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The Filtration Rate, Oxygen Consumption and Biomass of the Introduced Polychaete Sabella Spallanzanii Gmelin Within Cockburn Sound : Can it Control Phytoplankton Levels and is it an Efficient Filter Feeder?

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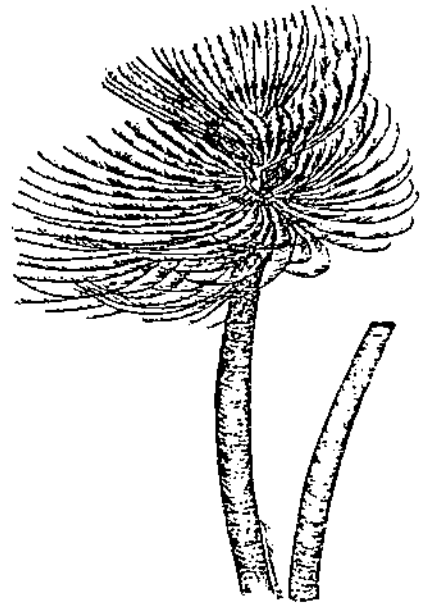

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ABSTRACT

The filtration rate, oxygen consumption and biomass of the introduced polychaete *Sabella spallanzanii*, within Cockburn Sound.

Sabella spallanzanii, a filter feeding, sabellid polychaete worm which is common in the Mediterranean Sea, was recently discovered in Cockburn Sound, Western Australia. The species has been in Port Phillip Bay, Victoria for about 10 years, where it has spread widely, competes with native species and has economic impacts on the local scallop fishery. In Cockburn Sound, *S. spallanzanii* has colonised a shallow, sandy area known as the Southern Flats, reaching a mean biomass of 258 gDW m^{-2}, as well as almost all artificial structures such as jetties and navigational marker pylons. A large biomass of this introduced filter feeder may have a considerable filtration capacity which could control levels of phytoplankton in the Sound.

This study measured the biomass and filtration rate of *S. spallanzanii* to determine its potential to effect phytoplankton levels in Cockburn Sound through filter feeding. The results suggest that these polychaetes have a substantial filtering capacity, capable of filtering the water-column above them at the Southern Flats (5m depth) 4.6 times daily.

To determine the feeding efficiency of *S. spallanzanii* (volume of water filtered per metabolic demand), the oxygen consumption and filtration rate were measured. Feeding efficiency, which may provide an indication of the potential spread of *S. spallanzanii* to less eutrophic waters, increased with temperature from 13°C, reaching an optimum at 22°C. Between 22 - 27°C the feeding efficiency decreased sharply, indicating that its upper temperature limit is approached. To meet its metabolic requirements, *S. spallanzanii* requires a phytoplankton concentration with a chlorophyll *a* level of 1.42 $\mu g\ L^{-1}$ in winter (17°C) and 0.73 $\mu g\ L^{-1}$ in summer (22°C). It was concluded that the feeding efficiency of *S. spallanzanii* may limit it to eutrophic harbours with a high level of phytoplankton.

DECLARATION

I certify that this thesis does not incorporate without acknowledgement any material previously submitted for a degree or diploma in any constitution of higher education; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

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Chapter 1

INTRODUCTION

Background

For many years much attention has been paid to the problems facing Australia from introduced species in terrestrial and freshwater environments. Meanwhile, invasions of our marine environment have gone largely unnoticed. The problems facing Australia from the introduction of marine pests have been highlighted by recent media attention to such pests as the northern Pacific seastar *Asterias amurensis*, the Japanese kelp *Undaria pinnatifida*, toxic dinoflagellates and the Mediterranean fan worm *Sabella spallanzanii*.

This concern over introduced marine species is not unjustified as many marine invasions are of such magnitude that they may be leading to profound ecological changes in the ocean (Carlton, 1989). With the advent of modern transport such as shipping, marine species have a mechanism to cross natural oceanic barriers, being deposited in a new environment where they may become a pest (Carlton and Geller, 1993). There are many examples of marine pests having serious ecological and economic impacts (e.g. Meinesz and Hesse 1991; Carlton and Geller, 1993; Nichols, Thompson and Schemel, 1990).

There are now over 70 species of introduced algae, invertebrates and fish which have been identified in Australian waters (Rainer, 1995). While not all of these species can be considered as pests, the CSIRO has compiled a list of species with sufficient potential for economic or ecological effects to justify them receiving immediate priority for study. *Sabella spallanzanii*, a filter feeding polychaete worm, is one of those species listed (CSIRO unpublished).

In 1994, large, dense beds of *Sabella spallanzanii* were discovered on the Southern Flats in Cockburn Sound, Western Australia (Lemmens, Clapin, Greenway, Lavery and Cary, in press). Further investigation found that it also covered most of the jetties throughout Cockburn Sound, north to Fremantle and in harbours at Bunbury and Albany (Clapin and Evans, 1995).

Sabella spallanzanii, which is common in the Mediterranean Sea, was first reported in Australia during the early 1980's in the Geelong arm of Port Phillip Bay, Victoria (Carey and Watson, 1992). It has now spread over most of the northern and western parts of Port Phillip Bay where it competes with native species and is causing serious problems for the commercial scallop fishery. As *S. spallanzanii* has only recently been found in Western Australia, it is not yet known whether it will have the same rapid rate of spread or impacts as seen in Port Phillip Bay. It is important, therefore, to determine the species' biological and ecological attributes, which may influence its further spread and potential impacts.

Significance and purpose of the study

One major difficulty with managing introduced species is the lack of knowledge on the ecological role and impact they will have in their new environment. This can only be partially predicted from knowledge of their biology and ecology in their region of origin (Carlton and Geller, 1993). Therefore, it is essential to the effective management of these pests to develop some knowledge on their biology and ecological role whilst insitu in their new environment.

Little is known about what impact *S. spallanzanii* will have in Cockburn Sound or whether it is capable of spreading outside the Sound and invading other near-shore environments. *S. spallanzanii* has already reached high densities on natural and artificial substrates in some areas of Cockburn Sound (Clapin and Evans 1995). The possible impacts may include

competition with native species for space and food, reduction in diversity, displacement of seagrass and impacts on commercial mussel farms. Because *S. spallanzanii* is a filter feeder, the combined filtration capacity of large beds in Cockburn Sound may have an impact by stripping phytoplankton from the water and if this is the case, then attempts to remove it could result in a localised reduction in water quality.

Amongst the more important questions which need to be addressed at this stage are:
What factors are important in the establishment and spread of *S. spallanzanii*. In particular, is its spread related to the availability of phytoplankton and the efficiency with which it feeds on that food source and what is its contribution to stripping the water column of phytoplankton.

Although there may be many other environmental factors which limit its density and distribution, in Australia *S. spallanzanii* has so far only been found at high densities in eutrophic harbours. Therefore, food availability and feeding efficiency may be important factors in limiting it to eutrophic areas which have levels of phytoplankton high enough to sustain its food requirements.

Sabelid worms such as *Sabella spallanzanii* gather food by filtering water to remove suspended particles or cells (Nicol, 1930). *S. spallanzanii* must be able to filter enough water to gather sufficient food to meet its metabolic requirements. If the food concentration in the water is low, it needs to filter more water to meet its needs than if the concentration of food is high. Two possibilities exist: if it is an efficient filter feeder and can filter a large volume of water compared to its metabolic requirements, then it can potentially live in waters with relatively low food concentrations. Should this be the case, it will have the potential to spread outside Cockburn Sound, unless it is limited by some other factor. If on the other hand, it is an inefficient feeder, then in order to meet its food requirements it may be restricted to areas where food concentrations are relatively high.

The purpose of this study therefore, is to increase our knowledge of the autecology of *Sabella spallanzanii* in Cockburn Sound. Specifically, the study will determine its feeding efficiency and whether this is a limiting factor and what impact *S. spallanzanii* could have on phytoplankton levels in Cockburn Sound. This will provide information to assist the management of *S. spallanzanii*. The study was carried out in conjunction with the CSIRO Coastal Zone Filter Feeder Project. This study also aims to highlight the importance of filter feeders in coastal waters and provide an opportunity for input to current coastal management models.

Components of the study

Feeding efficiency in filter feeders has not been measured as a single function. Instead, studies on other fan worms have inferred a level of feeding efficiency by comparing the oxygen consumption rate as a measure of metabolic rate, and therefore energy consumption, with filtration rate as a measure of food collection (Riisgård and Ivarsson, 1990; Shumway, Bogdanowicz and Dean, 1988). The present study followed a similar strategy, measuring the filtration rate and oxygen consumption rate of *S. spallanzanii* separately, and inferring a level of feeding efficiency from these two parameters. In addition, the amount of food required to meet the metabolic requirements per unit of water filtered was calculated. This could then be related to actual levels of phytoplankton in the natural environment to determine whether *S. spallanzanii* is potentially capable of spreading outside Cockburn Sound.

Because temperature is known to affect both filtration rate and oxygen consumption rate (e.g. Jørgensen, Larsen, and Riisgård, 1990; Schmidt-Neilsen, 1983) these parameters were each measured at a range of temperatures. This range of temperatures was also intended to detect either an optimum or the tolerable limits of *S. spallanzanii*, as this may give an indication of the environmental temperatures in which it can spread.

Since high algal cell concentrations are known to effect filtration rate (e.g. Petersen and Riisgård, 1992; Riisgård and Ivarsson, 1990), filtration rate of *S. spallanzanii* was also tested at various cell concentrations.

To determine the filtration capacity of the population of *S. spallanzanii* it was necessary to measure the biomass in Cockburn Sound, estimate the total area covered and relate this to the filtration rate per unit of worm body dry weight. Measurement of biomass also involved sampling different habitats where *S. spallanzanii* occurs and sampling seasonal variation.

Aims

The aims of this study were to:

1. Determine the filtration rate of *Sabella spallanzanii* and whether this rate is dependent on temperature or algal cell concentration.
2. Measure the oxygen consumption rate of *S. spallanzanii* and the effect of temperature on this rate.
3. Determine the biomass of *S. spallanzanii* in Cockburn Sound and whether the majority of that biomass is distributed on the Southern Flats area or on artificial structures such as jetties and navigational marker pylons. Secondly, to determine whether there is seasonal variation.
4. Using the filtration rate and oxygen consumption rate, to determine the feeding efficiency of *S. spallanzanii* and the concentration of food required to meet its metabolic requirements.
5. By combining the results of filtration rate and biomass, to determine the total filtration potential of the *S. spallanzanii* population in Cockburn Sound.

Review of literature

Introduced marine species.

There are numerous reports in the literature on the introduction and subsequent spread of marine species, often having both ecological and economic impacts on the area invaded. Since its introduction to the northern Mediterranean, the toxic tropical algá *Caulerpa taxifolia* has spread rapidly, significantly reducing or destroying seagrass populations and causing toxicity in fish rendering them unsuitable for human consumption or sale (Meinesz and Hesse 1991). Another case is the Asian clam *Potamocorubla amurensis* which has invaded San Francisco Bay, spread, and reached such high density that it has displaced the former community (Nichols, Thompson, and Schemel, 1990). These species have caused ecological changes through intense competition, changing community structure and reducing diversity. This is often facilitated by the absence of predators in their new environment as is the case with the toxic *Caulerpa taxifolia* (Meinesz and Hesse 1991).

Introduced species may also affect community structure through selective predation. The introduced European green crab, *Carcinus maenas* selectively preys on species of a certain size significantly reducing the abundance of several taxa and altering community structure in Bodega Harbour, California (Grosholz and Ruiz 1995).

There are not many reports of introduced filter feeders having an impact. One notable exception is the Asian clam *P. amurensis*, which has significantly reduced chlorophyll concentrations and the abundance of 3 common zooplankton species in the San Francisco Bay estuary (Kimmerer, Gratside and Orsi, 1994).

Carlton and Geller (1993) considered that bays, estuaries and inland waters where ships may take up and dump ballast water, are often disturbed by extensive urbanisation, rendering them especially susceptible to invasion. They concluded that these environments are the marine analogs of despoiled, highly invaded oceanic islands and they may be among the most threatened ecosystems on the planet. This conclusion is well supported by the literature as most of the reported invasions are in bays, estuaries, inland waters or harbours, including all the reported invasions of *S. spallanzanii* in Australia.

The importance of filter feeders

Although numerous studies have been conducted elsewhere (see below) there have been few studies in Australia on the filtration rate of locally occurring species (e.g. Lemmens, Kirkpatrick and Thompson, 1996). Van Senden (1994) points out that there is a general lack of information on local filter feeders and because of this, the filter feeder component of COASEC model is based on data from overseas literature. This highlights the need for studies on the filtration capacity of filter feeders in local waters. There are, however, some data which indicate that filter feeders are an important part of the coastal ecosystem. Wells and Threlfall (1980) found that 82.2% of the benthic fauna in the deep basin of Cockburn Sound were infaunal filter feeders. In Port Phillip Bay, filter feeders comprise about half the benthic macroinvertebrate biomass and may account for 42% of the total assimilation of organic material by benthic invertebrates (Wilson, Cohen and Poore, 1993).

Several studies have shown that filter feeders are important in controlling phytoplankton levels in shallow coastal bays (e.g. Officer, Smayda and Mann, 1982; Alpine and Cloern, 1992; and Hily, 1991). In South San Francisco Bay, Cloern (1982) showed that the abundance of filter feeding bivalves is sufficient to filter the entire volume of the bay daily, suggesting that this is the primary

mechanism controlling phytoplankton levels during summer and autumn. A similar result was demonstrated by Petersen and Riisgård (1992) for the ascidian *Ciona intestinalis*, which is sufficiently abundant in summer to filter the volume of a shallow fjord in Denmark daily. In Port Phillip Bay, filter feeders are estimated to filter the volume of the entire Bay in about 16.5 days (Wilson *et al.*, 1993).

Feeding efficiency

Jørgensen (1975) estimated that, in order to obtain enough food to meet its minimal energy requirements, a temperate zone, near coastal, marine filter feeder must filter more than 10 L of water per mL of oxygen consumed. Riisgård and Ivarsson (1990) found the water-processing capacity (feeding efficiency) of *Sabella penicillus* to be 354 L of water filtered per mL of oxygen consumed. The high feeding efficiency of *S. penicillus* suggests that this polychaete is adapted to live in waters with extremely low algal concentrations (Riisgård & Ivarsson, 1990). In comparison, the mussel *Mytilus edulis* filters only 15 to 50 L of water per mL of oxygen consumed (Riisgård, Randløv, and Kristensen, 1980). Riisgård and Ivarsson, (1990) concluded that *M. edulis* may not be able to live in localities with as low food concentrations as *S. penicillus*. This demonstrates that different species of filter-feeders can be adapted to different regimes of suspended food.

Although there has been previous work on both the filtration rate and oxygen consumption of other Sabellid polychaetes such as *Sabella penicillus* (Riisgård & Ivarsson, 1990), there are no appropriate results which can be used for *Sabella spallanzanii*.

Sabella spallanzanii

Sabella spallanzanii is common along the Italian coast and can be found in both the open ocean from 1 to 30m depth, as well as in eutrophic harbours, where it reaches high densities (Giangrande and Petraroli, 1994). So far, in Australia, *Sabella spallanzanii* has only been found at high densities in shallow, eutrophic harbours such as Port Phillip Bay and Cockburn Sound, (Carey and Watson, 1992; Clapin and Evans 1995). Cockburn Sound has had elevated levels of phytoplankton for at least the past 15 years (Chiffings and McComb, 1981; Cary, Simpson, and Chase, 1991; Cary, Masini, and Simpson, 1995). This suggests that, while it can live at low phytoplankton levels in the ocean, *S. spallanzanii* may need higher levels of food to establish a population of high density and therefore food levels may be a limiting factor.

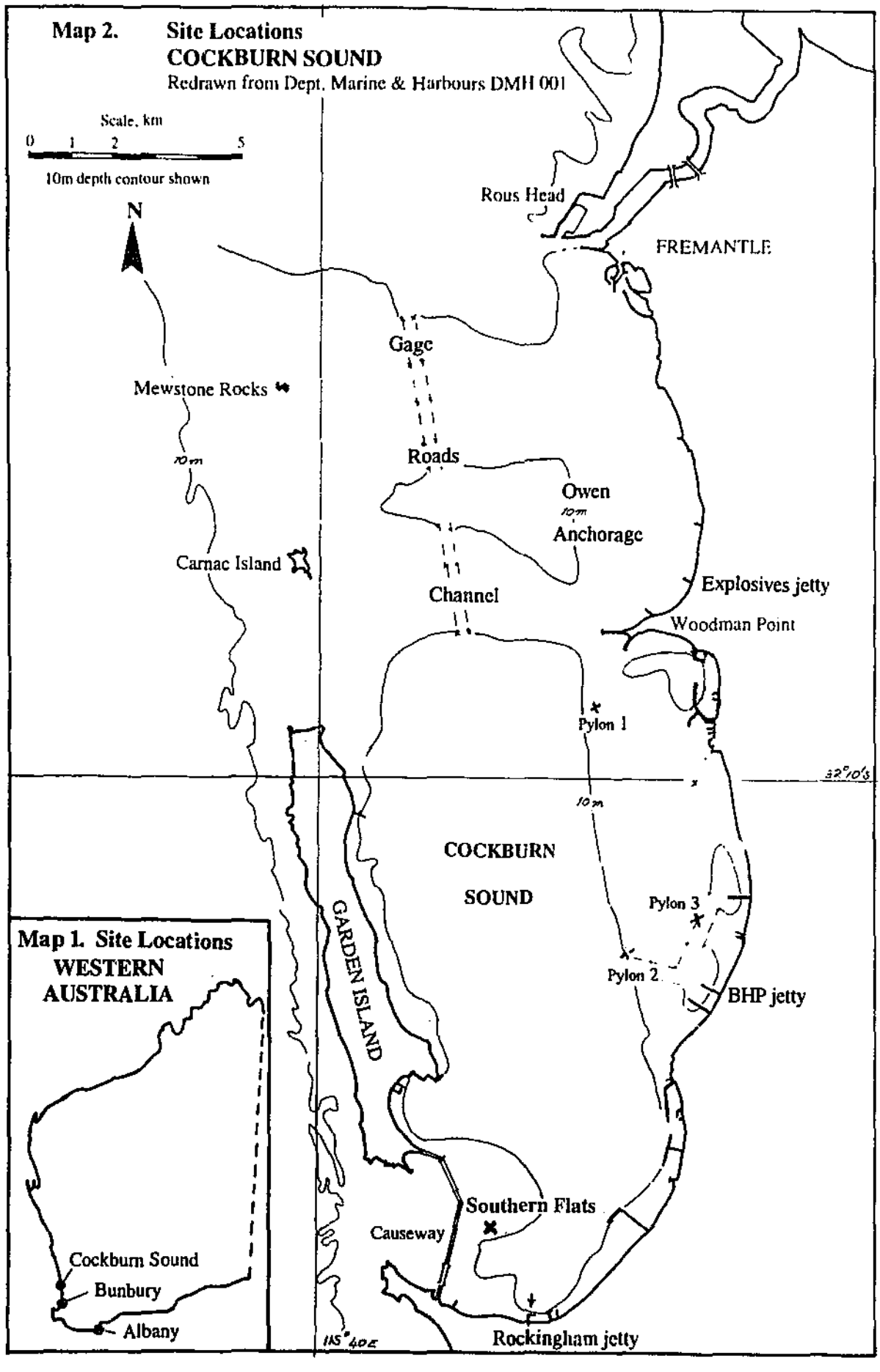
Structure of the thesis

Because there are several different components of this study, each with different methods and hypotheses, they will be dealt with in separate chapters. This chapter, (Chapter 1) will introduce the study, its various components and the general literature. Chapter 2 will cover the field study to determine the biomass of *S. spallanzanii* in Cockburn Sound. Chapter 3 covers the laboratory investigation of filtration rate, and Chapter 4 the laboratory measurements of oxygen consumption rate. Finally, the main results of the previous chapters will be amalgamated in Chapter 5, to determine the feeding efficiency and filtration capacity of *S. spallanzanii*, and the implications for the management of this introduced species. Each chapter will deal with the specific literature relevant to the topic, and the methodologies used. The discussions within the chapters on biomass, filtration rate and oxygen consumption rate will concentrate on the results and the technical aspects of the findings, whereas the final chapter will focus on a synthesis of results from the previous chapters and the implications for the management of *S. spallanzanii*.

Description of the study site: Cockburn Sound

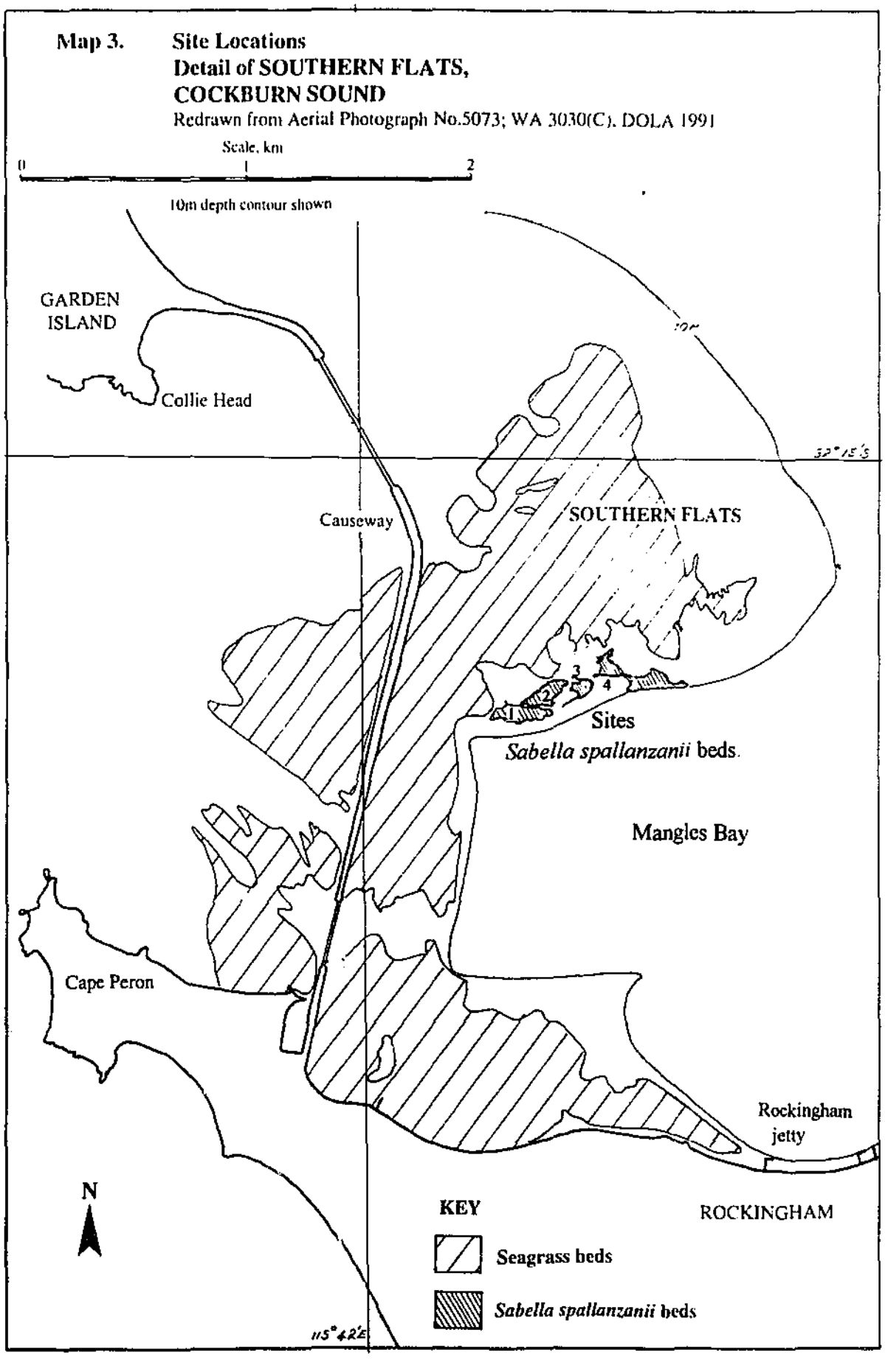
Cockburn Sound is a coastal embayment south of Fremantle, Western Australia. It is protected from the ocean by Garden Island on the western side and the shallow Parmelia bank on the northern side (Figure 1.1). The Sound has a deep central basin (18 to 20m), surrounded by shallow (< 8m) sediment platforms of 50m to 3km width. It is on one of these sediment platforms, the Southern Flats, that large beds of *S. spallanzanii* are located (Figure 1.2). The distribution of *S. spallanzanii* within Cockburn Sound is concentrated in beds on the Southern Flats. *S spallanzanii* covers an area of approximately 20 hectares on flat, shallow (4 to 5m) bottom (Clapin & Evans 1995). *S. spallanzanii* is also found on artificial structures such as jetty pylons, breakwaters, navigational markers and wrecks throughout the Sound and along the southern and eastern shore northwards to Fremantle (Clapin & Evans, 1995).

Figure 1.1. Site Location Maps 1 & 2.



Source, Clapin and Evans (1995).

Figure 1.2. : Site Location Map 3.



Source, Clapin and Evans (1995).

Chapter 2

BIOMASS IN COCKBURN SOUND

INTRODUCTION

In Cockburn Sound, large dense beds of *Sabella spallanzanii* occur on the flat, shallow (4 to 5m), sandy bottom of the Southern Flats (Clapin & Evans, 1995). *S. spallanzanii* also occurs on artificial structures such as jetty pylons, navigational marker pylons, marinas, breakwaters and wrecks. During a preliminary survey, Clapin and Evans (1995) found *S. spallanzanii* on all the jetties and pylons surveyed throughout the Sound, along the southern and eastern shore northwards to Owen Anchorage, the Gage Roads channel and Fremantle. During that survey, visual estimates of density were recorded but no quantitative data were collected, the only other data was a preliminary sample from one site on the Southern Flats when the species was first discovered (Lemmens, Clapin, Greenway, Lavery and Cary, in press). Thus, there were no data with which to determine the biomass of *Sabella spallanzanii* in Cockburn Sound. The present study quantified this biomass by sampling representative areas from the Southern Flats, jetties and navigational marker pylons.

Aims

The aims of this section were to determine the density and biomass of *Sabella spallanzanii* in Cockburn Sound and where the majority of that biomass is distributed: on the Southern Flats area or on artificial structures such as jetties and navigational marker pylons, so that the relative importance of these habitats can be established. Secondly, to determine whether there is seasonal variation in biomass or the size of individuals so that changes such as growth or recruitment may be detected and to give an indication of the dynamics of the population.

More specifically, the following questions are addressed in this section:

1. What is the biomass of *Sabella spallanzanii* in Cockburn Sound.
2. Is there a seasonal change in the biomass or size of *S. spallanzanii* in Cockburn Sound.
3. Whether the majority of that biomass is distributed on the Southern Flats area or on artificial structures such as jetties and navigational marker pylons.

METHODS

Study site

Sampling effort was concentrated on determining the density and biomass of the Southern Flats beds because they are the largest area of *S. spallanzanii* found on natural substrate. Clapin and Evans (1995) made a visual density estimate of these beds and indicated that there were differences. For this reason four of the large beds of *S. spallanzanii* on the Southern Flats were sampled (Fig. 1.1). Artificial structures however, cannot be ignored as an important substrate, so three randomly selected jetties and three navigational marker pylons were sampled on the southern and eastern edges of the Sound (Fig. 1.1). The sites on the Southern Flats had similar a depth of 4 to 5m and substrate of sand and shell, which was once covered by seagrass beds as there were remains of seagrass rhizomes. The jetties selected were the disused Explosives jetty just north of Woodman Point, the Broken Hill Pty. steel refinery jetty (BHP) and the Rockingham jetty in Mangles bay (Fig. 1.1). These jetties are well spaced from north to south in the Sound and each were considered representative of other nearby jetties in terms of size, structure and position. The pylons selected were located from the north, near Woodman Point, to Kwinana in the south (Fig. 1.1).

Preliminary sampling and determination of sample size

Because of the apparent clumping habit of *S. spallanzanii*, the distribution of individuals within large beds is very patchy. For this reason, preliminary sampling to determine an appropriate quadrat size and number of replicates was carried out early during the project (August 95) at the Southern Flats. Counts of individuals were made within ten random replicates of each of three quadrat sizes (0.25, 0.5 and 1.0m^2).

Precision (p = S.E. / mean count m^{-2}) was calculated for each quadrat size and increasing numbers of replicates. The unit (in this case, number and size of replicates) with the smallest value of p will give the most precise estimate (Andrew & Mapstone, 1987). The smallest quadrat size, 0.25m^2 (Fig. 2.1) gave the least precise result (p = 0.43, n = 10). There was a marked increase in precision for the 0.5m^2 quadrat (p = 0.15, n = 10) then only a small increase for the 1.0m^2 quadrat (p = 0.14, n = 10). Therefore, while there was an advantage in increasing the quadrat size from 0.25 to 0.5m^2, there was very little advantage in doubling the size again to 1.0m^2. A further advantage of the 0.5m^2 quadrat is that it was found to be an easily manageable size underwater, whereas the 1.0m^2 quadrat and the very large amount of material to be collected from it was much more difficult to handle. Therefore, the extra cost in time and effort was not worth the small gain in precision.

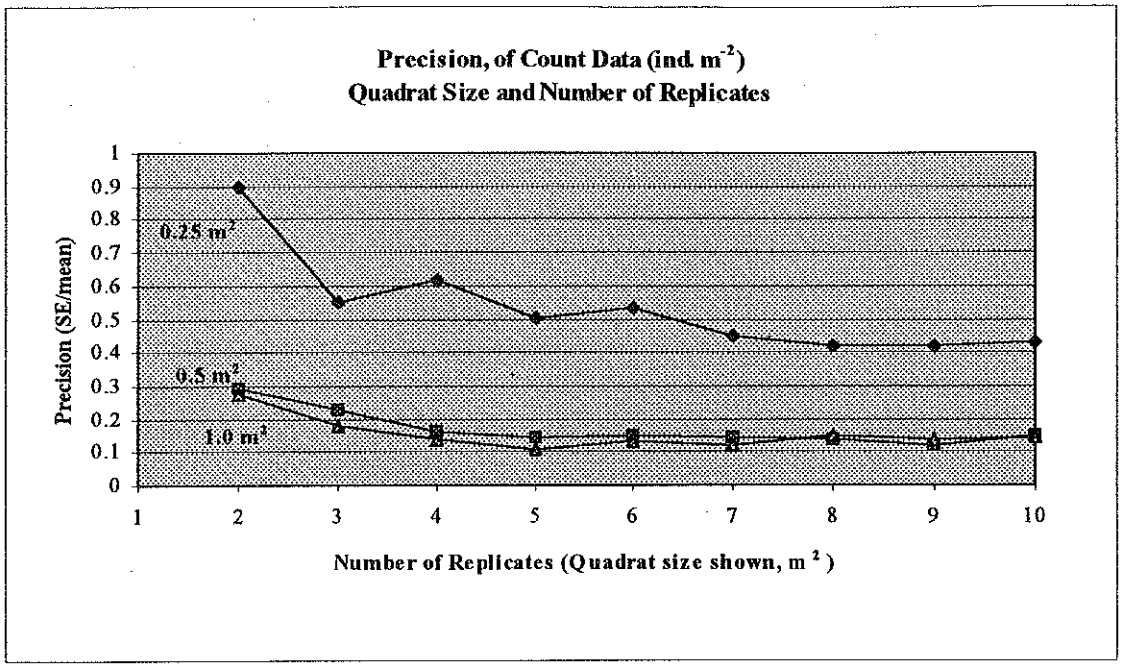


Figure 2.1. Precision (S.E. / mean of count data, ind. m^{-2}) with increasing number of replicates at each quadrat size 0.25, 0.5 and 1.0 m^2.

It can be seen from Figure 2.1 that with a $0.5m^2$ quadrat, there was no real gain in precision above 4 replicates ($p = 0.16$, $n = 4$, to $p = 0.15$, $n = 5$). Based on the precision comparison and ease of handling, 4 replicates of a $0.5m^2$ quadrat was chosen as the most efficient sample number and size.

Preliminary investigation to determine an appropriate measure of biomass

Because of the large size of each sample (3 to 15kg wet weight per replicate) and the limited time and furnace space available, an alternative was sought to using ash free dry weight (AFDW). The obvious choice was the dry weight (DW) of worms removed from their tube, as this would give a measurement of the living part of the worm without the possibility of errors from including inorganic material in the tube. This was also a convenient measurement to compare biomass with filtration rate and oxygen consumption rate in Chapters 3 and 4 of this study and has been used

by other researchers of filtration rate and oxygen consumption rate in fan worms (e.g. Riisgård and Ivarsson, 1990 and Shumway, Bogdanowicz and Dean, 1988).

A preliminary trial was conducted to test whether body dry weight (without the tube) would be a reliable measure compared to AFDW of the worm including the tube. Fifty worms were removed from their tubes and individually weighed, dried and ashed.

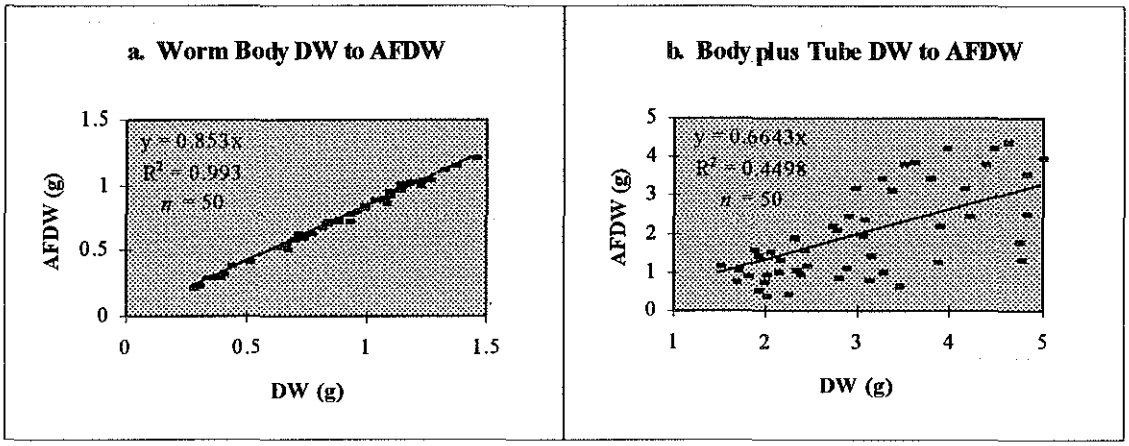


Figure 2.2. DW to AFDW regressions for: **a.** worm bodies and **b.** worm bodies plus tubes. The linear regression line and equations are shown on the figure.

The DW to AFDW of worm bodies produced a very strong positive linear regression with an R^2 of 0.993 (Fig. 2.2 a.) whereas the DW to AFDW of the worm bodies plus tubes produced a much poorer regression with an R^2 of 0.449 (Fig. 2.2 b.). The amount of inorganic material, such as sand and mud in the tube, varies between individuals and this causes a large variation in the ratio of DW to AFDW. As ashing of all samples collected was not possible, it was much more convenient and consistent to remove the worms from the tube and use DW of the worm body (including the crown) as a measure of biomass. A further useful measurement, mean DW per individual (body DW by total number in each replicate) may be used to detect changes in population structure such as growth or recruitment from one season to the next.

Sampling design, collection and processing

Sampling was carried out in winter 1995 (August - September), and in summer 1996 (January - February). This was expected to cover the range of seasonal conditions which may affect *S. spallanzanii* biomass and also permitted analysis of seasonal variation.

Using the rationale established above, four randomly located replicates of $0.5m^2$ were taken by a scuba diver from each of four sites on the Southern Flats. Because the jetties and pylons present a vertical substrate there was the possibility of stratification in biomass at different depths. To account for this possibility, sampling was stratified by taking three replicates at each of 3, 6 and 9 metres where depth was sufficient. Three jetties were sampled with three replicate pylons at each jetty and three navigational marker pylons were sampled, each being a replicate for a single 'pylon' category. Sampling on the jetties and marker pylons used a steel framed quadrat similar to that used on the Southern Flats, but which has been curved to wrap around the surface of the pylon to cover a surface area of $0.5m^2$. Because stratified sampling on jetties and pylons considerably increased the number of samples to be taken, the number of replicates was reduced to three. This helped keep the total number to a manageable size and should only slightly reduce the precision of sampling on the jetties and pylons.

For each quadrat, all *S. spallanzanii* and invertebrates were removed by hand, placed into plastic bags and labelled. Samples were transported in an insulated box to the laboratory and frozen (-20^0C) until processing. Specimens were carefully removed from their tubes, counted and placed into pre-weighed crucibles. The tubes were cleaned of any epifauna, counted and placed into trays. Epifauna and other invertebrates from each sample were placed in a freezer for storage until processing by a joint study (Lemmens, Clapin and Parker, in prep). Samples were dried in an oven at 80^0C until constant weight (5 to 8 days) and weighed for dry weight.

Area of *Sabella spallanzanii* coverage

The area covered by the beds of *S. spallanzanii* on the Southern Flats was determined by measurements from 1996 aerial photographs at a scale of 1:20000 (Department of Land Administration, Perth). It was not within the time or scope of this project to rectify the images however, to reduce errors caused by aberrations in the photographs, only images with the Southern Flats beds close to the centre point were used. Ground truthing was largely conducted during a preliminary study by Clapin and Evans (1995). However, additional field verification to check the extent of beds, was conducted using scuba, during this project. Photographs were overlain with a transparent sheet of 1mm graph paper and the area of *S. spallanzanii* beds was measured and calculated to square meters.

The surface area of pylons and jetties sampled was determined by counting the pylons and measuring their circumference and depth. The total area of jetties was estimated from multiplying the area of each jetty sampled by the number of jetties of a similar size in the region nearby (including the jetty sampled). There are 4 jetties of a similar size near the Rockingham jetty, 6 in the Kwinana region near the BHP jetty and 3 in the Owen Anchorage region near the Explosives jetty. This is possibly an underestimation because the other jetties in the Kwinana region, namely the Alcoa Australia, British Petroleum, Australian Steel Industries, Co-operative Bulk Handling and Kwinana Bulk Cargo jetties are all larger than the BHP jetty which was sampled.

The biomass of *S. spallanzanii* on jetties was calculated assuming that the other jetties in the same region each have approximately the same area and biomass. The biomass on the pylons was calculated using the mean biomass and mean pylon area from the 3 pylons sampled, multiplied by the number of similar sized pylons counted in Cockburn Sound (29) and the Gage Roads Channel (16).

Statistical analyses

Analysis of variance (ANOVA) was used to determine if there were significant differences in either biomass or worm size between sites and seasons. Then a Scheffe *F* test was used to identify where any significant differences occur. Prior to using ANOVA the data were checked for homogeneity using an F_{max} test and where necessary, transformed log (x+1) and checked again (Fowler and Cohen 1993).

RESULTS

Table 2.1. **Summary table: Area of *Sabella spallanzanii* coverage and biomass**

Measured area		Estimation of Total area		Mean Biomass	Total Biomass
Southern Flats	Area m^2		Area m^2	$g\ m^{-2}$	kg
Site 1	16800		16800	164	2760
Site 2	10000		10000	279	2787
Site 3	6000		6000	387	2323
Site 4	3200		3200	203	651
		Estimated Total area m^2	**36000**	**Total**	**8521 kg**
Jetties	Area m^2	Number of jetties of similar size		$g\ m^{-2}$	kg
BHP	4253	Kwinana area = 6	25518	57	1452
Explosives	918	Owen anchorage area = 3	2754	29	80
Rockingham	156	Rockingham area = 4	624	122	76
		Estimated Total area m^2	**33149**	**Total**	**1608 kg**
Pylons	Area m^2	Number of other pylons of similar size		$g\ m^{-2}$	kg
Area per pylon	23.9	Within Cockburn Sound = 29	693.1	Mean = 31	21
		Gage Roads Channel = 16	382.4		12
		Estimated Total area m^2	**1076**	**Total**	**33 kg**
				Total Biomass	**10162 kg**

Table 2.1. Summary table: Area of *Sabella spallanzanii* coverage and biomass. The total estimated area of Southern Flats *S. spallanzanii* patches, jetties and pylons is given in m^2. Mean biomass ($g\ m^{-2}$) for each site is the mean of all replicates, depths and seasons. Total biomass (kg) was calculated from the biomass of individual sites, mean of each site by the estimated area of coverage for that site.

The total area on the Southern Flats covered by *S. spallanzanii* is only slightly larger ($36000m^2$) than that of the jetties ($33149m^2$). However, because the mean biomass m^{-2} on the Southern Flats was considerably greater, the total biomass was much greater than the jetties (Table. 2.1). The

pylons represent a much smaller total area and biomass than the other two categories, with an area of 1076m^2 and a total biomass of only 33kg. The Southern Flats has a significantly higher mean biomass (258 ± 49 gDW m^{-2}) than either the pylons (31 ± 20) or jetties (69 ± 28) (ANOVA P = 0.0002, *df* = 2).

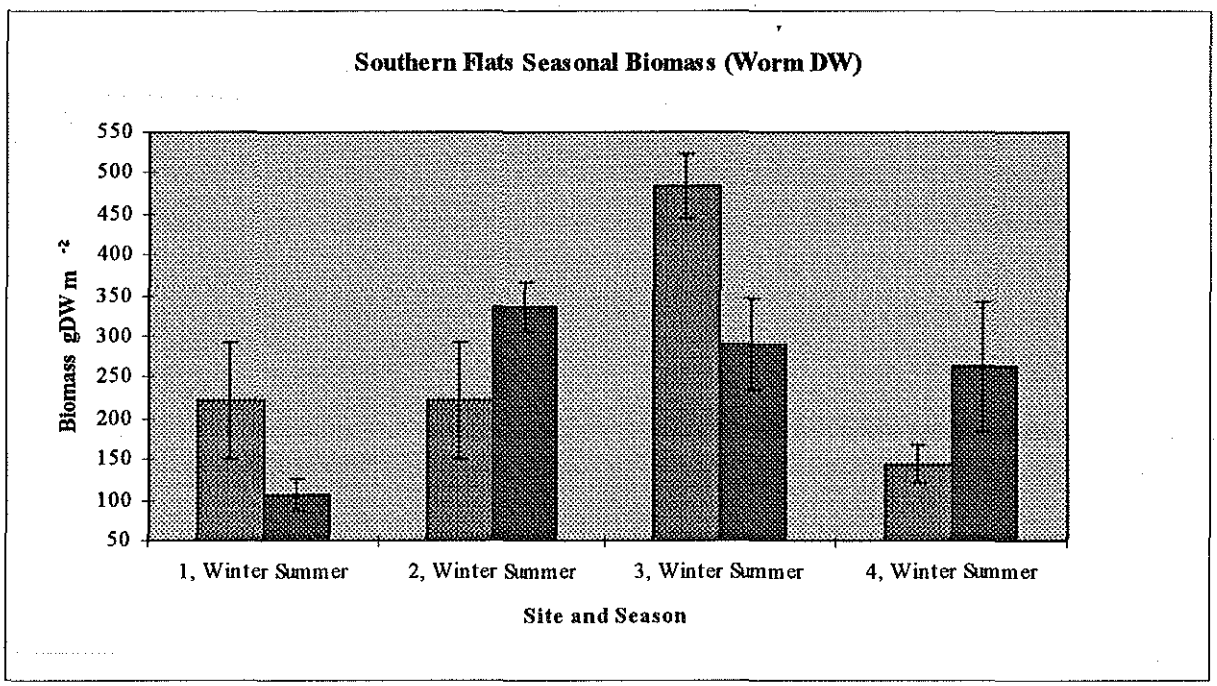


Figure 2.3. Seasonal mean biomass ± S.E. of *Sabella spallanzanii* for the Southern Flats. Biomass is given as mean worm body dry weight m^{-2} (excluding tubes) mean of 4 replicates at each site for winter and summer.

While there were noticeable spatial and temporal trends in biomass at the Southern Flats (Fig. 2.3.), these were not significant (two way ANOVA, between: seasons P = 0.733; sites P = 0.524; interaction P = 0.832, *df* = 1, 3 & 3 respectively). This is most likely due to high standard error and seasonal changes in biomass which did not follow a consistent trend between sites, while there was a decrease in biomass from winter to summer at sites 1 and 3, there was an increase at sites 2 and 4.

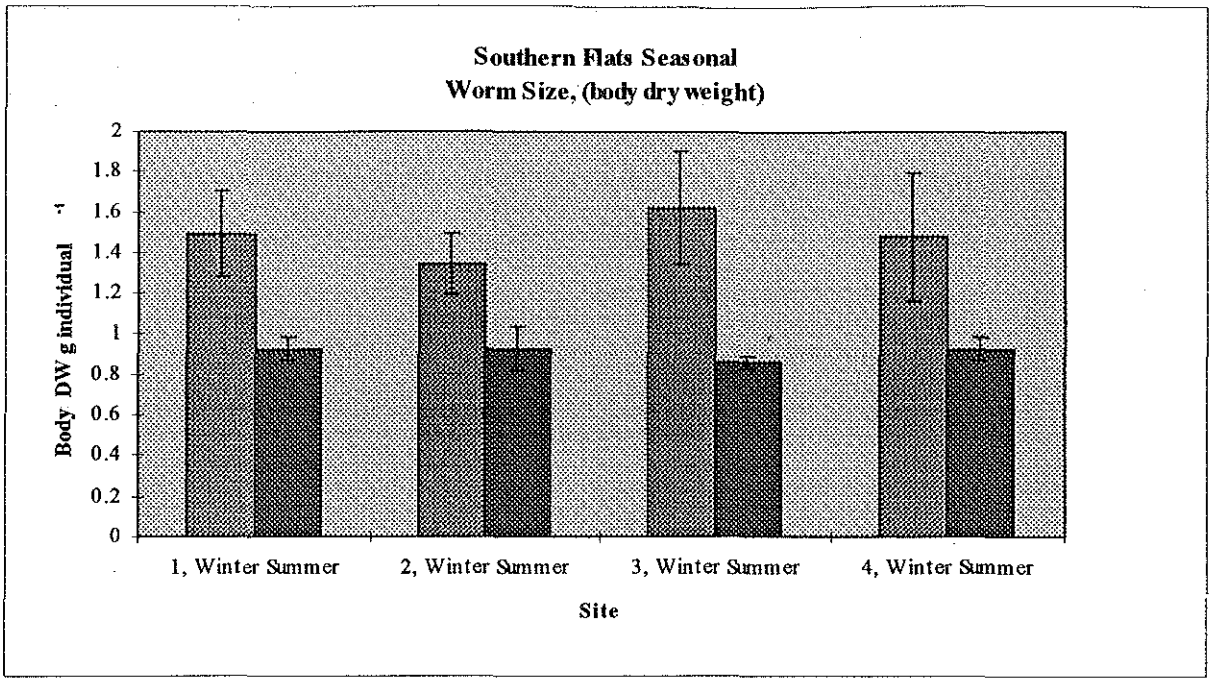


Figure 2.4. *Sabella spallanzanii*, seasonal worm body size g DW per individual at the Southern Flats. The worm body dry weight per individual (g DW ind^{-1} excluding the tube) shown is the mean ± S.E. of 4 replicates at each site for winter and summer.

Despite the inconsistent variation in biomass between sites and different seasons on the Southern Flats (Fig. 2.3), all sites showed a significant decrease in mean worm size from winter to summer (Fig. 2.4), (two way ANOVA, between: seasons $P < 0.0001$; sites $P = 0.657$; interaction $P = 0.31$, $df = 1, 3$ & 3 respectively).

This seasonal difference in mean worm size was not apparent on the jetties or pylons and there were no significant differences (ANOVA, between seasons: Explosives jetty $P = 0.92$; BHP jetty $P = 0.75$; Rockingham jetty $P = 0.78$; pylons $P = 0.82$).

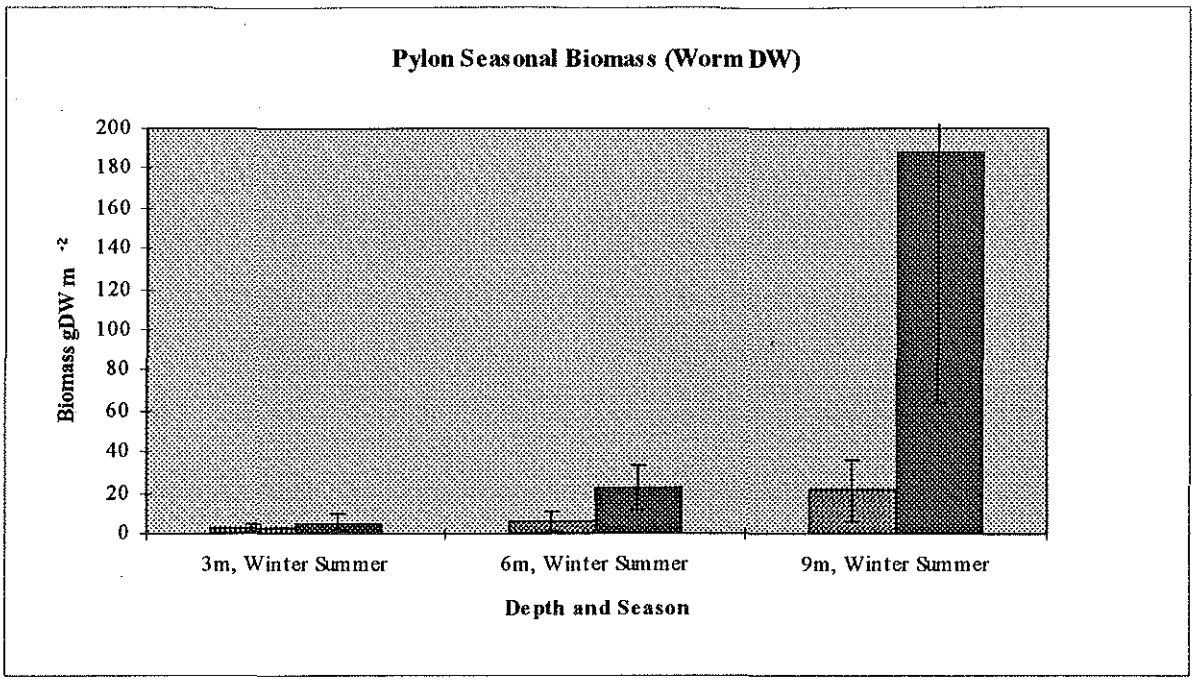


Figure 2.5. Seasonal mean biomass ± S.E. of *Sabella spallanzanii* for pylons. Biomass is given as mean worm body dry weight m^{-2} (excluding tubes), of 3 replicates at each depth for winter and summer.

Distinct spatial and seasonal patterns in biomass were noted for the pylons (Fig. 2.5). There was a clear increase in biomass with depth in both seasons and there was also an increase in biomass from winter to summer at all depths. However, because of large standard errors, these differences were not significant (ANOVA two way between: seasons P = 0.283; depths P = 0.287; interaction P = 0.49, *df* = 1, 2 & 2 respectively).

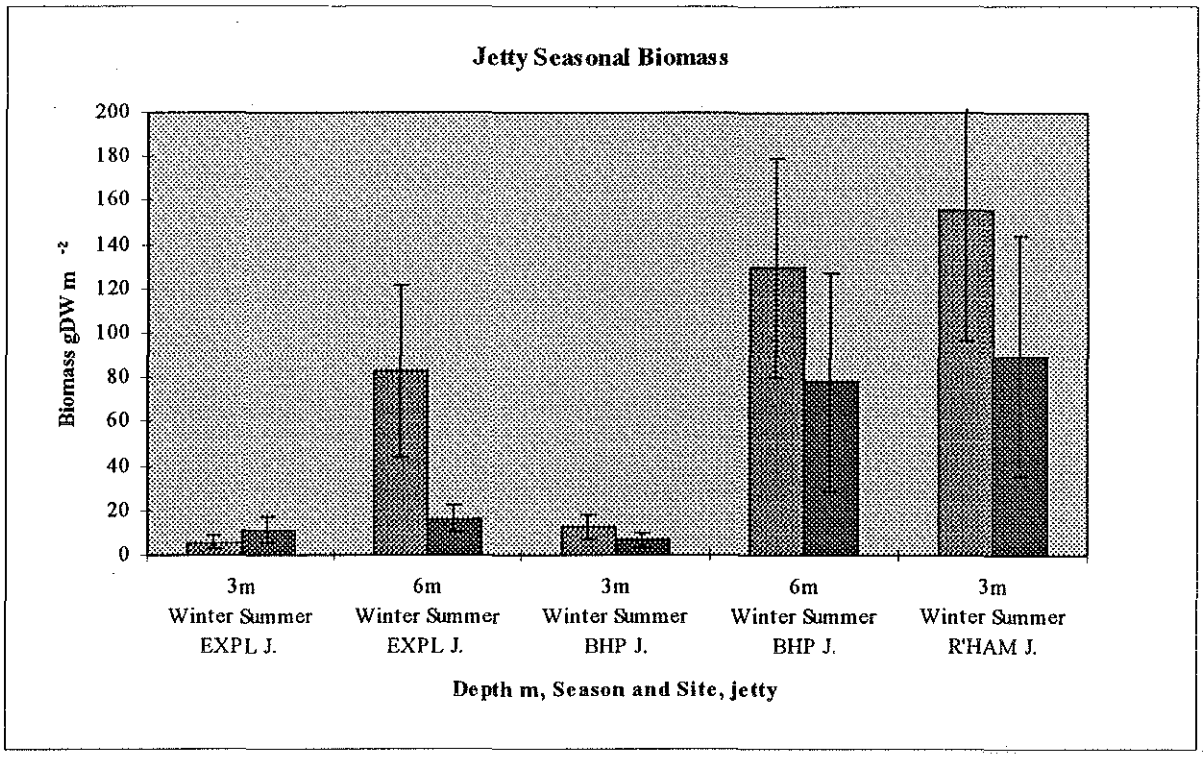


Figure 2.6. Seasonal mean biomass ± S.E. of *Sabella spallanzanii* for the Explosives jetty (Expl. J.), BHP jetty (BHP J.) and Rockingham jetty (R'ham J.). Biomass is given as mean worm body dry weight m^{-2} (excluding tubes) of 3 replicates at each depth for each jetty, winter and summer.

Biomass increased with depth at both the Explosives and BHP jetties (Fig. 2.6). While this difference in biomass between depths was significant at both jetties, it was not between seasons (two way ANOVA, between seasons and depths: Explosives jetty, seasons P = 0.416 and depths P = 0.013; BHP jetty, seasons P = 0.193 and depths P = 0.004). The Rockingham jetty was only sampled at 3m because of its limited depth, but has a greater biomass than either of the other jetties at 3m (one way ANOVA, P = 0.0002, *df* = 2). At 6m depth there was no significant difference in biomass between the BHP jetty and Explosives jetty (one way ANOVA, P = 0.14, *df* = 1).

DISCUSSION

Distribution of *Sabella spallanzanii* biomass within Cockburn Sound

Southern Flats vs artificial structures

The largest area and biomass of *Sabella spallanzanii* in Cockburn Sound are the dense beds on the Southern Flats. Although the Southern Flats beds are only slightly larger in area than the jetties (Table 2.1), the higher mean biomass m^{-2} on the Southern Flats yields a total biomass much greater than the jetties (8521 & 1608 kg respectively). The pylons represent a much smaller total area and biomass than the other two categories, with an area of $1076m^2$ and a total biomass of only 33kg. The Southern Flats therefore, represents the majority of the biomass of *S. spallanzanii* in Cockburn Sound and this may indicate that this shallow, but protected, sandy area is the preferred habitat of this species. This area has been greatly disturbed by the loss of seagrass (Cambridge, and McComb, 1984) and this disturbance may assist the invasion of *S. spallanzanii* through making space more available. *S. spallanzanii* makes up 94% of the total filter feeder biomass the Southern Flats whereas on the jetties and pylons it is only 3 to 9.5% of the total (Lemmens, Clapin and Parker, in prep.). The jetties are well covered with other invertebrates; space is not a readily available resource and therefore invasion of these habitats has been less successful (also see Chapter 5).

The estimated area of jetties does not include other artificial structures such as the Garden Island Causeway, Navy facilities, wrecks, marinas and rock breakwaters which could not be sampled within the constraints of this project. If the area of all jetties, pylons and other structures is considered, they are potentially a very important habitat for *S. spallanzanii* populations within Cockburn Sound. Additionally, the close proximity of these jetties to berthed shipping makes them ideal sources for further invasions of the species.

Patterns in distribution

Clapin and Evans (1995) found that *Sabella spallanzanii* occurs on the Southern Flats in an area of approximately 20Ha. The present study has found that within this area, dense beds which are visible in aerial photographs cover an area of approximately 3.6 Ha and there appears to be patterns in spatial distribution at several scales. Within the beds, distribution is patchy and although solitary individuals are common, worms tend to form large tangled clumps, attached to one common anchor which may be a piece of shell, a rock, an ascidian or one larger worm. On a larger scale, the patchy clumps form beds of 0.32 to 1.68 Ha, the density of these beds varing from 130 to over 437 ± 40 individuals m^{-2} and a biomass of 106 to 484 ± 39g body DW m^{-2}.

The distribution of *S. spallanzanii* biomass on artificial structures is also very patchy, but there does appear to be some trends in the data. On the jetties and pylons sampled, the biomass of *S. spallanzanii* increases with depth. This increase appeared to be very clear on the pylons but because of high standard error between replicates, was not significant (Fig. 2.5). These large differences between replicates were due to the randomly picked, replicate pylons being widely spaced in the survey area. Pylon 2 was closer to shore in a more protected position than the other pylons (Fig. 1.1) and it had 5 to 100 times their biomass. There was however, a significant increase in biomass with depth on the Explosives and BHP jetties (Fig.2.6). The Rockingham jetty was not deep enough to sample at 6m but the biomass at 3m was higher than that of the other jetties at 6m. This increase in biomass with depth may be partly related to shelter from wave action, as shallower depths would experience more vigorous disturbance from waves. This relationship to shelter is also seen when comparing the jetties. The Rockingham jetty which had a much higher biomass at 3m than the other jetties, is at the southern end of Cockburn Sound (Fig. 1.2) which is more sheltered than the positions of other jetties on the eastern side of the Sound. Pylons 1 and 3 are further from the shore and more exposed than the jetties (Fig. 1.1), they have a lower biomass at 3m and 6m than the jetties and the majority of their biomass is at 9m. This

may also hold true for the Southern Flats beds which are on the southern side of the Flats (Fig. 1.2 & 1.3). This location is well sheltered by the large shallow sand flats to the north, Garden Island and the Causeway to the west and to the south-west, Rockingham and Cape Peron.

Seasonal changes in biomass and worm size

It was expected that by sampling in different seasons, differences in biomass due to growth, mortality or recruitment could be detected. However, seasonal changes in biomass did not appear to follow any consistent trend. This result is quite different to that of Giangrande and Petraroli (1994) who found that *Sabella spallanzanii* in the Mediterranean Sea doubled in size from 10 to 20 cm in length over about one year. The population of *S. spallanzanii* on the Southern Flats consists of mostly large worms from 25 to 35 cm in length and very few or no small worms or juvenile recruits were observed during the sampling dives or in the samples. This would indicate that the Southern Flats population is one of large mature worms which are not growing at the rapid rate reported for small individuals by Giangrande and Petraroli (1994) and hence, no increase in biomass was observed.

Following from the above, comparison of worm body size at the Southern Flats between seasons gave an unexpected result. While no there was no significant difference between sites, there was a significant decrease in worm body size from winter to summer. Since there were no small worms or recruits in the population, this decrease in worm body size from winter to summer is not due to a recruitment event and therefore the larger worms must have reduced in biomass. It is possible that this decrease in body size is the result of spawning. Dales (1969) noted that in *Sabella spallanzanii* between two-thirds and three-quarters of the total body weight is expended annually in the form of gametes. There is no additional data such as gonad maturation or observations of spawning to back this up and hence, it can only be considered as a possibility. However, since no

other feasible explanation is apparent, this is considered the most likely possibility and warrants further investigation into the reproductive biology of the species in Cockburn Sound.

Differences in worm body size from winter to summer on the jetties and pylons did not show the same clear decrease as for the Southern Flats. Observations of the *S. spallanzanii* populations on the jetties and pylons indicated that there was a large range in size from 0.05 to over 3 g DW ind.$^{-1}$ with many large worms of 20 to 30 cm length and some very small recruits of only 3 to 5 cm. Whereas the Southern Flats population is of relatively even size, which may have resulted from one major recruitment event, the jetties and pylons support a population of varying size with evidence of some recent recruitment.

This large mature population of *S. spallanzanii* on the Southern Flats may act as the seed stock, releasing spawn which is carried away to the north-east by a counter clockwise summer current in the Sound (e.g. Steedman and Craig, 1983). This would help explain the lack of recruits on the Southern Flats and the opposite on jetties and pylons to the north-east. The biomass and density of *S. spallanzanii* reported here is 4 to 6 times that reported in the Mediterranean by Giangrande and Petraroli (1994) and in the absence of any other data on the density of mature populations in its place of origin, it is fair to suggest that this introduced species is thriving in the disturbed habitats of Cockburn Sound. The very large and widely distributed biomass of *S. spallanzanii* in Cockburn Sound will make any attempts to remove the species very difficult indeed, if not impossible. This data will be used in Chapter 5 to determine the filtration capacity of *S. spallanzanii* and as a base line which will be useful to assess future changes in its population in Cockburn Sound.

Chapter 3

FILTRATION RATE

INTRODUCTION

Filter feeders may have considerable potential to control phytoplankton levels by their filtering activity. This effect is particularly evident when there are large numbers of filter feeders in shallow waters (e.g. Officer *et al.* 1982; Alpine and Cloern, 1992; and Hily, 1991). *Sabella spallanzanii*, like other Sabellid polychaetes, feeds by extending its crown of fine filaments into the water column and with the action of beating cilia, pumps water through the filaments where particles are retained (Nicol, 1930; and Jørgensen, Kiørboe, Møhlenberg and Riisgård, 1984). The rate at which water is cleared of particles over time is often termed filtration or clearance rate (e.g. Jørgensen, 1966), (in this case the term filtration rate will be used). In order to determine the effect that *S. spallanzanii* may have in Cockburn Sound through filtering, it is necessary to know both its filtration rate per unit of biomass and the biomass of the population in Cockburn Sound. The biomass of *S. spallanzanii* in Cockburn Sound was reported in Chapter 2 and in this chapter the filtration rate is determined.

Evidence of effects of temperature and algal cell concentration on filtration rate.

Effects of temperature

Filtration rate generally increases with temperature. A positive relationship has been shown between filtration rate and temperature in the ascidian, *Ciona intestinalis* (Petersen & Riisgård, 1992), the mussel *Mytilus edulis* (Jørgensen *et al.*, 1990) and for *Sabella penicillus* (Riisgård & Ivarsson, 1990). If the same is true for *Sabella spallanzanii*, then it is likely that with seasonal

temperature changes there will be a difference in the filtration potential of the population in Cockburn Sound and hence a difference in the impact it has through filtering phytoplankton.

There is also evidence to suggest that filtration rate may reflect the limits of the tolerable temperature range of a filter feeder. Petersen and Riisgård (1992) found that the filtration rate of *C. intestinalis* increased linearly only within a certain temperature range, but then decreased rapidly when a critical temperature of 21°C was exceeded. The decrease in filtration rate above this range may be a response to the subject experiencing stressful temperatures. Jørgensen *et al.* (1990) found that in *M. edulis* there is a tolerable temperature range, above and below which the subjects did not remain fully open and filtering. The lower limit of this temperature range may also be important. Fiala-Medioni (1978) found that at low temperatures (7°C) filtration in the ascidian *Phallusia mammillata* had stopped or was too low to measure. The evidence indicates that there is a tolerable temperature range for these filter feeders which filtration rate is likely to reflect.

The tolerable temperature range is not known for *Sabella spallanzanii* from the literature, but it may have an important bearing on the extent to which it can spread to warmer or cooler waters.

Algal cell concentration

Recent studies on *Sabella penicillus* (Riisgård & Ivarsson, 1990) and *Ciona intestinalis* (Petersen & Riisgård 1992) found that filtration rate was constant with increasing algal cell concentration up to a point where the gut of the subject was filled (gut saturation point) then filtration rate decreased. The effect of algal cell concentration on the filtration rate of *S. spallanzanii* is not known from the literature but there could be a similar gut saturation effect. If this is the case then along with temperature, cell concentration will be an important parameter in determining the filtration capacity of the *S. spallanzanii* population in Cockburn Sound .

Aims and hypothesis

The aims of this section are firstly, to determine the filtration rate of *Sabella spallanzanii* and whether this is dependent on temperature and/or algal cell concentration. Secondly, to determine the most appropriate and practical measurement (i.e. body or crown size) to apply the filtration rate of *S. spallanzanii* to the population sampled in the biomass section of this study (Chapter 2).

The following hypotheses are addressed in this chapter:

1. That the filtration rate of *Sabella spallanzanii* increases with temperature within a tolerable temperature range.
2. That the filtration rate of *Sabella spallanzanii* decreases with high algal cell concentration above 10×10^3 cells mL^{-1}.

Definition of Terms

Since some important terms used in this thesis vary in the literature, they are defined here:

Filtration rate, (or clearance rate) is the volume of water filtered (cleared) of particles per unit time (e.g. Jørgensen, 1966), assuming that particles are retained 100% efficiently.

Pumping rate, is the actual volume of water pumped or processed per unit time.

Particle capture efficiency or retention efficiency, is the efficiency with which particles of a given size are captured and retained (Jørgensen *et al.* 1984). If this is less than 100% then filtration rate will be less than pumping rate.

Feeding efficiency, (water-processing capacity, e.g. Riisgård & Ivarsson, 1990) is an indirect measurement of the volume of water filtered per unit of metabolic demand (oxygen consumed).

Filtration capacity, is the volume of water, which can potentially be filtered per unit time, by a group or whole population of filter feeders.

Literature on filtration rate methodology

Direct and indirect measurement

The filtration rate of various filter feeders has been measured by numerous authors using either direct or indirect techniques (e.g. Dales, 1957; Jørgensen, 1966; Bayne, *et al.*, 1976; Shumway, *et al.*, 1988; Riisgård, 1991; Riisgård & Ivarsson, 1990).

Direct measurement involves measuring the actual volume of water pumped by the animal (pumping rate). Riisgård (1991) measured the pumping rate of the polychaete, *Nereis diversicolor* by placing the animal in a glass tube between two chambers, so that the volume of water pumped from one to the other could be measured directly. This technique may be appropriate for species which pump water through a tube such as *N. diversicolor* (Riisgård, 1991), or siphon such as an ascidian. Sabellid polychaetes however, extend their feeding mechanism (crown) of fine filaments out of their tube into the water (Nicol, 1930), therefore direct measurement of pumping rate is not feasible.

Indirect measurement of filtration rate involves measuring the decrease in the concentration of particles in the water surrounding the animal over time and inferring the volume of water filtered. Petersen and Riisgård (1992) defined filtration rate as "clearance of 100% efficiently retained particles" and measured this as the volume of water cleared of algal cells per unit time. This technique was also used to measure the filtration rate of *Sabella penicillus* in a glass beaker (Riisgård & Ivarsson, 1990). As *S. spallanzanii* is closely related and of similar morphology to *S. penicillus* (Ewer, 1946) it would seem that this technique is also appropriate to measure the filtration rate of *S. spallanzanii* and hence this method was adopted here.

Calculation of filtration rate

Filtration rate (F) is calculated as: $F = (V / n\ t) \ln (C_0 / C_t)$, where V is the volume of the experimental container, n is the number of worms, t is time and C_0 and C_t are the concentrations of algal cells at times 0 and t respectively (Riisgård & Ivarsson, 1990). The use of this formula requires the following assumptions to be made: particle capture or retention by the subject is 100% efficient,and there is instantaneous mixing of the total water volume (Riisgård & Ivarsson, 1990; Petersen & Riisgård, 1992).

Particle retention efficiency

Only if particle retention efficiency is 100% will filtration or clearance rate be equal to pumping rate, which is the total volume of water processed. If particle retention is less than 100% efficient, then more water will be pumped by the subject to filter the same number of particles, leading to filtration rate being an underestimation of pumping rate. As the present study intends to determine any impact which *Sabella spallanzanii* may have as a result of filtering phytoplankton, the volume of water which is cleared of particles (filtration rate) is a more relevant measurement than pumping rate.

Jørgensen *et al.* (1984) found that particle retention by *Sabella penicillus* was close to 100% at the optimum size of 3μm diameter, with little change in efficiency up to 8μm. However, with decreasing particle size retention, efficiency rapidly declined to about 30% for 1μm particles. The choice of particle size is therefore important in approaching the assumption of 100% retention efficiency. Because *S. spallanzanii* is closely related to *S. penicillus* it is likely to have a similar optimum size (3 to 8 μm) for maximum particle retention and this should be reflected in the choice of algal cells used in filtration experiments.

Mixing

With regard to the assumption of instantaneous mixing of the total water volume in the experimental chamber, Riisgård and Ivarsson (1990) noted that this condition is more easily approached with only one individual in the chamber. This condition could also be improved by providing internal circulation of the water with a pump or air bubbler.

Cell concentration

Riisgård and Ivarsson (1990) stressed the importance of performing laboratory filtration experiments at natural algal concentrations, because if the concentration is too high the gut capacity of the subject will be saturated, leading to a decline in filtration rate. At algal cell concentrations below 4 x 10^3 cells mL^{-1} the filtration rate of *S. penicillus* was found to be high and constant, but declined at higher cell concentrations (Riisgård & Ivarsson, 1990). Using dense suspensions of graphite, Dales (1957) recorded a filtration rate about 55 times lower for *S. penicillus* than the rate measured using lower algal concentrations by Riisgård and Ivarsson. Presumably, the low filtration rate resulted from gut saturation with a high concentration of inert particles.

Lag-phase

Petersen and Riisgård (1992) reported a lag-phase in the filtration rate of *Ciona intestinalis*. Filtration rate was initially low when the subjects were first fed, then increased until reaching a maximum and constant rate. Identifying this lag-phase may be quite important when measuring filtration rate, because if included, it may cause an underestimation of the true rate. Petersen and Riisgård noted that the lower filtration rate for *C. intestinalis* reported by Randløv and Riisgård (1979) may have resulted because they did not notice this lag-phase phenomenon. To avoid the lag-phase Petersen and Riisgård only used stable filtration rates which were obtained some time after the first addition of algal cells.

METHODS

Experimental design

In order to determine the filtration rate of *S. spallanzanii* and whether it is dependent on temperature or algal cell concentration, two separate experiments were conducted. In the first, filtration rate was measured at four temperatures: 13^0, 17^0, 22^0 and 27^0C at the same initial algal cell concentration of 2.5 to 5 x 10^3 cells mL^{-1}. The temperatures 17^0 and 22^0C were chosen because they approximate winter and summer means in Cockburn Sound (Pearse, 1986) as well as both 5^0C lower and higher, hence $13^{0\ [Note\ 1]}$ and 27^0C. Six replicates and two controls were measured at each temperature.

The second experiment measured filtration rate at 4 different initial algal cell concentrations at a constant temperature of 22 ± 0.1^{0}C. In each experiment, the filtration rate was first measured at a low initial cell concentration of 2.5 to 5 x 10^3 cells mL^{-1} to establish the base rate for each individual. The worms were allowed to graze the concentration down overnight, (14 hours) then measured again at a higher concentration in the ranges of 10 to 15, 15 to 20 and 20 to 35 x 10^3 cells mL^{-1} and the two rates compared. Nine replicates and three controls were measured at each algal cell concentration. Cell concentrations were randomly assigned to different worms until enough replicates had been measured at each concentration.

Controls

To control for changes in algal cell concentration during the experiment due to factors such as settlement, coagulation or growth, two control aquaria were set up and sampled in an identical manner, but with an empty worm tube.

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[1]. Unfortunately it was not possible to cool the constant temperature room sufficiently to achieve a stable water temperature of 12^{0}C, so 13 ± 0.2^{0}C was used as the lowest temperature.

Collection and care of specimens

Specimens of *S. spallanzanii* were collected from the Southern Flats in Cockburn Sound and transported in an insulated container with aerated sea water to the University of Western Australia Marine Biology Laboratory at the Bernard Bowen Fisheries Research Laboratories, Waterman (or the CSIRO Marine Laboratories at Marmion). The worms were kept in aerated, flow-through sea water aquaria in a temperature controlled room for several days to acclimate temperature and handling prior to experimentation. During acclimation the worms were fed on algal monocultures of *Thalassiosira pseudonana, Dunaliella marina* or *Rhodomonas sp.* as well as any natural particles in the seawater.

Phytoplankton selection and rearing

Algal monocultures of *Thalassiosira pseudonana*, *Dunaliella marina* and *Rhodomonas sp.* were cultured by The CSIRO Marine Laboratories at Marmion. Freely suspended cells were collected by carefully decanting or siphoning off the supernatant avoiding any coagulated or settled cells on the bottom. In this way, only suspended cells were used in the experiment, reducing the chances of settling or coagulation of cells leading to an error in the measurement of filtration rate. Only the larger (~5 to 8μm) *Rhodomonas sp.* were used in the experiments, because they were more easily measured above background interference and bacteria than the other smaller species cultured.

Preliminary investigation

A preliminary mortality experiment was conducted to determine if the subjects could be maintained in aquaria long enough to acclimate and for the experiments to be conducted (i.e. 10 to 14 days). Worms were kept in 18 L aerated, flow-through aquaria (10 to 60 in each) for one month at 17 to 22^{0}C without food. Worms were handled and counted regularly. No mortalities were recorded over 32 days, however signs of stress were observed after 14 to 20 days; some

worms had autotomised (dropped) their crowns. Observations also showed that if the worms were handled roughly or disturbed repeatedly, they would stop feeding, withdraw into the tube and produce a thick mucus secretion.

This led to several precautions being taken to avoid or reduce the possibility of stress problems interfering with experimental results. Fresh specimens were collected every 6 to 10 days. To avoid the production of excess mucus, worms were handled gently and were discarded if they began producing mucus or lost their crown. Only worms which were regularly open and filtering were used in experiments. Worms used for temperature experiments were adjusted slowly to that temperature over several days (< 2^{o}C change per day) then acclimated to that temperature for at least 48 hours.

A preliminary filtration rate trial indicated that the time length of the lag-phase in starved *Sabella spallanzanii* subjects was from 1 to 2 hours. To reduce the time required for recording a result, this lag-phase was avoided by starting feeding the subjects with a low concentration of cells (500 to 1500 cells mL^{-1}) for approximately 2 hours before starting the experimental measurements. This cell concentration was enough to stimulate the worms to feed, but much too low to cause gut saturation and hence have an effect on the experiment. Samples were taken every 30 minutes during this period to check that the worms were filtering. As per the procedure of Petersen and Riisgård (1992), only stable filtration rates were used, which were obtained some time after the first addition of algal cells (see calculation of filtration rate).

Experimental apparatus, set-up and testing

Tall aquaria (19 by 22cm wide and 45cm high) were specially constructed to accommodate worms in a natural upright position and without the extended crown touching the sides. Eight aquaria were set up in four 50 L plastic tubs, (two in each) within a constant temperature room. Julabo E-type temperature control units were connected to each bath and these units provided

water circulation within the baths (~15 L min^{-1}). Room temperature was set at 1 to 2 degrees lower than the desired water temperature and the water bath control units were used to maintain the aquarium water at the desired temperature. Aquarium water temperature was monitored for two days prior to each experiment and found to be stable within ± 0.1^{0}C for 17^0, 22^0 and 27^{0}C and ± 0.2 for 13 ^{0}C.

Mixing and aeration of the aquarium water was provided by an air bubbler. Mixing in the aquaria was first tested by adding a few drops of fluorocine and observing complete mixing. An air bubbler constructed from a glass pipette tip was found to create an approximately even mix within 10 to 15 seconds of introducing the dye. As the sampling interval for the experiment is 15 minutes, this mixing was considered to be as close to achieving the assumption of instantaneous mixing as was possible without disturbing the worm.

Procedure

The aquaria were filled with sea-water 36.5 ± 0.1‰ salinity, filtered to 1μm absolute (Cunuo filter No. PPK09NG010) to remove the bulk of the background particles in the range to be measured during the experiment (i.e. 3 to 10μm). Individual worms in their own tubes were placed in the aquaria and supported in a natural upright position with a stand made from PVC tubing. Worms were allowed to acclimate in the aquaria for approximately 24 hours. To reduce any effect from the build-up of waste products during this time, the aquaria were filled to near their maximum of 16 to 18 L. Faecal material was removed from the water prior to experimentation with a small siphon tube and the volume was topped up with clean filtered seawater.

Before starting the experiment the volume of each aquaria was reduced to a smaller known volume (6 to 10 L), because the change in algal cell concentration due to filtering by the worm

will be greater in a smaller volume, thereby increasing the accuracy. The volume was however, maintained large enough to permit the worm to fully extend its crown while feeding.

Phytoplankton cells (*Rhodomonas sp.*) were added to the water to reach the desired initial concentration. After allowing time for the water to be thoroughly mixed by the air bubbler, a 10mL sample was withdrawn and the initial water volume recorded. The first sample was measured for cell concentration at time zero (C_0). Subsequent samples were taken and measured every 15 minutes during the experiment. The cell concentration was returned to C_0 approximately each hour by adding *Rhodomonas sp.* cells and sampling was continued for up to 4 hours until a constant filtration rate was measured for each worm.

The concentration of algal cells mL^{-1} in each sample was counted on a Coulter Multisizer II model M/52RII with a 75μm aperture, set for a particle size range from 5.024 to 10.940 μm. This particle size range was selected to closely approximate the size of the *Rhodomonas sp.* cells used and to avoid measuring any smaller cells, particles or bacteria which would cause interference in the reading. To avoid any effect of settling or coagulation in the samples, all samples were measured within 15 minutes of sampling and each was thoroughly mixed by shaking before reading.

Calculation of filtration rate

Filtration rate (F) was determined by the reduction in algal cell concentration over time, as explained in the literature review on methods. However, as the lag-phase in the filtration rate of each worm varied, the mean rate over the entire experimental period would have been effected. As was explained earlier, to avoid the lag-phase, only stable filtration rates were used which were obtained some time after the first addition of algal cells (1 hour for concentration experiment). This was determined as the maximum filtration rate which was stable for at least 45 minutes (i.e. 4 of the 15 minute readings) where the $R^2 \geq 0.950$ for the slope of the concentration of algal cells.

This was verified during the experiment as a straight line on a semi-log plot (as per Riisgård and Ivarsson, 1990 p.251). The mean filtration rate over this 45 minute period was calculated by adapting the formula explained earlier $F = (V / n\,t) \ln (C_0 / C_t)$ to $F = - [(V \times slope) \times (60/1000)]$, where V is the aquarium volume (mL), *slope* is the slope of the ln (natural log) of cell concentration (cells mL^{-1}) as a function of time (min) and 60/1000 is used to convert to hours and litres. By checking the R^2 of *slope* this method has the advantage of giving an objective measure of whether the filtration rate of each worm has reached a level and remained stable.

An observation as to whether the worm was open or closed was made at the time each sample was taken. If during sampling, the worm was accidentally disturbed, had retracted into its tube and remained closed (not filtering) for more than a few minutes, the filtration rate would decrease. If this occurred then the R^2 of this data would be less than 0.95 and the data was excluded from the results until the worm was open and filtering normally again. This method did not exclude the worms' natural rhythm of opening and closing. It was quite common during the experiment to observe worms close then reopen and resume filtering without notably affecting the filtration rate.

Dry weight

At the end of the experiments the crown, body and tube of each worm were placed in separate pre-weighed crucibles and dried in an oven at 80^0C until constant weight. Weights were measured to 0.00001g on a Sartorias MC1 balance and the crucible pre-weight was subtracted to obtain dry weight (DW).

Crown measurement

The sum total length of crown-filaments was measured on a small sample of 12 worms. The spiralling crown of each was carefully dissected into small groups of filaments of relatively even length. The filaments were laid out on 1mm waterproof graph paper, counted and measured.

Statistical analyses

Temperature experiment

One way analysis of variance was used to determine if there were significant differences between filtration rates at different temperatures. Prior to using ANOVA, an F_{max} test was used to check for homogeneity of variance. The data were transformed log x+1 where necessary and checked again (Fowler & Cohen 1993). Because there was an uneven number of replicates, (only 2 at 13^0C and 6 at the other temperatures) a Tukey test could not be used (Fowler & Cohen). A Scheffe *F* test was used to identify where any significant differences occur between temperatures.

Cell concentration experiment

A 't' test for paired results (Fowler & Cohen 1993) was used to determine if there was a significant difference between filtration rate at a low cell concentration and each of the higher concentrations. As the 't'test assumes that the two samples have a similar variance, a two tailed F test was used to check for homogeneity, the data were transformed log x+1 where necessary and checked again (Fowler & Cohen).

RESULTS

Effect of temperature

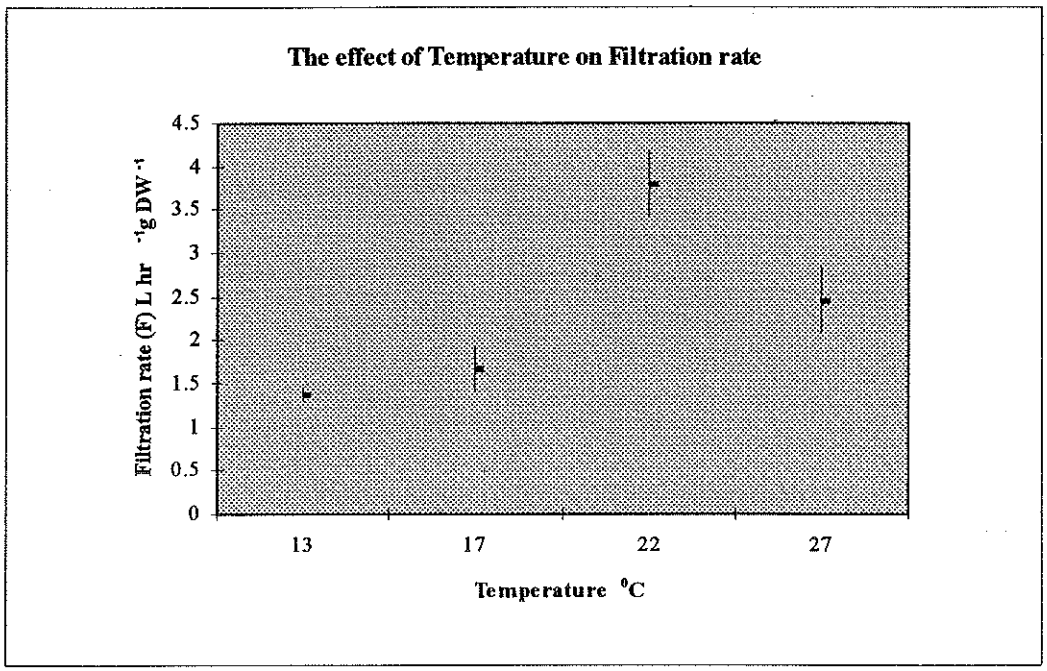


Figure 3. 1. The effect of temperature on filtration rate in *Sabella spallanzanii*. Mean filtration rate ± S.E. is shown at 13^0, 17^0, 22^0 and 27^0C ± 0.2^0C.

The effect of temperature on filtration rate is shown in Figure 3.1. Filtration rate per gram of worm body dry weight increases between 13^0 and 22^0C, then decreases sharply from 22^0 to 27^0C. Analysis of variance showed that there is a significant difference in filtration rate between temperatures (P = 0.0014). The source of this difference was between 17^0 and 22^0C as shown by a Scheffe *F* test (Scheffe P = 0.004). To reduce any effect of body weight on the temperature experiment, filtration rate has been normalised to per gram of body dry weight. The size of worms used as replicates at each temperature was not significantly different (ANOVA P = 0.713). The controls (not shown on the figure) showed that the effect on the results from changes in algal cell concentration due to growth, settlement, coagulation or errors in readings accounted for less than 0.66% of the mean filtration rate at each temperature (0.08% at 22^0 to 0.65% at 27^0C).

Effect of algal cell concentration

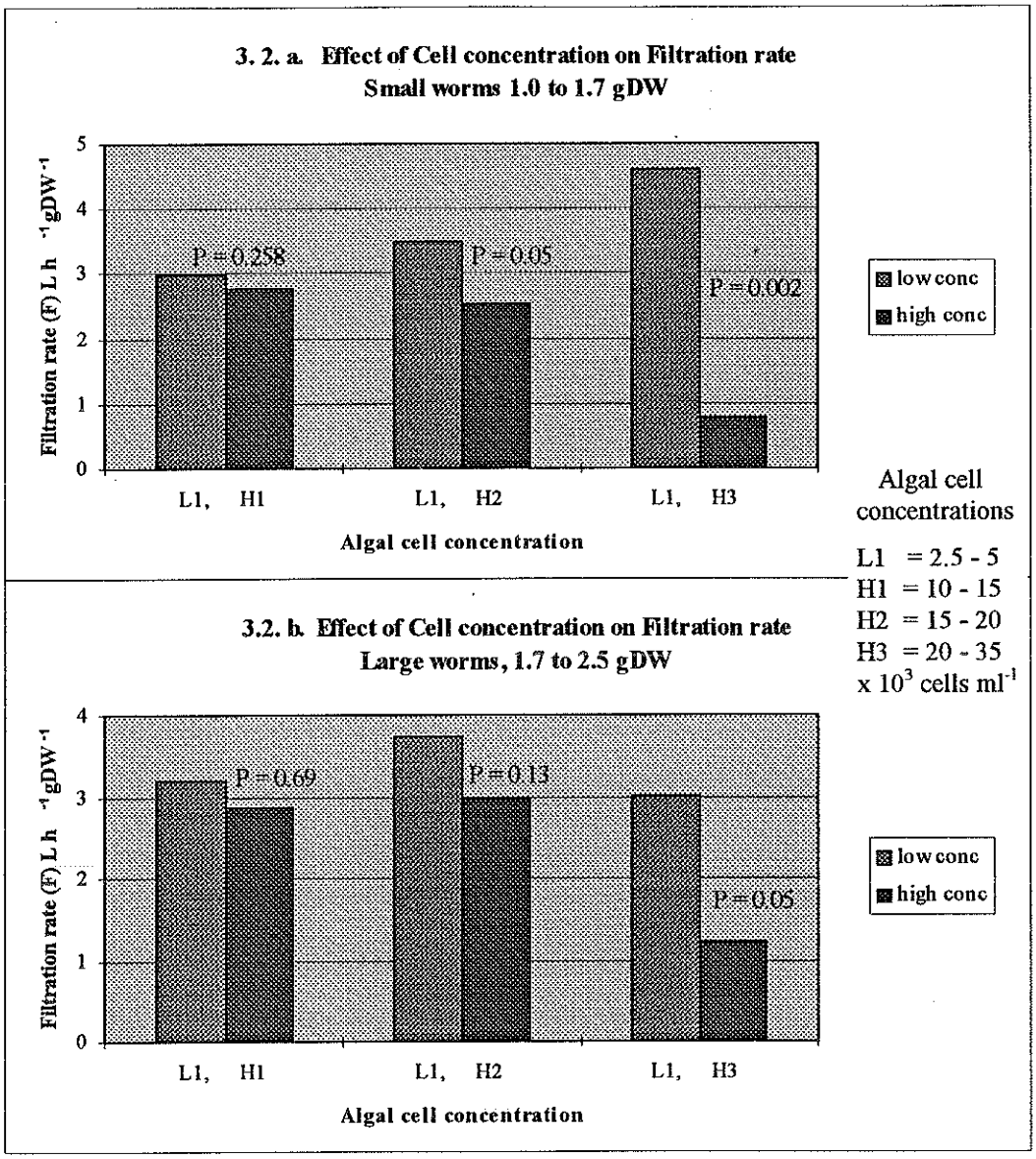


Figure 3.2. **a & b** *Sabella spallanzanii*. The effect of algal cell concentration on filtration rate in **a:** small worms (1.0 to 1.7 g body DW) and **b:** large worms (1.7 to 2.5g body dry weight DW). Each pair of bars shows the mean filtration rate (F, L h^{-1} gDW^{-1}) for the same worms at two different cell concentrations. The first bar in each pair is for a low cell concentration range of 2.5 to 5 x 10^3 cells mL^{-1} (L1), the second was at a higher concentration range of 10 to 15 (H1), 15 to 20 (H2) and 20 to 35 x 10^3 cells mL^{-1} (H3) respectively. For each pair the P value for a two tailed 't' test for paired results is given on the figure (Fowler & Cohen 1993).

The effect of algal cell concentration on filtration rate is shown in Figure 3.2 a and b.The mean filtration rate for the same worms is shown first for an initial low cell concentration (L1) then at a higher concentration (H1, H2 & H3), all measurements were started one hour after first dosing

with the desired algal cell concentration. When the results of all 9 replicates for each pair of concentrations were examined there was a significant decrease in filtration rate between the low (L1) and higher algal cell concentrations at both 15 to 20 and at 20 to 35 x 10^3 cells mL^{-1} (L1, H2 P = 0.007; L1, H3 P = 0.0004). When the results were compared by worm body size, larger worms (1.7 to 2.5 gDW, Fig. 3.2.b) did not show a significant decrease in filtration rate until experiencing the highest cell concentrations of 20 to 35 x 10^3 cells mL^{-1} (L1, H3 P = 0.05), while smaller worms (Fig. 3.2.a) showed a significant decrease in filtration rate at lower concentrations of only 15 to 20 x 10^3 cells mL^{-1} and at 20 to 35 x 10^3 cells mL^{-1} (L1, H2 P = 0.05; L1, H3 P = 0.002).

Effect of body and crown size

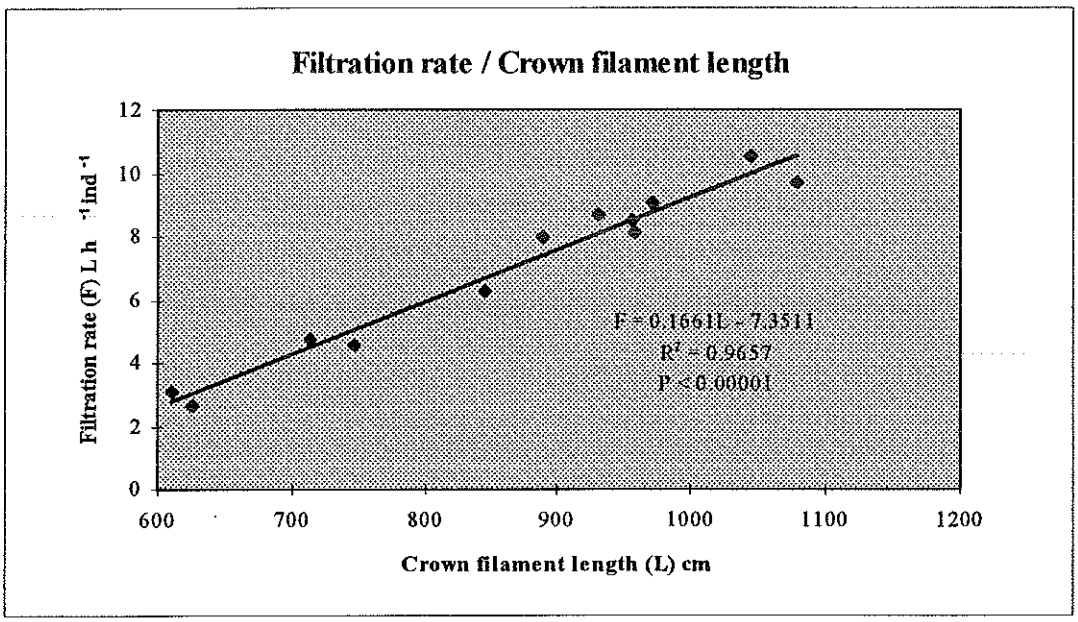


Figure 3.3. The relationship between filtration rate and crown-filament length of *Sabella spallanzanii* at 22°C. The equation and line of a linear regression are shown, along with the P statistic of an ANOVA for regression.

The total length of crown-filaments was measured on 12 worms whose filtration rate had been determined at 22°C and a cell concentration of ~2.5 x 10^3 cells mL^{-1}. There was a significant positive linear relationship between filtration rate and crown-filament length, (Figure 3.3., R^2 = 0.966 P < 0.0001).

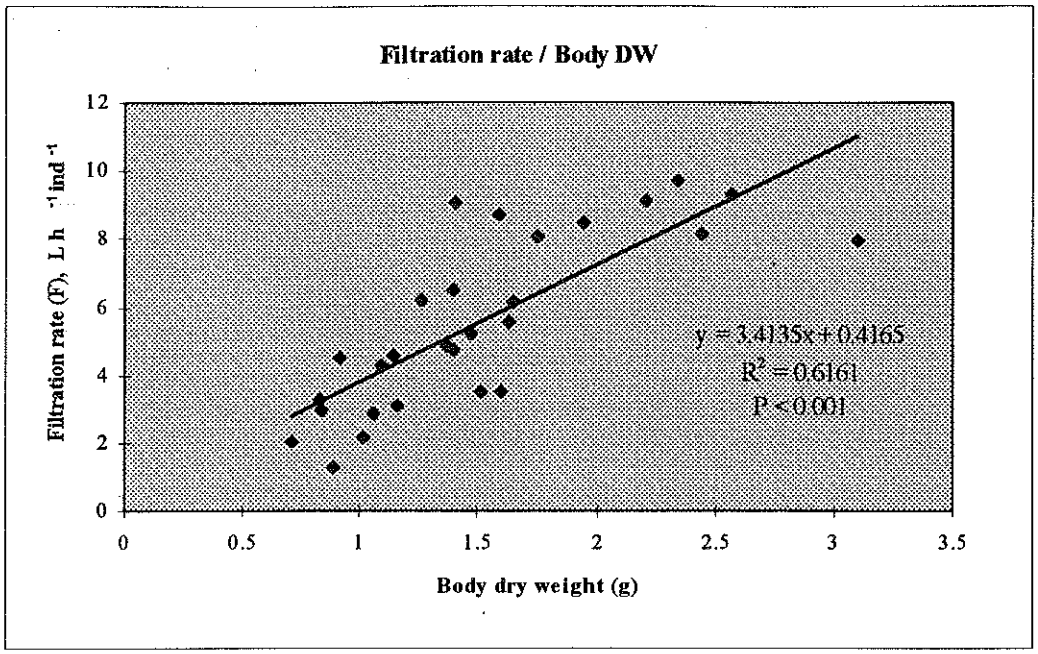


Figure 3.4. The relationship between filtration rate and body dry weight of *Sabella spallanzanii* at 22°C. The equation and line of a linear regression are shown, along with the P statistic of an ANOVA for regression.

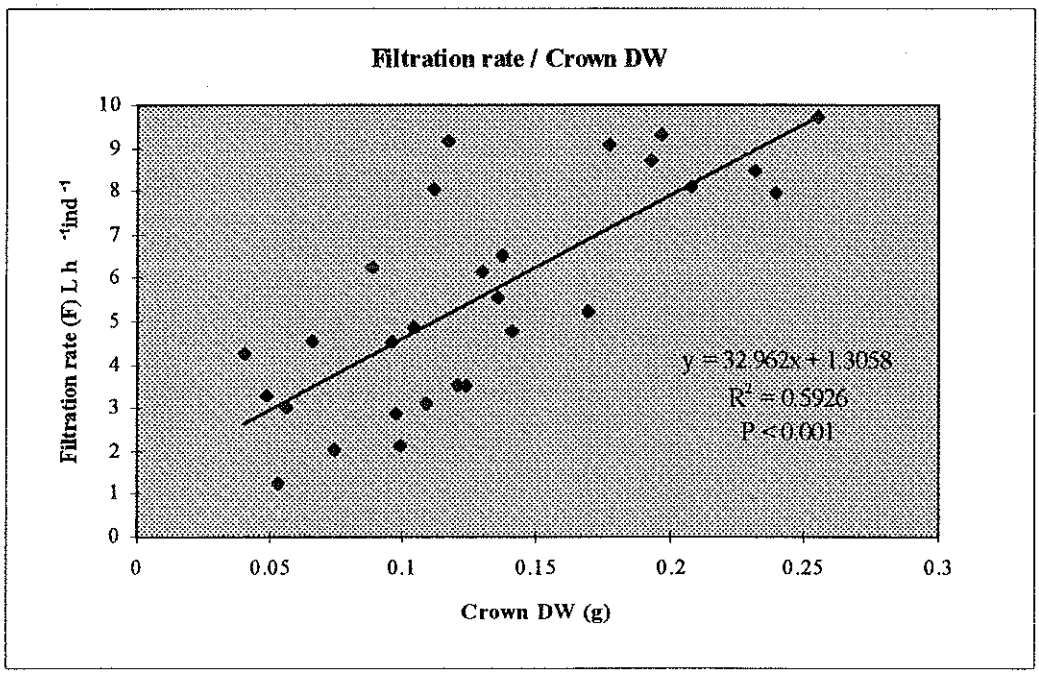


Figure 3.5. The relationship between filtration rate and crown dry weight of *Sabella spallanzanii* at 22°C. The equation and line of a linear regression are shown, along with the P statistic of an ANOVA for regression.

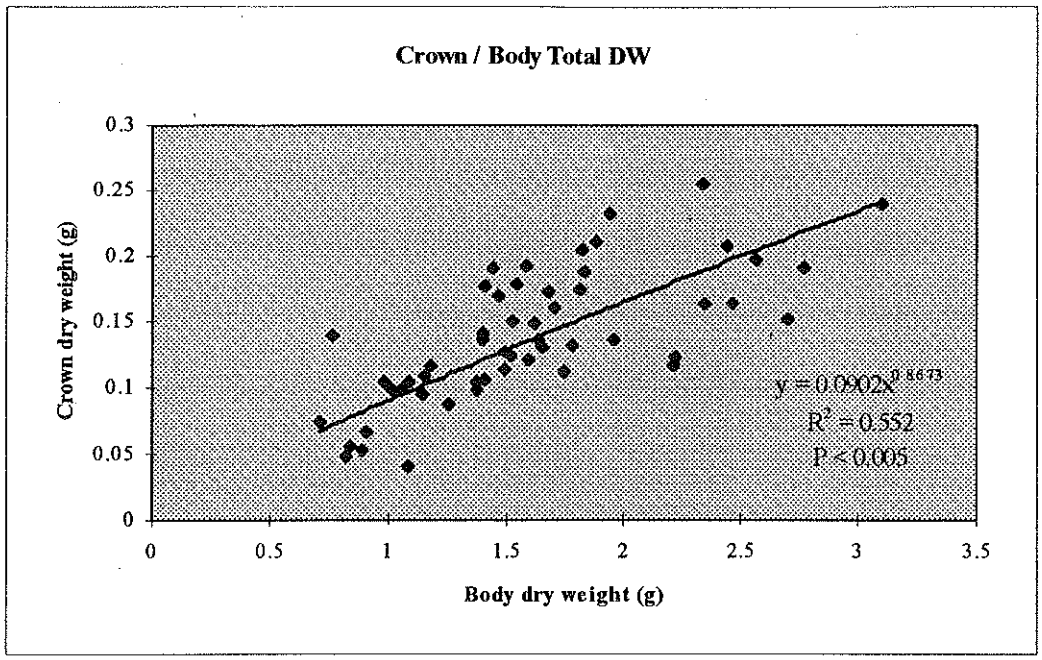


Figure 3.6. The relationship between crown and body (including crown) dry weight of *Sabella spallanzanii* at 22^{0}C. The equation and line of a power regression curve are shown, and the P statistic of an ANOVA for regression.

Body and crown dry weight

To help determine an appropriate measure with which to relate filtration rate to biomass, body and crown dry weight were each plotted against filtration rate of worms which had been measured at 22^{0}C and a cell concentration of 2.5 to 5 x 10^3 cells mL^{-1}. Figure 3.4. shows that there is a significant positive relationship between filtration rate and body dry weight (R^2 = 0.616, P < 0.001). Figure 3.5. shows the relationship between filtration rate and crown dry weight, while there is a significant positive relationship (R^2 = 0.593, P < 0.001), this is not as strong as that for filtration rate and crown filament length (shown earlier Fig.3.3). In both cases (Figs. 3.4 & 3.5), the line and equation of a linear regression are given because they showed the strongest R^2 value.

There is a positive relationship between crown dry weight and body dry weight (Fig. 3.6., R^2 = 0.552, P < 0.005). The equation for the regression line is expressed as a curve (power relationship) as this gave the strongest R^2 value.

DISCUSSION

Effect of temperature

The increase in filtration rate with temperature has been explained by various authors as due to either or both the decreasing viscosity of water with increasing temperature or by increasing metabolic rate with temperature. Riisgård and Ivarsson, (1990) found that for *Sabella penicillus* the correlation between filtration rate and temperature can be accounted for wholly by viscosity changes due to temperature and Jørgensen *et al.* (1990) found a similar result for *Mytilus edulis*. On the other hand, Petersen and Riisgård (1992) found that the ascidian *Ciona intestinalis* had a greater increase in filtration rate with temperature than that reported for either *S. penicillus* or *M. edulis*. They concluded that this increase suggests a substantial increase in ciliary activity with temperature and that it can not be explained solely by temperature-dependent viscosity. In the present study there is clearly an increase in filtration rate with increasing temperature in the range of 13^0 to 22 ^{0}C.

The increase in metabolic rate and hence activity with temperature is often modelled by the Q_{10} factor which is the proportional increase in metabolic rate over an increase of 10^0C. Riisgård and Larsen (1993) considered that the Q_{10} for biological processes is usually between 2 and 3 and thus if the Q_{10} for filtration rate is higher than 2 to 3 it indicates that physical effects including viscosity are making an impact. The Q_{10} for the filtration rate of *S. spallanzanii* is 3.08 for 13^0 to 22^0C and 5.1 for 17^0 to 22^0C, which is above the 2 to 3 suggested by Riisgård and Larsen, consequently both viscosity decrease and metabolic increase are likely to contribute to this filtration rate increase.

Filtration rate and tolerable temperature range

The first hypothesis was that, the filtration rate of *Sabella spallanzanii* will increase with temperature within a tolerable temperature range. The significant increase in filtration rate with temperature between 13^0 to 22 ^{0}C, tends to confirm this. Beyond the increase there was a marked decrease at a high temperature of 27^0C. Whether this is a gradual decline in filtration rate between 22^0 and 27^0C or a sharp cut off point as the lethal temperature is approached is unclear. However, 27^0C is very likely to be above the tolerable temperature for *Sabella spallanzanii*.

Observations at 13^0C found that the subjects did not stay open and filtering for long compared with higher temperatures; only 2 out of 8 worms acclimated to this temperature remained open for long enough to take measurements. Consequently, it was not possible to obtain measurements on the full 6 replicates at 13^0C and the result was calculated on only 2 replicates. Two possible scenarios could help to explain this: either 13^0C is below the optimum temperature range for *Sabella spallanzanii* or the acclimation period was insufficient. Even though the temperature was gradually decreased to 13^0C over four days, then held for 48 hours, this may have been insufficient to acclimate the subjects given that they were collected during summer from water of 20^0 to 22^0C. Unfortunately, time did not permit the experiment to be repeated with subjects collected during winter. *S. spallanzanii* is well established in Port Phillip Bay (Carey and Watson, 1992) where water temperatures are generally lower than in Cockburn Sound therefore, 13^0C is not likely to be below its tolerable range.

Effect of algal cell concentration

The second hypothesis was that, the filtration rate of *Sabella spallanzanii* will decrease with high algal cell concentration above 10 x 10^3 cells mL^{-1}, which the results confirm. However, the relationship does not appear to be a linear decrease, rather, filtration rate is high and relatively constant at low cell concentrations and then declines at concentrations around 15 to 20 x 10^3 cells mL^{-1}. Riisgård and Ivarsson (1990) suggested that the decrease in the filtration rate of *Sabella*

penicillus at cell concentrations above 4 x 10^3 cells mL^{-1} is because at higher concentrations the gut capacity is exceeded, thus leading to a lower filtration rate. Petersen and Riisgård (1992) found a similar relationship for the ascidian *Ciona intestinalis* and also found that the gut capacity was dependant on size; small ascidians reached gut saturation at lower cell concentrations (15 x 10^3 cells mL^{-1}) than large ascidians (20 x 10^3 cells mL^{-1}). The results of the present study tend to agree with these previous studies, with smaller worms showing a decrease in filtration rate at lower cell concentrations than larger worms (Fig. 3.2). As smaller worms are also likely to have a smaller gut capacity, the gut capacity explanation seems very likely to apply here.

Natural algal cell concentrations compared to experimental concentrations

The mean algal cell concentrations in Cockburn Sound from 1992 to 1994 were 160 ± SD110 cells mL^{-1} for summer and 184 ± SD118 cells mL^{-1} for winter, with the highest concentrations recorded in Mangles Bay (which is near the Southern Flats), with a mean of 260 cells mL^{-1} (pers. comm. Stuart Helleren, Curtin University). These cell concentrations are many times lower than the cell concentrations which caused a decrease in filtration rate of even the smaller worms and therefore, natural cell concentrations are very unlikely to cause gut saturation and effect the filtration rate of *S. spallanzanii* in Cockburn Sound.

It is possible that the results may have been affected by the difference between natural and experimental concentrations. Using lower concentrations was not possible as the accuracy of detecting a change above background noise was limited. An effect is unlikely because filtration rate was constant for up to 5 hours at cell concentrations of 2.5 to 5 x 10^3 cells mL^{-1}, indicating that the specimens were able to feed continually at this level without reducing the rate.

Effect of body and crown size

In order to find an appropriate measure to relate filtration rate to worm body size or biomass several parameters were explored. Body dry weight appears to be the most practical of the measurements to determine the potential filtration capacity of *Sabella spallanzanii* populations. This is also quite convenient, as body dry weight was an easy measurement to obtain from the large samples collected in the field component of this study to determine the biomass of *S. spallanzanii* in Cockburn Sound. Filtration rate per gram of body dry weight and biomass can thus be used to calculate the filtration capacity of the *S. spallanzanii* population per unit area.

Crown-filament length gave the strongest relationship with filtration rate (Fig. 3.3) which agrees with the comment of Riisgård and Ivarsson (1990) that crown-filament length may be regarded as an indirect measure of filtration rate. Measuring crown-filament length was quite a painstaking process, so despite this strong relationship, the measurement does not seem very practical to relate to the biomass study to determine the filtration potential of a *S. spallanzanii* population.

As the crown is the organ responsible for filtering, I would have expected crown dry weight (Fig. 3.5) to show a stronger relationship with filtration rate than body dry weight (Fig. 3.4), however, this was not the case. The explanation for this may be in the structure of the crown itself. The crown consists of two lateral lobes which bear the filaments (Nicol, 1930). The base of these lobes is quite fleshy and would account for a large portion of the crown weight compared to the fine filaments. If this base was removed from the filaments, the weight should have a much stronger relationship to the filtering unit, the filaments. Crown-filament dry weight would be a much easier measurement to obtain than crown-filament length. For future studies it may be useful to express filtration rates per unit of crown-filament length or dry weight to allow comparison of different sized worms and to normalise size differences between replicates when examining the effects of temperature or other factors on filtration rate.

A positive relationship was expected between crown dry weight, body dry weight and filtration rate, with larger worms generally having larger crowns and therefore a greater potential filtration rate. While this was the case, the relationship is a little weaker than expected. Looking at crown to body dry weight (Fig. 3.6) there is a reasonably large spread of crown size which may be due to the fact that the worms do occasionally autotomise their crown, often as a response to disturbance or predation. Consequently, there will be some worms at various stages of regenerating their crown. There may also be a physical limit on crown size, therefore it is likely that body size and crown size do not share a linear growth pattern, but rather crown size reaches a asymptote before body size. The equation for the regression line is thus expressed as a curve. None the less, the regression of filtration rate to body DW was significant and therefore, it was considered appropriate to relate filtration rate to biomass using F in L per g body DW per hour.

Comparison to *Sabella penicillus*

Riisgård and Ivarsson, (1990) considered that *S. penicillus* is well adapted to live in waters with low food concentrations, but this does not necessarily apply to *S. spallanzanii*, as the filtration rate, per gram DW, for *S. penicillus* is considerably higher (114.5 L as compared to 3.78 L gDW^{-1} hr^{-1}).

Chapter 4

OXYGEN CONSUMPTION

INTRODUCTION

Oxygen is consumed during respiration and the rate of oxygen consumption is a convenient measure for the overall metabolic activity of an animal (Schmidt-Nielsen, 1983). Riisgård and Ivarsson (1990) inferred a level of feeding efficiency in the fan worm *Sabella penicillus* by comparing the oxygen consumption rate as a measure of energy consumption and filtration rate as a measure of food collection. This approach was also used by Shumway *et. al.* (1988) which allowed them to calculate the concentration of food (algal cells) in the water filtered which is required to maintain the metabolic rate of the fan worm *Myxicola infundibulum*.

One important reason for measuring the oxygen consumption rate of *Sabella spallanzanii* is so that it can be related to filtration rate to calculate feeding efficiency. A second reason is to determine the effect of temperature on the metabolic rate of *S. spallanzanii* as this could help determine the range of tolerance for the species. This may be important in predicting the extent to which *S. spallanzanii* will spread into warmer or colder waters from its current range.

Metabolic rate generally increases with temperature. The classic notion is that as temperature increases within an animal's range of tolerance, the rate of oxygen consumption will increase in an exponential manner (Schmidt-Nielsen 1983). Above the range of tolerance the oxygen consumption may drop below the expected exponential relation with the drop becoming more pronounced until the lethal limit is reached (Schmidt-Nielsen). A similar relationship can be expected in *S. spallanzanii*. By testing a wide temperature range, the range of tolerance can be detected.

Aims and hypothesis

The aims of this section are to determine the oxygen consumption rate of *Sabella spallanzanii* and to determine the effect of temperature on oxygen consumption rate. A further aim is to determine what is the most appropriate and practical measurement (i.e. body DW or crown size) to relate the oxygen consumption rate of *S. spallanzanii* to the filtration rate (see Chapter 3). These data will then contribute to an analysis of feeding efficiency in Chapter 5 to help determine whether this or temperature could be factors controlling *S. spallanzanii* distribution.

Specifically, the hypotheses addressed in this section are:

1. That the oxygen consumption rate of *Sabella spallanzanii* will increase with temperature within a tolerable temperature range.
2. That at a given temperature, oxygen consumption rate will increase with worm size measured as body dry weight.

METHODS

The methodology of measuring oxygen consumption

The measurement of oxygen consumption rate in marine organisms is often conducted in a closed chamber so that the drop in oxygen concentration can be measured from the water surrounding the animal. There are several problems with this approach. Steffensen (1989) examined some errors in respirometry and found that the chamber volume needs to be sufficient and time short enough to avoid excessive decrease in ambient O_2 concentrations or build up of CO_2 and excretory products which might affect respiration. However, the volume must not be too large or the time too short, because then the accuracy of detecting a change in O_2 concentration will be impaired. Hence it is important to choose an appropriate respirometer volume for the size of each

specimen, allowing measurements of oxygen consumption over shorter time intervals (Steffensen, 1989). Riisgård and Ivarsson (1990) successfully used a chamber volume of 162 mL over a 2 hour period for measuring the respiration of a 120 mg (DW) worm.

Steffensen (1989) pointed out the problem of stratification of gas content in the water and suggested the use of an adequate pumping system for recirculating and mixing to solve this problem. A pump is also less likely to cause disturbance to the worm than other methods of mixing the water (e.g. stirring, or agitating the chamber), as was found in earlier experiments on *Sabella penicillus* by Ewer and Fox (1940).

Riisgård and Ivarsson (1990) measured the oxygen consumption of *S. penicillus* in a respiration chamber connected to a circulation pump which provided internal mixing and passed the water over an oxygen electrode connected to a recorder. The chamber and circulation system were immersed in a constant temperature bath to avoid any effects of small temperature changes on oxygen saturation levels and metabolic rate. All measurements were performed with oxygen saturation above 83% to minimise a decrease in respiration rate with lower oxygen levels, as was noted by Fox (1938).

After considering problems in the respirometry of aquatic organisms, Steffensen (1989) recommended the use of an intermittent flow system where the chamber is an open or flow through system which is then switched to closed circuit during measurements. This system avoids the problems of excessive decrease in ambient O_2 concentrations or build up of CO_2 and excretory products, but allows the ratio of chamber volume to specimen size to be small, which increases the accuracy of detecting a change in O_2 concentration over a short time. This approach was adopted in this study.

Experimental design

The oxygen consumption rate of *Sabella spallanzanii* was measured at four different temperatures: 13^0, 17^0, 22^0 and 27^0C (as for filtration rate) in filtered sea-water. Measurements were conducted on active, filtering worms which had their crowns open (i.e. routine metabolic rate) so that oxygen consumption could be related to filtration rate. The consumption of the electrode, water and empty tube was measured to control for any effect of background consumption (e.g. of the oxygen electrode, water, micro-organisms or the worm tube). Six replicates and six controls were measured at each temperature.

Collection and care of specimens

Specimens of *S. spallanzanii* were collected from the Southern Flats in Cockburn Sound and transported in an insulated container with aerated sea water to the University of Western Australia Marine Biology Laboratory at the Bernard Bowen Fisheries Research Laboratories, Waterman. The worms were kept in aerated, flow-through sea water aquaria in a temperature controlled room for several days to acclimate to temperature and handling prior to experimentation. During acclimation the worms were fed on algal monocultures of *Thalassiosira pseudonana, Dunaliella marina* or *Rhodomonas sp.* in addition to any natural particles in the seawater which was only coarse filtered.

Preliminary investigation

As described in Chapter 3, a preliminary mortality experiment indicated that the subjects could be maintained in aquaria long enough to acclimate and for the experiments to be conducted. Similar precautions were adopted here to avoid undue disturbance of the specimens which might effect the results.

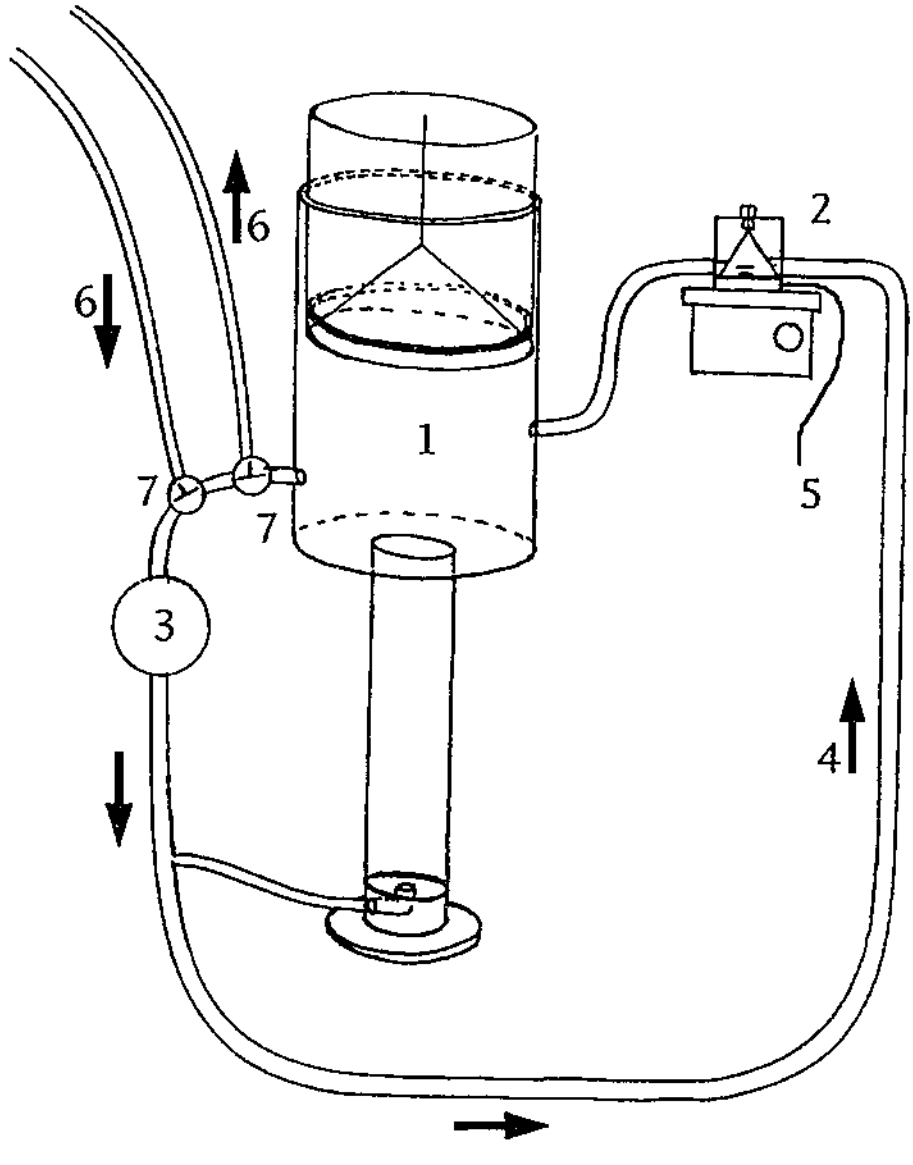
Experimental apparatus, design, construction, set-up and testing

To measure oxygen consumption of *S. spallanzanii*, two chambers were designed and constructed of clear, cylindrical acrylic (see diagram, Fig. 4.1.). The upper half had an internal diameter sufficient to accommodate the open crown of the specimen without it touching the sides (75 mm). To reduce the volume and thereby increase the accuracy of measurements, the lower half had a smaller diameter (28 mm) sufficient to accommodate the tube with minimum disturbance. An additional tubular insert was made to further reduce the chamber volume to accommodate smaller worms. O-ring seals ensured a good seal and allowed the segments to be separated while inserting the specimen. The top of the chamber had an o-ring sealed plunger with a tapered hole, leading to a fine capillary tube. The plunger was pushed down to exclude any air and to adjust the volume; the capillary tube allows air to escape and the internal pressure to be equalised with ambient pressure while minimising oxygen transfer into the chamber (Hansatech, 1993b). The volume of the chamber was adjustable from 200 to 750 mL including all tubing and the oxygen electrode housing.

Circulation system

Internal water circulation was maintained by a closed circuit and a small submersible pump (Rena C20). Water was pumped from one side of the chamber and the flow was divided, some being returned to the bottom of the chamber to ensure adequate circulation, while the rest passed over an oxygen electrode and returned to the top of the chamber. To allow flushing of the chamber with air saturated sea-water from a 30 L reservoir, the circulation system could be switched from internal to external circulation by means of two, three-way valves (Fig. 4.1).

Figure 4.1 : Respirometry Chamber diagram



1. Chamber with o-ring sealed, plunger lid.
2. O_2 electrode with magnetic stirrer.
3. Pump.
4. Circulation system pumps water through electrode.
5. O_2 data logged to computer.
6. Bypass through reservoir.
7. Three way valves.

Oxygen electrode

A small housing was constructed of clear acrylic to hold a Hansatech Clarke-type oxygen electrode (Delieu & Walker, 1972). A tapered hole with an o-ring sealed plug in the top of this housing allowed any air to be bled from the system. The electrode housing was connected to the chamber and pump with Nalgene premium grade VI tubing (1/4" ID). A small magnetic stirrer (follower) was placed in the housing to mix the water above the electrode to ensure complete mixing for maximal sensitivity and minimal noise (Hansatech, 1993b). The electrode was connected to a Hansatech CB1-D control box which allowed adjustment of the output voltage range, coarse and fine residual voltage back-off and showed a digital display of the output (Hansatech, 1993a). The output signal was interfaced to an IBM compatible computer with a CMA, 8 bit UIA analog to digital converter and recorded using IP Coach 4 software (CMA Foundation 1993). This system allowed two electrodes to be used simultaneously and graphically displayed the result on the computer during all calibration, experimental and control readings.

To control water temperature, the chamber, pump, tubing and 30 L reservoir were immersed in an insulated constant temperature water bath and the room was air conditioned. Cooling was provided by an external refrigeration unit and heating by a Julabo E-type temperature bath control unit. Circulation within the water bath was assisted by a submersible pump (Rena C40).

Testing

Mixing in the respirometry chamber was tested by adding a few drops of fluorocene die and observing complete dispersal. This was observed within approximately 10 to 15 seconds, indicating complete mixing. An initial trial was conducted while measuring the oxygen consumption of worms at 22^{0}C to check the methods and determine the appropriate volume of the chamber (these data are not included in the experimental results). The chamber volume was reduced to a volume where the change in oxygen concentration as a result of the worms

respiration was easy to detect above background consumption and noise. To avoid the associated problem of waste product and $C0_2$ build-up, the intermittent flow system suggested by Steffensen (1989) was adopted. To reduce any problems from electrical interference, the electrode was placed as far as possible from interference; the control box was run on batteries and the airconditioner, cooling unit and computer were run from power points outside the room.

Calibration

The oxygen electrode was assembled and connected to the computer as described by Walker (1987) and Lemmens (1994). The control box was switched on prior to calibration, allowing it to warm up and the electrode output voltage to stabilise. The electrode unit was flushed with a continuous stream of N_2 gas to remove all oxygen. Once the reading was stable the residual current was backed-off to zero (Walker, 1987, Hansatech, 1993a & b). The chamber was flushed with air-saturated seawater from the reservoir and once the voltage was stable, three 10mL samples were collected from the outflow for determination of oxygen concentration by Winkler titration (Grasshoff, Ehardt and Kremling, 1983). A third point of calibration was found by filling the chamber with water which had been partly deoxygenated by bubbling with N_2 gas. Again three 10mL samples were taken once the electrode had stabilised and the voltage recorded. To maintain the accuracy of measurements, oxygen electrode membranes were replaced every second day and calibrated daily. Winkler samples were taken at the beginning of each replicate and checked against the calibration values for voltage and oxygen concentration. The Winkler thiosulphate titrant was standardised against an Iodate standard daily (Grasshoff, *et. al.* 1983).

Acclimation

Worms used for temperature experiments were adjusted slowly to the experimental temperature over several days ($< 2^{0}C$ change per day) and then acclimated for at least 48 hours. Individual worms in their own tubes were transferred into the experimental chamber in a natural upright

position. Specimens were allowed to acclimate to the chamber for several hours, with the chamber circulation system switched to external flow of natural sea-water (36.5 ± 0.1‰ salinity). Additional sea-water was filtered through a Cuno 1μm absolute filter (No. PPK09NG010) to remove the bulk of the micro-organisms which might produce or consume oxygen and affect the experiment. The filtered water was allowed to stand for several hours in a 30 L reservoir in the temperature bath at the appropriate temperature to equalise oxygen saturation with ambient conditions and avoid oversaturation.

Procedure

Once the electrode had been calibrated and the specimen acclimated, the plunger lid was fitted to the chamber and pushed down slowly to set the chamber to a known volume and exclude all air. The electrode was connected and the chamber was flushed for 10 minutes with filtered sea-water from the reservoir. Once the worm had its crown fully open the chamber was switched to internal, closed circuit circulation, being careful not to disturb the specimen. The decrease in oxygen concentration was monitored by the electrode and recorded to computer over 30 minutes at 0.9 second intervals.

Several precautions were followed to ensure that the readings would represent the routine metabolic rate of the specimen while it was open and not disturbed. If the worm was accidentally disturbed and withdrew into its tube for more than a few minutes the reading was abandoned, the chamber was flushed until the worm opened again and then the experiment was restarted. This did not however, exclude the worm's natural, undisturbed rhythm of withdrawing for short periods of 30 seconds to 1 minute and reopening.

Measurements were only conducted between 100 and 80% oxygen saturation. If air was accidentally entrained in the chamber water, causing supersaturation, or if the saturation was allowed to fall to less than 80%, which may effect respiration rate (Fox, 1938), the chamber was flushed with saturated water from the reservoir and the experiment started again. By choosing the appropriate chamber volume for the size of the worm, and by carefully bleeding off air bubbles, these problems could largely be avoided.

Once a consistent oxygen consumption had been recorded over 30 minutes (as verified by a regression of O_2 levels over time), the worm was removed from its tube and the chamber was flushed for 10 minutes with filtered sea-water from the reservoir. The oxygen consumption of the electrode, water and empty tube was then measured for a further 30 minutes (the control).

Each worm, crown and tube were placed into pre-weighed crucibles and dried for the determination of dry weight, as described previously (Chapter 3).

Calculation of oxygen consumption rate

A regression of oxygen levels over time was calculated on the 2000 data points of each 30 minute reading to check that the reading represented a constant rate of consumption. In all cases R^2 was greater than 0.96, indicating that the oxygen consumption rate over 30 minutes was essentially linear. Voltage data were converted to oxygen concentration (mg L^{-1}) using the slope of the three point calibration. The oxygen consumption rate was calculated using the formula:

$\{(slope C_1 \times V) - (slope C_2 \times V) \times 60\}/ DW$, where $slope C_1$ is the slope of oxygen concentration from the experimental reading, $slope C_2$ is the slope from the control, V is the volume of the chamber, 60 is the conversion from minutes to hours and DW is the dry weight of the worm body including the crown but not the tube. This provides the consumption rate of the worm in mg

oxygen per hour per g DW corrected for the background consumption (of the tube, water, micro-organisms and the electrode). Mass (mg) of oxygen was used instead of volume (mL) because mass was the measurement produced directly from the calibration titrations and this also avoided any progressive inaccuracy from the extra step in converting to volume.

Statistical analyses

A one way analysis of variance was used to test if there was a significant difference in oxygen consumption rates between temperatures. An F_{max} test was used to check for homogeneity of variance prior to using ANOVA. The data were log (x+1) transformed where necessary and checked again for homogeneity (Fowler & Cohen 1993). A Scheffe *F* test was used to identify where significant differences occur.

RESULTS

Effect of temperature on oxygen consumption

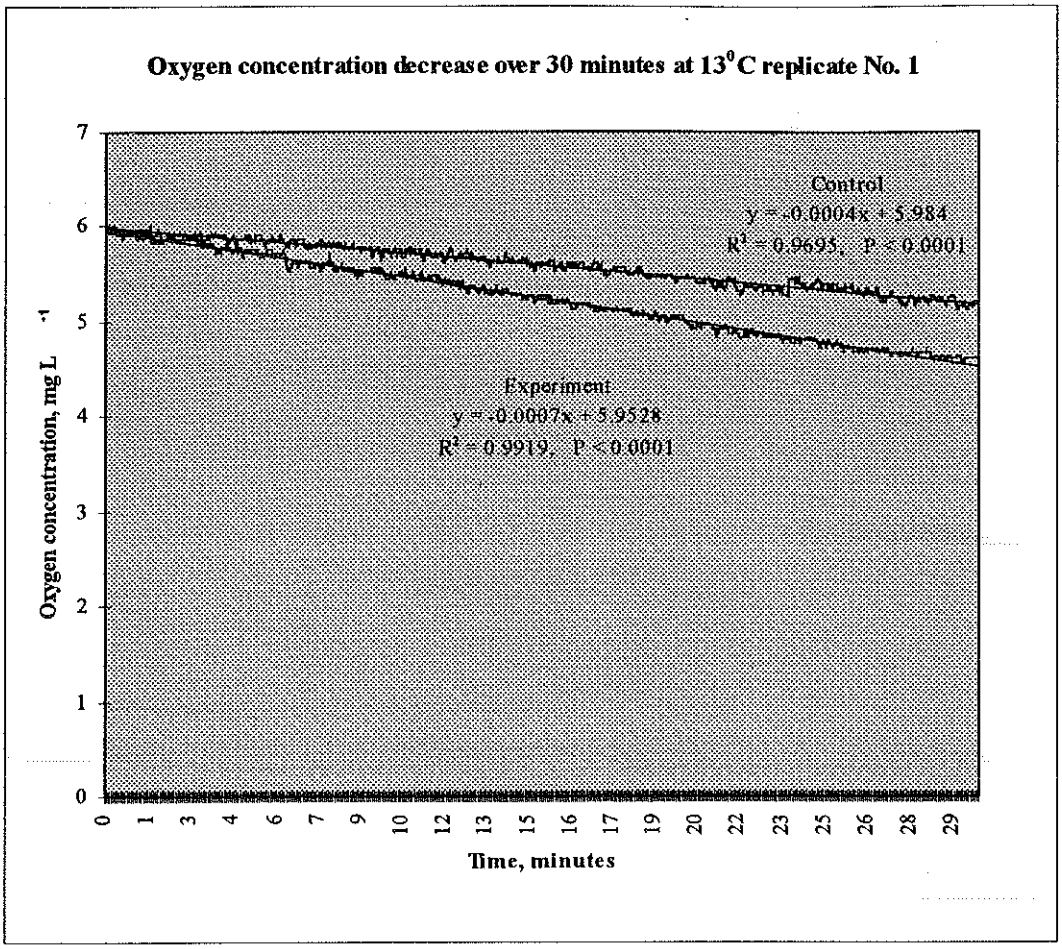


Figure 4.2. Typical oxygen consumption recording over 30 minutes (13°C, replicate 1), with the worm (experiment) and with the empty worm tube (control). Oxygen concentration is given in mg O_2 L^{-1} and the equation and line of a linear regression are shown next to each recording.

Figure 4.2 shows a typical recording of the decrease in oxygen concentration over 30 minutes in the experiment with the worm and the control with the empty tube. The oxygen consumption of the worm is represented by the difference between the slopes of the two linear regression lines, in all cases the slope of the experiment was greater than the control. Analysis of variance showed that there was a significant difference between the experiment and control, (ANOVA $P = 0.0006$, $df = 1$), therefore the consumption can be attributed to the worm.

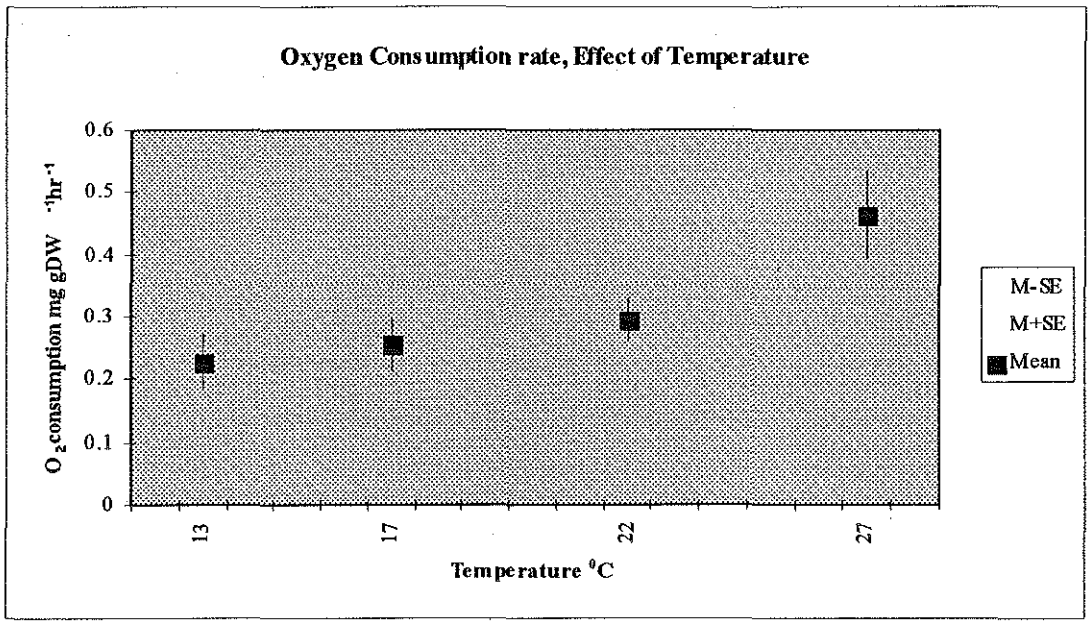


Figure 4.3. The effect of temperature on oxygen consumption rate in *Sabella spallanzanii*. The mean oxygen consumption rate of six replicates at each of 13^0, 17^0, 22^0 and $27^0C \pm 0.1^0C$ is given in mg O_2 $hour^{-1}$ g $DW^{-1} \pm$ S.E.

Figure 4.3 shows that there is an increase in mean oxygen consumption with increasing temperature (mean ± S.E. were: 0.228 ± 0.045; 0.254 ± 0.044; 0.294 ± 0.035 & 0.463 ± 0.069 at 13^0, 17^0, 22^0 and 27^0C respectively). Analysis of variance showed that there was a significant difference in consumption rates between temperatures (ANOVA P = 0.0117, $df = 3$). However, the source of this difference was between 13^0 and 27^0C as shown by a Scheffe F test (Scheffe P = 0.0239). The increase in oxygen consumption with temperature between 13^0 and 22^0 approximated an exponential increase; from 13^0 to 17^0C the Q_{10} was 1.3 and for 17^0 to 22^0C was only slightly higher, $Q_{10} = 1.34$. However, the increase from 22^0 to 27^0C was greater, which can be seen by the higher Q_{10} of 2.47 and in Figure 4.3.

Effect of body size, dry weight

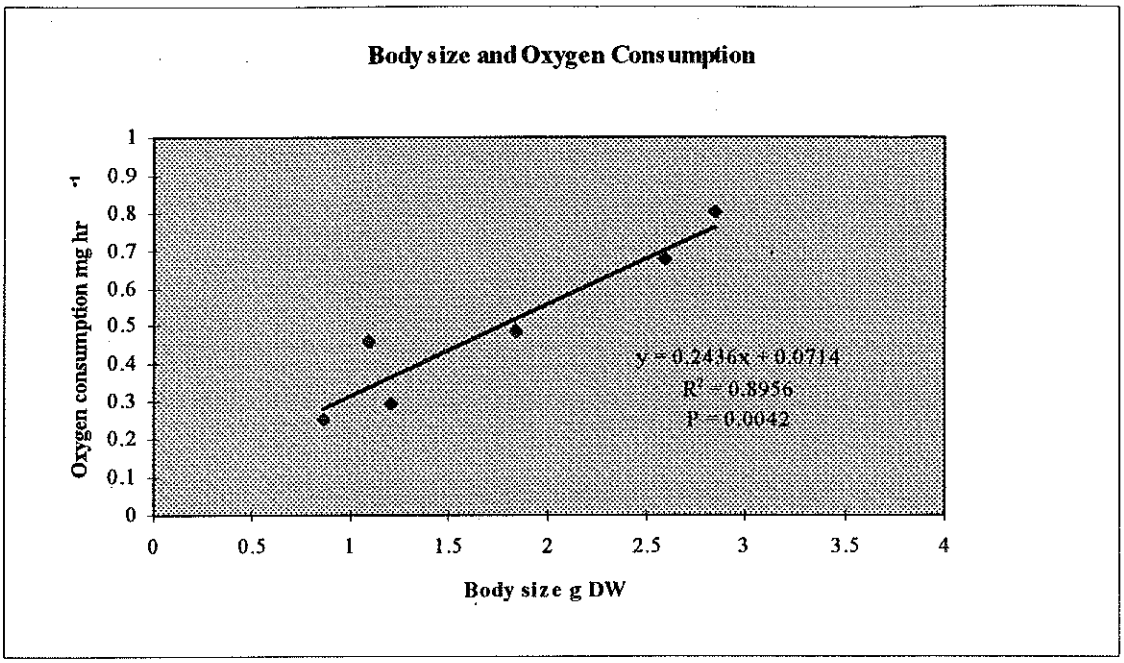


Figure 4.4. The relationship between body size DW and oxygen consumption rate in *Sabella spallanzanii*. Oxygen consumption rate in mg hour^{-1} at 22^{0}C is plotted against dry weight of the body including crown. The equation and line of a linear regression are shown.

It can be seen from Figure 4.4 , that for the six replicates measured at 22^{0}C there was a linear increase in oxygen consumption with body size as dry weight. Analysis of variance showed that this regression was significant (ANOVA 22^{0}C, P = 0.0042, n = 6).

DISCUSSION

Effect of temperature on oxygen consumption

The results of this experiment showed a significant increase in the oxygen consumption rate of *S. spallanzanii* with increasing temperature from 13^0 through to 27^0C which serves to confirm the first hypothesis, that the oxygen consumption of *Sabella spallanzanii* will increase with temperature within a tolerable range. Although the only significant difference shown by the Scheffe *F* test was between the two extreme temperatures of 13^0 and 27^0C and not between other combinations, this does not disprove the hypothesis and it is clear from Figure 4.3 that oxygen consumption rate increased with temperature.

Q_{10} is a commonly used expression of the proportional increase in oxygen consumption over a 10^0C increase in temperature. If the oxygen consumption rate were to increase in an exponential manner the Q_{10} will remain constant (Schmidt-Nielsen 1983). The increase in oxygen consumption with temperature between 13^0 and 22^0 approximates an exponential increase; the Q_{10} for 13^0 to 17^0C is 1.3 and for 17^0 to 22^0C is only slightly higher at 1.34. This follows what was expected within the range of temperature tolerance, but at higher temperature the Q_{10} was expected to drop below the exponential relation, with this drop becoming more pronounced until the lethal limit is reached (Schmidt-Nielsen 1983). The increase from 22^0 to 27^0C was greater than the expected exponential relation, with a Q_{10} of 2.47. The first possible explanation of this result is that 27^0C is not above the tolerable temperature for *S. spallanzanii* and the temperature range tested was not sufficient to detect the upper limit. Another possibility is that there could be a slight increase in respiration rate caused by stress, before it decreases, although this is not supported by the theory.

During acclimation at 27°C several specimens were observed producing mucus and some autotomised their crown, indicating that they were stressed. Although measurements were only taken on specimens which were not producing mucous and with the crown intact, this effect of stress may have resulted in the increase in metabolic rate detected by these results. Therefore it is likely that 27°C is approaching the upper limit for *S. spallanzanii* and it may not be able to successfully invade waters of this temperature or warmer (see Chapter 5 for further discussion).

Effect of body size on oxygen consumption

There was a significant increase in oxygen consumption rate with body size. This confirms the second hypothesis, that at a given temperature, the oxygen consumption rate of *S. spallanzanii* will increase with worm size measured as body dry weight. Body size does have an effect on oxygen consumption, as expected, larger specimens using more oxygen (Pandian and Vernberg, 1987). To compare to the results of Riisgård and Ivarsson (1990), their result was converted to per gram DW and the results given here are converted from mg to mL oxygen. In comparison, the oxygen consumption per gram of dry weight of *S. penicillus* is slightly higher, 0.323 mL gDW^{-1} hr^{-1} than that of *S. spallanzanii* 0.223 mL gDW^{-1} hr^{-1}.

A possible source of error in this experiment is from differences in the size of worms used as replicates at each temperature, because the worms were chosen at random. The results for mean consumption at each temperature have been normalised to oxygen consumption rate per gram of body dry weight to reduce any effect of body size. Analysis of variance was also used to test that there was no significant difference in mean body dry weight between temperatures ($P = 0.4898$ $df = 3$). These precautions should ensure that the mean increase in oxygen consumption rate per gDW with temperature was largely independent of worm size (Fig. 4.3).

Relation to the natural environment

The results of this experiment are likely to be representative of the oxygen consumption of *Sabella spallanzanii* in the natural (or invaded) environment. The specimens would be adapted to a certain level of disturbance in the Cockburn Sound environment, from waves or water motion and from the movements of fish or crabs. During the initial trial, some specimens were purposely disturbed by handling, as a result oxygen consumption was increased and became less stable, which could be detected in the resulting data. The results were improved by reducing disturbance to the specimens with careful handling and acclimation and by only using stable readings of oxygen consumption as displayed in Figure 4.2 with an R^2 of greater than 0.95 and in most cases around 0.99. When disturbed, specimens retracted into their tubes for long periods (10 minutes to hours) but when left undisturbed they would open and close in a natural cycle of 10 to 20 minutes open, then close for a short period of 30 seconds to 2 minutes and reopen. By only taking measurements when the specimens were opening and closing in their apparently undisturbed cycle, the effect of unnatural disturbance was minimal.

The other and probably more important reason for measuring open specimens, was so that the oxygen consumption measured was representative of an open, filtering worm, being the routine rather than basal metabolic rate. Although Riisgård and Ivarsson (1990) concluded that in *Sabella penicillus*, the beating of the cilia which are involved in filtering, contribute only marginally to the total respiration rate, I considered that it was important to measure respiration rate while filtering so that it could be related to filtration rate results from Chapter 3.

During the measurements, there was no detectable change in the respiration rate when the specimen periodically withdrew into its tube (for example Fig. 4.2). This agrees with the results of Riisgård and Ivarsson (1990) for *Sabella penicillus*, who also commented that this indicates the crown is for feeding only and is not a respiratory organ. This is in contrast to earlier work by

Ewer and Fox (1940) who found that the respiration rate of *S. spallanzanii* decreased by 36% when the crown was amputated and therefore concluded that the crown is a respiratory organ. Surely amputation of the crown would cause the specimen a great deal of disturbance which may itself effect the respiration rate. My own observations while keeping *Sabella spallanzanii* in aquaria indicate that when the crown is autotomised the worm can live for weeks without a crown while it is regrown. This alone indicates that it is not important as a respiratory organ. Wells (1951 & 1952) found that *Sabella spallanzanii* pumps water through its tube by peristaltic contractions of the body and that this and not the crown supplies the body with oxygen.

A limitation of this experiment is that is was not possible to test the oxygen consumption rate under identical conditions to the filtration rate experiments, with algal cells in the water being filtered. This was because the concentration of algal cells would be progressively reduced during the experiment by the filtering activity of the specimen, this would have made it difficult, or impossible to control for the background consumption or production of the cells. Riisgård and Ivarsson (1990) concluded that in *S. penicillus*, filtering contributes only marginally to the total respiration rate and therefore, it was considered that the oxygen consumption of *S. spallanzanii* would not be effected by the presence or absence of algal cells.

The results of this experiment are considered to be representative of the normal routine metabolic rate of *S. spallanzanii* while it is filtering water and therefore can be related to the filtration rate results to calculate the filtration efficiency at the various temperatures tested.

Chapter 5

FILTRATION CAPACITYand FEEDING EFFICIENCY - MANAGEMENT IMPLICATIONS

INTRODUCTION

The previous chapters have explored the biomass, filtration rate and oxygen consumption rate of *Sabella spallanzanii* in Cockburn Sound. This final chapter integrates those results and discusses the implications for management of this introduced species. The two main areas which will be discussed are the potential impacts which *S. spallanzanii* may have as a result of filter-feeding and its potential to invade new areas.

METHODS

Filtration capacity of the *Sabella spallanzanii* population in Cockburn Sound

The area of *Sabella spallanzanii* coverage and biomass (results from Table 2.1), were used with the mean filtration rate per gDW at each temperature (Chapter 3), to calculate the filtration capacity of *S spallanzanii* at each site at various temperatures. The filtration capacity of *S. spallanzanii* on the Southern Flats has been calculated separately for each site, then totalled, as this is more realistic than using the mean biomass and total area. The filtration capacity on jetties has been calculated assuming that the other jetties in the region nearby each have approximately the same area and biomass. For the pylons, the mean area and biomass of the three replicates was multiplied by the number of pylons in the Sound (see Chapter 2, Methods). Because there were no significant differences in biomass between summer and winter samplings, the means for each site includes data from both seasons.

Feeding efficiency of *Sabella spallanzanii*

There are several ways in which feeding efficiency has been calculated by different authors. To allow comparisons to be made, the methods of Riisgård and Ivarsson (1990) and Shumway *et al.* (1988) have been followed and an additional method has been devised to equate to the concentrations of chlorophyll *a* in Cockburn Sound.

Feeding efficiency in filter feeders has not been measured as a single function. Instead, Riisgård and Ivarsson (1990) inferred a level of feeding efficiency in the fan worm *Sabella penicillus* by comparing the oxygen consumption rate (energy consumption), and filtration rate (food collection). They then calculated the volume (L) of water filtered per mL of oxygen consumed, which they termed "water-processing capacity".

A similar approach was used by Shumway *et al.* (1988) which allowed them to calculate the concentration of food (algal cells) in the water filtered which is required to maintain the metabolic rate of the fan worm *Myxicola infundibulum*. This was calculated assuming an equivalent of oxygen consumed to calories required for respiration of 4.8 calories per mL O_2 and that 10×10^6 algal cells is approximately equal to 1 calorie (Shumway *et al.* 1988).

Feeding efficiency, volume filtered per unit oxygen consumption

The feeding efficiency of *S. spallanzanii* (Fig. 5.1) was calculated using the results of mean filtration rate per gram body DW, ± S.E. (Figure 3.1) and mean oxygen consumption rate per gram body DW, ± S.E. (Figure 4.3) at the various temperatures tested. The results are displayed as volume filtered (L) per mg of oxygen consumed (see Chapter 4, methods). The error bars displayed in Figure 5.1 represent the upper and lower limits calculated from the means ± S.E. for filtration rate and oxygen consumption. To allow comparison to other works, where necessary the

results of oxygen consumption in mg were converted to mL using standard temperature ($^0C + 273^0K$), pressure (1 atm), and the gas constant R (0.08206) (Zumdahl, 1993).

Food requirement, Chlorophyll *a* equivalent

To further evaluate the feeding efficiency of *S. spallanzanii*, the concentration of phytoplankton required as food was calculated, and converted to its equivalent in chlorophyll *a*. This is useful because data on the levels of chlorophyll *a* as a measure of phytoplankton abundance is readily available for Cockburn Sound (e.g. Chiffings, 1979; Chiffings & McComb, 1981; Cary *et al.* 1991). The food requirement (Chl *a* equivalent) of *S. spallanzanii* was calculated by converting the oxygen consumption (O_2; mg gDW^{-1} hr^{-1}) to the equivalent carbon requirement (C; mg gDW^{-1} hr^{-1}), assuming that in respiration, for each mole of oxygen (O_2) consumed there is one mole of carbon (C) required (Gnaiger, 1983). Therefore, C mg = O_2 mg x 12 / 32. The equivalent amount of chlorophyll *a* was calculated assuming a C:Chl *a* ratio of approximately 40:1 for phytoplankton (Parsons & Takahashi, 1973) and this was divided by the volume of water filtered to give the equivalent concentration of Chl *a* mg L^{-1} (and converted to μg L^{-1}) required to maintain the metabolic requirements of *S. spallanzanii*.

This calculation does make some broad assumptions, but the result tends to be conservative to avoid an overestimation. The oxygen to carbon consumption (RQ_i) for lipids and proteins is lower than the ratio of 1:1 for carbohydrate used in the above equation (Gnaiger, 1983). If we assume that the composition of phytoplankton is approximately 50% protein, 35% carbohydrate and 15% lipid (Parsons & Takahashi, 1973) then the RQ_i will be approximately 1 O_2 : 0.95 C (Gnaiger, 1983). This will result in the food requirement being 5% lower than that calculated above. On the other hand, if the C:Chl *a* ratio of 30:1 suggested by Strickland (1960) is used then the result will be approximately 25% higher than that calculated above.

RESULTS

Table 5.1. Filtration Capacity of *Sabella spallanzanii*: Summary Table				
Temp °C	**13°**	**Winter 17°**	**Summer 22°**	**27°**
	Volume filtered (megalitres per day)			
Southern Flats				
Total (sum of 4 sites)	280	341	772	503
Jetties				
Kwinana region	47.8	58.2	131.6	85.6
Owen anchorage region	2.6	3.2	7.2	4.7
Rockingham region	2.5	3.1	6.9	4.5
Total	52.9	64.4	145.8	94.8
Pylons				
Total	1.1	1.34	3.02	1.97
Cockburn Sound Total	**334**	**407**	**921**	**599**
	Mean Filtration rate (kilolitres per m^2 per day)			
Temp °C	**13°**	**Winter 17°**	**Summer 22°**	**27°**
Southern Flats	8.51	10.36	23.43	15.24
Jetties	2.29	2.78	6.29	4.09
Pylons	1.01	1.22	2.77	1.80

Table 5.1. Filtration capacity of *Sabella spallanzanii:* Summary table. The total filtration capacity was calculated from the estimated area of each site and the biomass for each site. The mean volume of water filtered kL m^{-2} day^{-1} is also given for comparison of each category: Southern Flats, jetties and pylons.

The filtration capacity of *S. spallanzanii* is highest in summer, with a water temperature of 22°C. It is estimated that the whole population of *S. spallanzanii* in Cockburn Sound can filter 921 megalitres per day in summer and 407 megalitres in winter (Table 5.1). The beds on the Southern Flats account for the major part of this filtration capacity (i.e. about 84%). On a per m^2 basis the beds of *S. spallanzanii* on the Southern Flats are capable of filtering 23.4 kL day^{-1} m^{-2}, on the jetties and pylons this is considerably less, 6.3 and 2.7 kL day^{-1} m^{-2} respectively, during summer.

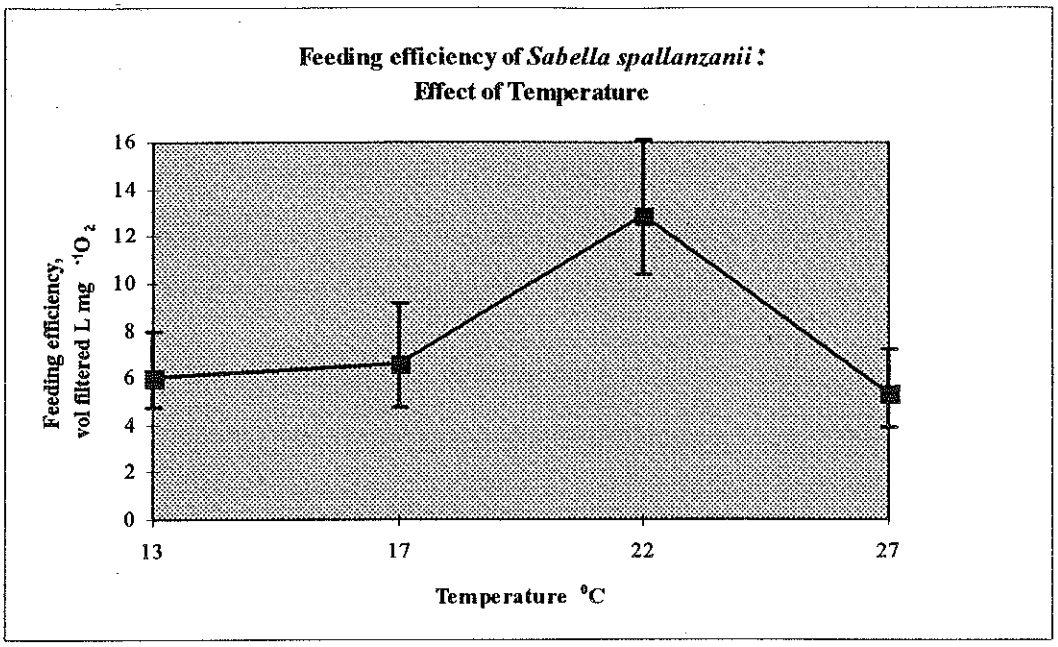


Figure 5.1. Feeding efficiency of *Sabella spallanzanii*: Effect of temperature. Feeding efficiency is given as the volume of water filtered in litres per mg of oxygen consumed at each temperature. Error bars represent upper and lower estimates by calculating feeding efficiency using mean ± S.E. oxygen consumption and filtration rates.

Table 5.2. **Feeding Efficiency of *Sabella spallanzanii*: Summary Table**

Temp °C	**13°**	**17°**	**22°**	**27°**
Filtration rate (L gDW^{-1} h^{-1}) from Chapter 3.	1.371	1.670	3.777	2.457
Oxygen consumption rate (mg gDW^{-1} h^{-1}) from Chapter 4.	0.228	0.254	0.294	0.463
Feeding efficiency (volume filtered L per mg of O_2 consumed)	**6.01**	**6.57**	**12.84**	**5.31**
Chlorophyll *a* (μg L^{-1}) food concentration required to meet metabolic demand measured as Chl. *a*	**1.56**	**1.43**	**0.73**	**1.76**
Feeding efficiency (volume filtered L per mL of O_2 consumed) for comparison to Riisgård and Ivarsson, (1990)	**8.20**	**8.84**	**16.97**	**6.91**
Algal cells required (cells mL^{-1}) for comparison to Shumway *et. al.*, (1988)	**585**	**543**	**283**	**695**

Table 5.2. Feeding efficiency of *Sabella spallanzanii*: Summary table. Feeding efficiency was calculated from the mean filtration rate and the mean oxygen consumption rate at each temperature tested. This is given as the volume of water filtered in litres per mg of oxygen consumed at each temperature (as for Fig. 5.1, above). Chlorophyll *a* required per litre of water filtered is given. For comparison to other work, feeding efficiency is also converted to L per mL of oxygen and to the algal cell requirement.

The feeding efficiency, measured as the volume of water filtered per unit of oxygen consumed, increased between 13°, 17° and 22°C (Fig. 5.1), then decreased to the lowest level of efficiency at 27°C (5.31 L mg^{-1} O_2). This trend was identical for efficiency expressed in litres mL^{-1} of oxygen (Table 5.2).

The algal concentration (expressed in chlorophyll *a* and/or cells mL^{-1}) required to meet the metabolic needs of *S. spallanzanii* is lowest at 22°C, because a greater volume of water is filtered per unit of oxygen consumed at this temperature (Table 5.2). At 27°C the filtration rate decreased while the oxygen consumption continued to increase, resulting in a lower feeding efficiency and thus, more food is required to meet its metabolic demand than at lower temperatures.

DISCUSSION

Filtration capacity of the *Sabella spallanzanii* population in Cockburn Sound

The population of *Sabella spallanzanii* in Cockburn Sound is capable of filtering a considerable volume of water per day. On the Southern Flats in summer (22°C), its mean filtration capacity is 23.4 kL day^{-1} m^{-2}. Given that the average depth on the Southern Flats is approximately 5m, and assuming complete mixing (see below), this equates to filtering the water column above each m^2 of *S. spallanzanii* 4.68 times per day (5m water depth ~ 5000L seawater per m^2). The density of *S. spallanzanii* on the jetties and pylons was generally less than on the Southern Flats. Consequently, they represent a lower filtration capacity. Despite this, *S. spallanzanii* is still able to reach a considerable filtration capacity at these sites. For example, *S. spallanzanii* on the Rockingham jetty has a potential mean filtration capacity of 11 kL day^{-1} m^{-2}. Therefore, the importance of these structures as artificial reefs supporting large numbers of *S. spallanzanii* should not be overlooked.

The filtration capacities estimated here assume that there is complete mixing, while in reality, stratification or boundary effects may limit access to a lesser portion of the water column. However, the shallow flats areas of Cockburn Sound where the majority of the *S. spallanzanii* population occurs, are likely to remain well mixed by wind driven currents in winter and convection currents in summer (Hearn, 1991; Steedman & Craig, 1983).

Effect of food concentration on filtration rate

The filtration rate of *S. spallanzanii* was shown to be high and constant at algal cell concentrations below 10 x 10^3 cells mL^{-1}. The rate did not decrease significantly until the specimens were exposed to high cell concentrations; above 15 x 10^3 cells mL^{-1} for small worms and 20 x 10^3 cells mL^{-1} for larger worms. Mean algal cell concentrations in Cockburn Sound range from approximately 160 to 260 cells mL^{-1} (pers. comm. Stuart Helleren). These cell concentrations are many times lower than the cell concentrations which caused a decrease in filtration rate of even the smaller worms and, therefore, natural cell concentrations are unlikely to effect the filtration rate and total filtration capacity calculated for *S. spallanzanii* in Cockburn Sound.

Effect of temperature

The filtration rate of *S. spallanzanii* is dependent on temperature, as was shown in Chapter 3. The mean filtration rate increased with temperature between 13^0 and 22^0C, then decreased when a high temperature of 27^0C was approached. The filtration capacity of the population is calculated using filtration rate, and it therefore, will also be dependent on temperature. In Cockburn Sound, *S. spallanzanii* is likely to experience a temperature range of approximately 15^0 to 23^0C with mean temperatures of 17^0 in winter and 22^0C in summer (Pearse, 1986). Given these mean

temperatures, in winter the filtration capacity of *S. spallanzanii* will be less than half that of summer and therefore, so will the impact on phytoplankton densities.

The total filtration capacity of the *S. spallanzanii* population on the Southern Flats in winter is estimated to be 341.5 megalitres per day and in summer 772.4 ML day^{-1}. The combined total potential filtration capacity for the jetties and pylons in winter is estimated to be 65.8 ML day^{-1} and in summer 148.8 ML day^{-1}. While this may not be large in terms of the whole of Cockburn Sound, the filtration capacity of *S. spallanzanii* may, at least locally, have a important effect on the phytoplankton population. As the population of *S. spallanzanii* increases and spreads in Cockburn Sound, this impact will become more pronounced. Filtration capacity was calculated from filtration rate which is the volume of water cleared 100% efficiently of particles per unit time, so the filtration capacities discussed here are the volumes completely cleared of cells.

Other studies have shown a similar potential for filter-feeders to filter large volumes of water and thereby exert a controlling effect on phytoplankton abundance in shallow water ecosystems (e.g. Cloern, 1982; Nichols, 1985; Hily, 1991; Alpine and Cloern, 1992). In Port Phillip Bay, filter-feeders are estimated to account for 42% of total assimilation of organic material by benthic invertebrates (Wilson, Cohen and Poore, 1993). By removing a large biomass of phytoplankton, incorporating it into their own biomass and releasing waste products, *S. spallanzanii* like these other filter-feeders, has become an important part of the trophic structure, however the contribution of this species was not a natural part of the undisturbed system.

This study has established that the filtration capacity of the *S. spallanzanii* population in Cockburn Sound is considerable, but we do not yet know the fate of this filtered organic material once it is ingested. *Sabella spallanzanii* may be involved in several ecological processes. Through filtration, it could be increasing the rate at which organic material is removed from the

water column and deposited to the sediments (e.g. Hatcher, Grant and Schofield, 1994). Another possibility is that it may interrupt the denitrification process by intercepting organic material before it can settle into the sediments. In areas such as Port Phillip Bay, denitrification is considered to play an important role in removing nitrogen from the system and releasing it to the atmosphere as N_2 (Skyring, Longmore, Chiffings & Crossland 1992) and therefore, any interruption of this process could have a severe impact. The large and increasing biomass of *S. spallanzanii* in Cockburn Sound, may be also acting as a nutrient sink, or an ecological dead end (e.g. Hopkinson, Fallon, Jansson & Schubauer 1991).

Kimmerer, Gratside and Orsi (1994) studied the impact of an introduced clam in San Francisco Bay and found that within a year of it becoming abundant, chlorophyll *a* levels and the abundance of 3 common zooplankton species had declined by 53% to 91%. They further concluded that the direct predation by this introduced filter-feeder may have an important effect on biomass and species composition of inshore zooplankton (Kimmerer, *et.al.* 1994). This suggests the so far overlooked possibility that an introduced filter-feeder such as *S. spallanzanii* may not only affect phytoplankton levels but could also affect zooplankton composition and abundance, further altering the natural trophic balance. The close proximity of large numbers of *S. spallanzanii* to commercial mussel farms in Cockburn Sound and at Albany is some cause for concern, as they may compete for both space and food. *S. spallanzanii* is already becoming a nuisance to commercial mussel farms, by attaching themselves to spat settlement ropes (pers. comm. Southern Ocean Fisheries Ltd. Albany).

An important impact from the introduction of *S. spallanzanii* may occur through competition with native species of filter feeders for food and space. The large filtration capacity of *S. spallanzanii* demonstrated in this work suggests that it could remove considerable amounts of food from the water-column and thereby create competition for food resources with other filter

feeders. *S. spallanzanii* has an additional competitive advantage of height over most of the other filter feeders where it occurs in Cockburn Sound; at 20 to 50cm long it can stand out further into the water than any other species observed during this study.

On the Southern Flats *S. spallanzanii* contributes 94% of the total filter feeder biomass, whereas on the jetties and pylons it is only 3% to 9.5% of the total (Lemmens, Clapin and Parker, in prep.). The Southern Flats area represents a disturbed habitat as it was once covered by seagrass (Cambridge and McComb, 1984). It appears that *S. spallanzanii* prefers this disturbed, shallow, sandy bottom habitat where it can out-compete other filter feeders. On the other hand, the jetties were densely covered with filter-feeders before the introduction of *S. spallanzanii* (pers. observations) and this may have helped resist the invasion. Disturbance is likely to increase the success of invasion and spread of *S. spallanzanii* and this should be seen as a caution against disturbing any new areas in the Sound as they are likely to be invaded. In Port Phillip Bay *S. spallanzanii* has spread rapidly across areas dredged by the local scallop fishery and while it may seem speculative, this disturbance is probably contributing to the spread.

A further impact is that once *S. spallanzanii* has invaded disturbed areas such as the Southern Flats which were once covered with seagrass, it might then prevent seagrass from re-colonising the area.

Feeding efficiency of *Sabella spallanzanii* and the effect of temperature

Temperature is an important factor in determining the potential of *Sabella spallanzanii* to spread to areas further north and south of its present distribution in Australia. The effects of temperature on *S. spallanzanii* were examined to help determine its tolerable temperature range. Both the filtration rate and oxygen consumption rate of *S. spallanzanii* increased with temperature from

13^0 to 22^0C. However, from 22^0 to 27^0C the oxygen consumption rate increased sharply while the filtration rate decreased sharply. Consequently, the feeding efficiency increased between 13^0 and 22^0C then decreased markedly at 27^0C (refer to Fig. 5.1). Feeding efficiency showed a much clearer change between temperatures than either oxygen consumption rate or filtration rate on their own. There was only a small increase in efficiency between 13^0 and 17^0C, however at 22^0C the efficiency is nearly twice that of the lower temperatures, suggesting that the optimum temperature for *S. spallanzanii* is approximately 22^0C.

At 27^0C the oxygen consumption of *S. spallanzanii* increased above the expected exponential relationship and the filtration rate declined, indicating that the specimens were suffering stress. 27^0C appears to be above the tolerable temperature of *S. spallanzanii* and this was further confirmed by observations of several specimens producing mucus or autotomising their crowns during acclimation to 27^0C. Observations during this study suggest that *S. spallanzanii* can survive short periods of extreme temperatures (12 hours at 4^0 or 30^0C), which would increase their chances of surviving transport through tropical or cold waters inside or on a ship's hull. However, because of the decrease in feeding efficiency and stress observed at 27^0C, it is considered unlikely that *S. spallanzanii* will successfully invade warm tropical waters. Even so, there is still a large area of the Western Australian coastline, at least north to Shark Bay where temperatures are below 27^0C (Prata, 1989). The next site north of Fremantle which seems most likely to be invaded would be Geraldton Harbour, as it has regular shipping visits and temperatures not much higher than Cockburn Sound.

At low temperatures and hence low feeding efficiency, *S. spallanzanii* will require more food in the volume of water filtered to meet its minimum energy requirements. Therefore, there must be a limit where even a high food concentration such as that in Cockburn Sound can not sustain its metabolic needs, and the combination of both low temperatures and food concentrations are likely

to limit its success and survival. To meet its metabolic requirements at 13°C, *S. spallanzanii* needs a phytoplankton level equating to a chlorophyll *a* concentration of approximately 1.56μg L^{-1}, at 17°C it requires 1.42μg L^{-1} whereas, at 22°C it only requires 0.73μg L^{-1}.

At 13°C *S. spallanzanii* was observed to react slowly to physical stimuli and some remained withdrawn into their tubes for longer periods than at higher temperatures, but they did not show the same signs of stress as at 27°C. Although the feeding efficiency of *S. spallanzanii* was low at 13°C, it can live at lower temperatures than in Cockburn Sound (eg. in Port Phillip Bay). Therefore, this is not below its tolerable temperature and providing it has an adequate food concentration it can thrive in temperatures of 13° to 22°C. However, it is still possible that even lower temperatures may limit the successful spread of *S. spallanzanii*, particularly if food concentrations are also low.

Comparison of the Feeding efficiency of *Sabella spallanzanii* to other filter feeders

Riisgård and Ivarsson (1990) calculated the water-processing capacity (feeding efficiency) of *Sabella penicillus* to be 354 L of water filtered per mL of oxygen consumed at 17°C. In comparison, *Sabella spallanzanii* filters only 17 litres of water per mL of oxygen consumed at 22°C and only 8.8 L per mL O_2 at a winter temperature of 17°C (Table 5.2). The feeding efficiency of *Sabella spallanzanii* is low compared to that reported for *Sabella penicillus*.

Because the highest efficiency for *S. spallanzanii* was at 22°C, and the results given by Riisgård and Ivarsson (1990) for *S. penicillus* are at 17°C, the following comparison assumes that these are the optimum temperatures for each species. To further assist the comparison, the results of Riisgård and Ivarsson (1990) have been converted from their 'standard' 65 mg DW worm to the equivalent of per 1g DW. The oxygen consumption of *S. penicillus* is slightly higher (0.323 mL O_2 gDW^{-1} hr^{-1}) than that of *S. spallanzanii*, (0.223 mL O_2 gDW^{-1} hr^{-1}), so this is not the cause

of the higher efficiency. However, the filtration rate of *S. penicillus* (114.5 L gDW^{-1} hr^{-1}) is considerably higher than for *S. spallanzanii* (3.78 L gDW^{-1} hr^{-1}).

Riisgård and Ivarsson (1990) concluded that the high feeding efficiency of *S. penicillus* suggests that this polychaete is adapted to live in waters with extremely low algal concentrations. They compared this to the mussel *Mytilus edulis* which filters 15 to 50 L of water per mL of oxygen consumed and commented that these two species seem to be adapted to different regimes of suspended food; *M. edulis* may not be able to live in the same localities (with low food concentrations) as *S. penicillus* (Riisgård & Ivarsson, 1990). Following this line of reasoning, *S. spallanzanii* would seem to be adapted to live in waters with much higher algal concentrations than either *S. penicillus* or *M. edulis*.

Shumway *et al.* (1988) measured an oxygen consumption rate of 0.221 mL gDW^{-1} hr^{-1} and filtration rate of 2.78 L gDW^{-1} hr^{-1} for the fan worm *Myxicola infundibulum*. Assuming an oxy-caloric equivalent of 4.8 calories per mL O_2 and that 10 x 10^6 algal cells is approximately equal to 1 calorie, they calculated that *M. infundibulum* requires a food concentration of 243.5 algal cells mL^{-1} to maintain its basal metabolic rate and 3816 cells mL^{-1} to maintain its routine metabolic rate (Shumway *et al.*, 1988). Using this same calculation method, *S. spallanzanii* would require a food concentration of 543 cells mL^{-1} to maintain its routine metabolic rate in winter and 283 cells mL^{-1} in summer. These algal cell concentration requirements are slightly higher than the concentrations in Cockburn Sound (160 to 260 cells mL^{-1}, pers. comm. Stuart Helleren). This raises the possibility that *S. spallanzanii* may not get all its food requirements from phytoplankton and could also feed on suspended organic material to meet its metabolic needs.

An alternative method to further evaluate the feeding efficiency of *S. spallanzanii*, is to calculate the concentration of chlorophyll *a* which equates to the minimal food requirements. This was

done by converting from oxygen consumption to the equivalent carbon requirement and equating this to the carbon to chlorophyll *a* ratio of phytoplankton, as described in the methods section. To meet its metabolic requirements, *S. spallanzanii* requires a phytoplankton concentration with a chlorophyll *a* level of 1.42 μg L^{-1} in winter and 0.73 μg L^{-1} in summer. Mean chlorophyll *a* levels of 1.82 to 2.22 μg L^{-1} in Cockburn Sound would satisfy this food requirement (Cary, Simpson and Chase, 1991; Lemmens *et al.* in press). Chlorophyll *a* levels outside Cockburn Sound are much lower, for instance, mean levels in Marmion Lagoon vary from 0.2 to 1 μg L^{-1} Chl *a* (Johannes and Hearn, 1985; Johannes *et al.*, 1994). These lower levels may not provide enough food to meet the minimum metabolic requirements of *S. spallanzanii* and for this reason alone it may not be able to live in these conditions.

The implication of the comparatively low feeding efficiency of *S. spallanzanii* is that if it requires a high concentration of food to meet its metabolic needs, then it may not be able to spread to areas with low food concentrations. If this is the case, then it would help to explain the present distribution of *S. spallanzanii* in Australia where it appears to be confined to eutrophic harbours such as Port Phillip Bay, Victoria, Princess Royal Harbour and Oyster Harbour at Albany, Bunbury Harbour and Cockburn Sound. The alternative is that it can spread outside these eutrophic harbours, but will not be likely to achieve the high population densities or biomass as it can under more favourable conditions.

The results of this study indicate that *Sabella spallanzanii* is most likely to successfully invade and reach high biomass in sheltered, shallow waters with temperatures between 13^0 and 22^0C where eutrophic conditions provide high levels of phytoplankton and in particular where human activity has created disturbance to natural or artificial habitats. In areas with lower temperatures than 13^0C and much less than 1 μg L^{-1} Chl *a*, *S. spallanzanii* would find it more difficult to prosper, while temperatures of 27^0C or above may limit its survival.

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