The life history patterns of the polychaete, *Terebrasabella heterouncinata*, a pest of cultured abalone

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Carol Anne Simon
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Abstract

Terebrasabella heterouncinata is a small K-selected sabellid polychaete. It is a simultaneous hermaphrodite with a semi-continuous mode of reproduction, producing relatively few large eggs that are brooded within the parental burrow until the larvae emerge, to settle on the growing edge of the abalone shell. Despite its low fecundity, this worm has become problematic on abalone farms in South Africa. The present study was conducted to gain an understanding of the life history patterns of *T. heterouncinata* to determine how they contributed to the success of these worms under altered conditions. This study demonstrated that conditions prevalent on abalone farms were conducive to enhancing the reproductive success of this worm, and suggests that larger, more fecund worms may have been selected for in the decade that these worms have been present on the farms. Increased nutrient availability, and possibly the increased stability of the farm environment relative to its natural environment, has led to a 1.5-fold increase in the average size of the worms. Body size was found to be positively correlated with brood size, and this resulted in worms on farms brooding 3 to 4.5 times more offspring at a time than worms from wild abalone. The ability to increase the number of eggs produced at a time may have been limited by the fact that these worms have only two ovaries. Thus, the increase in fecundity may have been related primarily to the increase in the rate at which the eggs were laid by the worms on the farms, and the increase in the coelomic space available for the storage of these rapidly developing eggs. The ability to increase the rate at which oocytes develop may be related to the vitellogenic mechanisms employed by these worms. Vitellogenic oocytes are able to incorporate high molecular weight yolk precursors from the surrounding coelomic fluid through endocytotic activity. This may allow the oocytes to increase the rate at which they incorporate yolk material under conditions of nutrient enrichment. The increase in fecundity did not occur at the expense of offspring size and, presumably, quality. The increased reproductive output on the farms was compounded by a proportionate increase in the number of reproducing worms within the population. In addition, these worms are long-lived (worms from farmed abalone reached a maximum age of approximately 40 months) and exhibit negligible senescence. Thus, their reproductive output did not change significantly with an increase in age. Furthermore, the proportion of the reproductive worms did not decrease with an increase in age. Thus, within the age range tested, worms of all ages have the potential to make equal contributions to population growth. While diet and abalone stocking density could not be identified as having a significant effect on reproductive output and infestation rate under intensive culture conditions, it was demonstrated that in a naïve abalone population, the total intensity of infestation increased exponentially with time. This increase may be a consequence of an increase in fertilisation success. These worms continuously produce ent-aquasperm that are released into the water column. The sperm are collected by other individuals that then store the sperm in a single spermatheca. The ability to store sperm relieves individuals of a

dependence on the synchronisation of spawning of eggs and sperm. As the population size and density increases, there could be more individuals releasing sperm into the water column, resulting in a continuous supply of sperm. The increased production of eggs would therefore not be constrained by a lack of sperm. The stored sperm are released into the brood chamber to fertilise eggs as they are laid, and this would probably increase the fertilisation success in the species. This study also provides evidence to suggest that reproduction in this worm has a seasonal component. Future studies should concentrate on measuring fertilisation success in greater detail, measuring the effect of season on reproduction, determining whether there are genetic differences between worms on farmed and wild abalone and determining whether wild worms have similar life-spans and age-related fecundity as worms on farms.

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"It was the best of times, it was the worst of times…"

Charles Dickens

CHAPTER 1

Introduction

"Humble thyself greatly, for the vengeance of the ungodly is fire and worms."

Sirach, 7:17

During the last decade, the South African abalone culture industry has suffered severe economic losses due to the infestation of the abalone, *Haliotis midae*, by an endemic sabellid, *Terebrasabella heterouncinata* Fitzhugh & Rouse 1999 (Fig. 1.1; Ruck and Cook, 1998; Fitzhugh and Rouse, 1999). In its natural environment, molluscan hosts are not heavily infested, yet on farms, infestation levels can be so high that they interrupt the natural growth patterns of the abalone, resulting in deformed, brittle shells (Fig. 1.2) and reduced abalone growth (Fitzhugh 1996; Oakes & Fields 1996; Culver et al., 1997; Ruck and Cook 1998). The proliferation of this worm on abalone farms therefore provides a unique opportunity to study the life history responses of this problematic worm under natural and altered conditions.

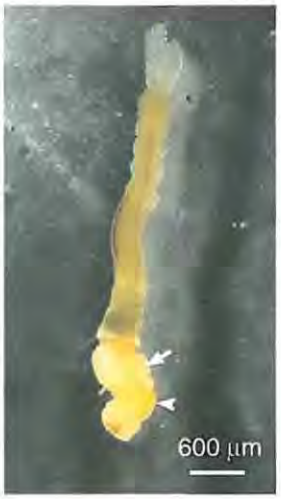

Figure 1.1. An adult *Terebrasabella heterouncinata*, showing the white male reproductive segment (arrow) filled with developing spermatids and the orange female reproductive segment (arrowhead) containing vitellogenic eggs.

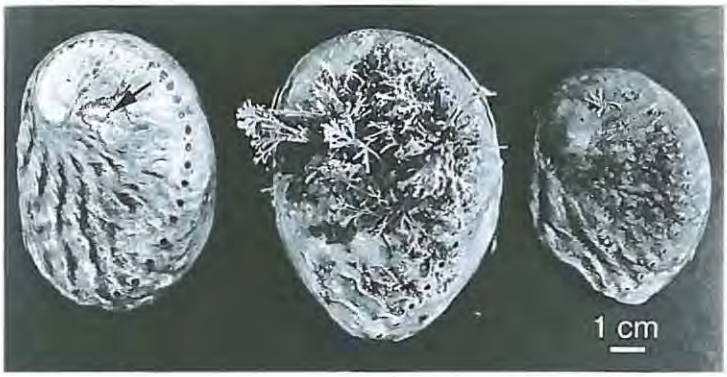

Figure 1.2. Abalone shells from different environments. From left to right: abalone shells from an abalone farm, the natural habitat close to the abalone farm effluent outflow, and the natural habitat, approximately 2 km from a farm. The arrow points to where the prismatic shell has come away from the shell due to weakening by infestation with *T. heterouncinata*.

An organism can become a pest or invader in an altered environment for several reasons. Invading organisms may escape their natural predators, diseases or parasites and other factors that control their population growth in their native environment (Bright, 1999). They may also be resistant to those parasites that control the populations of competitors in their new habitat (Calvo-Ugarteburu and McQuaid, 1998). Furthermore, many successful invaders and pests mature rapidly, have a high fecundity and thrive in disturbed environments (Mozley, 1960, in Grassle and Grassle, 1974; Bright, 1999). Such species therefore display life history traits similar to those of opportunistic or *r*-selected species (Grassle and Grassle, 1974; Levin, 1986; Zajac, 1986; Qian and Chia, 1991; Qian, 1994). This has been demonstrated in the invasive mussel species *Mytilus galloprovincialis* (Hockey and van Erkom Schurink, 1992) and *Dreissena polymorpha* (Zorpette, 1996) and the sabellid polychaete, *Sabella spallanzanii* (Currie et al., 2000). These species are characterised by a high fecundity and rapid population growth, all factors that contribute to their ability to successfully compete with local species. Similarly, shell-boring polychaetes that become pests of cultured shellfish have high levels of fecundity, with some species producing several thousands of planktonic larvae (Blake and Arnofsky, 1999; Lleonart, 2001). By contrast, *T. heterouncinata* displays many K-selected characteristics – it inhabits a stable environment; it has a relatively low fecundity, producing up to ten large eggs (240 x 130 μm) that are brooded until they develop into directly developing larvae which move to the growing edge of the host shell (Culver et al., 1997; Fitzhugh and Rouse, 1999; Gray, 2004). Furthermore, these worms breed repeatedly throughout the year (Culver et al., 1997). This disparity therefore raises the question of how an animal that broods only a few offspring simultaneously could become successful as a pest under altered conditions where hosts are plentiful, food is abundant and conditions are relatively stable and predictable.

Based on the life history traits described for *T. heterouncinata* (Culver et al., 1997; Fitzhugh and Rouse, 1999) and existing life history theories (e.g., Pianka, 1970; Stearns, 1976; Stearns and Hoekstra, 2000), it is possible to hypothesise how these animals might respond under altered environmental conditions such as those found on abalone farms. The increase in the relative stability of the environment (reduced water movement in the tanks compared to the subtidal region that they normally inhabit) predicts an increase in life span with a concomitant increase in the total number of clutches produced in the animal's life, but each clutch would consist of fewer progeny of a constant size (Pianka, 1970; Prevedelli and Zunarelli Vandini, 1998). Alternatively, an increase in habitat stability may lead to a reduction in the life span of an animal, but an increase in early reproduction (Prevedelli and Simonini, 2001). An increase in habitat availability and accessibility (increase in abalone density) predicts that fewer, larger progeny will be produced (Gotto, 1962, in Stearns, 1976). Finally, the effect of an increase in food quality and quantity (constant supply of degraded abalone food and faeces) may have several effects: 1) an increase in fecundity while egg quality remains the same (Grémare et al., 1988); 2) an increase in egg quality while fecundity remains unchanged (Qian, 1994); 3) an increase in both fecundity and egg quality (Bricelj et al., 1987); 4) a decrease in egg quality while fecundity increases (Qian and Chia, 1991; Qian, 1994) and 5) an increase in

egg quality while fecundity decreases (McKillup and Butler, 1979). However, under altered conditions it is not only fecundity that may be affected. Several studies have demonstrated the effect of habitat stability (Hart and Begon, 1982) and nutrient enrichment on the body size of invertebrates, including polychaetes (e.g., Levin, 1986; Qian and Chia, 1991; Qian, 1994), which may further influence fecundity. The testing of these predictions forms the basis of this study.

The manner in which *T. heterouncinata* responds to an altered environment may, however, be affected by the covariability of reproductive traits in polychaetes (Chia, 1974; Olive, 1985; Giangrande et al., 1994; Stearns and Hoekstra, 2000). Many polychaetes, including *T. heterouncinata,* differ from the proposed *r*- and K-scheme: large body size is usually associated with small planktotrophic eggs, large clutch size and no parental care, while small body size is associated with small clutches of large lecithotrophic eggs that are brooded (Westheide, 1984; Olive, 1985; Giangrande et al., 1994; Rouse and Fitzhugh, 1994). This relationship is particularly important with respect to changes in fecundity – in small-bodied polychaetes, such as *T. heterouncinata,* where space for the storage of developing oocytes might be limited (Hermans, 1979; Olive, 1985), reproductive output might be increased by decreasing the interval between reproductive episodes and not by increasing the number of offspring produced per reproductive episode.

Studies measuring the expression of life history responses under different environmental conditions concentrated on variables such as the number and size of offspring and the length of the intervals between reproductive episodes (e.g., Levin, 1986; Qian and Chia, 1991; Qian, 1994). These studies did not, however, consider that the degree of flexibility in the expression of life history responses is constrained by the structure of the ovary and species-specific mechanisms of egg production (Eckelbarger, 1983, 1994). Reviews by Eckelbarger (1983, 1986, 1994) revealed relationships between life history styles (particularly with respect to the length of the gametogenic cycle and the number of reproductive episodes per lifetime) and patterns of oogenesis in several invertebrate groups, including polychaetes. These reviews demonstrated that most life history traits depended on the location and complexity of the ovary and consequently the type of oogenesis, the site of yolk precursor production and storage, the nature of the precursor and the method by which the precursors are acquired by the oocytes (Eckelbarger, 1983, 1994). For example, in polychaetes that produce eggs rapidly, vitellogenic oocytes often occur in close association with proteosynthetically active follicle cells, within the ovary, or nurse cells, within the coelom, that supply the developing oocyte with high molecular weight yolk precursors. This has been demonstrated in the *r*-selected *Capitella jonesi*, *Phragmatopoma lapidosa* and *Streblospio benedicti* and the small-bodied *Ophryotrocha puerilis* and *Ophryotrocha labronica* (Emmanualsson, 1969; Eckelbarger, 1979, 1980; Eckelbarger and Grassle, 1982; Pfannenstiel and Grünig, 1982). By contrast, vitellogenesis in species that produce eggs slowly usually occurs without the intimate association of the developing oocyte with accessory cells, and has been demonstrated in the polychaetes *Nicolea zostericola*, *Sabella spallanzanii* and *Branchiomma luctuosum* (Eckelbarger, 1975; Giangrande et al., 2000; Licciano et al., 2002). An

investigation of the ultrastructure of the ovary and mechanism of vitellogenesis in *T. heterouncinata* was therefore undertaken to determine how it relates to the life history patterns of this sabellid.

The success of *T. heterouncinata* on farms may also be attributed to mechanisms of fertilisation. Finley et al. (2001) demonstrated that these worms are able to self-fertilise. Self-fertilisation in simultaneous hermaphrodites is, however, rare, while cross-fertilisation is considered the norm (Ghiselin, 1974). Comparison of the reproductive biology of *T. heterouncinata* with that of similar-sized Sabellidae with similar life histories, suggests that not only does this sabellid normally cross-fertilise, but that it probably also stores sperm from other individuals (see Rouse, 1996) although organs for sperm storage were not detected by Fitzhugh and Rouse (1999). Information concerning the fertilisation biology of a polychaete can be gleaned from the morphology and ultrastructure of the sperm. Franzén (1956) proposed that 'primitive' sperm (i.e., sperm with spherical heads) occurred in broadcast spawners while 'aberrant' sperm (i.e., sperm with elongate heads) occurred in invertebrates with a modified fertilisation biology. Rouse and Jamieson (1987), however, proposed that sperm should be classified according to their functional biology and not solely according to their morphology. Hence ect-aquasperm are spawned freely into the water where fertilization occurs; ent-aquasperm are released into the water but are gathered by females or hermaphrodites while introsperm never come into contact with the ambient water. Sperm morphology is not, however, sufficient to confirm cross-fertilisation (Rouse, 1999) and evidence of sperm storage is required. The ultrastructure of spermiogenesis and the sperm of *T. heterouncinata* are therefore described, and the polychaete was examined for structures of sperm storage to gain a better understanding of the fertilisation biology of this species.

The aim of this study was therefore to measure to what extent the life history traits of *T. heterouncinata* were able to change in an altered environment and how this could have helped this species in becoming so successful under mariculture conditions. This was done by measuring the degree of expression of life history traits under different farm, natural and laboratory conditions. In addition gametogenesis was described to determine how it contributes to the reproductive success of this worm. In Chapter 2 the expression of life history traits of worms from wild and cultured abalone are compared. Chapter 3 is a controlled laboratory study quantifying the effect of diet on the expression of life history traits. This chapter also investigates the nature of the relationship between the sabellid and its host to determine to what degree it is obligatory. Chapter 4 quantifies the combined effect of abalone diet and stocking density on abalone growth and the degree of infestation by *T. heterouncinata* under intensive mariculture conditions. This study also investigates the relationship between abalone growth rate and the rate and level of infestation. In Chapter 5 the effect of the worms' age on the reproductive traits is measured. Chapter 6 describes oogenesis and how that may influence the speed of egg production while Chapter 7 describes the ultrastructure of spermiogenesis, spermatozoa and spermathecae to gain a greater understanding of the fertilisation biology of this worm. Chapter 8 provides a synthesis of the results.

Chapters 2 to 7 are written in the form of papers, some of which have been published. There is

therefore a certain degree of repetition in the introductions concerning the natural history of the sabellid and its impact on the abalone industry.

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CHAPTER 2

The life history responses of *Terebrasabella heterouncinata* under natural and aquaculture conditions

Simon, C.A., Kaiser, H. and Britz, P.J. The life history responses of the abalone pest, *Terebrasabella heterouncinata*, under natural and aquaculture conditions. Marine Biology, in press.

"and down went
My Uncle
Sol
And started a worm farm."

e.e. cummings

Abstract

The sabellid, *Terebrasabella heterouncinata*, is a small (< 5 mm) intratubular brooder that lives in burrows within the host's shell matrix. It is a semi-continuous breeder and despite producing small numbers of large eggs, infestation by this animal has reached epidemic proportions on local abalone farms. The present study compared the morphometrics and reproductive characteristics of worms from farmed and wild abalone in the Walker Bay area of the south Western Cape Province of South Africa, to gain insights into why this animal has become so successful under aquaculture conditions. The farms designated FARM A and FARM B each had one 'on-farm' site, and two wild sites, while FARM C had two 'on-farm' sites and two wild sites. The wild sites were natural abalone habitats located within 2.5 km of the farms. Our results showed conclusively that environmental conditions prevalent on the farms enhanced the reproductive success of these worms relative to that observed in its natural environment. At FARMS B and C, worms occurred in significantly higher densities at the on-farm sites than in the corresponding wild samples, but at FARM A, density was equally low at the three sites. At all three farms, a greater proportion of the population was reproductively active in the on-farm samples than in the wild samples. Worms on farmed abalone had a higher instantaneous fecundity, brooded more clutches simultaneously and were larger than their conspecifics from the wild. There was a positive correlation between adult size and brood size and the number of clutches brooded simultaneously. Within the three on-farm sites there was a negative correlation between egg volume and brood size, indicating a trade-off between these traits. However, such a trade-off was not apparent between sites, with brood size being higher at the on-farm sites than at the wild sites, irrespective of egg size. This suggests that the stable nutrient enriched environment on the farm led to an increase in fecundity without compromising the size (and implicitly the quality) of the eggs. Worm density did not have a significant effect on body size or any other reproductive traits at most sites, and the density of *T. heterouncinata* was unaffected by the density of other shell-infesting polychaetes. The results suggest that the farm environment has selected for larger, more fecund worms that breed rapidly and have a high recruitment success as a consequence of abundant nutrients, high host density, habitat stability and a possible lack of predation and interspecific competition.

Introduction

The sabellid, *Terebrasabella heterouncinata*, is endemic to South Africa and infests the shells of various gastropods, including the commercially important abalone, *Haliotis midae* (Ruck and Cook, 1998). It is K-selected, producing few large eggs that are brooded in the parental burrow until their emergence as crawling, directly developing lecithotrophic larvae (Culver et al., 1997; Fitzhugh and Rouse, 1999). It reaches sexual maturity at three to four months (Ruck and Cook, 1998; Finley et al., 2001; Simon et al., 2002), after which it reproduces repeatedly, often brooding offspring of different ages simultaneously (Culver et al., 1997).

The intensification of abalone farming has allowed this sabellid to settle in an environment different from that in the wild. On abalone farms, abalone are cultured at high population densities (Cook, 1998) in systems with reduced water movement in comparison to the wild habitat. The farm environment provides the filter feeding sabellids with an abundance of nutrient rich, particulate organic matter originating from the faeces of the intensively fed abalone and degraded abalone food (Chalmers, 2002). It has also been shown that the use of pelleted abalone feed, as opposed to kelp (*Eklonia maxima*), results in abalone faeces with comparatively higher levels of protein and energy (Chalmers, 2002), promoting a higher rate of sabellid reproduction. *Terebrasabella heterouncinata* first became established as a pest on cultured abalone a decade ago (Ruck and Cook, 1998), and several generations of worms have grown up under farm conditions which have probably imposed certain selection pressures on these worms (cf. McKillup and Butler, 1979; Prevedelli and Zunarelli Vandini, 1998; Prevedelli and Simonini, 2001). The abalone farms, which support substantial abalone and *T. heterouncinata* populations, therefore provided an opportunity to investigate the degree to which the life history characteristics of this sabellid polychaete have changed in response to an altered environment.

Many successful invasive and pest species mature rapidly, have a high fecundity and thrive in disturbed environments (Mozley, 1960, in Grassle and Grassle, 1974; Bright, 1999) and display life history traits similar to those of opportunistic or *r*-selected species (Grassle and Grassle, 1974; Levin, 1986; Zajac, 1986; Qian and Chia, 1991; Qian, 1994). In the marine environment, this has been demonstrated in the invasive mussels *Mytilus galloprovincialis* (Hockey and van Erkom Schurink, 1992) and *Dreissena polymorpha* (Zorpette, 1996; Berkman et al., 1998) and the sabellid polychaete, *Sabella spallanzanii* (Currie et al., 2000). These species are characterised by a high fecundity and rapid population growth, all factors that contribute to their ability to successfully compete with local species for resources. Similarly, many problematic shell-infesting polychaetes belonging to the polydorid-complex, such as *Polydora websteri*, *Polydora ciliata* and *Boccardia proboscidea*, exhibit *r*-selected characteristics. These species produce up to 550, 4 000 and 190 000 relatively small eggs per spawn, respectively, and the larvae are planktonic (Blake and Arnofsky, 1999; Lleonart, 2001). These traits have contributed to them becoming pests under aquaculture conditions (Lleonart, 2001).

By contrast, *T. heterouncinata* only broods up to ten relatively large eggs that develop into crawling larvae (Culver et al., 1997; Fitzhugh and Rouse, 1999; Simon et al., 2002; Gray 2004). Despite these apparent reproductive limitations, the infestation of abalone by this worm has also become problematic under culture conditions.

While the degree of expression of life history responses of opportunistic, shell-infesting polydorid and invasive polychaete species is well documented (Levin, 1986; Zajac, 1986; Qian and Chia, 1991; Qian, 1994; Blake and Arnofsky, 1999; Currie et al., 2000), the quantification of the reproductive success of pest polychaetes under natural and altered environmental conditions has received scant attention. No studies have compared the growth and reproduction of *T. heterouncinata* from farmed and wild abalone, although Gray (2004) demonstrated a degree of variability in reproductive and morphometric characteristics of this worm sampled from four abalone farms in the Western Cape Province of South Africa. Gray (2004) also showed that the number of eggs brooded by worms on different farms ranged between one and ten. This is in stark contrast to some shell-boring polydorid polychaetes that show up to 100-fold differences in fecundity under different conditions (Blake and Arnofsky, 1999). The degree to which *T. heterouncinata* can increase its fecundity is probably limited by its small size due to limited coelomic space for storing developing eggs (Hermans, 1979; Olive, 1985), as well as limited storage space within the burrow. The high levels of infestation observed on cultured abalone (Oakes and Fields, 1996; Culver et al., 1997; Ruck and Cook, 1998; Gray, 2004) can probably be attributed to the short generation time, abundant resources, high larval survival owing to parental care and high recruitment success on abalone farms where the hosts live in close proximity to one another.

This study was therefore designed to compare the reproductive output of sabellids from abalone cultured on three farms with that of worms from wild abalone at varying distances from each of these farms. This was done in an attempt to understand the reproductive characteristics that determine the population growth of *T. heterouncinata* on cultured abalone.

Materials and methods

Sample sites

Sabellid-infested abalone with shell length ranging from 70 to 90 mm were collected between 26 September and 2 October 2003 from and in the vicinity of three abalone farms in the Walker Bay area of South Africa (Fig. 2.1). The identities of the farms have been withheld for the sake of propriety, and are referred to as FARMS A, B and C, respectively. FARMS A and B are situated close to Gansbaai, while FARM C is situated close to Hermanus. Abalone were collected on the farms, from the farms' effluent flow, and from the wild in the vicinity of each farm. The following codes will be used throughout the document to distinguish the different farms and their respective sampling sites:- FARM A-F and FARM B-F: abalone were sampled on-farm from the production raceways; FARM A-E and FARM B-E: at the point where the farm effluent entered the sea at the exit

of the outflow pipe; FARM A-W: at the wild site 2 km east of FARM A; FARM B-W: the wild site 1.5 km south of FARM B; FARM C-F1: On-farm, from the production raceways; FARM C-F2: the farm effluent channel; FARM C-W1: in the shallow sub-tidal zone, 60 m from the outflow of FARM C; FARM C-W2: at a wild site 2.5 km west of Farm C (Fig. 2.1).

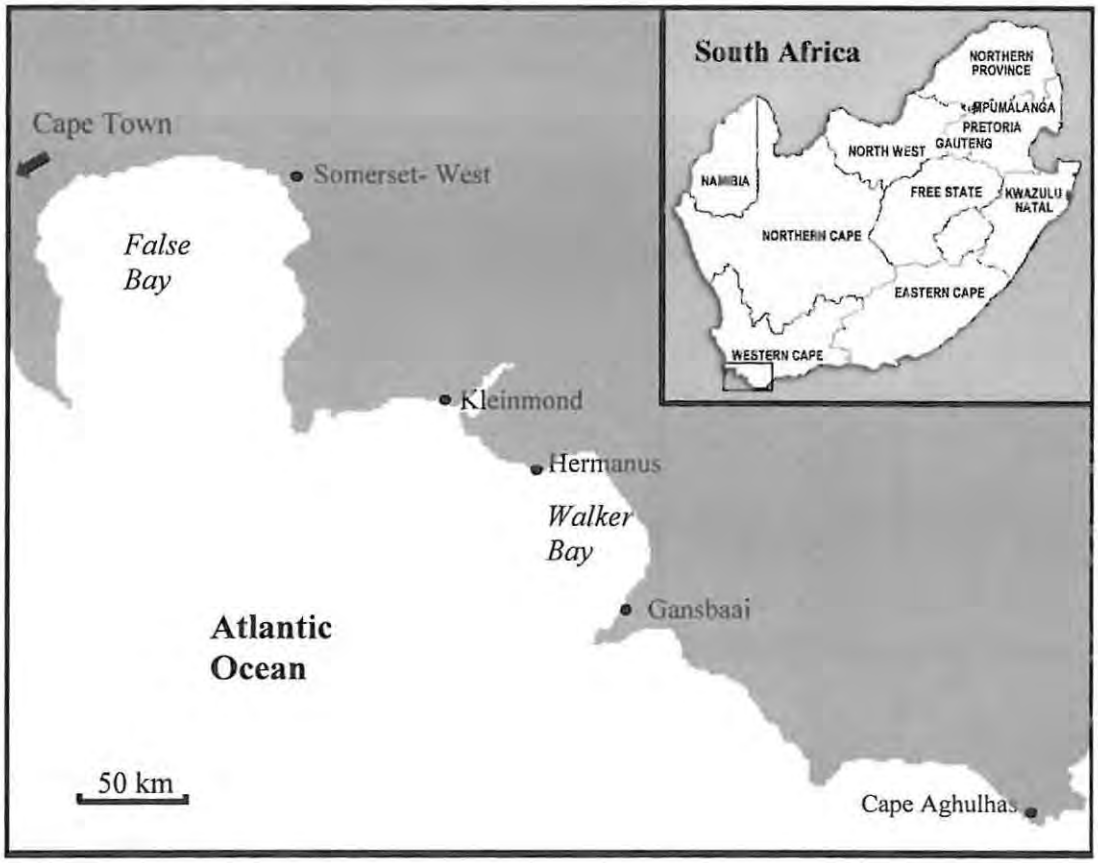

Figure 2.1. Walker Bay in the Western Cape Province, South Africa. FARMS A and B are situated close to Gansbaai while FARM C is in Hermanus.

The three farms employed land-based culture systems, rearing abalone in baskets that were suspended in raceways. The baskets contained vertical plastic plates which served as the attachment surface for the abalone. The dimensions and construction of raceways differed between farms (Table 2.1). Abalone were fed either freshly harvested kelp (*Eklonia maxima*), a major component of their natural diet, or the artificial pelleted feed, Abfeed™, with the frequency of feeding dependent on the food-type and farm management practise (Table 2.1). Abfeed™ was placed on feeder plates that were positioned horizontally across the top of the vertical plates. At night the abalone emerged to feed on the horizontal plates. Kelp was placed in the baskets between the vertical plastic plates. In these tanks the abalone had continuous access to food. Many management procedures differed between farms. Important differences were the stocking densities of abalone, sorting intervals to maintain a

constant stocking density and size variation, tank cleaning routine, and flow rate of water through the tanks. In addition, the average water temperature of the raceway differed between farms (Table 2.1). The three farms each produced an effluent flow of approximately 1000 m^3/h. The collection sites at the farm effluent outfalls and the nearby wild sites were typical juvenile abalone habitat (1-3 m depth), with a kelp (*Ecklonia maxima*) canopy, abundant sea urchins on the rocky substrate, and the cryptic juvenile abalone found under urchins and in rock crevices. At FARM C's effluent outfall the exposed, high relief rocky habitat supporting a dense *Pyura* covering did not appear suitable for juvenile abalone, and only a few large abalone were observed when a collection was attempted. Therefore abalone were sampled from within the concrete effluent channel on the farm itself.

Table 2.1. A summary of the environmental conditions and management procedures employed by FARMS A, B and C.

	FARM A	FARM B	FARM C
Abalone diet	Kelp	Abfeed	Kelp and Abfeed
Feeding regime	Every third day	Every second day	Daily
Size sorting	Every six months	Every four months	Every five months
Stocking density (% surface area)	30%	20 - 35%	18 - 20%
Flow rate (exchanges of water /hr)	1.5	2.2	3.3
Average temperature (May 2003 to October 2003)	Raceway: 16.4°C Sea: 14.9°C	Raceway: 16.1°C Sea: 14.9°C	Raceway: 15.4°C Sea: 15.5°C
Tank cleaning routine	information withheld	weekly	Abfeed: weekly Kelp: fortnightly
Tank volume and construction	7.3 m^3 Fibre re-enforced concrete	4 m^3 Concrete	3.5 m^3 Concrete

Removal of worms and measurements

Ten live abalone were sampled from each of the ten sites. The abalone were shucked and worms were preserved in the shells in 4% seawater formalin and transferred to 70% ethanol after one week. Blocks of shell measuring approximately one cm^2 were cut from the region 1.5 to 2 cm from the growing edge and immediately to the right of the respiratory pores, with the anterior part of the shell facing forwards. The blocks of shell were dissolved in 5% nitric acid in 70% ethanol for 12-24

hours. These softened shells were stored in 70% ethanol until the worms were removed. The total number of worms and number of worms brooding was determined for each block. From each block ten brooding worms were sampled for the following measurements:

1. Length of adult worms (mm), excluding the feeding crown, and pre-emergent larvae, i.e., larvae with eye spots, measured with a graduated eyepiece at 25 or 50 times magnification.
2. Egg volume (mm^3) calculated according to the equation $V = (4/3)\pi A^2 B$, where A is the short and B the long axis of the egg (Qian and Chia, 1991).
3. The number of offspring in each burrow (i.e., instantaneous fecundity).
4. The number of clutches per burrow. A clutch signifies all offspring at a similar developmental stage. The clutches are defined as a) developing oocytes visible within the body cavity, b) eggs without any signs of segmentation, c) larvae with visible segmentation but without eye spots and d) pre-emergent larvae with eye spots. Thus, up to four clutches could be observed in one burrow.

If there were less than ten brooding worms in a block, non-brooding worms were measured, to make the total up to ten. The number of polydorid polychaetes in each square was also determined.

Data analysis

To avoid pseudoreplication data from worms found on each abalone shell were averaged. Thus, there were up to ten values for each site and comparisons between sites were done using ranks as described below. For each shell, the average length was calculated for gravid and non-gravid adults, and pre-emergent larvae. Egg volume was averaged for each shell. Average instantaneous fecundity was calculated using only the values from tubes containing brooding worms. All values for the number of clutches per brood were used for the analyses (see below). Due to the differences in conditions between farms (Table 2.1) only data from each farm and its associated sites were compared with each other. The effect of the sampling site on the number of sabellids mm^{-2}, % sabellids brooding, egg volume, length of pre-emergent larvae and instantaneous fecundity was tested by using the Kruskal-Wallis ANOVA by ranks. This test was chosen due to the small sample sizes. If significant differences between sites were indicated at an error level of 5%, multiple comparisons of average ranks were done according to a method proposed by Siegel and Castellan (1988). The effect on adult length of the independent variables "site" and "reproductive state" (i.e., if the adult was gravid or not) was tested using a two-way ANOVA including a test for interactions between these two effects, followed by Tukey's (HSD) test for unequal sample sizes. The effect of sampling site on the number of clutches per brood were analysed using contingency analysis (Zar, 1999). Spearman Rank correlations were calculated between different variables related to the worm's morphometrics and reproduction. The minimum size at maturity was calculated as the average length of the 25% shortest mature worms per site.

Results

Density of worms

At FARM A there was no significant difference between the sampling sites with respect to the number of worms per mm^2 of shell (H=0.4, P=0.82, Table 2.2) with values averaging between 0.73 and 0.78 worms mm^{-2}. By contrast at FARM B the number of worms per mm^2 shell was three to five times higher in abalone sampled on-farm (H=15.74, P=0.004, Table 2.2) in comparison to the two associated sub-tidal sites. At FARM C the lowest number of worms (0.12 worms mm^{-2}) was recorded for the wild site (FARM C-W2), 2.5 km to the east, while the abalone sampled from the farm production raceways (FARM C-F1), the on-farm effluent channel (FARM C-F2), and 60m from the effluent outfall (FARM C-W1) had similar but much higher densities ranging from 0.71 (FARM C-W1) to 1.17 (FARM C-F2) worms mm^{-2} (H=23.85, P<0.00001, Table 2.2).

Table 2.2. Expression of various reproductive traits of *Terebrasabella heterouncinata* on FARM A, FARM B and FARM C, and wild sites at different distances from the farms. All values are average ± standard deviation, followed by the number of values. Identical letters denote no significant difference between averages.

		FARM A	
	FARM A-W	FARM A-E	FARM A-F
Density (worms.mm^{-2})	0.78 ± 0.72, 10^{a}	0.74 ± 0.56, 10^{a}	0.73 ± 0.23, 10^{a}
% Brooding	13.28 ± 12.34, 10^{a}	26.57 ± 25.69, 10^{b}	80.9 ± 5.39, 10^{c}
Adult size (mm)	1.36 ± 0.28, 16^{a}	1.74 ± 0.4, 15^{b}	2.41 ± 0.3, 20^{c}
Number of offspring per adult	1.95 ± 0.72, 8^{a}	2.11 ± 0.44, 7^{a}	8.76 ± 2.31, 10^{b}
Egg volume (mm^3)	0.024 ± 0.006, 8^{a}	0.033 ± 0.003, $7^{b,c}$	0.027 ± 0.002, $10^{a,c}$
Larvae length (mm)	0.42 ± 0.03, 5^{a}	0.46 ± 0.01, 5^{a}	0.49 ± 0.02, 10^{b}
Minimum size at maturity (mm)	1.2	1.58	1.67
		FARM B	
	FARM B-W	FARM B-E	FARM B-F
Density (worms.mm^{-2})	0.3 ± 0.3, 10^{a}	0.42 ± 0.48, 10^{a}	1.48 ± 0.43, 10^{b}
% Brooding	21.88 ± 29. 9, 10^{a}	12.24 ± 15.2, 10^{a}	62.14 ± 7.97, 10^{b}
Adult size (mm)	1.35 ± 0.26, 15^{a}	1.37 ± 0.31, 11^{a}	2.25 ± 0.21, 20^{b}
Number of offspring per adult	1.31 ± 0.41, 8^{a}	2.0 ± 1.2, 5^{a}	4.92 ± 0.92, 10^{b}
Egg volume (mm^3)	0.021± 0.006, 5^{a}	0.029 ± 0.003, 4^{a}	0.034 ± 0.003, 9^{b}
Larvae length (mm)	0.48 ± 0.03, 3^{a}	0.44 ± 0.04, 2^{a}	0.56 ± 0.04, 9^{b}
Minimum size at maturity (mm)	0.95	1.2	1.91

Table 2.2: continued.

	FARM C			
	FARM C-W1	FARM C-W2	FARM C-F1	FARM C-F2
Density (worms.mm^{-2})	0.71 ± 0.75, 10^{b}	0.12 ± 0.11, 10^{a}	0.8 ± 0.4, 10^{b}	1.17 ± 0.67, 10^{b}
% Brooding	15.59 ± 24.39, 10^{a}	19.1 ± 24.32, 10^{a}	67.19 ± 14.66, 10^{b}	58.6 ± 14.85, $10^{a,b}$
Adult size (mm): Gravid adults	1.61 ± 0.32, 6^{a}	1.74 ± 0.24, 5^{a}	2.29 ± 0.33, 10^{b}	2.55 ± 0.32, 10^{b}
Adult size (mm): non-gravid adults	1.33 ± 0.35, 9^{c}	1.35 ± 0.37, 10^{c}	2.08 ± 0.34, 10^{d}	2.39 ± 0.23, 10^{d}
Number of offspring per adult	1.8 ± 1.2, $6^{a,}$	2.33 ± 1.1, 3^{a}	5.26 ± 1.28, 10^{b}	5.48 ± 1.23, 10^{b}
Egg volume (mm^{3})	0.026 ± 0.005, 5^{a}	0.028 ± 0.003, 3^{a}	0.031 ± 0.003, 10^{a}	0.032 ± 0.003, 10^{a}
Larvae length (mm)	0.47, 1	0.47 ± 0.05, 2^{a}	0.47 ± 0.04, 10^{a}	0.55 ± 0.03, 10^{b}
Minimum size at maturity (mm)	1.52	1.24	1.67	2.1

Percentage of Reproductively Active Worms

For each farm, the percentage of brooding worms was significantly higher for the samples from the production raceways in comparison with the corresponding effluent outfall and wild sites (FARM A: H=19.76, P<0.0001; FARM B: H=15.74, P<0.0003; FARM C: H=19.6, P<0.0002, Table 2.2). At FARM A-F the average percentage of reproductively active worms was approximately 81% - about six times more than at FARM A-W and FARM A-E. Similarly, about 62% of the worms from the FARM B-F samples were brooding, and this was three to four times more than at FARM B-W and FARM B-E. Between 67 and 59% of the adult sabellids were brooding in the FARM C-F1 and FARM C-F2 samples, respectively, while the respective values for the two wild sites (FARM C-W1 and FARM C-W2) ranged between 16 and 19%, thus suggesting a positive effect of the farm environment on sabellid reproduction.

Body size and minimum size at maturity

There was a significant difference in adult length between all sampling sites at FARM A, but reproductive state did not affect length and there were no interactions between the main effects (site effect: F=45.2, P<0.0001; reproductive state effect: F=4, P=0.051, interaction term: F=1.5, P=0.23). Sabellid adult length was greatest on the farm, followed by the effluent, and wild site (Table 2.2). Similarly, adult length was significantly greater at FARM B-F than at FARM B-W and FARM B-E, and was not affected by reproductive state (site effect: F=61.2, P<0.0001; reproductive state effect: F=1.1, P=0.31; interaction term: F=0.57, P=0.57, Table 2.2). Thus, the values for adult length of both gravid and non-gravid worms from FARM A and FARM B were averaged for further analyses. Both site and reproductive state had a significant effect on adult size at FARM C (site effect: F=35.5, P<0.0001; reproductive state effect: F=9.8, P<0.003; interaction term: F=0.33, P=0.8). The worms at both FARM C-F1 and FARM C-F2 were longer than those at the two wild sites (FARM C-W1 and FARM C-W2) and gravid worms were significantly longer than non-gravid worms (Table 2.2). At all farms, the minimum size at maturity was greater in sabellids from farmed abalone than the respective wild sites (Table 2.2). There was a weak negative correlation between the number of worms mm^{-2} and adult length at the FARM A-W site (Table 2.4).

Size of offspring

Site affected the size of the offspring at FARM A and FARM B in different ways (Table 2.2). At FARM A the average sabellid egg size from animals sampled on farmed abalone was not significantly different from that at the wild sites, but sabellid eggs from the effluent site were significantly larger than eggs from the wild site (H=9, P<0.011, Table 2.2). Sabellid larvae on farmed abalone were larger than those from the wild sites (H=12.74, P<0.002, Table 2.2). At FARM B eggs and larvae on the farm were larger than those from the wild sites (H=11.18, P<0.004; H=7.58, P<0.02,

for the egg volume and larvae length, respectively). Site had no effect on the volume of eggs from FARM C, while the length of the larvae from FARM C-F2 was significantly larger than at the other sites ($H=7.3$, $P=0.06$; $H=14.14$, $P<0.001$, for egg volume and larvae length, respectively). At FARM C-W1 only one pre-emergent larva was measured, and this site was therefore excluded from the analysis.

Fecundity, number of clutches per burrow and investment per brood

For all farms instantaneous fecundity and the number of clutches per worm were significantly higher at the on-farm sites than at any of the corresponding wild sites (FARM A: $H=17.41$, $P<0.002$; $\chi^2=70.7$, $P<0.001$; FARM B: $H=16.83$, $P<0.0002$, $\chi^2=87.69$, $P<0.001$ and FARM C: $H=16.21$, $P<0.001$; $\chi^2=28.17$, $P<0.001$, for instantaneous fecundity and the number of clutches, respectively). There was a large variation in brood size. The fecundity of worms at the six wild sites ranged from four to eight offspring per brood. The largest brood of 21 eggs and larvae was recorded at FARM A-F. The maximum brood size on the farm at FARM C-F1 was 15, and 11 at FARM B-F. Up to 64% and 59% of the worms from the on-farm sites at FARM A and FARM B, respectively, brooded three or four clutches while up to 15% and 2.5% of the worms brooded that many clutches at the wild and effluent sites (Table 2.3). At FARM C the differences between the worms from the on-farm and wild sites were not as great; between 17% and 43% of the worms at the wild sites brooded more than two clutches, while 31% to 43% of the worms from the on-farm sites brooded that many clutches. Only 4% of the worms from the wild sites and up to 12% of the worms from the on-farm sites brooded four clutches.

There were significant positive correlations between adult size and instantaneous fecundity as well as number of clutches at most sites (Table 2.4). At FARM C there was no correlation between the length of non-gravid adults and instantaneous fecundity at both wild sites, and there was no correlation between non-gravid adult length and clutch number at FARM C-W1. Similarly, no correlation existed between these factors at the FARM B-W and FARM B-E sites. At all three on-farm sites there was a negative correlation between egg volume and brood size, while this relationship showed a positive correlation at FARM B-W (Table 2.4). Egg volume and the length of pre-emergent larvae were not significantly correlated with each other except at the FARM C-F2 site, where the correlation was positive (Table 2.4). Density and brood size were positively correlated at FARM C-W1, FARM A-E and FARM B-E. The same was found for density and number of clutches at FARM A-E. There were negative correlations between density and the number of clutches at FARM C-W2 and between density and brood size at FARM A-F.

Table 2.3. The frequency of the brooding of different numbers of clutches brooded simultaneously by *Terebrasabella heterouncinata* (with percentages in parentheses) on FARM A, FARM B and FARM C and wild sites at different distances from the farm.

	FARM A			
Number of clutches	FARM A-W	FARM A-E	FARM A-F	
1	29 (60%)	18 (38%)	8 (9%)	
2	12 (25%)	24 (50%)	25 (27%)	
3	7 (15%)	5 (10%)	42 (45%)	
4	0	1 (2%)	18 (19%)	
	FARM B			
	FARM B-W	FARM B-E	FARM B-F	
1	30 (75%)	13 (50%)	7 (7%)	
2	9 (22.5%)	13 (50%)	33 (33%)	
3	1 (2.5%)	0	48 (48%)	
4	0	0	11 (11%)	
	FARM C			
	FARM C-W1	FARM C-W2	FARM C-F1	FARM C-F2
1	13 (45%)	5 (22%)	15 (17%)	14 (15%)
2	11 (38%)	8 (35%)	47 (52%)	32 (33%)
3	5 (17%)	9 (39%)	18 (20%)	38 (39%)
4	0	1 (4%)	10 (11%)	12 (13%)

Density of polydorid spp.

The density of *Polydora* spp. at the three farms ranged between 0.007 and 0.09 worms mm^{-2}. There was no significant correlation between the density of polydorid spp. and *T. heterouncinata* at any of the farms.

Table 2.4. Correlations between various life history parameters of *Terebrasabella heterouncinata* at FARM A, FARM B and FARM C and wild sites at different distances from the farms. The statistics listed are the coefficients of determination (r), P and n. NS=not significant at P≤0.05. At FARMS A and B the length of gravid and non-gravid worms were not significantly different from each other, and the pooled data were used in the relevant analyses. At FARM C the lengths of gravid and non-gravid worms were different, and the relevant analyses were conducted separately for the 2 groups.

		FARM A	
	FARM A-W	FARM A-E	FARM A-F
Adult length vs. egg volume	NS	NS	-0.26, < 0.001, 87
Adult length vs. brood size	0.64, < 0.001, 48	0.49, 0.001, 48	0.8, < 0.001, 93
Adult length vs. number of clutches	0.48, 0.001, 48	0.51, 0.001, 48	0.52, < 0.001, 93
Log egg volume vs. brood size	NS	NS	-0.47, < 0.001, 87
Density vs. adult size	-0.35, 0.014, 48	NS	NS
Density vs. brood size	NS	0.31, 0.03, 47	-0.21, 0.048, 93
Density vs. number of clutches	NS	0.34, 0.018, 48	NS
		FARM B	
	FARM B-W	FARM B-E	FARM B-F
Adult length vs. egg volume	NS	NS	NS
Adult length vs. brood size	NS	NS	0.56, < 0.001, 99
Adult length vs. number of clutches	NS	NS	0.37, < 0.001, 99
Egg volume vs. brood size	0.19, 0.03, 19	NS	-0.21, 0.045, 87
Density vs. adult size	NS	NS	NS
Density vs. brood size	NS	NS	0.57, 0.002, 25
Density vs. number of clutches	NS	NS	NS

Table 2.4. continued:

	FARM C			
	FARM C-W1	FARM C-W2	FARM C-F1	FARM C-F2
Non-gravid adult length vs. egg volume	NS	-0.53, 0.01, 23	NS	NS
Gravid adult length vs. egg volume	NS	NS	NS	NS
Non-gravid adult length vs. brood size	NS	NS	0.73, < 0.001, 63	0.71, < 0.001, 64
Gravid adult length vs. brood size	0.85, < 0.002, 10	0.69, 0.013, 12	0.53, 0.002, 33	0.63, < 0.001, 33
Non-gravid adult length vs. clutch number	NS	0.52, 0.03, 17	0.52, < 0.001, 63	0.61, < 0.001, 64
Gravid adult length vs. clutch number	0.91, 0.001, 10	0.67, 0.016, 12	0.38, 0.029, 33	0.42, 0.032, 33
Egg volume vs. brood size	NS	NS	NS	-0.29, 0.01, 73
Egg volume vs. larvae length	NS	NS	NS	0.43, 0.04, 32
Density vs. non-gravid adult length	NS	NS	NS	NS
Density vs. gravid adult length	NS	NS	NS	NS
Density vs. brood size	0.47, 0.025, 21	NS	NS	NS
Density vs. number of clutches	NS	-0.4, 0.03, 29	NS	NS

Discussion

Environmental factors and reproduction

This study showed that the environmental conditions on the abalone farms studied enhanced the sabellid's reproductive success relative to that observed in the adjacent natural environment. On all three farms, a relatively greater percentage of sabellids were brooding offspring and adult worms were larger, had a higher instantaneous fecundity, and brooded more clutches simultaneously than those sampled from the corresponding wild sites. At two of the three farms, the density of worms that had successfully settled on the abalone shells was higher than at the respective wild sites. These findings, together with suggestions from other studies on the biology of this sabellid species (Kuris and Culver, 1999; Chalmers, 2002; Gray, 2004), suggest that the farm environment, with its high availability of food in the form of suspended organic material, stable habitat and high density of potential hosts, favours the reproduction and population growth of this species, enabling it to become a pest under aquaculture conditions.

The combined effects of the farm environment resulted in an increased average sabellid adult size as the worms from farms were 1.4 to 1.7 times larger than their wild conspecifics. These differences in adult size may be related to the increase in nutrients available on the abalone farms. Analogous differences in body size related to diet have also been observed in *Capitella* sp., where individuals fed *Ulva* were 1.6 times smaller than those fed squid egg capsules (Qian and Chia, 1991). The effect of reproductive state on adult size was not consistent across sites. At FARMS A and B the reproductive state, i.e., gravid and not gravid, respectively, did not have a significant effect on the length of the adults while at all FARM C sites, gravid worms were significantly larger than non-gravid individuals (cf. Simon et al., 2002). However, at all farm sites the average minimum size of reproductively active worms was generally larger than at the corresponding wild sites. This concomitant increase in the minimum size at sexual maturity with an increase in adult size was also demonstrated in laboratory-reared *T. heterouncinata* (Simon et al., 2002), *Capitella* sp. I (Bridges et al., 1994) and *Littorina rudis* (Hart and Begon, 1982). In the laboratory-reared *T. heterouncinata* (Simon et al., 2002) and *Capitella* sp. I (Bridges et al., 1994), the treatments that resulted in the greatest growth rate and adult size were associated with decreased age-at-maturity. This response is consistent with predictions made by Stearns and Koella (1986) for animals with different growth rates. Although the age-at-maturity of *T. heterouncinata* in the present study is not known, it is clear that worms on farms reach maturity at a size greater than the size at which they were observed to become reproductive in the wild. As worms on farms are exposed to increased nutrients, they may be growing more rapidly, and reaching maturity at the same or earlier age, than their wild conspecifics (cf. Stearns and Koella, 1986; Bridges et al., 1994; Simon et al., 2002).

In *T. heterouncinata* reproductive success may also be determined by instantaneous fecundity and the time interval between clutches. In this study the worms at the four on-farm sites were between

2.9 and 4.5 times more fecund than their wild conspecifics. This increase in fecundity was probably related to nutrient enrichment or a combination of nutrient enrichment and the stability of the environment, as has been demonstrated in the polychaetes *Capitella* sp. I (Bridges et al., 1994), *Dinophilus gyrociliatus* (Prevedelli and Simonini, 2001), *Polydora ligni* (Zajac, 1986), *Ophryotrocha labronica* (Prevedelli and Zunarelli Vandini, 1998) and *Capitella* sp. (Qian and Chia, 1991). The number of different age classes of larvae and embryos (i.e., the number of clutches) brooded simultaneously can be used as an indication of the rate at which eggs are being produced (Rouse, 1992). As *T. heterouncinata* living on farmed abalone tended to brood more clutches than their wild conspecifics, they probably produced eggs more rapidly.

Investment in reproduction and trade-off between offspring number and size

Investment in offspring is also determined by the size of the eggs or larvae. Worms from the on-farm sites did not always produce larger eggs and larvae than their wild conspecifics, suggesting that under altered conditions the worms do not adjust the investment per offspring. Furthermore, there was a weak positive correlation between egg volume and larvae length only at one of the on-farm sites at FARM C. Thus, the prediction that larger eggs lead to the development of larger larvae (McClintock and Pearse, 1986) does not always hold true.

In order to better understand the investment of energy into offspring correlations between the size of adults and the size and number of offspring were estimated. Findings were not consistent for all farms and sites. At most sites egg size was independent of adult size, i.e., these variables did not correlate. There was, however, a significant negative correlation between adult length and egg volume on FARM A. This was coupled with a negative correlation between brood size and egg volume, a positive correlation between adult size and brood size and a very high fecundity. This suggested that on average, larger adults produced more eggs while investing less energy into each egg as brood size increased.

At most sites, there was a positive correlation between adult size and fecundity, and adult size and number of clutches, respectively. Adult size and fecundity have been found to be positively correlated in the polychaetes *Polydora ligni* (Zajac, 1986), *Capitella* sp. (Qian and Chia, 1992; Qian, 1994), *Nereis arenaceodentata* (Moore and Dillon, 1993), *Capitella* sp. I (Bridges et al., 1994) and *Streblospio benedicti* (Levin, 1986) and the winkle, *Littorina rudis* (Hart and Begon, 1982). This may be related to an increase in space available for the storage of developing oocytes within the body cavity (Hermans, 1979; Olive, 1985). By contrast, there was no relationship between body size and number of spawns in *Capitella* sp. with planktotrophic development (Qian and Chia. 1992) and there was a negative correlation between body size and number of egg capsules in *Nassarius pauperatus* (McKillup and Butler, 1979). The positive relationship found in this study between body size and the number of clutches may have been due to an increase in food availability on the abalone farms that

simultaneously allowed sabellids to grow larger and produce eggs more rapidly.

A trade-off exists between the number of offspring produced and the amount of energy invested per offspring. If the amount of energy devoted to reproduction is to remain constant, an increase in fecundity will occur at the expense of the energy devoted to each offspring, but if energy devoted to reproduction is proportional to food availability, both fecundity and egg quality may change (Stearns, 1976; Qian, 1994). At all farms, the fecundity of worms on farmed abalone was greater than that of worms on wild abalone, irrespective of the mean size of the eggs at these sites. This was probably due to 1) the observed greater body size of the "farm" worms, and 2) a probable increase in nutrient availability in the abalone production systems providing the worms with relatively more energy to invest in reproductive output. At all four on-farm sites there was a negative correlation between instantaneous fecundity and egg volume. Similarly, egg volume has been negatively correlated with fecundity in *Capitella* sp. (Qian and Chia, 1992; Qian, 1994), and the molluscs *Littorina rudis* (Hart and Begon, 1982) and, in part, in *Nassarius pauperarus* (McKillup and Butler, 1979). This suggests that in some circumstances, reproductive output is limited either by available space in the body cavity, or by available energy, and that reproductive output is optimised by producing fewer, smaller eggs. The lack of a correlation between these variables in worms from the wild sites suggests that egg number may have been too low to compromise egg volume.

Reproduction and settlement success

At FARMS B and C, sabellid density, i.e., the number of sabellids per mm^2 of shell, was significantly greater on farmed abalone than on abalone from the related wild sites, and is presumably a consequence of the greater reproductive output or settlement success of the worms at these sites. By contrast, the densities of worms on abalone shells at the three FARM A sites were very similar, despite the observed reproductive output of the worms on the farmed abalone being significantly greater than that of worms on the wild abalone. This suggests a relatively low survival rate of worms at this site.

The relationship between sabellid density and their reproductive traits was not consistent across farms, with few significant correlations existing between sabellid density and adult size, brood size and the number of clutches, respectively. Where significant correlations existed, the coefficients of determination were low, and all but one significant correlation was found at the wild sites. Thus only a small percentage of the variation in reproductive traits could be explained by the variation in density. Linke-Gamenick et al. (1999) suggested that the negative impact of high density on most reproductive parameters may be related to food limitations. While this may partly explain findings related to the wild sites, food limitation is unlikely to have occurred at the on-farm sites (Chalmers, 2002). Overall, the potentially negative effect of sabellid density on their reproductive success was low, thus intraspecific competition may not have played an important role. This is in contrast to many

other sessile marine invertebrates where high settlement densities can lead to intraspecific competition which may affect the survival, growth and reproductive success of individuals within the population (Wilson, 1983; Levin, 1986; Zajac, 1986; Linke-Gamenick et al., 1999; Hills and Thomason, 2003). Densities of worms on farmed abalone were up to six times higher than in their wild conspecifics, and it is suggested that, under conditions of unlimited food and increased host availability, intraspecific competition was negligible in this species within the range of densities observed.

Interspecific competition may also influence the density and reproductive rate of competing organisms. For example, densities of the competing spionids, *Pygospio elegans* and *Pseudopolydora kempi* were inversely proportional to each other when they occurred at the same site (Wilson, 1983). In addition an increase in the density of *P. kempi* was also associated with a reduction in reproduction by *P. elegans* (Wilson, 1983). The abalone shells used in the present study were infested by both *T. heterouncinata* and several shell-boring polydorid polychaete species. The polydorids are much larger (between 10 and 15 mm in length, pers. obs.) than *T. heterouncinata* and could potentially compete with them for space. The density of polydorids was, however, comparatively low, ranging from 1 to 14 worms per block of shell, and there was no correlation between the density of the polydorids and the sabellids.

Changes in the expression of life history traits of the sabellid

Many studies have demonstrated the phenotypic plasticity of the expression of life history traits by polychaetes exposed to different environmental conditions (e.g., Levin, 1986; Zajac, 1986; Grémare et al., 1988; Qian and Chia, 1991, 1992; Bridges et al., 1994; Qian, 1994; Prevedelli and Zunarelli Vandini, 1998; Prevedelli and Simonini, 2001; Simon et al., 2002). In these studies, the responses to the environmental conditions were usually immediate, and were therefore probably not the result of a change in the genotype of the animal. Thus, the differences in the degree of expression of life history traits in *T. heterouncinata* from different environments might be phenotypic only. However, since its discovery on farmed abalone in 1994 (Ruck and Cook, 1998), *T. heterouncinata* has been present on South African farms for more than 40 generations and it is therefore reasonable to hypothesise that natural selection may have resulted in a possible genetic basis to the observed changes in the expression of life history traits (cf. McKillup and Butler, 1979). Anecdotal evidence suggests that the maximum number of eggs per brood recorded for worms from the on-farm sites has increased in recent years, as a previous study by Gray (2004) in 2001 recorded a maximum of 7 and 6 eggs per brood at FARMS A and C respectively, while in the present study, 13 and 9 eggs per brood were observed at these farms, respectively. These results suggest that the success of *T. heterouncinata* on the abalone farms may be related to the selection for large worms that produce more offspring more rapidly. This may be a result of abundant food resources, a possible lack of predation, low intra- and interspecific competition, and the increased availability of hosts.

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CHAPTER 3

The effect of diet and live host presence on the growth and reproduction of *Terebrasabella heterouncinata*

Simon, C.A., Kaiser, H., Booth, A.J. and Britz, P.J., The effect of diet and live host on the growth and reproduction of *Terebrasabella heterouncinata* (Polychaeta: Sabellidae). Invertebr. Reprod. Dev., 41(1-3) (2002) 277 – 286.

"Eye of newt and toe of frog,
Wool of bat and tongue of dog,
Adder's fork and blind-worm's sting,
Lizard's leg and owlet's wing,
For a charm of powerful trouble,
Like a hell-broth boil and bubble."

William Shakespeare

Abstract

The effect of diet type and the presence or absence of a live host on the growth and production of eggs and larvae by *Terebrasabella heterouncinata* was quantified. Diet was shown not to have a significant effect on the time in which the worms on live hosts reached their maximum size. Diet did, however, influence the maximum size and consequently the growth rate of worms, which were larger and grew faster on kelp-fed abalone than on those fed a commercial pellet diet. Despite diet having no effect on fecundity and offspring size, kelp-fed worms matured earlier. The maximum size of kelp-fed worms was unaffected by the absence of a live host, suggesting that the worms do not rely on the host for food. The absence of a live host reduced the growth and sexual maturation rates of worms. There was no difference in the size of offspring in the two treatments, but fewer worms matured sexually and fewer broods were produced on 'shells only' than on live hosts. The lower growth rate and reproductive output of worms on 'shells only' may be due to the diversion of energy from growth and reproduction to burrow expansion.

Introduction

The sabellid *Terebrasabella heterouncinata* is endemic to South Africa and infests the shells of several species of mollusc including the commercially important *Haliotis midae* (Ruck and Cook, 1998). The larva of *T. heterouncinata* settles on the growing edge of the host shell where the host covers it with nacre forming the tube (Culver et al., 1997). There is no evidence that other polychaetes employ the same method of burrow formation (Martin and Britayev, 1998) - most sabellids inhabit mucoid or sediment-encrusted tubes associated with soft sediments or gastropod shells (Fitzhugh and Rouse, 1999). Some species, such as *Caobangia* spp., bore into both living and non-living calcareous or carbonate substrates (Jones, 1974), while two as yet undescribed species of *Terebrasabella* from Australia bore into clean coral rock and tubes of spirobin polychaetes (A. Murray and G.W. Rouse, pers. comm.).

The infestation of abalone by *T. heterouncinata* has caused severe economic losses to the abalone industry in California and South Africa (Culver et al., 1997; Ruck and Cook, 1998). Heavy infestations result in the deformation of the shells which leads to a reduction in abalone growth rate and marketability (Culver et al., 1997).

Cultivated abalone are fed either kelp (*Eklonia maxima*) or a commercial high protein pellet diet, sold under the brand name Abfeed™ (Table 3.1). Some farmers noticed that abalone fed Abfeed™ appeared more prone to heavy infestations with sabellids than kelp-fed abalone. While it has been suggested that *T. heterouncinata* feed primarily on diatoms and protists (G.W. Rouse, pers. comm.), the possible differences in infestation levels of farmed abalone on different diets suggest that the worms also feed on fragmented abalone food and possibly abalone faeces. It is therefore hypothesised that the higher infestation levels observed in Abfeed™ -fed abalone was due to an increase in the reproductive output of sabellids in response to an increase in the nutrient load of the water in the abalone raceways.

Table 3.1. The proximate composition (% of dry matter) of kelp[a] and Abfeed™[b] fed to commercially reared abalone.

Nutrient	Kelp	Abfeed™
Crude protein	8.1	34.6
Carbohydrates	45.2	43.3
Crude lipid	0.5	5.3
Crude ash	25.3	5.7

[a] Kelp Products Pty. Ltd, Cape Town, in Britz 1995.

[b] Sea Plant Products Ltd, previously unpublished data.

Little is known about the interaction between boring polychaetes and their hosts (Caceres-Martinez et al., 1999; Martin and Britayev, 1998). Like true borers, *T. heterouncinata* continues to develop and reproduce in the absence of a living host (Jones, 1974; Culver et al., 1997; Caceres-Martinez et al., 1999). In contrast to true borers, *T. heterouncinata* larvae appear to depend on their hosts for the formation of

their burrows, suggesting that the relationship between the sabellid and its host is a more intimate one. Ruck and Cook (1998) did, however, note that while larvae will preferentially settle under abalone tissue, they will produce a mucoid tube around themselves and may metamorphose in the absence of a suitable substratum. It was therefore considered possible that in the absence of a live host, the larvae might settle in abandoned burrows (cf. *Dodecaceria caulleryi*, secondary borers that occupy abandoned burrows, Gibson and Clark, 1976). The only specialised relationship that has been documented between a boring polychaete and its host is that between the spionid, *Polydora commensalis* and its hermit crab host - the worm captures food particles that are suspended or stirred up by the host (Blake, 1996 in Caceres-Martinez et al., 1999).

An exploratory study was conducted to a) quantify the effects of diet on the growth and reproductive output of *T. heterouncinata* under controlled conditions, and b) measure the degree of dependence of the sabellid on a live host by comparing its growth and reproductive output in the presence and absence of a live host.

Materials and Methods

Experimental design

All experimental animals were supplied by Sea Plant Products (Ltd.), an abalone farm situated at Gansbaai, South Africa. The abalone that were to be infested for *Experiment 1* were 35 – 45 mm in length, as abalone are most susceptible to infestation at this size (A. Du Plessis, Aquafarm Development, pers. comm.). The heavily infested abalone used to infest the abalone in *Experiment 1* and in *Experiment 2* measured 60-80mm. All experimental animals were held in 76.5 L recirculating aquaria at $18^0C \pm 1^0C$ with a 12:12 light:dark cycle. The aquaria contained 23.4 L *in situ* downflow biological-mechanical filters composed of crushed molluscan shells. Twenty-five percent of the water was changed once a week.

Experiment 1: The effect of diet and the presence or absence of a live host on growth and reproduction

Abalone were infested with *T. heterouncinata* by keeping them for 24 hours in a tank with infested animals at a ratio of 1 infested to 10 uninfested abalone. The newly infested abalone were divided into two groups of 210 animals each: a) 'live host' and b) 'shell only'. For the latter group, abalone were left for a week for the new tubes to become established before killing and removing the abalone from the shells.

The experimental design is summarised in Fig. 3.1. The live abalone were fed dried kelp or *Abfeed* in excess of what they could consume. The control group was fed Abfeed™ once a month as a maintenance ration (i.e. to keep the abalone alive, but to limit its growth). For the 'shell only' treatments infested shells were attached to a sheet of plastic oyster meshing 18 cm x 40cm in size. The sheets were suspended vertically in the tank, with the bottom edge 8 cm from the bottom of the tank. Worms in the shells were fed either ground kelp (2.5 g dried kelp per tank per week, rehydrated in seawater and homogenised), ground Abfeed™ (0.7 g per tank per week), or they were not fed at all (control). Particles

were suspended by aerating the water with standard 2cm diameter airstones. Each treatment was conducted in duplicate. The experiment was run for nine months which is equivalent to approximately two worm generation times (i.e. the time from the settlement of an individual larva to the production of its first brood) at 18^{0}C (Finley et al., 2001).

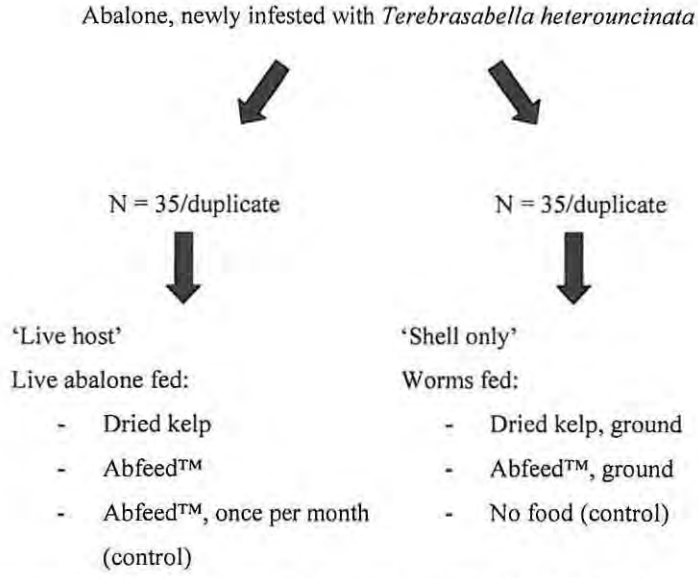

Figure 3.1. A summary of the experimental design to quantify the effect of diet and host presence on the growth and reproductive output of *T. heterouncinata*. Each treatment was done in duplicate.

Three shells or abalone per replicate were removed once a month for analysis of the sabellid population. The shells were preserved in 2.5% gluteraldehyde in seawater. The worms were removed by dissolving the shell in 6.5% HNO_3 in 70% ethanol. Where possible, ten worms were sampled per shell. When less than ten worms were present per shell, all the worms found were sampled. The following reproductive parameters were recorded:

1. Length of the worm (excluding the feeding crown), measured with a graduated eyepiece using a compound microscope.
2. Presence of oocytes in body cavity.
3. Presence and size of broods.
4. Volume of eggs and length of larvae.

The volume (V) of the ellipsoid eggs was calculated according to the following equation:

$$V = \left(\frac{3}{4}\right)\pi A^2 B$$

where A is the short axis and B the long axis of the egg (Qian and Chia 1991).

The growth data were fitted to a standard four parameter von Bertalanffy growth equation:

$$L_t = L_\infty \left[1 - e^{-K(t-t_0)}\right] + a\Phi$$

as well as an adjusted three parameter model:

$$L_t = L_\infty \left[1 - e^{-Kt}\right] + a\Phi$$

where L_t = size of the worm at time t, L_∞ = the theoretical maximum length (asymptotic length) of the worm, K = the time in which the asymptotic length is reached (Brody's growth coefficient), t_0 = age of the worm at zero length, a = difference in length of worms with and without oocytes within the body cavity, t = the age of the worm in months, and φ = a binary value indicating the presence of oocytes. No significant difference between the two growth models was found using a Likelihood Ratio test ($0.079 \leq P \leq 1.00$). The three-parameter model was therefore used to describe growth in length. The growth models and their parameters were compared using a Likelihood Ratio test. The size at maturity was compared using a one-way ANOVA followed by Tukey's (HSD) test for unequal sample sizes for contrasts given. The percentage of gravid worms (log transformed) was compared by a two-way ANOVA with time and treatment as the main effects, followed by the Tukey's (HSD) test for contrasts. The size of eggs and larvae were compared using a Kruskal-Wallis test. Further pair-wise comparisons were made using the Mann-Whitney U test with a Bonferoni adjusted *p* value. The frequency and size of broods were analysed with a contingency test (Zar, 1999). The Likehood Ratio tests were performed on Excel, and all other tests were performed using the STATISTICA statistical package.

Experiment 2: Settlement of larvae in abandoned burrows

Seventy-two infested abalone were shucked and the shells soaked in fresh water overnight, scrubbed and sun-dried, to provide shells with empty burrows. Eighteen cleaned shells were attached to oyster meshing on the inside of four shelters (90mm high x 450mm x 210mm) composed of black plastic. Each shelter was placed in a separate aquarium.

To determine whether larvae will settle in these abandoned worm burrows, the shells were exposed to either a) four live infested abalone or b) four shucked shells with live sabellids. Each treatment was conducted in duplicate. The abalone were free to move over the shells while the shucked shells were distributed randomly among the cleaned shells. Live abalone were fed Abfeed™ and the worms in the "shell only" treatments were fed ground Abfeed™ (0.7 g per tank per week). Once a month, three shells per replicate were examined for the presence of feeding crowns using a dissecting microscope.

Results

Experiment 1

Growth of worms

Diet had a significant effect ($P \leq 0.005$) on the growth coefficient K (the rate at which the worms reached their theoretical maximum size) of worms in the 'shell only' treatment, but not on those settled on

live abalone (P=0.51, Fig. 3.2, Tables 3.2 and 3.3). In the 'shell only' treatment, worms in the control group had the highest growth coefficient (i.e. these worms reached their theoretical maximum size the fastest), followed by those in the Abfeed™ and kelp treatments (Tables 2 and 3). In the 'shell only' Abfeed™ and control treatments, the number of worms decreased with time, until there were no survivors in the ninth month.

Diet had a significant effect on the size of worms in both the 'live host' and 'shell only' groups. In both groups worms on the kelp diet were the largest, followed by those in the Abfeed™ and control treatments (Fig. 3.2, Tables 3.2 and 3.3, $P<0.001$ for both 'live host' and 'shell only' groups). Although the growth coefficients in the 'live host' treatments were not different from each other, the realised growth was different because of differences in the asymptotic sizes of the worms. The onset of maturity had a significant effect on the size of the worms: gravid worms were significantly larger than reproductively inactive worms (Fig. 3.2). The mean degree of body expansion attributed to the maturing oocytes (a-value) did not differ between 'live host' treatments (Table 3.3).

The worms in the Abfeed™ and control groups were significantly larger in the 'live host' treatments than those in the 'shell only' treatments, but there was no significant difference in the length of kelp-fed worms in these treatments (Fig. 3.2, Table 3.3). The growth coefficient for the kelp-fed worms of the 'live host' treatment was greater than that for the 'shell only' treatment, while the relationship was reversed in the control groups. There was no difference in the growth coefficients between the two Abfeed™ groups.

Reproduction of worms

Worms were considered sexually mature when a band of oocytes was visible through the semi-translucent female reproductive segment. Worms in all 'live host' treatments reached maturity, but in the 'shell only' treatments only the kelp-fed worms developed eggs (Fig. 3.2). Due to the absence of sexually mature worms in the 'shell only' Abfeed™ and control treatments and the small size of these worms, it was concluded that these worms had starved. For this reason the data pertaining to these treatments have been omitted from the following analyses.

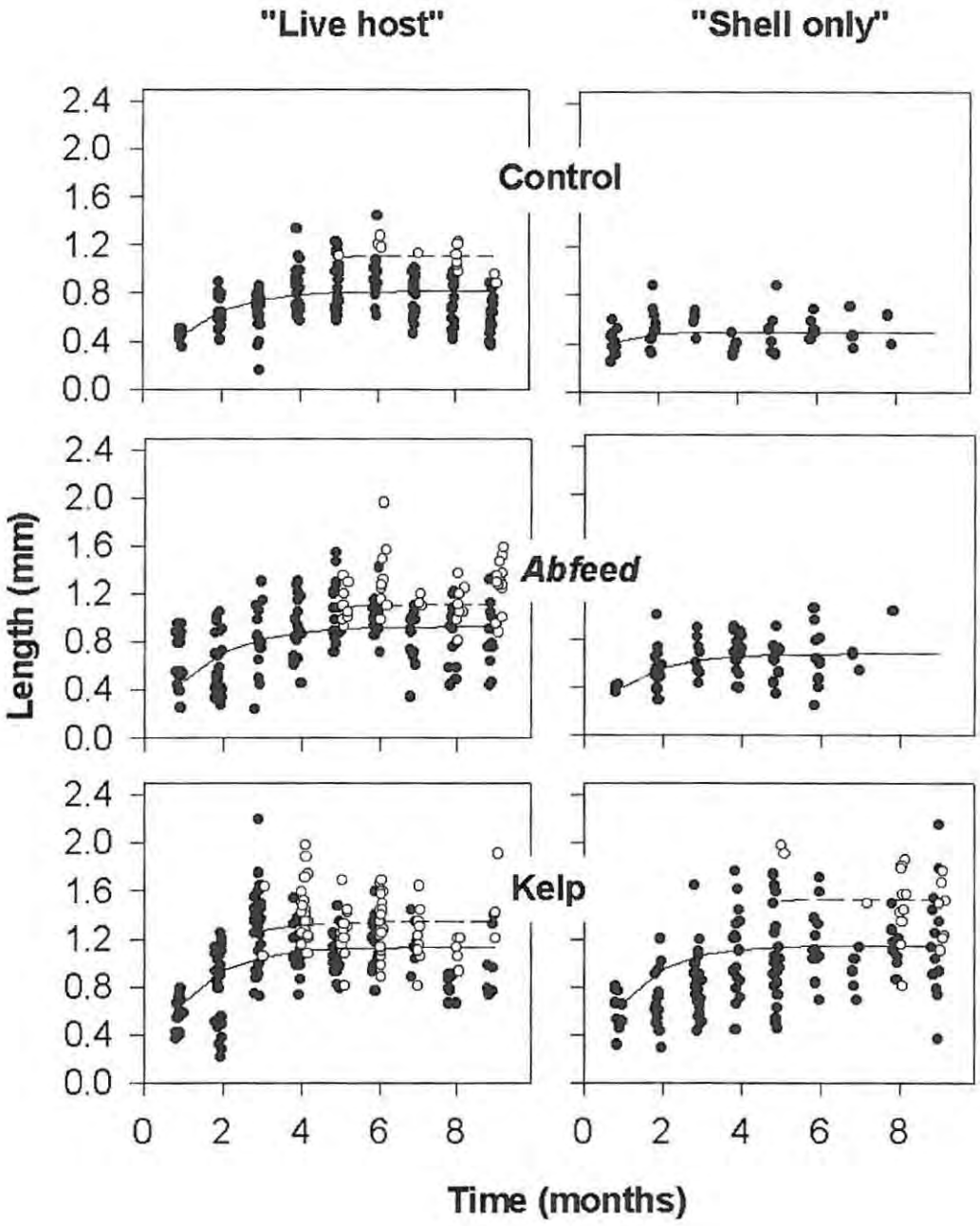

Figure 3.2. The growth of worms without oocytes and gravid worms on 'Live host' (A - C) and 'Shells only' (D - F), in control, Abfeed™ and kelp groups. (● = worms without oocytes, ○ = gravid worms, —— = predicted growth curve of worms without oocytes, – – = predicted growth curve of gravid worms)

Table 3.2. The von Bertalanffy growth parameters of worms on different diets and in the presence and absence of a live host, calculated according to $L_t = L_\infty(1 - e^{-Kt}) + a\varphi$, where L_∞ is the theoretical maximum length of the worm in mm, K is Brody's growth coefficient (the time in which the maximum size is attained), and 'a' is the extra length contributed by maturing oocytes. Asymptotic coefficients of variation are expressed as a percentage in parentheses.

Treatment	L_∞ (mm)	K	a (mm)
Live host			
Abfeed™	0.931 (3.34%)	0.73 (13.57%)	0.313 (15.16%)
Control	0.814 (2.32%)	0.808 (14.05%)	0.297 (17.22%)
Kelp	1.128 (2.93%)	0.882 (12.94%)	0.219 (18.47%)
Shell only			
Abfeed™	0.674 (4.53%)	0.873 (22.93%)	N/A
Control	0.498 (4.96%)	1.816 (32.52%)	N/A
Kelp	1.144 (5.18%)	0.489 (16.21%)	0.391 (21.29%)

Size- and age-at-maturity and proportion of gravid worms

In the 'live host' treatments no significant differences between the length at maturity of worms on kelp- and Abfeed™-fed abalone were found, but the worms on kelp-fed abalone were significantly larger than worms in the control group ($F=96.77$, $P<0.001$, Table 3.4, Fig. 3.2). There was no significant difference in the length at maturity of kelp-fed worms on live abalone and shells only ($F=0.3$, $P<0.59$, Table 3.4, Fig. 3.2).

Six percent of the kelp-fed worms in the 'live host' treatment had reached maturity three months after the initiation of the experiment. At five months 24%, 30% and 23% of the worms in the 'live host' Abfeed™ and control, and 'shell only' kelp groups, respectively, had reached maturity (Figs 2 and 3A). From the third to seventh month, the samples from the kelp 'live host' treatment had proportionately more gravid worms than the other treatments. Thereafter, the highest proportions were made up of worms from the 'shell only' kelp and 'live host' Abfeed™ treatments, respectively (Fig. 3.3A). Analysis of all the treatments indicated that treatment and time had a significant effect on the incidence of gravid worms (treatment: $F=6.63$, $P<0.002$, time: $F=4.94$, $P<0.002$, Fig. 3.2A), but there was no interaction between factors ($F=0.75$, $P=0.72$). Diet and time did not have a significant effect on the proportion of gravid worms in the 'live host' treatments, and there was no interaction between diet and time, using an adjusted *p* value (diet: $F=7.95$, $P=0.048$; time: $F=3.04$, $P=0.029$; diet x time: $F=0.83$, $P=0.623$). Host presence, but not time, had a significant effect on the proportion of gravid worms from the 'live host' and 'shell only' treatments, and there was no interaction between factors (host presence: $F=7.95$, $P<0.022$; time: $F=3.62$, $P=0.048$; host presence x time; $F=1.43$, $P=0.31$).

Table 3.3. The Likelihood Ratio test results, comparing the parameters of the growth models of worms on different diets and on 'live hosts' and 'shells only'. The rejection of the null hypothesis (i.e. a significant difference between treatments) is denoted by *.

Null Hypothesis	Wilks Λ	Degrees of freedom	P-value
Live host: Abfeed ™ vs. kelp vs. control			
H_1: L_∞ equal	66.28	2	≤ 0.001*
H_2 :K equal	1.34	2	0.51
H_3: a equal	2.98	2	0.23
H_4: L_∞, K and a equal	130.96	6	≤ 0.001*
Shell only: Abfeed ™ vs. kelp vs. control			
H_1: L_∞ equal	41.06	2	≤ 0.001*
H_2: K equal	10.52	2	≤ 0.005*
H_3: a equal	<0.0001	2	1
H_4: L_∞, K and a equal	116.72	6	≤ 0.001*
Live host vs. Shell only: Abfeed™			
H_1: L_∞ equal	11.58	1	≤0.001*
H_2: K equal	0.29	1	0.59
Live host vs. Shell only: Control			
H_1: L_∞ equal	39.18	1	≤ 0.001*
H_2: K equal	6.58	1	≤ 0.01*
Live host vs. Shell only: Kelp			
H_1: L_∞ equal	0.07	2	0.97
H_2: K equal	9.26	2	≤ 0.01*
H_3: a equal	3.79	2	0.15

Brood occurrence and size

Brooding (i.e. the presence of eggs and/or larvae in the burrow) occurred in all treatments where some worms had reached sexual maturity. In the worms on kelp- and Abfeed™ -fed abalone, broods appeared one month after gravid worms were observed. Usually no more than 40% of the gravid worms in each sample were brooding (Fig. 3.3), with the exception of the 'shell only' kelp treatment in the fifth month when both gravid worms were brooding. In the 'shell only' kelp treatment, the first brood was observed in the same month as the first gravid worms. In the 'live host' control group only one brood was observed, three months after the first mature adults appeared in the samples (Fig. 3.3).

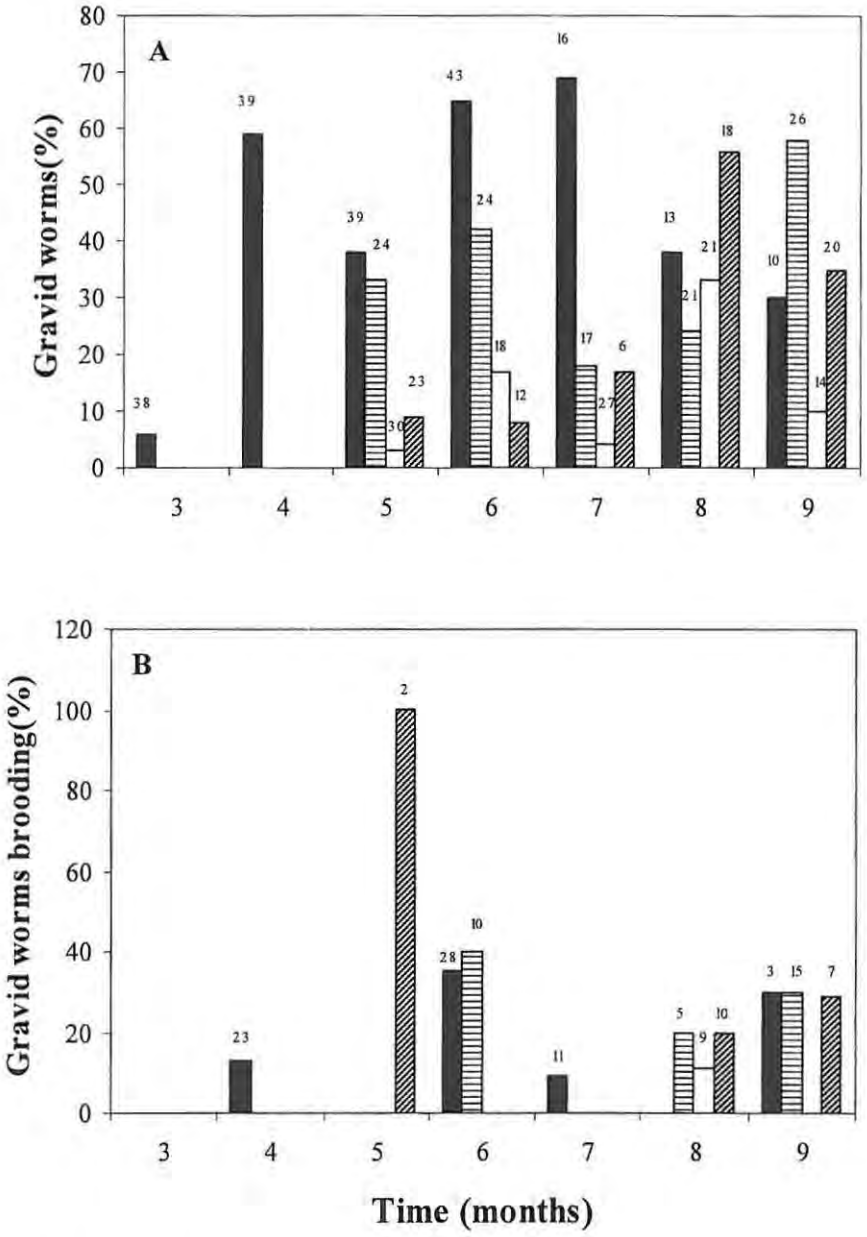

Figure 3.3. The proportion of **A** gravid, and **B** brooding worms in the 'live host' kelp, Abfeed™, and control, and 'shell only' kelp treatments. The values above the bars in **A** are the total number of worms in the sample, the values above the bars in **B** are the total number of gravid worms in the sample.

Table 3.4. Mean length (mm ± standard deviation) at maturity of sabellids in different treatments. Sample sizes are in parentheses.

Treatment	Mean length in mm ±SD (n)	Statistical relationships between treatments		
		All treatments*	Diet*	'Live host' vs 'Shell only'*
'Shell only': Kelp	1.51± 0.31 (20)	A		X
'Live host': Kelp	1.34 ± 0.24 (97)	A, B	R	X
'Live host': Abfeed™	1.24 ± 0.22 (42)	B, C	S	
'Live host': Control	1.11 ± 0.11 (15)	C	T	

All treatments: $F=11.24$; $P<0.0001$

Diet: $F=96.77$; $P<0.001$

'Live host' vs 'Shell only': $F=0.3$; $P=0.59$

* Values not significantly different from each other have the same letter (according to a Tukey's (HSD) test for unequal sample sizes).

Once the worms started brooding, broods were not always observed in each treatment. In the 'live host' kelp treatment there was a lag of one month between the first and second, and third and fourth broods. In the'live host' Abfeed™ treatment, there was a lag of one month between the first and second brood, and two months between the first and second broods in the 'shell only' kelp treatment (Fig. 3.3B).

Broods were composed of one, two or three eggs and/or larvae, but 80% of the broods had only one egg or larva (Table 3.5). When the data from all the treatments were analysed, treatment had an affect on the frequency of broods of different sizes ($\chi^2=13.781$, $P=0.05$, Table 3.5). In the 'live host' treatments, broods were composed of either one or three individuals, but there was no significant difference in the frequency of broods in worms on the different diets ($\chi^2=0.152$, $P=0.025$). Broods in the 'shell only' kelp treatment were composed of one or two individuals, and there was no significant difference in the frequency of broods in the 'live host' and 'shell only' treatments ($\chi^2=6.914$, $P=0.025$). In total, the worms in the 'live host' kelp treatment produced twice as many broods as those in the 'live host' Abfeed™ treatment, and 2.5 times as many broods as the worms in the kelp 'shell only' treatments (Table 3.5).

Table 3.5. The total number of broods and the number of eggs and larvae per brood observed in each treatment. The frequency of broods was analysed by a contingency test.

Treatment	Total broods	Number of eggs and larvae per brood		
		1	2	3
'Live host': Kelp[A, B, C]	15	13 (87%)	0	2 (13%)
'Live host': Abfeed™[A, B]	8	7 (87.5%)	0	1 (12.5%)
'Live host': Control[A, B]	1	1	0	0
'Shell only': Kelp[A, C]	6	3 (50%)	3 (50%)	0

[A]: All treatments: χ^2=13.781; P=0.05

[B]: Diet: χ^2=0.152; P=0.025

[C]:'Live host' vs 'Shell only': χ^2=6.914; P=0.025

Size of eggs and larvae

There was a significant difference in the volume of eggs in all the treatments combined (Table 3.6, H=6.16, P<0.046). No eggs were found in the 'live host' control samples. There was, however, no significant difference between the volume of eggs from worms on different diets in the 'live host' treatments (U=20, P=0.35) or between the volume of eggs in the kelp 'live host' and kelp 'shell only' treatments (U=17, P=0.57). All larvae measured were at the same stage of development (i.e. the latest stage before emergence from the burrow), and there was no significant difference in their lengths when the data from all treatments were analysed (H=2.43, P=0.49). Furthermore, there was no significant difference in the lengths of larvae on different diets in the 'live host' treatments (H=1.36, P=0.51), or between larvae from worms in the kelp 'live host' and kelp 'shell only' treatments (U=5.5, P=0.28).

Experiment 2: Settlement of larvae in abandoned burrows

After three months, no larvae had settled in the abandoned burrows in any of the treatments. Larvae were, however, observed crawling over empty shells.

Table 3.6. The mean volume (mm^3 ± standard deviation) of individual eggs and mean length (mm ± standard deviation) of larvae in the different treatments. The sample sizes are given in parentheses. The combined egg volume and length of larvae (^{A} and ^{R}, respectively) and length of larvae on different diets (^{S}) were analysed using the Kruskal-Wallis test. All other pair-wise analyses were conducted using the Mann-Whitney U test.

Treatment	Egg Volume in mm^3 (n)	Larvae length in mm (n)
'Live host': Abfeed™	0.014 ± 0.008 (8)$^{A, B}$	0.5 ± 0.11 (3)$^{R, S}$
'Live host': Kelp	0.027 ± 0.022 (7)$^{A, B, C}$	0.491 ± 0.081 (11)$^{R, S, T}$
'Live host': Control		0.431 (1)$^{R, S}$
'Shell only': Kelp	0.035 ±0.009 (6)$^{A, C}$	0.563 ± 0.025 (2)$^{R, T}$

Egg Volume: ^{A}: All treatments: H=6.16, df=2, n = 21; P<0.046,

: ^{B}: Diet: U=20, P=0.35

: ^{C}: 'Live host' vs 'Shell only' : U=17, P=0.57

Larvae length: ^{R}: All treatments: H=2.43, df=3, n = 17, P=0.49

: ^{S}: Diet: H=1.36, df=2, n=15; P=0.51

: ^{T}: 'Live host' vs 'Shell only' : U=55; P=0.28

Discussion

The limited growth and absence of sexually mature worms in the 'shell only' Abfeed™ and control treatments may have been due to starvation, rather than to the absence of a live host. This conclusion was based on several factors: 1) the growth and reproduction of worms in the 'shell only' kelp treatment were comparable to that of worms in the 'live host' kelp and Abfeed™ treatments, 2) the worms in the 'live host' control group, which were exposed to a limited amount of food, did not grow as well as the worms in the other 'live host' treatments, and although some of the worms did become gravid, only one worm produced a brood, and 3) most of the worms in the 'shell only' Abfeed™ and control treatments died after eight months. Thus the 'shell only' Abfeed™ and control treatments were disregarded in the following discussion. When discussing the effects of diet on growth and reproduction, only the results from the 'live host' treatments have been considered. When discussing the effects of a live host on growth and reproduction, only the results from the 'live host' and 'shell only' kelp treatments have been considered.

Effect of diet on growth and reproduction

Various criteria have been used to measure the reproductive responses of polychaetes to different environments and diets. These include growth rate and body size, age- and size-at-maturity, size and number of eggs and larvae and the reproductively active proportion of the population (Levin and Creed, 1986; Zajac, 1986; Grémare et al., 1988; Qian and Chia, 1991, 1992a, 1992b; Moore and Dillon, 1993; Qian, 1994). These studies have shown that growth and reproductive output respond to changes in the quality and quantity of food.

Diet had a significant effect on the growth of *T. heterouncinata*: although diet had no significant effect on the time in which the worms reached their maximum size, the worms on the kelp-fed abalone had a higher growth rate, as their maximum size was greater than the worms on abalone fed the high protein Abfeed™. This is in contrast to the results of Qian and Chia (1991) who demonstrated that individuals of *Capitella* sp. fed squid egg capsules (which have a high protein and lipid content) were twice as large as their siblings fed *Ulva*. The present results suggest that protein content is not the only dietary component contributing to the growth of *T. heterouncinata*. A similar conclusion was presented by Marsh et al. (1989) for *Capitella* sp. I. The authors found that the growth rates of juvenile *Capitella* sp. I were more closely correlated with the levels of the amino acids histidine and phenylalanine than with the nitrogen levels in the food.

Terebrasabella heterouncinata may change both its age- and size-at-maturity in response to different diets – worms that matured at an older age did so at a smaller size. This suggests that when conditions are favourable, the worms will grow rapidly and reproduce at an early age and large size. If, however, the food quality or quantity is limiting, the worms will reproduce at a smaller size and later age. This pattern differs from those observed in other polychaetes: 1) in some nereids the age-at-maturity depended on the growth rate of the worm (Olive et al., 1986, in Bentley and Pacey, 1992): when the

growth rate is low, the worms will delay reproduction until the following year, when their final size may in fact be greater than that of individuals that had a high growth rate and had reproduced during their first year; 2) polychaete species such as *Polydora ligni*, *Capitella* sp. with lecithotrophic development and *Nereis arenaceodentata* maintain a constant age-at-maturity, while changing its size-at-maturity on different food rations (Zajac, 1986; Qian and Chia, 1992a; Moore and Dillon, 1993) and this implies that in these species, the onset of reproduction is triggered by an internal rhythm which may override external factors; and 3) *Capitella* sp. with planktotrophic development changes its age- and size-at-maturity in response to changes in its diet (Qian and Chia, 1992a) but the worms that matured the soonest did so on the diet that provided the lowest growth rate. It is therefore clear that while the onset of sexual maturity is highly variable in polychaetes, it is controlled by different factors in different species.

The present data suggest that the energy invested in reproduction is equivalent for the different diets as the degree of expansion of the body due to the presence of maturing oocytes (parameter a) was the same in all treatments. This is further supported by the fact that there is no significant difference in the volume of eggs of worms on kelp- and Abfeed™-fed abalone.

There was no significant difference in the volume of eggs or length of larvae from worms on different diets. Furthermore, there was no difference in the brood sizes of worms fed the different diets. These worms have therefore maintained both their fecundity and offspring size when fed diets with different nutrient levels. This differs from Chia's hypothesis (1974) that marine invertebrates may produce fewer, but larger eggs in nutrient-poor environments, than in nutrient-rich ones, leading to the development of offspring with an increased chance of survival in nutrient-poor environments (Qian, 1994). It is, however, important to note that the number and size of offspring produced by *T. heterouncinata*, an intratubular brooder, may be phylogenetically constrained (Rouse and Fitzhugh, 1994). If this is the case, worms might increase their long-term fecundity by other means, such as by starting to breed sooner. This was demonstrated in the present study, where kelp-fed worms started to breed at a younger age than Abfeed™-fed worms. *Terebrasabella heterouncinata* and some other sabellid species lay eggs sequentially, and their broods are usually composed of eggs and larvae at different stages of development (Rouse and Fitzhugh, 1994; Fitzhugh and Rouse, 1999). *Terebrasabella heterouncinata* could therefore produce eggs continuously if conditions are favourable. This proved not to be the case in the present study as broods were not always present in the different treatments.

Neither diet nor time influenced the incidence of gravid worms. However, the use of an adjusted p value is a conservative approach, which makes the rejection of null hypotheses more rigorous. This result is contrary to the findings of Levin and Creed (1986) and Grémare et al. (1988) who found that food availability and nutrient profile can influence the number of gravid and brooding individuals in the population.

Based on the preliminary observations of the infestation levels in farmed abalone on different diets, it was expected that worms fed Abfeed™ would have a higher reproductive output than worms fed kelp.

This proved not to be the case. The results suggest that in spite of the higher protein content of Abfeed™, kelp was a 'better' diet for the worms. It is possible that this is only the case when either kelp or Abfeed™ are fed to the worms in isolation. On the farms, worms are exposed to degraded abalone food as well as other particulate organic matter. It is thus possible that the supplementation of Abfeed™ with other organic matter improves the quality of the diet of the worms on Abfeed™-fed abalone on farms.

Effect of the presence or absence of a live host on growth and reproduction

The present study showed that emerging larvae do leave their host shells, but do not settle in abandoned burrows. This study also demonstrates that although *T. heterouncinata* is able to dissolve shell, it cannot colonise bare calcareous substrates, unlike other boring sabellid species such as *Caobangia* (Jones, 1974). This means that if worms on a 'dead' shell continue reproducing, the larvae will either die or they have to disperse to find a suitable living host.

In the absence of a live host *T. heterouncinata* continued to grow and reproduce (present study and Culver et al., 1997). This indicates that once the worms have settled, they are not dependent on a live host for survival. The absence of a live host did, however, influence the growth rate and reproductive efficiency of the worms. Worms in the 'shell only' treatment grew to the same size as those in the 'live host' treatment, but the growth rate was significantly lower in the former treatment. The worms maintained a constant size-at-maturity, but those in the 'live host' treatment matured two months before the worms in the 'shell only' treatment which could potentially increase their long-term fecundity. The absence of a live host did not affect the volume of eggs, the length of the larvae or the frequency of broods. The interval between broods in the worms in the 'shell only' treatment was, however, greater than that between broods in the 'live host' treatment. These differences in growth rate and reproductive output might be related to the re-allocation of nutrients from somatic growth and reproduction to burrow expansion. When the abalone for the 'shell only' treatment were shucked, the burrows were large enough to accommodate week-old larvae. Under these circumstances, worms would either have to expand their burrows to accommodate their growing bodies or limit their growth. While Fitzhugh and Rouse (1999) suggested that after settlement *T. heterouncinata* extend their burrows to a limited extent, the worms in the present study expanded their burrows considerably. In the 'shell only' treatment the terminal end of the burrow extended into the thickness of the shell, forming an 'L'-shaped burrow, instead of lying in the same plane as the shell, as they do on live abalone.

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CHAPTER 4

Infestation of the abalone, *Haliotis midae*, by *Terebrasabella heterouncinata*, under intensive culture conditions, and the influence of infestation on abalone growth

Simon, C.A., Kaiser, K. and Britz, P.J., Infestation of the abalone, *Haliotis midae*, by the sabellid, *Terebrasabella heterouncinata*, under intensive culture conditions, and the influence of infestation on abalone growth. Aquaculture 232 (2004) 29-40.

"Once you open a can of worms,
the only way to re-can them
is to use a larger can."

Zymurgy's first law of Evolving Systems Dynamics

Abstract

Heavy infestation by the shell-infesting sabellid, *Terebrasabella heterouncinata*, has been shown to cause a reduction in the growth rate and marketability of cultured abalone. This worm is endemic to South Africa and the locally cultured abalone, *Haliotis midae*, are under constant threat of infestation. An understanding of factors that influence abalone growth and sabellid infestation levels under intensive culture conditions is therefore imperative to controlling infestation levels. Abalone were held on an abalone farm at stocking densities of 18%, 23% and 28% of the available surface area. They were fed kelp or a pelleted diet, Abfeed™. Their growth and infestation levels were monitored for 14 months. Within the range of abalone sizes used for this study kelp-fed abalone grew significantly better than Abfeed™-fed abalone ($F=7.99$; $P<0.005$), while stocking density had no effect ($F=1.41$; $P=0.25$) on growth. Abalone that became infested grew at the same rate as those that did not ($P=0.09$). No growth-limiting effect of infestation was detected. There was no effect on total intensity (i.e., the total number of worms per sample of 60 abalone; $P=0.41$and $P=0.17$), or prevalence (i.e., the percentage of infected abalone within a sample; $P=0.50$ and $P=0.19$) of infestation by diet and stocking density, respectively. There was a significant positive coefficient of correlation of 86.2% between the level at which an increase in total intensity starts, and the average increase in total intensity per day ($F=62.2$; $P<0.0001$). A regression model is presented to estimate total intensity based on prevalence. The greatest increase in total intensity levels coincided with the onset of spring, and a possible seasonal component to the reproduction of this sabellid is discussed.

Introduction

Commercial abalone aquaculture developed in South Africa during the 1990s and employs an intensive system in which abalone are reared at high densities in shore-based culture systems (Sales and Britz, 2001). The first serious health issue to affect production of the abalone, *Haliotis midae*, was the infestation of this abalone by a sabellid polychaete, *Terebrasabella heterouncinata* (Ruck and Cook, 1998; Fitzhugh and Rouse, 1999), an obligate symbiont which forms burrows in the shells of various gastropods. The species is a simultaneous hermaphrodite that broods clutches of up to seven offspring throughout the year if conditions are favourable (Culver et al., 1997). Upon leaving the parental burrow, the larva crawls to the growing edge where the host covers it with nacreous shell as it forms a new burrow (Culver et al., 1997). Although *T. heterouncinata* is endemic to South Africa (Ruck and Cook, 1998; Fitzhugh and Rouse, 1999), its potential to disrupt abalone production was first observed on Californian abalone farms in the early 1990's following its accidental introduction (Fitzhugh, 1996; Oakes and Fields, 1996; Culver et al., 1997). Heavy infestation of abalone leads to deformation and weakening of the shell, a reduction in growth, or the death of the abalone (Fitzhugh, 1996; Oakes and Fields, 1996; Culver et al., 1997; Ruck and Cook, 1998).

Efforts to treat worm infestation have met with varying success. Methods have included coating the shell with wax; exposing the worms to encapsulated toxins that can be ingested by them but not by abalone; anaesthetising the worms and then exposing them to fresh water or ultrasound; biocontrol agents and heat treatment (Culver et al., 1997; Leighton, 1998; Shields et al., 1998; Loubser and Dormehl, 2000; P. Truter, Sea Plant Products, pers. comm.). These methods were either too impractical to be implemented on a large scale, or the worms were resistant to the treatment (Oakes and Fields, 1996; Leighton, 1998) due to their ability to retract into the safety of their burrow. In California, an effective means of eradicating this worm from aquaculture facilities was by culling or isolating infested abalone (Oakes and Fields, 1996; Culver et al., 1997). This is, however, not a practical option in South Africa, due to continuous introduction of *T. heterouncinata* via the incoming seawater. Therefore, sabellid populations on South African abalone farms must be controlled and maintained at a level that does not negatively affect growth or appearance of the abalone. An understanding of the factors determining sabellid infestation rate is thus critical to formulating protocols for "managing" the problem.

Cultured molluscs are routinely infested by burrow-dwelling spionid and sabellid polychaetes, and their effect on the hosts' shell strength, tissue quality, growth and mortality rate is well documented (Kent, 1979; Laukner, 1983; Wargo and Ford, 1993; Oakes and Fields, 1996; Ruck and Cook, 1998; Read, 2001). The effect of environmental factors on the levels of infestation has, however, received little attention (Caceres-Martinez et al., 1998). Laukner (1983) suggested that salinity, water temperature and sediment composition appear to be the main factors influencing spionid abundance. A number of factors including abalone size, growth rate, diet, stocking density and season have been cited as influencing the infestation rate of *T. heterouncinata* (Oakes and Fields,

1996; Clayden, 2000; A. Du Plessis, Aquafarm Development, pers. comm.), but no quantitative studies on these effects have yet been published.

Previous reports by abalone farmers suggest that slow-growing abalone appeared most susceptible to infestation (Oakes and Fields, 1996; Clayden, 2000). Factors affecting abalone growth are diet, stocking density, and size and age of the abalone (Oakes and Fields, 1996; Culver et al., 1997; Clayden, 2000). Along the Western Cape coast of South Africa, where infestation by *T. heterouncinata* is high, cultured abalone are fed either a pelleted diet, Abfeed™ (Britz, 1995); *Eklonia maxima*, the abalone's natural kelp diet, or a mixture of these two feeds. Abfeed™ has a high protein and lipid content (Simon et al., 2002) and was specifically formulated to promote abalone growth (Britz, 1995). Abalone fed Abfeed™ seem to be more susceptible to sabellid infestation than kelp-fed abalone (Clayden, 2000). In addition, Chalmers (2002) showed that sabellids on Abfeed™-fed abalone displayed a higher production of eggs and larvae than those on kelp-fed abalone. This is likely to be related to the nutritional value of abalone faecal waste on which the sabellids presumably feed, as the faeces produced by Abfeed™-fed abalone contained significantly higher levels of protein and energy (Chalmers, 2002). A size effect also appears to be present as abalone larger than 45 mm in length appear to be more susceptible to infestation by *T. heterouncinata*, and grow better on kelp than on Abfeed™ (A. Du Plessis, Aquafarm Development, pers. comm.). Abalone growth is influenced by stocking density and increased stocking density has been shown to result in slower growth and a higher *T. heterouncinata* infestation level (Clayden, 2000). No experiments have been conducted to quantify sabellid infestation in abalone fed either pelleted feed or kelp, or combinations of the two at different abalone stocking densities.

The objectives of this study were to a) quantify the effect of diet and stocking density on the growth of the abalone, *H. midae*, and the intensity and prevalence of infestation by *T. heterouncinata*; b) investigate the relationship between abalone growth rate and infestation; and c) make predictions concerning the level of infestation under intensive farming conditions.

Materials and methods

The experiment was conducted at the abalone farm "Aquafarm Development" in Hermanus on the southwest coast of South Africa, from January 2001 to February 2002. The experimental animals, *Haliotis midae*, were maintained and fed according to farm production routine. Abalone with a shell length of 45-50 mm were randomly selected from one cohort. These animals had been fed Abfeed™ and were at the size at which their diet would have been changed to kelp.

Holding tanks

Abalone were held in baskets suspended in 2600 L fibreglass tanks. Each tank (4.2 m x 0.8 m x 0.8 m high) held seven baskets. The water supply to the tanks was filtered through a drum filter with a mesh size of 60 μm and was exchanged every 2.5 - 3 hours. The water in the tanks was aerated

through two air supply pipes installed along the length of the bottom of each tank. The baskets contained vertical plastic plates providing a surface area of 3.2 m^2 per basket. The abalone stocking density prior to the initiation of the experiment was 18%, i.e., 18% of the available plate surface area was covered by the abalone.

Experimental design

Kelp was placed into the baskets between the vertical plates, while the negatively buoyant and concentrated Abfeed™ pellets were placed on a 'feeder'-plate positioned horizontally across the top of the vertical plates. Abalone were held at densities of 18%, 23% or 28% for kelp and at 23% and 28% for the Abfeed™ treatment. Each treatment was duplicated. Based on the farmers' experience it was assumed that abalone fed Abfeed™ had a greater potential to become a source of worm infestation than kelp-fed abalone. Thus, by using only two stocking densities the number of baskets with potentially infested abalone was reduced. Experimental baskets were placed randomly among production baskets containing non-experimental animals, with one experimental basket per tank. The experimental abalone were kept close to an already infested group to allow transmission of sabellid larvae to the experimental animals.

Sixty abalone from each experimental basket were marked and measured, and the number of worms on the growing edge of the shells was counted. Subsequent sampling took place approximately every four months when the stocking density was adjusted to maintain the surface area covered by the abalone. At each sampling period all marked abalone were measured, and the worms on the growing edge counted. To compensate for lost labels, unlabelled abalone were randomly chosen from the experimental baskets and labelled. The experiment was concluded when the abalone reached a market size of approximately 84 mm shell length with a live animal mass of 100 g.

Terminology

For the purpose of this study, prevalence refers to the percentage of infested abalone in the sample. Intensity is defined as the number of burrows on the growing edge of an abalone (Margolis et al., 1982). Total intensity refers to the total number of worm tubes counted on the growing edge of all abalone in a sample.

Statistical analyses

Multiple regression analysis was used to identify the contribution of stocking density and diet to the variation in prevalence and total intensity. Correlation analysis was employed to describe the relationship between intensity and abalone growth (mm/d). Two-way ANOVA was used to test the effect of diet and stocking density on average growth (mm/d) of the abalone. To test the effect of sabellid infestation on abalone growth, only newly infested abalone were chosen for the analysis. Thus, the average growth (mm/d) of abalone before they were infested was compared to that of

animals that were never infested during the course of the 390-day experiment. This was done for each diet using pooled data from all stocking densities. Due to the small and unequal sample sizes, the non-parametric Mann-Whitney U-test was used to compare growth between a) the two dietary treatments and b) between abalone that had not been infested versus those that had been.

Results

The effect of diet, stocking density and sabellid intensity on abalone growth

Diet affected growth of the abalone (Table 4.1, $F=7.99$, $P<0.005$) with the growth rate of abalone fed on kelp (0.061 ± 0.0019 mm/d) being significantly greater than in those fed Abfeed™ (0.051 ± 0.0017 mm/d). Stocking density did not influence abalone growth over the range of experimental densities ($F=1.41$, $P=0.05$).

Due to the relatively high variation of infestation rate within diets and stocking density, the relationship between abalone growth (mm/d) and the number of worm tubes per cm of growing edge was analysed separately for abalone in each basket. The correlation between the number of tubes and abalone growth rate was very low, and in six of ten baskets it was insignificant at an error level of 5% (Table 4.1). Low but significant correlations between the intensity of sabellid infestation and abalone growth were found in the two treatment combinations with the highest prevalence, i.e., in the 'Abfeed™-fed 28% stocking density' and 'kelp-fed 23% stocking density' treatments (Table 4.1). Animals in these baskets showed a trend of increasing growth rate with an increasing number of tubes. Thus, no growth-reducing effect was found with sabellid infestation in any of the experimental baskets.

In both diet treatments, the average growth of abalone that did not become infested during the 390-day experiment did not differ significantly from that of abalone that were infested during this period ($P=0.09$). Uninfested Abfeed™-fed abalone grew at 0.04 ± 0.02 mm/d while those that became infested had an average growth rate of 0.03 ± 0.01 mm/d before they became infested. Uninfested kelp-fed abalone grew at 0.06 ± 0.02 mm/d while those that got infested had a growth rate of 0.04 ± 0.01 mm/d before the settling of sabellids first occurred.

Prevalence and intensity of infestation

There was a general trend for an increase in prevalence and total intensity over time in most treatments with the most pronounced increase occurring between days 139 and 259 (Figs 4.1 and 4.2, respectively). In baskets 3, 9 and 10 the increase in total intensity was apparent from day 139, while in the rest of the baskets, total intensity remained low for most of the experiment, with an increase from day 259 to day 390 (Fig. 4.2).

Table 4.1. The correlation coefficients (r) and levels of significance (P) between growth (mm/d) and intensity (number of tubes per growing edge) for different stocking density and diet combinations. * denotes a statistically significant correlation at $P \leq 0.05$ between these two variables.

Diet	Stocking density	Basket N^0	Growth (mm/d)	r	P
Kelp	18%	1	0.064±0.004	0.11	0.34
		2	0.061±0.005	0.14	0.27
	23%	3	0.075±0.005	0.32	0.009*
		4	0.059±0.004	0.22	0.04*
	28%	7	0.048±0.003	-0.18	0.15
		8	0.056±0.003	0.008	0.96
Abfeed™	23%	5	0.051±0.005	0.14	0.14
		6	0.057±0.008	0.11	0.32
	28%	9	0.050±0.003	0.32	0.004*
		10	0.050±0.004	0.30	0.007*

The development of prevalence and total intensity revealed large within-treatment variation (Figs 4.1 and 4.2). Thus, multiple regression analysis did not show a significant contribution by either diet or stocking density in predicting prevalence (P=0.05 and P=0.19, respectively) or total intensity (P=0.41 and P=0.17, respectively).

To describe the potential of the sabellid to increase its population size, data were pooled from all treatments, and only those periods showing an increase in total intensity of more than 5% over a 120 to 141-day period were used. The average increase in total intensity was 0.98 worms per day (n = 12) with a range of 0.02 to 4.6 depending on the level at which the increase in total intensity began. There was a significant positive coefficient of correlation of 86.2% ($F = 62.2$, $P<0.0001$) between the starting level of a rise in total intensity (S) and the average increase in total intensity per day (y). The model $y = 0.03\ S$ estimated the increase in total intensity per day for periods between 120 and 141 days as a function of the level at which the increase began. The intercept of this model was not significantly different from 0.

Figure 4.1. The change in prevalence (%) of infestation of cultured abalone, *Haliotis midae*, by the sabellid polychaete, *Terebrasabella heterouncinata*, over time under different culture conditions. Note that Y-axes scales are not standardised.

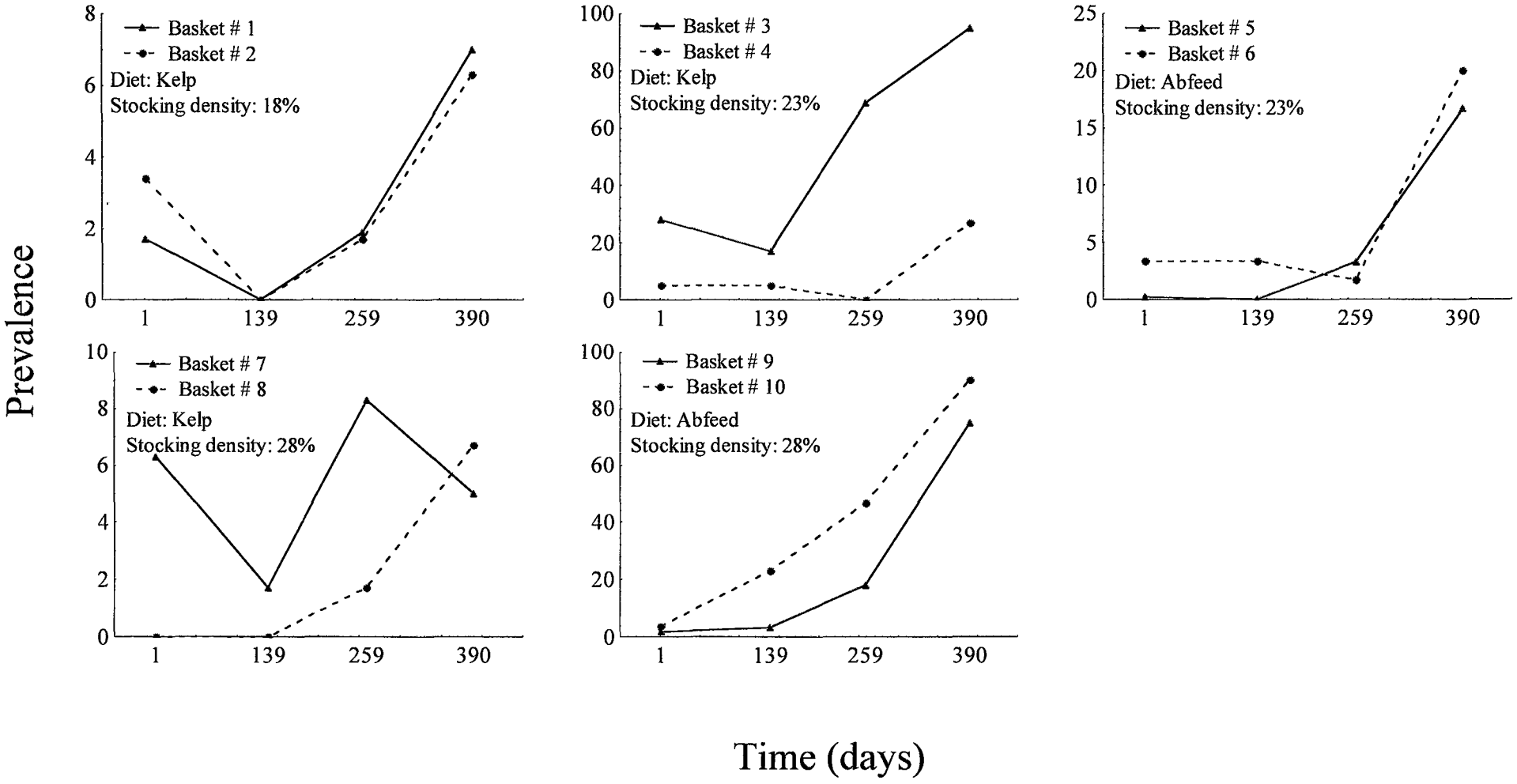
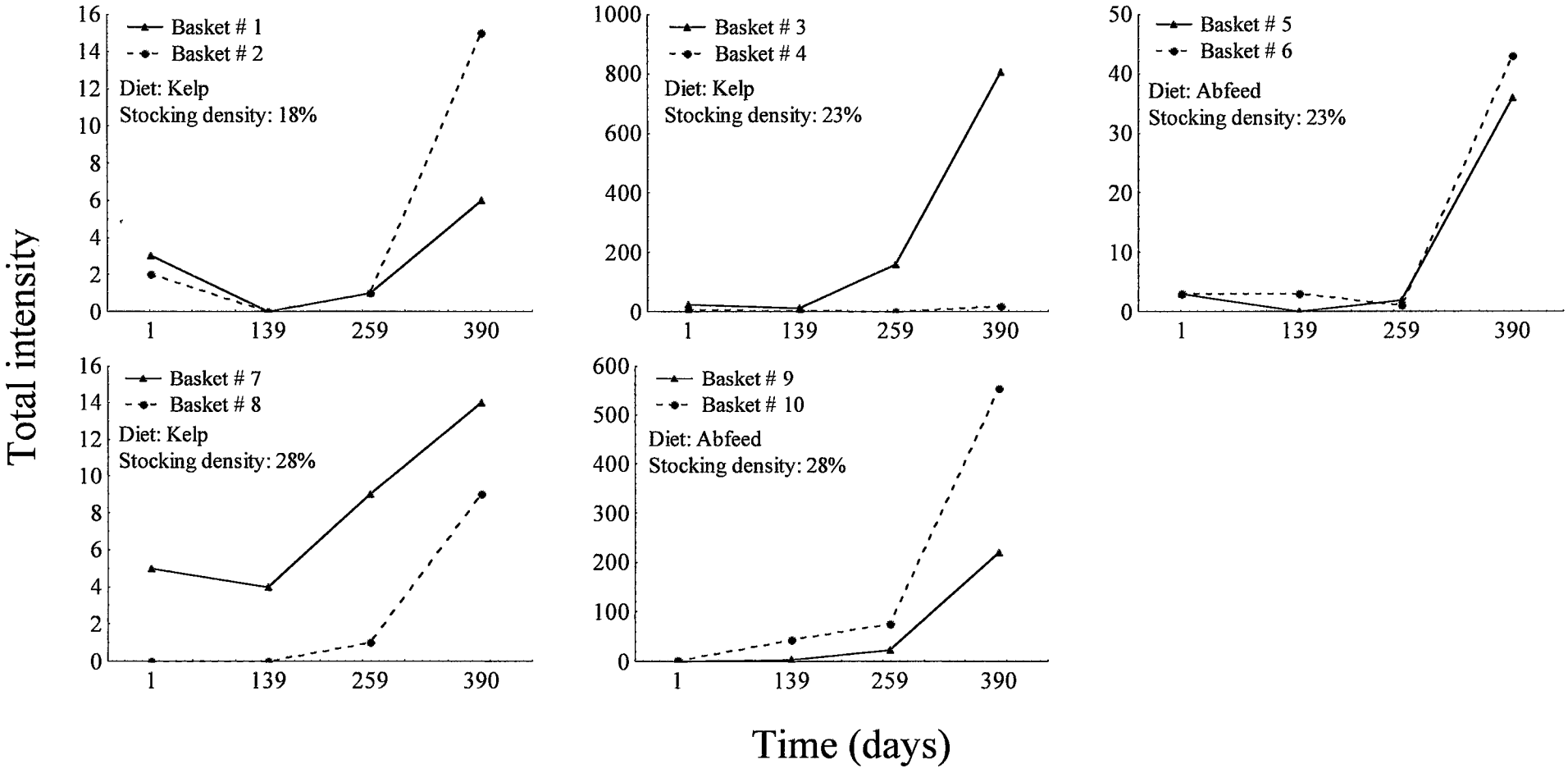

Prevalence
Basket # 1
Basket # 2
Diet: Kelp
Stocking density: 18%
Basket # 3
Basket # 4
Diet: Kelp
Stocking density: 23%
Basket # 5
Basket # 6
Diet: Abfeed
Stocking density: 23%
Basket # 7
Basket # 8
Diet: Kelp
Stocking density: 28%
Basket # 9
Basket # 10
Diet: Abfeed
Stocking density: 28%
1
139
259
390
Time (days)

Figure 4.2. The change in total intensity of the sabellid polychaete, *Terebrasabella heterouncinata* over time, on abalone, *Haliotis midae*, cultured under different conditions. Note that Y-axes scales are not standardised.


Total intensity
Basket # 1
Basket # 2
Diet: Kelp
Stocking density: 18%
Basket # 3
Basket # 4
Diet: Kelp
Stocking density: 23%
Basket # 5
Basket # 6
Diet: Abfeed
Stocking density: 23%
Basket # 7
Basket # 8
Diet: Kelp
Stocking density: 28%
Basket # 9
Basket # 10
Diet: Abfeed
Stocking density: 28%
Time (days)

The magnitude of the rise in prevalence was not related to the starting levels at which the values began to increase. Over an average period of 131 days the average increase in prevalence was 19% points ranging from 2% to 57%. Thus, within the range of stocking densities tested in this study, the sabellid had the potential to infest up to 57% of a population of abalone within this time period.

Intensity and prevalence (P) were closely correlated ($r^2 = 0.98$) and total intensity (I) could be predicted from prevalence using the model log (I) = -7.5 + 2.28 * log (P+0.5) (Fig. 4.3). If included as an additional variable into a multiple regression model, time (days after the start of the study) was not a significant predictor (P=0.36).

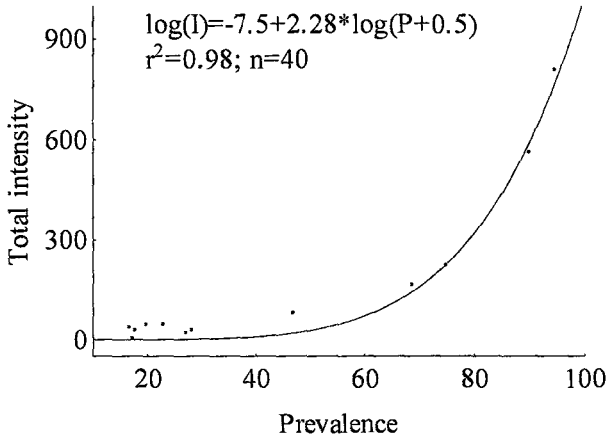
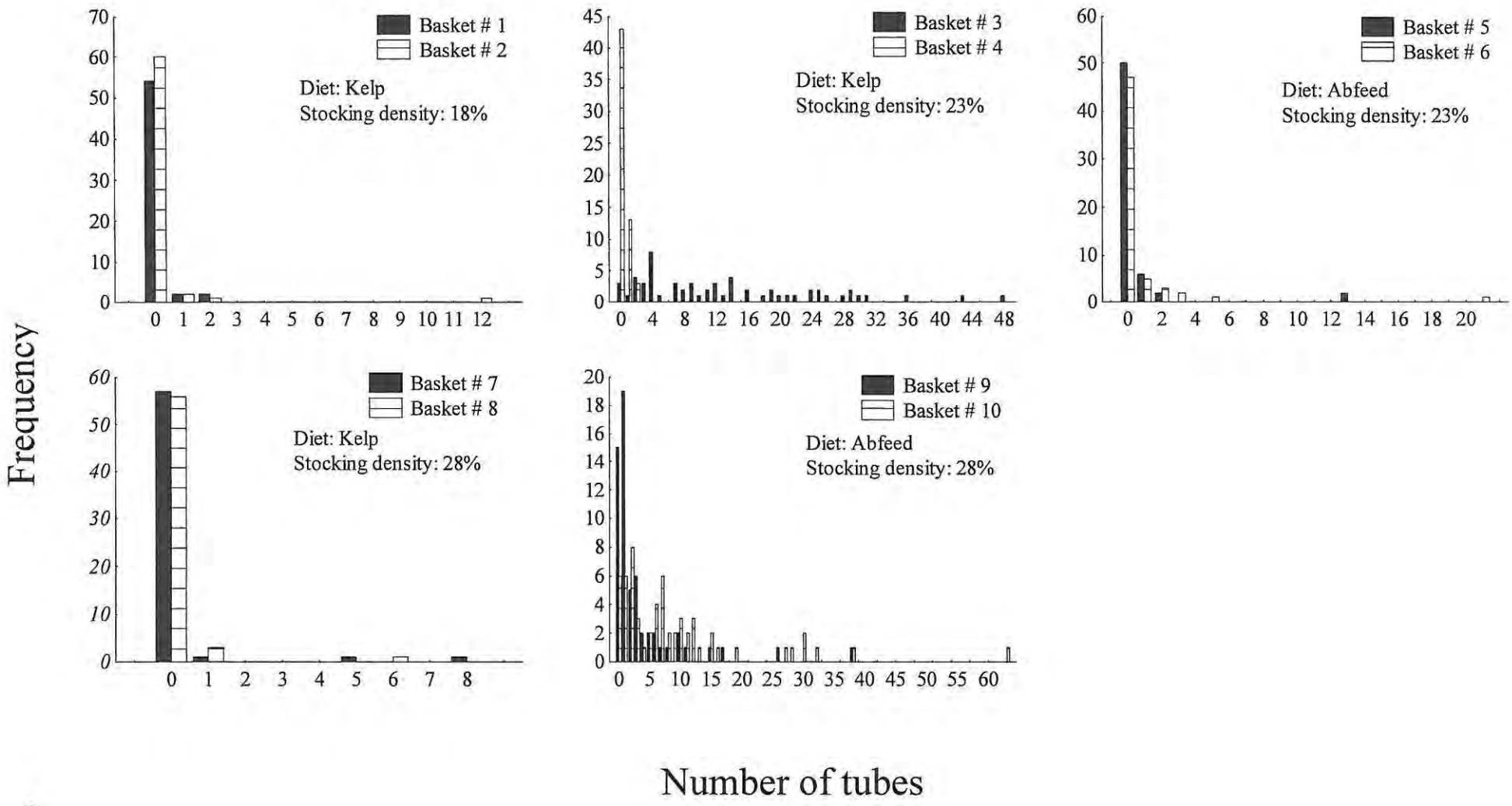


Figure 4.3. The correlation between intensity and prevalence. n = number of values used for the model (note that some data points overlap); r^2 = coefficient of correlation; log = decadian logarithm; I = Total intensity, and P = Prevalence (%).

Frequency distribution of intensity at the end of the study period

The number of worms per infested abalone ranged from one in all treatments to 63 in the 'Abfeed™ 28% stocking density' treatment (Fig. 4.4).

Figure 4.4. Frequency distribution of the number of sabellids, *Terebrasabella heterouncinata*, per abalone growing edge at day 390 of the experiment. Note that axes scales are not standardised.


Frequency
Basket # 1
Basket # 2
Diet: Kelp
Stocking density: 18%
Basket # 3
Basket # 4
Diet: Kelp
Stocking density: 23%
Basket # 5
Basket # 6
Diet: Abfeed
Stocking density: 23%
Basket # 7
Basket # 8
Diet: Kelp
Stocking density: 28%
Basket # 9
Basket # 10
Diet: Abfeed
Stocking density: 28%
Number of tubes

Seasonal variation in temperature and day length

The mean daily temperature (Fig. 4.5) ranged from 12.5°C to 16.6°C during the experimental period. Day length varied between 10 h and 14.5 h.

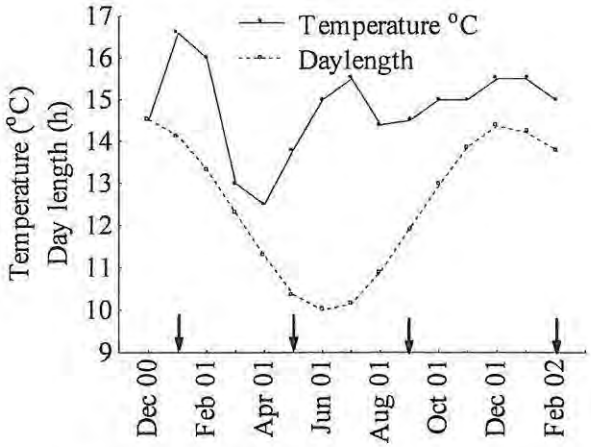


Figure 4.5. Monthly means of day length (h) and daily water temperature (°C) in abalone farm raceways at Aquafarm Development, Western Cape (South Africa) for the period December 2000 to February 2002. Arrows on the x-axis mark sampling dates.

Discussion

The worm infestation rates observed in this study were relatively low (0.02 to 9.53 tubes/cm shell growing edge) compared to levels typically observed in heavily infested cultured abalone in other studies (0.25 to 18.00 tubes/cm shell growing edge; Ruck and Cook, 1998; Gray, 2004), and no shell deformation was observed.

Abalone stocking density, within the ranges tested here, did not affect abalone growth. This differs from the results of a previous experiment that showed that abalone growth rate decreased when abalone were held at stocking densities higher than 18% (P. Pesch, Aquafarm Development, pers. comm.). Contrary to the suggestion by farmers, an effect of stocking density on the prevalence of infestation could not be shown in this experiment. Thus, other factors besides stocking density may play a role in the degree of transmission of larvae.

In this study kelp-fed abalone grew 33% faster than those fed Abfeed™, confirming the results of a previous experiment (A. Du Plessis, Aquafarm Development, unpublished data). According to Oakes and Fields (1996) slow-growing abalone were more prone to sabellid infestation. It would therefore be expected that the slower growing Abfeed™-fed abalone would be more susceptible to infestation than kelp-fed abalone. In addition, the quantity and quality of diet influences the reproductive output of several polychaete species, including *Terebrasabella heterouncinata* (Levin and Creed, 1986; Grémare et al., 1988; Qian and Chia, 1991; Qian, 1994; Chalmers, 2002; Simon et al., 2002). However, sabellid intensity could not be related to diet in the present study. Furthermore, pre-infestation growth was not a useful predictor of the susceptibility to infestation at the relatively low level of infestation found in this study.

Although the contribution of both diet and stocking density to infestation level could not be clearly identified due to large within-treatment variation, the pooled data from all treatments allowed predictions about the rate of increase in total intensity depending on the level at which it began to rise. It was necessary to use the starting level as an independent variable because intensity levels varied considerably between treatments. The model presented here can be used to predict the outcome of an increase in total intensity under conditions when the sabellid population finds a favourable environment. A more useful predictor for total intensity was prevalence. Total intensity increased exponentially as a function of prevalence, suggesting that the sabellid population reproduced increasingly better as more abalone became infested. This would allow a farmer to estimate a rise in sabellid population growth and the average number of tubes per abalone by determining the percentage of infested abalone rather than counting the number of tubes.

Within the range of sabellid intensities found in this trial, abalone growth was not reduced in infested animals. In those baskets with the highest levels of prevalence, a positive, albeit weak, correlation existed between the intensity of infestation and abalone growth. This differs from previous studies where abalone growth was depressed by *T. heterouncinata* infestation (Ruck and Cook, 1998; Day et al., 2000). It is probable that an intensity of 48 worms (the maximum intensity recorded in the

present growth study) was below the critical growth-reducing level. Day et al. (2000) found that the settlement of *T. heterouncinata* on *Haliotis rufescens* suppressed the deposition of prismatic calcite shell that leads to shell extension. Similar studies have not been conducted on *Haliotis midae* and it is possible that the initial response of the abalone to settlement leads to linear extension that is only later depressed as infestation levels increase. Further studies need to be conducted to test this hypothesis.

Time affected both total intensity and prevalence of infestation, independent of the treatment. In most treatments (including baskets with an overall low level of infestation) the prevalence, total intensity and intensity of infestation remained low until September 2001, about 259 days into the experiment. This increase coincided with the onset of spring, suggesting that season influences reproduction. This has been demonstrated in many other polychaete species (Garwood, 1980; Olive, 1984; Chu and Levin, 1989; Ivin, 1997). In particular, the generation time of *T. heterouncinata* held at a constant 12:12 dark:light cycle was inversely proportional to temperature, while brood size remained constant (Finley et al., 2001). For 11 of the 14 months of the present experiment, the mean daily water temperature ranged from 14.4 °C to 16.6 °C (Fig.4.5). Thus, based on this study and results by Finley et al. (2001), it can be estimated that the experimental period here was equivalent to two to three generation periods. However, intensity did not increase steadily throughout the duration of the experiment. Thus, if reproduction in *T. heterouncinata* was influenced by season, temperature could not have been the only environmental cue influencing population growth. This was previously demonstrated in the spirorbid polychaete *Circeis armoricana* that also breeds throughout the year, but with varying intensity (Ivin, 1997). In that species only 20.8% of the variation was attributed to the seasonal change in temperature, while no reference was made to changing photoperiod. Since the most marked increase in total intensity and prevalence of *T. heterouncinata* infestation occurred during the period of increasing day length, it is possible that photoperiod, or a combination of temperature and photoperiod, has a more profound effect on the reproductive activity of this sabellid than temperature alone. Similarly, brood size of the spionid *Streblospio benedicti* increased with an increase in day length at a constant temperature or when day length and temperature increased concurrently (Chu and Levin, 1989), while in *Harmothoe imbricata*, a species that spawns twice during spring, increasing day length accelerated the growth of the first cohort of oocytes (Garwood, 1980). The possibility of seasonal reproduction in *T. heterouncinata* is corroborated by the anecdotal report that egg numbers are highest, relative to adult numbers, in October and lowest in February (N. Dormehl, Irvin and Johnson, pers. comm.). Further research is thus required to understand the seasonality of reproduction in this polychaete better.

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CHAPTER 5

The effect of age on the reproductive output of *Terebrasabella heterouncinata*

Simon, C.A., Kaiser, H. and Britz, P.J., The effect of age on the reproductive output of the abalone pest, *Terebrasabella heterouncinata* (Polychaeta: Sabellidae: Sabellinae). Submitted to African Journal of Marine Science.

"That old saw about the early bird

just proves that the worm should have stayed in bed."

Robert Heinlein

Abstract

High levels of infestation by the polychaete, *Terebrasabella heterouncinata*, on cultured abalone, may be attributed to elevated levels of fecundity in comparison with conspecifics in their natural habitat. It is not, however, known if a high reproductive output is sustained throughout the animal's life. The present study therefore measured the effect of age on various reproductive parameters of *T. heterouncinata* from cultured abalone. Age did not have a significant affect on body size, fecundity or rate at which eggs were laid. Age did, however, affect the proportion of the population that was brooding offspring which was significantly lower in the youngest age group in comparison to the older groups. *Terebrasabella heterouncinata* are long-lived, surviving for over four years on abalone farms and can therefore spread their reproductive effort over an extended period, instead of restricting it to a limited period during its early life. The ability to maintain a high fecundity irrespective of age may contribute to the success of this animal on abalone farms.

Introduction

Terebrasabella heterouncinata, an obligate commensal of certain gastropods, is a small semi-continuous breeder that broods several clutches of offspring simultaneously within its burrow (Culver et al., 1997; Ruck and Cook, 1998; Fitzhugh and Rouse, 1999; Kuris and Culver, 1999). This sabellid has become a pest on cultured abalone where high levels of infestation lead to a reduction in the growth rate and market value of the abalone. These high infestation levels can be partly attributed to the high fecundity of the worms on farmed abalone, where the worms can have a fecundity that is about 3.5 times that of worms on wild abalone (Simon et al., unpublished data). It is, however, not known to what degree fecundity is sustained throughout the animal's life.

Several studies have demonstrated the age-dependency of reproduction in polychaetes that are semi-continuous breeders (Åkesson, 1976, 1982; Åkesson and Costlow, 1991; Qian and Chia, 1992; Levin et al., 1996; Prevedelli and Zunarelli Vandini, 1999). In most of these studies, fecundity peaked early in the animal's reproductive life, followed by a period during which eggs were produced in reduced numbers. This pattern in age-related fecundity may be due to differential adult and juvenile mortality, the need for the rapid establishment of a population in a new environment or senescence (Stearns, 1976; Qian and Chia, 1992; Vaupel et al., 2004). In a laboratory study, Simon et al. (2002) demonstrated that *T. heterouncinata* brooded offspring intermittently. However, the fecundity and incidence of brooding in that study was low compared to values from worms from farmed abalone (Simon et al., unpublished data), reflecting a possible restriction of nutrients available to worms in the laboratory study. The patterns observed in laboratory studies may therefore not reflect age-related reproductive activity in worms from farmed abalone.

Owing to the mode of burrow formation on the growing edge of the shell (see Culver et al., 1997 and Kuris and Culver, 1999 for detailed descriptions), and the fact that larvae do not settle in abandoned burrows (Simon et al., 2002), it is possible to determine a relative age for a worm, based on its position on the shell. Thus, worms closest to the growing edge are the youngest, while those further from the edge are older. It is therefore possible to measure the fecundity of *T. heterouncinata* of different ages in populations from cultured abalone.

The aim of this study was to determine the relationship between age and reproductive output in *T. heterouncinata* from farmed abalone. The findings will shed light on the life history strategy of *T. heterouncinata* and may help explain why this sabellid has become so successful on cultured abalone.

Materials and methods

Freshly shucked abalone shells infested with *T. heterouncinata* were provided by an abalone farm in Hermanus, on the south Western Cape coast of South Africa. The shells used were between 70 and 90 mm long. The worms from twelve shells were preserved in 4% seawater formalin and transferred to 70% ethanol after one week.

The linear growth rate of badly infested abalone, such as those used in this study, is between 1 and 1.2 mm per month (data available from abalone farmers). However, because the abalone grows on a spiral, the monthly linear growth equates to 2 to 3 mm on the curve of the shell. Six to eight blocks measuring approximately one cm^2 were cut from each shell in the region immediately to the right of the respiratory pores (Fig. 5.1). The first block was cut at the growing edge of the shell, and the last block incorporated the end of the infested part of the shell, furthest from the growing edge.

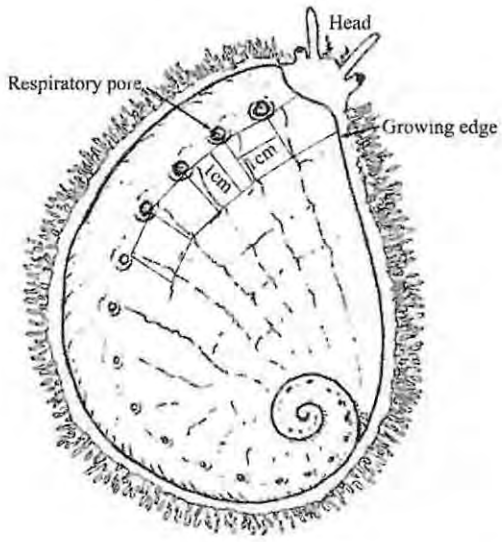

Figure 5.1. A diagram of an abalone, showing the positions of the first five blocks cut from the shell. The youngest worms are found in the block closest to the growing edge, while the oldest are furthest away. Adapted from Branch and Branch (1988).

The blocks of shell were dissolved in 5% nitric acid in 70% ethanol for 12-24 hours. The softened shells were stored in 70% ethanol until the worms were removed. The total number of worms and number of worms brooding eggs or larvae were counted for each block. From each block ten brooding worms were sampled for the following measurements:

1. The length of the adults in mm (excluding the feeding crown).
2. The total number of offspring in the burrow (instantaneous fecundity).
3. The number of clutches per burrow. A clutch signifies all offspring at a similar developmental stage. These stages were 1) eggs without any signs of segmentation, 2) larvae with obvious segmentation but without eye spots and 3) pre-emergent larvae with eye spots. Up to three clutches could be brooded simultaneously.

Data analyses

To avoid pseudoreplication, data from worms found on one block of shell were averaged. Thus, there were up twelve values for each age-group. The effect of age (distance from the growing edge) on the

number of sabellids per mm^2, % sabellids brooding and instantaneous fecundity, were tested by the Kruskal-Wallis ANOVA by ranks. The effect of age on the number of clutches brooded simultaneously was tested with contingency analysis (Zar, 1999).

Results

The lowest proportion of the population observed to be brooding eggs or larvae occurred within the first centimetre ($H=20.3$, $P<0.0049$; Fig. 5.2A). There was a significant increase from the first to second centimetre, after which the proportion of the population brooding (ranging between 50% and 87%) was not affected by the position of the sabellids on the shell. Distance from the growing edge had no effect on the mean length, instantaneous fecundity or the number of clutches brooded simultaneously ($H=5.5$, $P=0.6$; $H=8.3$, $P=0.3$; $\chi^2 = 23.685$, $0.05<P<0.25$, respectively. Fig. 5.2B and C, Table 5.1). Adult length ranged between 2.1 and 4.4 mm (Fig. 5.2B). The smallest brood observed comprised one offspring, and the largest, seven (Fig. 5.2C). With the exception of the oldest group, worms in all age-groups brooded between one and three clutches simultaneously (Table 5.1).

Table 5.1. The number of clutches brooded by worms at increasing distances from the shell growing edge.

	Number of observations							
Number of	Distance from the growing edge (cm)							
clutches	1	2	3	4	5	6	7	8
1	13	7	13	13	8	12	7	0
2	51	45	55	48	40	46	43	21
3	56	68	52	58	72	62	40	19
$\chi^2 = 23.685$, $0.05<P<0.25$								

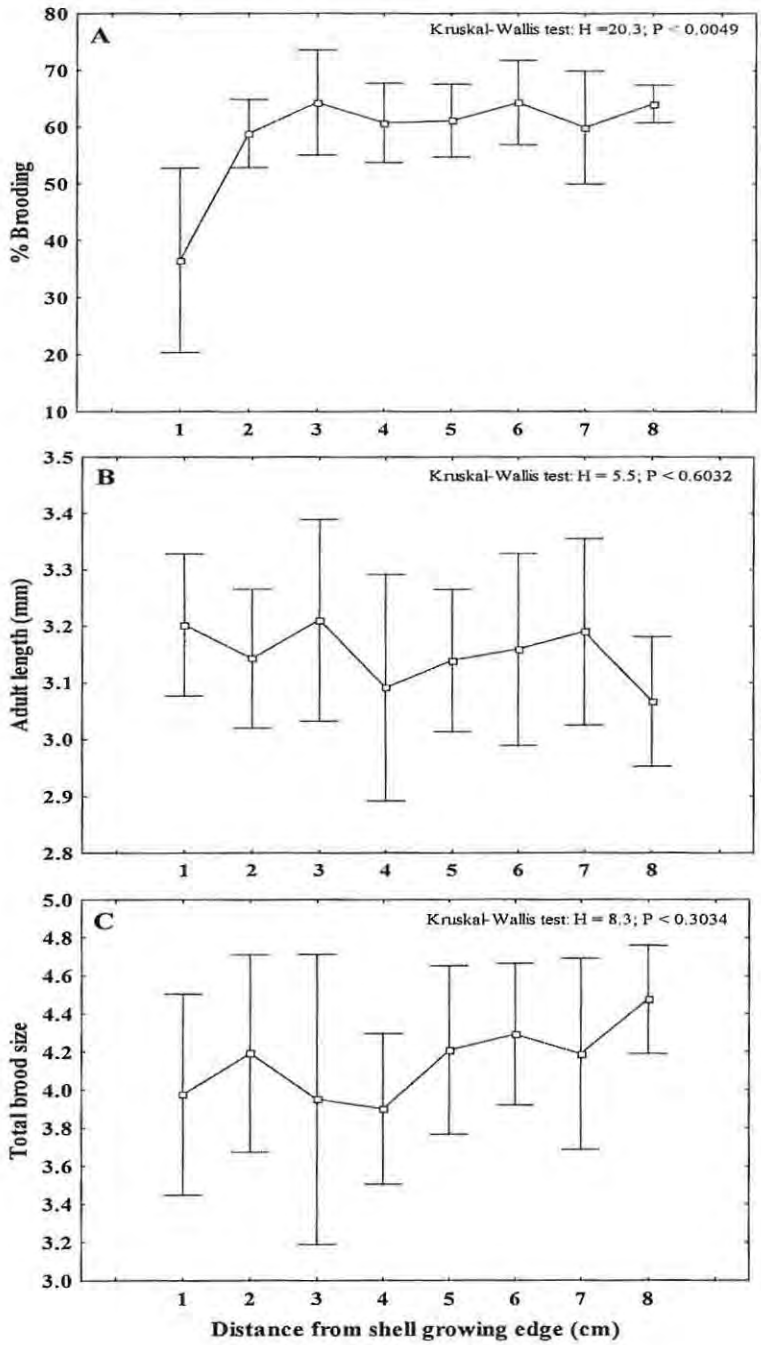

Figure 5.2. The effect of age (i.e., distance from the growing edge) on **A**) the proportion (%) of the population with broods, **B**) adult length (mm) and **C**) the total brood size (instantaneous fecundity) of *Terebrasabella heterouncinata* from the an abalone farm in Hermanus, South Africa.

Discussion

Several studies have investigated the effect of age on the reproductive output of vertebrates and invertebrates, including polychaetes (Pianka and Parker, 1975; Åkesson, 1976; Charlesworth and Leon, 1976; Rodhouse, 1978; Åkesson, 1982; Bayne et al., 1983; Peterson, 1986; Bricelj et al., 1987; Åkesson and Costlow, 1991; Qian and Chia, 1992; Levin et al., 1996; Prevedelli and Zunarelli Vandini, 1998; 1999; Prevedelli and Simonini, 2001; Vaupel et al., 2004). These studies showed that with age, fecundity or reproductive capacity may increase, increase to an optimum and then remain constant throughout the animal's reproductive life, or decrease. Vaupel et al. (2004) named these responses negative senescence, negligible senescence and senescence, respectively.

Terebrasabella heterouncinata is quite long-lived and demonstrates negligible senescence. Based on the growth rate of the infested abalone, each block of shell is equivalent to four to five months' growth. Thus, the maximum age of the worms in the present study is estimated at up to 40 months. Within the age range tested, age (i.e., distance from the growing edge) did not have a significant effect on the fecundity of *T. heterouncinata*. To date, negligible senescence has been demonstrated in only one other small-bodied polychaete, *Ophryotrocha labronica*, which showed no decline in fecundity in up to ten consecutive spawnings (Prevedelli and Zunarelli Vandini, 1998). This is in stark contrast to the patterns demonstrated in many other small iteroparous polychaete species that experience a decline in fecundity with age (Åkesson, 1976, 1982; Åkesson and Costlow, 1991; Qian and Chia, 1992; Levin et al., 1996; Prevedelli and Zunarelli Vandini, 1999; Prevedelli and Simonini, 2001). These polychaetes had life-spans ranging between 2.5 and 19 months, and fecundity usually peaked within the first third of the animals' reproductive lives.

Previous studies on the reproductive biology of polychaetes (e.g., Åkesson, 1976, 1982; Åkesson and Costlow, 1991; Levin et al., 1996; Prevedelli and Zunarelli Vandini, 1998, 1999; Prevedelli and Simonini, 2001) have not investigated the effect of age on the rate of egg production or on the proportion of the population that was breeding. In the present study, the number of clutches brooded simultaneously (i.e., the rate at which eggs were laid) did not appear to be dependent on age. Age affected the proportion of the population that were brooding, with fewer brooding individuals present in the youngest group (i.e., in the block of shell at the growing edge) than in the older age groups. Simon et al. (2002) demonstrated that age at first brooding is between three and four months. The youngest age group would therefore include juveniles and sub-adults that had not yet reached sexual maturity. Thus, once all individuals in an age group reached sexual maturity, most members of that group produced eggs at a consistent rate throughout their lives.

In many animals, including polychaetes, fecundity may be correlated with adult body size (Pianka and Parker, 1975; Charlesworth and Leon, 1976; Rodhouse, 1978; Bayne et al., 1983; Levin, 1986; Peterson, 1986; Bricelj et al., 1987; Moore and Dillon, 1993; Qian, 1994; Simon et al., unpublished data), and this may have consequences for the effect of age on fecundity (Charlesworth and Leon, 1976; Vaupel

et al., 2004). Thus, if an animal has indeterminate growth (such as certain molluscs, fish and reptiles) fecundity may increase indefinitely with age (Pianka and Parker, 1975; Charlesworth and Leon, 1976; Rodhouse, 1978; Bayne et al, 1983; Peterson, 1986; Vaupel et al., 2004). By contrast, Qian and Chia (1992) suggested that the reduced fecundity in older individuals of *Capitella* sp. may in fact be related to a reduction in adult body size with age. However, organisms that maintain a constant body size may experience a reduction in fecundity with an increase in age due to a "decline in vitality" (Vaupel et al., 2004). In the present study, age had no significant effect on the size of the reproductive worms or their fecundity, suggesting that the maintenance of body size in this species does not occur at the expense of fecundity.

Adult mortality in *T. heterouncinata* is likely to be low compared to larval mortality (the only time spent outside the burrow is as a larva, before settlement), and no predators of the adults have been identified (Kuris and Culver, 1999). This, together with the long life span and negligible senescence demonstrated in this study, suggests that *T. heterouncinata* does not need to limit its reproductive effort to a short period early in its life and thereby potentially compromising its long-term survival and future fecundity. Instead, it can maximise its life-time fecundity by producing many broods of a consistent (possibly small) size throughout its life (Stearns, 1976).

This study investigated the effect of age on the fecundity of *T. heterouncinata* on farmed abalone. These results suggest that the ability of *T. heterouncinata* to sustain high levels of fecundity throughout its reproductive life may have contributed to its success on abalone farms. Life history response experiments have shown that within species, the general relationship between age and fecundity usually remains the same, irrespective of the level of nutrient enrichment or stability of the environment (Levin et al., 1996; Prevedelli and Zunarelli Vandini, 1998, 1999; Prevedelli and Simonini, 2001). The variables that differ are the levels of fecundity, the age at maturity and life-span of the animal. Wild abalone can live for more than 13 years (Branch et al., 1994) while farmed abalone are sold when they are between three and four years old (André du Plessis, Aquafarm Development, pers. comm.). It is therefore possible that *T. heterouncinata* infesting wild abalone may be living for much longer than their farm conspecifics. Investigations on wild abalone could provide information that would help us to better understand the conditions affecting the relationship between age and fecundity.

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CHAPTER 6

Ultrastructure of oogenesis in the abalone pest, *Terebrasabella heterouncinata* (Polychaeta: Sabellidae: Sabellinae)

Simon, C.A., Ultrastructure of oogenesis in the abalone pest, *Terebrasabella heterouncinata* (Polychaeta: Sabellidae: Sabellinae). Invertebr. Reprod. Dev. In press.

"We are all worms, but I do believe I am a glow worm."

Sir Winston Churchill

Abstract

Terebrasabella heterouncinata is a small, semi-continuous breeder that has become a pest on cultured abalone. Its success on the farms may, in part, be related to an increase in fecundity and the rate at which eggs are produced in response to increased food availability. In this study, the ultrastructure of vitellogenesis was described to determine how this relates to the life history of this animal. A single pair of ovaries is attached to the anterior septum of segment nine. Early oocytes in the ovary are surrounded by follicle cells that contain few mitochondria and free ribosomes. The follicle cells associated with early vitellogenic oocytes in the ovary contain glycogen granules and dense bodies that resemble the putative yolk bodies present in these oocytes. Oogenesis is asynchronous, and most of the yolk bodies accumulate in the oocytes after their release into the coelom. Endocytotic activity (which presumably represents the rapid uptake of high molecular weight extracellular yolk precursors) is observed when the solitary oocytes are released into the coelom. During vitellogenesis, lipid droplets and glycogen granules also accumulate in the ooplasm. The yolk bodies and lipid droplets fill the ooplasm from the cortex towards the centre of the oocyte, with larger bodies accumulating in the centre. The results suggest that the bulk of the yolk bodies are produced heterosynthetically, but this could not be confirmed. The pattern of oogenesis demonstrated in *T. heterouncinata* is similar to that described for some semelparous or annual iteroparous sabellids.

Introduction

Polychaetes show a great diversity in life history traits, with few phylogenetic constraints on the evolution of the reproductive mode (Wilson, 1991; Rouse and Fitzhugh, 1994; Giangrande, 1997). Concomitant with this diversity is variability in the mechanisms of vitellogenesis (Olive, 1983; Eckelbarger, 1983, 1984, 1992). No relationship has been demonstrated between the mechanism of oogenesis or the type of ovary and egg size, and the mode of larval development (Eckelbarger, 1986). However, Eckelbarger (1994) proposed that different mechanisms of vitellogenesis lead to the differences in the rate of egg production and breeding frequency that are associated with semelparous or annual iteroparous and semi-continuous breeding modes, with the latter group relying more on the heterosynthetic production of yolk.

The Sabellidae includes species that are broadcast spawners of many small eggs, as well as brooders of a few large eggs (Wilson, 1991; Rouse and Fitzhugh, 1994; Giangrande, 1997). The former group of species are usually large animals that may reproduce seasonally, while the latter group tends to be small and reproduce semi-continuously (Rouse and Fitzhugh, 1994; Giangrande, 1997; Giangrande et al., 2000; Licciano et al., 2002). Although reproduction within Sabellidae has been well documented (Rouse and Fitzhugh, 1994; Giangrande, 1997), very few ultrastructural studies have been conducted on oogenesis in this family. Descriptions of the ultrastructure of oogenesis in the Sabellidae have been restricted to species that are seasonal broadcast spawners, some of which undergo vitellogenesis over several months (Dales, 1961; Giangrande et al, 2000; Licciano et al., 2002).

The sabellid *Terebrasabella heterouncinata* is a small (<5 mm) semi-continuous breeder that may brood several clutches of offspring simultaneously (Fitzhugh and Rouse, 1999; Simon et al., 2002). During the last ten years it has become established as a pest on South African abalone farms disrupting production and causing economic losses to the industry (Ruck and Cook, 1998). A recent study demonstrated that worms from farmed abalone were two to four times more fecund than their wild conspecifics, and may brood up to four clutches of embryos of different ages, simultaneously, with a maximum fecundity of 21 eggs and larvae per burrow (Simon et al., unpublished data). Although there is no information available on the precise rate at which eggs are laid, the life history characters of this sabellid suggest that egg production must be relatively rapid. The aim of the present study was therefore to describe the ovary structure and the ultrastructure of oogenesis in *T. heterouncinata* to determine how this may relate to the life history of this animal.

Methods and materials

Shells of *Haliotis midae* infested with *Terebrasabella heterouncinata* were provided by an abalone farm in Hermanus, South Africa, in August 2003. The shells were crushed and gravid worms were removed. The worms were kept in the refrigerator overnight to allow the animals to void their guts, and then anaesthetised by adding clove oil to the water and keeping the animals in the

refrigerator for a further hour. Specimens were fixed according to Eckelbarger et al. (2001). Whole worms were fixed in 2.5% glutaraldehyde in 0.2 M sodium phosphate buffer for 10 minutes at room temperature before removing the reproductive segments. The reproductive segments were fixed for a further 50 minutes. The samples were washed in 0.2 M sodium phosphate buffer and post-fixed in 1% OsO_4 with 1.25% sodium bicarbonate for 60 minutes at room temperature, washed in the same buffer, dehydrated through an ethanol series of ascending concentrations to 100% and embedded in Spurr's resin via propylene oxide (two washes of 10 minutes each). Ultrathin sections (silver/gold interface) and semi-thin sections (1 μm) were cut on an RMC MT-7 ultramicrotome with a glass knife. Ultrathin sections were mounted on uncoated copper grids and stained in lead citrate for 30 seconds, uranyl acetate for 1 minute and again in lead citrate for 30 seconds. The sections were viewed on a JEOL 1210 transmission electron microscope at 100 kv. Semi-thin sections were stained in 1% toluidine blue in 2.5% sodium carbonate. A total of six animals was examined.

Results

Oogenesis is asynchronous and yolk bodies appear mainly after the oocytes have been released into the coelom (Fig. 6.1a) - each ovary may contain up to ten previtellogenic and early vitellogenic oocytes, and the coelom up to seven oocytes at different stages of vitellogenesis.

Ovary

The ninth segment contains a pair of ovaries (one ovary seen in longitudinal section in Fig. 6.1a, b), each situated on either side of the ventral blood vessel. The ovaries extend posteriorly from the septum that separates the eighth and ninth segments into the coelom (Fig. 6.1a, b). The oogonia or youngest oocytes are situated closest to the septum while the previtellogenic and early vitellogenic oocytes that are ready to be released into the coelom are close to the edge of the ovary that protrudes into the coelom (Fig. 6.1c).

Previtellogenesis

Early previtellogenic oocytes are round to ovoid in shape (about 55 μm along the long axis) with spherical nuclei (about 30 μm in diameter; Fig. 6.1c). Late previtellogenic oocytes are more ovoid in shape and have a smooth oolemma (Fig. 6.1d). The cytoplasm contains mitochondria with poorly developed cristae, vesicles (that may be artefacts of fixation) containing an unidentified product, small amounts of rough endoplasmic reticulum (not shown) and free ribosomes (Fig. 6.1d). The RER appears to originate from the nuclear envelope. The oocytes are surrounded by flat follicle cells which contain ovoid nuclei (about 4.5 x 2 μm), mitochondria and free ribosomes (Fig. 6.1d). Follicle cell processes separate neighbouring oocytes and there are no obvious connections between these cells.

Figure 6.1. a and b Longitudinal sections through the female reproductive segment. **a** The ovary (ov) attached to the septum that separates the male (ma) and female segments. The coelom of the female segment contains late (lv) and mid-vitellogenic (mo) oocytes. Also visible are blood vessels (bv) and the ventral shield (vs). **b** The ovary at a greater magnification, with several previtellogenic oocytes (pv). **c** A longitudinal section through the ovary, along the frontal plane. The ovary contains several previtellogenic oocytes, oogonia (arrowheads) and an early vitellogenic oocyte (ev). The nucleus (n) and nucleolus (*) are clearly visible. **d** A previtellogenic oocyte closely associated with two follicle cells (fc). The oocyte contains poorly developed mitochondria (m), endoplasmic reticulum (er), free ribosomes (ri) and has a smooth oolemma (arrow). The follicle cells contain an ovoid nucleus (n), mitochondria and free ribosomes. **e** An early vitellogenic oocyte still in the ovary. The oocyte contains mitochondria and endoplasmic reticulum. Nascent electron dense and less electron dense yolk bodies (y; black and white arrow-heads, respectively) are visible along the oolemma where microvilli have developed. The neighbouring follicle cell contains dense bodies (db) that resemble the darker yolk bodies in the oocyte. **f and g** The early vitellogenic oocyte and neighbouring follicle cells showing the glycogen granules (g) and dense bodies in the follicle cells. The oolemma has become villous (arrows). Arrowheads point to the yolk bodies. **h** multi-vesicular bodies (vb) in the ooplasm of an early vitellogenic oocyte in the ovary. The vesicular body contains tiny vesicles that fuse to form condensing yolk bodies at the centre. **i** A membrane-bound yolk body with smaller yolk bodies joining (arrows).

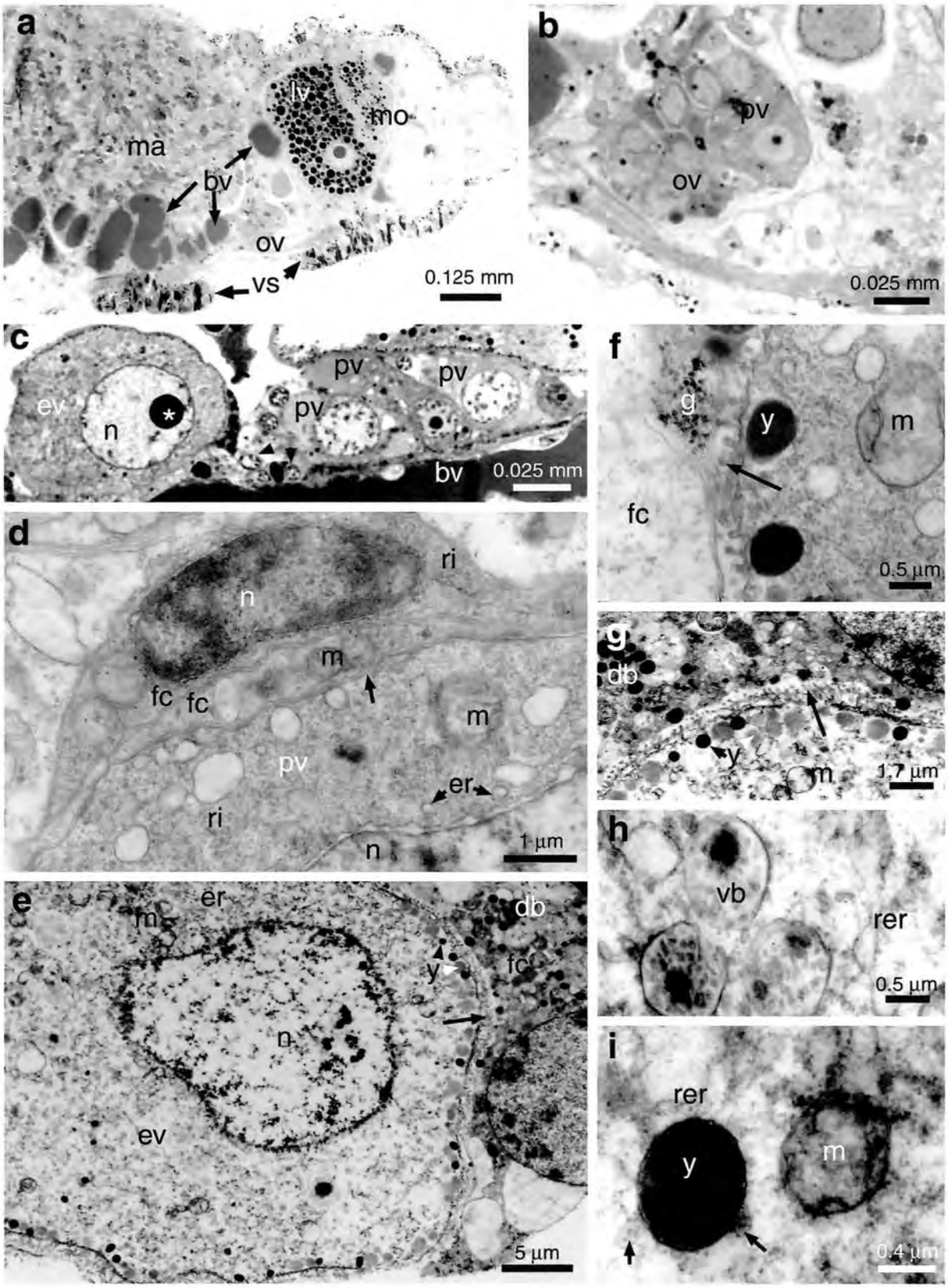
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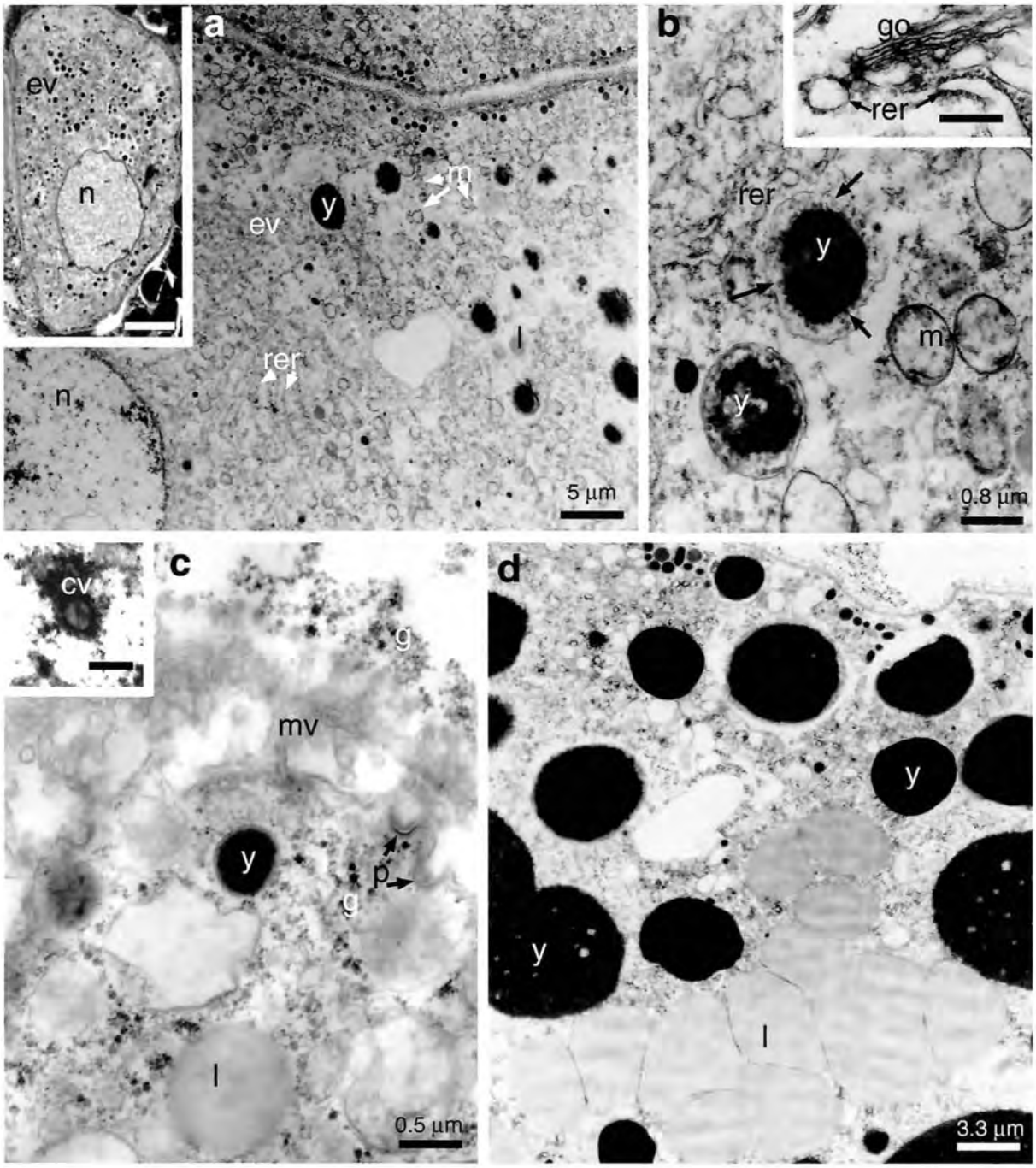
Figure 6.2. a An early vitelleogenic oocyte (ev) that has been released into the coelom. The cytoplasm contains numerous small mitochondria (m, arrows), and some rough endoplasmic reticulum (rer). The yolk bodies (y) towards the centre of the cell are larger than those along the oolemma. A few, small lipid droplets (l) appear. Inset shows a light micrograph of an early vitellogenic oocyte. (scale bar = 0.025 mm) **b** Yolk bodies at different stages of development. Vesicles may be fusing with the developing yolk body (arrows). Closely associated with these yolk bodies are distended cisternae of the rough endoplasmic reticulum. The inset shows a Golgi body (go) closely associated with the cisternae of the rough endoplasmic reticulum (scale bar = 0.5 μm). **c** The oolemma of an early to mid-vitellogenic oocyte, showing the endocytotic pits (p). Present in the ooplasm and the coelomic fluid surrounding the oocyte are glycogen granules (g). The inset shows a coated endocytotic vesicle (cv). (scale bar = 0.2 μm) **d** The ooplasm of a late vitellogenic oocyte, containing large yolk bodies and lipid droplets (l).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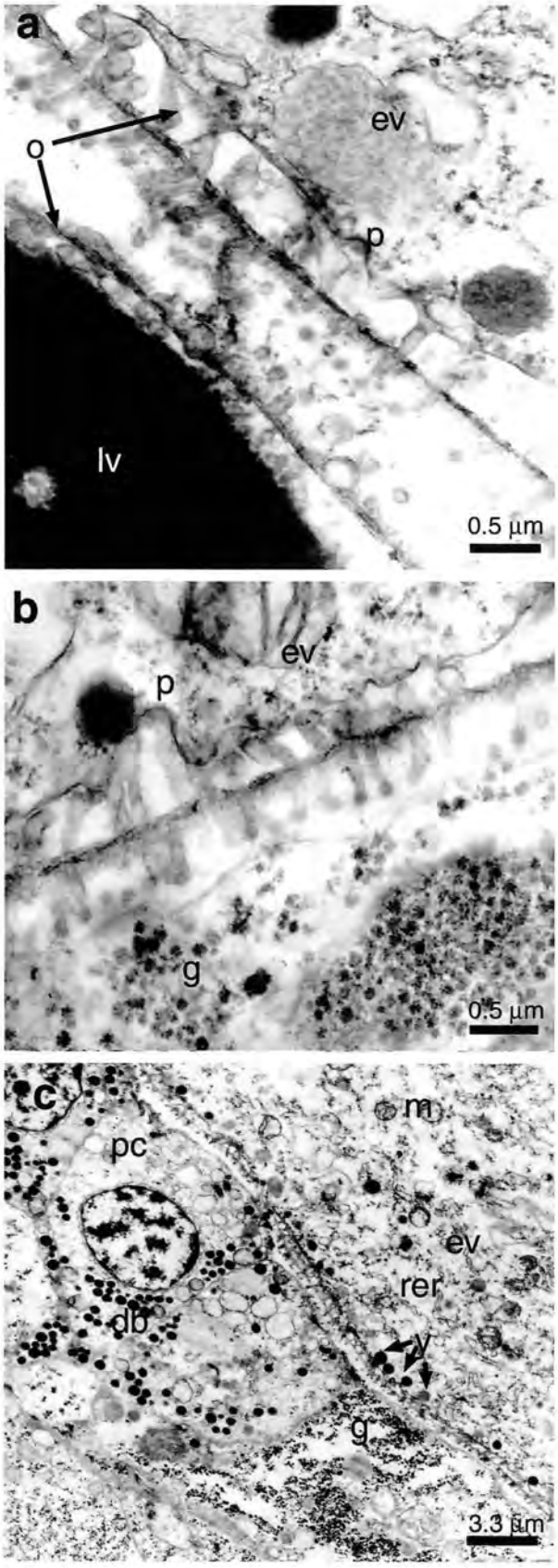
Vitellogenesis

Oogenesis starts in the ovary. Microvilli develop in groups along the oolemma as the oocyte separates from the surrounding follicle cells (Fig. 6.1e - g). At this stage the oocytes measure up to 100 μm, and the nucleus has become less regular in shape (up to 50 μm in diameter) (Fig. 6.1e). Two types of granules, presumably nascent yolk bodies, appear along the villus regions of the oolemma - one type is electron-dense with a smooth texture (0.39 - 0.73 μm diameter), while the second is less dense and more granular (0.65 - 1 μm diameter) (Fig. 6.1e - g, black and white arrow-heads, respectively). At this stage no endocytotic activity along the oolemma was observed (Fig. 6.1f, g). The ooplasm develops RER with distended cisternae containing material with low electron density, multi-vesicular bodies that may be immature yolk bodies and membrane-bound yolk bodies (Fig. 6.1h, i). Follicle cells are closely associated with the early vitellogenic oocytes and contain glycogen granules (Fig. 6.1f) and dense bodies similar in size and appearance to the darker presumptive yolk bodies in the oocyte (Fig. 6.1e, g).

The oocytes are released into the coelom when they are between 130 and 180 μm along their longest axes (Fig. 6.2a, inset). Endocytotic activity is evident in these oocytes, with electron-dense flocculent material present in coated pits (Fig. 6.2c) that are pinched off as coated vesicles (about 0.23 μm, Fig. 6.2c, inset). As vitellogenesis progresses, the number of yolk bodies increases and they fill the ooplasm from the cortex towards the centre of the cell (Fig. 6.2a). The yolk bodies in the cortex of the cell tend to be smaller (dark yolk bodies: 0.39 - 0.93 μm in diameter, light bodies: 0.57 - 1.1 μm in diameter) than those at the centre (dark yolk bodies: 1.2 - 3.9 μm in diameter, light bodies: 1.1 μm in diameter). The yolk bodies increase in size through the fusion of smaller yolk bodies (Fig. 6.2d). The RER is not well developed, and the interconnected cisternae resemble strings of beads (Fig. 6.2b). Golgi bodies are small and few in number and are associated with multi-vesicular bodies and the cisternae of RER (Fig. 6.2b, inset). At this stage, lipid droplets (0.47 - 1.58 μm in diameter) and glycogen granules begin to accumulate in the ooplasm (Fig. 6.2c). In late to mature vitellogenic oocytes, the yolk bodies (4 - 10 μm in diameter) and lipid droplets (4 – 8 μm in diameter) fill the ooplasm, with the latter being more abundant (Fig. 6.2d). The yolk bodies are all electron-dense, although some contain electron-lucent regions (Fig. 6.2d) - the paler bodies seen in early vitellogenic oocytes are no longer present. In late vitellogenic oocytes, endocytotic activity is no longer visible (Fig. 6.3a) and very few organelles are present. A few mature oocytes, that measure up to 300 μm, were observed. The cytoplasm is filled with yolk bodies and lipid droplets (Fig. 6.1a) and no organelles were observed.

The coelom is lined with cells that contain electron-dense bodies (0.34 - 0.61 μm) that resemble the smaller yolk bodies present in the oocytes (Fig. 6.3c). Some of these cells and the coelomic fluid contain glycogen granules (Fig. 6.3b, c).

Figure 6.3. **a** The oolemma (o) of two neighbouring oocytes. The oolemma of the early vitellogenic oocyte (ev) has endocytotic pits (p) that are absent from the late vitellogenic oocyte (lv). **b** The coelom contains high concentrations of glycogen granules (g) which are closely associated with the microvilli of the oocyte. **c** An early vitellogenic oocyte and the peritoneal cells (pc) that line the coelom. The peritoneal cells contain dense bodies that resemble the dark yolk bodies at the oolemma.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Discussion

A pair of ovaries is located in the ninth segment of *T. heterouncinata*. This limitation in ovary number is common in small polychaetes that produce few large eggs per reproductive episode and have a semi-continuous mode of reproduction (Olive, 1983). The ovaries are ventro-laterally attached to the anterior septum of the ninth segment, a position previously described in the small-bodied sabellid *Fabricia sabella* (Jaros, 1971, in Clark and Olive, 1973). The ovarian structure resembles that of other polychaetes that release their oocytes before or during the early phases of vitellogenesis - the youngest oocytes are closest to the septum while the older oocytes are closer to the terminal end of the ovary, and most of the accessory cells are not very prominent (Olive, 1983). The majority of the follicle cells in the ovaries of *T. heterouncinata* contain few organelles, except for a few mitochondria, glycogen granules and ribosomes. This paucity of organelles in the follicle cells suggests that their function at this stage is to provide the oocyte with mechanical support (Eckelbarger, 1992).

The production of yolk bodies by a combination of auto- and heterosynthetic processes has been inferred from fine structural studies in polychaetes and other invertbrates (see reviews by Eckelbarger, 1983, 1984, 1992, 1994), although yolk origin cannot always be determined by such techniques (e.g., Fischer and Dhainaut, 1985). In *T. heterouncinata,* yolk bodies first develop along the oolemma while the oocyte is still in the ovary. This suggests that the yolk precursors are heterosynthetic in origin, although no endocytotic activity is evident at this stage (cf. Fischer and Dhainaut, 1985). The follicle cells closely associated with the early vitellogenic oocytes contain glycogen granules and dense bodies that resemble the putative yolk bodies present along the oolemma. No proteosynthetic activity was detected in these follicle cells, suggesting that they serve as a site of nutrient storage and not production, as was demonstrated in the polychaetes *Phragmatopoma lapidosa* (Eckelbarger, 1979) and *Marenzellaria viridis* (Bochert, 1996). The close contact between the oocyte and follicle cell, as well as the development of microvilli along the oolemma, may facilitate the uptake of low molecular weight nutrients by the oocyte (Bochert, 1996).

Endocytotic activity, and the presumed rapid uptake of high molecular weight yolk precursors, is observed after the oocytes are released into the coelom. As vitellogenesis proceeds, the oocyte fills with yolk bodies from the cortical region towards the centre, where the larger bodies accumulate. Very little proteosynthetic activity is observed throughout vitellogenesis with only few, small Golgi bodies and poorly developed rough endoplasmic reticulum present in the ooplasm. The Golgi bodies are primarily associated with multi-vesicular bodies that are probably immature yolk bodies, and cisternae of the rough endoplasmic reticulum. The multi-vesicular bodies resemble those present in the nurse cells and oocytes of *Ophryotrocha labronica* (Emanuelsson, 1969). Emanuelsson (1969) suggested that these multivesicular bodies developed from transformed mitochondria that eventually formed the yolk bodies. The yolk bodies appear to increase through the fusion of small vesicles (presumably of Golgi origin) with the yolk bodies and the subsequent condensation of their

contents, resulting in a yolk body with an electron-dense core surrounded by the contributory vesicles, as was previously demonstrated in *Ophryotrocha puerilis* (Pfannenstiel and Grünig, 1982) and *O. labronica* (Emanuelsson, 1969). As vitellogenesis proceeds, the smaller yolk bodies fuse with each other, forming progressively larger bodies. Lipid droplets also accumulate in the ooplasm, increasing in size and number as vitellogenesis proceeds. The lipid droplets are, however, less numerous than the yolk bodies. The ultrastructural evidence suggests that the bulk of the yolk in the oocytes of *T. heterouncinata* is produced heterosynthetically. This cannot, however, be confirmed without supplementary physiological analyses, such as those conducted on nereids (e.g. Fischer, 1984; Fischer and Dhainaut, 1985). Thus, it is not possible to make any predictions concerning the rate at which *T. heterouncinata* produces its eggs.

The present study is the first to describe the ultrastructure of oogenesis in a small sabellid that reproduces semi-continuously. Several studies have been conducted on polychaetes with similar life histories, and many showed that vitellogenic oocytes of semi-continuous breeders tend to be associated with follicle or nurse cells or the circulatory system that provide the developing oocytes with yolk precursors (e.g., Emmanualsson, 1969; Eckelbarger, 1979, 1980; Eckelbarger and Grassle, 1982; Pfannenstiel and Grünig, 1982; Rouse, 1992), which presumably increases the rate at which vitellogenesis occurs. By contrast, the vitellogenic oocytes of *T. heterouncinata* occurred in the solitary state within the coelom. This pattern of oogenesis is often found in semelparous or annual iteroparous breeders, including all other sabellids examined to date, although vitellogenic oocytes are associated with coelomocytes in some species (Dales, 1961; Eckelbarger, 1975; Fischer, 1984; Fischer and Dhainaut, 1985; Giangrande et al., 2000; Licciano et al., 2002). It has, however, also been shown in *Spirorbis spirorbis* and three *Micromaldane* spp. (King et al., 1969; Rouse, 1992), which are small semi-continuous breeders. More studies therefore need to be conducted on semi-continuously breeding sabellids (particularly those measuring less than 10 mm long) to gain a clearer understanding of the vitellogenic processes involved, and how they may influence the rate at which the eggs are produced.

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CHAPTER 7

Ultrastructure of spermiogenesis, sperm and the spermatheca of *Terebrasabella heterouncinata*

Simon, C.A. and Rouse, G.W. Ultrastructure of spermiogenesis, sperm and the spermatheca of *Terebrasabella heterouncinata* (Polychaeta: Sabellidae: Sabellinae). Invertebr. Biol. In press.

"I would not enter on my list of friends
(though graced with polish'd manners and fine sense,
Yet wanting sensibility) the man
Who needlessly sets foot upon a worm."

William Cowper

Abstract

The sabellid polychaete, *Terebrasabella heterouncinata,* forms burrows in gastropod shells. It is a small, intratubular brooder that breeds semi-continuously. It has been shown to self-fertilise, but its reproductive biology suggests that some form of sperm transfer must occur between individuals. To gain an understanding of its fertilization biology, the ultrastructure of spermiogenesis and the sperm of *T. heterouncinata* was described, and the animal examined for sperm storage structures. Spermiogenesis occurs in clusters of eight spermatids. The mature sperm has an elongate nucleus and a bilaterally symmetrical acrosome with twisted subacrosomal spaces. The midpiece is short with three crescent-shaped mitochondria and forms a tight sheath around the axoneme. A single spermatheca, which opens on the inner ventral part of the crown near the buccal region, is present. It is a simple blind-ending duct that runs below the ventral nerve cord and is longer than 100 μm. This is the first record of a single spermatheca in Sabellidae. The shape of the sperm and the presence of a spermatheca confirm that *T. heterouncinata* produces ent-aquasperm and would normally cross-fertilise.

Introduction

The polychaete taxon Sabellida is currently thought to include Oweniidae, Siboglinidae, Sabellidae, Serpulidae and Sabellariidae (Rouse and Pleijel, 2001). These taxa exhibit a wide range of reproductive and fertilization patterns. All Sabellariidae and Oweniidae studied to date are broadcast spawners, whereas in Siboglinidae, the sperm or clusters of sperm are spawned into the water column and taken up by the females, the ova fertilised externally, and the larvae then brooded in the tubes or released into the plankton (Rouse and Pleijel, 2001). In both Sabellidae and Serpulidae there may be brooders or broadcast spawners with the former having lecithotrophic larvae only, whilst the latter shows both planktotrophy and lecithotrophy (Schroeder and Hermans, 1975; Wilson, 1991; Rouse and Fitzhugh, 1994; Giangrande, 1997; Rouse, 1999; Rouse and Pleijel, 2001).

Sabellidae comprises two main clades, Fabriciinae and Sabellinae (Rouse and Fitzhugh, 1994). All Fabriciinae studied to date are gonochoric and the females brood batches of directly developing larvae (Rouse and Fitzhugh, 1994; Rouse, 1995a,b). Sabellinae may be either gonochoric or hermaphroditic. They may be broadcast spawners or brooders that care for their directly developing larvae either within the tube, attached to the radiolar crown, or in a jelly mass or ring at the mouth of the tube (McEuen et al., 1983; Wilson, 1991; Rouse and Fitzhugh, 1994; Hsieh, 1997; Rouse, 1999). Some Sabellinae also reproduce asexually via scissiparity (Knight-Jones and Bowden, 1984; Gambi et al., 2000). Intratubular brooders are usually smaller than extratubular brooders and broadcast spawners in Sabellidae (Rouse and Fitzhugh, 1994). Also associated with small body size and brooding is repeated or semi-continuous breeding (Olive, 1985). Under these circumstances, sexual reproduction requires the storage of sperm by the female or hermaphrodite (Olive, 1985), and sperm storage has been demonstrated in several small Sabellidae and Serpulidae (Jones, 1974; Daly and Golding, 1977; Picard, 1980; Rouse, 1992b; 1995b; 1996; Rouse and Gambi, 1998).

Concomitant to this variability in reproductive patterns is variability in sperm morphology and ultrastructure (Franzén, 1956; Jamieson and Rouse, 1989; Rouse and Fitzhugh, 1994; Giangrande, 1997; Rouse, 1999). Although fertilization biology tends to correlate with sperm morphology (Franzén, 1956; Franzén and Rice, 1988), analysis of sperm type and reproductive mode in the Sabellidae and Serpulidae showed that no statistical relationship existed between these two factors when evolutionary events were taken into account (Rouse, 1999). Thus, the structure of the sperm alone cannot be used to predict the fertilization biology of an animal, as several brooding species produce sperm with spherical heads (Rouse, 1999). It is, however, interesting to note that many of the brooders with sperm with spherical heads are in fact extratubular brooders (McEuen et al., 1983; Rouse and Fitzhugh, 1994; Gambi et al., 2001).

Terebrasabella heterouncinata is endemic to South Africa, infesting several sub- and intertidal gastropods (Ruck and Cook, 1998) and became a pest on cultured abalone in South Africa and California in the early 1990's (Culver et al., 1997; Ruck and Cook, 1998; Fitzhugh and Rouse, 1999; Finley et al., 2001). The sabellid lives in burrows that form when the larvae settle on the

growing edge of the host shell and the host covers it with nacreous shell (Culver et al., 1997). It is a small (<5 mm long) simultaneous hermaphrodite, semi-continuous breeder and intratubular brooder (Fitzhugh and Rouse, 1999).

Finley et al. (2001) demonstrated that *T. heterouncinata* is able to self-fertilise. While self-fertilization has been demonstrated in several other polychaete species (Smith, 1958; Parenti, 1960; Gee and Williams, 1965; Hsieh, 1997), none are known to self-fertilise routinely (Knowlton and Jackson, 1993). Furthermore, comparison of the reproductive biology of *T. heterouncinata* with other Sabellidae (see Rouse, 1996), would suggest that some form of sperm transfer must occur, and that the allosperm (i.e. the sperm received from another individual) may be stored in spermathecae or epidermal cells although none were detected by Fitzhugh and Rouse (1999).

The aims of this study were therefore to gain an understanding of the fertilization biology of *T. heterouncinata* by examining the ultrastructure of spermiogenesis and sperm and determine whether sperm storage structures were present.

Materials and methods

Abalone shells infested with *Terebrasabella heterouncinata* were provided by the abalone farm, 'Aquafarm Development' in Hermanus, South Africa in November 2001. The shells were crushed, and six sexually mature worms were removed. The presence of sperm and spermatids was indicated by an enlarged, white eighth segment. Nomarski interference micrographs of sperm and spermatids were taken using a Leica DMR microscope. Specimens were left in the refrigerator overnight to evacuate their guts before anaesthetising in 7% MgCl in freshwater. The whole worms were fixed at 4°C in 2.5% glutaraldehyde in filtered seawater for approximately 12 hours, before removing the reproductive segments for further processing. The segments were then washed in 0.2 M sodium cacodylate buffer with 0.3 M NaCl (pH 7.4). The samples were post-fixed in 1% OsO_4 in 0.2 M sodium cacodylate buffer (pH 7) for 90 minutes at room temperature, washed in the same buffer, dehydrated through an ethanol series of ascending concentrations to 100% and embedded in Taab/Araldite resin via propylene oxide (Cross 1989). Other samples were fixed at 4°C in 3% glutaraldehyde in 0.2 M sodium cacodylate and 0.3 M sucrose overnight, washed in the same buffer, processed as before and embedded in Spurr's resin. Ultrathin sections (gold interface) and semi-thin sections (0.85 µm) were cut on an RMC MT-7 ultramicrotome with a glass knife. Ultrathin sections were mounted on uncoated copper grids and stained either in 1) lead citrate (30 seconds), uranyl acetate (1 minute), lead citrate (30 seconds) or 2) uranyl acetate (30 minutes) and lead citrate (5 minutes), and viewed on a JEOL 1210 transmission electron microscope at 100 kv. Semi-thin sections were stained in 1% toluidine blue in 2.5% sodium carbonate. For SEM sperm were fixed on a coverslip in 3% glutaraldehyde in 0.2 M sodium cacodylate and 0.3 M sucrose for 1 hour, washed in two changes of the same buffer (24 h each), dehydrated in increasing concentrations of acetone to 100%, critical point dried with CO_2 and sputter coated with gold. The samples were viewed on a

JEOL JSM 840 scanning electron microscope at 12 kv.

Results

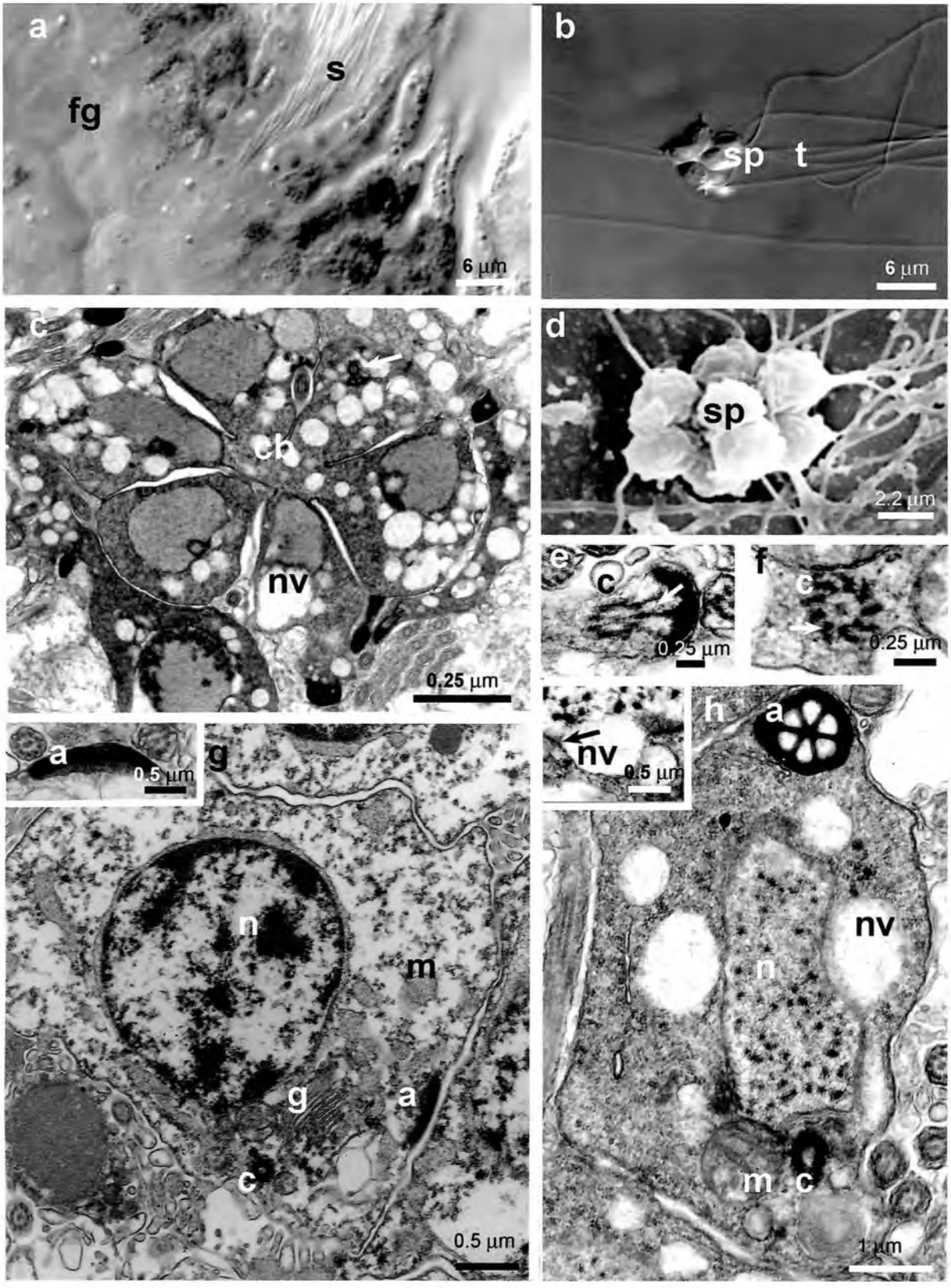
Spermiogenesis

Spermiogenesis in *Terebrasabella heterouncinata* is similar to published accounts of the process in other sabellids so only a brief description will be given here. Spermatids at varying stages of development were present within the coelom of the male reproductive segment. Spermiogenesis occurs in clusters of eight spermatids (Fig. 7.1b - d). Complete clusters of spermatids are shown via light microscopy (Fig. 7.1b) and SEM (Fig. 7.1d) but this was not possible with TEM. Figure 7.1c does show six connected spermatids with a small cytophore, or cytoplasmic bridge, in the middle. Late spermatids are clearly joined to the cytoplasmic bridge halfway along their length (Fig. 7.2a). Mature sperm were found only within the sperm ducts that run round the outside of the male segment and open into the fecal groove (Fig. 7.1a).

In early spermatids the nucleus is rounded with patches of condensed chromatin and small mitochondria are scattered in the cytoplasm, though most are clustered at one end of the nucleus (Fig. 7.1g). At this stage the acrosome is visible near the Golgi apparatus that lies near the centriole and mitochondria at the presumed posterior end of the nucleus (Fig. 7.1g). The acrosomal vesicle is initially flat, has a thin electron dense layer in the centre, and is closely apposed to the cell membrane (Fig. 7.1g, inset). A single centriole is present in early spermatids before extensive chromatin condensation has occurred (Fig. 7.1c, cf. Fig. 7.1e). The centriole has prominent satellite rays (Fig. 7.1e) where it is connected to the annulus. Otherwise it has prominent radial spokes (Fig. 7.1f).

While the acrosome migrates towards the anterior of the cell, it becomes more conical in shape and subacrosomal lacunae develop near its base (Figs 1h; 2e). Before nuclear elongation (Fig. 7.2e, h, l, m) begins, the nucleoplasm appears uniformly granular and numerous vesicles appear in the cytoplasm (Fig. 7.1c). Some of the vesicles are out-pocketings of the nuclear envelope (Fig. 7.1c, h, inset) while the remaining vesicles are completely separate from the nucleus. These vesicles are probably continuous with the vesicles still connected to the nuclear envelope. They are not arranged in any specific pattern and may represent fixation artefacts. As spermiogenesis proceeds the nucleus elongates and granules of condensed chromatin appear scattered in the nucleus (Fig. 7.1h). The mitochondria fuse to form three large spherical mitochondria that closely abut the nucleus at the putative posterior pole of the cell (Figs 1h; 2b, e), retaining their position and shape as the nucleus elongates. As the nucleus elongates and condenses, the acrosome migrates to its final position, while the Golgi apparatus remains visible at the posterior of the cell, close to the mitochondria (Fig. 7.2a).

Figure 7.1 a Interference contrast light micrograph of the sperm duct (with mature sperm (s)) opening into the ciliated fecal groove (fg). **b** Interference contrast light micrograph of a cluster of eight spermatids (sp) with their eight tails clearly visible. **c** A cluster of six early spermatids, with many nuclear vesicles (nv), around a central cytophore (cb). The arrow denotes the centriole attached to the annulus. **d** Scanning electron micrograph of a cluster of spermatids (sp). **e** Transverse section through the midpiece of a late spermatid showing the centriole (c) connected to the annulus by the satellite rays (arrow). **f** Transverse section through the centriole (c) with radial spokes (arrow). **g** Early spermatid with rounded nucleus (n) and patches of chromatin, early acrosome (a), Golgi body (g), centriole (c) and small mitochondria (m). Inset: early acrosome (a) showing electron dense center. **h** Mid-spermatid, with elongating nucleus (n), condensing chromatin and nuclear vesicles (nv). The acrosome (a) and mitochondria (m) are in their final positions. The inset shows a nuclear vesicle (nv) continuous with the nuclear membrane (arrow).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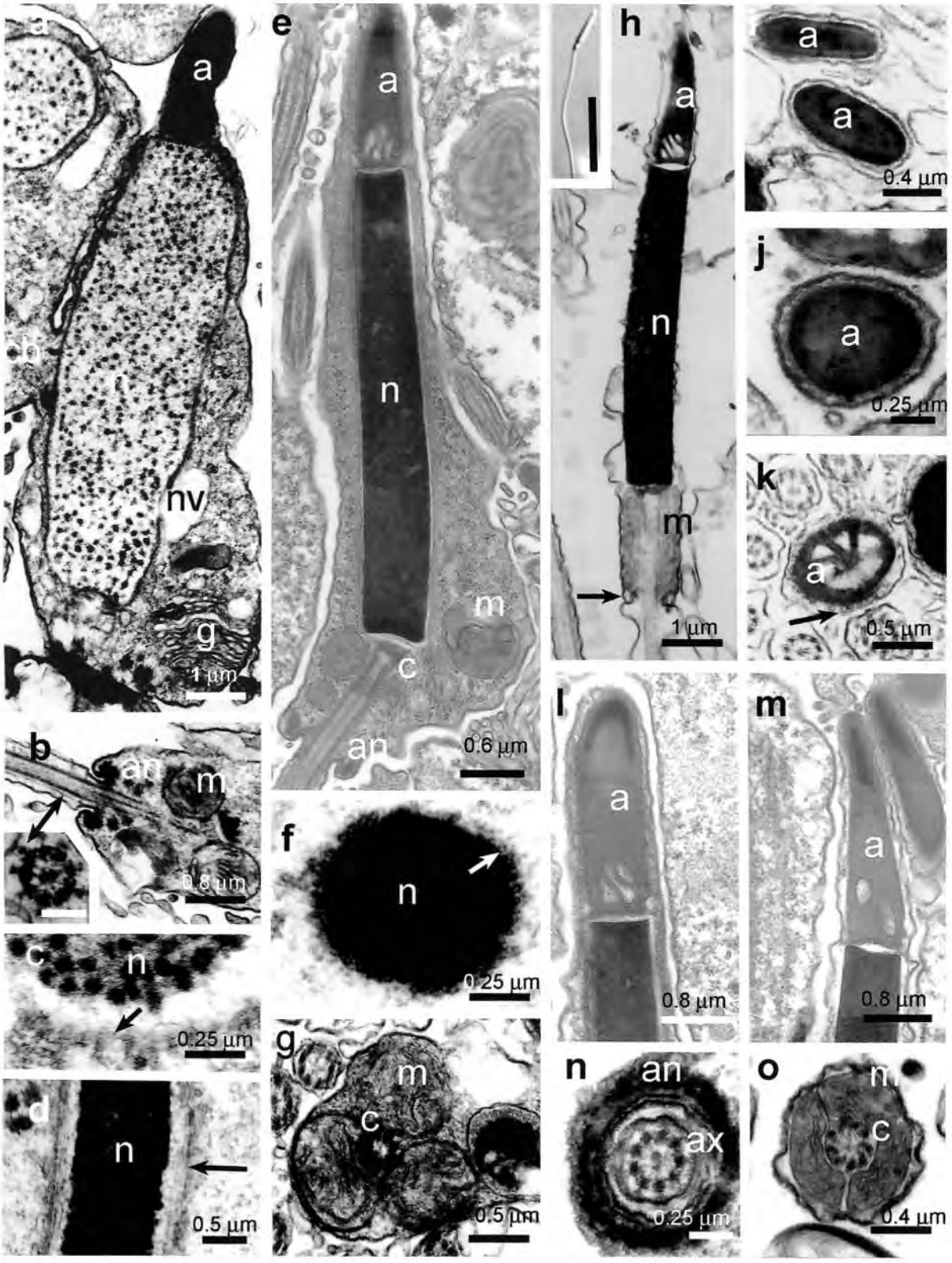
In mid- to late spermatids the nucleoplasm loses its grainy appearance, while the number of chromatin granules increase (Fig. 7.2a). As elongation proceeds, microtubules appear around the nucleus and acrosome (Fig. 7.2c, d, f, k), but not the mitochondria (Fig. 7.2g). In longitudinal section the centriolar complex consists of the single centriole and a semi-circular cap at the base of the nucleus (Fig. 7.2e). Oblique sections through the centriolar complex of the developing spermatids initially show the attachment of the satellite rays, possibly to the annulus (Fig. 7.1e). However, no sign of satellite rays or attachment of the centriole to either the mitochondria or the plasma membrane is visible in later spermatids. The annulus also appears to lose its connection with the centriole as spermiogenesis progresses (Fig. 7.2b, e, n).

The annulus forms a collar around the axoneme below the mitochondria once they have migrated to the posterior of the cell and the annulus remains in this position in the mature sperm (Fig. 7.2b, e). In late spermatids the nucleus becomes more densely granular and the cytoplasm around the nucleus withdraws, beginning at the anterior end of the cell (Fig. 7.2e). Also at this stage, the mitochondria surround the centriolar complex (Fig. 7.2g) and presumably begin to elongate. The flagellum emerges from the centriolar complex and has a 9 + 2 arrangement of microtubules, but just below the midpiece there is a ring of nine coarse fibers surrounding the nine doublets (Fig. 7.2b (LS), inset (TS)).

Spermatozoon

The head and midpiece of the mature spermatozoon averages 4.95 μm long (n = 5). The head, consisting of the acrosome (1.2 ± 0.1 μm) and nucleus (2.8 ± 0.17 μm), comprises the bulk of this length (n = 5). The midpiece (0.95 μm in length, ± 0.08 μm), is comprised of the three mitochondria, the centriolar apparatus and anterior axoneme and annulus (n = 5). The length of the flagellum was not established but is at least 83 μm long. The fully developed acrosome is conical when sectioned in the sagittal plane, spatula shaped in the frontal plane and the subacrosomal lacunae are distinctly twisted (Fig. 7.2e, h, l, m). In cross section, the acrosomal tip is oval with an electron-dense core, but becomes more rounded and uniformly electron-dense towards the base (Fig. 7.2i, j, k, respectively). The base of the acrosome is slightly concave (Fig. 7.2h, l, m). The nucleus of the mature sperm is cylindrical, and is very slightly invaginated at the anterior and posterior ends (Fig. 7.2h). The mitochondria are rod-shaped in longitudinal section and crescent shaped in transverse section and closely abutting to each other (Fig. 7.2h, o). In transverse section they clearly form a tight cylinder surrounding the centriolar apparatus and axoneme (Fig. 7.2o). At the posterior base of the mitochondria the annulus appears as an electron dense ring and marks the end of the midpiece of the sperm (Fig. 7.2h, n). The 9+2 axoneme initially has some connections with the plasma membrane (Fig. 7.1e) but other transverse sections suggest that more distally it is not connected (Fig. 7.2n).

Figure 7.2 a Mid-spermatid with elongate nucleus (n), nuclear vesicle (nv) and Golgi body (g) at the posterior of the cell. The spermatid is connected to the other cells in the cluster by the central cytophore (cb) half-way along its length. **b** Mid-spermatid stage showing midpiece with mitochondria (m) and axoneme (ax) with coarse fibers around the axonemal microtubules (arrows) and the annulus (an). Inset shows coarse fibers from outer axonemal doublets (arrow) in transverse section (Scale bar = 0.25 μm). **c** and **d** The nucleus (n) of mid- to late spermatids in TS and LS, respectively, showing microtubules (arrows). **e** Late spermatid. The chromatin has condensed completely but there is still residual cytoplasm present. Visible are the acrosome (a), nucleus (n), mitochondria (m), centriole (c) and annulus (an). **f** Cross section of the nucleus (n) of a late spermatid showing microtubules (arrow). **g** Cross section through the mitochondria (m) and centriole (c) of a late spermatid. **h** Mature sperm with acrosome (a), nucleus (n), transformed mitochondria (m) and annulus (arrow). Inset shows an interference contrast light micrograph of a mature sperm from the sperm duct (Scale bar = 6 μm). **i, j** and **k** transverse sections through the acrosome (a), from the tip to the base, respectively. Note the microtubules around the base of the acrosome (arrow) in **k.** Longitudinal sections through the fully developed acrosomes (a) along the sagittal **l** and frontal **m** planes. **n** Transverse section through the annulus (an) and axoneme (ax). **o** Transverse section through the transformed mitochondria (m) of a mature sperm and centriole (c).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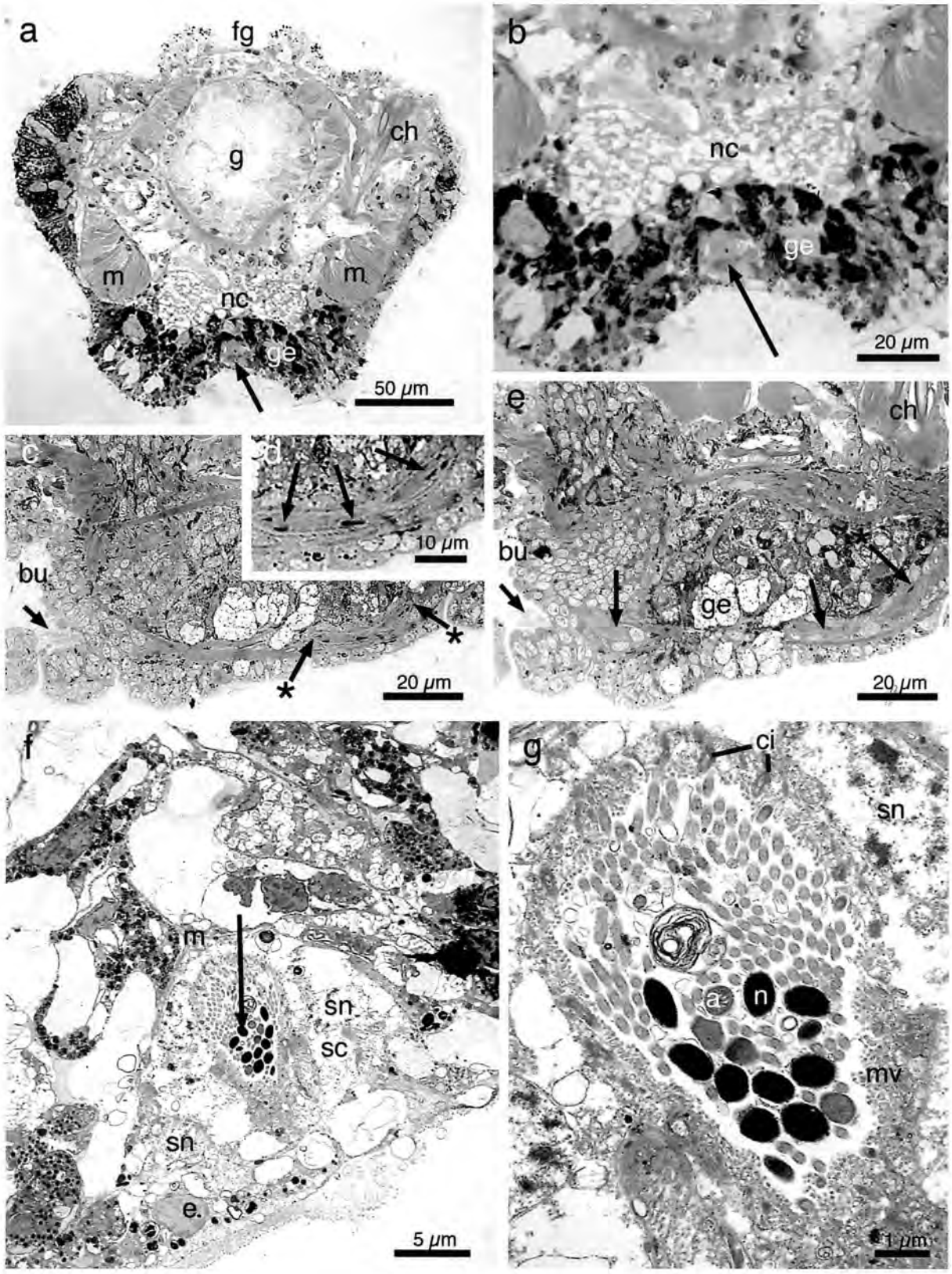
Sperm storage

A single spermatheca is present in each adult *T. heterouncinata* (Fig. 7.3a - g), though it is not visible with a light microscope in living specimens owing to small size, lack of pigmentation and being surrounded by a glandular epidermis. The spermatheca is a simple blind-ending duct with the entrance opening on the inner ventral part of the crown near the buccal region (Fig. 7.3c, e). The duct lies in the epidermal tissue and runs ventrally, below the ventral nerve cord (Fig. 7.3a, b), as far back as the first chaetiger, a distance of well over 100 μm (Fig. 7.3e). There is little obvious morphological differentiation of the spermatheca along its length and the maximum diameter of the lumen of the spermatheca is around 5 μm. The single layer of cells forming the spermathecal lining bear cilia and microvilli (Fig. 7.3f, g), and the duct appears to be ciliated along its entire length. The fixation of these spermathecal cells may not have been optimal since they are largely electron-lucent and only appear to contain nuclei and vacuoles (Fig. 7.3f, g). The sperm are mainly located in the distal part of the duct (Fig. 7.3c - e). No actual incorporation of spermatozoa into spermathecal cells is visible and the sperm all lie freely in the duct lumen (Fig. 7.3f, g). Although the spermatheca duct was well lined with microvilli no direct contact between these and sperm was visible either (Fig. 7.3g). A spermatheca has up to 15 spermatozoa lying in the duct in any one section along the distal third of the duct (Fig. 7.3c, d, f, g). The total number of sperm stored in a spermatheca at any one time was not accurately estimated, but would appear to be less than 100. The sperm do not appear to show any transformation from those in the sperm ducts and are not enclosed in any surrounding material. A thin layer of muscle cells appears to surround the spermatheca and there is an epidermal layer of cells between the spermathecal cells and the outside (Fig. 7.3f), suggesting that the spermatheca is an invagination of epidermal tissue.

Figure 7.3 Spermatheca of *Terebrasabella heterouncinata* **a** Transverse section (1 μm thick) through segment 1 of an adult specimen showing the fecal groove (fg) dorsally, the central gut (g) and ventral nerve cord (nc). The notochaetae (ch) are visible as are longitudinal muscle bands (m). The spermatheca is a small duct in the ventral epidermis (arrow) surrounded by a glandular epithelium (ge). **b** Detail of the same section showing the spermatheca (arrow) lying in the epidermis (ge) and below the ventral nerve cord (nc). A single sperm nucleus is visible in the lumen of the spermatheca. **c** Near to median sagittal section (1 μm thick) through the anterior of an adult specimen showing the region of the buccal opening. The gut is not visible owing to the specimen being sectioned slightly off axis. The spermatheca opening is at the point of the unmarked arrow and other parts of the spermathecal duct are marked with arrows and *. In this distal region sperm are clearly visible. **d** Detailed view of the distal region of the spermatheca showing the cilia lining the duct and three sperm nuclei (arrows). **e** Another section slightly further in than that in Figure 3c with arrows marking the position of the spermatheca from near the buccal region (bu) to the end point in the chaetiger where the notochaetae (ch) are clearly visible. The glandular epithelium (ge) masks the spermatheca part of the way. **f** Transmission electron micrograph of spermatheca in transverse section. Arrows point to sperm lying free in the spermathecal lumen. The spermathecal cells (sc) have large nuclei (sn) but are otherwise vacuolated and electron-lucent (possibly artefactual). A thin layer of muscle cells (m) surrounds the spermatheca and there are epidermal cells (e) between the spermathecal cells and the exterior. **g** Transmission electron micrograph of spermatheca in transverse section showing the lumen in detail. The sperm are clearly not in contact, in this region at least, with the cells or their microvilli (mv). The cells of the spermatheca are heavily ciliated (ci) and the sperm acrosomes (a) and nuclei (n) appear to be unaltered from their appearance in the male sperm ducts.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Discussion

Spermiogenesis and sperm structure

Reviews of polychaete sperm morphology and spermiogenesis (Franzén, 1956; Franzén and Rice, 1988; Jamieson and Rouse, 1989; Rice, 1992) have highlighted that there is extreme diversity in these annelids. Sabellidae is perhaps the best studied group of polychaetes, with the sperm structure of more than 50 species having been described to date (Rouse and Fitzhugh, 1994; Rouse, 1995a; Rouse and Gambi, 1998; Rouse, 1999; Gambi et al., 2000; Giangrande et al., 2000). While all Fabriciinae studied to date brood their larvae and have elongate-headed sperm, Sabellinae exhibits diversity both in reproductive mode and sperm morphology (Rouse and Fitzhugh, 1994; Rouse, 1995a; 1999).

Spermiogenesis in most polychaetes occurs in syncytial masses or clusters, joined by a central cytophore (Schroeder and Hermans, 1975), with the number of cells per cluster ranging from four to hundreds. While all Fabriciinae show the pattern of spermiogenesis with hundreds of spermatids connected to a cytophore, the situation in Sabellinae is more variable (Rouse and Fitzhugh, 1994; Rouse, 1999). Across most Sabellinae, including all plesiomorphic forms, spermiogenesis occurs in tetrads of synchronously developing spermatids. However, in some of the more apomorphic sabellins such as *Potamilla*, spermatids form clusters of hundreds, joined by a central cytophore, as in Fabriciinae (Rouse and Fitzhugh, 1994). Also in the derived *Amphiglena* and *Laonome* clade, Rouse and Fitzhugh (1994) described them as having spermiogenesis with sperm developing simultaneously in clusters of less than 100 cells. Fitzhugh and Rouse (1999) reported that in *Terebrasabella heterouncinata*, an apomorphic sabellin, spermiogenesis occurred in clusters of sixteen. Their analysis placed *Terebasabella* as part of the *Laonome/Amphiglena* clade. However, the present study showed that spermiogenesis occurs in clusters of eight. Further study on the cladistic placement on the position of *Terebrasabella* and the evolution of spermiogenesis in Sabellinae is needed to determine if the clusters of eight spermatids are a reduction from a condition of having more spermatids, or a doubling of the tetrad condition. For example, Rouse and Fitzhugh (1999) had a very broad character state 'spermiogenesis in clusters of 100 or less (but more than four)' and this may need some further refinement.

The structure of the acrosome of polychaetes is highly variable but the acrosomes of most sperm are radially symmetrical (Jamieson and Rouse, 1989). The acrosome of *T. heterouncinata* is unusual in having a bilaterally symmetrical component. This is only found in the sperm of two other sabellids, those of *Amphicorina brevicollaris* and *A. dentata* (Rouse, 1992a), though in these two the entire acrosome was flattened in one plane rather than just the anterior half as in *T. heterouncinata*. In fact, in *A. dentata* the whole sperm head was flattened in one plane. The acrosomes of *T. heterouncinata*, *A. brevicollaris* and also *A. mobilis* are similar in having an electron dense region in the anterior part of the acrosome vesicle. This may represent a store of membrane precursor as postulated by Dan (1970). *Amphicorina mobilis* also has spiral subacrosomal spaces as in *T.*

heterouncinata, while *A. brevicollaris* has distinct, separate subacrosomal spaces (Rouse, 1992a). The functional significance of the flattenened acrosome and the subacrosomal spirals is unclear. *Amphicorina* and *Terebrasabella* are not particularly close relatives according to the analysis of Fitzhugh and Rouse (1999). However, all are brooders of larvae and store sperm prior to fertilization, so the similarities in acrosome may represent convergences related to reproductive mechanisms. It is interesting though that *Amphiglena*, which is the closest relative to *Terebrasabella* and also a brooder, shows little similarity in sperm structure (see Rouse and Gambi, 1998).

In bivalves the development of elongate nuclei is thought to be related to the fertilization of large yolky eggs that are associated with lecithotrophy (Jamieson and Rouse, 1989). This relationship is not evident in polychaetes (Jamieson and Rouse, 1989), and in sabellids such as certain *Amphicorina* spp (Rouse, 1992a) and *T. heterouncinata* (Fitzhugh and Rouse, 1999), the elongation of the nuclei and mitochondria is probably more closely related to sperm movement and efficiency of sperm storage and packaging than the actual process of fertilization (Jamieson and Rouse, 1989; Rice, 1992; Rouse, 1992a).

A manchette of microtubules is present during spermiogenesis in several polychaete species that have elongate sperm (Postwald, 1967; Rice, 1992; Rouse, 1995b). These include members of Sabellida, such as the serpulids *Chitinopoma serrula*, *Simplaria potswaldi* and several Fabriciinae (Potswald, 1967; Franzén, 1975; Rouse, 1995a). In *T. heterouncinata* the microtubules do not extend into the midpiece, as they do in *S. potswaldi* and fabriciins (Potswald, 1967; Franzén, 1975; Rouse, 1995a), though this is not that surprising given the midpiece of the former is so short. The present study is the first record of microtubules associated with the nucleus of any sabellin.

During much of spermiogenesis in *T. heterouncinata* the sperm mitochondria are spherical, an arrangement that is similar to that found in many ect-aquasperm such as *Sabella spallanzanii* (Giangrande et al., 2000). In very late spermatids, the mitochondria elongate slightly down the axoneme, forming a tight sheath. This developmental pattern is also seen in the serpulids *C. serrula* (Franzén, 1982) and *S. postwaldi* (Potswald, 1967) and some sabellids. These include several species of *Amphiglena* (Rouse, 1993; Rouse and Gambi, 1998) and *Amphicorina* (Rouse, 1992a; Rouse, 1999). In *Amphiglena terebro*, *Amphicorina dentata, C. serrula* and *T .heterouncinata* the mitochondria remain straight, while the mitochondria of the remaining species eventually spiral around the axoneme of the mature spermatozoa.

The sperm flagellum is typically anchored by a pair of centrioles, the distal and proximal, situated at the base of the nucleus (Rice, 1992). In contrast, the flagellum of *T. heterouncinata* is anchored by a centriolar complex that is comprised largely of the distal centriole only. Among sabellids, single centrioles are also seen in *Fabricia stellaris* (as *F. sabella*, Franzén, 1975) and three species of *Amphicorina*, although the centrioles in these species are embedded in a centriolar fossa (Rouse, 1992a). According to Franzén (1975), the centrioles of elongate-headed sperm may disappear or become modified and the development of a centriolar complex can be correlated with the

development of a mitochondrial sheath, such as those present in the aforementioned species.

Sperm transfer and sperm storage structures

In *T. heterouncinata,* two lateral sperm ducts open into the dorsal ciliated faecal groove (Fitzhugh and Rouse, 1999) that is probably analogous to the dorsal sperm duct of male Fabriciinae that runs along the length of the body and opens behind the radiolar crown (Kahmann, 1984; Rouse, 1995a). The sperm move along the faecal groove towards the anterior of the worm (personal observation), where they are released into the water column. The sperm of *T. heterouncinata* are probably picked up by other individuals in their feeding currents or by some other means (Kahmann, 1984; Jamieson and Rouse, 1989; Rouse, 1992a; 1999) and directed to the spermatheca for storage. Thus they are clearly ent-aquasperm.

The ability to store sperm affords the animal with several advantages – the uptake of sperm can occur long before oviposition, thus eliminating the need for synchronising the spawning of sperm and egg maturation among individuals, and oviposition can take place over a prolonged period after a single uptake of sperm (Olive, 1985; Westheide, 1988). Fertilizing eggs in a brood chamber as they are laid, with stored sperm, also increases fertilization success. Westheide (1988) classified spermathecae according to their anatomical position and developmental origin – Type I spermathecae are epidermal infoldings of the body surface while Type II spermathecae are oviducal pouches. All spermathecae described in Sabellidae (including *T. heterouncinata*) and Serpulidae to date are Type I (Daly and Golding, 1977; Picard, 1980; Rouse, 1992b; 1996; Rouse and Gambi, 1998; present study).

The mechanisms of sperm storage within Fabriciinae are highly variable. For example, the spermathecae may be complex and composed of three distinct regions (Rouse, 1992b; 1995b; 1996), invaginations of the epidermis at the base of the radiolar crown (Rouse, 1993), simple sacs in the peristomium, or the sperm may be stored in epidermal cells below the dorsal lips of the radiolar crown or in the buccal cavity (Rouse, 1996). The spermathecae of *Caobangia abbotti* (a sabellid of uncertain affinity (Rouse and Fitzhugh, 1994)) open into the coelomic cavity (Jones, 1974). Spermathecae have been described in only two sabellin genera: *Amphicorina* and *Amphiglena* (Rouse, 1992b; Rouse and Gambi, 1998). The spermathecae in *Amphicorina* may be simple blind ducts or divided into two distinct regions (Rouse, 1992b). The spermathecae of *Amphiglena* spp. are narrow convoluted ducts or simple sacs (Rouse and Gambi, 1998). Spermathecae have been documented in eleven serpulid species, although only those of *Spirorbis spirorbis* and *Salmacina* sp. were described in any detail (Daly and Golding, 1977; Picard, 1980; Rouse, 1996). The spermatheca of *S. spirorbis* is bag-shaped, while *Salmacina* has simple ducts (Picard, 1980; Rouse, 1996). In all these species, the spermathecae are dorsally or dorso-laterally placed with respect to the mouth, and in all but *S. spirorbis* that have only one spermatheca, they occur in pairs.

The spermatheca of *T. heterouncinata* is unique among Sabellidae for two reasons; firstly, it occurs singly, and secondly, it is situated ventral to the mouth. In other respects it is not particularly

remarkable and is little more than a blind-ending ciliated tube. The closest relatives to *Terebrasabella*, *Amphiglena,* have all been shown to have a pair of spermathecae that are part of the radiolar crown (Rouse and Gambi, 1998). This also occurs in all other Sabellinae, except for *Amphicorina bicoloris* where the pair of spermathecae lies in the peristomial collar (Rouse, 1992b). The implication is that there has been a switch from paired spermathecae in the crown to a single spermatheca in the body of *T. heterouncinata.* For those sabellins with spermathecae in the crown it means that when it is lost, for example via predation, the worm cannot fertilise its eggs until it grows a new crown and gathers a new collection of sperm. Presumably the reason for the shift of sperm storage from the crown to the body may have something to do with this factor. The presence of sperm storage structures indicates that *T. heterouncinata* does cross-fertilise. Fertilization occurs externally in the burrow by sperm that are released from the spermatheca as the eggs are laid. How this relates to the fact that *T. heterouncinata* can self-fertilise (Finley et al., 2001) is interesting and experiments on the incidence of self-fertilization would be desirable.

Sperm structure and systematics

The cladistic analysis conducted on *Terebrasabella heterouncinata* by Fitzhugh and Rouse (1999) placed this genus in the clade (*Laonome (Terebrasabella, Amphiglena*)). The present study has shown that some of the reproductive characters were coded incorrectly. These include the structure of mitochondria (originally coded as spherical) and the presence of sperm storage structures (coded as absent), but it is unlikely that these characters would lead to a major revision of the systematic placement of this genus. The location of spermathecae could also be used as an additional character for future analyses. There are two undescribed *Terebrasabella* species in Australia (Rouse and Murray in prep.) and a description of their sperm and spermathecae will no doubt be useful in analyzing the evolution of this unusual group of sabellids.

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CHAPTER 8

Discussion

"Wollust ward dem Wurm gegeben.
Even the worm can feel contentment."

Friedrich Schiller

This study investigated factors that influence the reproductive characteristics of the sabellid, *Terebrasabella heterouncinata*, and identified what enabled it to become established as a pest on abalone farms. The study quantified the degree of expression of life history traits under natural and farm conditions in order to develop an understanding of how they are affected by changes in the environment. The reproductive output of worms on abalone held under intensive farming conditions and controlled laboratory conditions was quantified to identify the specific farm-related factors that may influence reproduction. The study also investigated aspects of oogenesis, spermiogenesis and sperm storage (that may be related to fertilisation biology) that could influence the reproductive success of this worm. It became clear that the sabellid responds to the favourable conditions on the farm by investing more effort into reproduction and that its ability to care for its young, combined with the ability to store sperm from other individuals (and to self-fertilise when isolated) made it well equipped to become a pest .

The shells of molluscs are routinely infested by shell-infesting polychaetes (see Martin and Britayev, 1998; Read, 2001 for reviews). In the natural environment these polychaetes seldom have a negative effect on the host (see Jones, 1969), but they often become pests when they infest cultured molluscs (Martin & Britayev 1998; Read 2001). Like other pests or invasive marine invertebrates, problematic shell-infesting polychaetes often have high levels of fecundity, planktonic larvae and opportunistic life styles, although there are exceptions (Table 8.1; Hockey and van Erkom Schurink, 1992; Lodge, 1993; Zorpette, 1996; Giangrande, 1997; Bright, 1999; Currie et al., 2000). An exception to this rule is *T. heterouncinata.* In spite of producing few offspring, infestation levels by this species reached epidemic proportions on some abalone farms (Oakes and Fields, 1996; Culver et al., 1997; Ruck and Cook, 1998). This is, however, not as incongruous as it may seem at first. Many of the problematic shell-boring polychaetes produce planktonic larvae after a brief period of brooding, or after fertilisation of eggs in the water column (Table 8.1). In these species, larval mortality will probably be high, and in the case of *Pseudopotamilla reniformis*, reproductive success might be further compromised by reduced fertilisation success (Chia, 1974). By contrast, brooders such as *T. heterouncinata* and *Caobangia* spp. fertilise few eggs that are retained within a brood chamber or internally, respectively, and that develop into crawling, lecithotrophic larvae (Jones, 1969; Culver et al., 1997; Fitzhugh and Rouse, 1999). In such species, fertilisation success is usually considered to be high while larval mortality is assumed to be low due to the parental care (cf. Chia, 1974). This may compensate for the low fecundity (Chia, 1974) and it is often adopted by small-bodied polychaetes (Westheide, 1984). The production of planktonic larvae leads to increased dispersal and mutation (Chia, 1974), and would be advantageous for invasive or pest species (cf. Lodge, 1993). The converse would be true for animals that produce benthic larvae. Low dispersal by *T. heterouncinata* larvae may, however, lead to increased local densities that could have a positive effect on fertilisation success (see below) and an increased level of adaptation to conditions on the farm (Strathmann, 1990). In addition, recruitment success of *T.* heterouncinata is probably high due to the production of

crawling larvae that can re-infect its host. Thus an increase is fecundity would be expressed as an increase in recruitment. By contrast, increased fecundity by broadcast spawners does not necessarily result in increased recruitment due to the unpredictability of environmental factors. These factors could all contribute to the success of *T. heterouncinata* under mariculture conditions.

Table 8.1. Life history traits of certain shell-boring polychaetes. All but *Caobangia* infest commercially important shellfish, and all are known to damage the host shell at high levels of infestation. This information was edited from reviews by Rouse and Fitzhugh, 1994; Giangrande, 1997; Martin and Britayev, 1998; Blake and Arnofsky, 1999; Lleonart, 2001. * data from present study. BR: brooding, BR.C: broadcast spawning, V: viviparous; Developmental mode = L: lecithotrophic, P: planktotrophic.

Family and species	N Eggs per spawn	Brood care	Developmental mode
Sabellidae			
Caobangia spp.	1	V	L
Pseudopotamilla reniformis	34 000	BR.C	L
*Terebrasabella heterouncinata**	1 – 13	BR	L
Spionidae			
Boccardia chilensis	1 300	BR	P
B. proboscidea	30 – 1 600	BR	L
B. proboscidea	304 – 190 000	BR	P
B. hamata	> 10 – 12	BR	P
Polydora ciliata	140 – 4 250	BR	P
P. hoplura	>150	BR	P
P. websteri	500 – 550	BR	P

The nature of the relationship between *Terebrasabella heterouncinata* and its host

A review of shell-boring polychaetes showed that most of these polychaetes have facultative relationships with their molluscan hosts (Martin and Britayev, 1998). In the families Spionidae, Cirratulidae and Sabellidae, there are 47 species (including *T. heterouncinata*) that routinely infest the shells of molluscs, but only nine are considered to form obligatory relationships with their hosts. However, the novel way in which *T. heterouncinata* forms its burrow (Culver et al., 1997) suggests that the relationship between the worm and its host is obligatory. The present study confirms this hypothesis for larval settlement. As emerging larvae do not settle and develop to maturity in abandoned burrows (Chapter 3), the position of the worm on the shell is an indication of its age, making it possible to measure the effect of age on reproduction in worms from farmed abalone (Chapter 5).

The flexibility in the expression of life history traits

The flexibility in reproductive traits of *T.* heterouncinata was compared by means of coefficients of variation, or as ratios (Table 8.2). To calculate the ratios, the smallest mean value for a particular trait at each farm is considered the baseline value (i.e., 1), and other values for that trait are calculated as a fraction of that value. The present study demonstrated that although *T. heterouncinata* was K-selected, it displayed a sufficiently high degree of flexibility in its reproductive traits which enabled the worm to increase its reproductive output (Table 8.2). Within sites, the variables displaying the highest variability (based on the coefficients of variation) were worm density and the proportion of the population brooding offspring, particularly at the wild sites (Table 8.2). When comparing worms from wild and farmed abalone, the most variable traits were the number of offspring per adult, the number of individuals that were brooding more than two clutches of offspring simultaneously and the proportion of the population brooding offspring (Table 8.2). At most farms, the mean values for these variables were between three and six times greater for the worms from farmed abalone than for those from the wild. There was, however, a 23-fold difference in the percentage of adults brooding more than two clutches of offspring on abalone from FARM B than from abalone at its respective wild site. The high variation in egg number observed in the present study has been demonstrated in several other polychaete species (e.g., Grémare et al., 1988, Qian, 1994; Prevedelli and Zunarelli Vandini, 1998; Prevedelli and Simonini, 2001), and supports the theory that egg number is not a conservative reproductive variable within species (Eckelbarger, 1986). As a consequence of high fecundity of individual worms and the population as a whole, worms generally occurred at higher densities on farms than they did in the wild, with the density of worms at most on-farm sites being between five and ten times the density at the respective wild sites.

There was comparatively less variability in the size of the adults and their offspring than in instantaneous fecundity and the rate of egg production (Table 8.2). It is, however, interesting to note that a 1.56 to 1.8-fold increase in adult size led to a 3.04 to 4.49-fold increase in instantaneous fecundity in the present study. At all farms, offspring size showed the least variability of all traits measured, although egg volume tended to be more variable in the wild than at other sites. This limited variation in the size of offspring has been observed in other polychaetes, and egg size is therefore generally thought to be a carefully controlled reproductive trait within species (Eckelbarger, 1986; Grémare et al., 1988; Rouse and Fitzhugh, 1994). However, under certain conditions, such as when there is increased competition between juveniles, there may be a trend towards the development of larger eggs and larvae (Stearns, 1976). There was no indication of such pressures in the present study, as there was no relationship between density and larvae and egg size. This may be related to the increased availability of settlement sites due to the high number of potential hosts available in the raceways on farms. The differences in the degrees of expression of life history traits demonstrated in the present study may have been due to the phenotypic plasticity of the reproductive traits, since such responses to different environments and nutrient levels could occur within one generation (cf. Levin,

1986; Bridges et al., 1994; Prevedelli and Zunarelli Vandini, 1998; Prevedelli and Simonini, 2001). However, evidence suggests that there has been a gradual increase in the fecundity (and possibly body size) of *T. heterouncinata* since their establishment on farmed abalone (Gray, 2004), suggesting that progressive selection for larger, more fecund worms may have occurred on the farms (cf. Prevedelli and Zunarelli Vandini, 1998; Prevedelli and Simonini, 2001). Thus it can be hypothesised that the differences in the degree of expression of life history traits observed in worms from wild and farmed abalone may have a genetic component (cf. McKillup and Butler, 1979; Hart and Begon, 1982). The limited dispersal of larvae would contribute to the retention of certain advantageous genes expressed on farms, further strengthening adaptation to local conditions.

This study raises interesting questions concerning the selection of genetic traits that may enable certain individuals to flourish within the farm environment leading to a gradual change in the genetic make-up of worms on farms. For example, is there a significant degree of genetic divergence between worms from farmed and wild abalone? Sequencing the mitochondrial DNA of worms from different environments might increase our understanding of the processes taking place. Such genetic studies can be supplemented by ecological studies, measuring the performance of wild worms infesting farmed abalone relative to that of worms that had been reared on the farm.

Table 8.2. The degree of variation in the life history characteristics of *T. heterouncinata* expressed as ratios and the coefficient of variation (in parentheses) where possible. The ratios were calculated as a fraction of the smallest value for each parameter using the data in Tables 2.2 and 2.3. (FARM A-W and FARM B-W were wild sites, situated 2 km and 1.5 km from the respective farms; FARM A-E and FARM B-E were situated at the exit of the outflow pipes; FARM C-W1 and FARM C-W2 were 60 m and 2.5 km from the farm; FARM A-F; FARM B-F; FARM C-F1 and FARM C-F2 were the on-farm sites at the three farms.)

		FARM A	
	FARM A-W	FARM A-E	FARM A-F
Density (worms.mm^{-2})	1.07 (92%)	1.01 (75.7%)	1 (31.5%)
% Brooding	1 (77.9%)	2 (96.3%)	6.09 (6.7%)
Adult size (mm)	1 (20.6%)	1.28 (23%)	1.77 (12.4%)
% worms with >2 clutches	1.25	1	5.33
Number of offspring per adult	1 (36.9%)	1.08 (20.9%)	4.49 (26.4%)
Egg volume (mm^3)	1 (25%)	1.38 (9%)	1.13 (7%)
Larvae length (mm)	1 (7%)	1.1 (2%)	1.17 (4%)
Minimum size at maturity (mm)	1	1.32	1.39
		FARM B	
	FARM B-W	FARM B-E	FARM B-F
Density (worms.mm^{-2})	1 (100%)	1.4 (114%)	4.93 (29%)
% Brooding	1.79 (136%)	1 (124%)	5.08 (12.8%)
Adult size (mm)	1 (19.3%)	1.01 (22.6%)	1.67 (9.3%)
% worms with >2 clutches	1	0	23.6
Number of offspring per adult	1 (31.3%)	1.53 (60%)	3.76 (18.7%)
Egg volume (mm^3)	1 (28.6%)	1.38 (10.3%)	1.62 (8.8%)
Larvae length (mm)	1.09 (6.3%)	1 (9.1%)	1.27 (7.1%)
Minimum size at maturity (mm)	1	1.26	2.01
		FARM C		
	FARM C-W1	FARM C-W2	FARM C-F1	FARM C-F2
Density (worms.mm^{-2})	5.92 (105.6%)	1 (91.7%)	6.67 (50%)	9.75 (57.3%)
% Brooding	1 (156.4%)	1.23 (127.3%)	4.31 (21.8%)	3.79 (25.3%)
Adult size (mm): Gravid adults	1 (19.9%)	1.08 (13.8%)	1.42 (14.4%)	1.56 (12.5%)
Adult size (mm): non-gravid adults	1 (26.3%)	1.02 (27.4%)	1.56 (16.3%)	1.8 (9.6%)
% worms with >2 clutches	1	2.53	1.82	3.06
Number of offspring per adult	1 (66.7%)	1.29 (47.2%)	2.92 (24.3%)	3.04 (22.4%)
Egg volume (mm^3)	1 (19.2%)	1.08 (10.7%)	1.19 (9.7%)	1.23 (9.4%)
Larvae length (mm)	1	1 (10.6%)	1 (8.5%)	1.17 (5.5%)
Minimum size at maturity (mm)	1.23	1	1.36	1.69

The quantification of factors contributing to the success of worms on farmed abalone

Several factors related to the unique farm environment have been suggested as having contributed to the success of the worm under aquaculture conditions. These include the increased availability and nutrient content of food, increased habitat stability and predictability (i.e., limited water movement and wave action and a constant, predictable supply of food) and increased host (habitat) availability. Quantifying the effects of each of these factors on the reproductive success of the sabellid in isolation under aquaculture conditions was not possible. The effect of diet, in isolation of other farm-related factors, was tested under laboratory conditions (Chapter 3). The results of this study were, however, anomalous. Contrary to predictions, worms fed the less nutritious diet (i.e., kelp) appeared to fare better than those on the nutritious Abfeed™, with worms on kelp-fed abalone being larger and maturing earlier than worms on Abfeed™-fed abalone. There are several possible reasons for this result: the relatively low level of particulate matter (degraded abalone food) in the Abfeed™ tanks compared to the kelp treatments (personal observation) may have resulted in less food available to the worms in the former treatment. Similarly, if the worms are supplementing their diets with abalone faeces, the faeces from the low density of abalone in the experimental tanks may not have provided adequate food for the worms. While diet did affect body size, it had no effect on offspring number or size. It is important to note that adult size, mean brood size, offspring size and the proportion of the sample that was reproductively active in the laboratory study were similar to values for wild worms (cf. Chapters 2 and 3). These results suggest that the worms in the laboratory study may not have had enough food available to them (Ruck and Cook, 1998). Therefore, the differences observed in worms from farmed abalone fed different diets should be considered more representative.

An attempt was then made to quantify the effect of both abalone stocking density and diet on the rate and level of infestation (as an indication of reproductive output) of worms under intensive aquaculture conditions. There was high variability in infestation levels, with prevalence levels increasing to as much as 80% in some treatments. This study could therefore not identify either abalone diet or stocking density as having a significant effect on infestation level in farmed abalone. Thus, it is difficult to predict under which conditions this worm will flourish. In the present study, infestation may have been limited by a limited source of infestation, as well as a seasonal reproductive pulse that occurred before the initiation of the study. Furthermore, since the discovery of the sabellid on abalone farms, farm managers have been adopting improved husbandry techniques, and in particular, have been minimising the particulate load of the water within abalone raceways (P. Pesch, Aquafarm Development, pers. comm.). It is therefore also possible that the low levels of infestation observed in this study were a direct consequence of improved management practices. This supports the findings of Chalmers (2002) and Gray (2004) who demonstrated that food availability is an important factor driving the reproductive success of this worm on abalone farms.

The sabellid population size (i.e., total intensity of infestation) increased exponentially as the prevalence of infestation (i.e., the number of infested abalone in the sample) increased. Thus there was an increase in both the size of the population as well as the spatial distribution of the worms within the population, as the experiment progressed. The greatest increase in infestation levels observed in this study occurred at the onset of spring. Season may influence reproduction in *T. heterouncinata,* as it does in several other polychaete species (reviewed by Olive, 1984). This hypothesis is supported by the fact that generation time in this polychaete is temperature-dependent (Finley et al., 2001), although photoperiod may play a role. This pattern in infestation levels may, however, also be related to increased fertilisation success as a consequence of the increase in population size and spatial distribution of individuals within the population (Strathmann, 1990; Claereboudt, 1999; Yund, 2000) and will be discussed in greater detail elsewhere. Further studies are required to measure the effect of population density and size on fertilisation success and the effect of season on reproduction in this species.

Reproduction in worms of different ages

On farms, age had no effect on fecundity, or on the rate of egg production or the proportion of the population brooding in those age groups in which all individuals had reached sexual maturity, for *Terebrasabella heterouncinata* that were up to 40 months old. Thus, once the sabellids reach sexual maturity, worms in all age groups contributed to population growth, which would further contribute to the success of these worms on abalone farms. The apparent negligible senescence demonstrated here is not typical for polychaetes. With the exception of *Ophryotrocha labronica* (Prevedelli and Zunarelli Vandini, 1998), other polychaetes exhibited senescence (*sensu* Vaupel et al., 2004), i.e., reproductive output decreased with age (Åkesson, 1976, 1982; Åkesson and Costlow, 1991; Qian and Chia, 1992; Levin et al., 1996; Prevedelli and Simonini, 2001). This difference in reproductive output with an increase in age depends (among other factors) on differential adult and juvenile mortality and possibly the life span of the animal (Charlesworth and Leon, 1976; Vaupel et al., 2004). In animals exhibiting senescence, early sexual maturity coupled with high fecundity usually leads to a reduction in the life span of the animal, and is common in species that experience high adult mortality relative to juvenile mortality. These species are often short-lived and are therefore under pressure to reproduce as early as possible, and to produce as many offspring as possible. Senescence is therefore likely to be common in animals that live in unstable environments or under conditions where the rapid establishment of a population is required (Charlesworth and Leon, 1976; Stearns, 1976). By contrast, long-lived animals that experience low adult mortality relative to juvenile mortality, such as *T. heterouncinata,* are not under such pressures, particularly if they inhabit stable environments (Stearns, 1976). These animals can therefore spread their life-time reproductive output over a longer period, investing more energy per offspring, thus ensuring greater survival of those offspring. This study could not, however, give any indication that offspring produced by worms of different ages were of a

consistent quality, as that will also affect the contribution by worms of different ages to population growth (Qian and Chia, 1992).

A change of environmental conditions can influence both the life-span and the life-time fecundity of polychaetes (Åkesson, 1976, 1982; Åkesson and Costlow, 1991; Qian and Chia, 1992; Levin et al., 1996; Prevedelli and Zunarelli Vandini, 1998; Prevedelli and Simonini, 2001). Most of these studies concentrated on the effect of nutrient enrichment, and few have measured the effect of artificial environments on life-span and fecundity. Two studies demonstrated that laboratory-rearing may lead to either an increase or decrease in the life-span of some polychaetes (Prevedelli and Zunarelli Vandini, 1998; Prevedelli and Simonini, 2001). The species tested differed in their respective life-span and reproductive responses to age. *Ophryotrocha labronica* was the longer-lived species, lived longer under laboratory conditions than it did in the wild and showed no change in brood size with an increase in age (Prevedelli and Zunarelli Vandini, 1998). Such responses to increased habitat stability confirm predictions made by life history theories (Pianka, 1970; Stearns, 1976). By contrast, the shorter-lived *Dinophilus gyrociliatus* had a shorter life-span under laboratory conditions and produced most of its offspring early in its life (Prevedelli and Simonini, 2001). Based on these studies it is possible to make suggestions as to how the farm environment has affected the life-span and life-time fecundity of *T. heterouncinata*. This sabellid is a long-lived (up to 40 months under aquaculture conditions), K-selected animal that exhibits negligible senescence. It is therefore possible that under conditions of increased habitat stability, worms from farmed abalone could live longer, producing more clutches than their wild conspecifics, if their hosts are allowed to live as long as they do in the wild (Chapter 5). Further studies are required to estimate the maximum age of wild worms, and to determine how age influences the reproductive output in these worms.

The consequences of oogenesis for life history strategies

The extent to which an animal can translate increased nutrient availability and other environmental factors into reproductive output depends on the vitellogenic mechanisms employed and ovary structure and number (Eckelbarger, 1983, 1986, 1994). Polychaetes such *Capitella* sp. and *Streblospio benedicti* can increase their fecundity by an order of a magnitude under conditions of nutrient enrichment and can produce more than a thousand eggs per spawn (Qian and Chia, 1991; Qian, 1994; Levin et al., 1996). This may be related to the fact that these species have several reproductive segments (Eisig, 1887, in Clark and Olive, 1973; Levin, 1986). By contrast, *T. heterouncinata* has only one female segment, and two ovaries. Thus, their ability to increase the number of eggs produced at a time is limited compared to that of *Capitella* sp. and *S. benedicti*. The relatively higher fecundity observed in *T. heterouncinata* from farmed abalone could therefore depend more on the degree to which the body size, and consequently coelomic space for the storage of developing oocytes, increased.

When storage space for developing oocytes is limited, small-bodied polychaetes may rely on the acceleration of egg production to further increase fecundity. This was demonstrated for *T. heterouncinata* that increased both the egg number and the rate of egg production under favourable conditions (Chapter 2). Differences in fecundity and the speed of egg production that are correlated with the nutritional state of the adult may be related to the mechanisms by which yolk precursors are synthesised, transported and incorporated into the oocytes. This would enable polychaetes that incorporate high molecular weight precursors through high levels of endocytotic activity to exploit periods of increased nutrient availability, resulting in an increased rate of egg production (Eckelbarger, 1980, 1986; Levin, 1986). This has been suggested for species of *Phragmatopoma, Capitella, Streblospio* and *Polydora* that have of the shortest vitellogenic periods reported, while showing intensive endocytotic activity during oogenesis (Eckelbarger, 1986). In addition, the speed of vitellogenesis in *Phragmatopoma, Capitella,* and *Streblospio* was further increased through the incorporation of extracellular yolk precursors that are produced or stored by the ovarian follicle cells and blood vessels with which the oocytes are associated. In *T. heterouncinata,* oogenesis is solitary, but vitellogenic oocytes do appear to incorporate high molecular weight yolk precursors endocytotically which may enable the sabellid to increase the rate at which it incorporates yolk precursors during periods of nutrient enrichment (Levin, 1986). To date, oogenesis has only been described in semelparous or annual iteroparous sabellids which have vitellogenic periods lasting several months (Dales, 1961; Giangrande et al., 2000; Licciano et al., 2001). It is therefore interesting that in these species and *T. heterouncinata,* oogenesis is solitary and occurs in the coelom. Further studies are therefore required to gain a clearer understanding of the relationship between life history patterns and vitellogenic mechanisms in sabellids.

The consequences of fertilisation biology for reproductive success

Sessile animals, such as *T. heterouncinata*, face several problems with respect to fertilisation. Such animals must release sperm into the water column (e.g., Chia, 1974; Yund, 2000), while isolated sessile simultaneous hermaphrodites may be able to self-fertilise (Gee and Williams, 1965; Hsieh, 1997; Finley et al., 2001). Self-fertilisation is, however, rare (Ghiselin, 1974) as it often leads to inbreeding depression and a reduction in fecundity and survival of offspring (Gee and Williams, 1965; Jarne and Charlesworth, 1993). Studies conducted by Finley et al. (2001) demonstrated that *T. heterouncinata* is probably able to self-fertilise, although the authors did not discount the possibility that the worms were reproducing by parthenogenesis. However, the present study indicates that this sabellid routinely cross-fertilises, as it produces ent-aquasperm and possesses an organ of sperm storage. The fact that *T. heterouncinata* cross-fertilises does not imply that fertilisation success would be reduced compared to self-fertilisation. Sperm storage and the fertilisation of eggs within the burrow as they are laid may lead to increased fertilisation success and individuals can fertilise several

batches of eggs after a single sperm-collecting episode, thus reducing the need for the synchronisation of the spawning of eggs and sperm between individuals (Chia, 1974; Westheide, 1984; Olive, 1985).

The fertilisation success of sedentary species is often influenced by sperm limitation, (i.e., the dilution of sperm) (Strathmann, 1990; Claereboudt, 1999, Yund, 2000), particularly as they are not able to congregate during spawning episodes. Sperm limitation is inversely proportional to the density and size of a population, suggesting that population growth would be slow when individuals are far apart (Claereboudt, 1999; Yund, 2000). *Terebrasabella heterouncinata* are probably able to minimise the possible effects of sperm limitation by storing sperm and through their presumed ability to filter sperm from their feeding currents. However, sperm limitation may still influence the fertilisation success of wild sabellids due to the low density of wild worm populations owing to the dispersed distribution of their hosts. By contrast, the high population density and size that occurs on farms might minimise the effect of sperm limitation, leading to increased fertilisation success and population growth. Examination of the male segment showed that spermatids at different stages of development were present, suggesting a continuous supply of sperm. The effects of this constant supply of sperm would be further enhanced when the number of spawning individuals in the population increases. The incidence of sperm limitation in *T. heterouncinata* requires further investigation.

Concluding remarks

This investigation demonstrated a high level of plasticity in the degree of expression of life history traits by *T. heterouncinata*, further contributing to results of studies conducted by Chalmers (2002) and Gray (2004). By virtue of this plasticity, the worms were able to increase their reproductive output and become established on farms at high densities under conditions of unlimited food supply and host availability. Owing to the increased body size of worms from farmed abalone, these sabellids may have been able to increase their fecundity. In addition, the conditions on the farms allowed for the acceleration of egg production and an increase in the number of individuals in the population that were reproducing. This increased rate of reproduction by worms on farms is consistent among worms from different age groups, allowing all the adults in the population to contribute to population growth. The ability of the worms to respond to nutrient enrichment (and other farm-related conditions), by increasing the rate at which eggs are produced, may be related to vitellogenic mechanisms that allow for the rapid incorporation of yolk precursors from extracellular sites into the developing oocytes. The increase in reproductive success is likely to be further enhanced by increased fertilisation success owing to an increase in intraspecific density which could reduce sperm limitation, and through the sabellid's ability to store sperm, and fertilise eggs as they are laid within the burrow.

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