2000

Structure and dynamics of sponge-dominated assemblages on the temperate reefs: variability associated with anthropogenic disturbance

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STRUCTURE AND DYNAMICS OF SPONGE-DOMINATED ASSEMBLAGES ON TEMPERATE REEFS: VARIABILITY ASSOCIATED WITH ANTHROPOGENIC DISTURBANCE

A thesis submitted in fulfilment of the requirements for the award of the degree

DOCTOR OF PHILOSOPHY

from

UNIVERSITY OF WOLLONGONG

by

Daniel E. Roberts

Department of Biological Sciences
2000
FRONTISPIECE: Sponge-dominated assemblage from a temperate reef at 50m depth (Sydney, NSW, Australia).
DECLARATION

I, Daniel E. Roberts, declare that this thesis, submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the Department of Biological Sciences, University of Wollongong, is wholly my own work unless otherwise referenced or acknowledged. The document has not been submitted for qualifications at any other academic institution.

Daniel Edward Roberts

November 2000
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Acknowledgements

As the year 2000 drew to a close, I seriously considered changing the title of this thesis to “2001: A Sponge Odyssey”. In retrospect, I was quite naïve about the significant amount of time that I would need to devote to this research. Some said that trying to do a PhD whilst working full time would be very difficult if not impossible. At times I had to agree, especially when it came to the amount of time that I needed to spend on the most tedious of tasks, and especially when I realised just how much I actually did not know. Many difficult but enjoyable times were ahead, and I met many new friends and colleagues along the way. The support of these colleagues and friends made the task achievable and worthwhile.

Most of the chapters in this thesis have now been published or are in the process of being published and the contributions of co-author(s) in each of the various papers are duly acknowledged: Andy Davis, Sharon Cummins, Steve Fitzhenry, Steve Kennelly, Tony Roach, Peter Scanes, Adam Smith and Penny Ajani.

Much of the earlier work done in this thesis was logistically possible because of the support given by management and colleagues from the NSW Environment Protection Authority (EPA). In particular I would like to thank Gary Henry, Tony Church, Peter Scanes, Klaus Koop, Ross Higginson, David Leece, Robin Macdonald and Neil Philip. Colleagues from the Water Studies Section (EPA) are also thanked for their valuable assistance over the years, in particular, Scott Carter, Peter Scanes, Tony Roach, Stuart Puckeridge, Martin Krogh, Penny Ajani, Graham Sherwin, Rob Smith and Chris Pangway. Last, but not least, I would particularly like to thank Sharon Cummins for her advice and assistance throughout the work done in this thesis, and her expertise in fending off the white pointer sharks and the marauding yachties.
The following colleagues have also, at some stage, either assisted in reviews of manuscripts or been substantially involved with some aspect of the research done in this thesis and they are all acknowledged for their valuable contributions: Dan Fitzhenry, Paul McGaw, Steve Fitzhenry, Adam Fitzhenry, Yvette Eckersley, Adam Smith, John Hooper, Steve Kennelly, Tony Underwood, Marcus Lincoln-Smith, Alan Jones, David Ayre, Rob Whelan, Alan Butler, Brian Bayne, Clive Wilkinson, Peter Fairweather, Phil Colman, Kirsten Benkendorff, Geoff Sainty, Paul Somerfield, Ken Zimmerman, Shane Murray, Bob Diaz, Karla Casey, Sian Towel.

I would also like to thank my “fish-widow” mum (Jan Roberts) and “so long and thanks for all the fish” dad (Bob Roberts), for inspiring in me a love of the sea and all those smelly wriggly bits in it. Dad is also thanked for his advice and help in constructing the treatment cages for the experimental work. The support and significant contributions of my wife (Jan) and children (Nicholas, William, Ella and Ethan) throughout this thesis was most appreciated, as I know they gained a “sponge nerd” for a few years.

Before starting my PhD, I was given a fundamental piece of advice - “what determines whether your PhD is typical or exceptional will be somewhat reflected by the expertise, enthusiasm and wisdom of your supervisor – so get a good one”. I certainly took that advice when I chose my supervisor - I sincerely thank Associate Professor Andy (Aza) Davis whom through his sage advice, genuine enthusiasm and good humour made the whole thing possible (although I am still a little confused about the relevance of underwater goats in subtidal assemblages).
Papers and Manuscripts Associated with Thesis Chapters

CHAPTER 2 - General Methods


CHAPTER 3 - Patterns of Variability


CHAPTER 4 - Patterns Associated with Anthropogenic Disturbance


CHAPTER 5 – Processes Associated with the Effects of Sewage


Papers and Reports Associated with Thesis


Abstract

The structure and dynamics of sponge-dominated assemblages living on both shallow and deep coastal and estuarine reefs in temperate NSW, Australia were quantified by examining their spatial and temporal variability at a number of scales. The effect of the discharge of sewage effluent on these assemblages was also examined. Quantifying the natural spatial and temporal variability in these assemblages has received inadequate attention because of the difficulty in sampling these habitats. Following assessment of the efficacy of photoquadrats and appropriate levels of taxonomic resolution, I sought to determine i) spatial and temporal patterns of variability and ii) assess the effects of point source sewage pollution on these assemblages.

A reliable quantitative technique using photo-quadrats was therefore developed and tested for sampling the macrobenthic assemblages living on hard substrata. No loss in the accuracy of the data was detected whilst the increased efficiency of the method enabled these assemblages to be sampled at appropriate spatial and temporal scales. Both univariate and multivariate techniques determined that sufficient taxonomic resolution was attainable at the level of phylum for assemblages in kelp forests, whilst the level of order was necessary for assemblages in crustose habitats. Furthermore, as taxonomic resolution increased towards species level, the detection of differences associated with sewage became less likely.

Patterns and processes in deep-water macrobenthic assemblages have largely been inferred from the study of such assemblages on shaded artificial structures or in relatively shallow water. Patterns in the diversity and abundance of sponges on the coastal reefs off Sydney were examined using photo-quadrats to provide estimates of species richness and percentage cover. Spatial and temporal variability was measured by sampling three nested sites within each of three reefs at each of three depths (20, 30 and 50 m) on three occasions. Sponge richness generally
increased with depth, as did the number of erect or massive forms. In contrast, cover decreased with depth particularly for encrusting sponges. Univariate and multivariate analyses revealed considerable small-scale spatial and temporal variation in sponge distribution and abundance. A significant positive relationship between richness and cover was also apparent. In general, there were greater temporal changes in the patterns of abundance for the shallow reef assemblages at 20 m, relative to those at 30 m and 50 m. The structure and dynamics of sponge-dominated assemblages were further examined by contrasting these assemblages on both exposed and sheltered reefs at the same depth. Four sheltered estuarine and four open coastal reef locations were examined where three nested sites were sampled within each location over time. Generally, richness of erect species was greater for the assemblages on sheltered reefs whilst there was greater temporal variability on reefs exposed to wave energy.

The effect of discharging sewage effluent on shallow, nearshore and deep-water macrobenthic assemblages was examined at various spatial and temporal scales. In shallow water kelp forests, the assemblages were sampled according to an experimental design that had two sites nested within each of three outfall and three reference locations. Apart from the richness of sponges, univariate and multivariate analyses determined that there were no obvious patterns associated with the discharge of sewage effluent from these low volume and highly mixed cliff-face outfalls. In general, the assemblages showed natural spatial patchiness that could not be attributed to the effects of sewage.

In contrast, using a ‘Beyond BACI’ experimental design, the cover and the number of encrusting macrobenthic species inhabiting a nearshore reef in the vicinity of an ocean outfall changed rapidly following the discharge of secondary treated sewage effluent. Within 3 months, significant reductions in the cover of crustose and foliose algae were apparent when compared to reference locations. The cover of several species of sponge, including *Cymbastela concentrica*, *Geodinella* sp. and *Spongia* sp. also underwent marked declines coincident with the
commissioning of the outfall. Multivariate analyses revealed marked shifts in the structure of the assemblage at the outfall relative to the reference locations. The overall composition of the community at the outfall changed from one in which algae and sponges were well represented to an assemblage dominated by silt and ascidians.

Finally, the macrobenthic assemblages living on reefs in the vicinity of a newly commissioned deep-water ocean outfall were sampled using an experimental design that had three sites nested within each of three reef locations. After 18 months, significant effects of the outfall were detected for the total number of species, bryozoans, cnidarians and the abundances of two bryozoan species and a non-descript silt matrix. Small-scale interactions among sites through time also occurred for many taxa which represented inherent 'noise' and some evidence for the effects of the outfall at smaller spatial scales.

To partition out variation associated with the discharge of sewage, a manipulative field experiment was done to examine some of the processes that may be causing some of the observed patterns within sponge-dominated assemblages. The phototrophic sponge, *Cymbastela concentrica* was used to test its response to increased shade, silt and nutrients. Furthermore its response to a salinity gradient was also examined. The results indicated that sponge growth and reproductive status were influenced by shading and siltation, and the relationship between the host sponge and its macroalgal symbiont were also affected. Nutrients did not affect the sponge in any way, whereas a decreasing salinity gradient affected the growth, reproductive status and symbiotic algae within the sponge.
In general, the structure and dynamics of sponge-dominated assemblages was found to be highly variable at many scales with natural and anthropogenic disturbance playing a critical role in the dynamism of the assemblages. Water authorities with the responsibility of managing the disposal of sewage effluent to the marine environment commonly use poorly designed “post-commissioning” sampling programs to assess environmental impact. The utility of monitoring programmes that do not sample at appropriate spatial and temporal scales is compromised because of the considerable variability inherent within sponge-dominated assemblages.
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Figure 45. Mean (± SE) concentration of chlorophyll-a (µg/g) in the sponge Cymbastela concentrica in (a) shade, (b) silt, (c) nutrients and (d) salinity experiments.
CHAPTER 1: GENERAL INTRODUCTION

Anthropogenic disturbance has the potential to alter the structure and dynamics of marine communities (Warwick, 1993), which can manifest as increased variability in the diversity and abundance of marine organisms at different spatial and temporal scales (Warwick and Clark, 1993a). The detection of such change has been the subject of considerable discussion in the marine literature (see Warwick, 1986; Clarke and Green, 1988; Underwood and Peterson, 1988; Warwick and Clarke, 1993a; Underwood, 1994; Underwood, 1996; Underwood, 2000a). It is generally agreed that marine assemblages can experience significant natural spatial and temporal variability and to be able to detect environmental impact associated with anthropogenic disturbance, sampling designs must be capable of accounting for and explaining this variability (Andrew and Mapstone, 1987; Warwick, 1993; Underwood, 1996; Underwood, 2000b).

The discharge of sewage effluent into ocean waters has been found to alter, at various spatial scales, marine assemblages living in soft sediment habitats (Pearson and Rosenberg, 1978; Gee et al., 1985; Warwick et al., 1987; Warwick, 1988a; Austen et al., 1989; Anderlini and Wear, 1992; Otway et al., 1996a) and on hard substrata (Warwick, 1993a; Chapman et al., 1995; Tegner et al., 1995; Smith, 1996a). The amount of disturbance that an assemblage of organisms will experience will depend on the distance from the point of discharge, and the quantity and quality of the sewage effluent (Otway, 1995). The amount of change to variables such as abundance and diversity will also depend on whether any anthropogenic disturbance is sustained (press) or episodic (pulse) (Bender et al., 1984; Underwood, 1993; Otway, 1995).

In temperate regions, research into the effects of sewage effluent on assemblages living on hard substrata has generally focused on intertidal reef habitats and specifically those changes
associated with the diversity, distribution and abundance of macroalgae (Borowitzka, 1972; Murray and Littler 1978; May 1985; Brown et al., 1990; Fairweather, 1990; Lopez Gappa et al., 1990; Doblin and Clayton, 1995; Bellgrove et al., 1997). There are very few studies that have examined attributes such as mortality, growth and recruitment in determining the structure of intertidal reef communities exposed to sewage effluent (Hindell and Quinn, 2000). Those studies that have been done have acknowledged sewage as modifying algal and faunal communities (Littler and Murray, 1975; Bellgrove et al., 1997), reducing the dominance of mytilids (Lopez Gappa et al., 1990) and altering the rate of growth of macrofauna (Tablado et al., 1994).

Underwood et al. (1991) have suggested that before we can understand the processes affecting populations of subtidal marine organisms, we must first identify their “natural” spatial and temporal patterns of distribution and abundance. Establishing the effects of the discharge of sewage on subtidal assemblages living on hard substrata, or for that matter quantifying their natural spatial and temporal variability, has been problematic due to the difficulty in collecting these data at depth (Friedrich, 1969).

There is a considerable amount of “grey literature” describing the effects of sewage on sessile assemblages living on hard substrata, however few studies make use of appropriate spatial and temporal sampling designs with multiple controls or reference locations (Chapman et al., 1995). The lack of appropriately scaled and replicated designs has reduced the power to make predictions and generalisations about the effects of sewage on the structure and dynamics of these assemblages. Furthermore, where sewage outfalls have high standards of effluent quality (e.g. tertiary treatment) and/or low discharge volumes, adverse effects to these assemblages have been reportedly difficult to detect (Smith and Simpson, 1992; 1993; Smith, 1994; Roach et al., 1995).
In depths below 15m, sponges and ascidians generally dominate the sessile encrusting assemblages on temperate reefs (Ayling, 1981; Butler, 1986; Underwood et al., 1991; Butler, 1997; Davis et al., 1997; Andrew; 1999). Spatial and temporal data, which describe the natural structure and dynamics in these assemblages, are quite limited (Underwood et al., 1991), therefore making predictions about the effects of disposing sewage effluent into these habitats difficult, if not impossible (Underwood and Chapman, 1996). To place this lack of understanding in perspective, the long-held view that assemblages of organisms living on temperate reefs (Ayling, 1983a) and artificial structures (Kay and Butler, 1983; Butler and Chesson, 1990) are “temporally stable” has been used to reject the general hypothesis of increased variability associated with anthropogenic disturbance (Chapman et al., 1995), proposed by Warwick and Clarke (1993a). Generalising about temporal stability in these assemblages may lead to incorrect assumptions about their structure and dynamics and predictions about the way in which anthropogenic disturbance alters these patterns.

In this thesis I set out to investigate four general questions:

1. Can subtidal sponge-dominated assemblages living on hard substrata be reliably quantified at appropriate spatial and temporal scales, taking into account their taxonomic uncertainty and the difficulties associated with collecting quantitative data in these types of habitats (Chapter 2)

2. What are the “natural” patterns of spatial and temporal variability in sponge-dominated assemblages associated with depth and degree of exposure on subtidal temperate reefs? (Chapter 3)

3. What (if any) are the impacts of discharging sewage effluent to shallow, mid and deep-water sponge-dominated assemblages? (Chapter 4)

4. What potential processes, associated with the discharge of sewage, could cause any observed patterns in these assemblages? (Chapter 5)
To answer the first question, a reliable and efficient technique was required that enabled the collection of data at appropriate scales and that was scientifically rigorous. A remotely operated camera rig was developed, which allowed the collection of these data in both shallow and deep-water, and an experiment was done to test its efficacy. The second part of this question was answered by examining the level of taxonomic sufficiency required to detect differences between assemblages at outfall and reference locations.

The second question was investigated by quantifying the spatial and temporal distribution and abundance of sponge-dominated assemblages associated with three depth gradients (20 m, 30 m and 50 m), at three locations off the Sydney coastline. Furthermore, the differences in sponge-dominated assemblages between exposed and sheltered reefs were also quantified at a number of spatial and temporal scales.

The effect of anthropogenic disturbance (sewage) on the structure and dynamics of sponge-dominated assemblages was investigated using the general null hypothesis that there would be no significant differences in the spatial and temporal patterns of abundance or diversity of sponge-dominated assemblages associated with the disposal of sewage. Assemblages were examined near outfalls that discharged sewage effluent into three general depth ranges, i.e. 6-10 m, 18-20 m and 50-60 m.

Finally, some of the processes through which sewage effluent may alter sponge-dominated assemblages were investigated in a series of field-based experiments. The null hypotheses that shading, siltation, nutrients and salinity would not affect the rate of growth, reproductive status and associations with symbiotic algae were tested using the phototrophic sponge *Cymbastela concentrica*. 
CHAPTER 2: GENERAL METHODS

STUDY AREA

All studies and experimental work described in this thesis were done in subtidal habitats along the eastern coastline of New South Wales, from Cronulla in the south to Port Stephens in the north (Fig. 1). Shallow-water habitats were generally in the depth range 6-20 m, whilst the deep-water habitats were from 20-60 m. The reefs sampled during this research ranged in depth and degree of exposure to disturbance from ocean storms. The types of assemblages found on different reefs varied, however the dominant assemblage on any one reef was generally similar (Table 1). General descriptions of the subtidal assemblages inhabiting some of these habitats can be found in Underwood et al. (1991) and Underwood and Chapman (1997). A recent review of the ecology of assemblages on temperate subtidal reefs in NSW can be found in Andrew (1999).

<table>
<thead>
<tr>
<th>Depth Range (m)</th>
<th>Sheltered reef</th>
<th>Exposed reef</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-12</td>
<td>Kelp</td>
<td>Kelp</td>
</tr>
<tr>
<td>12-15</td>
<td>Crustose Algae</td>
<td>Crustose Algae</td>
</tr>
<tr>
<td>15-20</td>
<td>Sponge/Ascidian</td>
<td>Sponge</td>
</tr>
<tr>
<td>20-40</td>
<td>-</td>
<td>Sponge</td>
</tr>
<tr>
<td>40-60</td>
<td>-</td>
<td>Sponge</td>
</tr>
</tbody>
</table>

The subtidal reefs in very shallow water (i.e. 6-12 m) were generally dominated by the kelp *Ecklonia radiata* Agardh (Table 1 and Table 2) and have been described by Kennelly (1987a) and Kennelly and Underwood (1992). The reefs between 12-15 m were generally...
composed of red crustose coralline algae (urchin grazed barren grounds), whilst the
dominant sessile encrusting species were sponges and ascidians (Underwood *et al.*, 1991;
Andrew, 1999). On reefs between 15-20 m the dominant sessile macrobenthic organisms
were sponges and ascidians (Butler, 1997; Andrew, 1999), whilst sponges dominated the
reefs greater than 20 m depth (Roberts and Davis, 1996; see Chapter 3).

Table 2. Locations of the subtidal reefs studied along the NSW coastline and some of their general attributes.

<table>
<thead>
<tr>
<th>Location</th>
<th>Aspect</th>
<th>Proximity to Coast</th>
<th>Depth (m)</th>
<th>Dominant Assemblage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tomaree Head</td>
<td>Exposed</td>
<td>Adjacent</td>
<td>18-20</td>
<td>Sponge</td>
</tr>
<tr>
<td>Point Stephens</td>
<td>Exposed</td>
<td>Adjacent</td>
<td>18-20</td>
<td>Sponge</td>
</tr>
<tr>
<td>Boulder Bay</td>
<td>Exposed</td>
<td>Adjacent</td>
<td>18-20</td>
<td>Sponge</td>
</tr>
<tr>
<td>Norah Head</td>
<td>Exposed</td>
<td>Adjacent</td>
<td>6-12</td>
<td>Kelp</td>
</tr>
<tr>
<td>North Avoca</td>
<td>Exposed</td>
<td>Adjacent</td>
<td>6-12</td>
<td>Kelp</td>
</tr>
<tr>
<td>Winnie Bay</td>
<td>Exposed</td>
<td>Adjacent</td>
<td>6-12</td>
<td>Kelp</td>
</tr>
<tr>
<td>Lyon Island</td>
<td>Sheltered</td>
<td>Adjacent</td>
<td>12-15</td>
<td>Sponge</td>
</tr>
<tr>
<td>Bungan Head</td>
<td>Exposed</td>
<td>Adjacent</td>
<td>6-12</td>
<td>Kelp</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>18-20</td>
<td>Sponge</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Nearshore</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Offshore</td>
<td></td>
</tr>
<tr>
<td>Turrimetta Head</td>
<td>Exposed</td>
<td>Adjacent</td>
<td>6-12</td>
<td>Kelp</td>
</tr>
<tr>
<td>Long Reef</td>
<td>Exposed</td>
<td>Adjacent</td>
<td>18-20</td>
<td>Sponge</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nearshore</td>
<td>30-40</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Offshore</td>
<td>40-60</td>
<td></td>
</tr>
<tr>
<td>North Head</td>
<td>Exposed</td>
<td>Adjacent</td>
<td>18-20</td>
<td>Sponge</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nearshore</td>
<td>30-40</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Offshore</td>
<td>40-60</td>
<td></td>
</tr>
<tr>
<td>Quarantine Head</td>
<td>Sheltered</td>
<td>Adjacent</td>
<td>18-20</td>
<td>Sponge</td>
</tr>
<tr>
<td>South Head</td>
<td>Sheltered</td>
<td>Adjacent</td>
<td>18-20</td>
<td>Sponge</td>
</tr>
<tr>
<td>Cape Banks</td>
<td>Exposed</td>
<td>Adjacent</td>
<td>18-20</td>
<td>Sponge</td>
</tr>
<tr>
<td>Henry Head</td>
<td>Sheltered</td>
<td>Adjacent</td>
<td>18-20</td>
<td>Sponge</td>
</tr>
<tr>
<td>Inscription Point</td>
<td>Sheltered</td>
<td>Adjacent</td>
<td>18-20</td>
<td>Sponge</td>
</tr>
<tr>
<td>Cape Solander</td>
<td>Exposed</td>
<td>Adjacent</td>
<td>12-15</td>
<td>Crustose</td>
</tr>
<tr>
<td>Cronulla</td>
<td>Exposed</td>
<td>Adjacent</td>
<td>6-12</td>
<td>Kelp</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>12-15</td>
<td>Crustose</td>
</tr>
</tbody>
</table>
Figure 1. Location of the study area along the NSW coastline, Australia.
QUANTIFYING SUBTIDAL ASSEMBLAGES

Introduction

Since the advent of SCUBA (Self Contained Underwater Breathing Apparatus), marine biologists have examined *in situ* assemblages of species living on subtidal hard substrata. For quantitative sampling in depths below 30m, however, SCUBA has only limited utility due to the small maximum bottom times permitted and the need for expensive diving equipment. Other methods which sample these assemblages such as dredges, grabs and settlement plates have had limited success due to their destructive nature and their preclusion of *in situ* sampling (see Friedrich, 1969; Kennelly, 1983). Because of these problems, assemblages of species living on hard substrata (here defined as hard substrata macrobenthos - HSM) in deeper water have been largely ignored. These assemblages are an important component of marine ecosystems because of their size, range, diversity and richness, and it has become necessary to develop techniques that permit their cost-effective sampling.

Underwater photography has been used with some success to quantify HSM assemblages in deeper water. Its first reported use was by Louis Boutan in the Bay of Banyuls in 1893 (Friedrich, 1969; Holme, 1984; see Ewing *et al.*, 1967 for a discussion of the history of underwater photography in scientific investigations). SCUBA divers have used cameras to photograph the sea floor to depths of 80 metres whilst remotely operated vehicles (ROV's) and specialised diving suits have used photography to depths of 3000 metres (Friedrich, 1969; Hawkes, 1983; Nuytten, 1983). The macrobenthos on sublittoral hard substrata in Sweden and Norway has been studied by Sandnes and Gulliksen (1980) using photographic methods described by Torlegard and Lundalv (1974) and the diversities and abundances of fish and crabs have been quantified, using ROV's with video and/or still cameras (Russel and Serafry, 1986; Conan and Maynard, 1987; Green and Alevison, 1989). Many unpublished reports describe the use of ROV's and tethered cameras in sampling biological communities in deep water, but there are few published papers describing deep-water assemblages living on hard substrata (see Logan, 1988; Barthel *et al.*, 1991).
Underwater photographic methods have been compared with conventional methods for sampling epifauna and HSM (e.g. direct sampling using SCUBA, dredges, nets etc. - Vever, 1952; McIntrye, 1956; Laughton, 1959; Emery et al., 1965; Torlegard and Lundalv, 1974).

A consistent conclusion from these comparisons was that photographic methods were usually comparable with conventional methods and, in many cases, were the only effective means for retaining a permanent record of data collected in these habitats. Another conclusion was that photographic methods may underestimate abundances of small and cryptic individuals (see Bohnsack, 1979; Logan, 1988; Foster et al., 1991).

Few papers dealing with subtidal HSM assemblages in Australian waters have been published and these were generally done in shallow water using SCUBA or settlement plates (for review see Underwood and Kenneily, 1990). The only quantitative study that has been done on deep-water HSM assemblages in Australia is an unpublished report by Jones (1977), which involved photography by a "jump camera" (a remotely operated camera on a frame which is "jumped" along the bottom), SCUBA divers and settlement boxes. Although this study provided some information on the taxonomy and distribution of deep-water HSM organisms off Sydney, NSW, problems in experimental design precluded the valid comparison of alternative methods.

Recently there has been an increased demand for quantifying the deep-water HSM assemblages off Sydney, because of environmental concerns over the extension of Sydney's sewage outfalls into these habitats. To address this demand, a photographic sampling system was developed involving a jump camera. Here the technique, its accuracy and efficiency were assessed by comparing data gathered from the camera with data gathered using conventional quadrat sampling in situ by divers using SCUBA. The time required to gather data using the two techniques was also compared.
Methods

\textit{Jump Camera}

The jump camera system (Fig. 2) used for this work consisted of an aluminium frame (A) supporting a 35mm underwater camera (B). Zinc sacrificial anodes (C) on the base of the frame protected the frame from corrosion and approximately 15kg of lead weights (D) kept the frame upright underwater ensuring that the trip leg (see below) came into contact with the substratum. A rear mounted directional fin (E) kept the rig from twisting whilst operating in strong currents.

A 35mm Sea & Sea Motor Marine-2 underwater camera was suspended from the cross member (F) and trigger arm support (G) at the top of the frame, allowing it a full view of the area encompassed by the base of the frame. This camera had an automatic film advance mechanism, which permitted all frames in a roll of film to be taken in succession. The frame and camera was accompanied by a Sea & Sea Yellow Sub-50 MM-11 flash strobe unit (H), which was synchronised with the camera shutter and offset at approximately 15 degrees to maximize illumination and reduce back scatter from particulate matter in the water.

The frame was lowered to the sea floor by 16mm rope. Upon reaching the substratum, the upward thrust provided by the settlement of the frame and its trip leg (I) on the bottom was translated into a horizontal movement of the trigger arm (J), depressing the shutter button on the camera, thus taking a photograph. After each photograph, the frame was lifted approximately 2m off the bottom and held for at least 30 seconds while the boat drifted. After this time, the strobe was recharged and, because the film had automatically advanced, the system was ready to be lowered again or "jumped" to a new location for another photograph. This procedure was continued until the film was finished (i.e. 36 exposures), then the jump camera was lifted to the surface, the film and batteries for the camera and strobelight were replaced and the whole system was made ready for re-deployment. The
date, time and satellite-navigation fixes using the Global Positioning System (accuracy of ± 10 m) were recorded onboard the vessel during each deployment of the jump camera. A watertight timepiece, depth gauge and compass were attached to the frame (K) within the camera's field of view for comparison with readings taken on the vessel.

**Analysis of Photographs**

The photographs taken by the jump camera (area 0.45m$^2$) were analysed using a Bell and Howell black box projector. An overlay plastic grid of 100 regularly-spaced points was placed on the screen so that the numbers and percentage covers of individual organisms or colonies in each photograph could be recorded. Where possible, organisms were identified to species in the field or from the photographs. Some organisms could only be identified to genus and some had to be classified as a complex or component taxon. Data are presented as the mean percentage covers for each component or individual species analysed and the mean numbers of species.

**Comparison of Jump Camera with in situ Sampling**

To assess whether data from photographs taken by the jump camera gave a good estimate of the deepwater HSM assemblages off Sydney, a field experiment was done to quantify any differences between photo-quadrats and conventional quadrats sampled by divers using SCUBA. This comparison had to be done in relatively shallow water (<15 m) so that SCUBA divers could sample quadrats of the same dimensions (0.45m$^2$) as those taken by the jump camera at the same location and time. At the HSM deep water sites off Sydney (see Fig. 3), flat encrusting primary (including encrusting algae and fauna) and erect secondary space (including massive and erect faunal species) have been identified (Roberts and Henry, 1992). This experiment was done near Sydney Harbour at North Head (Fig. 3) where habitats resembled those found in deeper water (40 - 60 m). To test whether the jump camera and SCUBA divers sampled these two habitats similarly, two locations nested within each type of habitat were sampled (Table 3). At each location, the jump camera was lowered
to the bottom and ten haphazardly-placed photo-quadrats were taken. Similarly at each location, SCUBA equipment was used to sample in situ, ten haphazardly-placed 0.45m² quadrats. In each quadrat, the number of species and their percentage covers were estimated. To assess the cost-effectiveness of the two techniques, the time required to do each type of sampling was assessed.

Photo-quadrats and diver-sampled quadrats were examined for the percentage covers of each taxon and numbers of taxa. These variables were examined using the appropriate 3-factor analysis of variance where habitat-type was treated as fixed, locations were nested within each habitat and the technique used was considered a fixed factor. Prior to analysis, the data were examined using Cochran's test for homogeneity of variances (Winer, 1971; Underwood, 1981) and where variances were heterogeneous, data were transformed to log (x + 0.5) for richness and arc-sine for percentage covers (Winer, 1971). If variances could not be stabilized at $p = 0.05$ but could be stabilized at $p = 0.01$, the analysis of variance was done using the $p = 0.01$ probability level (Underwood, 1981). Where significant differences were found in the analysis of variance or where variances could not be stabilised, Student-Newman-Keuls (SNK) multiple comparisons were done to determine differences among means (Winer, 1971).

Table 3. Experimental design of experiment comparing photo-quadrats taken by the jump camera and in situ quadrats sampled by SCUBA divers (JC – jump camera, D – diver).

<table>
<thead>
<tr>
<th>Habitat</th>
<th>Flat</th>
<th>Erect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Technique</td>
<td>JC</td>
<td>D</td>
</tr>
<tr>
<td>Replicates</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>
Figure 2. Specifications for the jump camera showing (A) the aluminium frame, (B) 35mm underwater camera, (C) zinc anodes, (D) lead weights, (E) directional fin, (F) cross member, (G) trigger arm support, (H) strobe light, (I) trip leg, (J) trigger arm and (K) timepiece, depth gauge and compass.
Figure 3. Location of the experimental site (▼) and deep-water locations at North Head (●), Long Reef (◇) and Bungan Head (△).
Results

Four major groups of animals were identified using the jump camera: the Porifera, Cnidaria, Bryozoa and Asciidiacea. Other taxa that were less common included the Echinodermata, Mollusca and Annelida. The dominant algae found at the deep-water sites were encrusting Corallinacea from the Order Cryptonemiales (Roberts and Henry, 1992). A silt matrix was also found at the deep-water sites. In shallow waters nearby, this material has been described as a mixture of algae, sediment and micro-organisms (Kennelly, 1983).

Table 4 summarises the results from the analysis of variance for all taxonomic groups analysed in the experiment comparing data gathered from the jump camera with that sampled by SCUBA divers in situ. Generally, the number of species and percentage covers for all taxa were greater in habitats that consisted of erect secondary space. A significant habitat x technique interaction occurred for total species richness (including all faunal and algal components except the silt matrix) and the numbers of cnidarians (Table 4; Fig. 4a & 4i). SNK tests of these variables showed that in erect habitats, divers recorded more taxa than did estimates from photo-quadrats.

Sponges were the dominant faunal group found at the experimental site, both in terms of numbers of species and percentage covers. They included both encrusting and erect species and commonly included *Tethya cortica* Lendenfeld, *Chondropsis* sp., *Mycale mirabilis* Lendenfeld, *Phyllospongia caliciformis* Carter, *Echinoclathria gigantea* Lendenfeld, *Ophlitaspongia tenuis* Dendy, *Clathria* spp. and *Haliclona* spp. *T. cortica* (Fig. 4b) did not occur in flat habitats and its abundances were not significantly different among locations or sampling techniques (Table 4). The sponges *Chondropsis* sp., *M. mirabilis* and *P. caliciformis* were all found to exhibit highly significant differences among habitats and, like *T. cortica*, were only found in habitats that consisted of erect secondary space (Fig. 4c,d,e; Table 4). These latter results should be treated with caution, however, because the variances
could not be stabilized. Significant habitat differences were found for the number of sponges (Fig. 4f; Table 4), and for individual sponges (see Fig. 4c,d,e), but no differences were found between the techniques used (Table 4). The erect habitats were significantly richer in numbers of sponges than were the flat habitats. The total percentage covers of sponges (Fig. 4g) were not significant for any of the three sources of variation tested (Table 4).

Cnidarians were found in both habitat types and included species of *Sertularia*, *Anthothoe* and *Culicia*. Analyses of the percentage covers of the small coral *Culicia* sp. (Fig. 4h; Table 4) and all cnidarians (Fig. 4j; Table 4) showed no significant differences between habitats, locations or techniques. As mentioned above, a significant interaction occurred between habitat and sampling technique for the numbers of cnidarian taxa (Fig. 4i; Table 4).

Bryozoans within both habitats included species of *Membranipora*, *Triphyllozoan* and *Iodictyum*. The lace bryozoan *Triphyllozoan* sp. was commonly found in the erect habitat (Fig. 4k) and its percentage cover was not significantly different for any sources of variation (Table 4). Total numbers of bryozoa (Fig. 4l) and their percentage covers (Fig. 4m) were not significantly different ($P > 0.01$) for any sources of variation (Table 4).

Ascidians were found in both habitats and included species of *Polycarpa*, *Pyura* and *Botrylloides*. *Pyura spinifera* Quoy and Gaimard (Fig. 4n) was found only in the erect habitat whilst a club ascidian *Polycarpa* sp. (Fig. 4o) was found in both. No differences between the two sampling techniques were detected for either species (Table 4). Numbers of ascidians (Fig. 4p) and their percentage covers (Fig. 4q) were not significantly different among habitats, locations or techniques (Table 4).

Non-sessile invertebrates such as molluscs (Fig. 4r) and echinoderms (Fig. 4s) were found in both habitats but in very small numbers. The molluscs included small numbers of nudibranchs and limpets such as *Patella chapmani* Tennyson Woods and *Patelloida mufria*
Hedley, while the echinoderms included sea urchins such as *Centrostephenus rogersii* A. Agassiz. No significant differences were found for any of the sources of variation analysed for these taxa (Table 4).

Encrusting coralline algae were present in all habitats (Fig. 4t). In flat habitats, these algae accounted for approximately 45% of the total cover whilst in erect habitats they accounted for only 15%. These algae comprise a number of species from the order Cryptonemiales but, because taxonomic differentiation for these species is difficult, data from these various species were combined. No significant differences were found among all sources of variation for the percentage covers of this group (Table 4). Turfing algae were present at all locations and averaged around 25-30% of the total cover in both habitats (Fig. 4u). No significant differences were found among the sources of variation for this group (Table 4).

As described previously, a silt matrix was evident at all locations. This matrix averaged around 25% in the erect habitat and 2% in the flat habitat (Fig. 4v). Because of heterogeneous variances (Table 4), however, significant differences between habitat types must be viewed with caution. Comparisons of means showed that erect habitats had greater percentage covers of the silt matrix than flat habitats.

The total time taken for each technique is shown in Table 5. Quadrats sampled by divers required no interpretation as the organisms were identified and counted in situ. The jump camera data includes the time required in the field, plus analysis and interpretation of the photographs in the laboratory. On average, diver quadrats took 1.5 minutes per quadrat in flat habitats and 3 minutes per quadrat in erect habitats whilst the jump camera took 2.0 minutes per quadrat in flat habitats and 3 minutes in erect habitats. To collect 40 diver quadrats it took approximately 180 minutes, whilst the jump camera photo-quadrat estimation required approximately 200 minutes.
Figure 4. Mean richness and abundances (± SE) for the major groups and species of macrobenthos collected by jump camera photo-quadrats and in situ diver-sampled quadrats. Each histogram depicts data for both flat and erect habitats (clear – jump camera; shaded – diver).
Figure 4. (continued)
Table 4. Summaries of $F$ ratios from analyses of variance of data comparing the jump camera method and *in situ* sampling using SCUBA. nil = no transformation required; ns = not significant ($P > 0.05$); ns+ = not significant ($P > 0.01$); * = significant ($P < 0.05$); ** = significant ($P < 0.01$).

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>% cover Teorya cortica</th>
<th>% cover Chondropsis sp.</th>
<th>% cover Mycale minibus</th>
<th>% cover Phylospogia calificormis</th>
<th>% cover Culisia sp.</th>
<th>% cover Trphylozoan sp.</th>
<th>% cover Pyura spinifera</th>
<th>% cover Polycarpa sp.</th>
<th>Total number of species</th>
<th>No. of Poriferan species</th>
<th>No. of Cnidarian species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Habitat</td>
<td>1</td>
<td>1.78ns</td>
<td>592.1**</td>
<td>128.44**</td>
<td>36.00*</td>
<td>13.44ns</td>
<td>2.47ns</td>
<td>3.24ns</td>
<td>0.11ns</td>
<td>150.7**</td>
<td>164.0**</td>
<td>67.6*</td>
</tr>
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<td>0.07ns</td>
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<td>1.45ns</td>
<td>1.39ns</td>
<td>0.73ns</td>
<td>0.24ns</td>
</tr>
<tr>
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<td>1.62ns</td>
<td>0.56ns</td>
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<td>2.86ns</td>
<td>14.4ns</td>
</tr>
<tr>
<td>H X T</td>
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<td>0.44ns</td>
<td>0.58ns</td>
<td>1.62ns</td>
<td>0.56ns</td>
<td>3.24ns</td>
<td>0.04ns</td>
<td>0.01ns</td>
<td>0.06ns</td>
<td>31.25*</td>
<td>0.36ns</td>
<td>32.4*</td>
</tr>
<tr>
<td>L X T(H)</td>
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<td>0.62ns</td>
<td>0.96ns</td>
<td>1.86ns</td>
<td>0.83ms</td>
<td>1.24ms</td>
<td>1.05ns</td>
<td>1.15ns</td>
<td>0.14ns</td>
<td>0.65ns</td>
<td>0.24ns</td>
</tr>
<tr>
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<td>1.45</td>
<td>2.83</td>
<td>6.96</td>
<td>5.24</td>
<td>0.52</td>
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</tbody>
</table>

Cochran's test

** ns ns ns ns ns ns NIL ns
Transform

ns+ nil nil nil nil nil nil **
Table 5. Summary of time taken (minutes) to complete 40 quadrats of each technique.

<table>
<thead>
<tr>
<th>Task</th>
<th>Collection in Field</th>
<th>Interpretation in Laboratory</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Flat</td>
<td>Erect</td>
</tr>
<tr>
<td>Habitat</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diver</td>
<td>60</td>
<td>120</td>
</tr>
<tr>
<td>Jump Camera</td>
<td>20</td>
<td>20</td>
</tr>
</tbody>
</table>

Discussion

The jump camera developed for quantifying macrobenthic assemblages living on hard substrata in deep water proved to be an effective tool that is relatively simple and inexpensive to construct and can be deployed and recovered successfully in most sea states and in strong currents. The rig can be effectively used in depth ranges from 5 to 100 m. For depths below 100 m, a more sophisticated underwater camera and flash system would be required. Other logistic advantages of using the jump camera include its ability to sample in low visibility, at night and from a reasonably small vessel.

The deep-water photo-quadrats enabled consistent and reliable recognition of many macrobenthic organisms (Roberts and Henry, 1992). There was some difficulty, however, in recognising individuals or colonies smaller than about 4-5 mm. This was to be expected, however, because Foster et al. (1991) compared point-quadrats and photo-quadrats on intertidal hard substrata and found that taxonomic resolution was difficult for organisms smaller than 2-3 cm. The taxonomy of subtidal marine organisms from deep water is not well documented for the east coast of Australia and this, (not the use of photography to sample them) precluded the identification of many taxa to species. Although previous workers have collected these organisms, much of the material has not survived recent
taxonomic revisions. The sponges are probably the most neglected of the invertebrate phyla from these subtidal habitats due to their difficult taxonomy (Barthel et al., 1991).

The comparison between data collected by the jump camera and by SCUBA divers for the numbers of species and percentage covers of individual taxa indicated that no advantages were gained by using a diver to collect HSM data. This was also the case in terms of the time required for the two techniques and the non-destructive identification of species. Where significant differences between habitats occurred, there were no differences between the two techniques, implying that for those habitats, a jump camera yielded as much information as a SCUBA diver operating in situ.

Advantages of the jump camera technique over diver-collected data include the permanency of the data recorded and the ability to sample across a range of depths. It is also considerably less dangerous than SCUBA diving operations and, when compared with other types of quantitative sampling, it does not damage the sampled assemblage. Disadvantages of the technique involve those situations when the assemblages to be sampled live on vertical sides of rock walls or reefs. In these situations, the camera frame cannot sit upright, precluding an estimation of the size of the quadrat sampled. SCUBA diving is advantageous over a jump camera when biological specimens need to be collected in situ for taxonomic purposes or when particularly cryptic organisms need to be sampled. It is also easier for a diver to quantify organisms inhabiting primary space when they are covered or hidden by secondary space macrobenthos (eg. kelp canopies, large erect sponges, etc.). When studying processes that occur at small spatial scales, such as recruitment and settlement, the jump-camera method would be inappropriate and in situ sampling using underwater microscopy or settlement plates would be necessary (see Kennelly, 1983; Kennelly and Underwood, 1984).
In the present study, significantly greater percentage covers of the sponges *Chondropsis* sp., *Mycale mirabilis* and *Phyllospongia caliciformis* occurred in erect habitats. Usually these sponges are found at depths greater than 15 m where wave energy and turbulence are considerably reduced, when compared with the flatter habitats that occur in shallow areas. The types of sponges that were sampled in these latter areas are usually flat, encrusting or small and include species such as *Haliclona* and *Tethya*. Although *Tethya cortica* was not found in the flat habitat in this study, its range extends from deep water up to the lower intertidal zone. Total species richness and the numbers of sponges and cnidarians were also significantly greater in erect habitats. As stated previously, wave energy and turbulence is considerably less in these locations and species seem to exhibit more varied morphologies and greater numbers of species. Abundances of the silt matrix were significantly greater in erect habitats and this may be related to silt being trapped in areas of greater micro-topographical heterogeneity.

The time required to sample areas using each technique was greater in erect habitats due to greater species diversity and habitat complexity. On average the jump camera took slightly longer to sample a site, as the vessel was required to maintain a relatively stable position. In general, however, collecting a photo-quadrat is virtually instantaneous with only a small delay between photographs whilst the batteries of the strobelight recharge. Interpretation of a photo-quadrat should take approximately the same time as a diver-collected quadrat.

Interpretation of the results discussed in this paper, in terms of the structures of deep-water assemblages, should be done with caution. From shallow to deep water, the numbers of species and the relative covers of the major taxa are at least comparable for those places examined with the jump camera (Fig. 2; see also Roberts and Henry, 1992). Changes in species composition with depth will occur (Jones, 1977; Logan, 1988), making the level of taxonomic discrimination an important factor when determining spatial and temporal variation in these assemblages. Kennelly and Underwood (1992) concluded that for
quantifying patch dynamics in kelp forests, data is required on a species-specific level because small-scale variability can be masked at higher taxonomic levels. For large-scale pollution monitoring or ecological assessment, the jump camera technique yields data at a reasonable level of taxonomic discrimination. Warwick (1988a & b) suggests that anthropogenic effects which modify the composition of a community were more detectable at high taxonomic levels and that in some cases, the lower the taxonomic level, the greater the masking by natural variation. Ferraro and Cole (1990) suggest that the use of higher (coarser) taxa is more suitable for studying effects of enrichment on marine assemblages. For shallow and deep-water HSM assemblages in Australian waters the taxonomic levels required to differentiate natural spatial and temporal variations from those caused by human physical disturbances such as sewage outfalls have yet to be determined.

The jump camera method has been compared with diver-sampled quadrats and found to be as accurate and efficient in the collection of data. The many logistic advantages of the jump camera over the use of SCUBA divers in deep water gives this method substantial advantages in quantifying the structures of deeper water assemblages living on hard substrata. These techniques were used in the first quantitative assessment of spatial and temporal variations in the deeper water assemblages off the east coast of Sydney, Australia.
TAXONOMIC DISCRIMINATION

Introduction

In pollution monitoring or marine ecological studies, ecologists generally attempt to quantify and describe assemblages using the finest achievable taxonomic unit, i.e. species. This level of taxonomy is sought because it is believed to be the level at which organisms respond to their environment in terms of their behaviour, reproductive capabilities and tolerance to both natural and human disturbances (Krebs, 1978). Most of our knowledge of how organisms function is generally at the level of species whilst coarser taxonomic groupings such as phyla, although convenient in classifying biota, do not have any ecological meaning (Hutchings, 1999).

The use of species level compared with coarser taxonomic level data in identifying the effects of pollution and disturbance has been examined for assemblages that live in marine soft sediments (Ellis, 1985; Herman and Heip, 1988; Warwick, 1988a; 1988b; Ferraro and Cole, 1990; 1992; James et al., 1995; Chapman, 1998), within kelp holdfasts (Smith and Simpson, 1993) and in upland streams (Wright et al., 1995). Generally, these studies indicated that coarser taxonomic levels were often a better measure of community stress in polluted areas. In some cases, no extra information was gained from identifying organisms to the species level when examining stress on a community or whilst attempting to detect an impact.

For diverse and complex assemblages the identification of individuals to species level can result in considerable costs being incurred and differentiation at coarser taxonomic levels may thus reduce sorting costs (Hutchings, 1999). Further, the taxonomic status of marine invertebrates from temperate subtidal reefs can only be described as poor, making species identifications almost impossible for the non specialist (Davis et al., 1999).
In studying the macrobenthic assemblages that live within sediments, the samples are generally collected in the field and sorted and identified in the laboratory at a later date (Pearson and Rosenberg, 1978). Studying subtidal macrobenthic assemblages that live on hard substrata can be problematic due to the difficulties of destructive-sampling techniques in these habitats, and are usually sampled by SCUBA divers (Kennelly, 1987a). These types of studies can only be done when there is a good understanding of the taxonomy and identification of these organisms in situ or when destructive sampling is done, for example collection of kelp holdfasts (Smith et al., 1996). Where depth restricts the amount of time a diver may spend sampling underwater, comprehensive collections can be made in conjunction with quantitative photo-quadrat sampling.

In NSW, Australia, most ocean sewage outfalls discharge onto shallow water subtidal rocky reefs (Fairweather, 1990). In depths ranging from 0-12m, these habitats are generally dominated by the kelp *Ecklonia radiata* (C. Agardh) J. Agardh, which can form dense canopies under which live benthic algae, invertebrates and fish (Kennelly and Underwood, 1992; Smith et al., 1996). From 12-18m depths, assemblages are generally comprised of red crustose coralline algae, sponges, sea urchins, anemones, ascidians, and mobile and cryptic fish (Fletcher, 1987; Underwood and Kennelly, 1990; Underwood et al., 1991; Smith et al., 1999).

Studies associated with sewage outfalls on intertidal rocky shores indicated significant effects on the distribution of macrobenthic assemblages (Littler and Murray, 1975, 1978; Murray and Littler, 1978). Kindig and Littler (1980) discussed various effects of primary, secondary and secondary-chlorinated sewage effluent on the long-term growth and primary productivity of marine intertidal macrophytes. Along the east coast of Australia, sewage outfalls have been linked with changes to the macrobenthos (Borowitzka, 1972; Fairweather,
1990) with an absence of "typical" intertidal zonation and reduced species diversity. In subtidal habitats, responses to sewage have been recorded depending on the magnitude and quality of the effluent, the depth at which the discharge occurs and the type of assemblage being examined. Chapman et al. (1995) found relatively few differences in the time courses of assemblages at a decommissioned cliff-face outfall compared with reference locations, whereas Roberts (1996a; 1996b; see Chapter 4) demonstrated a reduction in the number of species following the discharge of sewage from a deep-water outfall. Furthermore, Smith and Simpson (1993) reported differences between faunal assemblages in kelp-holdfasts close to sewage outfalls whereas Roberts and Scanes (2000) could not detect differences in the assemblages of fauna and algae within kelp forests at three secondary-treated shoreline sewage outfalls (see Chapter 4).

Apart from studies on assemblages from within kelp holdfasts (Smith and Simpson, 1993), there have been no published accounts on the taxonomic sufficiency required to detect impacts associated with sewage-related discharges on assemblages that live on hard substrata. Here the differences between subtidal macrobenthic assemblages in the vicinity of a shoreline sewage discharge were contrasted with reference locations. Where differences were found, the taxonomic sufficiency required to detect sewage-correlated effects using both univariate and multivariate statistical approaches was examined.
Methods

The shoreline ocean outfall at Cronulla, NSW, Australia (Fig. 5) discharges approximately 60 megalitres per day of primary-treated sewage effluent onto shallow (6 m deep) subtidal rocky reef habitat. The sewage plume from the outfall is highly visible at the surface and can be extensive in its coverage. The direction of the plume is generally to the south, however this can vary depending on the currents, tides and winds.

At the outfall and at reference locations (Fig. 5), two depths/habitats were sampled during March 1993. At each location, SCUBA divers sampled the macrobenthic assemblages at 6 m (kelp habitat) and 15 m (crustose coralline habitat). Because there were two depths/habitat (kelp or crustose), different techniques and experimental designs were used for each. Within kelp forests, five replicate 27 x 27 cm quadrats were sampled in each of two randomly located sites, approximately 50 m apart and within 50 m of the discharge pipe. The richness and abundance of macrobenthic fauna living under the kelp canopy was estimated by recording their percentage covers and the number of species in each quadrat. Kennelly (1987c) discussed the limitations and advantages of using small quadrats that could physically fit between individual kelp plants and justified their size for use in kelp habitats.

Within the crustose habitats, five replicate 0.25 m² quadrats were sampled using the same methods outlined above, in each of three randomly located sites, approximately 50 m apart and 100 m from the outfall pipe. The reference locations for this study were different for each habitat type. The reference locations for the kelp habitat were situated at North Avoca, Bungan Head and Cape Banks (Fig. 5). The reference locations for the crustose habitat were situated to the north of the Cronulla outfall at Cape Solander and Inscription Point (Fig. 5). No suitable reference locations could be found to the south of the outfall, because the sewage plume more often than not travelled in that direction.
Figure 5. Locations of the reefs sampled to determine the effects of sewage and taxonomic resolution (Cronulla outfall - ●, kelp reference locations - ○, crustose coralline reference locations - △).
Univariate Assessment

Assemblages were examined for richness (number of species) and abundance (percentage cover or number of individuals). The richness data were aggregated into the taxocenes (sensu Ferraro and Cole, 1992) of phylum, class, order, family, genus and species. These derived variables were examined using analysis of variance, where locations were treated as fixed and sites were nested within each location. Prior to analysis, the data were tested for homogeneity of variances using Cochran's test (Winer, 1971; Underwood, 1981) and where variances were heterogeneous, data were transformed to log $(x + 0.5)$. If variances could not be stabilised at $P = 0.05$ but could be stabilised at $P = 0.01$, the analysis of variance was done using the $P = 0.01$ probability level (Underwood, 1981). Where significant differences were found in the analysis of variance or where variances could not be stabilised, Student-Newman-Keuls (SNK) multiple comparisons were done to determine differences among means (Winer, 1971).

A test of the non-centrality parameter (Winer, 1971) was also done to examine post-hoc statistical power of the taxonomically aggregated richness data. These tests indicate the probability of erroneous retention of the null hypothesis when applied to non-significant results (Andrew and Mapstone, 1987). This was done primarily to examine whether the power of the statistical test showed different patterns with increasing taxonomic resolution. The power required to detect statistical differences was determined from the treatment means, the number of replicates and the mean square residual from the results of each analysis of variance (Winer, 1971). After calculating the non-centrality parameter, or $phi$ value, to extract $beta$ from the non-central $F$ distribution tables, it was found that $beta$ in most cases was so low that no value could be calculated. The magnitude of making a Type-II error (Underwood, 1997) is represented by $beta$, and the power of a test is numerically equal to $\text{Power} = 1 - beta$. In all cases $beta$ was so low that power approached, or was equal...
to, one. This indicates that the power to detect differences in this study was very good. Because the power values were either equal to or approaching one, the calculated phi values were plotted against each taxonomic unit for both kelp and crustose habitats.

**Multivariate Assessment**

Multivariate statistical techniques were also used to examine the effect of taxonomic level on the description of spatial pattern of the deep and shallow communities. Comparisons of the composition of these communities among locations and sites were done for each taxonomic level using the procedures in the software package PRIMER (Plymouth Routines in Multivariate Ecological Research - Clarke and Warwick, 1994). The data were transformed to the double-square-root of their original value and similarity matrices were calculated using the Bray-Curtis similarity index (Clark and Warwick, 1994). Non-metric multidimensional scaling (nMDS) was used to obtain a multivariate representation of the data. Ordination plots were used to visually examine variations in the relationships among sites and locations at each taxonomic level. Pairwise rank correlations, between the similarity matrices at each taxonomic level used to create the ordinations, were also examined using the program RELATE (Somerfield et al., 1995). Percent similarity analyses (SIMPER) were used to compare changes in the dissimilarity among locations and similarity within locations, at each taxonomic level. Analysis of similarities (ANOSIM) was also used to examine differences between locations and sites. Where there were insufficient permutations to test for significant differences among locations, these analyses were interpreted using the R statistic (Clarke and Warwick, 1994), which provided a measure of discrimination among locations and sites.
Results

Six major groups of macrobenthic fauna were identified from within the kelp and crustose habitats: Porifera, Bryozoa, Cnidaria, Crustacea, Mollusca and Asciidiacea. The sponges (Porifera) were the most numerically dominant phylum in terms of their richness and abundance in both habitats and at all locations.

Three encrusting sponges were identified from within the kelp forests, *Psammopemma* sp., *Tedania* sp., and *Haliclona* sp. Eighteen species of sponge were identified from within the crustose habitats; *Tedania* spp., *Haliclona* spp., *Chondropsis* spp., *Dendrilla rosea* Lendenfeld, *Halichondria* sp., *Psammopemma* sp., *Chondrilla* sp., *Polymastia* spp. and *Ophlitaspongia tenuis* Dendy.

Bryozoans within the kelp forests included three species of encrusting *Membranipora* and a *Bugularia* sp., whilst *Membranipora membranacea* Linnaeus was the only bryozoan found within crustose habitats. Cnidarians were found at all locations within kelp forests and included two species from the Hydrozoa; *Sertularia* sp. and *Sertularia tenuis* Bale and two species from the Anthozoa; *Anthothoe albocincta* Hutton and *Oulactis muscosa* Dana. Within the crustose habitat four cnidarian species were found; *A. albocincta*, *Culicia tenella* Dana, *O. muscosa* and *Actinia* sp.

Crustaceans at kelp-dominated locations were represented by two species of barnacle, *Austrobalanus imperator* Darwin and a small *Balanus* sp. whilst *A. imperator* was the only crustacean found within the crustose habitat. Molluscs represented at all kelp locations were predominantly two species of limpet, *Patella chapmani* Tennyson Woods and *Patelloida mufria* Hedley. One species of chiton, *Onithochiton quercinus* Gould, was also found at the outfall location. Seven species of molluscs were found within the crustose habitat;
Patelloida mufria, Patella chapmani, Thais orbita Gmelin, Australium tentoriformis Jonas, Cabestano tabulata Menke, Aplysia sydneyensis Sowerby and Aphelodoris varis Abraham.

Ascidians were common at the Cronulla outfall location and in kelp forests and included Botrylloides magnicoecum Hartmeyer, Pyura gibbosa gibbosa Herdman and Didemnum moseleyi Herdman. Four species of ascidian were found within the crustose habitat; Botrylloides nigrum Herdman, B. magnicoecum, Botrylloides leachi Savigny and D. moseleyi.

Univariate Approach

Taxonomic resolution within kelp forests

Significantly greater numbers of taxa within all categories were found within the kelp forests at the outfall location compared with the reference locations (Fig. 6; Table 6). No significant differences were found among sites nested within locations for any of the taxonomic levels examined (Fig. 6; Table 6). The same general pattern was found to occur from the level of phylum down to species, indicating that for this variable, the same result occurred. That is, no matter which taxocene was examined we end up with the same result of correlative evidence for the enriching effect of the outfall.

Taxonomic resolution within crustose coralline habitats

The richness data from within crustose habitats were also subjected to the same aggregation procedures as described previously. For phylum, class and order there were significant differences between the outfall and the reference locations with greater richness at the outfall. From the level of family down, the pattern of richness changed with the reference location at Inscription Point having significantly lower numbers compared with the outfall.
and the Cape Solander locations (Fig. 7, Table 6). Increasing taxonomic resolution reduced the potential differences between locations.

**Statistical power and taxonomic resolution**

For kelp habitats, $\phi$ increased with increasing taxonomic resolution to the level of order, after which it plateaued and no further power was achieved through family down to the level of species. For the crustose habitats, $\phi$ started high and decreased with increasing taxonomic resolution down to the level of order, after which it also plateaued (Fig. 8). In both habitats, $\phi$ was approximately the same at the level of class (Fig. 8).

In the kelp habitats, the power to detect patterns of richness between outfall and controls remained reasonably constant with changing taxonomic resolution (Fig. 6). There was, however, a clear increase in the magnitude of the difference between the outfall and the controls with increasing taxonomic resolution. This trend was also reflected in the statistical power calculations (Fig. 8).

In the crustose coralline habitats, the opposite trend occurred (Fig. 7). As taxonomic resolution increased, the differences in the magnitude between locations decreased, which again was reflected in the plot of statistical power versus increasing taxonomic resolution (Fig. 8).
Table 6. Summary of analyses of variance of richness for taxocenes within (a) kelp forests and (b) crustose coralline habitats from the Cronulla ocean outfall and reference locations (ns - not significant \( P > 0.05 \); * - significant \( P < 0.05 \); ** - significant \( P < 0.01 \); \( F \) – ratios in bold were calculated after pooling).

<table>
<thead>
<tr>
<th>(a) Kelp forests</th>
<th>Phylum</th>
<th>Class</th>
<th>Order</th>
<th>Family</th>
<th>Genus</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Source</td>
<td>df</td>
<td>MS</td>
<td>( F )</td>
<td>MS</td>
<td>( F )</td>
<td>MS</td>
</tr>
<tr>
<td>Location</td>
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<td>9.49</td>
<td>7.75*</td>
<td>12.57</td>
<td>8.67*</td>
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<td>1.68ns</td>
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<tr>
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<td>0.83</td>
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<td>1.08</td>
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</table>

<table>
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<th>(b) Crustose habitats</th>
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<th>Family</th>
<th>Genus</th>
<th>Species</th>
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<td>MS</td>
<td>( F )</td>
<td>MS</td>
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<td>Location</td>
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<td>8.57*</td>
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<tr>
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<td>0.26ns</td>
<td>0.2ns</td>
<td>0.23ns</td>
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</tbody>
</table>

35
Figure 6. Mean richness (± SE) for each taxocene within kelp forests at the Cronulla outfall (Cr) and at the three reference locations, Bungan Head (Bh), North Avoca (Na) and Cape Banks (Cb).
Figure 7. Mean richness (± SE) for each taxocene within crustose coralline habitats at the Cronulla outfall (Cr) and at the two reference locations, Cape Solander (Cs) and Inscription Point (Ip).
Figure 8. Power ($\phi$) versus taxonomic resolution within kelp forests (•) and crustose habitats (○).

**Multivariate Approach**

**Taxonomic resolution within kelp forests**

The ordination plots of the data aggregated to the different taxonomic levels showed a relatively similar pattern (Fig. 9). Generally samples from Bungan Head and North Avoca were most intermingled whereas samples from Cronulla and Cape Banks were relatively more separated. Greatest separation of the sewage-affected location, Cronulla, is seen in the -species, -family and order-level ordinations. The ordination plots also indicated, however, that the samples from Cape Banks were also different from the other two control locations.
Pairwise comparisons of the similarity matrices from each level showed that, as the data sets were more highly aggregated, the correlation between the species level data set and those from the coarser levels became markedly less, although there remained a statistically significant relationship between data sets (Table 7). The SIMPER analysis at each level showed that the average within-site similarity and average among-site similarity increased with increasing aggregation, whilst average among group dissimilarity decreased (Table 8). The rank order of the average site similarity also changed with taxonomic level. Bungan Head and North Avoca were the most similar at all levels but the comparison showing the greatest dissimilarity switched between the North Avoca and Cape Banks comparison and that for Cronulla and Cape Banks (Table 8). Further, the Bungan Head and Cape Banks comparison became relatively more similar with aggregation.

Despite the fact that aggregation increased the among-location similarity, decreased similarity of the ordinations, and changed the rank order of the dissimilarity of the comparisons, ANOSIM showed that the differences between locations detected at lower taxonomic levels were also found in the phylum level analysis (Table 9). Aggregation increased the $R$ statistic for the global test among locations above that for species analysis for each coarser taxonomic level. In contrast, the global among-site variation was less than that for the species level analysis for the same analyses. The $R$ statistics for the comparison among specific locations either remained the same or increased with aggregation. The initial design did not permit sufficient permutations to test differences among specific locations without pooling the samples from the sites. The species-level analysis showed significant among-site variation, which precluded pooling. The aggregation of the data to family-level had the effect of reducing the among-site variation allowing pooling and specific tests among locations. These comparisons found that, with the exception of the Bungan Head versus North Avoca test, all locations were significantly different and that these differences were evident at all taxonomic levels.
**Taxonomic resolution within crustose coralline habitats**

The ordination plots of the samples from the crustose coralline habitats showed a similar pattern at each of the taxonomic levels (Fig. 10). The samples from the outfall location (Cronulla) were interspersed with those from Cape Solander but those samples from Inscription Point were generally more separated from the other locations. The stress of the ordination plots decreased with increasing aggregation.

The pairwise comparisons among similarity matrices showed a decrease in their correlation with the increasing difference in aggregation (Table 7). The within-location and among-location similarities also decreased with taxonomic level (Table 8). In contrast with the analysis of the data from the assemblages in the kelp forest, the rank order of the dissimilarity among locations did not change with taxonomic level (Table 8).

The R statistic from the global test for differences among locations in the ANOSIM decreased and its level of significance increased from species ($P = 0.01$) to phylum ($P = 0.004$) level (Table 9). A similar pattern was observed with the global test for differences among sites. The trend with taxonomic level for the test among sites was opposite to that observed in the analysis of the data from the kelp habitats.
Table 7. Pairwise comparisons of similarity matrices for (a) kelp forests and (b) crustose habitats using the RELATE procedure.

<table>
<thead>
<tr>
<th></th>
<th>(a) Kelp forests</th>
<th></th>
<th>(b) Crustose habitats</th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Family</td>
<td>Order</td>
<td>Phylum</td>
<td>Family</td>
</tr>
<tr>
<td>Species</td>
<td>0.905</td>
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<td>Family</td>
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<td>Order</td>
<td></td>
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<td>0.864</td>
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Table 8. Average (a) within group similarity and (b) among group dissimilarity for kelp forests and crustose coralline habitats at the Cronulla outfall and reference locations (Cr – Cronulla, Bh – Bungan Head, Na – North Avoca, Cb Cape Banks, Cs – Cape Solander, Ip – Inscripton Point).

<table>
<thead>
<tr>
<th></th>
<th>(a) Average Within Group Similarity</th>
<th></th>
<th>(b) Average Among Group Dissimilarity</th>
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<tr>
<td></td>
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<td>Species</td>
<td>Family</td>
<td>Order</td>
</tr>
<tr>
<td>Kelp forests</td>
<td>Cr</td>
<td>39.43</td>
<td>42.47</td>
<td>53.33</td>
</tr>
<tr>
<td></td>
<td>Bh</td>
<td>45.87</td>
<td>45.87</td>
<td>50.35</td>
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<tr>
<td></td>
<td>Na</td>
<td>32.05</td>
<td>37.27</td>
<td>39.01</td>
</tr>
<tr>
<td></td>
<td>Cb</td>
<td>41.05</td>
<td>45.52</td>
<td>46.66</td>
</tr>
<tr>
<td>Cr v Bh</td>
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<td>Cr v Na</td>
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<td>Bh v Cb</td>
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<tr>
<td>Na v Cb</td>
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Table 9. Summaries of analysis of similarities (ANOSIM) comparing differences in the structure of the assemblages in both (a) kelp forests and (b) crustose habitats (nc = not calculated because of insufficient permutations; ns = p > 0.05; * = p < 0.05; p = calculated after pooling non-significant sites; Cr – Cronulla, Bh – Bungan Head, Na – North Avoca, Cb Cape Banks, Cs – Cape Solander, Ip – Inscription Point).

### (a) Kelp forests

<table>
<thead>
<tr>
<th>Among Locations</th>
<th>Species</th>
<th>Family</th>
<th>Order</th>
<th>Phylum</th>
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<tbody>
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<td>R-Stat</td>
<td>%Sig.</td>
</tr>
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<td>*</td>
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<td>*p</td>
</tr>
<tr>
<td>Cr v Cb</td>
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<td>0.500</td>
<td>*p</td>
</tr>
<tr>
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<td>*p</td>
</tr>
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<td>Na v Cb</td>
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<td>nc</td>
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<tr>
<td>Among Sites</td>
<td>0.128</td>
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### (b) Crustose habitats

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<tr>
<td>Cr v Cs</td>
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<td>0.416</td>
<td>*</td>
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<tr>
<td>Cr v Ip</td>
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<td>nc</td>
<td>0.370</td>
<td>nc</td>
</tr>
<tr>
<td>Cs v Ip</td>
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<td>nc</td>
<td>0.630</td>
<td>nc</td>
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<tr>
<td>Among Sites</td>
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<td>nc</td>
<td>0.407</td>
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</table>
Figure 9. MDS ordination for the assemblages found within kelp forests: Cronulla – circle; Bungan Head – square; North Avoca – triangle; Cape Banks - diamond. Stress: species = 0.2; family = 0.21; order = 0.19; phylum = 0.18.

Figure 10. MDS ordination for the assemblages found within crustose habitats: Cronulla – circle; Cape Solander – square; Inscription Point - diamond. Stress: species = 0.21; family = 0.2; order = 0.19; phylum = 0.14.
Discussion

The univariate analyses revealed that the pattern of diversity within the assemblages dominated by the kelp *Ecklonia radiata* was consistent between taxocenes, and significant differences between outfall and controls were found at all taxonomic levels. Therefore, no matter which taxonomic level was examined, the same result would be achieved. The power to detect differences using these variables was high, whilst the variability at all spatial scales was low.

Within the crustose coralline algae habitats the patterns were not so clear. At coarse taxonomic levels, differences between the outfall and the references were apparent. This pattern changed as the taxonomic resolution increased, resulting in reduced magnitudes of differences in taxonomic richness between the outfall and the references at the family, genus and species level. If this study had been done comparing the number of species, then a result of no significant differences between outfall and controls would have occurred. This pattern was also represented in the power comparisons, with decreasing power associated with increasing taxonomic resolution.

In terms of detecting impacts from the outfall, the multivariate analyses were less emphatic than the univariate. In the analyses of the kelp-dominated habitat, Cronulla appeared different from the references but the natural variation among the reference locations, particularly for Cape Banks, appeared as great. The pairwise comparisons showed that the communities at the outfall location along with many of the reference locations were significantly different from most other locations. This makes it difficult to attribute any differences associated with Cronulla solely to pollution.
These differences in the two types of analyses also highlight differences in the nature of community level statistics and measures, which are used to represent community structure. Statistics such as diversity, the number of species and individuals ignore the actual composition of the communities and create the possibility of having reference locations with very different community structure but similar number of species and individuals (see Warwick and Clarke, 1994); which appeared to have occurred in this study.

In terms of the level of taxonomic resolution required to describe patterns, the univariate and multivariate analyses were in general agreement. The results can be similarly interpreted at most levels except in the analysis of the crustose coralline assemblages at the level of phylum. In that analysis, the ordination plots show a markedly different pattern among locations and sites when compared to those plots of analyses done with less aggregation. Further, the $R$ statistic for the pairwise comparison between Cronulla and Cape Solander was much lower in this analysis and the comparisons of the similarity matrices (RELATE) also showed a much lower correlation coefficient when compared with the other analyses.

The results obtained here indicated that species level identification may not be necessary to detect sewage-related impacts on hard substratum macrobenthic assemblages. Furthermore, it would appear that within kelp assemblages, the number of phyla is as good a measure as the number of species. Ferraro and Cole (1992) suggested that as toxic stress increased, the adaptability of firstly the individual, then the species, genus, family etc. is exceeded, resulting in increasing stress being manifested at coarser taxonomic levels. This however, does not explain the increases in species richness, possibly due to sewage and nutrient enrichment, which was found in this study (Pearson and Rosenberg, 1978).

Ellis (1985) described taxonomic sufficiency as the level of identification necessary to meet a study's objectives. In terms of the amount of time and the costs involved in studying
subtidal macrobenthic assemblages, identifying organisms to levels that are finer than required is wasteful of resources that could be redirected into increasing the power of the scales of interest (James et al., 1995; Chapman, 1998). Moreover, it appears that data gained at coarser taxonomic levels has lower inherent variability, making detection of smaller perturbations more probable (Warwick, 1988a).

In studies on marine macrobenthos inhabiting hard substrata, samples are generally collected in situ by suitably trained divers using quadrats or photographic methods. In both cases identifying organisms to species level is both difficult and, for some taxa, impossible (Davis et al., 1999). Roberts et al. (1994) compared diver-collected quadrats with photo-quadrats and found that no extra information or time was gained from using either method. The use of photo-quadrats to identify organisms to species level can result in some difficulties. For some species identifications, specimens must be examined at the microscopic level making diver-collected or photographic methods inadequate if only field identifications are used.

The valid use of coarser taxonomic identifications, therefore, becomes an important tool when large monitoring studies are required to detect impacts on assemblages on hard substrata. Ferraro and Cole (1992) suggested that any proposed monitoring program must include not only information on sample size, number of replicates, power etc., but also information on the taxonomic sufficiency required for the proposed sampling program. This can only be achieved by examining different habitats and doing similar types of experiments to the one presented here (Warwick, 1988a & b). Ferraro and Cole (1992) conceded that species level data from old impact assessment and monitoring programs could be “reworked” and analysed to gain valuable information on taxonomic sufficiency for various habitats and systems.
The majority of studies on taxonomic resolution have concentrated on using multivariate techniques to identify taxonomic sufficiency (see Warwick, 1988a; Ferraro and Cole, 1992) where the evidence suggested taxonomic sufficiency at the level of family or higher. In this study, the macrobenthic assemblages living on hard substrata in the vicinity of outfalls were found to be different, at coarser taxonomic levels, in the response to sewage effluent. In kelp habitats influenced by sewage, greater richness was found at all the taxonomic levels examined. It should be remembered that the results presented here are correlative in terms of whether or not the outfall caused the differences in assemblages from one place to another. To determine if these patterns were really caused by sewage from the outfall, sampling would need to be done at different temporal scales to determine whether consistent patterns emerged.
CHAPTER 3: PATTERNS OF NATURAL VARIABILITY

DEPTH RELATED PATTERNS IN SPONGE ASSEMBLAGES

Introduction

In the absence of foliose macroalgae and macrophytes, assemblages of sessile encrusting invertebrates generally dominate hard substrata at depths greater than 30 m (Lewbel et al., 1981; Lissner and Dorsey, 1986). The difficulties associated with the use of SCUBA at these depths have seen much of our understanding of the processes within these communities developed from the investigation of sessile assemblages associated with shaded artificial substrata in relatively shallow water, such as pier pilings (Sutherland and Karlson, 1977; Kay and Butler, 1983; Russ, 1982). Hence the ecology of these deeper reefs represents a significant gap in our knowledge. Moreover, where shallow rocky reef assemblages (< 20 m) have been examined directly (Ayling, 1981; Sebens, 1986; Underwood et al., 1991; Chapman et al., 1995), it would seem inappropriate to extrapolate these patterns and processes to deeper water (> 20 m) communities.

A further hindrance to our understanding of these communities stems from the taxonomic uncertainty that surrounds marine invertebrates; particularly sponges (Davis et al., 1999). A further impediment is the propensity of sponges to adopt a variety of growth forms under different environmental conditions (e.g. see Warburton, 1960; Trammer, 1983; Palumbi, 1986). For example, it is not clear whether the predominance of massive or erect growth forms with increasing depth stems from a simple change in growth form for shallow water encrusting species or a suite of different species (Barthel, 1991).
In temperate Australia, there are no quantitative published accounts of deeper water sponge assemblages. An understanding of the structure and dynamics of such assemblages is timely as local authorities in south-eastern Australia are transferring the near shore disposal of sewage effluent to deeper coastal reefs. Environmental impacts associated with the disposal of effluent in deep water will be difficult to assess in the absence of data describing spatial and temporal variation in the distribution and abundance of organisms in these communities (see Chapter 4; Roberts, 1996a; Roberts, 1996b). Because light, siltation and turbulence all change with depth (Wilkinson and Cheshire, 1989; Barthel and Gutt, 1992; Liddell and Ohlhorst, 1987; Alcolado, 1994; Hardin et al., 1994) extrapolating from shallow water studies must only be undertaken with caution.

Here, a combination of SCUBA, remote cameras and a remotely operated vehicle (ROV) were used to describe variation in the distribution and abundance of sponge assemblages on three coastal reefs off Sydney, Australia. Specifically I, (i) made collections of voucher material for identification purposes, (ii) analysed photo-quadrats at three depths (20 m, 30 m and 50 m) in order to examine differences in the relationship between sponge species richness and the percentage of the rocky substratum covered by sponges and (iii) categorised sponges in relation to their growth form, noting how cover and richness changed with depth.

**Methods**

**Study Sites and Sampling**

Sponge assemblages were sampled from three locations; North Head, Long Reef and Bungan Head near Sydney on the south-eastern coast of Australia (Fig. 11). Rocky substratum was examined at three depths (20 m, 30 m and 50 m) using a 'jump' camera (see Chapter 2; Roberts et al., 1994) in the deeper sites and a diver-operated camera-rig in the shallow sites. The
assemblages were sampled on three occasions, April 1993, August 1993 and April 1994. Within-location variation was determined by haphazardly photographing five replicate 0.45m² quadrats at each of three random sites nested within each location. Each site was at least 50 m in diameter and was separated from other sites by at least 100 m. The three depths at each location were approximately 2 km apart (Fig. 11). The species richness, percentage cover and morphotype of sponges were estimated from the photo-quadrats. Prostrate or encrusting sponges were defined by the absence of any structural complexity and were limited to primary cover on the substratum. Erect or massive sponges were defined to be those with a distinct structural shape such as massive, cups, fans and branching forms.

To help determine the identity of sponges recorded in quantitative photo-quadrats, a comprehensive field collection of the sponges from deep and shallow locations was done. A modified Remote Operated Vehicle (ROV) was used to collect sponges at depths ranging from 30 to 60 m whilst above 30 m, SCUBA was used. Voucher collections were lodged with the Queensland Museum, Brisbane, Australia.

Statistical Treatment of Data

Multivariate statistical techniques were used to describe the variation between species abundance and composition on each reef location at each sampling time using the PRIMER software package (Plymouth Marine Laboratories, UK). All replicate quadrats for each site were pooled and the abundance data were double-square-root transformed. The Bray-Curtis similarity matrix was calculated and used to generate 2-dimensional plots using non-metric multi-dimensional scaling (Clarke, 1993). Two-way nested ANOSIM tests (Clark and Warwick, 1994) were done on each matrix to examine differences among locations and depths (see Clarke, 1993). The ANOSIM and MDS procedures were used to analyse differences between sites but do not indicate which species are responsible for these differences. The SIMPER procedure was
therefore used to identify the contribution of individual species to the similarity measure employed. This resulted in a list of species (ranked in order of importance), which contributed most to the similarities within a group or site (Clark and Warwick, 1994).

The null hypothesis that sponge cover and richness does not change with depth or through time was tested by ANOVA, where depth was treated as a fixed factor, locations were treated as random, sites were nested within locations and time was considered to be random. It should be noted that one of these reefs, North Head, supports a deep-water sewage outfall at 50 m depth (Roberts, 1996a). In this study however, sites were always sampled a considerable distance (2-3 km) away from the outfall diffusers. Prior to the analysis, the homogeneity of variance assumption was examined using Cochran's test (Winer, 1971) and where variances were heterogeneous, data were transformed to log (x) + 0.5 for species richness and arc-sine transformed for percentage cover following the recommendations of Winer (1971).

To increase the power of these univariate analyses, post hoc pooling procedures were used, but only when there were no significant 2nd-order interactions at $P = 0.25$ (Winer, 1971). Where significant differences were found in the analysis of variance and where contrasts were generally required, Student-Newman-Keuls (SNK) multiple comparisons were done to determine differences among means (Winer, 1971).

To establish the degree of association between species richness and cover for both erect and encrusting forms at different depths, the percentage cover, pooled for each location and depth, was plotted against species richness and Pearson’s product-moment correlation coefficients ($r$) were calculated (Sokal and Rohlf, 1981).
Figure 11. Locations of the reefs sampled off the Sydney coast (North Head: • 50m, • 30m, • 20m; Long Reef: ◊ 50m, ◊ 30m, ◊ 20m; Bungan Head: △ 50m, △ 30m, △ 20m).
Results

Species Distribution and Composition

Sponges dominated the reefs at all locations in terms of faunal richness and percentage cover. Encrusting and erect or massive forms were found at all depths at all locations. Over 50 species were identified, representing 8 orders (21 families) of Demospongia and 2 orders (2 families) of Calcarea (see Roberts and Davis, 1996). The Axinellidae (Order Halichondrida) and the Ircinidae (Order Dictyoceratida) were the best represented families. Generally, the distribution of the most common sponges was widespread across the depth ranges sampled (Table 10).

*Spirastrella* sp., *Ircinia* sp. 1 and *Tedania* sp. 1 were found at all depths at all locations, while *Tethya* sp., *Hamigera* cf. *kirki* (Shaw) and *Cymbastela concentraria* (Lendenfeld) were found at most depths and locations except for some of the 20 m reefs (Table 10). The deeper reefs generally supported all the common species listed except for *Desmapsamma* cf. *kirki* (Bowerbank) and *Chondropsis* sp. In contrast, *Thorecta freija* (Lendenfeld), *Antho* (*Isopenectya* chartacea) (Whitelegge), *Axinella* sp. 1 and *Jasps* sp. 1 were never found shallower than 30 m, perhaps owing to their relatively ‘fragile’ structure. This may also be the case for *Ceratopsis aurantiaca* (Lendenfeld), which was also restricted to deeper reefs with the exception of a single occurrence at 20 metres on Long Reef (Table 10). There was little evidence of variation in morphotype with depth within a species although there were exceptions. For example, *Spirastrella* sp. was generally encrusting in shallow water but developed massive forms in deeper water.

The species composition and abundance of sponges within these assemblages was found to be highly variable, with the structure or patterns changing between different locations, depths and at different times.
Table 10. Mean percentage cover of the most abundant species of sponge at three depths (20 m, 30 m, 50 m) from three locations, *n* = 45; NH, North Head; LR, Long Reef; BH, Bungan Head; • < 0.1%; • 0.1 - 0.5%; • 0.5 - 1%; • 1 - 3%.

<table>
<thead>
<tr>
<th>Location</th>
<th>NH (20)</th>
<th>LR (20)</th>
<th>BH (20)</th>
<th>NH (30)</th>
<th>LR (30)</th>
<th>BH (30)</th>
<th>NH (50)</th>
<th>LR (50)</th>
<th>BH (50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cymbastela concentrica</td>
<td>•</td>
<td>•</td>
<td>•</td>
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<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>●</td>
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<tr>
<td>Jaspis sp. 1</td>
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<tr>
<td>Tethya sp.</td>
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<tr>
<td>Holopsamma arborea</td>
<td>●</td>
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<tr>
<td>Axinella sp. 1</td>
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<tr>
<td>Ceratopsis aurantiaca</td>
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<tr>
<td>Antho (Isopenectya) chartacea</td>
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<tr>
<td>Tedania sp. 1</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
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<tr>
<td>Ircinia sp. 1</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>.</td>
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<tr>
<td>Chondropsis sp.</td>
<td>.</td>
<td>●</td>
<td>●</td>
<td>.</td>
<td>.</td>
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<tr>
<td>Hamigera cf. dendyi</td>
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<tr>
<td>Desmapsamma cf. kirki</td>
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<tr>
<td>Spirastrella sp.</td>
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<tr>
<td>Thorectafreija</td>
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</table>
The multivariate ANOSIM tests showed no significant variation between reef locations at any time ($P > 0.05$: April 93 - $R = 0.05$; August 93 - $R = -0.1$; April 94 - $R = 0.01$), however significant differences between depths were detected at each time ($P < 0.01$: April 93 - $R = 0.62$; August 93 - $R = 0.77$; April 94 - $R = 0.56$). The power to differentiate between depths was low, due to significant variation among sites and the small sample sizes involved, which reduces the number of possible permutations in the analysis (see Clark and Warwick, 1994).

Multi-dimensional scaling ordinations also revealed considerable variation among reefs and depths and for sites nested within locations (Figs. 12a-c). The simplest indicator of how well the among-sample relationships have been preserved using this technique is to examine the stress value associated with each MDS plot (Clarke, 1993). The stress value for the April 1993 plot was considered to be good (Stress = 0.1) with no real risk of drawing false inferences, whilst those for August 1993 (Stress = 0.03) and April 1994 (Stress = 0.04) were considered to be an excellent representation of the spatial relationships with no prospect of misinterpretation (Clarke, 1993).

The SIMPER analysis identified species, ranked in order of importance, which contributed to the similarities within a location. No matter which reef was examined, the dominant contributing species changed depending on the time, location or depth (Table 11). It is noteworthy that *Tedania* sp. 2 was not among the 15 most abundant species (see Table 10), yet was ranked first for more than 50% of depths and locations during the first sampling period using the SIMPER analysis (Table 11).
Figure 12. nMDS plots for the abundance of sponges for each sample time (a) April 1993 (b) August 1993 and (c) April 1994 (North Head: ● 50m, ● 30m, ● 20m; Long Reef: ◊ 50m, ◊ 30m, ◊ 20m; Bungan Head: Δ 50m, Δ 30m, Δ 20m).
Table 11. Species of sponge (ranked in order of importance as determined by the SIMPER procedure), which contributed most to the similarities within a location (NH, North Head; LR, Long Reef; BH, Bungan Head).

<table>
<thead>
<tr>
<th>Species</th>
<th>April 1993</th>
<th>August 1993</th>
<th>April 1994</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NH (50)</td>
<td>NH (30)</td>
<td>NH (20)</td>
</tr>
<tr>
<td>Cymbastella concentrica</td>
<td>8</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Spirastrella sp.</td>
<td>6</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Ircinia sp. 1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Tedania sp. 2</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Tedania sp. 1</td>
<td>10</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Axinella sp. 1</td>
<td>1</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Ceratopsis aurantiaca</td>
<td>7</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Thorectia freija</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Hamigera cf. dendyi</td>
<td>2</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Chondropsis sp.</td>
<td>1</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>Desmopsamma cf. kirkii</td>
<td>5</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>Antho (isopenectya) chartacea</td>
<td>3</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>Jasps sp. 1</td>
<td>3</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Tethya sp.</td>
<td>4</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Holoppsamma arborea</td>
<td>6</td>
<td>5</td>
<td>2</td>
</tr>
</tbody>
</table>
Total Sponge Richness and Cover

In general, sponge species richness increased with depth. For example, the maximum average richness of $7.9 \pm 0.8$ species ($\pm$ SE) per $0.45m^2$ plot was observed on reefs at 50 m. Reefs at 30 m supported a maximum of $5.1 \pm 0.3$ species, while reefs at 20 m supported up to $4.3 \pm 0.4$ species (Fig. 13a). Bungan Head and Long Reef 30-m reefs sometimes supported smaller species richness than the reefs at 20 m. No significant fluctuations in richness occurred through time on most reefs however richness did show significant variation between locations on the 50-m and 30-m reefs in April 1993 and on the 30-m and 20-m reefs in August 1993 (Table 12).

The total cover of sponges was always greatest on the 20-m reefs (maximum cover of $32.4 \pm 4.1$ %) whilst the total cover at 30 m (maximum cover of $10.6 \pm 2.1$ %) and 50 m (maximum cover of $12.0 \pm 1.4$ %) was quite similar (Fig. 13b). Generally, the cover of sponges did not fluctuate on the 50-m and 30-m reefs whereas on the 20-m reefs cover was quite variable among locations through time (Fig. 13b; Table 12). Bungan Head consistently supported the greatest sponge cover at the 20 m and 50 m depths. Inexplicably, it generally had the smallest cover at 30 m.

Comparison Between Erect and Encrusting Forms

Erect sponges were better represented (maximum of $7.2 \pm 0.8$ species) on the deeper (50 m) reefs than on the shallower reefs (Fig. 14a). This was more than two fold the maximum number of encrusting sponges observed ($3.4 \pm 0.3$) at 50 m. Generally, the species richness of erect sponges increased with depth (Fig. 14a) whilst the number of encrusting sponge species did not show a consistent pattern with depth (Fig. 15a; Table 12). The cover of erect sponges was highly variable across all depths (Fig. 14b). Extremely low cover was detected at 20 m at North Head ($0.7 \pm 0.2$ %) while the maximum cover was also apparent on the 20-m reef ($13.3 \pm 1.9$ %) at Long Reef.
Generally, the cover of encrusting sponges decreased with increasing depth (Fig. 15b). The reefs at 50 m had a maximum cover of 4.7 ± 0.9 %, whereas the reefs at 30 m and 20 m had maximum covers of 7.2 ± 1.1 % and 25.7 ± 3.7 % respectively. At North Head and Bungan Head, there were significantly lower covers of encrusting sponges in the deeper 50-m and 30-m reefs on all sample occasions. At Long Reef, however, there were no differences between shallow and deeper locations in the cover of encrusting sponges (Fig. 15b; Table 12). Generally, variation in the cover of encrusting sponges through time was limited on the 50-m and 30-m reefs. In contrast the cover of encrusting sponges fluctuated significantly through time on the 20-m reefs (Fig. 15b; Table 12).

For species richness and percentage cover, significant 2nd-order interactions in the ANOVA’s meant that 1st-order interactions and main effects could not be interpreted (Underwood, 1981). Small-scale patchiness was evident within these assemblages and was reflected by significant time x site (depth x location) interactions for total species richness, erect sponge richness and the cover of erect sponges. Significant location x time interactions occurred for encrusting sponge richness, whilst depth x location interactions were detected for the total cover of sponges, the cover of encrusting sponges, and the species richness of encrusting sponges (Table 12). Variances for the cover of encrusting sponges could not be stabilised and therefore these results should be treated with caution (Table 12).

For erect sponges, species richness was positively correlated with percentage cover at all depths; $r$: 50 m = 0.83, 30 m = 0.87, 20 m = 0.78 (Fig. 16a). In contrast, the relationship between species richness and cover was poor for encrusting sponges (Fig. 16b). The 50-m reefs showed a significant relationship but on the 30-m and 20-m reefs the richness and cover were relatively poorly correlated ($r$: 50 m = 0.67, 30 m = 0.38, 20 m = 0.30).
Figure 13. (a) Mean species richness and (b) mean percentage cover for all types of sponges \( (n = 15, \pm SE) \) from the coastal reefs off Sydney:
- North Head;
- Long Reef;
- Bungar Head.
Table 12. Summaries of $F$-ratios from analyses comparing spatial and temporal variation in the number and cover of sponges at North Head, Long Reef and Bungan Head, NSW. nil, no transformation required; ns, not significant ($P > 0.05$); *, significant ($P < 0.05$); **, ($P < 0.01$)

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>MS</th>
<th>$F$</th>
<th>$F$</th>
<th>MS</th>
<th>$F$</th>
<th>MS</th>
<th>$F$</th>
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<td>37.79</td>
<td>1089.4</td>
<td>7.16</td>
<td>4099.2</td>
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<tr>
<td>Location</td>
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<td>14.38</td>
<td>452.09</td>
<td>3.76</td>
<td>983.87</td>
<td>15.89</td>
<td>1038.4</td>
<td>9</td>
<td></td>
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<tr>
<td>D x L</td>
<td>4</td>
<td>39.57</td>
<td>220.41</td>
<td>3.99**</td>
<td>8.56</td>
<td>1201.5</td>
<td>10.94</td>
<td>4.45*</td>
<td>757.71</td>
<td>6.24*</td>
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<tr>
<td>Site</td>
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<td>3.36</td>
<td>40.35</td>
<td>0.9ns</td>
<td>0.51</td>
<td>69.73</td>
<td>2.28</td>
<td>1.59ns</td>
<td>70.35</td>
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<td>104.74</td>
<td>2.50ns</td>
<td>2.52</td>
<td>52.57</td>
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<td>0.29ns</td>
<td>382.59</td>
<td>3.81ns</td>
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<tr>
<td>D x T</td>
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<td>9.03</td>
<td>302.71</td>
<td>3.47ns</td>
<td>1.32</td>
<td>64.82</td>
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<td>221.83</td>
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<tr>
<td>L x T</td>
<td>4</td>
<td>1.4</td>
<td>41.88</td>
<td>0.93ns</td>
<td>1.46</td>
<td>100.28</td>
<td>4.69</td>
<td>3.66**</td>
<td>100.48</td>
<td>1.34ns</td>
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<tr>
<td>D x L x T</td>
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<td>6.45</td>
<td>87.21</td>
<td>1.94ns</td>
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<td>1.71ns</td>
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<td>T x S (D x L)</td>
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<td>44.92</td>
<td>0.84ns</td>
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<td>69.37</td>
<td>1.79**</td>
<td>1.44</td>
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<td>2.32</td>
<td>53.21</td>
<td>0.33</td>
<td>38.81</td>
<td>1.21</td>
<td>76.21</td>
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</table>

Cochran's test

Transform nil ns ns ns nil *
Figure 14. (a) Mean species richness and (b) mean percentage cover for erect or massive sponges ($n = 15, \pm SE$) from the coastal reefs off Sydney: • North Head; ◊ Long Reef; △ Bungan Head.
Figure 15. (a) Mean species richness and (b) mean percentage cover for encrusting sponges ($n = 15, \pm \text{SE}$) from the coastal reefs off Sydney:

- North Head; ◊ Long Reef; △ Bungan Head.
Figure 16. Species richness versus percentage cover (each plot $n = 27$) for (a) erect (50 m, $r = 0.83$; 30 m, $r = 0.87$; 20 m, $r = 0.78$) and (b) encrusting sponges (50 m, $r = 0.67$; 30 m, $r = 0.38$; 20 m, $r = 0.30$).
Discussion

This work represents the first quantitative comparison of shallow and deep-water sponge assemblages from temperate Australia. Generally, sponge species richness was found to increase with depth, particularly for the erect or massive species, whilst the percentage cover of encrusting sponges decreased with depth. This general pattern has been observed in studies on sponges elsewhere (Logan et al., 1983; Liddell and Ohlhorst, 1987; Wilkinson and Evans, 1989; Zea, 1993a; Hardin et al., 1994).

Sponge species were generally widespread over the depth range sampled, but some differences in their distribution with depth, possibly related to their physical structure, were observed. Erect or massive sponges dominated the deeper reefs, where water turbulence is presumably lower and siltation rates are higher than in comparable shallow water locations. Trammer (1983) found that erect sponges could be physically damaged by turbulence and were able to survive in calm conditions because their shape allowed them to resist clogging by siltation. This may account for the dominance of massive or erect forms in deeper water (Kluijver, 1993). Encrusting sponges, in contrast, exhibited the highest cover on the shallow reefs, and this may reflect their ability to better resist strong turbulent water flow and scour (Pettie and Hoare, 1981). Encrusting species and recruits may also be particularly susceptible to smothering by silt in deeper water (Zea, 1993a).

The sponge assemblages on the reefs at 20 m, generally showed greater temporal variation in their patterns of abundance, compared with those on the deeper reefs. The increased dynamism associated with these shallow reefs may reflect variation in the intensity and duration of coastal processes such as wave action and turbulence (Chapman et al., 1995). Sponges are considered to be slow-growing and long-lived (Dayton, 1979; Ayling, 1983b), however for encrusting species on these shallow reefs this may not be the case as various biological and physical disturbances can alter the patterns of richness and cover over relatively short time periods (Moran, 1980; Kay and Butler, 1983; Keough and Butler, 1983).
For shallow locations on temperate reefs, these findings are in general agreement with those of other workers. For example, Ayling (1981) reported percentage covers of sponges between 20-30% in comparable 20 m habitats and up to 45% at shallower depths (12 m) for encrusting species. Underwood et al. (1991) described deep reef habitat (> 10 m) along the coast of NSW with a combined average cover of sponges, ascidians and bryozoans of approximately 6% cover but they do admit that these habitats were probably underestimated because the deeper reefs extended past their sampling limits. In deeper temperate reef habitats ranging from 100 to 200 m, Hardin et al. (1994) found that the cover of the most abundant sponges was 1.3% on low relief and 3.3% on high relief substratum.

On tropical hard substrata (between 15-56 m) Liddell and Ohlhorst (1987) found that sponges showed striking depth-related trends where cover increased with depth from 1.7-14.5%. On Caribbean reefs, Zea (1993b) noted that the average number (10-18.3) and cover (14.5-58%) of sponges increased with depth from 12 m down to 36 m.

Small-scale patchiness in both the distribution and abundance of sponges was apparent in this study and may, in part, account for the number of significant interactions detected in the analyses. In these assemblages short distance dispersal of larvae, the release of oocytes in mucus strings, or the action of predators, may act to produce aggregations of individuals or ramets over relatively small spatial scales (Barthel and Gutt, 1992; Zea, 1993b). Large erect species like Holopsamma arborea, Mycale (Arenochalina) flammula and Cymbastela concentrica all exhibited an aggregated distribution, which contributed to the patchiness observed within the assemblage.

Significant interactions between the main sources of variation were a common feature of the analyses that were presented. This is not surprising given that any effect due to depth may vary significantly between locations, perhaps due to water clarity for example. The effect of depth is unlikely to wholly account for these interactions however, as other studies of shallow subtidal
reef communities in NSW also reveal considerable spatial and temporal variation (see Kennelly and Underwood, 1992). A number of factors may be acting to produce this variation, such as larval supply, recruitment, physical disturbance and biological interaction (Underwood et al., 1991; Chapman et al., 1995).

These results have direct implications for the management of sessile hard substratum assemblages that may be subjected to anthropogenic disturbance. Because sessile marine assemblages are highly variable at different spatial and temporal scales, monitoring designs must be sufficiently sensitive to discriminate anthropogenic disturbance from high levels of natural variability (Andrew and Mapstone, 1987; Underwood, 1993).

In addition, the data indicate that a useful estimate of the species richness of erect sponges may be obtained at all depths by quantifying the cover of these sponges; $r$ values varied between 0.78 and 0.87. This may provide a useful way of circumventing taxonomic problems associated with working on sponge assemblages. Unfortunately, the correlation between the cover of encrusting sponges and their species richness was quite poor, particularly on 20 and 30-m reefs; $r < 0.4$. This poor correlation probably reflects the striking variation in the sizes of ramets and genets of encrusting sponges (Wright, 1992).

Understanding the mechanisms that produce the patterns of distribution for sponges on these temperate reefs awaits investigation and provides the focus for ongoing work. This includes examining the role of variation in light, siltation and nutrients as well as the relationships between sponges and other members of these temperate reef assemblages.
STRUCTURE AND DYNAMICS ON EXPOSED AND SHELTERED REEFS

Introduction

The role of biological disturbance and interaction in the structure and dynamics of assemblages on shallow subtidal reefs is well documented (Jackson, 1977; Moran, 1980; Russ, 1980; Ayling, 1981; Choat, 1982; Butler, 1986; Fletcher, 1987; Kennelly, 1987b; Jones and Andrew, 1990; Underwood and Kennelly, 1990; Kennelly, 1991; Ceccherelli and Cinelli, 1998; Davis and Ward, 1999). In contrast, for similar assemblages in deeper water (> 20 m) very little is known (see Andrew, 1999). In temperate NSW Australia, spatial and temporal patterns of distribution and abundance in these assemblages have largely been ignored because of the difficulties associated with obtaining quantitative data at these depths (see Chapter 2; Roberts et al., 1994).

The sessile fauna found at these depths are comprised of assemblages of sponges, ascidians, bryozoans and cnidarians (Underwood et al., 1991; Davis et al., 1997; Andrew, 1999), whilst the algae consists of red crustose and foliose forms, many of which are also found in shallow water (Underwood and Kennelly, 1990; Davis et al., 1997). Andrew (1999) termed this habitat “Deep Reef” and described it as being covered with colourful sheet, erect and branching sessile organisms but highlighted that very little was known about their ecology.

In studying marine assemblages, ecologists are generally constrained in their attempts to describe patterns and processes because of considerable spatial and temporal variability at sometimes very small scales (Underwood et al., 2000). The collection of quantitative data, which takes into account this variability, is therefore a necessary first step in understanding the ecology of subtidal encrusting assemblages (Underwood et al., 2000).
On shallow temperate reefs, both physical (Kennelly, 1987a) and biological (Fletcher, 1987; Davis et al., 1997) disturbance has been found to alter patterns of distribution and abundance, sometimes quite rapidly. The importance of storm-generated disturbances and their severity in altering the structure of assemblages of intertidal (Denny, 1995; Underwood, 1998; 1999a) and subtidal (Dayton and Tegner, 1984; Wulff, 1995; Posey et al., 1996) plants and animals has also been well documented. Deeper, subtidal reefs also routinely experience significant exposure to storms and ocean waves (Short and Trenaman, 1992), which has the potential to play an important role in structuring the assemblages at various scales. Natural physical processes (e.g. storms) may account for a large proportion of the variation in differences that are observed between habitats (Underwood et al., 1991, Underwood et al., 2000).

In more sheltered locations, e.g. the subtidal reefs at the entrances to estuaries and embayments, the frequency and severity of the physical effects of storm waves will be greatly reduced. In these habitats, strong tidal currents may play a significant role in some of the processes determining the structure and dynamics of sponge-dominated assemblages (Kaandorp, 1999; Bell and Barnes, 2000; Ginn et al., 2000). For example, episodic pulses of freshwater after heavy and prolonged rainfall will also cause fluctuations in the concentration of salinity at the mouths of estuaries. Mass mortalities (sponge dieback) of marine sponges have been recorded where changes in temperature and water-flow rates have stimulated bacterial and fungal infections (Hummel et al., 1988).

Temperate subtidal encrusting assemblages living on hard substrata in shallow water have also been reported as temporally stable at some scales (Ayling, 1981; Ayling, 1983a; Kay and Butler, 1983; Butler and Connolly, 1996; Underwood and Chapman, 1996) but dynamic at others (Kennelly and Underwood, 1992). Temporal changes in the assemblages of sessile organisms living on subtidal reefs should, in theory, be greater where they are exposed to higher energy associated with wave action (Underwood et al., 1991). Storm-related physical disturbance should lead to a form of “instability” in the structure of the assemblage by
making new space available for settlement and recruitment (Kennelly, 1987a; Dayton et al., 1992; Warwick and Clarke, 1993b). Furthermore, subtidal assemblages on exposed reefs should have greater abundances of prostrate species than those on sheltered reefs at the same depth due to the effects of wave energy. To test these predictions, the structure and dynamics of sponge-dominated assemblages were examined by quantifying their spatial and temporal patterns of variability on exposed and sheltered reefs at depths of 18-20 m, in temperate NSW, Australia.

Methods

Study Locations and Sampling Design

Spatial and temporal patterns in subtidal sponge-dominated assemblages were determined by sampling exposed and sheltered reefs, from Botany Bay to Broken Bay, NSW Australia (Fig. 17). Four exposed and four sheltered estuarine reef locations were sampled at depths of approximately 18-20 m (Fig. 17). Some of these habitats have been described previously (Fletcher, 1987; Underwood et al., 1991; Underwood and Chapman, 1996; Davis et al., 1997; Andrew and O’Neill, 2000).

The assemblages on these reefs were sampled using a diver operated camera rig that supported a 35 mm Sea & Sea Motor Marine-2 underwater camera and strobe. The assemblages at each location were sampled a total of 7 random times from April 1993 to September 1995. Within-location variability in the assemblages was determined by haphazardly photographing five replicate quadrats (photo-quadrat dimensions - 0.8 m x 0.56 m, total area 0.45 m$^2$) at each of 3 sites nested within each location. At each location, the 3 sites (approximately 50 m in diameter and 50 m apart) were randomly selected each time.
Figure 17. Exposed (○) and sheltered (●) reefs sampled along the Sydney coastline: BH – Bungan Head; LR – Long Reef; NH – North Head; CB – Cape Banks; QH – Quarantine Head; SH – South Head; HH – Henry Head; IP – Inscription Point.
Analysis of Photographs and Taxonomic Discrimination

The photo-quadrats were analysed using a Bell and Howell ‘black box’ projector. An overlay plastic grid of 100 regularly spaced points was placed on the screen and an estimate of the percentage cover and number of species was recorded from the photo-quadrat. Many of the crustose coralline and foliose macroalgae could not be identified to species in the photographs. In those cases, they were grouped into morphotaxa, termed foliose macroalgae and crustose coralline. To help differentiate the invertebrate taxa recorded in photo-quadrats, specimens were collected at all locations. An in-situ, close-up 35 mm colour photograph was taken of each specimen prior to collection as a permanent record of the habit of the organism. Many invertebrates (especially sponges) lose colour and shape once out of the water so another photograph was taken on the surface and the samples were labelled and immediately frozen for later identification. This voucher collection was lodged with the Queensland Museum, Australia.

Univariate Statistical Analyses

Prior to analysis, the data were examined for homogeneity of variances using Cochran's test (Winer, 1971). Where variances were heterogeneous, data were transformed to log \((x + 1)\) for number of taxa and arcsine for percentage cover (Winer, 1971). Where transformations did not result in homogeneous variances, analyses were done on the untransformed data (Underwood, 1981). If variances could not be stabilized at \(P = 0.05\) but could be stabilized at \(P = 0.01\), the analysis of variance was done using the \(P = 0.01\) probability level (Underwood, 1981). Where significant differences were found in the analysis of variance, Student-Newman-Keuls (SNK) multiple comparisons were done at the appropriate alpha level to determine differences among means (Winer, 1971).
Multivariate statistical techniques were used to identify patterns in assemblages at each location and between habitats at each time using the PRIMER software package (Plymouth Marine Laboratories, UK). Bray-Curtis measures of similarity were used to examine the data matrices, which were double-square-root transformed to reduce the weighting given to abundant taxa and increase the weighting given to rarer taxa (Clarke and Green, 1988). Dendrograms (produced by cluster analyses) and non-metric multi-dimensional scaling (nMDS) ordinations were constructed to graphically illustrate relationships between samples. The significance of any apparent difference among the scales of interest was determined using analysis of similarity (ANOSIM) tests (Clarke and Warwick, 1994). The similarity of percentages (SIMPER) procedure was used to identify the contribution of taxa to the similarities (or dissimilarities) among locations and between habitats (Clarke, 1993).

Patterns of temporal variability in the composition of assemblages at exposed and sheltered reefs were also examined after calculating the average of all replicates (i.e. the centroids) for each habitat for each time \( n = 60 \). Patterns were illustrated using the nMDS ordination and the Index of Multivariate Dispersion (IMD) technique (Warwick and Clarke, 1993a) was used to measure the amount of variability among times for each habitat. The index ranges from -1 to +1 with a value close to +1 indicating that there is more variability between times at the exposed habitat compared to the sheltered habitat. A value of -1 indicates that there is more variability at sheltered reef habitats and values near zero imply no difference between habitats. The IMD technique does not provide a statistical framework to formally test hypotheses of comparable variability between habitats (Clarke and Warwick, 1994).
Results

Erect and encrusting sponges were the richest and most abundant taxon encountered on both sheltered and exposed reefs, with a total of 82 species identified. Of the other major phyla, 14 species of ascidians, 12 bryozoans and 12 cnidarians were recorded. The most abundant algae were crustose Corallinacea and a mixture of macroscopic foliose species.

Changes in Richness and Cover

The cover of macroalgae was generally greatest on the exposed reefs (Fig. 18), however the number of species and their covers fluctuated at various spatial and temporal scales (Fig. 19; Table 13). A matrix of silt, consisting of a non-specific mixture of micro-flora and -fauna, silt and micro-organisms, was the dominant primary cover on exposed reefs, and at times, the dominant cover on sheltered reefs (Fig. 18). The total number of species and cover of all the fauna combined, fluctuated significantly at the smallest scale examined (Table 13), however, there were generally greater numbers of species and covers of fauna on the sheltered reefs (Fig. 19). Sponges were the dominant faunal cover on both exposed and sheltered reefs (Fig. 18). A significant time x habitat x location interaction was detected in the richness and cover of sponges (Table 13; Fig. 19). Generally, there were greater numbers and covers of sponges on the sheltered reefs, compared with the exposed reefs (Figs. 18, 19 & 20). Erect sponges were responsible for the major cover and richness on sheltered reefs, whereas encrusting sponges had the greatest cover and richness on the exposed reefs (Fig. 20). The cover and richness of ascidians, bryozoans and cnidarians also fluctuated at various spatial and temporal scales (Table 13), however their contribution to primary cover was very small in both types of habitat when compared with the sponges (Fig. 18).
Table 13. Summaries of $F$-ratios from analyses comparing spatial and temporal variation in the number and cover of taxa at locations in habitats on exposed and sheltered reefs in NSW. nil, no transformation required; ns, not significant ($P > 0.05$); *, significant ($P < 0.05$); **, ($P < 0.01$).

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Cochran's test, ns, no transformation required; Ascidian Cover ns, not significant ($P > 0.05$); *, significant ($P < 0.05$); **, ($P < 0.01$).
Figure 18. Mean cover estimates at each time for the four major components of the subtidal encrusting assemblage on exposed and sheltered reefs.
Figure 19. Mean number of species and cover (± SE) for (a-b) algae, (c-d) fauna and (e-f) sponges from (○) Bungan Head, (▲) Long Reef, (●) North Head, (□) Cape Banks, (◆) Quarantine Head, (▲) South Head, (★) Henry Head, and (■) Inscription Point (n = 15).
Figure 20. Mean number of species versus mean percentage cover for (a) total, (b) encrusting, and (c) erect sponges on exposed and sheltered reef locations.
Changes in Community Structure

The non-metric multidimensional scaling (nMDS) ordinations clearly demonstrated that assemblages on exposed reefs were considerably different from those on sheltered reefs (Fig. 21). Generally, there was also a clear separation of samples into groups that readily distinguished among the eight locations (Fig. 21). The high stress values were of concern (Fig. 21), so groups from the cluster analyses were superimposed upon the ordination to check the adequacy of the 2-dimensional plots (Clarke and Warwick, 1994). Agreement between the two techniques indicated that the ordinations were an accurate representation of the sample relationships (Clarke and Warwick, 1994). The ANOSIM tests confirmed that there were significant differences in the structure of assemblages between habitats and amongst each of the locations (Fig. 21). The SIMPER procedure consistently ranked the silt matrix, the crustose coralline algae, and the sponge, *Tedania digitata*, as contributing most to community structure within habitats (Table 14) and locations.

Thirty-five sponges were ranked within the top 10 species, although only six (*Dendrilla* sp., *Desmopsamma kirki*, *Hymedesmia* sp., *Mycale* (mycale) sp., *Mycale flammula*, *Spirastrella* sp. and *Tedania digitata*) ranked consistently each time. Seven algal species, 7 ascidians, 5 cnidarians, 3 bryozoans and the marine worm, *Galeolaria caespitosa*, were also ranked as important contributors to community composition. With the exception of *Spirastrella* sp. (Table 14) and the cnidarian, *Capnella* sp., which was only recorded at South Head, few species of importance were ranked exclusively at either exposed or sheltered reefs or at a particular location. This suggested that dissimilarities were primarily due to differences in the relative abundances of taxa rather than the presence or absence of specific taxa. The green algae, *Caulerpa scalpelliformis* (R. Brown ex Turner) C. Agardh, was ranked as the second most important species at Inscription Point in September 1995 (Table 14), and had not previously been recorded at this location in Botany Bay, NSW (Davis *et al.*, 1997).
Figure 21. Non-metric MDS plots for the abundance of sponge-dominated assemblages for each time, (●) Bungan Head, (▲) Long Reef, (○) North Head, (□) Cape Banks, (•) Quarantine Head, (▲) South Head, (●) Henry Head, and (■) Inscription Point, and through time for exposed (▼) and sheltered (▲) habitats.
A non-metric MDS ordination was also used to illustrate temporal variation in the composition of assemblages on exposed and sheltered reefs, using the centroid of all replicates in each habitat (Fig. 21). The nMDS plot separated the samples into two clear groups, which distinguished between habitats, and ANOSIM found the two groups were significantly different (Fig. 21). The ordination implied that there was greater variation through time in the assemblages on the exposed reefs compared with those on sheltered reefs (Fig. 21). This observation was also supported by the positive IMD result (+0.68).

Table 14. Summary of SIMPER analysis indicating the average rank (1-10 presented only) contribution of taxa to the similarities within a habitat during each sample period (E = Exposed reefs; S = Shelter reefs).

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<td></td>
<td></td>
</tr>
<tr>
<td>Anthothoe</td>
<td></td>
<td>10</td>
<td>10</td>
<td>5</td>
<td>5</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Calicia sp.</td>
<td></td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
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<tr>
<td>Worms</td>
<td>7</td>
<td>8</td>
<td>6</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Galeolaria caespitosa</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Discussion

Sponges were the dominant faunal group (greatest richness and cover) on both exposed and sheltered reefs, whilst ascidians were the second most important. In terms of the cover of primary space, foliose and crustose macroalgae and a silt matrix were also important in contributing to the structure of the assemblage. The silt matrix was generally responsible for the greatest primary cover on the exposed reefs, whilst sponges had the greatest cover on sheltered reefs. The cover of macroalgae (foliose and crustose) was generally greatest on exposed reefs whereas the covers of the other fauna (ascidians, bryozoans and cnidarians) were generally similar in terms of the amount of space they occupied in both habitats.

The greater richness and cover of sponges on sheltered reefs, was primarily due to the presence of massive or erect forms, e.g. Spirastrella sp., Desmapsamma kirki and Mycale spp. Whilst these species were also found on the exposed reefs, their morphology or form of growth tended to be more prostrate. This pattern of increased cover and richness of prostrate sponges with depth has been described for temperate (Bell and Barnes, 2000; Ginn et al., 2000) and tropical reefs (Wilkinson and Evans, 1989). In general, small basal areas to volume ratios may render species of sponges unsuitable for high-energy environments (Wulff, 1995; Bell and Barnes, 2000). The greater richness and cover of encrusting species on the exposed reefs was consistent with the greater water turbulence anticipated in this habitat. These “depth-related” patterns have also been described for other types of subtidal assemblages (Schmahl, 1990; Clarke et al., 1993; Bell and Barnes, 2000).

Changes in sedimentation, light and water turbulence associated with depth gradients have all been identified as important in structuring the distribution and abundance of assemblages of sessile invertebrates on temperate (Underwood et al., 1991; Zea, 1993a; Smith 1996b; Bell and Barnes, 2000), tropical (Wilkinson, 1981; Liddell and Ohlhorst, 1987; Wilkinson
and Cheshire, 1989; Wilkinson and Evans, 1989) and polar (Barthel, 1991) subtidal reefs. In this study on temperate reefs however, patterns of distribution and abundance of sponges may not entirely be related to depth gradients, because there were consistently different community structures found between the two habitat types and among locations, at the same depth. Underwood et al. (1991) described a mosaic of subtidal habitat types along the NSW coastline, with the distribution of assemblages related to depth, wave exposure and herbivory, however their study did not generally extend into the habitats below 15 m.

In shallow subtidal habitats, grazing sea urchins and molluscs have been found to effectively maintain the structure of coralline algal crusts in barren ground habitats (Fletcher, 1987; Jones and Andrew, 1990; Andrew and Underwood, 1993; Davis and Ward, 1999). Habitat related differences have also been described at smaller spatial scales (Wright et al., 1997) where differences in the structure of sponge assemblages between two adjacent habitats at the same depth, were related to the ability of some sponges to withstand predation by apparently using chemical defence mechanisms (see Turon et al., 1998). At this stage however, the processes that interact to determine the structure of subtidal assemblages on reefs at depths greater than 18-20 m remain untested (Underwood et al., 1991).

The Index of Multivariate Dispersion (Clarke and Warwick, 1994) test indicated that the structure and dynamics of sponge-dominated assemblages on exposed reefs were more temporally variable than those on sheltered reefs. The application of this index however suffers from a lack of any statistical framework to test the hypothesis about the variability between the two habitats, and Clarke and Warwick (1994) advise caution in the use of the procedure. In general, the multivariate and the univariate analyses supported the hypothesis of increased variability for the assemblages on the exposed reefs. Warwick and Clarke (1993a) identified increased variability in meiobenthic, macrobenthic, coral reef and fish assemblages associated with different types of disturbance in natural habitats and concluded
that this variability may be a symptom of stress. Wave energy or storms are considered to be
natural physical disturbances to temperate subtidal reefs within embayments, estuaries and
on exposed coastlines (Kennelly, 1987a; Underwood, 1999a). The greatest changes in
assemblages will occur where the disturbance is not one that the assemblage normally
experiences, and will depend on its type and magnitude, the pre-disturbance structure of the
community and the morphological and physiological adaptations of its members
(Schratzberger and Warwick, 1999). The results obtained in this study indicated that there
was greater spatial and temporal variability of the assemblages on the exposed reefs and, to
some degree, supports the general hypothesis of increased variability associated with
disturbance or stress proposed by Warwick and Clarke (1993a).

Models associated with exposure and others regarding physical, chemical and biological
factors which could potentially determine the structure and dynamics of these subtidal
assemblages still need to be tested using appropriate manipulative experiments. The
potential physical distinctions between the habitats studied here include increased wave
energy (Short and Trenaman, 1992) and light penetration on exposed reefs (Rendell and
Pritchard, 1996), and stronger tidal currents and increased siltation on sheltered estuarine
reefs (Middleton et al., 1996). There would also be greater loads of nutrients and
fluctuations in salinity on the reefs in the estuarine locations (Middleton et al., 1996; Rendell
and Pritchard, 1996). Differences related to predation (Ayling, 1981; Fletcher, 1987;
Kennelly, 1991; Maldonado and Uriz, 1998), recruitment and settlement (Ayling, 1980;
Keough; 1983; Butler, 1986), and competition for space (Jackson, 1977; Russ, 1980; 1982;
Ayling, 1983a; Davis et al., 1997) are also likely to be important in determining the structure
and dynamics of assemblages within these two types of habitat. These processes are likely
to be interactive at a number of spatial and temporal scales and so hypotheses derived from
the models outlined above must be tested by manipulation of a range of different types of
disturbances (Underwood, 2000b).
The work described here represents the first quantitative assessment of spatial and temporal patterns of variability in sponge-dominated assemblages on temperate reefs in Australia, at depths of 18-20 m. Presenting these results was an important, logical, first-step in developing an understanding of the ecology of sponge-dominated assemblages on subtidal reefs at these depths (Underwood et al., 1991). Research into the ecology of subtidal assemblages living on hard substrata has unfortunately been “depth limited” with most effort spent on those shallow-water assemblages, which are easily sampled using SCUBA techniques (Underwood and Kennelly, 1990; Underwood et al., 1991; Davis and Ward, 1999; Witman and Grange, 1998) or by examining assemblages on artificial structures (Sutherland and Karlson, 1977; Russ, 1982; Kay and Butler, 1983; Connell and Glasby, 1999; Glasby, 1999a). In recent years, marine ecologists have rightfully focussed on experimental tests of hypotheses about processes to explain patterns of variability, however descriptive studies are still a necessary pre-cursor to experimental manipulative analyses (Underwood et al., 2000). For sponge-dominated assemblages on deeper temperate reefs, experimental tests of hypotheses about the processes producing their structure and dynamics, were considered to be premature until spatial and temporal variability had been quantified at appropriate scales.
CHAPTER 4: PATTERNS ASSOCIATED WITH SEWAGE DISPOSAL

SHALLOW-WATER ASSEMBLAGES IN KELP FORESTS

Introduction

A common problem faced by many coastal communities is the need to dispose of a variety of waste products. Historically, the oceans and estuaries have been viewed as convenient and safe places to dispose of liquid wastes such as sewage. This has led to the construction of many sewage outfalls discharging effluent of varying qualities to coastal waters. In the coastal area of New South Wales, between Sydney and Norah Head (100 km to the north), there are ten sewage ocean outfalls. The three largest are deep-water outfalls discharging primary-treated sewage and have been extensively studied (Scanes and Philip, 1995). The other seven outfalls discharge either primary or secondary-treated sewage into shallow (0-10 m) coastal waters.

There are few published accounts of the impacts of the discharge of secondary-treated sewage effluent on subtidal marine macrobenthos living in shallow kelp forests in New South Wales (Smith and Simpson, 1992; 1993; Roach et al., 1995; Smith, 1994; 1996a). Smith and Simpson (1993) reported differences between the faunal assemblages living within holdfasts of the kelp *Ecklonia radiata* C. Agardh close to sewage outfalls compared with sites further away. In contrast, Roach et al. (1995) found that for invertebrate assemblages living in kelp holdfasts, the effects of low-volume sewage effluent could not be separated from natural variability.

Effects of sewage outfalls on intertidal macrobenthic assemblages living on hard substrata have been examined in the Sydney region (Borowitzka, 1972; Fairweather, 1990). These
studies found that "typical" zonation patterns were absent and that species diversity was reduced close to the outfalls. Fairweather (1990) described increased cover of the algae Ulva lactuca Linnaeus and Enteromorpha intestinalis Linnaeus in close proximity to the outfalls. Studies on rocky shores in the USA have also shown significant effects on the distribution of macrobenthos because of the effects of sewage outfalls (Littler and Murray, 1975; 1978; Murray and Littler, 1978). Kindig and Littler (1980) reported enhanced growth rates of intertidal marine algae in response to additions of primary and secondary-treated sewage effluent, whilst secondary-chlorinated effluent caused negative short term growth.

Here spatial patterns in the subtidal macrobenthos at three secondary-treated shoreline sewage outfalls and at control or reference locations in the Sydney and Central Coast regions of New South Wales are described. Furthermore, differences between assemblages exposed to chlorinated and non-chlorinated sewage effluent were examined. This was done by measuring attributes such as the cover and the richness of species in places that were subjected to sewage and at reference places that were not subjected to sewage. The rationale behind such comparisons is that differences in biological assemblages between outfall and reference locations would provide correlative evidence for the effects of outfalls (Fairweather, 1990). If present, such differences in assemblages at outfall locations may be attributed to the toxic effects of effluent on adult species, differences in the settlement and survival of recruits into the community and/or physical disturbances caused by siltation, nutrient enrichment, changes in light regimes and decreased salinity.
Methods

Macrobenthic assemblages were sampled during March 1993 at six subtidal locations along the coast of central New South Wales (Figure 22, Table 15). The six locations have similar physical attributes in terms of their geomorphology and degree of physical exposure to waves and currents. Three of the locations (Norah Head, Winnie Bay and Warriewood) were situated at secondary-treated shoreline ocean outfalls. The quantity and quality of the sewage effluent at each outfall indicates their relative similarity in terms of potential anthropogenic disturbance (see Table 16 - data from MHL, 1997).

At each location, two randomly selected sites were sampled in approximately 6-10 m depths. At the three outfall locations, these sites were situated within 50 m of the discharge pipe and within the sewage plume. At each site, the numbers of adult and juvenile kelp (Ecklonia radiata) plants were counted by SCUBA divers in five random 1 m² quadrats. In addition, the abundance of macrobenthos living under the kelp canopy was estimated by recording their percentage covers (using the point-intersect method) and the number of species in 5 randomly placed quadrats (27 x 27 cm). To ensure independence of data, separate quadrats were sampled for algae and fauna. Mobile species such as molluscs were sampled by counting the number of individuals in a quadrat. The limitations and advantages of using such a small quadrat, which could physically fit between individual kelp plants and holdfasts, were addressed by Kennelly (1987c). Furthermore, the size of the sampling unit and the number of replicates has also previously been justified by Kennelly (1987c) for shallow subtidal kelp forests in temperate New South Wales.

The richness and abundance of all major taxa were examined, as were dominant or common species. A number of these variables were analysed using analysis of variance where
treatments were fixed, locations were nested within treatments and sites were nested within locations (Tables 17 and 18). Prior to analysis, the data were examined using Cochran's test for homogeneity of variances (Winer, 1971; Underwood, 1981). Where variances were heterogeneous, data were transformed to log \((x + 0.5)\) for the number of taxa or individuals and arcsine for percentage cover (Winer, 1971). Where transformations were unsuccessful, analyses were done on the untransformed data (Underwood, 1981). If variances could not be stabilised at \(P = 0.05\) but could be stabilised at \(P = 0.01\), the analysis of variance was done using the \(P = 0.01\) probability level (Underwood, 1981). Where significant differences were found in the analysis of variance, Student-Newman-Keuls (SNK) multiple comparisons were done at the appropriate alpha level to determine differences among means (Winer, 1971).

Multivariate statistical techniques were used to examine the variation between species abundance and composition at each location using the PRIMER software package (Plymouth Marine Laboratory, 1997). Abundance data for each site \((n = 5)\) were double-square-root transformed to reduce the weighting given to abundant taxa and increase the weighting given to rarer taxa. The Bray-Curtis similarity matrix was used to generate 2-dimensional plots using the non-metric multi-dimensional scaling (nMDS) technique (Clarke, 1993). Plots were produced for the entire assemblage (algae and fauna), the algal assemblage and the faunal assemblage. Analysis of similarities (ANOSIM) was used to examine the differences between locations and sites (Clarke and Warwick, 1994) for each of these assemblages. The similarity-percentages (SIMPER) procedure was used to identify the major species that contributed to similarities within a location and differences among locations (Clarke, 1993).
Figure 22. Outfall locations at Norah Head (●), Winnie Bay (■) and Warriewood (▲), and reference locations at North Avoca (□), Bungan Head (○) and Cape Banks (▲).
Table 15. Experimental design used for sampling macrobenthos at outfalls and reference locations along the central coastline of NSW (NH - Norah Head, WB - Winnie Bay, WW - Warriewood (Chlorinated), BH – Bungan Head, NA - North Avoca, CB - Cape Banks).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>NH</th>
<th>WB</th>
<th>WW</th>
<th>BH</th>
<th>NA</th>
<th>CB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Locations</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sites</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Replication</td>
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<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

Table 16. Summary of sewage effluent characteristics between the three outfall locations (source MHL, 1997).

<table>
<thead>
<tr>
<th>Outfall</th>
<th>Treatment</th>
<th>Discharge Volume (ML/Day)</th>
<th>Discharge Rate (L/Sec)</th>
<th>Nearfield Dilution (Within 50m)</th>
<th>Farfield Dilution (Within 100m)</th>
<th>Average Nitrogen (mg/L)</th>
<th>Average Phosphorus (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norah Head</td>
<td>Secondary</td>
<td>30</td>
<td>700</td>
<td>20</td>
<td>100</td>
<td>7.4</td>
<td>3.6</td>
</tr>
<tr>
<td>Winnie Bay</td>
<td>Secondary</td>
<td>38</td>
<td>640</td>
<td>4</td>
<td>900</td>
<td>30.2</td>
<td>9.3</td>
</tr>
<tr>
<td>Warriewood</td>
<td>Secondary</td>
<td>15</td>
<td>173</td>
<td>50</td>
<td>350</td>
<td>40.2</td>
<td>7.8</td>
</tr>
<tr>
<td></td>
<td>(chlorination)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Results

Univariate Analyses

Algal assemblages

A dense canopy of the kelp *Ecklonia radiata* was found at every location. The density of adult kelp plants was significantly greater at Cape Banks (reference location) than at any other location (Fig. 23a, Table 17, Table 19). No differences were detected among treatments or sites nested within locations (Table 17). The density of juvenile kelps was significantly \((P < 0.01)\) greater at the Norah Head outfall location than at other locations (Fig. 23b, Table 17, Table 19). The outfall at Warriewood had lower numbers of juvenile kelps than all other locations. There were no differences between treatments or sites nested within locations for the number of juvenile kelp plants (Table 17).

The richness and cover of algae were not significantly different for any of the sources of variation tested (Figs. 23c and 23d, Table 17). Algal richness and cover within the various kelp understories varied between locations with no obvious trends apparent among treatments.

The articulated calcified alga, *Amphiroa anceps* Lamark, was more abundant at Cape Banks than at all other locations (Fig. 23e). Another articulated calcified alga, *Corallina officinalis* Linnaeus, was most abundant at Norah Head and North Avoca. The next greatest abundance of this species occurred at the Warriewood location and it was almost absent from Winnie Bay, Cape Banks and Bungan Head (Fig. 23f).

Red crustose coralline algae included *Porolithon* sp. (Fig. 23g) and *Lithothamnium* sp. (Fig. 23h). The crustose alga, *Peyssonnelia* sp. was most abundant at Winnie Bay and North
Avoca (Fig. 23i). Porolithon sp. was abundant at Norah Head, Bungan Head and Warriewood. Covers at Warriewood were smaller and no Porolithon sp. was found at Winnie Bay, North Avoca or Cape Banks (Fig. 23g). Winnie Bay and Cape Banks had greater covers of Lithothamnium sp. compared with other locations (Fig. 23h). Greater covers of the brown alga Zonaria crenata J. Agardh were found at the three southern locations, Warriewood, Bungan Head and Cape Banks (Fig. 23j).

**Faunal assemblages**

Six major groups of macrobenthic animals were identified in the kelp assemblages: Porifera (sponges), Bryozoa (sea mosses), Cnidaria (hydroids, sea anemones), Crustacea (barnacles), Mollusca (gastropods, limpets) and Ascidiacea (sea squirts). Total richness of macrobenthic faunal species and their covers were not significantly different for any of the sources of variation tested (Figs. 24a and 24b, Table 18).

Encrusting sponges were the most abundant faunal group found at all locations and included Psammopemma sp. (Fig. 24c) and Tedania sp. (Fig. 24d). Outfall locations had a significantly greater richness of sponges than the reference locations (Fig. 24e, Table 18, Table 19) whilst no significant effects were detected for the cover of sponges (Fig. 24f, Table 19).

The bryozoans included three species of encrusting Membranipora and one of Bugularia. Cape Banks had greater covers of Membranipora membranacea Linnaeus than all other locations (Fig. 24g). The total richness and cover of bryozoans was significantly greatest at this location (Figs. 24h and 24i, Table 18, Table 19).

Cnidarians included Sertularia tenuis Bale (Fig. 24j) and a species of Hydroid. There were no differences in the richness or cover of cnidarians (Figs. 24k and 24l) among locations.
There were no significant sources of variation for molluscan richness or abundance (Table 18, Figs. 24m and 24n). The molluscs included two species of limpet, *Patelloida mufria* Hedley and *Patella chapmani* Tennyson Woods. The richness and cover of crustaceans (Figs. 24o and 24p) were represented by a single barnacle species, *Balanus* sp.

Ascidians were found only at the northern locations and included species of *Botrylloides* and *Pyura*. *Botrylloides magnicoecum* Hartmeyer (Fig. 24q) was only found at the two outfall locations at Norah Head and Winnie Bay. The total richness of ascidians was significantly different at sites within locations at Norah Head, Winnie Bay and North Avoca (Fig. 24r, Table 18) but not among locations or treatments. No significant differences were found for the total cover of ascidians for any sources of variation (Fig. 24s, Table 18).

Table 17. Summaries of F ratios from analyses of variance for richness and cover of algae and fauna, and the number of adult and juvenile kelp plants. T = Treatment; L = Location; nil = no transformation required; ns = not significant (P > 0.05); ns+ = not significant (P > 0.01); * = significant (P < 0.05); ** = significant (P < 0.01); Transform = results of Cochran’s test after transformation.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Algal Richness</th>
<th>Algal Cover</th>
<th>No. Kelp Adults</th>
<th>No. Kelp Juveniles</th>
<th>Faunal Richness</th>
<th>Faunal Cover</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>1</td>
<td>0.10ns</td>
<td>0.70ns</td>
<td>0.47ns</td>
<td>0.01ns</td>
<td>0.37ns</td>
<td>0.32ns</td>
</tr>
<tr>
<td>Location (T)</td>
<td>4</td>
<td>1.95ns</td>
<td>0.61ns</td>
<td>12.85**</td>
<td>11.04**</td>
<td>3.57ns</td>
<td>0.71ns</td>
</tr>
<tr>
<td>Sites (L x T)</td>
<td>6</td>
<td>1.19ns</td>
<td>1.53ns</td>
<td>0.65ns</td>
<td>1.64ns</td>
<td>1.49ns</td>
<td>1.24ns</td>
</tr>
<tr>
<td>Residual</td>
<td>48</td>
<td>0.062</td>
<td>253.88</td>
<td>99.7</td>
<td>65.37</td>
<td>1.43</td>
<td>39.78</td>
</tr>
<tr>
<td>Cochran’s</td>
<td></td>
<td>Ns</td>
<td>*</td>
<td>*</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Transform</td>
<td></td>
<td>ns+</td>
<td>**</td>
<td>ns</td>
<td>nil</td>
<td>nil</td>
<td>nil</td>
</tr>
</tbody>
</table>
Table 18. Summaries of $F$ ratios from analyses of variance for selected faunal taxa. T = Treatment; L = Location; nil = no transformation required; ns = not significant ($P > 0.05$); ns+ = not significant ($P > 0.01$); * = significant ($P < 0.05$); ** = significant ($P < 0.01$); Transform - results of Cochran’s test after transformation.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Poriferan Richness</th>
<th>Poriferan Cover</th>
<th>Bryozoan Richness</th>
<th>Bryozoan Cover</th>
<th>Molluscan Richness</th>
<th>Molluscan No. Ind.</th>
<th>Ascidian Richness</th>
<th>Ascidian Cover</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>1</td>
<td>8.65*</td>
<td>0.68ns</td>
<td>0.46ns</td>
<td>0.65ns</td>
<td>0.29ns</td>
<td>0.13ns</td>
<td>0.89ns</td>
<td>1.30ns</td>
</tr>
<tr>
<td>Location (T)</td>
<td>4</td>
<td>1.37ns</td>
<td>1.09ns</td>
<td>6.52*</td>
<td>10.42**</td>
<td>2.5ns</td>
<td>2.28ns</td>
<td>1.47ns</td>
<td>2.91ns</td>
</tr>
<tr>
<td>Sites (L x T)</td>
<td>6</td>
<td>0.53ns</td>
<td>1.74 ns</td>
<td>2.57*</td>
<td>1.77ns</td>
<td>1.19ns</td>
<td>1.34ns</td>
<td>3.17*</td>
<td>1.37ns</td>
</tr>
<tr>
<td>Residual</td>
<td>48</td>
<td>0.6</td>
<td>36.07</td>
<td>0.18</td>
<td>7.59</td>
<td>0.31</td>
<td>0.83</td>
<td>0.1</td>
<td>4.82</td>
</tr>
<tr>
<td>Cochran’s</td>
<td>ns</td>
<td>*</td>
<td>Ns</td>
<td>*</td>
<td>ns</td>
<td>*</td>
<td>ns</td>
<td>*</td>
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<td>nil</td>
<td>ns</td>
<td>nil</td>
<td>ns</td>
<td></td>
</tr>
</tbody>
</table>

Table 19. Summary of SNK tests for taxa where significant differences were found among locations. Locations are ranked from the lowest to the highest mean. An underlined location indicates no significant differences between adjacent underlined locations.

<table>
<thead>
<tr>
<th>Algae</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ecklonia radiata</em> (Adults)</td>
<td>NA, WW, BH, NH, WB, CB</td>
</tr>
<tr>
<td><em>Ecklonia radiata</em> (Juveniles)</td>
<td>WW, BH, WB, CB, NA, NH</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Fauna</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Poriferan Richness</td>
<td>CB, NA, BH, WW, NH, WB</td>
</tr>
<tr>
<td>Bryozoan Richness</td>
<td>WW, BH, NA, NH, WB, CB</td>
</tr>
<tr>
<td>Bryozoan Cover</td>
<td>WW, BH, NA, WB, NH, CB</td>
</tr>
</tbody>
</table>
Figure 23. Mean (+ SE) number of *Ecklonia radiata* plants and percentage covers of various algal taxa ($n = 10$, i.e. sites are pooled; NH - Norah Head, WB - Winnie Bay, WW - Warriewood, BH - Bungan Head, NA - North Avoca, CB - Cape Banks).
Figure 24. Mean (± SE) number of species and percentage covers of faunal taxa (n = 10, i.e. sites are pooled; NH - Norah Head, WB - Winnie Bay, WW - Warriewood, BH - Bungan Head, NA - North Avoca, CB - Cape Banks).
Figure 24. Continued
Multivariate Analyses

The multivariate statistical procedures nMDS and ANOSIM confirmed that differences among locations could not be related to the effects of sewage outfalls (Fig. 25). A nMDS ordination (stress = 0.2) and ANOSIM test (Treatment: \( R = -0.26 \); Location: \( R = 0.84 \)) on the combined macrobenthic assemblage determined that there were no significant differences between outfalls and controls, however there were significant differences among locations (Fig. 25a). The nMDS plot indicated differences between locations and the pairwise comparisons confirmed this pattern with all locations being significantly different from each other (Fig. 25a). The stress value for the ordination was 0.2, indicating a potentially useful 2-dimensional picture (Clarke and Warwick, 1994).

Similar patterns, found in the analysis of the combined data (algae and fauna) were also found in the analyses of the assemblages of algae. The stress value for the ordination and the ANOSIM results (Fig. 25b) were typical of those found in the results of the combined assemblage (Fig. 25a). The locations at Cape Banks, Warriewood and North Avoca were clearly separated from the other locations in the ordination (Stress 0.17), and there were significant differences among locations \( (R = 0.86) \) according to the ANOSIM test (Fig. 25b). The same patterns were also found in the pairwise comparisons for each location.

No significant differences were found between treatments \( (R = -0.11) \) for the faunal assemblage (Fig. 25c). According to the ANOSIM test there were significant differences among locations \( (R = 0.26) \). The pairwise comparisons indicated that the Cape Banks location was significantly different from all other locations, whilst Warriewood was significantly different from Winnie Bay and Norah Head outfalls (Table 20). The North Avoca location was also significantly different from Winnie Bay and Warriewood outfall locations (Table 20).
The SIMPER procedure ranked, in order of importance, those species that contributed most to the similarities within a location (Table 21) and those that contributed most to the dissimilarities among locations (Table 22). It is clear that there was significant variation associated with the species considered important in structuring the assemblage at each location (Table 21). In general, there were no patterns associated with the structure of assemblages between outfall and reference locations for either algae or fauna (Tables 21 and 22).

The relative importance of each species within a location varied, however *Porolithon* sp. and *Ecklonia radiata* were generally the species that contributed most to the structure of the algal assemblage at each location (Table 21a). The contribution of each species of algae to the average dissimilarity was also found to vary among locations (Table 22). Species will contribute to the dissimilarity between two locations by having either greater or lesser abundance of the same species. For example *Lithothamnium* sp., *Porolithon* sp. and *Corallina officinalis* were important in distinguishing Norah Head from Winnie Bay, Warriewood and Cape Banks, whereas *Polysiphonia* sp. and *Rhodymenia* sp. were important in distinguishing Norah Head from North Avoca and Bungan Head (Table 22).

The encrusting sponge *Psammopemma* sp. was most “important” at all locations, with the exception of Cape Banks, where *Membranipora membranacea* was ranked highest (Table 21b). The limpet, *Patella chapmani* consistently appeared as an important contributor to the assemblage at each location (Table 21b). In general, *P. chapmani*, *Tedania* sp. and *Psammopemma* sp. were most important in contributing to the dissimilarities among locations for the faunal assemblage (Table 22). *M. membranacea* was consistently most important in distinguishing Cape Banks from all other locations (Table 22).
Figure 25. Non-metric multidimensional scaling (nMDS) plots and summary of the ANOSIM tests for (a) all species (b) algal species and (c) faunal species at each location: outfalls – Norah Head (●), Winnie Bay (■), Warriewood (▲), controls - North Avoca (○), Bungan Head (●̄) and Cape Banks (▲̄).
Table 20. Summary of $R$ statistics for pairwise comparisons between faunal assemblages at each location. ns = not significant ($P > 0.05$); * = significant ($P < 0.05$).

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<tr>
<td>WW</td>
<td>0.22*</td>
<td>0.33*</td>
<td>-</td>
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<td>0.23*</td>
<td>-</td>
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<td>0.11ns</td>
<td>-</td>
</tr>
<tr>
<td>CB</td>
<td>0.37*</td>
<td>0.29*</td>
<td>0.47*</td>
<td>0.49*</td>
<td>0.29*</td>
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Table 21. (a) Species of algae and (b) fauna ranked in order of importance, which contributed to the similarities within a location as determined using the SIMPER procedure.

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<td>4</td>
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<tr>
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<td>7</td>
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Table 22. Species of algae and fauna listed in order of importance (1-5), which contributed to the dissimilarities among locations as determined using the SIMPER procedure.

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Discussion

No patterns associated with sewage effluent at Norah Head, Winnie Bay and Warriewood ocean outfalls were found in the assemblages of algae or fauna living in the shallow kelp forests. The richness of sponges was the only derived variable that showed any significant treatment (outfall versus control) effect. This was contrary to other studies on subtidal assemblages, which have found reduced richness of sponges associated with sewage effluent. The richness and abundance of cnidarians, molluscs and crustaceans and the cover of some sponges and ascidians were found to exhibit patterns that could not be attributed to the proximity of the outfalls.

Kennelly and Underwood (1992) reported that juvenile *Ecklonia radiata* had relatively consistent natural fluctuations on large spatial and temporal scales that may make them potentially good indicators of anthropogenic disturbances. Spatial differences were observed for the juvenile kelp *E. radiata*, however no evidence could be found to verify their prediction. This may indicate that disturbance due to these outfalls is insignificant compared with natural fluctuations in kelp settlement, recruitment and early survival. Ajani *et al.* (1999), using a “Beyond BACI” experimental design demonstrated that there were no spatial or temporal patterns in the abundance or recruitment of *E. radiata* associated with a sewage outfall.

Many studies on intertidal and subtidal hard substrata reported effects due to sewage outfalls. These effects have included changes to species richness, diversity and abundance as well as changes to the overall structure of the community (e.g. Kindig and Littler 1980; May, 1985; Fairweather, 1990; Smith and Simpson, 1992; Roberts *et al.*, 1998). As an example, Kindig and Littler (1980) found that certain species of algae (including species similar to those found in the present study) showed enhanced rates of growth in the presence of primary and
secondary treated sewage effluent. They also reported that secondary chlorinated effluent had only a short-term negative effect on the growth of algae. It should be noted that Kindig and Littler (1980) did their studies under controlled laboratory conditions, the results of which can only be extrapolated into the field with caution. Conversely, there have been many studies where there were no detectable effects of sewage outfalls (e.g. Wharfe et al., 1981; Chapman et al., 1995; Roach et al., 1995; Underwood and Chapman, 1996).

Here outfalls that can discharge up to 38 ML/day of sewage effluent were examined. The question remains as to why there are no apparent effects of sewage on these subtidal assemblages directly within the receiving waters. At least one of the treatment plants (Norah Head) is operated in a way that could potentially minimise the effects of its discharge; i.e. it commonly discharges its effluent at night. The responses of a subtidal assemblage effected by sewage could include (1) increased growth catalysed by elevated nutrients (increased primary productivity), (2) decreased light caused by shading (reduced primary productivity), (3) increased siltation (smothering of encrusting organisms) and (4) reduced salinity (death of stenohaline species). For this particular outfall, the reduction in light caused by shading is not an issue, because it is dark when the sewage is discharged and the plume is generally gone by daylight. The elevated nutrients associated with the discharge of the effluent go largely unused by the primary producers, because nutrient uptake is generally lower during their dark cycle. It should also be noted that these shallow high-energy habitats are generally chosen as suitable outfall locations because of the considerable mixing that occurs. Under these conditions, the effects of decreased salinity and increased siltation are most likely minimised.

Another relevant question with respect to the present study is whether the experimental design provided sufficient statistical power to be able to detect differences in the subtidal assemblages. The issues regarding appropriate power to detect environmental impacts have
received considerable attention in the ecological literature (Green, 1989; Peterman, 1990; Fairweather, 1991; Mapstone, 1995; Underwood, 1997). When a statistical test is used, there is always the chance of making two potential errors. Type I errors occur when we reject the null hypothesis when it is actually true, i.e. we claim to have determined an impact when in reality there was no impact. Type II errors occur when we accept the null hypothesis when it is false, which equates to claiming no impact when in fact there is one (Underwood, 1997). In assessments of potential anthropogenic disturbance, Type II errors can be detrimental because we may be lulled into a false sense of security.

Many significant results were found in this study, which by definition proves that there was sufficient statistical power (Fairweather, 1991). For results where no significant differences could be found, post hoc power was evaluated and mixed results were found, ranging from low to medium power. There can be confidence in the interpretation of no impact because both the univariate and multivariate approaches resulted in similar conclusions. The experimental design was also considered sufficient because a similar study successfully detected the effects of a discharge of phenols and PAH's in similar habitats using the same methods employed here (Scanes and Roberts, 1993).

The spatial patterns of the macrobenthic assemblages reported in this study indicated considerable variation within and among locations. Underwood et al. (1991) described the subtidal reefs along the coast of NSW as a mosaic of habitats related to depth, wave exposure and a number of biological processes. Kennelly and Underwood (1992) reported complex spatial patchiness at various scales in assemblages of organisms living within kelp forests. Given that these subtidal assemblages are naturally highly variable it is doubtful, unless the anthropogenic disturbance is large, that we will ever be in a position to tease out the effects of sewage from that caused by natural processes.
Here biological attributes such as richness and cover of subtidal macrobenthos were used in an attempt to quantify the impact of sewage discharges on the shallow marine environment. The results indicated no evidence for differences in macrobenthic assemblages associated with sewage outfalls. This may mean that the variables examined were either not suitable for detecting these impacts or there were no impacts to detect. There were no consistent patterns or measurable impacts associated with these outfalls and increased spatial and/or temporal replication would not necessarily have improved the ability to detect an impact.
NEARSHORE SPONGE-DOMINATED ASSEMBLAGES

Introduction

The spatial and temporal variation inherent in natural systems can make the detection of human impacts extremely difficult. This subject has drawn considerable attention in marine systems and has been the subject of considerable discussion (Clarke and Green, 1988; Underwood and Peterson, 1988; Underwood, 1992; Warwick and Clarke, 1993a). The development of the Before-After, Control-Impact (BACI) design (Green, 1979) and more recently the 'Beyond BACI' design (Underwood, 1991a; 1992; 1993; 1994) has led to significant advances in the detection of impacts associated with anthropogenic disturbance. The 'Beyond BACI' approach clearly acknowledges the importance of spatial and temporal sampling designs that ensure un-confounded detection of anthropogenic disturbance.

In coastal regions around the world, it has been convenient to discharge sewage effluent into the marine environment. In many instances, sewage is discharged onto subtidal rocky reefs exposed to high levels of water movement to ensure dilution. Sessile encrusting assemblages, including sponges and ascidians, often dominate the rock surfaces under these circumstances and because they are fixed to the substratum have a high probability of responding to the effects of sewage (Warwick, 1993).

Temperate subtidal encrusting assemblages living on natural substrata are reputedly temporally stable (Ayling, 1983b) and those living on artificial substrata may be quite stable at medium scales, although highly dynamic at small scales (Kay and Butler, 1983; Butler and Connolly, 1996). Recent studies on assemblages living on natural substrata indicate that they are much more dynamic, particularly in shallow water (Kennelly and Underwood, 1992) and therefore the response of the assemblage to sewage-induced disturbance may be very rapid.
Many studies into the effects of sewage on subtidal rocky reef assemblages have been done after the potential disturbance has already occurred. These ‘post hoc’ studies abound (Littler and Murray, 1975; Lopez Gappa et al., 1990; Muricy, 1991; Smith and Simpson, 1992; 1993; Smith, 1994; Chapman et al., 1995) however, their numbers are insignificant when compared with the “grey literature” examining patterns associated with sewage. If we ignore that inappropriate design and or logic (for example only one control) confounded many early studies, they are still generally inadequate, because pre-disturbance data were not collected. In practice, pre-disturbance data may be difficult (or impossible) to collect and the subsequent utility of monitoring programs that cannot, or do not, incorporate pre-impact data and appropriate controls is limited. Moreover, there is always the potential that any monitoring program will merely measure pre-existing differences between locations.

In November 1993, the nearshore ocean outfall at Boulder Bay, NSW Australia, began discharging approximately 3 megalitres per day of secondary treated sewage effluent, from two diffuser heads into subtidal rocky reef habitat at a depth of 20 m. Here the effects of discharging sewage on sessile macrobenthic assemblages using both (i) a multivariate approach (Clarke and Warwick 1994) and (ii) a univariate asymmetrical analysis incorporating a “Beyond BACI” experimental design (Underwood, 1994) were used.
Methods

Study Locations and Sampling Design

To study the effects of sewage from the Boulder Bay outfall, assemblages were sampled at the outfall location (Boulder Bay - exposed to effluent) and at 2 control or reference locations (Point Stephens and Tomaree Head - no effluent) (Fig. 26). In general, the 3 locations were situated on rocky reef to a depth of approximately 20 metres and the benthic community was dominated by erect sponges. Underwood et al. (1991) described similar subtidal habitat for coastal waters in NSW, Australia.

The outfall location was situated within a 100 m radius of the diffusers and the assemblages were sampled using a diver operated camera rig that supported a 35 mm Sea & Sea Motor Marine-2 underwater camera and strobe. Investigations of the inshore and offshore circulation patterns around the outfall area were done to identify potential sewage plume movements from the outfall and to assess the suitability of controls. It was concluded from these investigations that the closest control location at Point Stephens (see Fig. 26) was independent of the effects of sewage (Lee and Wallace, 1993). It is also worth noting that there were no suitable accessible reference locations south of the outfall i.e. subtidal rocky reef at 18-20 metre depth.

The assemblages at each location were sampled a total of twelve times over an 18 month period from June 1993 to February 1995. The temporal design incorporated 4 random times within each of 3 periods, 1 before and 2 after the discharge of sewage. Because the temporal design was unbalanced, separate balanced analyses (i.e. Period 1 versus Period 2 and Period 1 versus Period 3) were done on all derived variables. Only the P1 versus P3 analyses are presented, however, the P1 versus P2 analyses are referred to in the text where relevant.
Within-location variability in the assemblages was determined by haphazardly photographing 10 replicate quadrats (photo-quadrat dimensions - 0.8 m x 0.56 m, total area 0.45m²) at each of 3 sites nested within each location. At each location, the 3 sites (approximately 50 m in diameter and 50 m apart) were randomly selected each time.

*Analysis of Photographs and Taxonomic Discrimination*

The photo-quadrats were analysed using a Bell and Howell ‘black box’ projector. An overlay plastic grid of 100 regularly spaced points was placed on the screen and estimates of the percentage cover and number of species were recorded from the photo-quadrat.

To help differentiate the taxa recorded in quantitative photo-quadrats, invertebrate collections were made at all locations. An *in-situ*, close-up 35 mm colour photograph was taken of each specimen prior to collection as a permanent record of the habit of the organism. Many invertebrates (especially sponges) lose colour and shape once out of the water so another photograph was taken on the surface and the samples were labelled and immediately frozen for later identification. This voucher collection was lodged with the Queensland Museum, Australia.
Univariate Statistical Analyses

Since there was only one outfall location, asymmetrical analyses of variance were used to examine the differences between the outfall and the two control locations (Underwood, 1992; 1993). Asymmetrical, before/after-control/impact (Beyond BACI) designs, their mechanics and potential for detecting both temporal and spatial disturbances have been discussed in detail by Underwood (1991a; 1992; 1993; 1994). An example of the model used here can be found in Table 23, whilst the logic associated with interpreting these types of analyses are fully discussed by Underwood (1993; 1994). In this case, there were 2 fixed periods, with 4 random times within each period. The outfall versus controls term was considered fixed whilst between controls was random. Sites were randomly nested within locations. The asymmetrical analyses repartition a fully orthogonal model, and allow \textit{a priori} comparisons between the outfall and the average of the controls (Underwood, 1992). The data associated with both the outfall and controls were analysed using a fully orthogonal design in which sites were nested within locations and time was considered a random factor within periods. A second analysis was then done on only those data associated with the controls. The asymmetrical component, or outfall versus control partition, was calculated by subtracting the sums of squares of the second analysis from those of the first. What was left was the variance associated with the potentially impacted outfall location. The various factors within the analysis were calculated using the same logic, as were the partitioned interactions associated with the various main effects (see Underwood, 1993). Appropriate F-tests were constructed (see Table 23) using the principles outlined in Underwood (1981).

As Underwood (1981) notes, significant higher order interactions, e.g. Time (Period) x Sites (Location), mean that lower order interactions and main effects generally cannot be interpreted and should not be reported. Where significant Time (Period) x Sites (Location) interactions
occurred, a 2-tailed $F$ test was used to compare the temporal variability among sites within control locations, with the temporal variability among sites at the outfall (Underwood, 1992). This allowed a direct comparison of the effects of the outfall at these smaller spatial scales. *Post hoc* pooling procedures were used when the Time (Period) x Sites (Location) or Time (Period) x Location interactions were found to be non-significant at $P = 0.25$; this allowed appropriate $F$ tests to be constructed for lower order interactions and main effects (Winer, 1971). Furthermore, where significant differences occurred in the Time (Period) x Sites (Location) and the Time (Period) x Location interactions (and their partitions), the Period x Outfall versus Control interaction was reported so that the trend above these higher order interactions could be examined (Underwood et al., 1993). It should be noted that this is not generally done because the significant higher order interactions warn that the lower order interactions are not independent (Underwood, 1981). It should also be noted that when many repeated significance tests are done there is always a danger of increasing the probability of making Type I errors (Clarke and Warwick, 1994).

Prior to analysis, the data were examined for homogeneity of variances using Cochran's test (Winer, 1971). Where variances were heterogeneous, data were transformed to log $(x + 0.5)$ for number of taxa and transformed to arcsine for percentage cover (Winer, 1971). Where transformations did not result in homogeneous variances, analyses were done on the untransformed data (Underwood, 1981). If variances could not be stabilized at $P = 0.05$ but could be stabilized at $P = 0.01$, the analysis of variance was done using the $P = 0.01$ probability level (Underwood, 1981). Where significant differences were found in the analysis of variance, Student-Newman-Keuls (SNK) multiple comparisons were done at the appropriate alpha level to determine differences among means (Winer, 1971).
**Multivariate Statistical Analyses**

Multivariate statistical techniques were used to analyse the variation in species abundance and composition at each location and at each time using the PRIMER software package (Plymouth Marine Laboratories, UK). Abundance data for each location and time \((n = 30)\) were double-square-root transformed to reduce weighting given to abundant taxa and increase the weighting given to rarer taxa. The Bray-Curtis similarity matrix was used to generate 2-dimensional plots with the non-metric multi-dimensional scaling (nMDS) technique (Clarke, 1993). Two-way analysis of similarity (ANOSIM) tests were used to examine the differences between periods and locations, while the SIMPER procedure was used to identify the major species contributing to the similarity measure obtained (Clarke and Warwick, 1994).

**Results**

Sponges were the most species rich and abundant taxon encountered at all locations. Over 100 species were identified, including erect and encrusting forms. Of the three other major phyla, 37 ascidian species, 17 bryozoan species and 17 species of cnidarian were recorded. The most abundant algae were crustose Corallinacea from the Order Cryptonemiales and a mixture of macroscopic foliose species. Since the algae were not readily identifiable from the photographs, they were grouped as foliose or crustose taxa.
Changes in Cover and Richness

The richness and cover of many of the taxa observed in this study fluctuated at various spatial and temporal scales. As judged by their response to the outfall, taxa may be split into three groups: (i) those for which no significant change in cover or richness occurred, (ii) those that showed a decrease and (iii) those that showed an increase. For the sake of brevity, only selections of the taxa are presented here. The means and standard errors presented (Fig. 27) were calculated after the three sites within a location were pooled, i.e. \( n = 30 \). This makes the values for locations at each time look far more precisely determined than they actually were.

Significant differences among sites were found and ideally all sites should have been plotted. This was not done because the sites were randomly nested within each location and therefore plotting a time coarse for each of the random sites would be pointless and generally makes the figures uninterpretable.

Taxa which showed little change at the outfall

Following the commissioning of the outfall, no significant differences were detected in the richness or cover for total fauna or sponges (Figs. 27a-d, Table 23). Significant differences in richness were detected in the Time (Period) x Sites (Location) interaction; however, this variation was primarily associated with differences among control sites (Table 23). Whilst the cover of the total fauna appeared to decrease after the discharge of sewage (see Fig. 28), by period 3 it had recovered to similar levels found in period 1. Although not statistically significant (Table 23), this reduction could represent a press disturbance to 30% of the original cover. Sponges were the major faunal contributors of both richness and cover in the assemblage, and their spatial and temporal patterns are therefore very similar to the total fauna (Figs. 27a-d). The location at Tomaree Head had the greatest richness and cover of
sponges (Figs. 27c-d) perhaps as a result of being closer to and under the influence of stronger tidal currents from the estuary at Port Stephens (Fig. 26).

*Taxa which decreased at the outfall*

The abundance of several species of sponge decreased significantly in response to the commissioning of the outfall (Tables 23 & 24). The number of individuals of the sponge *Cymbastela concentrica* (Lendenfeld) was significantly reduced by approximately one third (Fig. 27e, Table 23) after the outfall came on line. By period 3, they had been further reduced by another third (Fig. 27e, Table 23 & 25). A significant Time (Period) x OvsC interaction was also detected (Fig. 27e, Table 23) because of a reduced number of individuals recorded in the August 1994 sampling. The cover of *C. concentrica* also declined significantly (~50%) once the outfall was commissioned (Fig. 27f, Table 23 & 25) however no differences were detected between period 2 and period 3 using the SNK procedure (Table 25). Significant small-scale spatial and temporal variation occurred for both the number and cover of *C. concentrica* and 2-tailed F tests indicated that this variation was associated with the outfall (Table 23). In general, no significant fluctuations in the number or cover of this species occurred at either of the control locations (Figs. 27e-f, Table 23 & 25).

The sponges *Geodinella* sp. (Fig. 27g) and *Spongia* sp. (Fig. 27h) almost disappeared completely from the outfall location after the discharge of sewage began (Table 24). No such changes occurred in the cover or number of these species at the control locations (Figs. 27e-h, Table 25). Furthermore, the 2 tailed F-tests confirmed that the significant higher order interactions were due to greater variation associated with the outfall sites through time (Table 24).

At all locations the richness of algae was low, with most of the cover attributable to foliose and crustose corallines. Generally, the cover of algae was greater at the outfall and the Point
Stephens control compared to Tomaree Head (Fig. 27i). The total cover of algae fluctuated significantly at the outfall and control locations through time (Fig. 27i, Table 24). However, the mean cover of algae at the outfall location in period 2 and period 3 was significantly lower than the mean cover in period 1 (Table 24 & 25, refer to Fig. 28). No significant differences between periods were found in the cover of algae at the control locations (Table 25). Although the cover of algae fluctuated significantly within periods, the overall mean was generally reduced after the discharge of sewage (Fig. 28).

The cover of foliose algae at the outfall was almost reduced to zero by the end of the study (Fig. 27j). It was detected as a significant Period x OvsC interaction (Table 24), with period 1 having significantly greater cover than either period 2 or 3 (Table 25). The cover of foliose algae declined at all locations following the August 1993 sampling, i.e. prior to sewage discharge (Fig. 27j); however, the overall mean trajectories at the control locations did not show a general decrease and there were no significant differences between periods (Table 25).

The cover of crustose coralline algae was significantly reduced after the discharge of sewage at the outfall (Fig. 27k, Table 24). There were no significant differences between periods in the mean cover of crustose algae at the controls (Table 25). A significant Time (Period) x OvsC interaction occurred at the outfall because of an increase in the cover of crustose algae in July 1993, i.e. prior to sewage discharge (Fig. 27k, Table 24). A 2-tailed $F$ test found that a significant Time (Period) x Site (Location) interaction was associated with increased variability at the outfall location (Table 24).
Table 23. Summary of analyses of variance and 2-tailed F tests (details in text) for selected taxa at the outfall and the control locations (Period 1 versus Period 3) using a Beyond BACI sampling design (repartitioned sources of variation are indented and the associated degrees of freedom are in brackets).

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>F-ratio</th>
<th>m.s.</th>
<th>F</th>
<th>% Fauna</th>
<th>m.s.</th>
<th>F</th>
<th>% Sponges</th>
<th>m.s.</th>
<th>F</th>
<th>% Sponges</th>
<th>m.s.</th>
<th>F</th>
<th>% Cymbastela</th>
<th>m.s.</th>
<th>F</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Period</td>
<td>1</td>
<td>No Test</td>
<td>188.1</td>
<td>13.2</td>
<td>30</td>
<td>520.2</td>
<td>46.3</td>
<td>456.</td>
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<tr>
<td>Time (Period)</td>
<td>6</td>
<td>T(P) x OvsC</td>
<td>51.9</td>
<td>1090</td>
<td>14.1</td>
<td>507</td>
<td>1.5</td>
<td>4.8</td>
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<tr>
<td>Location</td>
<td>2</td>
<td>Not Used</td>
<td>350.4</td>
<td>2422</td>
<td>494.8</td>
<td>35146</td>
<td>180.4</td>
<td>1232</td>
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<tr>
<td>Outfall versus Controls</td>
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<td>No Test</td>
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<td>2414</td>
<td>130.8</td>
<td>15080</td>
<td>351.1</td>
<td>2301</td>
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<td></td>
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<tr>
<td>Between Controls</td>
<td>(1)</td>
<td>No Test</td>
<td>369.3</td>
<td>2429</td>
<td>858.7</td>
<td>55212</td>
<td>9.6</td>
<td>163.</td>
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<tr>
<td>Sites (Location)</td>
<td>6</td>
<td>T(P) x S(L)</td>
<td>7.4</td>
<td>187.1</td>
<td>78.4</td>
<td>311.5</td>
<td>0.8</td>
<td>4.1</td>
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<tr>
<td>Sites (Outfall)</td>
<td>(2)</td>
<td>T(P) x S(L)</td>
<td>0.6</td>
<td>53.4</td>
<td>232.1</td>
<td>93.3</td>
<td>2.2</td>
<td>3.2</td>
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<tr>
<td>Sites (Controls)</td>
<td>(4)</td>
<td>T(P) x S(L)</td>
<td>9.7</td>
<td>253.9</td>
<td>1.5</td>
<td>420.7</td>
<td>0.1</td>
<td>4.6</td>
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<td>Period x Location</td>
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<td>Not Used</td>
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<td>1377.</td>
<td>108.2</td>
<td>714.7</td>
<td>38.9</td>
<td>259.</td>
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<tr>
<td>Period x OvsC</td>
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<td>No Test</td>
<td>121.9</td>
<td>38</td>
<td>115.6</td>
<td>207</td>
<td>77.9</td>
<td>19.9**</td>
<td>503.</td>
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<tr>
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<td>115.1</td>
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<td>100.8</td>
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<tr>
<td>Period x Sites (Location)</td>
<td>6</td>
<td>T(P) x S(L)</td>
<td>6.0</td>
<td>172.6</td>
<td>3.6</td>
<td>95.7</td>
<td>3.6</td>
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<td>34.3</td>
<td>1.0ns</td>
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<tr>
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<td>506.9</td>
<td>4.5</td>
<td>155.6</td>
<td>10.8</td>
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<td>80.1</td>
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<td>5.4</td>
<td>3.1</td>
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<td>347.4</td>
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<td>277.8</td>
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<tr>
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<td>T(P) x</td>
<td>34.3</td>
<td>0.8ns</td>
<td>157.2</td>
<td>0.3ns</td>
<td>12.7</td>
<td>0.6ns</td>
<td>156.1</td>
<td>0.4ns</td>
<td>3.9</td>
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<tr>
<td>Time (Period) x Controls</td>
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<td>T(P) x S(L)</td>
<td>41.6</td>
<td>4.3**</td>
<td>537.6</td>
<td>2.7*</td>
<td>23.1</td>
<td>3.1*</td>
<td>399.5</td>
<td>2.5*</td>
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<tr>
<td>Time (Period) x Sites</td>
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<td>Residual</td>
<td>9.7</td>
<td>199.2</td>
<td>0.9ns</td>
<td>7.4</td>
<td>2.2**</td>
<td>159.2</td>
<td>0.9ns</td>
<td>4.5</td>
<td>2.6**</td>
<td>34.3</td>
<td>3.9**</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time (Period) x Outfall</td>
<td>(12)</td>
<td>Residual</td>
<td>8.9</td>
<td>199.2</td>
<td>0.9ns</td>
<td>5.6</td>
<td>1.7ns</td>
<td>103.4</td>
<td>0.6ns</td>
<td>12.9</td>
<td>7.4**</td>
<td>85.5</td>
<td>5.5**</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time (Period) x Sites</td>
<td>(24)</td>
<td>Residual</td>
<td>10.2</td>
<td>235.7</td>
<td>1.1ns</td>
<td>8.3</td>
<td>2.5**</td>
<td>187.1</td>
<td>1.0ns</td>
<td>0.3</td>
<td>0.2ns</td>
<td>8.7</td>
<td>0.6ns</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Residual</td>
<td>648</td>
<td></td>
<td>4.8</td>
<td>211.6</td>
<td>3.3</td>
<td>179.3</td>
<td>1.8</td>
<td>15.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

# = number of; % = percentage cover of; not significant ns (P > 0.05); significant * (P < 0.05); ** (P < 0.01);
F-ratios in bold were calculated after post hoc pooling; No Test – without pooling.
Table 24. Summary of analyses of variance and 2-tailed F tests (details in text) for selected taxa at the outfall and the control locations (Period 1 versus Period 3) using a Beyond BACI sampling design (repartitioned sources of variation are indented and the associated degrees of freedom are in brackets).

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>F-ratio</th>
<th>m.s.</th>
<th>% Geodinella</th>
<th>% Spongia</th>
<th>% Algae</th>
<th>% Foliose</th>
<th>% Crustose</th>
<th>% Silt matrix</th>
</tr>
</thead>
<tbody>
<tr>
<td>Period</td>
<td>1</td>
<td>27.4</td>
<td>16.5</td>
<td>8928.8</td>
<td>2353.5</td>
<td>1830.4</td>
<td>1746.9</td>
<td>304.3</td>
<td>11014</td>
</tr>
<tr>
<td>Time (Period)</td>
<td>6</td>
<td>T(P) x</td>
<td>6.9</td>
<td>0.5</td>
<td>1.</td>
<td>2147.6</td>
<td>5213</td>
<td>2441.6</td>
<td></td>
</tr>
<tr>
<td>Location</td>
<td>2</td>
<td>Not Used</td>
<td>34.9</td>
<td>15.1</td>
<td>17692.</td>
<td>2095.6</td>
<td>7746.9</td>
<td>10956</td>
<td></td>
</tr>
<tr>
<td>Outfall versus Controls (1)</td>
<td>No Test</td>
<td>61</td>
<td>30.2</td>
<td>11088.</td>
<td>235.2</td>
<td>2679.1</td>
<td>19163</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between Controls (1)</td>
<td>No Test</td>
<td>8.7</td>
<td>0.00</td>
<td>24296.</td>
<td>3956</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sites (Location)</td>
<td>6</td>
<td>T(P) x S(L)</td>
<td>2.8</td>
<td>0.8</td>
<td>124.5</td>
<td>100.6</td>
<td>17.2</td>
<td>236.2</td>
<td></td>
</tr>
<tr>
<td>Sites (Outfall)</td>
<td>(2)</td>
<td>T(P) x S(L)</td>
<td>7.4</td>
<td>2.3</td>
<td>297.5</td>
<td>210.4</td>
<td>6.8</td>
<td>231.2</td>
<td></td>
</tr>
<tr>
<td>Sites (Controls)</td>
<td>(4)</td>
<td>T(P) x S(L)</td>
<td>0.6</td>
<td>0.05</td>
<td>37.9</td>
<td>45.8</td>
<td>22.3</td>
<td>238.7</td>
<td></td>
</tr>
<tr>
<td>Period x Location</td>
<td>2</td>
<td>Not Used</td>
<td>25.5</td>
<td>16.4</td>
<td>6479.1</td>
<td>3224.1</td>
<td>676.4</td>
<td>12342</td>
<td></td>
</tr>
<tr>
<td>Period x OvsC (1)</td>
<td>No Test</td>
<td>50.8</td>
<td>32.6</td>
<td>11805</td>
<td>16.8*</td>
<td>6379.8</td>
<td>1153.8</td>
<td>24660</td>
<td>76.1**</td>
</tr>
<tr>
<td>Period x Controls (1)</td>
<td>No Test</td>
<td>0.2</td>
<td>0.04ns</td>
<td>0.2</td>
<td>1153.2</td>
<td>68.3</td>
<td>0.1ns</td>
<td>198.9</td>
<td>23.9 0.02ns</td>
</tr>
<tr>
<td>Period x Sites (Location)</td>
<td>6</td>
<td>T(P) x S(L)</td>
<td>5.7</td>
<td>0.9ns</td>
<td>0.6</td>
<td>163.3</td>
<td>1.1ns</td>
<td>102.2</td>
<td>90.2 1.7ns</td>
</tr>
<tr>
<td>Period x Sites (Outfall) (2)</td>
<td>T(P) x S(L)</td>
<td>9.4</td>
<td>1.6ns</td>
<td>184.1</td>
<td>1.3ns</td>
<td>235.1</td>
<td>1.2ns</td>
<td>231.2</td>
<td>4.2ns 1524.9</td>
</tr>
<tr>
<td>Period x Sites (Controls) (4)</td>
<td>T(P) x S(L)</td>
<td>3.9</td>
<td>0.7ns</td>
<td>0.1</td>
<td>152.9</td>
<td>1.0ns</td>
<td>35.8</td>
<td>19.7</td>
<td>474.7 1.8ns</td>
</tr>
<tr>
<td>Time (Period) x Location</td>
<td>12</td>
<td>No Test</td>
<td>3.5</td>
<td>0.5</td>
<td>714.6</td>
<td>584.8</td>
<td></td>
<td>164.5</td>
<td>651.8</td>
</tr>
<tr>
<td>Time (Period) x OvsC</td>
<td>(6)</td>
<td>T(P) x</td>
<td>2.5</td>
<td>0.5ns</td>
<td>0.9</td>
<td>13.9**</td>
<td>703.5</td>
<td>0.9ns</td>
<td>285.5 4.5*</td>
</tr>
<tr>
<td>Time (Period) x Controls (6)</td>
<td>T(P) x S(L)</td>
<td>4.6</td>
<td>0.8ns</td>
<td>0.1</td>
<td>725.8</td>
<td>4.9**</td>
<td>600.6</td>
<td>3.2*</td>
<td>63.6 1.2ns</td>
</tr>
<tr>
<td>Time (Period) x Sites</td>
<td>36</td>
<td>Residual</td>
<td>5.8</td>
<td>1.1ns</td>
<td>2.6</td>
<td>2.2**</td>
<td>147.8</td>
<td>1.9**</td>
<td>190.8 5.5**</td>
</tr>
<tr>
<td>Time (Period) x Sites (Outfall) (12)</td>
<td>Residual</td>
<td>10.8</td>
<td>2.0*</td>
<td>7.8</td>
<td>6.5**</td>
<td>192.2</td>
<td>2.6**</td>
<td>360.9</td>
<td>10.4* 3.5**</td>
</tr>
<tr>
<td>Time (Period) x Sites</td>
<td>(24)</td>
<td>Residual</td>
<td>3.4</td>
<td>0.6ns</td>
<td>0.1</td>
<td>125.5</td>
<td>1.7*</td>
<td>105.8</td>
<td>3.1** 0.4ns</td>
</tr>
<tr>
<td>Residual</td>
<td>648</td>
<td></td>
<td>5.4</td>
<td>1.2</td>
<td>74.9</td>
<td>34.7</td>
<td></td>
<td>37.8</td>
<td>239.5</td>
</tr>
</tbody>
</table>

Two-Tailed F-test

# = number of; % = percentage cover of; not significant ns (P > 0.05); significant * (P < 0.05); ** (P < 0.01);
F-ratios in bold were calculated after post hoc pooling; No Test - without pooling.
**Taxa which increased at the outfall**

The percentage cover of the silt matrix was found to increase significantly at the outfall location following the discharge of sewage (Fig. 27i & 28, Table 24). This matrix consisted of a non-specific mixture of micro flora and fauna, silt and microorganisms and has been described previously (see Chapter 2). The silt matrix increased rapidly and dramatically from an average cover of around 35% at the outfall and control locations, to around 55% at the outfall location following its commissioning (Fig. 27i, Table 25). A significant Time (Period) x Controls interaction (Table 24) was due to a reduction in silt matrix at the Point Stephens control location (Fig. 27i) in April (period 2) and August 1994 (period 3). The amount of bare space at the outfall location appeared to be covered by the silt matrix during periods 2 & 3 (Fig 28).

**Table 25. Summary of SNK tests on the means (number of individuals (#) and percentage covers of taxa) of each of the three periods (P1 - before sewage discharge, P2 – immediately after, P3 – approximately 1 year after) at each of the three locations (Boulder Bay - outfall, Point Stephens and Tomaree Head – controls).**

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Boulder Bay</th>
<th>Point Stephens</th>
<th>Tomaree Head</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Cymbastela concentrica</em> (#)</td>
<td>P1 &gt; P2 &gt; P3</td>
<td>P1 = P2 = P3</td>
<td>P1 = P2 = P3</td>
</tr>
<tr>
<td><em>Cymbastela concentrica</em></td>
<td>P1 &gt; P2 = P3</td>
<td>P1 = P2 = P3</td>
<td>P1 = P2 = P3</td>
</tr>
<tr>
<td><em>Geodinella</em> sp.</td>
<td>P1 &gt; P2 = P3</td>
<td>P1 = P2 = P3</td>
<td>P1 = P2 = P3</td>
</tr>
<tr>
<td><em>Spongia</em> sp.</td>
<td>P1 &gt; P2 = P3</td>
<td>P1 = P2 = P3</td>
<td>P1 = P2 = P3</td>
</tr>
<tr>
<td>Total Algae</td>
<td>P1 &gt; P2 = P3</td>
<td>P1 = P2 = P3</td>
<td>P1 = P2 = P3</td>
</tr>
<tr>
<td>Foliose Algae</td>
<td>P1 &gt; P2 = P3</td>
<td>P1 = P2 = P3</td>
<td>P1 = P2 = P3</td>
</tr>
<tr>
<td>Crustose Algae</td>
<td>P1 &gt; P2 = P3</td>
<td>P1 = P2 = P3</td>
<td>P1 = P2 = P3</td>
</tr>
<tr>
<td>Silt Matrix</td>
<td>P1 &lt; P2 = P3</td>
<td>P1 = P2 = P3</td>
<td>P1 = P2 = P3</td>
</tr>
</tbody>
</table>

= Means not significantly different at $P = 0.05$. 


Figure 27. Mean number of species and % cover (± SE) for taxa analysed from Boulder Bay ●; Point Stephens ○; and Tomaree Head Δ; (n = 30).
Figure 28. Mean cover estimates of the 4 major components of the subtidal encrusting assemblage (total fauna and algae, silt matrix and bare space) at (A) the outfall and (B) the average of the controls (Period 1 = before sewage discharge, Periods 2 & 3 = after sewage discharge).
Changes in Community Structure

The multivariate analyses clearly demonstrated that the assemblages at the three locations were significantly different from each other during the first sampling period and that rapid changes occurred to the assemblage at the outfall following the discharge of sewage. The analysis of similarities (ANOSIM) detected significant differences among all three locations and all periods at the outfall (Table 26). In contrast, no significant differences were found between periods at the Point Stephens location, but period 1 at Tomaree Head was significantly different from period 3 (Table 26).

The SIMPER procedure ranked, in order of importance, those species that contributed most to the similarities within a location for a given time period. Although only the species ranked in the “top ten” are included in Table 27, it is clear that species of importance were usually quite different among locations but were, in general, more consistent among time periods within a location. In the first period at the outfall location, six sponges featured within the top ranked species, however, they did not rank at all in periods 2 or 3 (Table 27). This indicated that their relative abundance at the outfall location had decreased or became less important in terms of representing community structure.

Changes were also apparent in the relative importance of ascidians at the outfall location during periods 2 and 3. During period 1 at the outfall, no ascidians were ranked within the top 10 (Table 27). However, by periods 2 and 3 one colonial, Didemnum sp., and two solitary ascidians, Pyura spinifera (Quoy and Gaimard) and Cnemidocarpa pedata (Herdman), had increased their relative importance as contributors to the similarity at the outfall (Table 27). It should be noted that this increase in the importance of ascidians is due to a decrease in the importance of sponges.
The dramatic and rapid changes to the structure of the benthic assemblages at the outfall location are clearly portrayed in the nMDS ordination (Fig. 29). The 2-dimensional plot reveals clear separation among all locations, yet considerable overlap is apparent for points from all time periods at the control locations. In contrast, at the outfall location the data points representing pre-commissioning (period 1) are clearly separated from the post-commissioning points (period 2 & 3). There is also a degree of separation between the second and third periods (Fig. 29).

Stress for this ordination was considered to be good (Stress = 0.11) with no real risk of drawing false inferences (Clarke, 1993). An example of these changes can be observed in photo-quadrats taken at the outfall location before and after it was commissioned (Fig. 30).

### Table 26. Summary of two-way analysis of similarities (ANOSIM) comparing differences in the structure of the assemblages at each location and period. The first period (P1) was in the pre-commissioning phase whilst P2 and P3 were post-commissioning periods.

<table>
<thead>
<tr>
<th>Location</th>
<th>BB</th>
<th>R</th>
<th>PS</th>
<th>R</th>
<th>TH</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>BB vs PS</td>
<td>0.93*</td>
<td>P1 vs P2</td>
<td>0.53*</td>
<td>P1 vs P2</td>
<td>0.19ns</td>
<td>P1 vs P2</td>
</tr>
<tr>
<td>PS vs TH</td>
<td>1.0*</td>
<td>P2 vs P3</td>
<td>0.64*</td>
<td>P2 vs P3</td>
<td>0.24ns</td>
<td>P2 vs P3</td>
</tr>
<tr>
<td>TH vs BB</td>
<td>1.0*</td>
<td>P3 vs P1</td>
<td>0.71*</td>
<td>P3 vs P1</td>
<td>0.54ns</td>
<td>P3 vs P1</td>
</tr>
</tbody>
</table>

BB - Boulder Bay; PS - Point Stephens; TH - Tomaree Head; P - Period; ns - not significant \((P > 0.05)\); * - significant \((P < 0.05)\).
Table 27. Species ranked in order of importance (1-10 presented only), which contributed to the similarities within a location/period as determined using SIMPER.

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Species</th>
<th>B1</th>
<th>B2</th>
<th>B3</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Porifera</td>
<td>Cymbastela concentrica</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Spirastrella sp.</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ceratopsis aurantiaca</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Xytopsene sp.</td>
<td>6</td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Spongia sp.</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cliona (Raphyrus) hixonii</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Tedania sp.</td>
<td>4</td>
<td>3</td>
<td>9</td>
<td>3</td>
<td>6</td>
<td>8</td>
<td>8</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ircinia sp.</td>
<td>10</td>
<td>3</td>
<td>10</td>
<td>9</td>
<td>6</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Tedania digitata</td>
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<td>7</td>
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<td>8</td>
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<tr>
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<td>Cribralina sp. 1</td>
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<td></td>
<td>3</td>
<td>4</td>
<td>7</td>
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<tr>
<td></td>
<td>Stylinos sp.</td>
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<td>8</td>
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<tr>
<td></td>
<td>Thorecta sp.</td>
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<tr>
<td></td>
<td>Acarnus sp.</td>
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<td>2</td>
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<tr>
<td></td>
<td>Phoriospongia sp.</td>
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<td>5</td>
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<td></td>
<td>Clathria (Dendroica) pyramidia</td>
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<tr>
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<td>Calyspongia sp.</td>
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<td>8</td>
<td>4</td>
<td>4</td>
<td>9</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Mycale (Arenochalina) flammula</td>
<td></td>
<td>2</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Mycale (Aegogropila) sp.</td>
<td></td>
<td></td>
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<td>7</td>
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<tr>
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<td>Hymedesmia sp.</td>
<td>5</td>
<td>7</td>
<td>4</td>
<td>9</td>
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<tr>
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<td>Phoriospongia sp. 2</td>
<td>5</td>
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<tr>
<td></td>
<td>Geodinella sp.</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Antho (Isopenectya) chartacea</td>
<td></td>
<td>5</td>
<td>7</td>
<td>9</td>
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B = Boulder Bay, P = Point Stephens, T = Tomaree Head, 1 = Period 1, 2 = Period 2, 3 = Period 3.
Figure 29. nMDS plots for the abundance of species at each location and time (data points are averages for the 3 sites at each time - Boulder Bay - outfall: Period 1 •; Period 2 •; Period 3 •, Point Stephens - control: Period 1 〇, Period 2 〇; Period 3 〇, Tomaree Head - control: Period 1 △; Period 2 △; Period 3 △).
Figure 30. Example of photo-quadrats taken (top) immediately before the discharge of sewage and (bottom) 3 months after, at the Boulder Bay outfall location.
Discussion

Here, rapid changes to sessile macrobenthic assemblages were found following the commissioning of an ocean outfall at Boulder Bay, New South Wales, Australia. Within three months of the release of sewage effluent into the environment, the effects on the community were far reaching, with statistically significant reductions in the abundance of encrusting invertebrates and algae. A number of species of sponge almost disappeared completely from the outfall location. Moreover, the SIMPER analysis indicated that the community in the vicinity of the outfall changed from one dominated by sponges to one in which ascidians became an important component of the fauna.

Drastic reductions in species richness and increases in the abundance of a small number of tolerant species have been recorded previously in the vicinity of ocean outfalls (May, 1985; Lopez-Gappa et al., 1990; Fairweather, 1990). Few studies have had the opportunity to compare pre and post-impact data and hence most are likely to have missed many of the subtle short-term changes reported here. The study also benefited from a combination of multivariate and univariate analyses to examine changes in the structure of the encrusting assemblages. The effects of ocean outfalls on biological assemblages have been described using multivariate (Clarke, 1993; Smith and Simpson, 1993) and univariate techniques (Otway et al., 1996a), however, the use of a combination of these techniques is rare (however see Chapman et al., 1995; Otway et al., 1996b). Underwood and Peterson (1988) and Warwick (1993) emphasise the need to use all available techniques of analysis, as different approaches examine different components of the assemblage and are therefore more likely to detect effects.
The importance of appropriate temporal scales to measure variation in these dynamic communities cannot be overlooked and may be more important than examining smaller scale spatial patterns. In this study many higher-order, small-scale temporal and spatial interactions occurred. These interactions warn us that main effects and lower order interactions may be unreliable and are not independent (Underwood, 1981). Small-scale within-site variation was found for most taxa in this study, and understanding the mechanisms leading to this variability would greatly assist in identifying the effects of anthropogenic disturbance. The contrasts that were made here between small-scale spatial and temporal variability at the outfall and the controls, demonstrated that the outfall did affect within-site variability in the cover and number of a range of taxa. This is important because it indicates that sewage discharged into these habitats can affect them on very small spatial scales. Warwick and Clarke (1993a) examined various assemblages and concluded that increased variability among samples may be a general feature exhibited by assemblages under stress.

In reporting changes in pattern associated with the commissioning of an ocean outfall, it is instructive to consider the potential mechanisms that may be operating. A discharge of primary or secondary treated sewage has the potential to increase nutrients and suspended solids (silt) and decrease the salinity in the vicinity of the discharge. There is evidence that ocean outfalls increase the level of suspended solids in the water column at or near the point of the discharge (Coade, 1995) and may reduce the amount of photosynthetic active radiation at the bottom (Kirk, 1983).

The direct effects of sedimentation on the structure and dynamics of encrusting communities have rarely been reported, but it is clear that some species of sponge are sensitive to burial beneath sediment (Wulff, 1997). Moreover, reductions in the pumping rate of sponges may occur when the concentration of sediment increases (Gerodette and Flechsig, 1979). There is
also correlative evidence that the structure of encrusting communities change along sedimentation gradients (Carballo et al., 1996; Naranjo et al., 1996). Field and flume studies of the morphology of some invertebrates indicate sensitivity to high rates of sedimentation (Riegl et al., 1996). Some members of the benthic assemblage examined share this morphology, e.g., *Cymbastela concentrica*, and may be vulnerable to increased sedimentation.

Sewage plumes often act to shade the bottom, and this reduction in light may limit the growth of benthic algae (Vadas and Steneck, 1988). These effects may not be restricted to algae however. Many invertebrates, including *Cymbastela concentrica*, play host to symbiotic micro-algae, which can account for a major part of their nutritional requirements (Wilkinson, 1983; Cheshire et al., 1995). Increased concentrations of nutrients within sewage can alter the growth rates of macro-algae (Borowitzka, 1972), whilst low salinity can decrease the diversity and abundance of sponge-dominated assemblages (Storr, 1976). Clearly, the processes producing change in the assemblages and at the species level at the outfall are likely to be complex and require detailed experimental examination.

The data imply that the paradigm that encrusting assemblages from temperate reefs are relatively stable through time needs to be re-examined. It is acknowledged that episodic storm events can produce rapid change in these communities, particularly in shallow water (e.g. Kennelly, 1987a). In deeper or more sheltered habitats benthic assemblages have been considered to be relatively stable, although they may be highly dynamic at a small scale (Ayling, 1983b; Kay and Butler, 1983). More recently, Chapman et al. (1995) concluded that the decommissioning of a large shoreline sewage outfall produced no significant difference in an encrusting assemblage when compared to two control locations. They noted that their study began three months after the flow of sewage ceased but believed that their interpretations were valid because of the slow changes experienced by these types of
assemblages. In contrast, I described significant temporal fluctuations within 4 months (see Chapter 3) in shallow temperate reef sponge assemblages in both shallow and deep water off the East Coast of Australia. Davis et al. (1997) reported that sponge-dominated assemblages from similar habitat types at the mouth of an embayment responded quickly to biological disturbance.

This study emphasises a persistent problem with assessing impacts on communities, and that is, the selection of appropriate reference or control sites. The structure of the assemblages at the three locations investigated in this study differed markedly from the outset. As Underwood (1992) notes, the control locations do not have to be identical but an important requirement is that they come from a population of apparently similar locations. In this study the locations were spatially independent, so that there was no spatial autocorrelation among locations confounding the ability to identify impacts of the outfall.

Here, what might be termed a significant "press perturbation" was correlated with the commissioning of an outfall (see Glasby and Underwood, 1996 for a review of this topic). The changes at the outfall can be attributed to the release of sewage, as no such changes were observed at the control locations. In addition, the rapid increase in the cover of the silt matrix suggests that the outfall is the causative agent. However, in the absence of direct experimental evidence, namely, changes in the survivorship of sensitive species following their reciprocal transplantation to an outfall location and replicated reference locations, it is not possible to infer causality.
SPONGE-DOMINATED ASSEMBLAGES IN DEEP-WATER

Introduction

Ocean outfalls generally discharge treated sewage effluent into shallow subtidal rocky reef habitats (Fairweather, 1990). Such outfalls can have significant effects on the patterns of distribution of macrobenthic assemblages (Littler and Murray, 1975; 1978); on the long-term growth and primary productivity of marine intertidal macrophytes (Kindig and Littler, 1980); on the diversity and abundance of algae and invertebrates (Lopez Gappa et al., 1990) and on species diversity of sponges (Muricy, 1991). In Australia, there are few published descriptions of the effects of sewage on macrobenthic assemblages living on hard substrata (Smith, 1994; Chapman et al., 1995). For intertidal communities, the only published accounts from New South Wales (NSW) record an absence of ‘typical’ zonation patterns and a reduction in species diversity close to large shoreline sewage outfalls (Borowitzka, 1972; May, 1981; 1985; Fairweather, 1990). The effects of sewage effluent on shallow subtidal macrobenthic assemblages in NSW have focused on the holdfast macrofauna of kelp (Smith and Simpson, 1992; 1993; Smith, 1994) where natural variability in this macrofauna was far greater than any potential variability caused by a tertiary-treated outfall. Shallow subtidal assemblages inhabiting vertical cliff-faces in the vicinity of a newly de-commissioned nearshore ocean outfall were found to have significant differences in the mean abundances and variances of some taxa when compared with control locations (Chapman et al., 1995). Although there have been some studies (see Jones, 1977), no published accounts exist concerning the effects of sewage effluent on marine macrobenthos living on hard substrata in deeper waters off the NSW coast.

In December 1990, the deep-water ocean outfall at North Head, NSW, commenced discharging sewage effluent from a line of diffuser heads at a depth of 60 m. Near the diffuser line, the sea bottom is predominantly rock with gullies and channels where sediments can accumulate. The
rocky reef habitats support a diverse range of organisms that include fish, coralline algae, ascidians and invertebrates.

Three years of sampling at the North Head deep-water outfall and at two control locations were recorded. This represents the first published assessment of the effects of sewage on these macrobenthic assemblages and the first quantitative spatial and temporal assessment of subtidal assemblages on deeper coastal reefs in temperate Australia.

**Methods**

To study the effects of sewage from the North Head outfall, assemblages were sampled in areas exposed to effluent (North Head) and in controls (or reference areas) that were not exposed (Long Reef and Bungan Head) (Fig. 31). The outfall location was situated around the diffusers and the assemblages were sampled on nine occasions over a three-year period from March 1991 to April 1994 using a jump camera (Roberts and Henry, 1992; Roberts *et al.*, 1994). Within-location variability in these assemblages was determined by photographing ten replicate quadrats (photo-quadrats - area 0.45m$^2$) at each of three sites nested within each location. At each location the three sites were randomly selected on each occasion. The jump camera supported a 35 mm Sea & Sea Motor Marine-2 underwater camera and flash, which were triggered when the camera frame settled on the bottom. At each site, 36 random photo-quadrats (Fig. 32) were taken of which 10 were randomly selected for analysis.

The photo-quadrats were analysed using a Bell and Howell ‘black box’ projector. An overlay plastic grid of 100 regularly-spaced points was placed on the screen to estimate the diversity (number of species) and abundance (percentage covers) of each taxon and the abundance of individual species.
Since there was only one outfall location, asymmetrical analyses of variance were used to examine the differences between the outfall and the two control locations (Underwood, 1992; 1993). Asymmetrical, before/after-control/impact (BACI) designs and their potential for detecting disturbances have been fully discussed by Underwood (1991a; 1992; 1993). However, since there were no pre-commissioning data for this study an after-control/impact (ACI) design was used. Whilst this design has considerably less power to detect an impact, it could be argued that any changes that were found in the assemblages at the outfall location through time (compared with those at the control locations) could be ascribed to the outfall. A potential impact would be detected as an interaction between the outfall versus control locations through time that was different from the among controls through time, i.e. the time courses for the outfall location would be different from the time courses for the average of the controls (Underwood, 1993).

These asymmetrical analyses effectively repartition a fully orthogonal model allowing a priori comparisons between the outfall and the average of the controls (Winer, 1971; Underwood, 1992). Firstly, the complete data set associated with both the outfall and the controls was analysed using a fully orthogonal design, where sites were nested within locations and time was treated as a random factor. A second analysis was then done using only those data associated with the controls at Long Reef and Bungan Head. The asymmetrical component or outfall versus control partition was calculated by subtracting the sums of squares of the second analysis from the first analysis. What remained was the variance associated with the potentially impacted location at North Head. The various factors of the analysis were calculated using this same logic as were the partitioned interactions associated with the various main effects (for an example of these calculations see Underwood, 1993). All mean square estimates and $F$ ratios were calculated using the appropriate methods for a nested design (Winer, 1971).
Significant time x site (location) interactions meant that higher order interactions and main effects should not be interpreted (Underwood, 1981). However, where significant differences were found in the time x site (location) interaction, the time x location interaction was reported to examine the trend above the level of sites (Underwood et al., 1993). Furthermore, where these significant time x site (location) interactions occurred, a 2-tailed F-test was used to compare the temporal variability among sites within control locations with the temporal variability among sites at the outfall (Winer 1971). This allowed a direct comparison of the differences in the variability at these smaller scales and the potential effects of the outfall. Post hoc pooling procedures were used when the time x site (location) or time x location interactions were found to be non-significant at $P = 0.25$, which allowed appropriate F-tests to be calculated for the main effects (Winer, 1971).

Prior to analysis, the data were examined using Cochran's test for homogeneity of variances (Winer, 1971; Underwood, 1981) and where variances were heterogeneous, data were transformed to log $(x + 0.5)$ for number of species and transformed to arcsine for percentage cover (Winer, 1971). If variances could not be stabilised at $P = 0.05$ but could be stabilised at $P = 0.01$, the analysis of variance was done using the $P = 0.01$ probability level (Underwood, 1981). Where significant differences were found in the analysis of variance or where variances could not be stabilised, Student-Newman-Keuls (SNK) multiple comparisons were done at the appropriate alpha level to determine differences among means (Winer, 1971).
Figure 31. Locations of the deep-water reefs sampled off Sydney.
In order to improve the discrimination of the various taxa recorded in quantitative photo-quadrats, qualitative *in situ* sampling of the major taxonomic components was done at the deep-water outfall and control locations. A modified Remote Operated Vehicle (ROV) was used to collect specimens of sponges (Fig. 33) and other taxa at depths ranging from 40-60 m. Before each specimen was collected, a close-up 35 mm colour photograph was taken by the ROV (Fig. 34). The specimen was then collected by a manipulator arm on the ROV and placed in a collection bag attached to the vessel's anchoring system. Continuous colour video footage was also taken during this process and detailed descriptions of colour, habit, depth and location were recorded for each specimen. Once the samples were brought to the surface, a number of colour photographs of each specimen were taken and the samples were labelled and immediately frozen for later identification. The voucher collection was sent to Queensland Museum for lodgement and identification.
Figure 33. The ROV used to collect sponges and other taxa from the deep-water reefs off Sydney.

Figure 34. Sponges were photographed *in situ* prior to being removed from the reef using the manipulator arm on the ROV.
Results

Four major faunal taxa were identified on the deep-water reefs: Poriferans, Cnidarians, Bryozoans and Ascidians. Other taxa that were less common included Echinoderms, Molluscs and Annelids. The dominant algae were encrusting Corallinacea species from the Order Cryptonemiales. A non-specific mixture of algae, sediment and microorganisms was also found and this is referred to as the 'silt matrix'.

There were no significant differences in the total number of species between the outfall and control locations for the first five sample periods (i.e. March 1991 - August 1992) but in December 1992, a significant decrease in the total number of species occurred at the outfall location and has remained through all subsequent sample periods (Table 28; Fig. 35a). Significant time x site (Outfall versus Control) interactions occurred in the number of species, and variability among sites at the outfall was greater compared with the controls (Table 28). Significant time x site (Outfall versus Control) interactions also occurred in the total abundance of all fauna (Table 28; Fig. 35b), however variation among outfall sites and control sites through time were not different using a 2-tailed $F$ test (Table 28).

Sponges were the dominant fauna found at all locations; they represented the greatest number of species and were the most abundant taxon. The sponges included both encrusting and erect forms and over 50 species belonging to 10 orders and 22 families of Demospongia were identified. Families with the most species were the Axinellidae and the Ircinidae, which belong to the orders Halichondrida and Dictyoceratida respectively. Species that were relatively common included *Cymbastela concentrica* Lendenfeld, *Jaspis* sp., *Tethya* sp., *Mycale (arenochalina) flammula* Lamarck, *Holopsamma arborea* Lendenfeld, *Axinella* sp. and *Ceratopsis aurantiaca* Lendenfeld. The abundances of the sponges *C. concentrica* (Fig. 35c), *Tethya* sp. (Fig. 35d) and *Jaspis* sp. (Fig. 35e) fluctuated between locations. The abundance of
the sponge *M. (arenochalina) flammula* (Fig. 35f) fluctuated through time and among control locations (Table 28). The total abundance of sponges fluctuated through time and among sites (locations) but no differences were found in the variances at the outfall and control sites through time (Table 28; Fig. 35g). Fluctuations in the mean abundances of sponges at all locations appeared to be relatively constant and generally followed the patterns that were found in the total faunal abundance (see Fig. 35b). A significant time x Among Controls interaction occurred in the total number of sponges with fluctuations between locations depending on which time they were examined (Table 28; Fig. 35h).

Bryozoans were found at all locations and included *Triphyllozoon* sp., *Iodictyum phoeniceum* Busk and *Membranipora* sp. A significant time x Outfall versus Control interaction was detected for the abundances of *I. phoeniceum* (Table 28; Fig. 35i) and *Triphyllozoon* sp. (Table 28; Fig. 35j). There was no difference in the abundance of *I. phoeniceum* between outfall and control locations (Fig. 35i) for the first four sample periods (i.e. March 1991 - April 1992) but by August 1992, *I. phoeniceum* had disappeared from the outfall location and since that time has not returned, whereas, the abundance of *Triphyllozoon* sp. fluctuated depending on which time it was examined (Fig. 35j).

Total bryozoan abundances fluctuated through time with no trends found in these patterns (Table 28; Fig. 35k), whereas significant time x location and time x site (Outfall versus Control) interactions were detected for the total number of bryozoans (Table 28; Fig. 35l). Generally, the number of bryozoans at each location were not different from March 1991 to August 1992. In December 1992, significant differences occurred between the outfall and the two control locations, but by April 1993 all locations were different. In August 1993, the number of bryozoans at the Bungan Head location was greater than at the other locations. In April 1994, there were significantly greater numbers of bryozoans at the Long Reef location compared with
the outfall and Bungan Head locations (Fig. 35f). No differences were found in the time x site (location) interaction using the 2-tailed \( F \) test procedure (Table 28).

Cnidarians were found at all locations and commonly included *Balanophyllia* sp., *Mopsea australis* Thompson and Mackinnon and *Primnoella australasiae* Gray. Abundances of *M. australis* and total cnidarians fluctuated among locations at various times with no apparent temporal trends (Table 28; Figs 35m and 35n). A significant time x Outfall versus Control interaction occurred in the number of cnidarians where, after December 1992, reduced numbers were found at the outfall compared with the controls (Table 28; Fig. 35o). A significant time x Among Controls interaction occurred in the abundance of the cnidarian *P. australasiae* and the 2-tailed \( F \) test indicated that variation among the control sites was significantly greater through time compared with the outfall sites (Table 28; Fig. 35p).

Ascidians were found at all locations and included *Pyura spinifera* Quoy and Gaimard, *Botrylloides magnicoecum* Hartmeyer, *Didemnum* c.f. *moseleyi* Herdman and *Polycitor giganteus* Herdman. Significant differences for time x Among Controls were found for abundances of *D. moseleyi* indicating that variation in the controls was different than at the outfall (Table 28; Fig. 35q). A significant time x site (Outfall versus Control) interaction occurred for the ascidian *P. giganteus* and 2-Tailed \( F \) tests indicated that increased variation occurred among sites at the outfall (Table 28; Fig. 35r). The interaction between time x Among Controls was significant for the abundance and number of ascidians (Table 28; Figs 35s and 35r). The abundance of ascidians was greater at the North Head location in March and August 1991, whereas, in April 1994 abundances were greater at the Long Reef location. The number of ascidians at North Head were greatest in March 1991, however no differences occurred between locations until April 1994, at which time all three locations differed.
Crustose coralline algae were present in all locations and represent a number of species from the order Cryptonemiales, but, because taxonomic differentiation could not be done from photoquadrats, data were combined to give total coralline algae abundance (Roberts et al., 1994).

Significant differences occurred in the time x Among Controls interaction (Table 28; Fig. 35\textit{a}). Generally, the outfall location had lower abundances of algae in December of each year with an increase in August of each year. These same increases and decreases occurred at the controls, but the pattern was not as distinct as that which occurred at the outfall location (Fig. 35\textit{u}).

The silt matrix component found at all locations has been described in Roberts and Henry (1992) and Roberts et al. (1994) and for shallow water locations around Sydney in Kennelly (1987b).

The time x Outfall versus Control and Among Controls interactions were significant because the abundance of the silt matrix was similar at all locations from March 1991 until April 1992, after which the three locations differed (Table 28; Fig. 35\textit{v}). Silt matrix generally increased at all locations from August 1992 until April 1993. This general trend reversed in August 93 when abundances decreased at the North Head location to the same level as the Long Reef location. The silt matrix at Bungan Head also decreased and was lower compared with the other two locations. At the last sample period in April 1994 the silt matrix had again increased at the outfall and Bungan Head locations, but had reduced its abundance significantly at the Long Reef location (Fig. 35\textit{v}). No differences were found in the time x site (location) interaction using the 2-tailed $F$ test procedure (Table 28).
Figure 35. Mean number of species and percentage cover (± SE) for the major taxa and species of macrobenthos analysed from the deep-water reefs (North Head •, Long Reef □, Bungan Head △; Mar 91 - 1, Aug. 91 - 2, Dec. 91 - 3, Apr. 92 - 4, Aug. 92 - 5, Dec. 92 - 6, Apr. 93 - 7, Aug. 93 - 8, Apr. 94 - 9).
Figure 35. Continued.
Table 28. Summaries of analyses comparing spatial and temporal variations in macrobenthos at North Head, Long Reef and Bungan Head, NSW (OvC = outfall versus controls; AmC = among controls; nil = no transformation; ns = not significant (P > 0.05); ns+ = not significant (P > 0.01); *= significant (P < 0.05); ** = significant (P < 0.01); F ratio's in bold have been calculated after pooling).

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Transform

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Table 28. Continued
Discussion

The assemblages associated with the deep-water ocean outfall at North Head have undergone some changes since its commissioning in December 1990. The number of species at the outfall location had decreased by December 1992 and has remained significantly lower than control locations in all subsequent sampling periods. This reduction in the mean number of species around the outfall may be indicative of a press disturbance (Bender et al., 1984) within the assemblage, with less tolerant species declining and being replaced over time by opportunistic or more tolerant species. Long-term press disturbances are considered to be difficult to detect by monitoring programs unless the disturbances are of considerable magnitude (Underwood, 1992). If the assemblages at North Head were experiencing some type of long term temporal reduction in species richness, no information has been gathered which determines what may be happening at smaller temporal scales. Underwood (1992) recommends that, whilst nested hierarchical spatial sampling should be done to determine effects of disturbances like sewage outfalls, these should incorporate different time scales so that both press and pulse episodes of disturbance can be identified.

Significant time x site (locations) interactions occurred for many of the taxa analysed and illustrate natural patterns of distribution and abundance within a location at small spatial scales and at different times. Whilst interactions at the level of sites within a location are ecologically important and show how assemblages experience patchiness in their distributions through time, they contribute little to the general considerations of diversity and abundance when comparing at the larger scale of outfall and control locations. However, if the variations in these interactions are examined it is possible to determine whether the small-scale patterns among locations have been changed by the outfall. Doing this also helps to identify the sources of potential variation that could influence the observed larger scale patterns and so provide confidence when generalising about the distribution and abundance for these assemblages (Andrew and Mapstone, 1987).
The taxonomic status of subtidal marine macrobenthos from deeper temperate coastal reefs in Australia is poor and this precluded placing species names on many of the taxa. In a study that compared *in situ* SCUBA diver-collected quadrats with jump camera photo-quadrats, no significant differences were found between techniques for the number of species nor abundances of individual taxa or higher taxa (Roberts *et al.*, 1994). It was concluded from this work that for macrobenthos, estimates of the number of species and their abundances could be adequately assessed using photo-quadrats.

Sponges were the dominant fauna found at all locations and included many species from a range of orders and families. The abundances of the sponges *Cymbastela concentrica*, *Tethya* sp. and *Jaspis* sp. did not fluctuate through time whilst *Mycale (arenochalina) flammula* underwent significant fluctuations as did the mean number and abundance of sponges. The ability to counteract physical smothering and clogging by siltation is an important mechanism in some sponges (Barthel and Gutt, 1992). This ability is important for deep-water species where siltation during periods of low current velocity can occur or where there is the potential for excessive siltation from sewage outfalls. In the deep-water locations off Sydney, siltation does occur and has been observed in this study and reported by Jones (1977). Such siltation can cause physical smothering and the death of some species, which may lead to patchy distributions within these assemblages (Barthel and Gutt, 1992). Patchiness has been observed in different sponge assemblages and occurred on the deep-water reefs in the present study. Jones (1977) found that sponges varied widely in their responses to siltation and this resulted in different distributional patterns for some species.

Underwood *et al.* (1991) described a mosaic of shallow subtidal rocky habitats in temperate NSW which were related to depth, wave exposure and herbivory. In deeper (15-20 m), more sheltered areas I have noted that sponges were more abundant and invertebrate grazers were rare.
Alcolado (1979) found conspicuous zonation of sponges related to illumination, turbulence, sedimentation and the type of substratum, whereas Wilkinson and Evans (1989) found that depth was the major discriminating factor for sponges on the Great Barrier Reef, Australia.

The reproductive strategies of some sponges include the production of large internal embryos or eggs that settle in the near vicinity of the "parent" sponge colony. This leads to small individuals being clumped close to adult colonies, which was observed in this study whilst doing ROV collections and in the photo-quadrats. Large erect species like Holopsamma arborea, Mycale (arenochalina) flammula and the cup sponge Cymbastela concentrica were all found to exhibit this type of aggregation. These reproductive strategies could have direct implications for organisms that are affected by some type of localised perturbation. When adults die no juveniles are able to recruit back into that area unless it is by wider larval dispersal mechanisms.

Bryozoans were common and included Membranipora sp., Iodyctium phoeniceum and Triphyllozoon sp. Depth and current are considered to be important in determining bryozoan diversity and abundances, but food supply is considered to be the most important factor in determining growth (Shepherd and Thomas, 1982). Jones (1977) reports that lattice bryozoans were found at various locations in deep-water off Sydney and supporting settlement studies showed that certain species had very rapid growth rates. The variable growth rates, reproductive behaviour and paucity of life history data for Australian species makes any interpretation of changes due to the effects of sewage difficult. The loss of the bryozoan I. phoeniceum may be due to a recruitment failure at the outfall location, however due to the correlative nature of the evidence other factors may have caused the subsequent disappearance of this species.

Cnidarians found on the deep-water reefs included hydroids, anemones, corals, sea pens and sea whips. Species that were common at all locations included Primnoella australasiae, Mopsea australis and Balanophyllia sp. There were no spatial or temporal trends apparent in the
abundance of *M. australis* or total cnidarians whereas, the number of cnidarians and the abundance of *P. australasiae* fluctuated among locations.

Ascidians were found at all locations. Some ascidians have been known to have long life spans and show slow changes in abundances (Svane and Lundalv, 1982), however this study showed small-scale changes in the abundances of ascidians. Jones (1977) reports ascidians as being a minor element on the deep-water reefs off Sydney with comparable diversity and abundances to those found in this study.

The crustose coralline algae found in the deep-water assemblages were a combination of species whose individuals could not be identified from photo-quadrats. Temporal changes in the mean abundances of this group showed that at the North Head location there was a peak in abundance in August of each year, after which abundances decreased and stabilised from December through to April. Whether the abundances of these crustose coralline algae change at smaller temporal scales (i.e. less than 3 months) cannot be determined from this study. At the Long Reef and Bungan Head locations, the same general patterns occurred but were not as distinct as at the North Head location. The abundances of coralline algae were always lower at the outfall location compared with the control locations.

There are few published accounts describing the spatial and temporal abundances of any macroalgae in Australia and this is especially the case for crustose coralline algae in deeper water habitats (see Underwood and Kennelly, 1990). Scanes (1986), Fletcher (1987) and Andrew and Underwood (1993) demonstrated that sea urchins and some grazing molluscs are responsible for the maintenance of crustose coralline habitats in shallow-water. The results from these studies may not be applicable to the maintenance of crustose algae in deeper water habitats, because they were not observed here, however, fish may take over the role of invertebrate grazers at these depths (see Kennelly, 1991).
The silt matrix component found in the deep-water habitats was found to be a mixture of fine silt, microscopic invertebrates and microorganisms, similar to that described for nearby shallow-water habitats (Kennelly, 1987b). This silt matrix was the most abundant component at all three deep-water locations. Jones (1977) described a silt/sediment component in deep-water off Sydney that increased in abundance with increasing depth. In the present study, the abundance of silt matrix was relatively similar among locations but some temporal differences occurred.

Considerable variation in the mean number of species and abundance of most organisms were apparent on the deep-water reefs. This variability has the potential to mask effects due to sewage discharge from the outfall. This does not mean that there were no changes, but rather, natural fluctuations in the number of species and their abundances may be greater than those caused by any potential effects of sewage (see Smith, 1994). As there were no pre-commissioning data for this study any detectable differences found are only correlative in nature and the spatial and temporal fluctuations at the outfall location may merely represent any number of factors not necessarily associated with the sewage discharge. Nevertheless, the challenge to marine ecologists faced with no before-data is in their ability to utilise the available information to be able to determine the effects of so-called human disturbance. In reality, many types of studies on the effects of human perturbation generally do not have data prior to the event (for example an oil spill) so that designs and monitoring of the types described in this thesis must be used to assess ecological effects. Detecting these effects on marine communities can be difficult if the large-scale natural variability that is characteristic of these systems is not examined by sampling at appropriate spatial and temporal scales. These scales were unknown at the start of this project. Biological processes which lead to natural variability can include fluctuations in larval settlement and recruitment, predation and competition. Physical factors not associated with sewage outfalls can also cause such natural fluctuations in marine assemblages and may include currents, upwelling, light penetration and storms. Despite such natural fluctuations, human perturbations
(for example the sewage plume) may also affect the species diversity at an outfall location via decreased light penetration, increased siltation and abundant nutrients.

In addition to monitoring and describing the effects of the deep-water ocean outfall on the macrobenthos this study has resulted in a better understanding of the temporal and spatial variation for deeper water temperate reef assemblages off Sydney, NSW.
CHAPTER 5: SEWAGE-RELATED PROCESSES

Introduction

The discharge of sewage effluent into the ocean can alter the chemical and physical nature of the receiving water (Baker et al., 1995) leading to changes in the structure of a wide range of biological communities (Pearson and Rosenberg, 1978; Gee et al., 1985; Austen et al., 1989; Smith, 1993; Otway et al., 1996b; Smith et al., 1999; Hindell and Quinn, 2000). Reported effects of sewage effluent on the structure and dynamics of sessile subtidal assemblages on temperate reefs have included changes to (i) the fauna inhabiting the holdfasts of the kelp Ecklonia radiata in shallow water (Smith, 1996a), (ii) sponge and algal dominated assemblages close to the shoreline at 15-20m depth (Underwood and Chapman, 1996; Roberts et al., 1998) and (iii) sponge-dominated assemblages on deep-water reefs to 60m depth (Roberts, 1996a; 1996b). In temperate Australia, there is no research into processes by which sewage can potentially cause these changes (Hindell and Quinn, 2000).

Sewage can increase the level of suspended solids, and therefore turbidity, in the water column at or near its point of discharge (Coade, 1995), which has the potential to reduce the amount of light that reaches the substratum (Kirk, 1983). Reduced light availability is a limiting factor for the growth of subtidal primary producers such as seagrasses (Fitzpatrick and Kirkman, 1995), benthic algae (Vadas and Steneck, 1988), and symbiotic micro-algae (Maldonado and Young, 1998). The structure of shallow subtidal assemblages can be altered due to shading by kelp canopies (Kennelly, 1989) and artificial structures (Kay and Butler, 1983; Glasby, 1999b; Glasby, 1999c), whilst depth and light limitation have been correlated with changes to the community structure of assemblages in coral reef (Wilkinson and Vacelet, 1979).
Increased sedimentation can also alter the structure and dynamics of subtidal assemblages (Schiel and Foster, 1986; Rogers, 1990; Airoldi and Cinelli, 1997; Wulff, 1997), and there is correlative evidence that community structure can change along sedimentation gradients (Carballo et al., 1996; Naranjo et al., 1996). As well as reducing light availability, sewage plumes will increase the rate of siltation to the substratum (Baker et al., 1995), resulting in the burial or smothering of encrusting species and indeed, increased cover of silt has been documented close to sewage outfalls (see Chapter 4). Field and flume studies of the morphology of some invertebrates indicate sensitivity to high rates of sedimentation (Riegler et al., 1996) and increased siltation may lead to the occlusion of inhalant pores of many filter feeders (Gerodette and Flechsig, 1979; Cerrano et al., 1999).

On temperate shallow subtidal reefs, the encrusting assemblages are generally comprised of algae and invertebrates (Underwood et al., 1991), and changes to the structure of the assemblage because of sewage effluent may be caused by the excessive growth of primary producers or through modifications to phototrophic invertebrates (Roberts et al., 1999). Due to its very nature, sewage contains significant concentrations of nutrients that are needed for plant growth; i.e. nitrogen and phosphorus. Nitrogen is generally the limiting nutrient for algal growth in marine waters (Rhyther and Dunstan, 1971) and if other growth limiting factors are optimal, e.g. light and temperature, then “blooms” of macroalgae can occur in the presence of sewage effluent (Borowitzka, 1972; May, 1985; Fairweather, 1990; Beilgrove et al., 1997).

Since sewage effluent is primarily fresh-water, it has the potential to reduce the salinity at its point of discharge (Baker et al., 1995). The degree of mixing between fresh and salt water at this point will depend on the quantity of effluent, the depth at which it is discharged, and the local wave energy and current regimes (Baker et al., 1995). Given that freshwater can be toxic to marine organisms, there are surprisingly few studies which examine or discuss the
role of reduced salinity in causing stress at either the community or population level (Storr, 1976; Hoegh-Guldberg and Smith, 1989).

The commissioning of a new sewage outfall at Boulder Bay, NSW (see chapter 4) provided an opportunity to examine changes in the nature of a sponge-dominated assemblage using pre and post "impact" data. The discharge of sewage onto shallow subtidal reef led to rapid changes from a sponge/algal dominated assemblage to one that was dominated by silt and ascidians (see Chapter 4 and Roberts et al., 1998). The dominant sponge on the impacted reef was *Cymbastela concentrica* (Lendenfeld), which decreased in abundance within 3 months of the outfall being commissioned (see Chapter 4). A number of competing models were proposed about the mechanisms, which altered the structure of the assemblage and caused the decline of *Cymbastela concentrica* and other sponges (see Chapter 4; Roberts et al., 1998). *Cymbastela concentrica* is a phototrophic sponge (Wilkinson, 1983; Seddon et al., 1993; Cheshire et al., 1995; Roberts et al., 1999), which has a symbiotic relationship with micro-algae that live within its peripheral skeleton (Hooper and Bergquist, 1992). These types of symbiotic relationship have proven to be advantageous for additional nutrition in some species of sponge (Wilkinson et al., 1999).

A reduction in the amount of light reaching the substratum could therefore affect the symbiotic algae leading to a reduction in nutrition for the host sponge and resulting in decreased growth and reproductive potential. Siltation may also affect the pumping and filtration activity of sponges, which could result in decreased growth and reproductive activity. Increased nutrients in the sewage plume may also affect any symbiotic relationship or cause epiphytes to grow on the surface of the sponge. Decreased salinity may have a direct toxic effect on *Cymbastela concentrica*. To test these hypotheses, the rate of growth and reproductive status (number of larvae, eggs and sperm) of *C. concentrica* and the abundance of symbiotic algae (concentration of chlorophyll-a) were measured in experiments that manipulated shade, siltation, nutrients and salinity.
Methods

Study Sites and Sponge Handling

*Cymbastela concentrica* is an erect, cup-shaped, lamellate sponge, common on coral reefs (Hooper and Bergquist, 1992) to 30 m and temperate reefs at depths of 8 to 60 m (see Chapter 3; Roberts and Davis, 1996). To determine the relative importance of reduced light, increased salinity, siltation and nutrients on the phototrophic sponge *C. concentrica*, four manipulative field experiments were established for a period of at least ninety days beginning in September 1998. Three previous attempts at these experiments on exposed reefs met with disaster, as frequent storms destroyed the experimental units. The experimental site was therefore located away from the effects of storms in shallow water at the entrance to Brisbane Waters on the Central Coast, NSW Australia (Fig. 36). SCUBA divers randomly collected individuals from a natural population of *C. concentrica* on a reef (depth 12-15 m) at Lyon Island (Fig. 36). On the surface, each sponge was blotted dry with a towel and any epiphytes removed. Individuals were weighed (nearest gram using a field balance), tagged and randomly allocated to the experimental units. To determine the reproductive and symbiotic status of the natural population of *C. concentrica*, control sponges were also collected at the beginning, during and at the end of the experiments. Once deployed, weekly visits to the experimental site were made to remove any epiphytes or silt that may have settled on the experimental units. At the end of each experiment the sponges were collected, field weighed (nearest gram) and appropriately preserved (see below) for further analysis.
Figure 36. Study locations at Lyon Island (natural populations of *Cymbastela concentrica*) and Brisbane Water (1 - 5 represents the locations where sponges were deployed), NSW.
Experimental Design

Each experiment was designed to examine the effects on the growth, reproductive status and symbiotic algae in the lamellate sponge *Cymbastela concentrica*. In the shade experiment, three treatments that consisted of rectangular metal frames were placed on the bottom at a depth of approximately 3m in each of three random sites. The dimension of a frame was 0.8 x 0.6 x 0.8 m and a wire basket was attached beneath, so that one sponge in each basket was kept at least 0.3 m off the substratum at all times. The shade-frame was topped with black perspex, and the sides were covered from the top to half way down the side, which allowed water to circulate through the unit, whilst reducing the amount of available light to the sponges (Fig. 37a). Two procedural controls were used; one control frame was covered with clear perspex (same as the shade treatment), which allowed light to enter, whilst the other control was made of a frame with no perspex (to determine whether perspex caused artefacts that could confound the experiment). The amount of light under each experimental frame was measured *in-situ*, using a Li-cor (LI-185) light sensor to establish whether the units significantly reduced the light reaching the sponges.

The experiment where silt was manipulated had three treatments: (i) silt (ii) no silt and (iii) cage control at each of three random sites. The sponges were placed into small wire cages, which were welded to the top of a 0.8 m length of aluminium pipe (Fig. 37b). The cage control consisted of a wire mesh base to which the sponge was secured using a small cable tie. The pipe was pushed 0.3 m into the substratum, at a depth of approximately 3 m, keeping the cages at least 0.5 m off the bottom. Using a small plastic shovel, approximately 5g of fine silt were added to the “silt-treatment” sponges at least once per week. The control sponges were carefully fanned to free any silt that may have settled since the last site visit.
The “nutrient-addition” experiment had four treatments: (i) nitrogen; (ii) phosphorus; (iii) nitrogen + phosphorus and (iv) control (no nutrient) at each of three random sites. The same type of cage was used as described in the siltation experiment. The sponges were placed into the cages with a small hessian bag containing 50g of “slow-release” fertilizer, i.e. nitrogen or phosphorus, or a mixture of both. A hessian bag was also placed in the control treatment cages.

In the final experiment, the effect of salinity was examined by deploying sponges at five locations along a decreasing salinity gradient in the estuary (Fig. 36). The sponges and cages were deployed as described in the previous experiments, however there were three random sites nested within each of the locations.

**Laboratory Procedures**

The growth rate (FW g/day) of each sponge was calculated by subtracting its initial weight from its final weight and dividing by the number of days deployed (determined for each experiment). Sponges used for analysis of reproductive status were fixed in a gonad fixative, FAACC (100ml: 37-40% formaldehyde solution: 5ml glacial acetic acid: 1.3g calcium chloride dihydrate: 85ml distilled water) for 48 hours, and then transferred to 75% ethanol (Fromont, 1999). Thin sections from each sponge were cut (8μm) using a microtome and stained with haematoxylin-eosin, mounted and examined under the light microscope for the presence of larvae, eggs and sperm (Fromont, 1999). The number of larvae and oocytes was estimated from the average of three haphazardly placed “fields of view” (400x) on each section, whilst spermatocytes were counted in three random 4 x 3μm sub-quadrats. The concentration of chlorophyll-a was determined for each sponge by homogenising sub-samples using a mortar and pestle and extracting the chlorophyll using 90% acetone for 30 minutes on ice and in the dark. A Shimadzu UV1601 spectrophotometer was used to measure the concentration of chlorophyll-a within each sponge (Clesceri et al., 1985).
Analyses of Data

The growth rate of each individual, the number of larvae, oocytes, spermatocytes and the concentration of chlorophyll-α were used as the response variables in two-factor analysis of variance models for each experiment. In the experiments on shading, siltation and nutrients, sites were considered random and treatments were fixed. In the experiment examining a salinity gradient, locations were considered fixed and sites were nested within location. Post-hoc pooling procedures were used when the Site x Treatment interaction was found to be non-significant at $P = 0.25$; this allowed appropriate $F$-tests to be constructed for main effects (Winer, 1971). Prior to analysis, the data were examined for homogeneity of variance using Cochran's test (Winer, 1971). Where variances were heterogeneous, data were transformed to log $(x + 1)$ (Winer, 1971). Where transformations did not result in homogeneous variances, analyses were done on the untransformed data (Underwood, 1981). Where significant differences were found in the analysis of variance, Student-Newman-Keuls (SNK) multiple comparisons were done at the appropriate alpha level to determine differences among means (Winer, 1971). It should be noted that data for the number of larvae had many zeros, and was not normally distributed. Analysis of variance is generally robust in terms of violating this assumption (Underwood, 1981), however these results should be treated with caution.

Figure 37. (a) Frames used in shade and (b) cages used in silt, nutrient and salinity experiments.
Results

Sponge Growth

*Cymbastela concentrica* lost weight (after 90 days) under the shade treatments whilst there was significant growth in the sponges in the perspex and open controls (Table 29; Figure 38a). There were no significant differences in the growth of sponges in the two procedural controls and there were no significant differences for sponge growth among the three sites. The specimens in both procedural controls appeared “healthy” and were similar in colour and consistency to specimens collected on the reef at Lyon Island at the end of the experiment (Fig. 39a). The sponges under the shaded treatments had a “bleached” appearance and showed evidence of necrotic tissue around the edges (Fig. 39b). The amount of light (measured in micro-Einsteins) reaching the sponges in each of the shade, clear perspex control and frame control treatments was $12.8 \pm 0.2$ (mean $\pm$ SE), $265.0 \pm 3.5$ and $293.3 \pm 4.1$ respectively. This represented a reduction of over 90% in available light under the shade treatment, which is typical of the amount of shading that may be produced by a sewage outfall plume (see Chapter 4).

*Cymbastela concentrica* also lost weight (after 90 days) in the treatment where silt was added, whilst there was significant growth in the sponges in the controls (Table 30; Figure 38b). There was no significant difference in the growth of sponges in the procedural controls or among sites. As reported in the shade experiment, the sponges in the “silt-addition” treatment had a “bleached” appearance and showed evidence of heavy necrosis, and in some replicates the surface colour of the sponge was black (Fig. 39c). The sponges in both control treatments were generally “healthy” and appeared to be similar in colour and consistency to specimens collected on the reef at Lyon Island at the end of the experiment.
Nitrogen, phosphorus, and a combination of both, did not affect the growth of *Cymbastela concentrica*. Whilst there was growth, there were no significant differences between any treatments including those in controls (Table 31; Fig. 38c). There was also no significant difference in the growth of sponges among sites. All sponges in the nutrient-addition experiment were generally “healthy” and appeared to be similar in colour and consistency to specimens collected on the reef at Lyon Island at the end of the experiment.

There was correlative evidence to suggest that lower salinity had a negative effect on the growth of *Cymbastela concentrica* (Table 32; Fig. 38d). There were no significant differences between the rate of growth at locations 1, 2 and 3, however there was negative growth at locations 4 and 5, where the salinity was on average 2-3 ppt lower than the locations closer to the mouth of the estuary (Fig. 40). There was no significant difference in sites nested within locations and generally the sponges at all locations appeared healthy. It is important to note that this experiment was correlative and there could be potential confounding effects from other water quality gradients (e.g. turbidity) in the estuary.

**Reproductive Status**

*Cymbastela concentrica* randomly collected from the natural populations near Lyon Island were reproductively active in that they all possessed larvae, oocytes and spermatocytes. For each of these variables, mean numbers were smaller in the “natural” sponges collected at Lyon Island at the end of the experiment compared with those collected at the start (Table 33). The mean number of larvae, oocytes and spermatocytes were generally greater in the natural population at the end of the experiment compared with those in the procedural controls (Table 33). The diminished reproductive activity in the translocated sponges may be due to the effects of handling (e.g. depth changes, movement, stress). A procedural control that examined the effects of translocation (sponges collected from the reef at Lyon Island, moved and replaced back at
Lyon Island) would have been useful, however as previously mentioned this was not possible due to storms.

Sponges that were placed under the shade treatment were not as reproductively active as those within the control treatments. There were significantly lower numbers of oocytes and spermatocytes within sponges kept in the shaded treatment (Table 29; Fig. 41) compared with both control treatments, however there were no significant differences in the number of sponge larvae in all three treatments (Table 29; Fig. 41).

Sponges that had a treatment of silt were generally not as reproductively active as the control sponges. There were significantly lower numbers of spermatocytes within sponges to which silt had been added (Table 30; Fig. 42) compared with controls, however there was no significant difference in the number of sponge larvae or oocytes among the treatments (Table 30; Fig. 42).

The sponges in the nutrient-addition experiment were all reproductively active. There were no significant differences in the number of larvae, oocytes and spermatocytes within any of the treatments (Table 31; Fig. 43). A significant Site x Treatment interaction was detected for the number of oocytes. Specifically both the nitrogen and phosphorus addition treatments at site 3 were significantly lower than those treatments at the other two sites (Table 31; Fig. 43).

The sponges in the salinity experiment were all reproductively active, however, there was a significant difference between locations for the number of larvae, oocytes and spermatocytes (Table 32; Fig. 44). Location 1 was the only location that had sponges with larvae, whilst oocytes were absent at location 5 and spermatocytes were significantly lower in location 5 compared with all other locations.
Symbiotic Algae

Natural populations of *Cymbastela concentrica* near Lyon Island generally had a higher concentration of chlorophyll-α than those within the procedural controls (Table 34). The concentration of chlorophyll-α within these natural populations did not change through time (Table 34). The lower chlorophyll-α concentrations in the translocated sponges could be due to handling or relocation stress. The major difference in the concentration of chlorophyll-α was evident in the nutrient and salinity experiments, where all concentrations were considerably lower than in the other two experiments (Table 34).

There was a significantly lower concentration of chlorophyll-α in sponges within the shade treatment compared with the procedural controls (Table 29; Fig. 45a) and site 1 had significantly greater concentrations than the other two sites (Table 29; Fig. 45a). Sponges that had silt added had a significantly lower concentration of chlorophyll-α compared with the procedural controls (Table 30; Fig. 45b), whilst there were no significant differences between sites. There were no significant differences in the concentration of chlorophyll-α in any treatments in the nutrient (Table 31; Fig. 45c) and salinity experiments (Table 32; Fig. 45d). As previously mentioned, the concentration of chlorophyll-α was generally quite low for all treatments in these last two experiments and it may be that some "uncontrolled factor" has affected the overall symbiosis of the sponges at a scale that is probably greater than that of the experiment.
Figure 38. Mean (± SE) rate of growth in the sponge *Cymbastela concentrica* in (a) shade, (b) silt, (c) nutrients (N – nitrogen, P – phosphorus, C- control) and (d) salinity treatments (Location 1 – High; Location 5 – Low) at the experimental locations in Brisbane Waters.
Figure 39. Example of (a) healthy, (b) necrotic and (c) blackened *Cymbastela concentrira*.

Figure 40. Average (± SE) salinity (ppt) along an estuarine gradient in Brisbane Waters (*n* = 30): Location 1 - Lobster Beach, Location 2 - Hardy’s Bay, Location 3 - St Huberts Island, Location 4 - Saratoga, Location 5 - Gosford.
Figure 41. Mean (± SE) number (per field of view) of (a) larvae, (b) oocytes and (c) spermatocytes in the sponge *Cymbastela concentrica* in the shade-reduction experiment.
Figure 42. Mean (± SE) number (per field of view) of (a) larvae, (b) oocytes and (c) spermatocytes in the sponge *Cymbastela concentrica* in the siltation experiment.
Figure 43. Mean (± SE) number (per field of view) of (a) larvae, (b) oocytes and (c) spermatocytes in the sponge *Cymbastela concentrica* in the nutrient-addition experiment (N – nitrogen, P – phosphorus, C – control).
Figure 44. Mean (± SE) number (per field of view) of (a) larvae, (b) oocytes and (c) spermatocytes in the sponge *Cymbastela concentrica* in the salinity experiment.
Figure 45. Mean (± SE) concentration of chlorophyll-a (µg/g) in the sponge *Cymbastela concentrica* in (a) shade, (b) silt, (c) nutrients and (d) salinity experiments.
Table 29. Summary of analysis of variance on the effects of shade on the rate of growth, reproductive status (larvae, oocytes, spermatocytes) and symbiotic algae (chlorophyll-a) in *Cymbastela concentrica*. *F* ratios in bold have been calculated after pooling; nil, no transformation required; ns, not significant (*P* > 0.05); *, significant (*P* < 0.05); **, (*P* < 0.01).

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Table 30. Summary of analysis of variance on the effects of siltation on the rate of growth, reproductive status (larvae, oocytes, spermatocytes) and symbiotic algae (chlorophyll-a) in Cymbastela concentrica. F ratios in bold have been calculated after pooling; nil, no transformation required; ns, not significant (P > 0.05); *, significant (P < 0.05); **, (P < 0.01).

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Cochran’s Test       | ** | *     | **     | ns     | *      |        |
Transformation        | ** | *     | **     | nil    | ns     |        |
Table 31. Summary of analysis of variance on the effects of nutrients on the rate of growth, reproductive status (larvae, oocytes, spermatocytes) and symbiotic algae (chlorophyll-α) in *Cymbastela concentrica*. *F* ratios in bold have been calculated after pooling; nil, no transformation required; ns, not significant (*P > 0.05*); *, significant (*P < 0.05*); **, (*P < 0.01*).

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Table 32. Summary of analysis of variance on the effects of salinity on the rate of growth, reproductive status (larvae, oocytes, spermatocytes) and symbiotic algae (chlorophyll-a) in *Cymbastela concentrica*. *F* ratios in bold have been calculated after pooling; nil, no transformation required; ns, not significant (*P > 0.05*); *, significant (*P < 0.05*); **, (*P < 0.01*).

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<td>0.0052</td>
<td>0.99ns</td>
<td>0.003</td>
<td>0.5ns</td>
</tr>
<tr>
<td>Residual</td>
<td>30</td>
<td>0.0053</td>
<td>0.005</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td>Cochrans Test</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td></td>
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</tr>
<tr>
<td>Transformation</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 33. Mean (± SE) number (per field of view) of larvae, oocytes and spermatocytes in natural populations of *Cymbastela concentrica* at Lyon Island (*n* = 9) and in the procedural controls for shade (*n* = 18), silt (*n* = 18), nutrients (*n* = 9) and salinity (*n* = 9) at the end of the experiments.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Lyon Island</th>
<th>Procedural Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Start</td>
<td>End</td>
</tr>
<tr>
<td>Larvae</td>
<td>0.1 ± 0.1</td>
<td>0.04 ± 0.04</td>
</tr>
<tr>
<td>Oocytes</td>
<td>1.5 ± 0.8</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td>Spermatocytes</td>
<td>9.4 ± 0.6</td>
<td>6.7 ± 0.9</td>
</tr>
</tbody>
</table>

Table 34. Mean (± SE) chlorophyll-a concentrations (µg/g) in *Cymbastela concentrica* collected from the natural population at Lyon Island (*n* = 9) and in the procedural controls for shade (*n* = 18), silt (*n* = 18), nutrients (*n* = 9) and salinity (*n* = 9) at the end of the experiment.

<table>
<thead>
<tr>
<th></th>
<th>Lyon Island</th>
<th>Procedural Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Start</td>
<td>Middle</td>
</tr>
<tr>
<td></td>
<td>145.1 ± 26.8</td>
<td>150.1 ± 13.9</td>
</tr>
</tbody>
</table>
Discussion

Experimental shading and siltation were found to reduce the growth and reproductive status of the sponge *Cymbastela concentrica* and significantly alter its symbiotic relationship with micro-algae. A decrease in salinity of around 2ppt also affected the rate of growth and reproductive status of *C. concentrica*, but did not alter the symbiotic relationship. The addition of "key nutrients" responsible for the growth of algae, i.e. nitrogen, phosphorus and a combination of both, did not cause any changes to the rate of growth, reproductive status or symbiotic relationship in *C. concentrica*.

In the experiment where light was manipulated to mimic the effects of shading by a sewage plume, the symbiotic algae (measured as the concentration of chlorophyll-\(a\)) within the test sponges were significantly reduced. From the results obtained, it would appear that *Cymbastela concentrica* could rely heavily on its relationship with symbiotic micro-algae for at least some of its nutritional requirements. Sewage plumes and suspended particulate matter can alter the intensity and spectral quality of light reaching organisms on subtidal reefs (Baker et al., 1995), which in turn can affect metabolic processes (Rogers, 1990). The growth of symbiotic micro-algae (Cheshire et al., 1997) may therefore be limited by the amount of light available for photosynthesis.

There is sufficient evidence that many tropical marine sponges are ‘phototrophic’ and that symbiotic algae can provide the bulk of their carbon energy requirements (Wilkinson, 1983; Larkum et al., 1988; Cheshire and Wilkinson, 1991; Wilkinson et al., 1999). Cheshire et al. (1995) demonstrated that *Cymbastela* sp. from reefs in southern temperate Australia were probably phototrophic, whilst Roberts et al. (1999) provided evidence that a surprisingly high proportion of temperate reef sponge species (including *C. concentrica*), also had this potential. It could therefore be argued that the ability of *C. concentrica* to obtain the
required nourishment from symbiotic algae was diminished under the conditions of these experiments, thus affecting its rate of growth and reproductive status. In coral communities, Rogers (1990) found that the net productivity of coral assemblages was reduced when light was experimentally manipulated to mimic the shading associated with increased turbidity, whilst Tomascik and Sander (1985) found that the concentration of suspended particulate matter was the strongest single estimator of growth in corals.

Studies of the effects of shading on whole assemblages have included those associated with seagrass meadows (Fitzpatrick and Kirkman, 1995), kelp forests (Kennelly, 1989), coral reefs (Rogers, 1990), subtidal caves (Cinelli et al., 1977) and artificial structures (Kay and Butler, 1983; Glasby, 1999b). In temperate estuaries, Glasby (1999b) found significant effects of reducing light on the cover of sessile macrobenthic assemblages on artificial structures that was consistent with the findings of other workers (Reed and Forster, 1984; Kennelly, 1989; Rogers, 1990; Fitzpatrick and Kirkman, 1995). On artificial substrata, Glasby (1999b) reported that assemblages of sponges did not differ between shaded and unshaded treatments, presumably because the variability in shaded treatments was very large. Glasby (1999b) postulated that given more time he would have observed a greater abundance of sponges in shaded areas as previously reported by others (Wells et al., 1964; Sutherland and Karlson, 1977; Kay and Butler, 1983; Cinelli et al., 1977; Bibiloni et al., 1989).

Increased amounts of silt were found to reduce the growth and reproductive status of *Cymbastela concentrata*. Field and flume studies of the morphology of some invertebrates indicate sensitivity to high rates of sedimentation (Riegl et al., 1996). Heavy siltation has been shown to affect the ability of some sponges to pump water (Gerodette and Flechsig, 1979), whereas some species are sensitive to burial beneath sediment (Wulff, 1997). The ability to counteract this smothering and clogging is believed to be an important mechanism in many sponges and those
species that cannot tolerate increased sedimentation will be disadvantaged (Barthel and Gutt, 1992). Coping with increased sedimentation is important for deep-water species where siltation during periods of low current velocity can occur or where there is the potential for excessive siltation from sewage outfalls (Barthel and Gutt, 1992). Siltation has also been shown to alter the structure and dynamics of encrusting communities (Airioldi et al., 1995) and there is correlative evidence that their structure changes along sedimentation gradients (Carballo et al., 1996; Naranjo et al., 1996). Here, increased silt also altered the symbiotic relationship between the sponge and its micro-algae. It is conceivable that burial would effectively stop any photosynthetic activity by micro-algae, whilst clogging would reduce water flow and therefore nutrients. The sponge may therefore loose its nutritional bonus from its symbiotic micro-algae and as a result of decreased or complete shutdown of normal pumping activities.

The nutrient-addition experiment provided no evidence to support the hypothesis that nutrients enhanced symbiotic micro-algal growth, i.e. the symbiotic algae were not nutrient limited under the conditions of the experiment. Increased concentrations of nutrients in sewage can increase the growth rates of macroalgae (Borowitzka, 1972) and potentially could have altered the biomass of micro-algae in *Cymbastela concentrica* by providing additional nutrition. There were, however, no changes to the rate of growth or the reproductive status of *Cymbastela concentrica* with respect to different additions of nutrients. The growth rates were also similar to those obtained in the procedural controls in the other two experiments. Given the results obtained here it can be concluded that the “key growth nutrients”, nitrogen and phosphorus, which are typically found in sewage effluent, did not enhance symbiotic algal growth and thence sponge growth.

A decrease in the salinity by approximately 2ppt resulted in negative growth rates and lower reproductive activity in *Cymbastela concentrica*. Marine sessile organisms are generally stenohaline, i.e. they have a low tolerance to changes in salinity, and reduced salinity has been shown to decrease the diversity and abundance of sponge-dominated assemblages.
Maldonado and Young (1998), using a transplant experiment, relocated keratose sponges to depths where there was a pycnocline of lower salinity, temperature and oxygen. This resulted in the death of their sponges, which they suggested was due to changes in temperature and loss of nutrient producing symbionts.

The low chlorophyll-a readings I obtained in the salinity and nutrient experiments reflected an overall change in the symbiotic relationship of the test sponges compared with the natural population collected from Lyon Island. These two experiments started and finished a month later than the experiments examining shading and siltation. The only factor that changed significantly within the estuary over this period was an increase in water temperature. Increased water temperature and heat stress has been correlated with changes to the photosystem of symbiotic algae in corals (Jones et al., 2000). The thermal sensitivity of symbiotic algae is believed to be the cause of the “coral bleaching phenomenon”, where the tissue whitens as symbiotic algae “die-off” during periods of elevated water temperature (Hoegh-Guldberg, 1999). Interestingly, there were no differences in the rate of growth of *Cymbastela concentrina* in the earlier experiments compared with the later ones where reduced abundances of symbiotic algae were recorded. If the reduction in the symbiotic algae was caused by an increase in water temperature then this must have occurred close to the end of the experiment, thus the rate of growth of the sponges was not significantly affected during that time. Nevertheless, it is advisable to interpret the results of the two sets of experiments in isolation because some uncontrolled factor certainly affected the symbiotic relationship between the sponge and its micro-algae.

In this series of experiments, increased shading and siltation were the primary abiotic processes that resulted in reduced growth, reproductive status and symbiosis. A reduction in salinity also affected these variables, however it was considered to be purely correlative and further field-based experiments are required.
CHAPTER 6: GENERAL DISCUSSION

SPATIAL AND TEMPORAL VARIABILITY

To gain an understanding of the natural and anthropogenic processes that can modify development in the structure and dynamics of sponge-dominated assemblages on temperate reefs, it was essential that patterns of spatial and temporal variability be examined first (Underwood et al., 2000). Unfortunately, for the assemblages that I studied here, there were no data available, let alone measures of variability collected at appropriate spatial and temporal scales. This made generalising about their subsequent ecology or developing predictive or conceptual models about the types of changes that might occur following disturbance difficult. Chapter 3 in this thesis therefore represents the first quantitative assessment of spatial and temporal patterns of variability in subtidal sponge-dominated assemblages living on deeper reefs in temperate Australia.

There is a plethora of evidence to suggest that the patterns of abundance and distribution of sessile marine assemblages are related to changes associated with depth (Schiel et al., 1986; Wilkinson and Evans, 1989; Clarke et al., 1993). These changes in the structure of assemblages have also been correlated with the degree of exposure to wave energy or strong currents (Kaandorp, 1999). In chapter 3, I found that the species richness and abundance of erect sponges increased with depth, whilst encrusting sponges were more abundant as depth decreased. This pattern of increased richness, abundance and morphological complexity is likely to be a response to reducing wave energy as depth increases, which can facilitate more complex and intricate morphologies of the species within the assemblage (Bell and Barnes, 2000; Ginn et al., 2000). These depth-related patterns of richness and abundance have been well described in assemblages of marine organisms from tropical (Wilkinson and Evans, 1989; Baynes, 1999) and polar waters (Barthel et al., 1990; Barthel, 1991), on artificial
structures (Keough, 1983; Connell, 1999; Glasby, 1999a) and on the walls of submarine caves (Cinelli et al., 1977; Southward et al., 1996).

The structure of the sponge-dominated assemblages (including fauna and algae) at 20 m was found to be different on exposed reefs compared with sheltered reefs at the same depth. Therefore it may not be depth per se that is instrumental as a factor in structuring the assemblage, but depth modifies the extent of physical energy and other water quality variables (Short and Trenaman, 1992). For example, the quality and quantity of light is reduced as depth increases, which can affect the photosynthetic activity of primary producers such as macroalgae (Vadas and Steneck, 1988; Foster and Schiel, 1993). For many subtidal species, this depth-limitation to the euphotic zone is either because they are photosynthetic (algae) or are indirectly reliant upon the photosynthetic activity of co-occurring species (symbiotic). On deeper reefs, green and brown algae are replaced with red coralline species which have photosynthetic pigments that can utilise those wavelengths of light which are capable of penetrating into deeper water (Schiel and Foster, 1986; Vadas and Steneck, 1988; Roberts, 1996a). This is important because in very shallow water, foliose algae can out-compete sessile sponges and other invertebrates, so the greater success of sessile fauna in deeper water may also be due to the inability of green and brown algae to grow at these depths.

The rate of siltation to the substratum may also be an important determinant in structuring subtidal assemblages on temperate reefs (Airoldi and Cinelli, 1997). Siltation is generally greatest in low energy environments, so that as depth increases, or the degree of exposure decreases, so too does the rate of deposition (Wilson et al., 1995). In chapter 3, I reported that the morphology of many species were structurally more complex with increased depth and lower degrees of exposure. Variations in the supply of sediment to the substratum can significantly influence the structure of assemblages by excluding species that are less
tolerant to siltation (Kennelly, 1983; Airoldi and Cinelli, 1997). Excessive silt has the ability to clog the inhalant pores of many sessile invertebrates (Gerodette and Flechsig, 1979). The predominance of species which have adapted to withstand heavy siltation can be seen in the morphology of these species in deeper water, i.e. branching or erect instead of a flattened morphology which is easily smothered (Wulff, 1997). For sponge-dominated assemblages living on reefs in deep-water, the effects of different rates and amounts of siltation on encrusting species requires experimentation.

As previously discussed, the structure of sponge-dominated assemblages varied between depths and the degree of exposure to wave action. At the spatial scale of locations or reefs, significant differences in the structure of the assemblage were also noted. For example, the three reef locations, sampled at Port Stephens (within 3 km of each other and at a depth of 20 m) generally had different community structures. The abundances of some taxa were similar on the three reefs, however many individual species were commonly found on one reef but not on another. These differences, at the scale of reefs within a few km of each other, represent the influence of both physical and biological processes acting within a particular location. Wave action, currents, herbivory and variations in the supply of larvae, settlement and subsequent recruitment, all help to produce these mosaics of different habitats at these scales (Underwood et al., 1991; Kennelly and Underwood, 1992). Within any location, and at the scale of sites, the structure of the assemblage was generally homogeneous, i.e. similar numbers and abundances of the same types of species. These patterns were also observed in the structure of the assemblages from one side of an estuary location to the other.

Much of our understanding about processes in subtidal assemblages has been achieved by studying them in either very shallow water (Kennelly, 1987a, b & c; Andrew, 1999) or on assemblages that live on artificial structures (Kay and Butler, 1983; Keough, 1983; Butler, 1991; Connell and Glasby, 1999; Glasby, 1999c; Smith and Witman, 1999). Furthermore,
complex biological interactions are important in determining the structure of marine assemblages and a great deal of research has also been done, but again only in shallow water (Menge and Sutherland, 1976; Sutherland and Karlson, 1977; Ayling, 1981; Kay and Keough, 1981; Keough and Downes, 1982; Keough, 1984; Butler, 1986; Davis and Butler, 1989; Davis et al., 1991; Uriz et al., 1992; Aerts and Van Soest, 1997; Hill, 1998; Maldonado and Uriz, 1998; Baynes, 1999). Whilst these studies have aided our understanding, they should not be used to infer patterns or processes for subtidal assemblages on reefs in deeper water.

Significant temporal variability was found in the abundances of sponge-dominated assemblages at the scales that were measured. The hypothesis that sponge-dominated assemblages on temperate subtidal reefs are temporally stable (Ayling, 1983a; Kay and Butler, 1983; Chapman et al., 1995) needs to be formally tested (Butler and Connolly, 1996). I found significant temporal fluctuations in the richness and abundance of these assemblages at the scale of reefs, between depths and among different types of habitat. These finding may be in contrast to those reported for assemblages on artificial structures in temperate South Australia, where a form of “dynamic stability” at the scale of sites and reefs has been reported (see Butler and Connolly, 1996; Butler and Connolly, 1999). As Butler and Connolly (1999) noted, we know very little about the long-term development of these assemblages, which may lead to an “established” state. Butler and Connolly (1999) argue that this established state may take much longer than hitherto first believed, however it is conceivable that it may never reach that point.

The dynamism produced in different temperate reef habitats and assemblages can be triggered by biological (Ayling, 1981; Choat, 1982; Butler, 1986; Kennelly, 1991; Davis et al., 1997; Wright et al., 1997; Underwood, 1998) or physical disturbance (Dayton et al., 1984; Kennelly, 1987a; b & c; Dayton et al., 1992; Posey et al., 1996; Underwood, 1999a).
Generalising about the findings from one system and extrapolating to another, albeit a similar system, can be problematic. As an example, Chapman et al. (1995) explored the hypothesis by Warwick and Clarke (1993a) that increased variability may be a symptom of stress in subtidal assemblages. Chapman et al. (1995) refuted the hypothesis, because they did not find this variability within assemblages at a decommissioned sewage outfall compared with reference locations. The outfall in question had been decommissioned for 4 months before they started sampling. They used the often-quoted evidence for this “temporal stability” hypothesis as a justification for not detecting any effects of the outfall, in terms of the variables they measured or the hypothesis proposed by Warwick and Clarke (1993a). It is conceivable that they did not find a significant difference between their potentially impacted location and control locations because the assemblage responded rapidly to the new conditions in water quality. Clearly, more research is required to unravel many of the processes of long-term development and stability of subtidal encrusting assemblages.

**EFFECTS OF SEWAGE**

In NSW Australia, sewage pollution is a potentially significant source of anthropogenic disturbance to subtidal assemblages (Koop and Hutchings, 1996), and whether they respond in an adverse manner will depend on whether this disturbance is greater than that caused by natural disturbance (Underwood, 1999b). In this thesis, I reported a number of responses by sponge-dominated assemblages on temperate reefs to sewage pollution (Chapter 4). For assemblages within shallow water, where there was sufficient water turbulence, mixing and a great amount of localised energy, the effects of sewage were masked by natural variability (Roach et al., 1995). At slightly greater depths, where sewage has the ability to alter the physical nature of the water column (e.g. effects of shading by plumes or silt smothering the bottom) the response of the assemblage was rapid (see Chapter 4). The changes to these
assemblages at 20 m occurred within 3 months of a relatively small quantity of sewage effluent (3 ML/day) being discharged. In deeper water (50 m), the response of the assemblage was much slower (Roberts, 1996a; 1996b), apparently not because the organisms were any more resilient or "temporally stable" but because the sewage plume was diluted and rose to the surface quickly following its discharge (Schroeter et al., 1993). In this case, the effect of sewage disposal on the sponge-dominated assemblages was not detected until 18 months after the discharge began. In the course of this study, however, large storms occurred which caused measurable changes to some components of the assemblage. The effects of episodic natural disturbances such as storms can therefore potentially alter these assemblages at scales that are greater than that caused by sewage effluent (Wulff, 1995).

The discharge of sewage effluent may be considered as an anthropogenic "press" disturbance whilst storms may be considered to be natural "pulse" disturbances (Bender et al., 1984; see Glasby and Underwood (1996) for a recent review of this topic).

In this thesis, I report various patterns associated with the effects of sewage outfalls. In the case of the nearshore outfall at Boulder Bay (see Chapter 4), changes to the structure of the assemblage were rapid, i.e. from a sponge and algae dominated assemblage to one dominated by silt and ascidians. It was hypothesised that the processes that may have led to these changes could be a reduction in light and salinity or an increase in siltation or nutrients (see Chapter 5). The sponge (*Cymbastela concentrica*) used to test hypotheses regarding these processes was considered to be phototrophic (Roberts et al., 1999) and along with other sponges (e.g. *Geodinella* sp.) had declined in abundance following the discharge of sewage. My experiments with *C. concentrica* confirmed that shading and siltation can act to reduce sponge growth, reproductive status and reliance on symbiotic algae (Chapter 5). Salinity was also correlated with a decline in its growth, however nutrients were not that important (Chapter 5). Light and siltation have certainly been implicated in structuring assemblages of plants and animals in shallow estuarine systems within temperate NSW.
(Kennelly, 1987a, b & c; Kennelly, 1989; Glasby, 1999b) and in other parts of the world (Maldonado and Young, 1996; Baynes, 1999). Whilst the experiments reported in this thesis give us important information about the potential mechanisms for a single species, the results should not be extrapolated to an entire assemblage and it would be constructive to repeat these types of experiments at the scale of the assemblage (Airoldi and Cinelli, 1997).

A simple conceptual model of how sewage alters some of the processes within these assemblages can be formulated. Firstly, the sewage plume shades the substratum, which causes the photosynthetic components of the assemblage to be affected, i.e. photosynthesis is reduced. As light is reduced, the cover of foliose macroalgae within the assemblage declines, and for subtidal assemblages, any increase in nutrients from the sewage plume may not benefit macroalgal growth, i.e. nutrients are not a limiting factor (Smith, 1994; Ajani et al., 1999). This pattern may be the reverse for assemblages of macroalgae on intertidal reefs (Murray and Littler, 1978; May, 1985; Fairweather, 1990). A large proportion of sponges on subtidal reefs in temperate NSW may also contain symbiotic algae and many may be phototrophic (Roberts et al., 1999), so that any shading has the potential to impinge on their growth and reproductive status (Chapter 5; Maldonado and Young, 1998; Wilkinson et al., 1999).

With the addition of increased siltation from the sewage plume, the assemblages can become smothered and for some filter feeders like sponges, inhalant pores can become clogged leading to an inefficient or highly reduced ability to pump water and nutrients through the colony (Gerrodette and Flechsig, 1979). The organism becomes "stressed" so that growth and reproduction is impaired. If we extrapolate these simple explanations to the assemblage as a whole, then sponges, many of which are less tolerant to siltation, will disappear allowing greater space for recruitment of more tolerant species (Airoldi and Cinelli, 1997).
MANAGEMENT CONSIDERATIONS

The discharge of sewage effluent into the ocean is a common means of waste disposal around the world (Dubinski and Stambler, 1996) and in NSW, Australia there are 35 sewage outfalls which discharge effluent into the coastal marine environment (MHL, 1997). These outfalls have a great range in the discharge of their effluent quantity (3 - 100 ML per day; MHL, 1997) and quality (tertiary-treated to primary-treated; MHL, 1997). Agencies responsible for the maintenance of ocean outfalls include local coastal councils and independent water authorities, eg. Sydney Water. The NSW coastline is quite rugged and generally from the shoreline to depths of 18m, subtidal reef is common (Andrew and O’Neill, 2000). The human population tends to congregate close to the sea and disposal of sewage into it, under current technologies, is necessary (Koop and Hutchings, 1996; Leadbitter, 1996; Pritchard, 1997). The disposal of sewage along the coastline is therefore nearly always onto subtidal reef and not soft-sediment habitats (however see Koop and Hutchings, 1996). In the past, many sewage outfalls discharged into estuaries and from smaller cliff-face outfalls, however, the current general policy is to discharge into either shallow turbulent marine waters, which is cost effective and aids in dilution, or to dispose of the sewage via deep-water ocean outfalls, which are a few kilometres off the coastline (Otway, 1995; Scanes and Philip, 1995; Koop and Hutchings, 1996; Pritchard, 1997).

In this thesis, significant issues were identified that may have direct implications for the management of sewage outfalls in NSW coastal waters, specifically the way in which existing and future monitoring programs are designed and implemented. For ocean outfalls, which currently discharge sewage into NSW marine waters, monitoring programs exist, which have supposedly been designed to establish their effects on both marine assemblages and on human health. These programs are generally done by consultants with the production
of the usual colossal 'grey literature' report of which two thirds consists of unanalysed raw data in the form of appendices, whilst at least half of the other third comprises the consultants' resume and capability statement. Whilst this candid and generalised criticism does not relate to all consultants in this field, it is nonetheless common. The fault however, rests not with the consultants, but with the water managers and engineers who write the project briefs for these programs, and unfortunately have no concept regarding spatial and temporal variation in ecology or how to design and implement programmes that can detect impacts in the marine environment (see Underwood, 1991b).

As indicated in chapter 4 of this thesis, the problems relate to the way in which the sampling programmes are designed, i.e. inadequate or inappropriate spatial or temporal scales are measured, e.g. impacted locations are contrasted with one control location, or worse, no controls at all (Underwood, 2000a). The utility of these monitoring programs is very limited, if we add to these poor designs the fact that no pre-disturbance data are usually collected. In this thesis, sessile marine assemblages were found to be highly variable at different spatial and temporal scales, so any monitoring designs must be sufficiently sensitive to discriminate anthropogenic disturbance from high levels of natural variability (Underwood, 1993; Warwick, 1993; Andrew and Mapstone, 1987; Underwood, 2000b). Impacts that stem from point sources such as sewage, should in theory, be easy to detect if one or more impacted locations are compared with several reference locations. Some studies have detected effects associated with outfalls, but without pre-impact data, these remain only correlative in nature.

Many millions of dollars are spent annually in NSW on monitoring the effects of ocean outfalls on the marine environment (Koop and Hutchings, 1996). For sessile marine assemblages however, it is questionable whether much of this monitoring is justified. If the mixed results obtained in this thesis are examined, it becomes clear that if we wish to assess the impact of an existing ocean outfall using inadequate biological monitoring programs, we
are doomed to failure. Many such monitoring programs are currently being done either as part of state government license discharge conditions or because of some "moral" environmental obligation on behalf of the responsible water authority (see Chapter 4; Roberts and Scanes, 2000). In reality it is the community who pays for these studies but the question of whether they are getting their money's-worth remains. There is the potential that any monitoring program will merely be measuring pre-existing differences among locations that occurred within a very short time of an outfall being commissioned. It is also questionable whether much of this monitoring is justified and perhaps the money that is spent on monitoring should be channelled into gaining a better understanding of the processes of sewage-related disturbance in subtidal marine assemblages.
REFERENCES


