affecting sabellid infestation under culture conditions include abalone growth, diet and stocking density, water quality,

temperature, and management procedures (Culver *et al.*, 1997; Clayden, 2000; Chalmers, 2002). Two diets are primarily used in South African abalone culture. These are kelp harvested from the ocean and an artificially formulated pelleted diet, Abfeed (Simon *et al.*, 2002). During abalone grow-out any of these diets may be used as the only diet or sequentially with kelp being fed to larger animals. The two diets differ in their proximate analysis (Chalmers, 2002; Simon *et al.*, 2002) and this was highlighted as a potentially important factor influencing reproductive output of the sabellid worm, and the success of larval settlement.

Most brooding sabellids have young that leave the burrow as pre-adults with the branchial crown already formed so they can feed and commence building their own tube (Fitzhugh, 1996). The young of *T. heterouncinata* leave the burrow as larvae, without a developed branchial crown. Therefore, they are not able to feed until they have settled on their host. At this stage the larva has yolk granules in the body and there is no evidence of gut formation (Fitzhugh & Rouse, 1999). The larvae can only leave the burrow once segmentation is complete and the "bristles" or setae are visible (Culver *et al.*, 1997). Now, the larvae, complete with two eyespots and sensory tentacles at the anterior end, crawl out of the burrow using the setae. This motile phase is the infesting stage and the larvae exit the burrow and go in search of a new host or a suitable area to settle on the same host. The larvae normally crawl, using a band of cilia on the ventral surface, over the host's shell and settle under the lip of the shell, in contact with the mantle tissue (Fitzhugh & Rouse, 1999). Consistent with larval behaviour in other species, sabellid larvae seem to have the ability to locate suitable areas on the shell to settle, but this is an area in which more research needs to be conducted (Caceres-Martinez *et al.*, 1999; Ruck, 2000). Within a few hours of leaving the burrow, the larvae usually settle on the

underside of the abalone shell along the growing edge margin. They may also settle on the outer lip or around the respiratory pores (Culver *et al.*, 1997). Within the first day of exiting the adult burrow, the larvae secrete a thin mucous sheath with the anterior end open at the shell margin.

The host abalone responds by depositing a thin transparent calcified layer over the ensheathed larvae, forming a calcareous tube around it (Kuris & Culver, 1999). Thus, in the immediate vicinity of each newly settled worm, the abalone's mantle is temporarily retracted which results in the prevention of prismatic deposition at the growing margin of the shell aperture (Ruck, 2000). After a period of time the nacreous layer deposited over the larval tube is too thick to be seen through the laminar layers of the shell (Kuris, 1997). It is only at this time that the abalone resumes prismatic shell growth (Culver *et al.*, 1997). Shell growth is therefore most visibly disturbed in the area below the worm. As infestations continue the abalone shell takes on a characteristic domed-shaped appearance (Kuris & Culver, 1997; Culver *et al.*, 1999; Ruck 2000) and without continued linear extension of the shell, the abalone ceases to grow. The respiratory pores are also sites of sabellid larval settlement (Culver *et al.*, 1997; Kuris & Culver, 1999; Ruck, 2000). Pores can become clogged with newly settled worms and formation of pores at the growing edge is impaired (Culver *et al.*, 1997). Although missing and deformed pores do not cause instant mortality, abalone growers have reported greater mortality in those abalone that lack respiratory pores for several months or more (Culver *et al.*, 1997).

Chalmers (2002) showed that sabellid infestation is significantly influenced by abalone diet. He showed that kelp and Abfeed differ in the size range of particulates produced in

commercial raceways as well as their protein and energy content. He hypothesised that differences in sabellid intensity were related to the different properties of the two diets.

Sabellid intensity and settlement success are closely related. As the adults are sessile, the sabellid worm relies solely on the short, motile larval phase for the distribution of progeny (Finley *et al.*, 2000). Thus, it is essential to gain a better understanding of the factors that influence sabellid larval settlement success on abalone shells. However, in previous studies the settlement success of larvae around the shell edge has not been investigated. For example, Simon *et al.* (2003) counted the number of tubes on the growing edge region of abalone fed kelp or Abfeed. Their experimental abalone were kept in baskets in raceways, and measurements were taken every four months. Chalmers (2002) sampled abalone from raceways of different farms and counted the number of larval tubes on the growing edge only once. Therefore, the settlement success of larvae during the first days could not be quantified. Although diet has been suggested as a factor influencing the intensity of sabellid infestation (Chalmers, 2002; Simon *et al.*, 2003), results vary. One possible reason is the difficulty in counting newly settled larvae and distinguishing them from established ones. For example, all authors encountered problems in attempting to count the number of newly settled larvae as it was not always clear which larvae were new due to the opacity of the nacre laid down by the abalone. When counting the larvae on the growing edge it is important to count only larval tubes from the same shell layer to avoid counting larvae of different ages. Representative results for a given area of the shell can only be achieved if all tubes of a given age are counted. A possible solution to this problem was identified during a pilot study conducted as part of the research of this thesis. Whilst marking areas on the growing edge of an abalone shell with a pencil, it was noticed that after drawing the pencil lines and leaving

the abalone in the tanks for several days, sabellid larvae had settled over the pencil lines. The larvae were easy to identify as newly settled on the dark background created by the pencil lines. It was concluded that the use of pencil areas might be a quick and effective means of describing settlement of larvae on the growing edge of abalone. Thus, using this method, this study was conducted to quantify settlement success and distribution of larvae on the shell edge of abalone fed different diets.

Therefore, this is the first study that investigates larval settlement success in abalone fed on different commercial diets. The aim of this study was to determine if abalone diet an influenced the success of sabellid larval settlement and the distribution of settled larvae around the shell edge. This study was also able to show population growth during the first 36 days of new infestation.

To achieve this aim the number of sabellid larvae settled around the shell edge of abalone fed two different diets was quantified.

The following hypotheses were identified:

H_o: Diet of the host abalone does not influence larval settlement success.
H_a: Larval settlement success is related to diet.

H_o: Larvae will settle equally well on all parts of the shell.
H_a: Larvae will show a preference for some areas of the shell.

MATERIALS AND METHODS

Preferential settlement trial- A pilot study

Before studying the pattern of larval settlement, it was necessary to determine the potential effect of pencil marks on the inner margin of an abalone shell edge on larval settlement. Six uninfested and ten heavily infested abalone were sampled from Aquafarm development and Sea Plant Products, respectively. The mean shell length ± standard deviation of the abalone sampled was 80.1 ±4.3 mm. All abalone sampled had been fed on Abfeed. The two groups of abalone were kept in commercial abalone baskets in separate experimental tanks at Aquafarm development, and were fed Abfeed once a day. After acclimatising the abalone for seven days, the six uninfested abalone were removed and anaesthetised in a solution of 7% magnesium sulphate in seawater for 20 minutes. Once anaesthetised the abalone were placed, ventral surface up, on a moist sponge. The mantle was then pulled back to reveal the margin of the inner shell. Seven lines were drawn with a pencil, perpendicular to the shell edge, five millimeters apart along the growing edge between the spire and the first respiratory pore. A template, made from cutting a 25 mm^2 area out of a hard plastic card, was used to draw the lines. Every alternate square on the shell margin was penciled in with a Staedler HB pencil, resulting in three blank and three 25 mm^2 marked areas on the growing edge. The uninfested abalone were placed in a basket together with the infested abalone. These animals were left for 96 hours to allow larvae to infest the previously uninfested abalone. After this time, the experimental abalone were removed from the basket and anaesthetised. Once anaesthetised, each abalone was placed under a binocular dissecting microscope and the number of newly settled larvae, on both the pencilled and the blank areas of the growing edge, were counted and recorded.

Main Experiment

To repeatedly determine the success of larval settlement and the distribution of larvae on the shell edge, infested and uninfested abalone were kept in experimental tanks to allow for new infestations to occur. The uninfested abalone had pencil blocks drawn along the perimeter of the shell edge and the number of larvae settled on each block was quantified every 96 hours for 36 days. At each recording the pencil marks were covered by new ones.

Uninfested abalone

Twenty four uninfested abalone between 62 and 76 mm shell length were sampled from Aquafarm Development on the 4th February 2002. Each animal was examined for sabellid infestation by viewing the shell under a dissecting microscope. A tag with an identification number was glued onto the dorsal surface of each of the uninfested abalone. They were split into four groups of six abalone and each group of six was placed into separate abalone baskets in separate experimental tanks at Aquafarm development, where they were left to acclimatise for seven days. Each group of abalone had a constant supply of kelp to feed on for the duration of the experiment. Once a week all uneaten kelp was removed and replaced with fresh kelp.

Infested abalone

Two groups of ten abalone infested with sabellids were sampled from both I&J and Sea Plant Products on the 5th of February 2002. The level of infestation was determined by judging the appearance of the shell and by counting the number of larvae on the inner margin of the growing edge of the abalone shell. The mean size ± standard deviation of these sampled abalone was 72.6 ±3.6 mm and 70 mm ±5.1 mm for I&J and Sea Plant products, respectively. The abalone from I&J had been fed kelp and the Sea Plant

Products abalone were fed Abfeed. These four groups of abalone were placed in separate baskets in separate experimental tanks at Aquafarm development. For the duration of the experiment, the I&J abalone were fed kelp and the Sea Plant animals were fed Abfeed. These infested abalone were left in the experimental tanks for seven days to acclimatise.

Experimental infestation

After acclimatisation, each uninfested abalone was anaesthetised in a 7% solution of magnesium sulphate in seawater and placed under a binocular dissecting microscope. Using a template, 25 mm^2 pencil blocks were drawn 5 mm apart along the inner shell margin (Figure 6.1). A line was taken from the spire to the first respiratory pore (Line "A"; Figure 6.2). The first block was drawn on the growing edge, perpendicular to this line off the spire (Block 1; Figure 6.1). The blocks were numbered in a clockwise direction from the block opposite to the spire. Six uninfested animals marked with pencil blocks were placed into each of the four baskets containing ten infested abalone from either I&J or Sea Plant Products. The abalone were left for 96 hours to allow for infestations to occur on the previously uninfested abalone. After this time, the marked animals were anaesthetised and removed from the baskets. Each animal was placed under a binocular dissecting microscope. The mantle was pulled back to reveal the inner shell margin of each shell. Each numbered pencil block was examined and the number of settled larvae on each block was counted. Counting took place, in a clockwise direction from the first block, the entire way around the shell edge. Both occupied and unoccupied tubes per block were counted. If any part of a sabellid tube was covering a part of a block, it was included in the count. After counting was complete, the blocks were marked again, covering all larvae that had previously settled. The abalone were placed back in their respective tanks and left for another 96 hours. After this time the newly settled larvae

were again counted on each block and the pencil blocks were redrawn. Measurements were taken every 96 hours for 36 days.

Each shell edge was divided into three regions to facilitate analysis (Figure 6.2). A line was taken from the spire to the first respiratory pore. The first region began perpendicular to this line off the spire and continued to the point where the abalone's respiratory pore line met with the shell edge. This region is referred to as the growing edge (GE). The second region was taken from the respiratory pore line to the point at which the shell margin thickens, this region shall be referred to as the pore edge (PE). The third region was the remainder of the shell perimeter, and is termed the thick edge (TE).

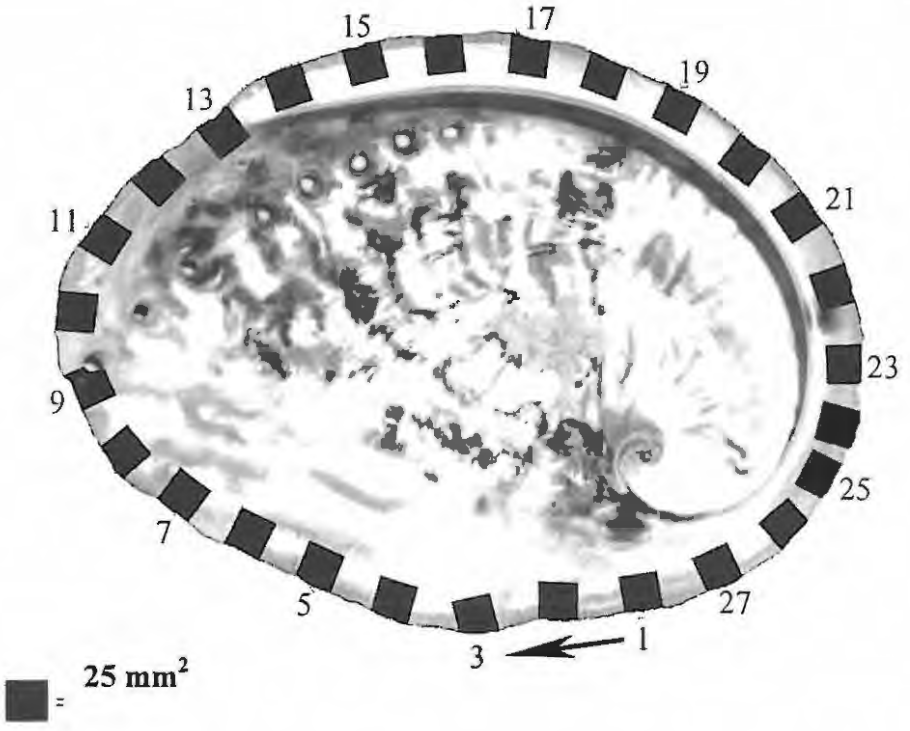

Figure 6.1: A diagram illustrating the position of the pencil blocks used for measuring the number of larvae settled. The blocks were numbered in a clockwise direction around the entire shell edge. Each block represents a 25 mm² area.

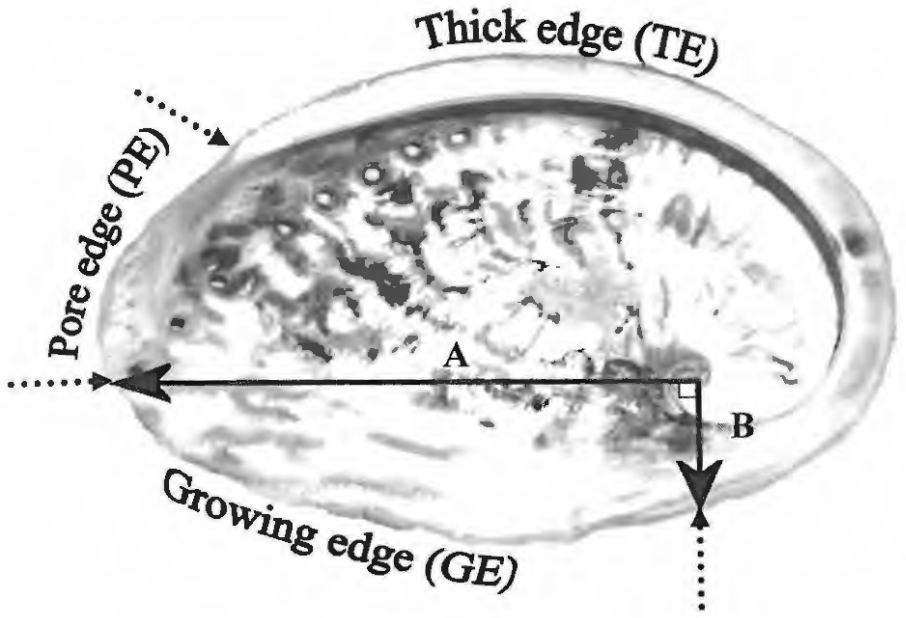

Figure 6.2: A diagram of an abalone shell indicating the regions of the shell that were used for analyses. The dotted arrows indicate the boundaries of the various regions. Arrow "A" is the line taken from the spire to the first respiratory pore and 'B' was taken perpendicular to 'A' to give the position of the first pencil block (see block 1; Figure 6.1)

Statistical analysis

The Mann-Whitney U-test was used to test if larvae settled on the pencil marks in preference to the blank areas of the shell edge. The Mann-Whitney U-test was used to test if there was significant difference in the number of sabellid larvae settled and the number of tubes deposited between different host abalone diets. After testing for significant differences between the number of larvae settled in each shell region with the Kruskall-Wallis ANOVA, the Bonferroni correction was applied and the different regions were tested pair-wise with the Mann-Whitney U-test. For this, the level of significance was determined by dividing 0.05 by the number of pre-planned pairwise comparisons. For all data tested with the Mann-Whitney U-test, the null hypotheses were rejected at $p \leq 0.05/3 = 0.017$. The H-statistic refers to the results obtained for comparisons between variables

using the Kruskall- Wallis ANOVA by ranks test and the Z-statistic refers to the Mann-Whitney U-test results.

Regression analysis was used to determine the relationship between the number of settled larvae and the tidal height. Analysis of Covariance was used to test the difference between the cumulative number of larvae over the 36 days for the different diets.

RESULTS

Preferential settlement trial- Pilot study

Sabellid larval settlement was not significantly affected by the presence of pencil markings on the growing edge of abalone shells (Z =0.66; p= 0.5) (Figure 6.3).

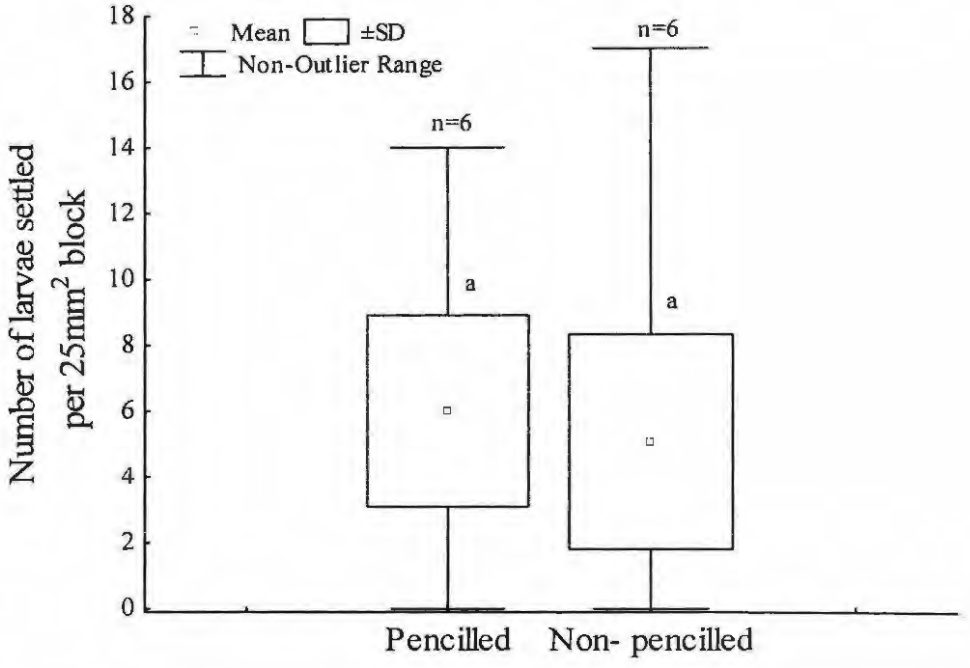

Figure 6.3: The number of sabellid larvae settled on the pencilled and non-pencilled areas of the abalone shell edge during the preferential settlement trial.

Larval settlement

Abalone diet had no effect on the mean number of larvae settled per block per 96 hours (Z= 0.88; p= 0.55) and the mean number of sabellid tubes deposited per block on the entire shell edge per 96 hours (Z=0.78; p= 0.48) (Table 6.1). The occupation level of tubes ranged between 85% and 87% for both diets. There was no significant difference in the occupation level of tubes between diets (Z= 0.69; p= 0.64) indicating that larval mortality is not influenced by diet within the first 96 hours after settlement.

The effect of shell region and diet on settling success was evaluated separately for each of these factors. Shell region had a significant influence on the mean number of settled larvae per 25 mm^2 block per 96 hours ($H= 12.3$; $p \leq 0.0001$) (Figure 6.4). The mean number of larvae settled per block per 96 hours in each region was not influenced by diet ($H= 0.88$; $p= 0.38$) (Figure 6.4). The greatest number of larvae settled in the pore edge region and the thick edge had a significantly lower number of worms settled than any of the other regions (Table 6.2 and Figure 6.4).

The residuals of the cumulative mean number of larvae settled per abalone as a function of time was normally distributed indicating sabellid population growth was linear. The relationship between the number of larvae settled on the shells and the number of days is shown in Figure 6.5. This information allows the estimation of the number of worms in a population on an abalone shell within 36 days subsequent to contact with sabellids. There was no significant difference between the cumulative number of larvae settled over the 36 days for the different diets ($F= 0.01$; $p= 0.93$).

The settlement of larvae was correlated with the tidal height (Figure 6.6). The mean number of larvae settled per 4 days and the corresponding lunar phases and tidal height are shown in Figure 6.7.

Table 6.1: Mean and range of the number of larvae settled and tubes deposited per pencil block per 96 hours for Abfeed- and kelp-fed host abalone. Diet did not significantly influence the mean number of larvae or tubes per block per 96 hours ($p \leq 0.05$).

	Larvae settled on kelp-fed abalone (n= 264)	Larvae settled on Abfeed-fed abalone (n= 264)	Tubes deposited on kelp-fed abalone (n= 264)	Tubes deposited on Abfeed-fed abalone (n= 264)
Mean number per block per 96 hours	0.52	0.64	0.6	0.74
Range (per block)	0-11	0-12	0-12	0-12

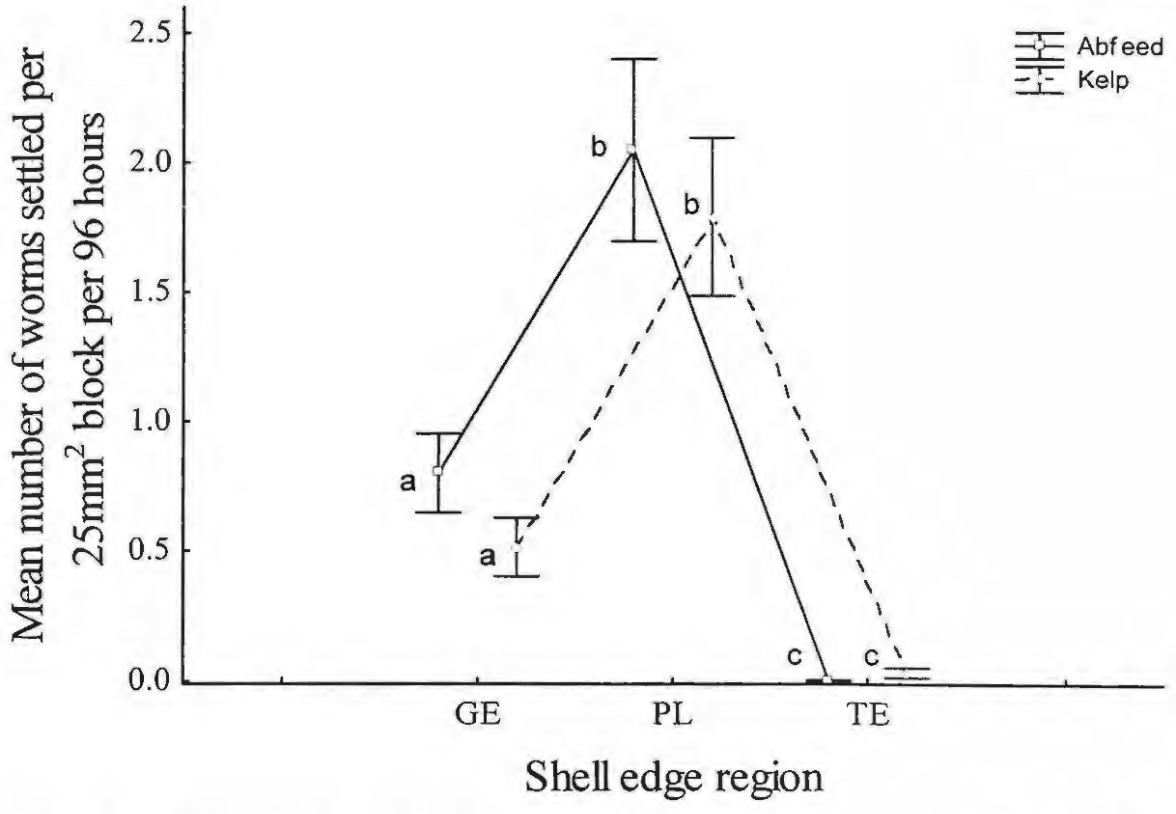

Figure 6.4: A comparison of the mean number of worms settling per block with different abalone diets for the three shell regions. The p-value at which pairwise comparisons were considered significant was set at $p \leq 0.013$ due to the Bonferroni-correction; different letters indicates significant differences (GE=growing edge (Z= 0.43; p= 0.26) PE= pore edge (Z=0.79; p= 0.42); TE= thick edge (Z= 1.9;p= 0.057).

Table 6.2: A comparison of the settled larvae per region as a percentage of the total number of larvae settled during the study and the corresponding percentage perimeter of the region as a percent of the total shell edge perimeter.

Shell Region	Larvae settled per region as a percentage of total number of larvae settled during the study (%)	Perimeter of region as a percentage of total perimeter of shell edge (%)
Growing edge	35 (n per abalone = 38)	31
Pore edge	63 (n per abalone = 68)	21
Thick edge	2 (n per abalone = 2)	48

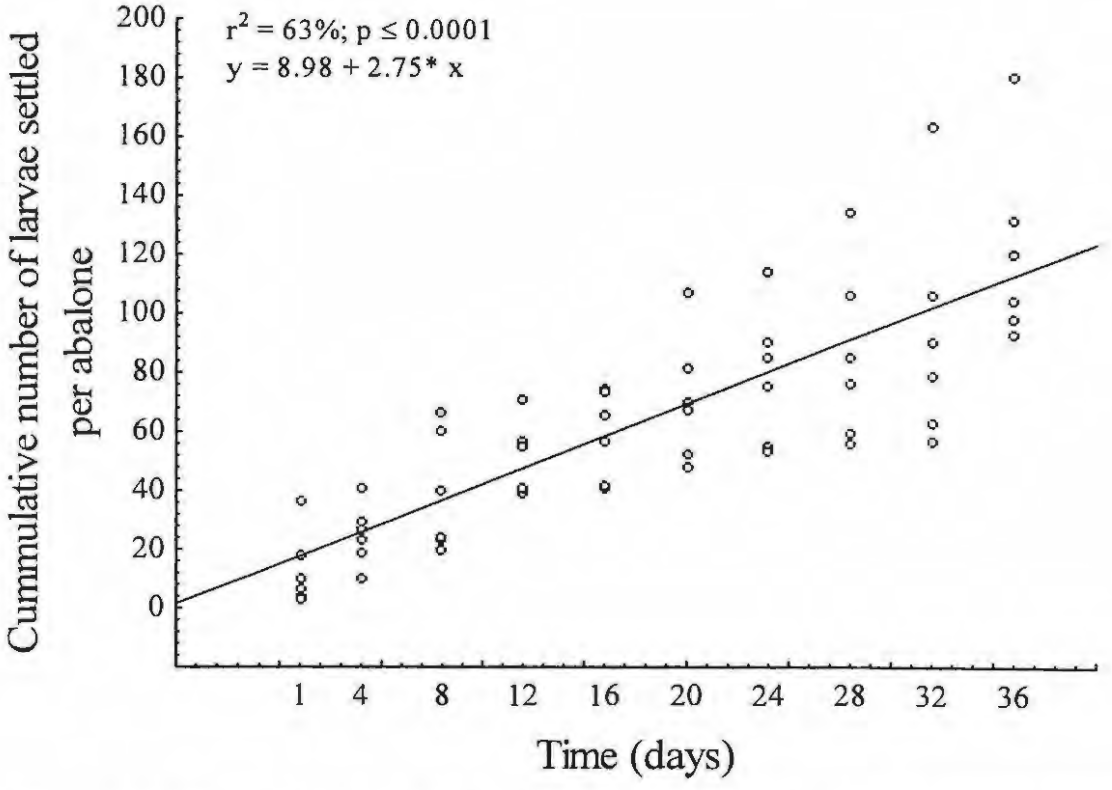

Figure 6.5: The relationship between the cumulative number of larvae settled on abalone shells over the 36-day study for sabellids from Abfeed and kelp fed abalone. The p-value indicates the slope of the relationship was significantly different from 0.

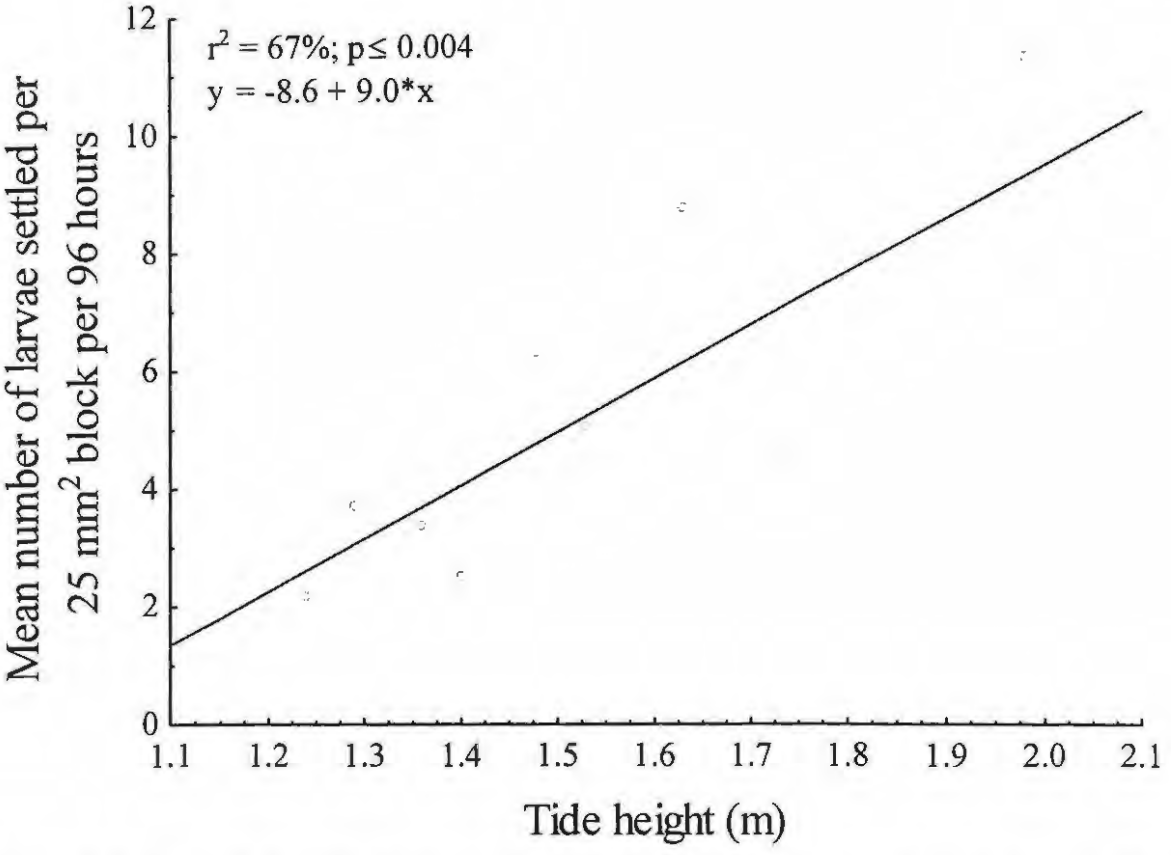

Figure 6.6: The relationship between the mean number of larvae deposited every 96 hours per 25 mm^2 block on the abalone shell edge and the tidal height.

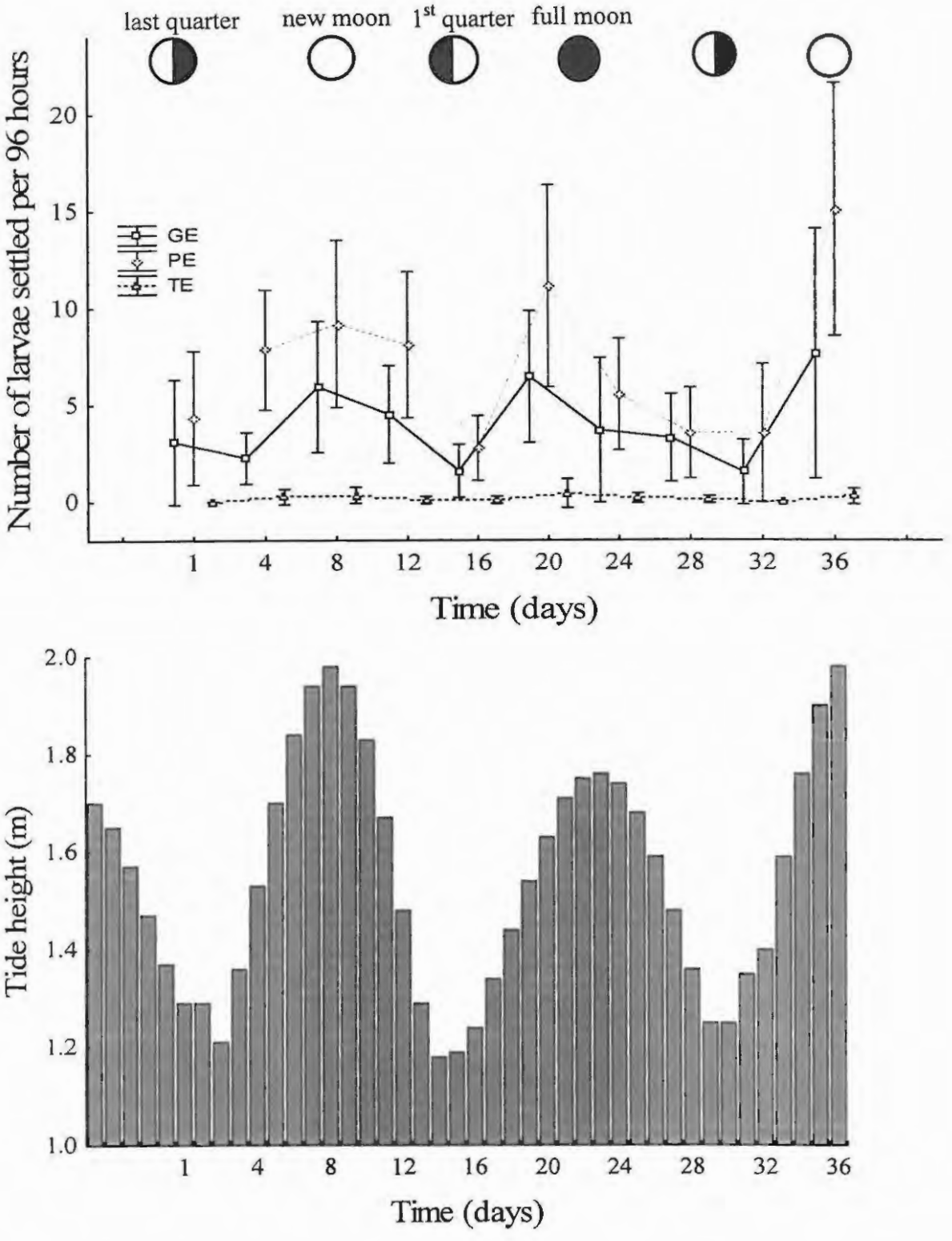

Figure 6.7: A comparison of the mean number of new larvae settled per 96 hours for the three shell edge regions and the corresponding tide heights and lunar phases. Bars in the upper graph indicate a 95 % level of confidence.

DISCUSSION

Host abalone diet had no influence on the success of larval settlement success within the first 96 hours after settlement. This suggests that sabellid population characteristic is possibly a result of post-settlement mortality. This suggestion is supported by data from Chapter 4 and 5 which showed that occupation level was not influenced by diet or abalone growth within the first week after larval settlement. Examples of factors affecting post-settlement mortality of other sessile marine invertebrates include predation (Connell, 1961; Zamarano *et al.*, 1995) and competition for food or space (Connell, 1961; Young & Chia, 1984). Other studies have suggested that pre-settlement processes regulate survival of settled larvae. Examples of pre-settlement processes influencing larval survival in marine invertebrates include larval settlement preferences (Grosberg, 1981), larval behaviour (Young & Chia, 1985) and larval predation (Gaines & Roughgarden, 1985). No studies have investigated factors affecting pre- and post- settlement mortality of *T. heterouncinata*. Of the post-settlement factors potentially influencing sabellid larval survival predation appeared to be an unlikely factor regulating sabellid numbers as no predators were observed in the tanks. Kuris and Culver (1999) introduced a wide range of predatory species into tanks containing infested abalone and concluded that none of these potential natural predators caused detectable mortality of sabellids.

The high stocking density of abalone under culture conditions may result in damage to the larval tubes on the shell edge when abalone come in contact with each other (Ruck, 2000). No data are available to corroborate this hypothesis and it is unknown whether larval survival is influenced once the larval tube is damaged. Sabellid larvae are not able

to inhabit burrows created by conspecifics (Simon *et al.*, 2002) and it is not known if a larva is able to re-infest a shell if the larval tube is damaged prior to metamorphosis.

The role of competition for food or space has not been evaluated in this species. The juveniles possess a functional feeding crown approximately seven days after settlement (Culver *et al.*, 1997). Competition for food can not be a factor influencing survival until the larvae have metamorphosed into adults and begin exogenous feeding. Only then can the abundance of particulates or space available for feeding become a limiting factor. Therefore, the influence of resource competition on settlement would not have been discernible in this study as larvae less than four days subsequent to settlement were used. In previous studies in this thesis the occupation level was not influenced by abalone growth rate or diet history within the first week after settlement suggesting competition may only play a role in regulating population size after this time.

Effect of diet on settlement success

Food particle supply has been shown to affect the spacing between neighbouring polychaetes of the same species (Taghon, 1992). Kelp and Abfeed produce different quantities and sizes of particulates within abalone raceways (Chalmers, 2002). Abalone diet had a significant effect on sabellid reproductive characteristics and growth (Ruck, 2000; Chalmers, 2002). In this study abalone diet had no influence on the number of sabellid larvae settled on host abalone shells. Chalmers (2002) hypothesised that differences in diet quality may have resulted in the production of poor quality eggs for sabellids from kelp- fed abalone. He suggested that the production of low-quality eggs may not provide the emerging larvae with sufficient energy to sustain itself until it was able to locate a suitable settlement site on the shell edge (Chalmers, 2002). However, he

did not have data to support this hypothesis. Data from this study indicate that the energy content of eggs produced by sabellids on different diets may be similar. This was deduced from information on egg size rather than chemical composition. The similarity in the number of larvae settled on abalone fed different diets suggested that the nutritional value of the sabellid eggs may not be influenced by the diet history to the extent that larval mortality is influenced within the first 96 hours. To contribute to this topic there is a need to investigate the energy content of sabellid eggs rather than estimating it from egg volume.

The linearity of the increase in the number of larvae settled over the 36-day-period provides an estimation of the daily number of sabellids infesting an abalone within this period. The number of larvae settled on abalone shells up to 36 days can be estimated from the following equation:

$$\mathbf{L = 8.98 + 2.75 * d}$$

Where: L= number of larvae settled
d= number of days since start of infestation

This estimation could not have resulted from previous research on the sabellid since it could only be made due to the technique of marking the growing edge. The estimation is based on the number of larvae settled on the growing edge and does not take into account any larval mortality that occurs 96 hours post-settlement. Sabellid population growth on abalone shells was linear. The natural growth of a population is most often exponential (Campbell, 1995). Simon *et al.* (2002) indicated that it takes approximately four months for the sabellid to mature and begin producing offspring. This experiment was run for only 36 days. Thus, it covers only the initial phase of settlement. In future studies, the growth of a reproducing population could be tested using the same method.

Settlement preference

The highest number of worms infested the pore edge, where the shell edge was thinnest. The thick edge had the lowest infestation level, and was the thickest region of the shell edge. This observation was similar for both diets. Settlement of the larvae of the spirorbid tube worm *Neodexiospira brasiliensis* was influenced by the presence of settlement cues (Hamamoto & Mukai, 1999). In this study there was a preference for larvae to settle around the thinnest area of the shell edge, and the thicker edge of the shell had lowest number of settled larvae. The thinner the growing edge of a shell, the faster the growth of the abalone and hence the greater the rate of shell deposition (Pesch, *pers. comm.*). It may be hypothesised that the larvae settle in areas of the shell with the greatest rate of deposition as it may provide an environment ensuring fast encapsulation. A study investigating the relationship between shell edge thickness and rate of larval settlement may contribute towards this topic. In addition the larvae settled most readily around the respiratory pores. Caceres *et al.* (1999) described a similar observation for the relationship between the burrowing worm *Polydora* sp. and the black clam, *Chione fluctifraga* Showerby. They observed that *Polydora* sp. tend to settle preferentially around the siphon area of the clam and suggested that this is advantageous due to the increased water flow from the clam inhaling and exhaling (Caceres *et al.*, 1999). The fact that larvae settle in certain shell areas in preference to others suggests the presence of settlement cues. He & Mai (2001) have shown differences in the mineralogy and elements present in the prismatic and nacreous shell of abalone. Larvae appear to settle most readily on prismatic shell. It should be tested if the potential difference in chemical or physical properties between prismatic shell and nacreous shell may be detectable to sabellid larvae and provide a cue for the larvae to locate settlement sites. This could be

achieved by placing pieces of prismatic and nacreous shell in seawater, introducing sabellid larvae and observing the number of larvae settled on each shell type.

Periodicity of larval settlement

There was a positive correlation between the tide height and the settlement of sabellid larvae. It is not clear from these data whether there were a greater number of larvae released during the high tides or whether settlement success was higher during these periods. Other studies have shown greater numbers of polychaete larvae were released with increasing tidal height (Knight-Jones, 1951) and no studies have reported increased settlement success at different tidal heights or lunar phases. The highest larval release of a species is generally around periods when the survival of the larvae is maximised. The timing of larval release may be cued by either external cues or by endogenous rhythms. Examples of external factors influencing marine invertebrate species include tidal cycles, lunar phases and photoperiod (Neumann, 1978; Forward *et al.*, 1986). As the abalone were kept in tanks on-shore a direct influence of tidal cycles would not be present. A likely cue for the timing of larval release appears to be the lunar cycle. There was peak settlement of larvae at both the new and full moons suggesting that the sabellid was not cueing larval release according to patterns of moonlight intensity. From the literature available, this is the first example of larval release at both the new and full moon for marine invertebrate larvae. It may be hypothesised that the sabellid is cued by the moon's quarters, releasing the lowest number of larvae at these phases. There is however no data or information available to support this hypothesis. Morgan (1995) stated that the timing of larval release will continue according to the periodicity of the environmental cycle several times after the cycle is removed, after which time the rhythm will eventually decay without further reinforcement. Therefore, if the sabellids were kept in complete darkness for several lunar cycles it should be tested if the periodicity of the larval

settlement would weaken, thus indicating the sabellid settlement is cued by the phases of the moon. If there were still periodicity to larval settlement then it would suggest that the sabellids are using other cues. A study conducted over several months would provide data to better understand periodicity of larval release.

This study indicates that larval settlement success and survival are not influenced by diet within the first 96 hours after settlement. This is supported by findings in Chapter 4 and 5 which showed that diet or abalone growth does not influence sabellid intensity or occupation level within the first week subsequent to settlement. The study was the first to show synchronicity to larval settlement and appears that settlement is cued to the tidal cycle, with peak settlement occurring at high tides.

CHAPTER 7

GENERAL DISCUSSION

T. heterouncinata had received attention from abalone farmers as worm infestations had reached a level where they were threatening the economic viability of abalone farming in South Africa. As the sabellid is endemic to South African waters, its complete eradication from the farms appeared unlikely. Hence there was a need for basic research to identify factors that influence the infestation of farmed abalone. Abalone diet, growth rate and farm management had been suggested by farmers as those factors that most influence reproduction and growth of the sabellid (AFASA, pers. *comm.*). The infestation of farmed abalone indicated that the sabellid had found a niche in which it benefited from the farm environment.

The main objective of this study was to investigate the response of *Terebrasabella heterouncinata* to abalone diet history and abalone growth rate under commercial abalone culture conditions using sabellid morphometrics and offspring counts. To accomplish this, data on the life-history and degree of expression of traits were obtained in an initial study aimed at comparing sabellid populations from different environmental conditions. The success of settlement and settlement preference of sabellids on the shell edge under different dietary conditions was investigated.

The influence of farm conditions on sabellid reproduction and morphometrics

A comparison of reproductive characteristics with other polychaete species revealed that the sabellid is essentially a *K*- strategist. *T. heterouncinata* is characterised by a high degree of parental care (intratubular brooding), a few relatively large eggs and larvae, iteroparous breeding and lecithotrophic larvae. This is the first study to place the sabellid's reproductive characteristics on the *r-K* continuum.

Sabellid reproductive and morphometric characteristics were influenced by the farm from which infested abalone were sampled. Each farm had a unique suite of management-related environmental conditions. The comparison of sabellid life history characteristics between farms was carried out, not to try and isolate factors responsible for influencing the sabellid, but rather to determine the degree of variation exhibited by the sabellid under presumably different environmental conditions. Plasticity of reproduction and development has been well documented for several polychaete species (Grahame & Branch, 1985; Qian & Chia, 1991; Giangrande *et al.*, 1994; Qian, 1994; Giangrande, 1997). In this study *T. heterouncinata* exhibited treatment-dependent variation in morphometric and reproductive characteristics. Variability was expressed as the coefficient of variation. The coefficient of variation ranged from 120% for the number of larvae per egg to 16% for the stage 2 larvae length. The coefficient of variation for the number of eggs and larvae per adult ranged from 77 to 120%. Coefficient of variation values for the size of the life history stages were all less than 33%. Thus, it appears that the sabellid responds to different environmental conditions by changing the number rather than the size of the various life stages. Furthermore, the size of the larvae and eggs was similar between farms, while the number of eggs and larvae showed greater variability between farms. This flexibility is possibly a means by

which the sabellid modifies the expression of life history traits in order to maximize its fitness. There was sufficient variability in the morphometric and reproductive characteristics of *T. heterouncinata* to show sensitivity to different conditions. This study provides data on offspring counts and morphometrics to which future studies can be compared. The range of egg and larvae counts and morphometrics of *T. heterouncinata* for this and other studies are provided in table 7.1. Winter (2001) crushed whole shells and used the ratio of eggs and larvae to adults and did not count eggs and larvae specific to adults. This may account for the lower offspring counts compared to this study. Ruck (2000) observed a lower abundance of particulates suspended in aquaria under laboratory conditions compared to raceways. Simon *et al.* (2002) conducted their study under laboratory conditions and the lower particulate load may have resulted in smaller adults and a lower number of offspring per adult than observed in sabellids from farms.

Table 7.1: A comparison of the range of sabellid morphometrics and offspring counts between this and other studies.

Variable	**This study**	**Simon *et al* (2002)**	**Winter (2001)**	**Chalmers (2002)**
Occupation level (% tubes occupied)	33 - 89		0 - 85	6 - 63
Intensity (tubes per cm)	0.25 - 18			3 – 15
Adult length (mm)	0.94 – 4.6	1.1 – 1.5		1.7 – 5.5
Base width (mm)	0.1 – 0. 55			0.1 – 0.73
Stage 1 larvae length (mm)	0.3 – 1.1			
Stage 2 larvae length (mm)	0.47 – 1.54			
Egg Volume (mm^3)	0.01 – 0.06	0.01 – 0.04		
Number of stage 1 larvae per adult	0 - 7		0 - 3	
Number of stage 2 larvae per adult	0 - 7	0 - 3	0 – 1.5	
Number of eggs per adult	0 - 10	0 - 3	0 - 3	
Number of larvae per egg	0 - 6		0 – 1.4	

The influence of a diet change on sabellid reproduction and morphometrics

Diet history of the host abalone influenced the reproductive and morphometric characteristics of *T. heterouncinata*. Diet had been suggested as an important factor affecting infestation of sabellids under farm conditions (AFASA, *pers. comm.*). Thus, the importance of abalone diet history on sabellid reproduction and morphometrics was investigated. According to Chalmers (2002) particulates originating from Abfeed raceways had a higher energy and protein level than suspended matter in kelp raceways (Chalmers, 2002). It was hypothesised that sabellids from the Abfeed raceways may be larger or more productive. In the current study the diet of the abalone appeared to exert a stronger influence on the size of the sabellid life stages than on the number of eggs and larvae produced per adult. This observation differed from that in Chapters 3 and 5, where the conditions on the farms influenced the number of offspring more than their size. This may be related to the fact that this experiment was run for 185 days. It may thus be hypothesised that the sabellid responds to a change in conditions by initially altering the size of the offspring after which time the number of offspring changes. It should be tested if the number of offspring becomes influenced by a change in conditions more than 1.5 generations after the change has occurred. Only three of the morphometric and reproductive characteristics of the sabellid were different between the Abfeed- only and kelp- only group. These were base width, egg volume and intensity of infestation. Chalmers (2002) reported a higher sabellid intensity on abalone fed Abfeed which is similar to results obtained for this study. Chalmers (2002) was the only author to have measured base width for the sabellid. The results are, however, not comparable as he investigated the combined influence of abalone growth and diet on the base width and was not able to isolate the factor influencing the base width. Simon *et al.* (2002) reported no difference in the volume of sabellid eggs from abalone fed

kelp and Abfeed. Their study was conducted in aquaria under laboratory conditions. The suggested lower abundance of particulates in tanks kept in a laboratory when compared to commercial raceways (Ruck, 2000) may have diminished the response of the sabellid population to different diets.

Farmers reported a reduction in the level of infestation when abalone diet was shifted from kelp to Abfeed. In this study there was no significant difference in the sabellid intensity or occupation level of tubes from abalone that had their diet changed from Abfeed to kelp compared to the sabellids from abalone that were fed kelp, Abfeed or kelp then Abfeed. The intensity of infestation was only different between the kelp- only and Abfeed- only groups indicating that diet had a small effect on regulating the number of tubes on the shell edge. However, a comparison of the sabellid intensity of the previously infested abalone at the start and end of the study revealed that the number of sabellid tubes on the shell edge showed the greatest decrease in the Abfeed-kelp treatment. This corroborates the farmers' observation of a decrease in sabellid intensity with a change in abalone diet from Abfeed to kelp. Abalone fed Abfeed throughout the study had a slower growth rate and a higher sabellid intensity than abalone fed kelp. It has been suggested that fast growing abalone are able to deposit shell at a rate which smothers the larvae settled on the shell edge, reducing the survival of the larvae (Ruck, 2000). As the importance of abalone growth on sabellid reproduction and morphometrics was not clear, a study to investigate this factor in isolation was conducted.

The influence of abalone growth on sabellid reproduction and morphometrics

Previous studies had been conducted to show the influence of abalone size on sabellid population characteristics (Chalmers, 2002; Simon *et al.*, 2003). It was, however, not investigated if slow-growing abalone were most prone to sabellid infestation or if the presence of sabellids on the abalone resulted in reduced growth. This study attempted to isolate the potential effect of abalone growth on the success of sabellid settlement and its population growth.

The sabellid intensity and occupation level were not influenced by the growth rate of the abalone for abalone fed kelp or Abfeed. Thus, from these data the hypothesis that slow growing abalone are more susceptible to sabellid infestation could not be supported. However, there were differences in reproduction and morphometrics of the sabellids on abalone of different growth. Thus, it may be hypothesised that the environment in which the sabellid matures is modified by abalone growth rate.

Sabellids from Sea Plant Products were more affected by abalone growth than I&J sabellids. Abalone growth influenced the reproductive and morphometric characteristics of the sabellids. There was a decrease in the expression of all life-history- related variables on fast growing abalone. The slow growing abalone from Sea Plant had larger sabellids, produced larger and more eggs and larvae than the faster growing abalone. In sabellids from I&J, only the egg volume was significantly different between the two abalone size classes. Thus, the response of the sabellid populations to abalone growth was evident on Sea Plant but not on I&J. In many other species it has been shown that animals respond differently to one factor in the presence or absence of another (Tave, 1993). It is possible that abalone growth influenced sabellid population

on abalone fed Abfeed. Chalmers (2002) showed a positive influence of Abfeed on sabellids. He showed that abalone fed Abfeed had greater sabellid intensity and contained larger adults with a greater base width than sabellids from abalone fed kelp (Chalmers, 2002). Thus, the combined effect of slow-growing abalone fed a more nutritious diet may have synergistically affected sabellids in combination with slow growth.

Larval settlement

This study was the first to investigate larval settlement in *T. heterouncinata*. Larval mortality was shown to be similar between diets within the first 96 hours after settlement. Variable settlement between treatments would indicate that pre-settlement factors such as settlement preference and larval settlement influenced sabellid populations. Thus, it appears that post-settlement mortality of *T. heterouncinata* is a more important factor regulating the population on a shell than larval settlement. Chapter 5 and Chapter 6 made a contribution to this topic. It was shown that larval settlement depended more on the choice for an area on the shell than on the growth of the abalone. Chapters 4, 5 and 6 showed that occupation level was not influenced by diet or abalone growth within the first week after larval settlement. Chalmers (2002) hypothesised that competition between sabellids may influence survival. However, in this study only larvae less than seven days after settlement were considered. Competition for food can only influence sabellids that have formed a feeding crown and have begun exogenous feeding. Therefore, it was not possible to determine the influence of competition for food or space between sabellids.

Sabellid population estimation

The new technique used in this study allowed the estimation of population increase of sabellids within the first 36 days of infestation under the conditions found on the farms. The number of larvae settled on abalone shells up to 36 days could be estimated from the following equation:

$$L = 8.9 + 2.7 * d$$

Where: L= number of larvae settled
d= number of days since initial infestation

This study was conducted under farm conditions with a low abalone stocking density (16 abalone per basket). Future studies using this technique should test if a higher abalone stocking density influences the sabellid population growth. This estimation could only be made due to the technique of marking the growing edge with pencil lines. It appears that the use of pencil areas on the shell is a quick and effective means of describing settlement of larvae on the growing edge of abalone.

Larval settlement preference

There was a preference by larvae to settle around the thinnest area of the shell edge. Settlement preference of *T. heterouncinata* larvae had not been investigated by other authors. The thinner the growing edge of a shell, the faster the growth of the abalone and hence the greater the rate of shell deposition (Pesch, *pers. comm.*). It may be hypothesised that the larvae settle in areas of the shell with the greatest rate of deposition as it may provide an environment ensuring fast encapsulation. Thus, the heterogeneous distribution of sabellid larvae on the shell edge indicated the presence of settlement cues. The respiratory pores may be attractive to the sabellids as the increased movement of water through the pores during respiration may lead to better food

availability than other shell areas. Differences in shell mineralogy and composition have been shown for different types of abalone shell (He & Mai, 2001). In this study larvae appeared to settle most readily on prismatic shell. Differences in chemical or physical properties between prismatic shell and nacreous shell may be detected by sabellid larvae and may have provided a cue to locate settlement sites. Thus, these data and the data from Chapter 5 allow for conclusions to be made regarding larval settlement strategy. Sabellids to do not appear to choose the abalone they infest as settlement was uninfluenced by abalone growth. However, once the larvae have located a host abalone they appear to select a region of the shell on which they preferentially settle.

Periodicity of settlement

Larval settlement of *T. heterouncinata* was related to tidal height, being greatest at high tides. It is not clear whether the liberation of larvae was greatest at high tides or if settlement success was influenced by the height of the tide. Knight-Jones (1951) observed peak liberations of *Spirorbis borealis* larvae at high tides. As the influence of the tides would not have been present on the farm, it may be hypothesised that sabellid larval settlement may be cued by the lunar cycle. However, peak settlement occurred at both the new and full moon, suggesting that the sabellid may not be using moonlight intensity to cue larval release. Thus, it is not clear whether the peak settlement of larvae is cued by the lunar or tidal cycle and future studies should examine potential changes in water chemistry on farms during low and high tides.

Therefore, from the combined results several conclusions can be drawn and hypotheses proposed. The greater the variation in the expression of life history traits the greater

was the influence of environmental conditions on the sabellid. Potentially the greatest variability in environmental conditions of all studies conducted was between farms. Sabellids from abalone that experienced a diet change exhibited less variation in the expression of traits than sabellids from the four farms but more than sabellids from abalone of different size. If it is assumed that the greater the variability in a population, the greater the influence of a particular factor on that population, then it appears that abalone diet is more influential than abalone growth on the sabellid morphometrics and reproductive characteristics. This is supported by data which shows that the variation of seven of the 11 life-history- related variables as a result of change in diet was greater than the variation due to abalone growth. The difference in variation was greater than 20 % when comparing the influence of a change in diet and abalone growth for the number of stage 1 and stage 2 larvae per adult, sabellid intensity, larvae per egg and occupation level. The suggestion that diet is more influential than abalone growth is supported by the lack of response of sabellids to abalone growth rate from abalone fed kelp. If abalone growth was an important factor regulating sabellid populations then the response of sabellids to abalone of different growth rates would have been similar for abalone fed Abfeed and kelp.

Under the wide range of conditions sabellids were exposed to during these studies there were similarities in the response of the sabellids. Under different farm conditions, abalone diets and abalone growth rates the same life-history traits were most variable. These were the number of larvae per egg, number of stage 1 and stage 2 larvae per adult, number of eggs per brood and sabellid intensity. This observation has been reported by Qian and Chia (1991) who indicated that the number of eggs and larvae

were more variable than the size. It appears that the sabellid is able to maximise its fitness by varying the number of offspring rather than their size.

Sabellid intensity and occupation level of tubes was not influenced by a change in host diet or abalone growth. However, these variables were significantly different between some of the farms. Although it was not possible to isolate factors responsible for differences between farms, this information suggests that there may be confounding factors which influence the sabellid populations. This suggestion is corroborated by data which showed that the combination of abalone growth and the feeding of Abfeed had more pronounced effects on sabellid populations than the influence of abalone growth and the feeding of kelp. Slow growth of abalone in combination with feeding Abfeed appears to positively influence sabellid populations, resulting in larger adults and greater numbers of larger offspring and eggs than sabellids from fast growing Abfeed- fed abalone. Thus, from these data it appears that sabellid populations were not strongly influenced by abalone diet or growth rate in isolation, as has been suggested by farmers, but rather the combination of these two factors.

The greater difference in sabellid morphometrics and reproduction between fast and slow growing abalone fed Abfeed when compared to abalone fed kelp indicates that Abfeed is resulting in a greater response by sabellids than kelp. This suggestion is supported by data obtained on sabellid population exposed to a change in diet. It was shown that sabellid morphometrics and reproductive characteristics were more influenced in sabellids that experienced a change in diet from kelp to Abfeed than those from abalone fed Abfeed then kelp. Since there could have been no difference in the particulate composition available to sabellids from each abalone size class the

differences in sabellid morphometric and reproductive characteristics may not have been due to the quality of the feed particles.

The similarity between the expression of sabellid life history traits between Hermanus Abalone and HIK indicates that farm management practises may play an important role in regulating the sabellid populations. These farms are located next door to one another, share the same source of water and have similar management procedures. Therefore, it appears that water quality or management procedures may play an important role in determining the extent of sabellid infestations on farms. Water quality parameters such as pH, ammonia, nitrite, nitrate and temperature may influence sabellid populations and appear to be the most suitable factors for future investigation. Some farms have managed to curtail the spread of sabellids within the farm and the sabellid does not pose as great a threat as previously reported. However, other farms are still experiencing great difficulty in dealing with the sabellid infestation and their economic viability is still under threat. Thus, it follows that a greater amount of collaboration between farmers needs to be implemented in order to ensure economic viability of the South African abalone industry.

In conclusion, this research was successful in developing a technique for the removal of adult sabellids and the offspring and eggs specific to individual adults. The use of pencil markings on the shell edge to count the number of larvae settled allows for a more accurate determination of sabellid intensity and occupation level. Furthermore, the number of larvae settled in a known period of time could be determined for the first time. It was shown that abalone growth rate did not influence the number of larvae settled or survival of larvae on the shell edge, suggesting that slow growing abalone

were not more susceptible to infestation by sabellids, as had been suggested in past studies. The reproductive and morphometric characteristics of the sabellid were influenced by both abalone diet and growth rate however, the responses of the sabellids were varied and inconsistent. It appears that there may be several factors acting simultaneously, producing the different reproductive and developmental characteristics seen in sabellid populations under different environmental conditions. Finally the data on settlement allowed the presentation of a hypothesis regarding choices made by the sabellid. It may be hypothesised that sabellid larval settlement is principally determined by abalone shell region, then by a change in diet, and least importantly by abalone growth.

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