Effects of Wastewater Effluent on Macrobenthic Infaunal Communities at Christies Beach, South Australia

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Declaration

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Abstract

The effects of an outfall discharging secondarily treated wastewater effluent into the southeast region of Gulf St Vincent, South Australia, were evaluated. The environmental assessment involved the sampling of macrobenthic infaunal communities, which were then analysed by a variety of methods ranging from multivariate (classification and ordination) to univariate (number of species, abundance, diversity and evenness) and distributional/graphical (log normal and ABC comparison) techniques.

The multivariate analyses of community structure indicated that the Christies Beach Wastewater Treatment Plant effluent outfall had induced changes to the macrobenthic infaunal communities. Whereas there were significant spatial and temporal variations at both the outfall and control sites, differences between the compositions of the macrobenthic infaunal communities at the outfall site and the control site were still evident. Furthermore, the expected gradient response with increasing distance from the outfall was also observed. Differences were consistent with an increased organic and nutrient loading on the benthos resulting from discharge from the Christies Beach Wastewater Treatment Plant.

In measuring community stress, the Abundance-Biomass Comparisons (ABC) showed intermediate to moderately disturbed conditions for stations in close proximity to the outfall. These stations appeared to have communities increasingly dominated numerically by very small opportunistic species (especially spionid polychaetes). Similarly, the neutral model analysis indicated disturbed conditions with reduced diversity for stations close to the outfall. Although plots of abundance of individuals among taxa (log-normal plots) showed that both the outfall and control sites had uneven distribution of abundance groups, interpreted as being indicative of the effects of moderate organic enrichment, the outfall site had more abundance groups, suggesting the possibility of higher organic enrichment at the outfall site. Furthermore, indicator abundance groups at the outfall site had taxa such as capitellid polychaetes, which were responsive to organic enrichment while taxa that characterised the control site (e.g. gammaridean amphipods) were more pollution sensitive. The changes in the various community descriptors (e.g. abundance and diversity) along a gradient of organic enrichment were generally observed to follow models such as the Pearson-Rosenberg model.

The results of this research were also compared with work on other macrobenthic communities in Gulf St Vincent, to address the problem of a single control site. In addition, a more detailed analysis of the infauna data, addressed temporal (inter-annual differences) and spatial variability (resolution of the gradient response) at the outfall site. These detailed analyses showed that the single control site (Moana) was typical of shallow water gulf sites along the metropolitan coast and therefore an appropriate basis for the comparisons in this study. The comparison within the outfall site in terms of spatial resolution of the gradient response and inter-annual variability indicated that regardless of the variability, the macrobenthic infaunal communities appeared to change with increasing distance from the discharge point.

Benthic respirometry was used to quantify the rate of total sediment oxygen consumption at the outfall site and at two control sites. The results showed that the mean rate at the southern control site was lower than the outfall site and the northern control site. However, there were no differences between the northern control site and the outfall site. These differences in rates were probably due to the supply of organic carbon to the sediment from the effluent discharged at the outfall site and a storm water drain at the northern control site, while there were no such discharges in the immediate vicinity of the southern control site.

Overall, the results of this study supported the hypothesis that the Christies Beach effluent outfall has induced significant and persistent changes to the structure of the macrobenthic infaunal communities, which increased in severity in relation to the proximity to the outfall. Furthermore, the temporal patterns of community structure indicated that, over and above the seasonal variations, communities at the outfall site were still different from communities at the control site and appeared to relate primarily to distance from the discharge point. These differences were consistent with the environmental effects one would expect from an effluent discharge.

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Preface

The rationale

The oceans have historically been viewed as boundless in size, able to absorb all wastes and still be able to feed a hungry world. However, it has become evident over the last decade that this is not the case. The Group of Experts on the Scientific Aspects of Marine Pollution (GESAMP), reporting to the United Nations in 1990 on the health of the world's oceans, stated "chemical contamination and litter can be observed from the poles to the tropics and from beaches to abyssal depths". If nothing is done, pollution will lead to "global deterioration in the quality and productivity of the marine environment.... We fear, especially in view of the continuing growth of human populations, that the marine environment could deteriorate significantly in the next decade unless strong, co-ordinated national and international action is taken" (GESAMP 1990b).

The need to ensure the healthy condition of the oceans is now generally recognised. Covering 70% of the earth's surface, the oceans play a dominant role in the biogeochemical processes on local, regional and global scales. The uses of oceanic and coastal areas are multiple, serving many diverse purposes. These include the production of food, as sources of energy, minerals and other raw materials, as a base for tourism, recreation, settlement and transport and it is also the location for both aquatic and shoreline farming.

In the world today, 65% of cities with populations above 2.5 million inhabitants are coastal and this is still increasing. In Australia, the potential problem of a growing population of approximately 18 million with the majority living in the narrow coastal strip extending from Brisbane to Adelaide is recognised. Coastal waters are becoming degraded due to pollution from various sources, especially from the coastal land developments of ports, harbours, towns or cities. With such developments there will be an increase in the discharge of wastewater as sewage effluent, industrial wastes and storm water.

South Australia has two gulfs (Gulf St Vincent and Spencer Gulf); the city of Adelaide is situated on the eastern seaboard of Gulf St Vincent and has a population of approximately 1 million people. The discharge of effluent from the city's wastewater treatment facilities is considered to be a major environmental concern for the State. The South Australian government is expending around $280 million to upgrade existing wastewater treatment facilities in order to meet Environment Protection Authority discharge guidelines by the end of 2001. There are four treatment facilities serving metropolitan Adelaide including the Christies Beach Wastewater Treatment Plant, which serves the southeast region. After treatment, the reclaimed water (effluent) is discharged from this plant into the nearshore southern region of Gulf St Vincent.

Both Gulf St Vincent and Spencer Gulf are inverse estuaries. The high rate of evaporation coupled with very low freshwater input into the marine environment has resulted in the gulf waters being relatively more saline than the waters outside the gulfs (Bye 1976). The gulfs therefore represent a unique environment that may respond differently to various disturbances. There are few data from elsewhere in the world for comparisons or for environmental management decisions to be based on. This present research thus aims to address the paucity of such information in South Australia.

The aims

The aim of this research is to determine the effects of wastewater effluent on benthic communities at Christies Beach with the specific objectives of:

1. characterising the physical, biological and chemical environment of Gulf St Vincent and the region around the study area,

2. ascertaining the extent to which the effluent outfall has affected the macrobenthic infaunal community structure,

3. characterising the structure of macrobenthic infaunal communities in the near shore water of the Adelaide metropolitan coast, and

4. determining the functional response through measurements of sediment community respiration to these changes.

The thesis layout

The first chapter provides an overview of marine pollution. The review concentrates on wastewater effluent as a source of pollution to the marine environment and the effects of effluent on marine communities. Also included is a general review on the use of benthic invertebrates for assessing and monitoring the effects of wastewater effluent. Chapter two gives a description of the general environment of Gulf St Vincent with a more detailed description of the study area based on information from literature, meta-data and some collected data. The selection of sampling sites, the sampling design and an outline of the sampling methods used both in the field and laboratory are given in Chapter three. This is followed by the results of the study assessing the effects of wastewater effluent from the Christies Beach outfall on macrobenthic infaunal communities in chapter four.

Chapter five compares the results of this research with work on other macrobenthic infaunal communities in Gulf St Vincent, addressing the problem of a single control site. In addition, chapter five gives a more detailed analysis of the infauna data at the outfall site, addressing temporal (inter-annual differences) and spatial variability (resolution of the gradient response). Chapter six gives the results of the preliminary work on using benthic respirometry to assess organic and nutrient enrichment from the wastewater effluent. Finally, chapter seven synthesises the results from this research.

Chapter 1 Introduction

> **"Marine pollution is the introduction by man, directly or indirectly, of substances or energy into the marine environment (including estuaries), resulting in such deleterious effects as: harm to living resources; hazards to human health; hindrance to marine activities including fishing; impairing the quality for use of seawater and reduction of amenities". (Intergovernmental Oceanographic Commission, IOC, cited in Gerlach 1981).**

Sources of marine pollution

Inputs to the marine environment may arise from both natural sources or from human activities. Contaminants are substances from anthropogenic inputs in seawater, sediments or organisms where the concentrations are above the natural background level for both that area and the organisms. Contaminants enter the marine environment via a variety of pathways including direct outfalls into estuaries, inshore waters, bays and open coastal areas from coastal towns and industries (GESAMP 1990b, Clark 1997). Rivers are also sources of inputs flowing via their estuaries to the sea, carrying potential pollutants like organic wastes, pesticides, fertilizers, petroleum and oils. In the course of trade, ships carrying oil, liquefied natural gas and industrial chemicals are another source of inputs into the marine environment. The dumping of a variety of materials including dredge spoils, sewage sludge and other wastes such as fly ash constitute offshore inputs. Offshore exploration and extraction of materials also result in various inputs into the sea. Discharges to the atmosphere returning to the land or the sea in rain or particulate fallout constitute atmospheric inputs, with gaseous wastes dissolving directly in the sea at its surface (GESAMP 1990b, Clark 1997).

Disposal of sewage into the sea dates back to the earliest of civilisations (Shepherd and Cannon 1989) and now poses problems for developed and developing countries because of the world's increasing population (Duedall *et al.* 1983). Many major coastal cities in the world discharge their wastewaters to the ocean. In urban areas, both sewage and industrial effluent are often mixed in sewerage systems and reach the sea together as municipal wastewater. Depending on local ocean conditions and the configuration of the sewage discharge, effluent may be rapidly dispersed and diluted with little visible trace, or it may remain in a highly visible and relatively undiluted plume in the vicinity of a discharge point (GESAMP 1990b). Disposal into the sea is one of the most serious, and increasingly common sources of disturbance in the marine environment (Gerlach 1981).

Effects of wastewater effluent

The effects of wastewater effluent are varied depending on whether it is viewed from an anthropocentric or ecocentric point of view. Anthropocentrically, health risks from the presence of sewage pathogens are a major impact from effluent discharges (GESAMP 1990b). Other health risks are from the various contaminants in seafood, which are a consequence of the bioaccumulation of toxins from effluent in marine organisms. Ecocentrically, eutrophication associated with effluent discharges due to nutrients and organic carbon affect marine organisms (GESAMP 1990b, Clark 1997). In addition, pollutants in the effluent may also be directly toxic to the organisms (GESAMP 1995). In this present study, the effects of wastewater effluent were assessed from an ecocentric point of view.

Wastewater effluent is primarily organic in nature and is usually broken down by bacteria (Gerlach 1981, Clark 1997). This process results in the oxidation of organic matter to inorganic compounds (Gerlach 1981), thereby reducing the concentration of oxygen in the water (Clark 1997). Although there is oxygen replenishment from the air, anaerobic oxidation may occur at a slower rate than aerobic oxidation, which results in an accumulation of wastes. The end products of anaerobic oxidation, hydrogen sulphide, ammonia and methane, also exacerbate the depletion of oxygen in the water (Clark 1997).

Besides causing oxygen depletion, effluent also contains contaminants, which can affect marine organisms. Eight major contaminants were identified by GESAMP in their report on the state of the marine environment (GESAMP 1990b). These contaminants include trace metals, microorganisms, organic materials affecting oxygen balance, nutrients, synthetic organic compounds, petroleum-related compounds, particulate matter/ sediments and heat.

Effluents usually contain a mixture of contaminants with varying composition. A specific agent or compound from any of the above contaminants can have different degrees of impact on the marine environment. The contaminants may be toxic to organisms and may alter various aspects of an organism's biochemical, cellular and physiological functions (Addison 1988, Capuzzo 1988, Moore 1988). The magnitude of the impact is dependent upon the chemical and biological characteristics of the compound, the total amount and concentration, bioaccumulation, persistence, recycling potential and the characteristics of the receiving environment (GESAMP 1990b, Connell 1993). The impacts of effluent on marine communities therefore have both spatial and temporal components. Spatially, impacts may extend for some kilometres (Reish 1980, Mearns and O'Connor 1984, Fry and Butman 1991) or alternatively may be restricted to the immediate areas surrounding the outfall (Littler and Murray 1978, Fairweather 1990, Anderlini and Wear 1992). Changes may also occur along similar spatial gradients in time, either as effluent discharge is increased or abated (Pearson and Rosenberg 1978, Spacie 1986, Clark 1997).

The effects of effluent pollution can occur at the cellular, individual, population or community levels of organisation, but the vast majority of effects have been documented at the community level (Underwood and Peterson 1988). Benthic organisms are directly affected by sewage and sewage sludge dumped in the sea. There are a whole host of biological responses of benthic organisms to the various contaminants from wastewater effluent (GESAMP 1995). For example, benthic communities exposed to the sedimentation of particulate matter rich in nutrients and bacteria, may result in changes in the number of species, species abundance, biomass, diversity, mean size, spatial distribution and temporal variability, composition of trophic groups, fecundity, mortality, recruitment, etc. More sensitive species may be reduced as a consequence of smothering by particulate matter and the reduction of oxygen concentration from enhanced bacterial activity (Pearson and Rosenberg 1978). However, species that are more tolerant may flourish because of the input of extra nutrients (Pearson and Rosenberg 1978, GESAMP 1990b, GESAMP 1995, Clark 1997).

Studies assessing the effects of wastewater effluent

Many studies have been undertaken, both internationally and in Australia, assessing the impacts of wastewater effluent on individual organisms as well as whole communities. Changes in community structure such as a decrease in species diversity, dominance by a particular species and an increase in variability may all result from pollution by wastewater effluent. Increases in nutrient levels from effluent can also lead to loss of habitats adjacent to the outfall. For example, work in Oslofjord, Norway, showed a decline in species number and abundance, due to the increased organic loading and resulting decreased oxygen levels after the commissioning of a sewage treatment plant (Aschan and Skullerud 1990). In Florida, USA, the polychaete fauna of a sandy intertidal habitat receiving effluent from a sewage outfall was dominant, having higher number of individuals, total biomass, and average species numbers (Dauer and Conner 1980). In the Arabian Gulf, Ismail (1992) showed that diversity was higher in response to organic enrichment from sewage dumping with the appearance of opportunistic species such as the polychaete *Capitella capitata*. The work by Anderlini and Wear (1992) in New Zealand indicated that benthic macrofauna community structure within a radius of approximately 500 m of a sewage outfall was affected by the discharge of effluent.

Similar work on the impacts of wastewater effluent has also been carried in Australia. Fairweather (1990) in a study in New South Wales showed that there was a reduction in species diversity of seashore biota with a shift in dominance to ephemeral green algae and an absence of animals. Another study, also in New South Wales, showed that there was increased variability of shallow subtidal assemblages of organisms (Chapman *et al.* 1995). A longer term monitoring study over eight years at Coffs Harbour (New South Wales) indicated that impacts on algal species richness and changes to the structure of invertebrate communities living in kelp holdfasts, were restricted to within approximately 300 m of the outfall, but that the cover of the ephemeral green alga *Ulva lactuca* was significantly greater than at reference sites up to a distance of 500 m from the point of discharge (Smith 1996). In Victoria, work by Brown *et al.* (1990) on intertidal macroalgae; found that there were fewer species at sites close to the outfall. There was also less vertical stratification near the outfall with the loss of canopy-forming species of algae.

Locally in South Australia, loss of seagrasses has been attributed to increased nutrient levels from effluent outfalls (Neverauskas 1987, Burns 1992). It was argued that increased nutrient levels from effluent discharge led to excessive growth of epiphytes on the seagrasses, causing shading and ultimately death of the seagrasses (Neverauskas 1987). The increased nitrate levels in the vicinity of an effluent outfall was found to be toxic to seagrasses, altering the morphology of shoots of *Zostera muelleri*, thereby decreasing the biomass of the seagrasses (Burns 1992). Wastewater effluent also indirectly affected mangroves where increased drift algae and seagrass detritus, a consequence of effluent impact, affected the survival of mangrove propagules and the subsequent establishment of seedlings (Fairhead 1995).

Environmental assessment of the effects of wastewater effluent

Marine environmental assessment is "the orderly process of gathering information about exposure and effect in a possibly stressed system and determining the significance and causes of any observed changes" (GESAMP 1995). The assessment process ideally consists of three steps (GESAMP 1995).

- A study prior to disturbance where the evaluation of the consequences of loss or alteration of a population, community or habitat is carried out.
- The prediction of the extent of such losses or alterations based on this prior study.
- An assessment after the disturbance where various measurements are carried out.

A disturbance is "a chemical or physical process caused by humans that may or may not lead to a response in a biological system within an organism or at the level of whole organisms or assemblages" (GESAMP 1995). The discharge of wastewater effluent is a press disturbance where any changes to the environment are chronic and temporally sustained. In contrast, pulse disturbances are acute disruptions that are not sustained through time (such as in an oil spill). Other impacts may have characteristics of both types of disturbances, such as commercial harvesting, where the activity may be a one-off pulse disturbance or a series of repeated pulses that may manifest as a continuous stress (Bender *et al.* 1984, Keough and Quinn 1991). When a disturbance evokes a response, it becomes a stress (GESAMP 1995). Effluent discharged into the sea alters both the physical and chemical nature of seawater (Baker *et al.* 1995) and is a major source of stress for marine communities.

Chemical assessment and monitoring has been the most commonly used technique to investigate anthropogenic disturbance and stress in the marine environment (Maher and Norris 1990, Connell 1993, Norris and Norris 1995). However, chemical surveys have limitations in that they give no indication of biological effects (Lack and Johnson 1985). Therefore, in the past two decades, biological assessments have increasingly been used because they evaluate biological consequences, integrating synergistic and antagonistic effects of pollutants over time (Maher and Norris 1990, Connell 1993, Warwick and Clarke 1993a). In addition, although the sampling cost and effort may be perceived to be higher for biological assessments, this might not necessarily be the case. Overall, the effort and cost of collecting may be lower if the same standards of accuracy and precision (with regards to variability and replication) are applied (Norris and Georges 1986) or if rapid approaches are used (Plafkin *et al.* 1989).

There are a multitude of biological assessment methods in use, ranging in levels from cellular, to individuals, populations or whole communities (Bayne *et al.* 1988, ANZECC 1992, GESAMP 1995). Biochemical, cytological, and physiological measures of stress, such as cellular indices based on histological changes (Moore 1988, Nowak 1996), and physiological indices such as scope for growth (Bayne *et al.* 1979, Lack and Johnson 1985, Capuzzo 1988, Widdows and Johnson 1988), have been used extensively to measure the type and magnitude of pollution responses. Ecological surveys based on differences in the measures of abundance, diversity or biomass indices of benthic macro- and meiofauna (e.g. Warwick 1986, Warwick *et al.* 1987, Warwick 1988b, Austen *et al.* 1989, Aschan and Skullerud 1990, Anderlini and Wear 1992, Agard *et al.* 1993, Underwood and Chapman 1996) between polluted and unpolluted areas have also been extensively used to assess pollution impacts.

All of these biological assessment techniques will only be useful in the assessment of impacts in the marine environment if they are able to detect differences between natural and anthropogenically induced characteristics (McIntyre 1977, Boesch *et al.* 1990). To differentiate any observed change in parameters being caused by the presence of a pollutant from the natural fluctuations inherent in the marine environment, is the main problem facing biological assessments (McIntyre 1977, Butler 1984, Miller 1984, Boesch *et al.* 1990, Warwick 1993). There is therefore the need for baseline measurements to understand these inherent fluctuations (Miller 1984, Maurer *et al.* 1993). However, frequently insufficient or no baseline data are available (Sheehan 1984, Warwick 1993), although an appropriately designed sampling programme may alleviate this problem (GESAMP 1995).

The minimum ideal sampling design is the BACI (Before/After, Control/Impact) design (Green 1979). This design constitutes the taking of a sample before and a sample after the potential disturbance. Essentially, it is a symmetrical design with a single putatively impacted location and a single non-replicated control location. Newer designs, such as beyond BACI (Underwood 1991), involve an asymmetrical contrast of a single supposed impacted location with a set of control locations. This design may include temporal replications; that is, samples are taken at both the impacted and the set of control locations several times before and after the potential disturbance. Beyond BACI designs are more useful, enabling studies of disturbances that affect temporal and spatial variations (Underwood 1991).

Although ideal, it is unusual to have data from the site under investigation prior to the pollution event. Spatial control sites may be used in such cases where environmental factors other than the impact under study are considered to have negligible or known effects (Warwick 1993, Cheshire 1999). These are then compared with the impacted site. However, in a heterogeneous environment, good spatial control samples may also be difficult to obtain (Cheshire 1999). Hence, the use of absolute measures, which do not require an extensive series of temporal and spatial control samples, can be useful in complementing any comparative studies (Warwick 1993).

The choice of biological variables to be measured is a crucial component in any environmental assessment and primarily depends on the questions being asked. In addition, the selection of appropriate variables has to be relevant to the study area in terms of the abiotic and biotic components of the system and must be feasible with acceptable precision, accuracy and cost (Maher *et al.* 1994). Due to the spatial and temporal variability of the marine environment (Morrisey *et al.* 1992a, Morrisey *et al.* 1992b) and the different specificity of biological variables to contaminants (Bayne *et al.* 1988), it would thus be advantageous to use a whole suite of measurements (from cellular to community) to cover these differences (Peterson 1993). Using all the techniques for environmental assessment is ideal but rarely possible. Therefore when little is known about the ecosystem suspected of being disturbed, examining a larger component of the ecosystem such as at the community level has the advantage of minimising the risk of missing any effects because a community integrates conditions over a period of time (Warwick 1993).

Benthic communities in the assessment of the effects of wastewater effluent

Benthic communities are diverse, representing almost every phylum of invertebrates in the animal kingdom. These organisms may be grouped by size, by mode of life, or by feeding habits (Mann 1976). An easy division commonly used by benthic ecologists is: (a) infauna, organisms that live within the bottom sediments, and (b) epifauna, those that live on or above the sea floor. Important components of the infauna include polychaete worms, burrowing crustaceans and burrowing clams, while mussels, seastars, sea urchins and large crustaceans are examples of epifauna. The infauna is often further divided by size into macrobenthos, meiobenthos and microbenthos and the dividing lines are roughly 0.5 mm and 63 μm. The present study concentrated on elucidating differences in the structure of communities of macrobenthic infauna associated with an effluent outfall.

Advantages

The macrobenthic infauna is the component of benthic communities about which most is known and they are increasingly being used as indicators of environmental degradation or restoration (Clarke and Green 1988, Austen *et al.* 1989, Warwick *et al.* 1990, Agard *et al.* 1993).

There are several advantages of using macrobenthic infauna (Bilyard 1987). They are easier to sample and sort than the smaller forms (meio- or microbenthos). More importantly, macrobenthic infauna are sedentary and closely associated with the sediments. Some live within the sediment and are directly dependent on the nature of the sediment. Others, like suspension feeders, may just depend on the sediment for support. The sedimentary environment, in which macrobenthic infauna live, is defined by the distribution of sediments, together with water movement, turbulence and suspended load. Therefore the infauna must either adapt to any environmental stress or become extinct.

Being diverse, macrobenthic infauna encompasses a wide range of sizes, reproductive strategies, feeding behaviours and life histories. Therefore, they include both sensitive and tolerant species, which will have a range of responses to any disturbances (Pearson and Rosenberg 1978, Boesch and Rosenberg 1981).

As prey to many animals, macrobenthic infauna has the potential to mediate the transfer of toxic substances to higher trophic levels, thereby initiating pathological responses in predators (Mann 1976). They are also mediators of nutrient recycling from the sediments into the water column (Rhoads 1974, Nowicki and Nixon 1985).

Macrobenthic infauna is also relatively immobile and persistent when compared to the pelagos or epibenthos; therefore they are useful in assessing local effects. The macrobenthos is also capable of integrating conditions over a period rather than just reflecting the conditions at the time of sampling. Essentially macrobenthic infaunal communities are able to respond to localised conditions and therefore the community structure at any one site can be viewed as an integrated response to the environmental conditions at that site (Warwick 1993). With such diverse characteristics, the responses of macrobenthic infauna to sediment contamination thus allow for the spatial definition of impacts.

Measurement

There are several indices of community structure that measure ecological responses to anthropogenic stresses. Univariate indices involve the measurement of a single variable (intrinsic univariate) or the combined properties of more than one variable (contrived univariate), responding to an environmental stress (GESAMP 1995). Loss of species is a commonly used intrinsic univariate measure for assessing environmental stress. The loss of primary species often results in the loss of dependent species (Pearson and Rosenberg 1978). Diversity is a contrived univariate measure where the number of species and the number of individuals are integrated into a single value. Diversity decreases in response to various stresses (Pearson and Rosenberg 1978, Gray *et al.* 1990, Olsgard and Hasle 1993), but only significantly under severe stress (Gray *et al.* 1990, Warwick and Clarke 1991). Changes in several aspects of community structure can also be measured and these include changes in the type or abundance of dominant species and even biomass. Under severe stress, dominance by a few species will increase and these are the so-called opportunistic species (Pearson and Rosenberg 1978). Examples include dominance by capitellid and spionid polychaetes under extreme organic enrichment (Pearson and Rosenberg 1978) and in response to mine waste discharge (Olsgard and Hasle 1993). Biomass dominance patterns also change with increased stress; thus comparisons of dominance patterns of abundance and biomass changes can be used to detect environmental changes (Warwick 1986, Warwick *et al.* 1987, Warwick and Clarke 1994). Other measures such as spatial distribution of individuals, temporal variability, fecundity, age or size-specific patterns of mortality and rates of birth and recruitment can all be indicators of population well being.

Multivariate measures represent the range of properties of an ecosystem more completely because they essentially retain the information about more than one variable. It is a valuable technique especially for assessing change due to stress in a complex assemblage of many species (GESAMP 1995, Clark 1997). Changes in abundance patterns and species differences among sites are incorporated, thus integrating many of the properties of univariate measures. Multivariate analyses based on species abundance have been shown to detect subtle effects of stress on natural systems when univariate measures could not (Gray *et al.* 1990, Warwick and Clarke 1991, Warwick and Clarke 1993b).

The problem at Christies Beach

The Christies Beach Wastewater Treatment Plant serves the southeast region of metropolitan Adelaide (see Fig. 2.2 in Chapter 2). There is little information on the impact of this wastewater effluent outfall on the immediate environment. Water quality around the outfall has been monitored since 1972 with particular attention to levels of inorganic nitrogen and phosphorus in the effluent and receiving waters (Steffensen 1985). However, there have been no biological surveys except for a one-day survey in 1978 undertaken by officers of the responsible management agency (formerly the Engineering and Water Supply Department). The biological survey comprised some collection of algae and sessile animals with a subjective visual assessment of relative abundance of flora and fauna in the area immediately around the outfall. These programmes concluded that the outfall and effluent discharged only had a localised effect on the water chemistry and that the biological communities were largely unaffected and were generally similar to comparable unpolluted areas (Steffensen 1985).

Subsequent studies have found results contrary to those reported by the management agency, including evidence of a possible extended effect of the wastewater effluent on benthic communities in the region (Cheshire and Kildea 1993, Cheshire *et al.* 1996, Cheshire and Miller 1999). A study of the effects of beach sand replenishment dredging by the University of Adelaide surveyed nine sites that extended north and seaward of the outfall (Cheshire and Kildea 1993, Cheshire *et al.* 1996, Cheshire and Miller 1999). This work comprised a series of quantitative surveys of both epibenthic and infaunal communities, which concluded that the site nearest the outfall appeared to be degraded, with a reduced abundance of most taxa relative to the other sites in the study. March (1996) carried out a detailed analysis of the communities in this region using the data collected by the University of Adelaide in 1992 and 1993. Her work demonstrated that there were differences in the infaunal communities at the site closest to the outfall but argued that these differences were not consistent with what was known about responses to nutrient and organic enrichment. The conclusion from these studies was that more work was required to characterise both the nature and variability of communities associated with the wastewater effluent discharge at Christies Beach.

This led to the present study, designed specifically to assess the effects of wastewater effluent discharged at Christies Beach. The macrobenthic infaunal community was used in the assessment to enable comparisons with previous studies (Cheshire and Kildea 1993, Cheshire *et al.* 1996, Cheshire and Miller 1999). The Christies Beach Wastewater Treatment Plant has been in operation since 1971, which implies that the macrobenthic infauna in the area has been subjected to chronic disturbance since then. As no prior sampling of the macrobenthic communities had been done, it was not possible to implement the ideal BACI or beyond BACI sampling design in this study at Christies Beach. Consequently, in the present study, the community structure at the impacted site (Christies Beach) was compared with communities from non-impacted 'control' sites.

This included an investigation of the structure of communities both over a series of sampling periods and at a range of spatial scales.

Objectives

There are four main objectives in this study.

1. To characterise the physical, biological and chemical environment of the Gulf and the region around the outfall and control sites (Chapter 2).
2. To compare and contrast the macrobenthic infaunal communities between the outfall and the control sites (Chapter 4).
3. To compare communities from the outfall and control sites with other communities in the Gulf, addressing spatial and temporal variability (Chapter 5).
4. To quantify the physiological responses of the sediment communities (in terms of oxygen exchange rates) at both the outfall and control sites (Chapter 6).

Chapter 2 The Environment of Gulf St Vincent

With an understanding of the approaches that are available for analysing macrobenthic infauna data (Chapter 1), this chapter will present the particular context of the effluent outfall at Christies Beach within the Gulf environment. The chapter aims to present this information in five parts, which include (i) a description of the physical environment, patterns of flow and exchange and hydrology of Gulf St Vincent, as drawn from existing literature, (ii) a description of the biotic environment, also drawn from existing literature, (iii) a general description of the Christies Beach Wastewater Treatment Plant and other discharges into the area, (iv) an analysis of other environmental data (meta-data) which were made available to this study but have not been synthesised elsewhere, and (v) an analysis of some data collected in this present study relating to sediment grain size and organic carbon content. This information is included at this point in order to provide a coherent summary of all the environmental data but there is the need to acknowledge that a full rationale for site selection will not be detailed until Chapter 3.

Physical environment of Gulf St Vincent

Gulf St Vincent is located on the southern coastline of Australia and is adjacent to Adelaide, the capital of South Australia. Yorke Peninsula and Fleurieu Peninsula define the western and eastern bounds respectively. It is a shallow marine basin with a surface area of 6800 km^2 and a mean depth of 21 m (Bye 1976). Investigator Strait to the southwest and Backstairs Passage to the south, connect it to the ocean, with Kangaroo Island situated at the mouth of the Gulf between the two straits (Figure 2.1).

Tidal motions and circulation

The tides in the Gulf are affected by the dual connection with the open sea. As such, the time of high water within the Gulf is almost identical everywhere with typical tidal amplitude of 1 to 1.5 m. In addition, a "dodge" tide, where there is no net movement of water for a whole day occurs every fortnight in the Gulf because of the near equality of the semi-diurnal lunar and solar tidal constituents (Bye 1976).

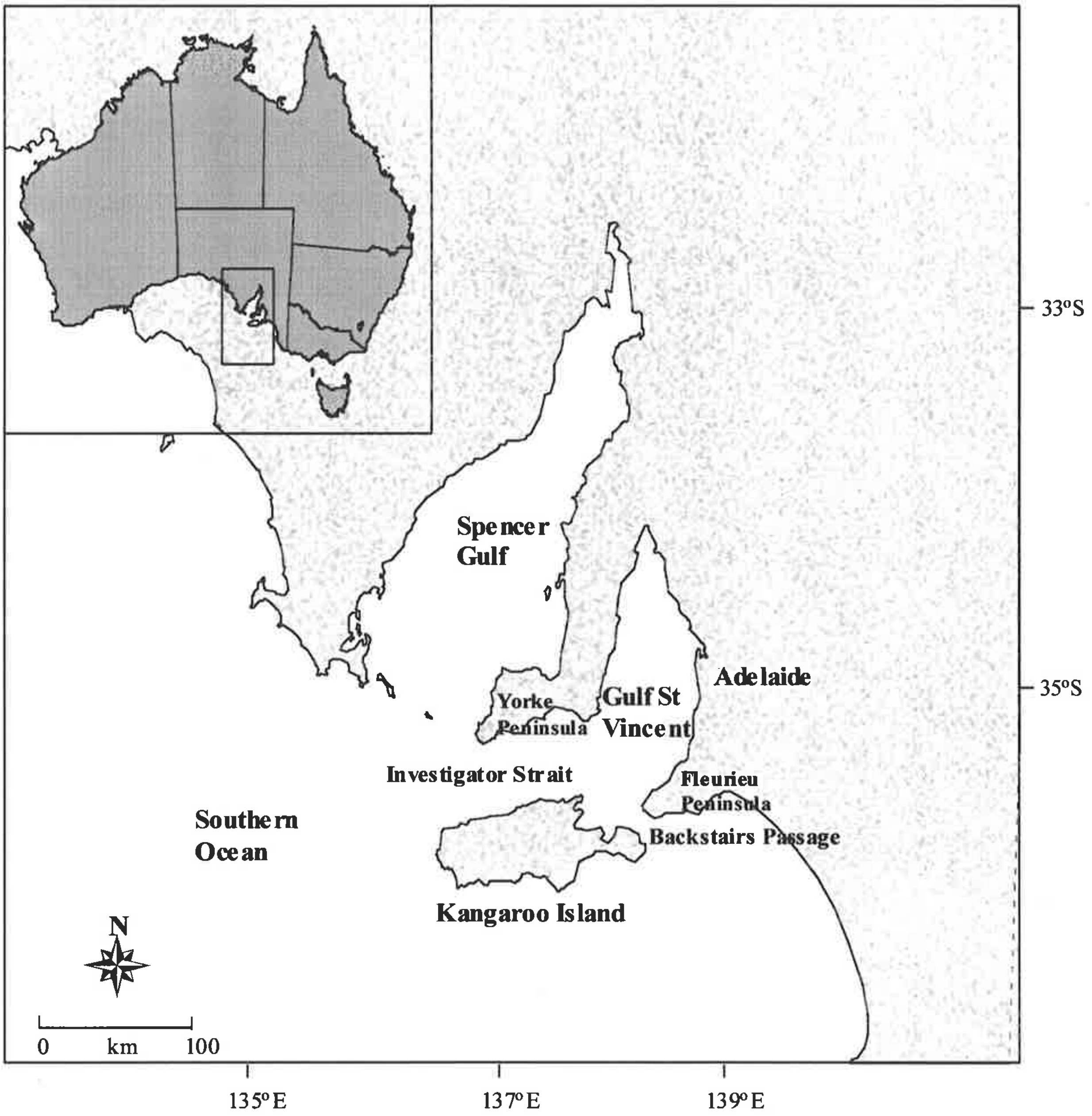

Figure 2.1 **Map showing the southern coastline of South Australia and the straits and peninsulas defining Gulf St Vincent.**

The currents within the Gulf are mainly tidal, with the general circulation affected by local wind, exchange of heat and water across the sea surface and the circulation in the deep ocean adjacent to the Southern Ocean (Bye 1976).

The continuous inflow into the Gulf is along the west, with an eastward flow on the north coast of Investigator Strait. The outflow is in the central part, which either goes eastward through Backstairs Passage or returns as a counter-current in Investigator Strait, occurring especially in summer. There is also an eddy-like circulation adjacent to the Fleurieu Peninsula (Bye 1976). The tidal currents in the coastal waters offshore from Adelaide have their principal axis in the longshore direction with speeds up to 0.2 - 0.3 m s^{-1} (Bye 1976). This results in a north-south flow along the metropolitan coast in the Gulf predominantly northwards in summer and southwards in winter.

The flushing time, defined as the time required for complete renewal of waters for Gulf St Vincent, oscillates around a mean of about half a year with minimum in the winter months and maximum in late summer and early autumn (Tippins 1996).

Hydrology

The Gulf lies in an area where evaporation significantly exceeds rainfall. A result of this, coupled with its shape and the location of Kangaroo Island, is that the Gulf behaves as an inverse estuary. Salinity values in the Gulf, commonly between 36 and 38 ppt around the Adelaide area, are higher than typical oceanic salinity. A salinity gradient from the mouth to the head of the Gulf also exists (Tippins 1996). The balance between water exchange and net evaporation generates salinities as high as 47 ppt at the head of the Gulf (Bye 1976). Vertical mixing brought about by wind and tidal current result in a well-mixed vertical profile for salinity or temperature. However, there are seasonal adjustments in temperature and salinity that are associated with the net effects of radiation, evaporation and rainfall (Tippins 1996). The northern region of the Gulf has a greater annual variation due to its shallowness, smaller volume and greater evaporation. The southern region, on the other hand, has a greater thermal capacity, responding slower to summer heating and hence has smaller variations. Consequently, the northern region of the Gulf is denser with higher salinity during summer and lower temperature during winter.

Biotic environment of Gulf St Vincent

Shepherd and Sprigg (1976) comprehensively described the biotic communities in Gulf St Vincent, basing their descriptions on dominant species. Three different communities were described, which included seagrass communities, algal communities and animal assemblages. The various seagrass communities were defined on dominance by particular species, which is in turn dictated by water movements and substrate type. The *Posidonia* *australis* community occurred in extensive meadows while the *Amphibolis antarctica* community occurred in small stands where there was stronger currents and more mobile substrate. *Heterozostera tasmanica* and *Halophila ovalis* were widespread but sparsely distributed. Algal communities occurred mainly on subtidal cliffs and rocky bottoms in the lower part of the Gulf to a depth of about 15 m. This distribution of algae is largely dictated by the availability of suitable substrates for attachment, light reaching the habitat and water movement. The distribution of animals is dependent upon the kind of substrate, depth, light and hydrological conditions. Based on these factors, Shepherd and Sprigg (1976) described six distinct assemblages, which included *Pinna*-holothurian assemblage, Ascidian-scallop assemblage, Bryozoan assemblage, *Malleus/Pinna* assemblage, *Heterozostera/Lunulites* assemblage and Sponge/bryozoan assemblage.

The study area in the southeast region of Gulf St Vincent is different from the northern part of the Gulf. Unlike the northern region, where seagrass meadows are extensive, the southeast region is dominated by rocky reefs and algal communities and only has patchy seagrasses occurring in sandy areas (Cheshire and Miller 1996). The reef at Hallett Cove is to the north of the outfall (Figure 2.2), comprising of several long narrow rock strips parallel to the shore in a north-south direction. There is patchy algal cover dominated by *Sargassum* spp. and *Ecklonia radiata* (Cheshire and Turner 2000). Horseshoe reef is located immediately to the south of the outfall (Figure 2.2) and has slopes and platforms covered by patches of mixed fucoid communities (Cheshire and Turner 2000). Aldinga and Noarlunga reefs, located 3-4 km from the outfall (Figure 2.2), have been aquatic reserves since 1971. These reefs are dominated by fucoids such as *Sargassum* spp. and *Cystophora* spp. and the kelp *Ecklonia radiata* (Cheshire *et al.* 1998, Cheshire and Turner 2000). Further south, at 8 km from the outfall is Moana reef (Figure 2.2), which consist of boulders and holes with high abundances of phaeophycean macroalgae, fucoids and kelp (Cheshire and Turner 2000).

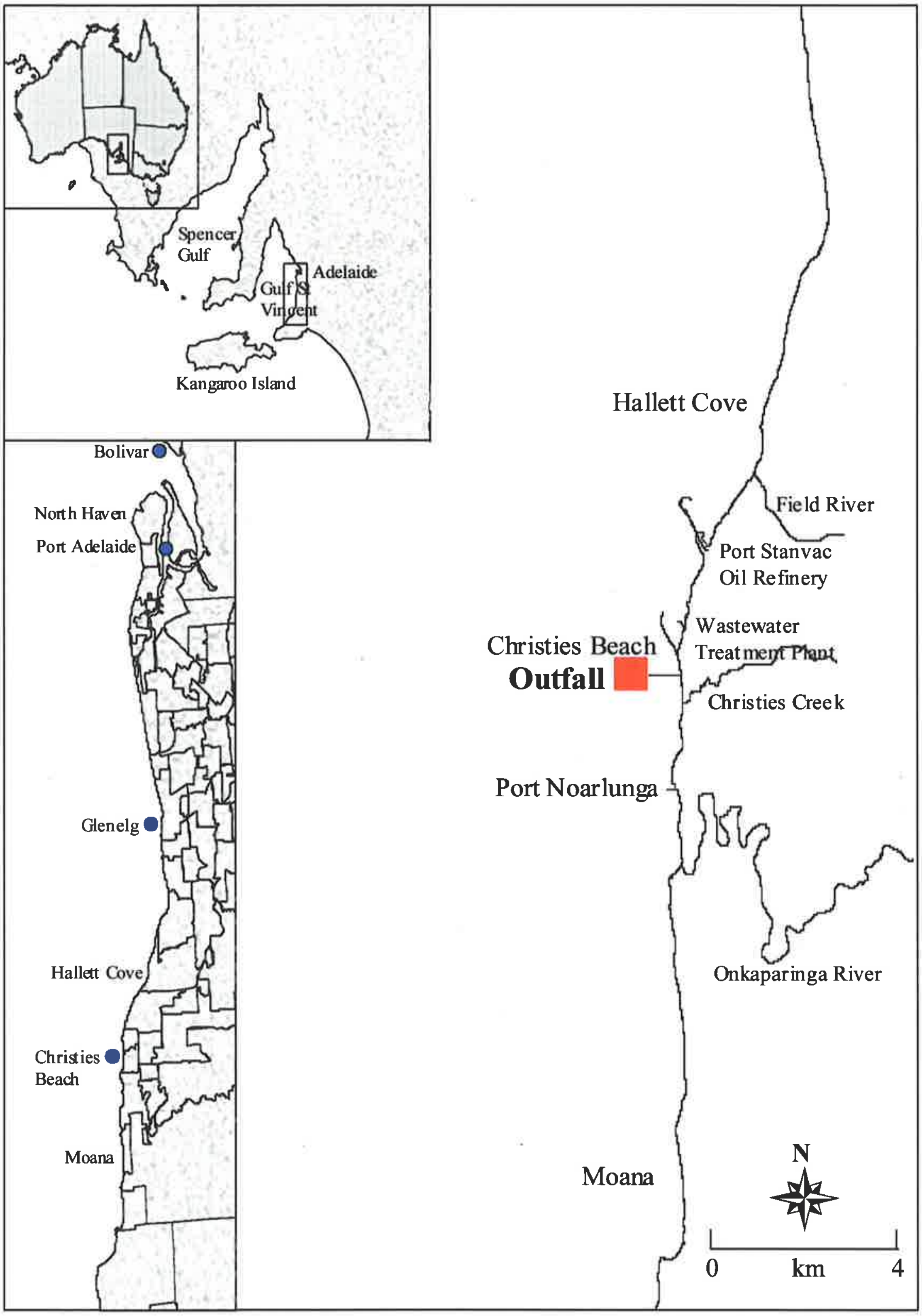

Figure 2.2 Map of the southeast coastline of Gulf St Vincent showing the location of the four outfalls (●) serving metropolitan Adelaide. Shown in detail are the location of the outfall at Christies Beach (🟥) and other sources of discharge into the area.

The Christies Beach Wastewater Treatment Plant and its environs

The sewerage system of metropolitan Adelaide is divided into four drainage areas with each being served by a wastewater treatment plant (Figure 2.2).

The Christies Beach Wastewater Treatment Plant serves the southeast region of metropolitan Adelaide (Figure 2.2). The drainage area covers approximately 130 km^2 (Gutteridge Haskins & Davey Pty Ltd 1994). The plant was established in two phases, with the first phase commissioned in 1971 and the second in 1979. The plant currently serves over 200,000 people plus industry with industry contributing 8 to 10% of the overall flow to the plant and between 20 to 25% of the total organic load (Gutteridge Haskins & Davey Pty Ltd 1994).

Wastewater arriving at the treatment plant goes through secondary treatment, which includes screening, grit removal and pre-aeration, primary sedimentation and scum removal, diffused air activation of sludge, secondary sedimentation and chlorination. After treatment, an average of 27 ML per day of the reclaimed water (effluent) is discharged from the plant into the near shore southeast region of Gulf St Vincent via an outfall (United Water 1997, United Water 1998). The outfall is a 675 mm open-ended pipe extending for 200 m from shore to a depth of about 6 m at low tide. The outfall opens into an area of bare sand, with patchy algal growth on the outfall pad and parts of the pipe that are not buried under sand (Gutteridge Haskins & Davey Pty Ltd 1994).

Other sources of discharge into the study area

Other land based discharges via rivers or storm water pipes have small and irregular flows in the southeast region of Gulf St Vincent. Discharges in the vicinity of the outfall include Christies Creek at Christies Beach and Field River at Hallett Cove (Figure 2.2). These two rivers periodically discharge large volumes of turbid water over short periods. Christies Creek is known to be the second largest discharge in the area, with an annual flow of 4900 ML contributing a large volume of total dissolved solids and a significant amount of nutrients (March 1996). However, there are generally no flows in the summer months and the mouths of both Christies Creek and Field River become closed with sand. Thus, the impact of these rivers on the study site would be minimal compared to the amount of wastewater effluent discharged at Christies Beach (9600 ML in 1997).

The Onkaparinga River is located 4 km from the Christies Beach outfall (Figure 2.2). It has an extensive catchment area and flow into the sea fluctuates through time and is at least partly controlled by a reservoir (Mount Bold Reservoir) in the upper reaches of the river. Consequently the load of organic and inorganic waste input into the area although significant, also fluctuates (Lewis 1975, Manning 1985). Even though the prevailing current is net northerly (see above), the distance of the river away from the study area would limit its potential impact on the present study. The only significant input into the sea in this area is therefore via the effluent outfall from the Christies Beach Wastewater Treatment Plant.

Meta-data

Additional data, which have not been synthesised elsewhere, were obtained from various sources to supplement the data collected in this study. These meta-data included temperature data from the National Tidal Facility, rainfall data from the Bureau of Meteorology and water quality data from United Water International/South Australian Water Corporation.

Temperature and rainfall

Data obtained from the National Tidal Facility showed seasonal fluctuations in seawater temperature. The mean monthly temperatures were relatively lower from 1994 to 1996, with the widest range of 12.7°C to 22.4°C in 1995. In 1997 and 1998, higher temperatures were recorded, ranging from 12.7°C to 23.3°C in 1997 and 11.9°C to 25.8°C in 1998. The last quarter of 1997 especially, had higher mean monthly temperature and remained high until April 1998 (Figure 2.3). This higher temperature has been attributed to the phenomenon known as El Niño Southern Oscillation (ENSO).

The ENSO phenomenon also correlates with the amount of rainfall. Data obtained from the Bureau of Meteorology showed that highest rainfall was generally recorded in winter months (June - August), however in 1997, highest rainfall was recorded from the end of winter (August) to spring (September - November) (Figure 2.4).

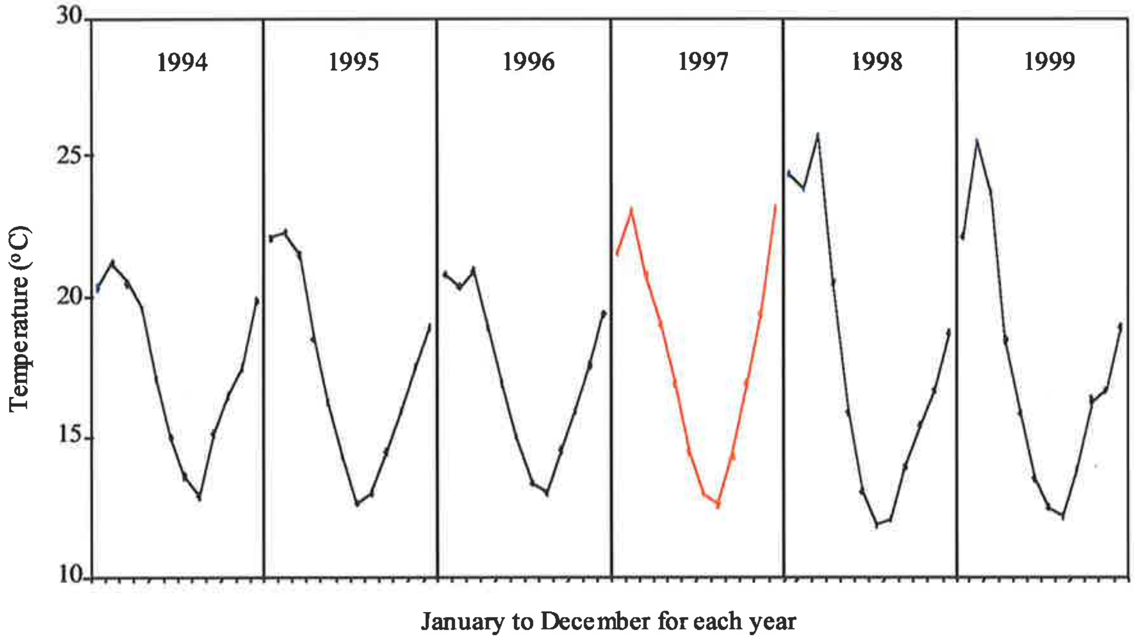

Figure 2.3 Mean monthly temperatures (January to December) for 1994 to 1999 with higher temperatures recorded from November 1997 to April 1998 (1997 in red indicates year of sampling).

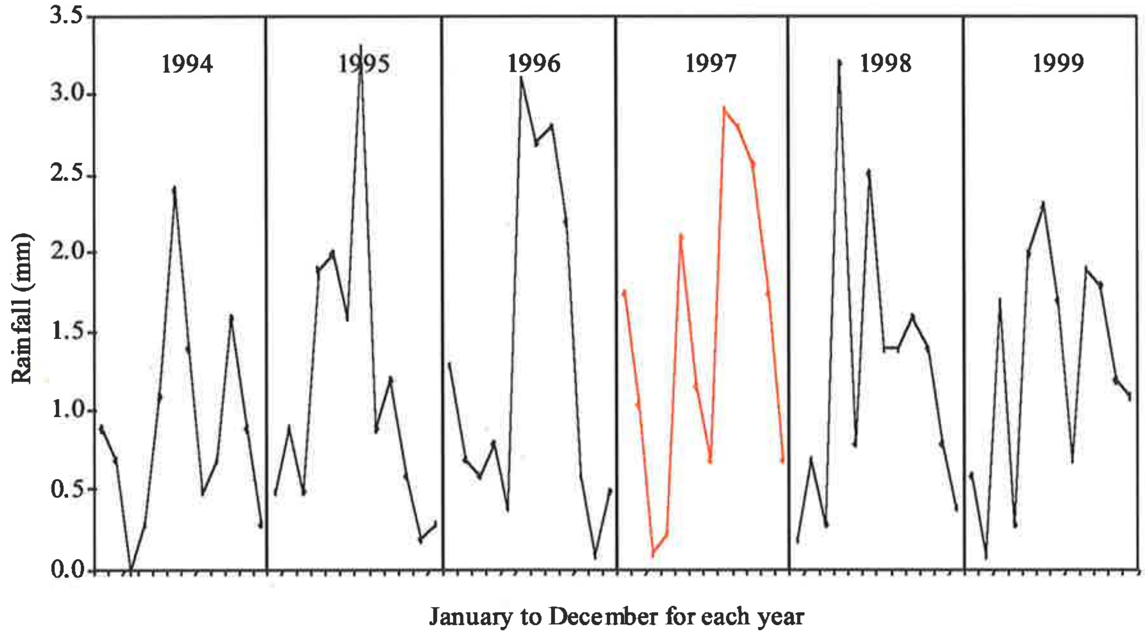

Figure 2.4 Mean monthly rainfall for 1994 to 1999 showing higher rainfall in winter months for most years except for 1997 where highest rainfall was recorded mostly in spring (1997 in red indicates year of sampling).

Water quality

The only known ambient water quality assessment carried out in the Gulf was that of the metropolitan bathing waters between February 1995 and December 1996 (Environment Protection Authority 1997). Other water quality monitoring were those carried out as part of licensing for discharges into the marine environment. The South Australian Environmental Protection Authority requires the licensee (United Water International/ South Australian Water Corporation) of the various wastewater treatment plants serving metropolitan Adelaide, to monitor water quality of the effluent (at point of discharge) and receiving waters (within or beyond the dispersion zone). Various physical and chemical parameters were measured *in situ* and water samples were collected at a depth of 0.5 m and sent to the Australian Water Quality Centre for analysis (United Water 1997, United Water 1998). The South Australian Guidelines for Licensing Discharges to the marine environment were adopted from the United States Environment Protection Authority (USEPA), Environment Canada or the ANZECC Guidelines (Environment Protection Authority 1993). A detailed summary of the various parameters measured and synthesised for 1997 (year of present study) is given in Appendix I. Contaminants highlighted here are nutrients, heavy metals and microbiological agents.

Nutrients

There is a general lack of information on the nutrient status of Australian marine environments (Brodie 1995) and Gulf St Vincent is no exception. The status of nutrients of Australian marine waters depends on the hydrodynamics of the area, such as flushing times and sources of input (upwelling or effluent discharges) (Brodie 1995). The ratio of nutrient elements in the marine waters is important for primary production (GESAMP 1990a, Brodie 1995). The rate of primary production of new organic matter is generally limited by nutrient availability and by light intensity and temperature (GESAMP 1990a). The principle nutrients associated with primary production are carbon (C), nitrogen (N) and phosphorous (P) but others such as calcium, silicon, iron, copper and manganese may play a supplementary role (GESAMP 1990a, Lobban and Harrison 1994). A moderate input of nutrients may lead to increases in primary production. Excess nutrients may result in extravagant growth of plants and the subsequent bacterial decay of dead plant material may result in oxygen depletion. Both excessive plant growth and oxygen depletion (signs of eutrophication) can lead to alterations of community structure (GESAMP 1990a, Brodie 1995, Clark 1997).

Waters in Gulf St Vincent are generally regarded as oligotrophic, with very low natural levels of nitrate, phosphate and ammonia (Butler *et al.* 1997). This is typical for South Australian waters and has been attributed to the nutrient-rich waters from the sub-Antarctic not reaching as far north as Australia (Connell 1993). In addition, Australian soils are low in phosphorus with low surface runoff to the sea (Brodie 1995) and also low rainfall for most months except during winter. Nutrients (nitrogen and phosphorus) entering the Gulf are therefore mainly of terrestrial origin such as sewage treatment works, urban storm water containing soil and fertilisers and industrial discharges rather than upwelling.

TKN (Total Kjeldahl Nitrogen) is a measure of organically bound nitrogen, and includes both dissolved and particulate forms, while both oxidised nitrogen and ammonia are dissolved forms of nitrogen. Oxidised nitrogen includes nitrate and nitrite, but nitrite concentrations are usually very low in well-oxygenated waters. The prevailing chemical and physical environment influences the form of nitrogen that is most commonly found in the environment. In anaerobic sediments, ammonia is produced under microbial activities, which converts to nitrate in aerobic conditions. Total phosphorus includes both dissolved and particulate forms of phosphorus. The rate at which bacteria and other microorganisms degrade organic phosphates depends on temperature, pH and oxygen. Physical disturbance or lowered salinity can affect the release and re-absorption of phosphates by suspended matter (Brodie 1995).

Expectedly the nutrient levels found in the effluent from the Christies Beach Wastewater Treatment Plant were high, with little variations over seasons. TKN levels ranged from a quarterly mean of 20.4 to 24.6 mg L^{-1}, while oxidised nitrogen (nitrates and nitrites) concentrations ranged from 13.4 to 22.1 mg L^{-1} and ammonia levels were between 16.1 and 22.1 mg L^{-1} (Figure 2.5). Total phosphorus levels ranged from a quarterly mean of 8.5 to 8.8 mg L^{-1} (Figure 2.5).

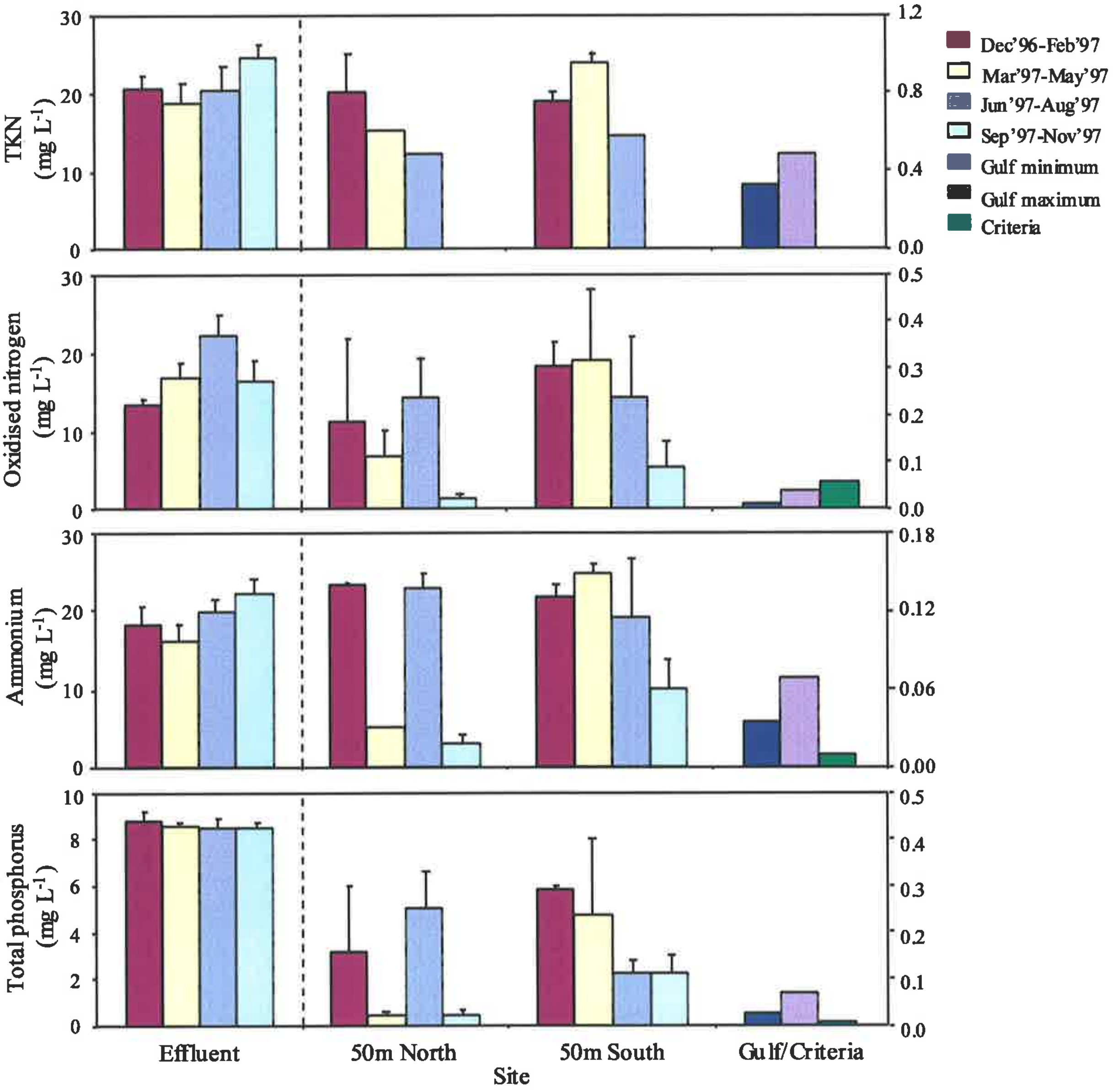

Figure 2.5 Quarterly means of various nutrient concentrations in effluent (left-hand y-axis) which were reduced at least ten times on discharge as seen in values for receiving waters 50 m north and south of the outfall (right-hand y-axis), but both receiving waters and ambient nutrient concentrations in the Gulf (right-hand y-axis) were still higher than criteria values. Missing histogram bar means values were below detectable limits or no data (refer to Appendix I).

Although these levels were generally reduced ten-fold on discharge to the sea, nutrient levels analysed from receiving waters 50 m to the north and south of the outfall were still higher than the criteria values (generally < 0.01 mg L^{-1}) set in the Guidelines (Environment Protection Authority 1993) (Figure 2.5). The receiving waters north of the outfall had TKN levels ranging from 0.5 to 0.8 mg L^{-1}, oxidised nitrogen from 0.02 to 0.19 mg L^{-1} while ammonia concentrations were between 0.018 and 0.140 mg L^{-1} and phosphorus levels ranged between 0.023 to 0.157 mg L^{-1}. The nutrient levels of the receiving waters to the south of the outfall were also above criteria values with TKN concentrations ranging from 0.58 to 0.95 mg L^{-1} and oxidised nitrogen levels were between 0.09 and 0.32 mg L^{-1}. Ammonia and phosphorus concentrations were also high ranging between 0.06 and 0.15 mg L^{-1} and 0.11 and 0.29 mg L^{-1} respectively. Even the ambient water quality in the Gulf was described as moderate and one of the reasons was elevated nutrients, in particular, ammonia concentrations (0.035 to 0.068 mg L^{-1}), at all sites sampled (Environment Protection Authority 1997).

Heavy Metals

Heavy metals are found in particulate and dissolved forms, and even though some are biologically essential, all have the potential to be toxic when above certain concentrations. Along the metropolitan coast of Adelaide, heavy metal contamination originates mainly from urban runoff and industrial sources such as manufacturing plants, power stations, port facilities and sewage treatment works (Environment Protection Authority 1997).

Concentrations of a number of metals were monitored for the effluent, however only copper, mercury, nickel and zinc were measured in 1997 for receiving waters. Hence only these metals are compared here. The concentrations of copper (0.020 to 0.029 mg L^{-1}) in the effluent being discharged from the Christies Beach Wastewater Treatment Plant were at least ten times higher than chronic criteria level (0.0029 mg L^{-1}) set out in the guidelines for marine discharges (Environment Protection Authority 1993). However, upon discharge these concentrations were reduced to approximate chronic criteria level (Environment Protection Authority 1993) (Figure 2.6).

The concentrations of mercury in both the effluent and the receiving waters were mostly below the detectable limits of analysis (< 0.0001). Nickel and zinc concentrations were more variable with nickel concentrations occasionally below detectable limits of analysis. The levels in the receiving waters measured 50 m from the outfall were below the chronic criteria values (Environment Protection Authority 1993) (Figure 2.6). The general water quality in the Gulf based on concentrations of heavy metals was rated from moderate to good and values were generally below criteria values (Environment Protection Authority 1997).

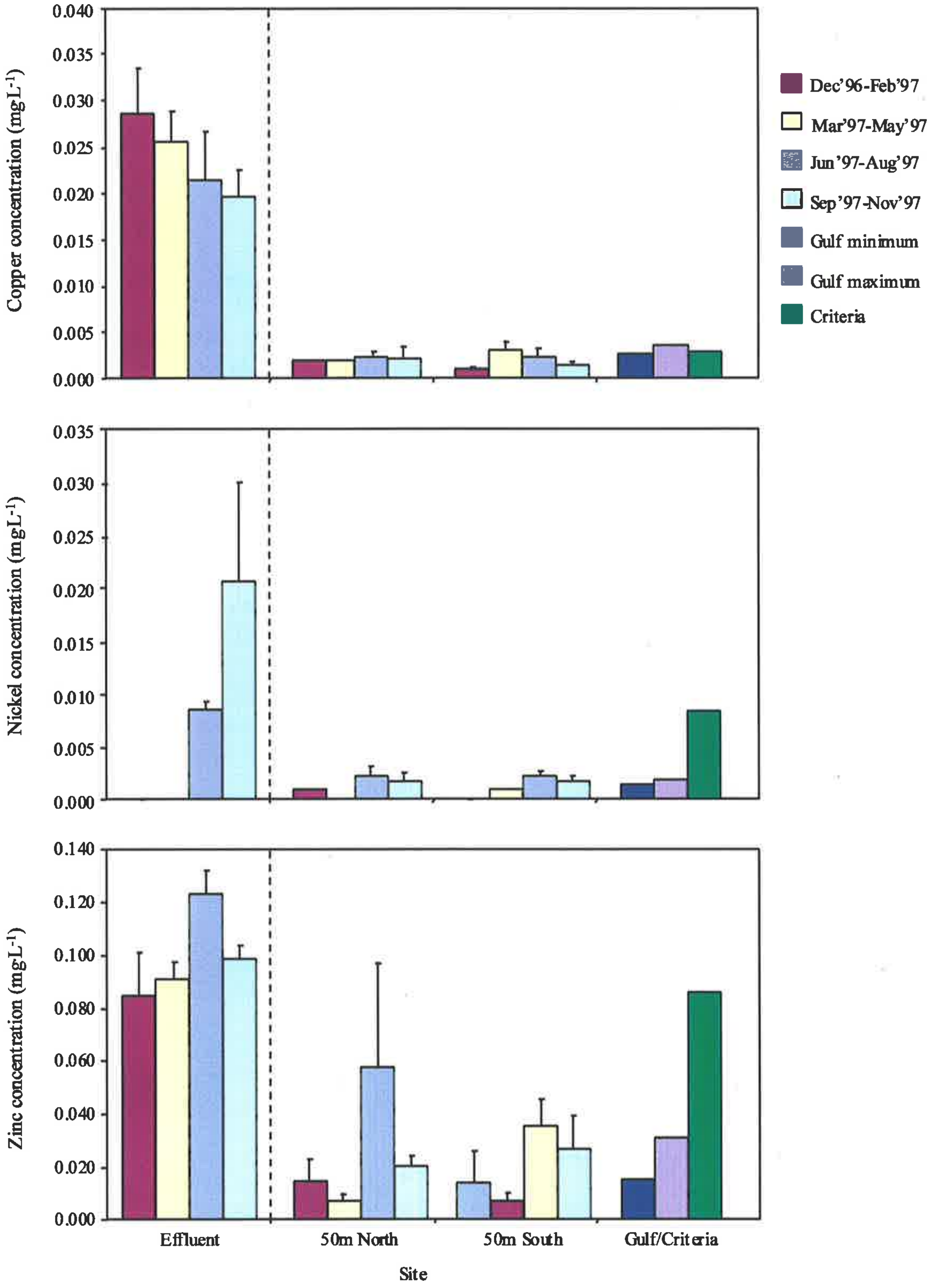

Figure 2.6 Quarterly means of copper, nickel and zinc concentrations of effluent, receiving waters 50 m north and south of the outfall and the ambient range (minimum and maximum) in the Gulf showing high copper concentrations in the effluent reducing to chronic criteria values while nickel and zinc concentrations were more variable. Missing histogram bar means values were below detectable limits or no data (refer to Appendix I).

Microbiological agents

Microorganisms in marine waters are important from an anthropogenic point of view in terms of health. Contact with seawater polluted by microbes and ingestion of seawater containing pathogens from domestic sewage may lead to infections and diseases. Due to this causal link between human disease and contaminated seawater, microbial standards based on indicator faecal bacteria were established and stringent monitoring and control were put in place internationally (GESAMP 1990b).

Sewage effluent is known to contain high levels of faecal coliform and Christies Beach effluent conforms to this pattern. Faecal coliform counts in the effluent ranged from 7.2 to 23.9 per 100 ml and this is reduced to below 5 counts per 100 ml in the receiving water 50 m from the outfall (Figure 2.7).

Microbiological quality in Gulf waters was moderate at some sites monitored, however, they were generally good and all sites monitored met the National Health and Medical Research Council requirements for primary contact recreation (Environment Protection Authority 1997).

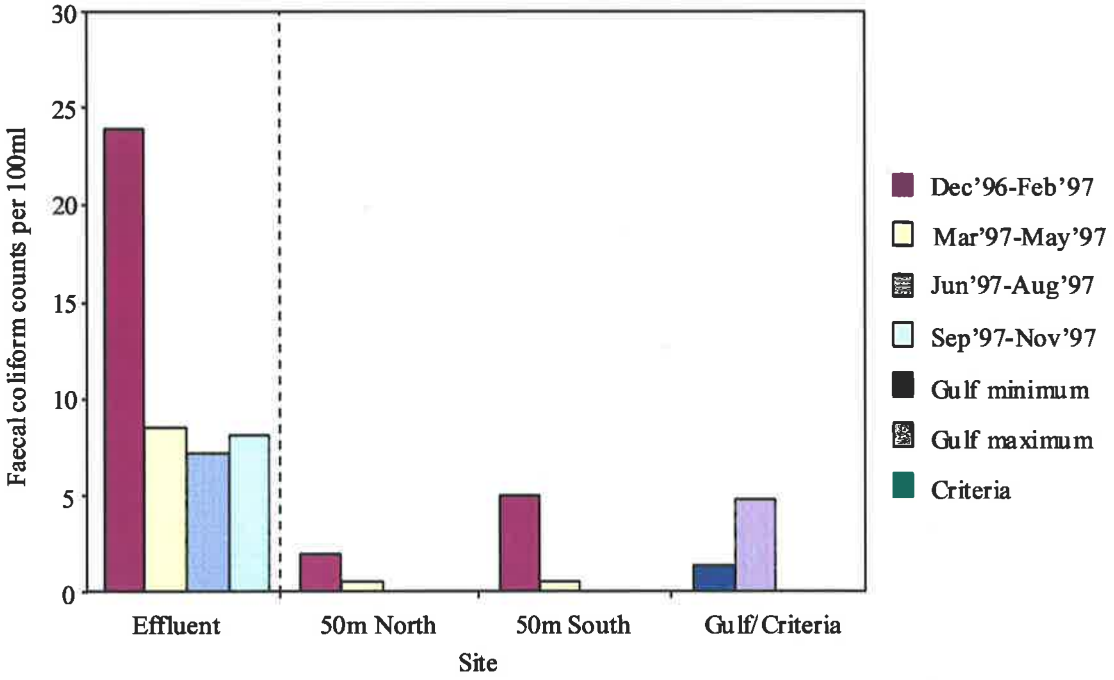

Figure 2.7 **Faecal coliform counts per 100ml of effluent, receiving water 50 m north and south of the outfall and ambient counts (minimum and maximum) for the Gulf. Missing histogram bar means values were below detectable limits or no data (refer to Appendix I).**

Collected data

Sediment grain size

The overall pattern of sediment distribution in terms of grain size in Gulf St Vincent shows that the finest products (less than 0.1 mm) have settled mainly into the deeper waters of the central northern part of the Gulf and at the eastern end of Investigator Strait. The coarser sediments (1 - 2 mm diameter) are concentrated about the southern, headlands of the Gulf, across the western part of Investigator Strait, and also in the zone beyond the intertidal of the eastern coastal beaches. Tidal currents, wave action and proximity to headlands or erosional rip channels control the supply of coarser sand and silt (Shepherd and Sprigg 1976). The study area, located in the south-eastern part of the Gulf, is a primarily carbonate sedimentary province overlying a rock basement (comprising limestone reefs) along with shell-bed platforms. Aeolianite dune systems contribue a range of fine to coarse grained sands comprising quartz and clay particles (Shepherd and Sprigg 1976).

Sediment size analysis was carried out on the sediments from both the outfall site and a control site[1] (located 8 km south of the outfall site at Moana; Chapter 3). The detailed procedures of this work are presented in Appendix II and only the major results are highlighted here.

The sediments at Christies Beach (outfall site) and Moana (control site) were essentially similar, both having predominantly fine sand according to the Wentworth classification scale (Buchanan 1984). Both sites had median grain size between +2 and +3 phi (fine sand class of the Wentworth classification scale). However, the median grain size of the sediment at the outfall site was significantly lower than that at the control site, Moana ($p<0.05$). The median grain size at the outfall site ranged from 2.31 to 2.91 phi while those of Moana was between 2.44 and 3.13 phi (Figure 2.8).

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[1] Details of the sampling protocol are given in Chapter 3; this includes a detailed rationale of the choice of sites and stations at the outfall and control sites. In brief, samples were collected at stations at increasing distances to the north and south of the discharge point of the outfall (1, 5, 10, 15, 30, 60, 120, 300 and 500 m) and this pattern was repeated at the control site, which was located 8 km south of the outfall site at Moana.

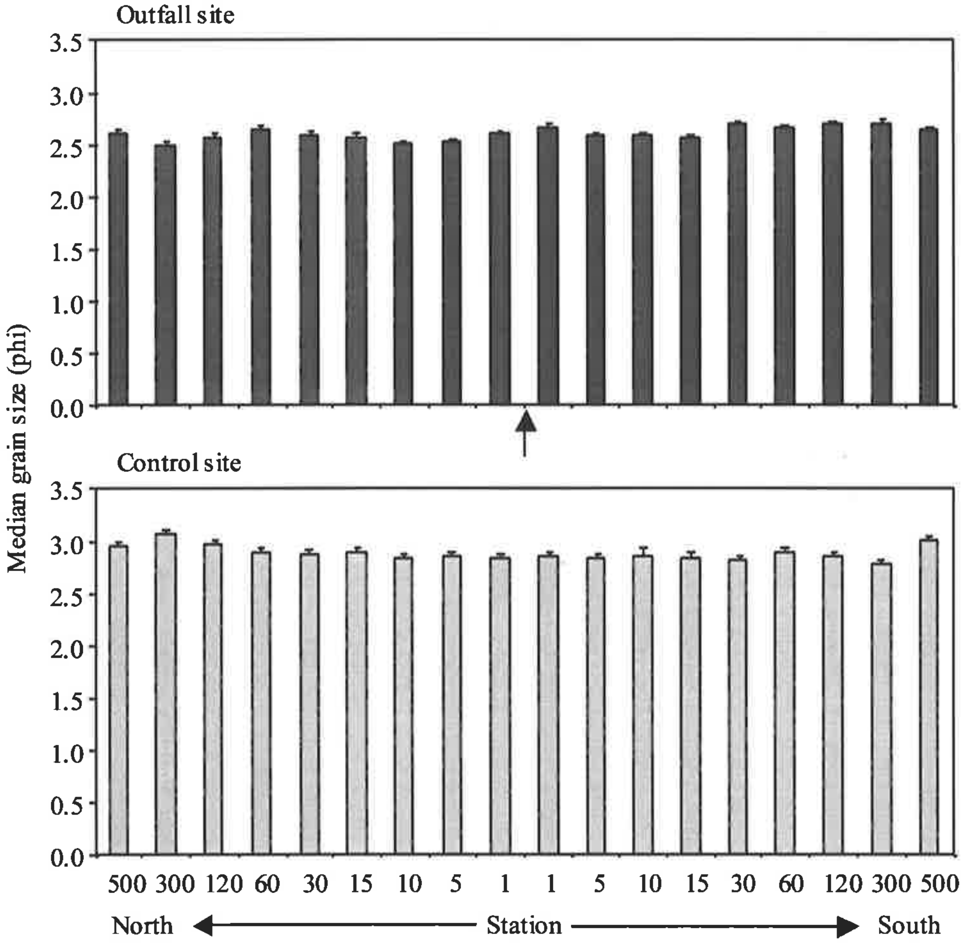

Figure 2.8 **Mean median grain size (± SE) according to the Wentworth classification scale for the outfall site (Christies Beach) and control site (Moana) for all stations to the north and south. Arrow indicates discharge point at the outfall site.**

Sedimentary organic carbon content

Sewage disposed at sea affects the water column in the short term, but in the longer term sediments are also affected (McIntyre 1990). Consequently sediment samples were collected from both the outfall site and the control site at Moana to ascertain the organic carbon content in sediments. The sediments were dried at 100°C until a constant weight was reached, then analysed for carbon concentration using a Skalar Primacs Solid Carbon Analyser. Results were expressed in percentage dry weight of sediment per total carbon and inorganic carbon. The concentration of organic carbon was calculated from the difference between percentage of total carbon and inorganic carbon.

The organic carbon concentration in sediments showed that the outfall site was more variable than the control site. The mean organic carbon content of the outfall sediments had concentrations ranging from 0.7 to 1.1 % dry weight whereas sediments from the control site had carbon concentrations ranging from 0.8 to 0.9% dry weight (Figure 2.9).

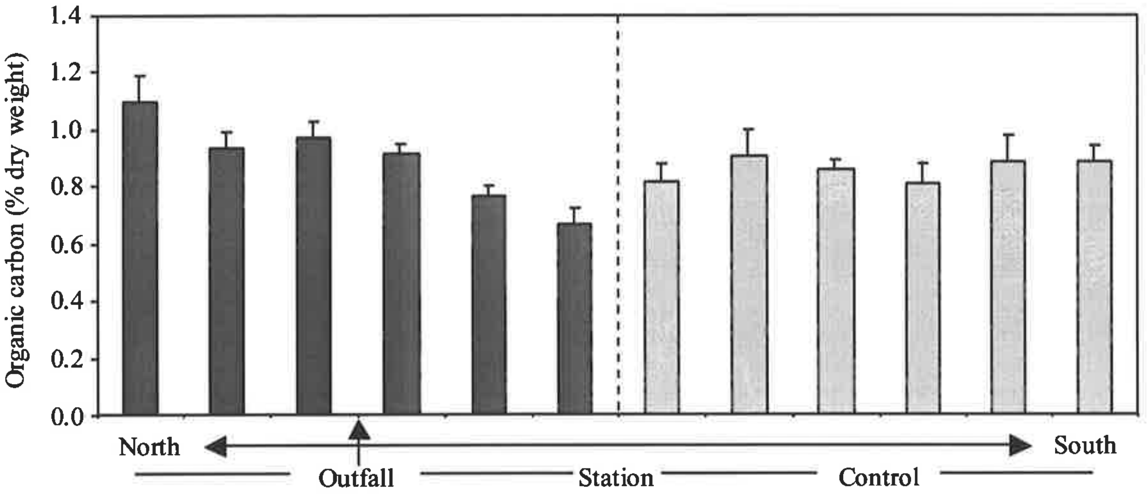

Figure 2.9 Mean organic carbon concentration (± SE) of sediments for the outfall and control sites. Stations are arranged in increasing distance from the outfall moving both northwards and southwards. Arrow indicates discharge point.

Seasonally, carbon concentrations are usually at a minimum during autumn and winter (Sampou and Oviatt 1991). At the outfall site, organic carbon content for the April and July surveys (autumn and winter) were marginally lower than the other two survey times (Table 2.1). However, this was not observed at the control site where organic carbon concentrations were higher in the April and July surveys (Table 2.1). This may be due to other sources such as detrital material from nearby reefs and seagrass beds.

Table 2.1 Mean sedimentary organic carbon concentration (± SE) in % dry weight of sediment for the outfall and control sites for each of the surveys carried out in 1997.

Survey	Outfall site	Control site
January	0.91 ± 0.10	0.77 ± 0.08
April	0.88 ± 0.06	0.96 ± 0.04
July	0.86 ± 0.10	0.95 ± 0.06
October	0.96 ± 0.08	0.83 ± 0.08

Implications

The physical and chemical conditions of the Gulf presented above describe the environment in which the macrobenthic infaunal communities under study exist and the nature of the stresses to which they may be exposed. It is an environment that changes seasonally in terms of temperature and salinity, with some degree of inter-annual variability due to long-term climate forces. There is also an apparent north-south flow in the Gulf with a huge influx of nutrients and heavy metals from the effluent outfall. Although the mixing and dispersion of the effluent brought nutrient concentrations to background levels for the receiving waters within 50 m of the outfall, organic carbon was highly variable in the sediments around the outfall site and heavy metals concentrations in particular copper, remained at chronic levels.

Studies have shown that the physical, chemical and biotic environment influences the structure of any biological communities (Snelgrove and Butman 1994). In an aquatic environment, organisms in a community are adapted to one another and the environmental conditions in which they exist. There may be variations both spatially and temporally in their distributions but they occur according to some pattern of natural relationships (Morrisey *et al.* 1992a, Morrisey *et al.* 1992b, Connell 1993, Thrush *et al.* 1994). Changes in the abiotic environment will lead to related modifications to the biota. The first resistance to any environmental disturbance involves physiological, behavioural tolerance or adaptability of individual organism (Boesch and Rosenberg 1981). Only the better-adapted individuals of the species will survive with increasing stress. Beyond this, replacements by different genera or families can occur (Pearson and Rosenberg 1978).

Therefore any patterns of change detected in the subsequent analyses of the macrobenthic infaunal data have to be interpreted against the natural variability of the community as influenced by this environment.

Chapter 3 The Sampling Protocol

This chapter will outline the sampling protocol used in this study, which will include the sampling programme, the selection of sampling sites, the determination of sampling design and sample size, the field and laboratory methods used and the data sets used in subsequent analyses.

Sampling programme

Preliminary work was undertaken in March and August 1996 to select appropriate sites; test the sampling technique; determine the sampling size and familiarise the researcher with the infauna of the area. Subsequently, a full sampling was carried out using SCUBA in January (summer), April (autumn), July (winter) and October (spring) of 1997.

Sampling sites and sampling design

The ideal sampling design would be a BACI (before/after-control/impact or Beyond BACI design (see Chapter 1). However, because the Christies Beach Wastewater Treatment Plant has been in operation since 1971, this was not possible, and an after-control/impact design was used. Although this has less power to detect an impact, any differences that were found in the macrobenthic infaunal communities at the outfall site when compared to those at the control site may be ascribed to the outfall (Warwick 1993).

The study site at Christies Beach extends for a distance of 500 m to the north and south from the discharge point of the outfall. Moana, located 8 km to the south of Christies Beach was the closest appropriate control site because of similar substrate type. The other control site at Hallett Cove (4 km north of Christies Beach) was less than ideal but was included for the benthic respirometry work (Figure 3.1).

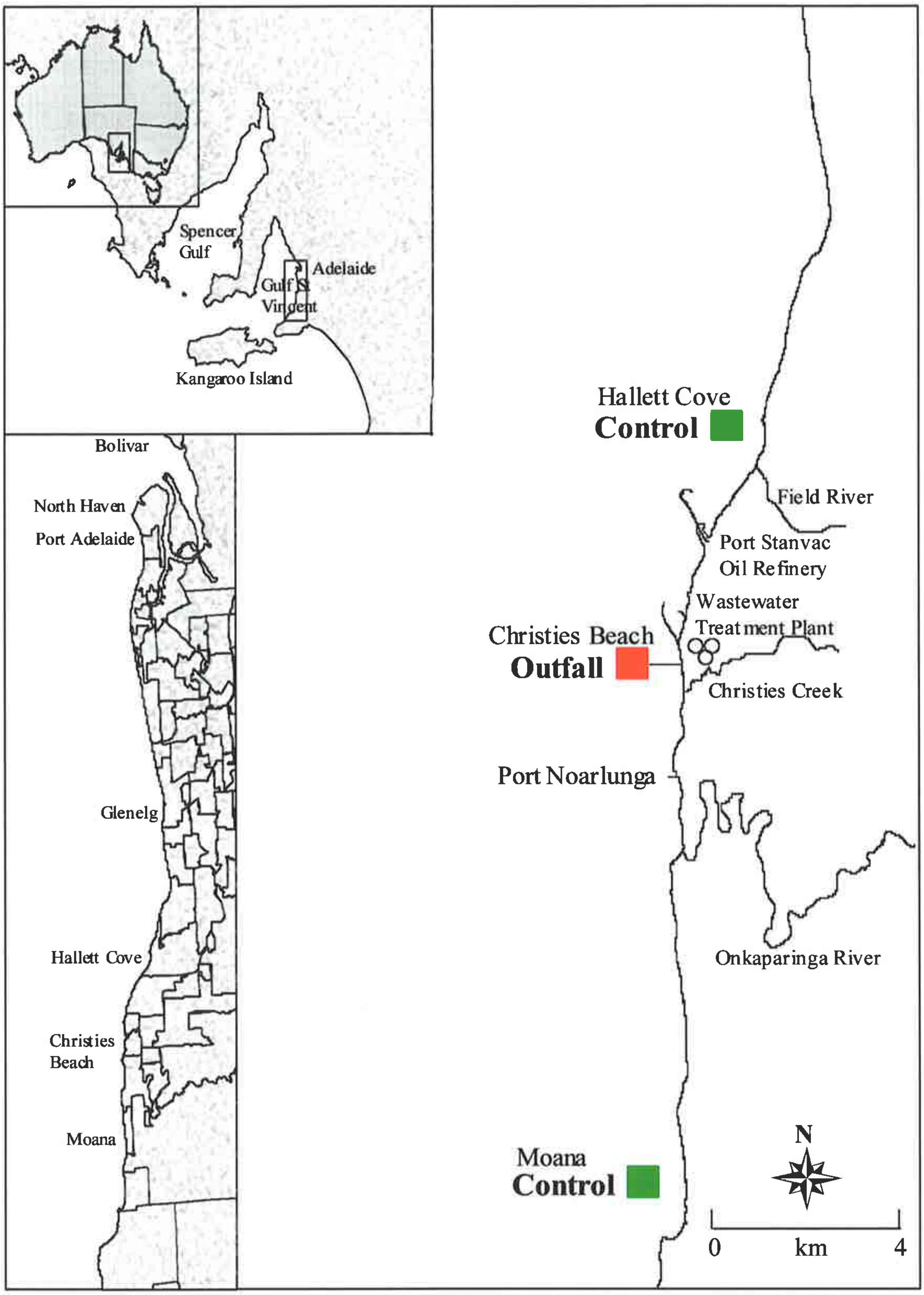

Figure 3.1 Map of the southeast coastline of Gulf St Vincent showing the location of the outfall (🟥) at Christies Beach and the control sites (🟩) at Moana and Hallett Cove.

The choice of control sites was problematic because the site needed to be close enough to the outfall to be representative of the region but also not impacted. Along this coast in the southeast region of Gulf St Vincent, few sites fulfil these criteria. Moana is the closest possible control site to the south that has a similar substrate type to the outfall site with no other significant anthropogenic impacts. Similarly when moving north of the outfall, there is the Port Stanvac Oil Refinery, which might have an impact on the area. Therefore the nearest possible control site in the north was at Hallett Cove, located beyond the refinery but was less than ideal because of much coarser sandy substrate and scattered rocks. Moreover, seagrass patches and anthropogenic impacts from urban development would potentially confound any sites further north of Hallett Cove. Hence, only Moana was used as the control site for the infaunal study, although a more extensive comparison was made with data collected for a range of other sites in the Gulf (see Chapter 5).

For the infaunal study, nine stations were established to the north and south of the outfall along a similar depth gradient. This design was intended to account for the two major environmental gradients, depth and longshore position, which were likely to influence the communities along the coast. The work by Cheshire and Miller (1999) at Port Stanvac (in the vicinity of Christies Beach in Gulf St Vincent) showed that the distributions of epibenthic and infaunal communities were affected by these two natural gradients.

The stations were therefore fixed at 1 m (that is, immediately adjacent to the outfall pad), 5, 10, 15, 30, 60, 125, 300 and 500 m both northwards and southwards of the outfall at an approximate depth of 7 m. The positioning of the sampling stations was to account for a gradient of the putative impact of the effluent, that is, the dilution of the effluent with increasing distance from the discharge point. This sampling design was mirrored at the control site, Moana (Figure 3.2).

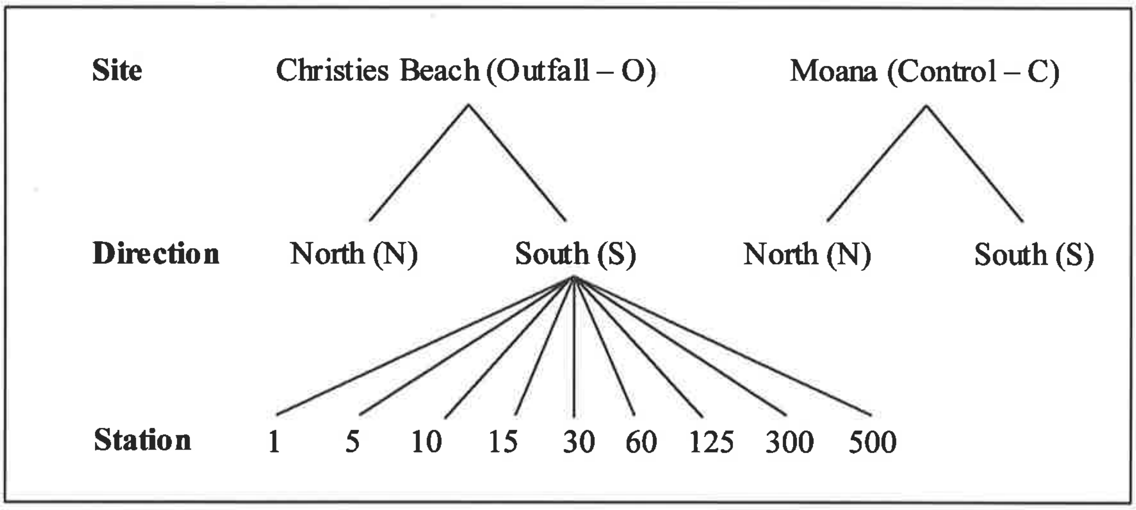

Figure 3.2 Sampling design for the outfall and control sites and codes used for sites, transect directions and stations.

Sample size

An essential component of any experimental design is the use of an appropriate number of replicates (sample size). It is necessary to ascertain the optimum number of samples required to detect a minimum difference between sample means, especially if the response is small. Conversely, an excessively large number of samples may give the required power, but will consume large amounts of time to collect and process the samples. To determine the optimum sample size, a minimum detectable difference among treatments needed to be determined *a priori*. There are many techniques for the computation of appropriate sample size (e.g. Andrew and Mapstone 1987, Bros and Cowell 1987, Clarke and Green 1988, Eberhardt and Thomas 1991, Fairweather 1991, Ferraro *et al.* 1994, Zar 1996); however, for environmental studies involving field sampling and when studies are carried out after a perceived impact, it is not always possible to specify, *a priori*, a minimum detectable difference between treatments. In such situations, large numbers of samples could be collected or a minimum desired difference might be selected arbitrarily. Since it was not ideal to implement either of these alternatives, an integration of both was carried out for this present study.

The data from the preliminary study were used to evaluate both the resolving power and the feasibility in terms of effort. In the preliminary study, 15 core samples were collected at each station of the outfall site. The standard error-sample size function (as recommended by Bros and Cowell 1987) was determined using these data. The relationship between the standard error (SE) and sample size is a decreasing asymptotic function that approaches zero. The rate of change in the slope of tangents to the function is small at very small or very large sample sizes and larger at some intermediate region of the curve. This region of greatest change in slope is a result of the relationship between the sample variance and the true (population) variance. The minimum acceptable sample size is recommended to be beyond this region of maximum change in slope of the SE function (Bros and Cowell 1987).

A sample size of ten cores was decided on, taking into consideration the feasibility of the effort for processing each sample and the resolving power (at least 0.8). This sample size was indicated by graphs of the SE-sample size function to be adequate (Figure 3.3).

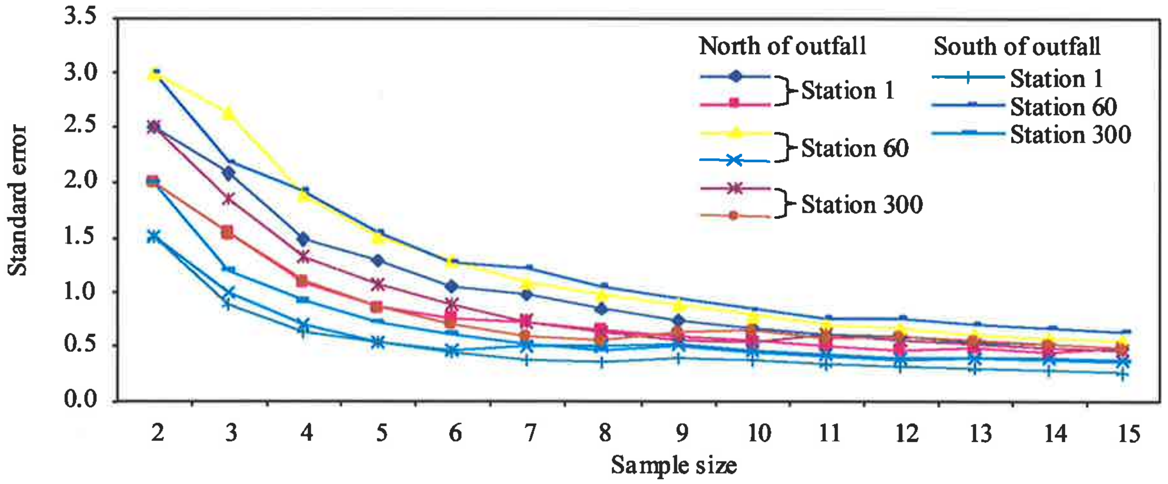

Figure 3.3 **Standard error as a function of sample size for stations from the outfall site.**

Field sampling

In the field, ten samples were collected for infauna analysis at each station using a PVC corer (42 mm diameter and 400mm length). Corers with larger diameters were trialled in the preliminary surveys, but difficulties were encountered. Larger corers offered greater resistance when pushing into coarser sediment and sediments tended to fall out when the corers were withdrawn. The 42 mm diameter corers, albeit small, had the advantage of ease of handling underwater, enabling more samples to be collected and more representative sampling of the area. This also reduces statistical error by allowing greater degrees of freedom.

In the field, each corer was pushed into the sediment until the depth of penetration was 200 mm. A rubber stopper was pushed into the open end at the top of the corer. The corer was then gently extracted and the bottom end sealed with another rubber stopper to prevent loss of sediment. The ten filled corers were then placed in a plastic basket and hauled into the boat after the divers had surfaced. The core samples were emptied into jars on site. Upon return to the laboratory, the samples were fixed with 10 % buffered formalin until further processing.

Laboratory processing

In the laboratory, the formalin in the jars was decanted before the samples were processed. The samples were gently washed and carefully screened using stacked 500 µm and 1.0 mm sieves. These mesh sizes were chosen as they were most commonly used for studies of marine macrofauna (Eleftheriou and Holme 1984, Kingston, 1989 #172). In addition, the choice of mesh size is dependent upon the coarseness of the sediment, which affect the amount of sediment retained and thus the sampling and sorting time. The use of coarser mesh size (1 mm instead of 500 µm) has been suggested for environmental impact studies of macrofauna (James *et al.* 1995). This reduces the sample processing (sorting and identification) time with little loss of data for site discrimination. The most appropriate mesh sizes for the sieves (500 µm and 1 mm) were chosen for use in this study after sample processing time and handling feasibility were determined from the preliminary work. Moreover, over 60 % of the fauna were consistently retained on the 500 µm sieve.

After most of the sediment had been washed from the sieves, the retained material was chased down to one side of the sieve with a gentle stream of water applied to the outside of the sieve. The material from each sieve was then washed into separate trays. The animals were sorted out with the aid of a lighted magnifier (MAGGYLAMP®). These animals were subsequently identified and counted. Fresh weight of all taxa was obtained after blotting on filter paper and measured to the nearest 10^{-4} g. When a single animal did not register a reading on the balance, multiple individuals of the same taxa were combined to obtain a mean fresh weight for each individual. The animals were then preserved in 70% ethanol for storage.

The taxonomic level to which macrobenthos are identified depends on the aim of the study and it is only necessary to identify organisms to a taxonomic level that is sufficient to meet the objectives of the study (Ellis 1985). The organisms obtained in this study were initially sorted to pseudo-species, which were then identified to families or pseudo-family levels while the less common taxa were identified to phyla, sub-phyla, class or order. The taxonomic resolution was deemed appropriate for this study where the main aim was to examine the effects of wastewater effluent on macrobenthic infaunal communities and for reasons outlined below.

There is a general lack of information on South Australian marine invertebrates (Shepherd and Sprigg 1976). Consequently, identification to species level is time consuming and expensive. In addition, species identification is more prone to error than identification to higher taxa (Ellis and Cross 1981, Ellis 1985) and correct identification to a higher taxon may be preferable, both biologically and statistically, than incorrect identification to species (Green 1979). Studies have shown that patterns of spatial variation arising from anthropogenic disturbances are often similar both for species and broader taxonomic levels of macrofauna (Ellis 1985, Warwick 1988c, Warwick 1988a, Ferraro and Cole 1990, Gray *et al.* 1990, Warwick *et al.* 1990, James *et al.* 1995, Underwood 1996). This has been attributed to the masking of pollution effects by natural environmental variables (Warwick 1988c) and natural variability in the numerical abundances of common macrobenthic species (Eagle 1975, Nichols 1985). Generally, anthropogenic disturbances tend to affect communities at a higher taxonomic level (family to phyla) as compared to natural disturbances, a consequence also of the physiological responses of taxa to disturbances (Warwick 1988c, Ferraro and Cole 1990).

Samples used

Analysis of the data from the preliminary surveys indicated that there was evidence of differences between the communities at the outfall stations and the control stations. Moreover, there were also differences in communities with increasing distance from the outfall. This gradient was evident even when analyses were done on a reduced number of stations from each transect. Therefore, the total number of samples processed could be reduced. All the samples from the survey carried out in January 1997 were processed to examine at higher resolution the gradient effect of the effluent (see Chapter 5). For the subsequent surveys carried out in April, July and October 1997, only samples from Station 1 (adjacent to the outfall), Station 60 (60 m from the outfall) and Station 300 (300 m from the outfall) were processed although samples were collected at all stations. These reduced the data set to three stations from each north and south transect from both the outfall and control sites, culminating in a total of 720 core samples processed excluding those from the preliminary surveys.

Chapter 4 The Impact

With an understanding of the environment in which the macrobenthic infaunal communities exist (Chapter 2), analyses of the infauna data was carried out. Any patterns of change detected were interpreted against the natural variability of the community as influenced by the environment. This chapter will give the results of these analyses and interpretation of the macrobenthic infauna data collected.

Introduction

Typically the pattern of species abundances changes along a pollution gradient (Pearson and Rosenberg 1978); this is a consequence of each species responding to the pollutant at different levels. Essentially, species will respond to the pollutant by either tolerating, moving or dying. The most frequent response for a community is that some species will increase in abundance, many decrease and others remain unaffected. The patterns of species abundance should therefore reflect the effects of the pollutant integrated over time. Consequently, these patterns are widely used to monitor the effects of pollutants in the marine environment (e.g. Pearson and Rosenberg 1978, Gray *et al.* 1988, Austen *et al.* 1989, Ritz *et al.* 1989, Aschan and Skullerud 1990, Anderlini and Wear 1992, Zmarzly *et al.* 1994, Simboura *et al.* 1995).

The objective of this present study was to determine the degree of disturbance or stress inflicted on the macrobenthic communities by the wastewater effluent discharge at Christies Beach. There are broadly four stages in the investigation of disturbances in benthic communities, (i) analysing the patterns of community structure and discrimination between sites, (ii) measuring community stress, (iii) characterising the communities and (iv) correlating measured environmental variables (Gray *et al.* 1988, Clarke 1993).

To execute the four analysis stages, there is a whole range of techniques that may be used to extract information from collected data. The techniques ranged from univariate, graphical/distributional to multivariate, using counts of individual species, counts of indicator species, ratios of important groups of species to more complex derived statistics such as diversity and equitability, through to multivariate classification and ordination techniques. The following will discuss the use and application of these techniques for the assessment of the responses of infaunal community.

The first analytical stage involves the use of multivariate techniques, which have become widely used to elucidate patterns of community structure. Commonly used multivariate methods include classification and ordination (Field *et al.* 1982, Clarke 1993, Warwick 1993). Similarity matrices are the starting point for most of these techniques. The Bray-Curtis similarity coefficient (Bray and Curtis 1957) is a reliable measure for biological data on community structure and is not affected by joint absences that are commonly found in infaunal data (Field *et al.* 1982, Clarke 1993). This similarity matrix is then subjected either to classification (such as agglomerative classification and linkage methods like Ward's or flexible beta) or ordination (such as non-metric multidimensional scaling or principal component analysis). The results of classifications are displayed in dendrograms while those of ordinations are in a two or more dimensional plot.

When there are differences in the patterns of community structure between sites, the next logical step would be to determine whether the differences are due to community stress. Again there are various methods for measuring community stress where community descriptors such abundance, number of species, diversity and evenness are commonly used. Distributional/graphical methods such as abundance/biomass comparisons (ABC), neutral model analysis and log-normal plots have also been used.

Although community descriptors have been commonly used, their use in impact assessments has been widely debated in the literature, especially diversity indices (Sheehan *et al.* 1984, Magurran 1988, Warwick and Clarke 1993a, Begon *et al.* 1996, Chapman *et al.* 1996). However, when used together with multivariate analyses, they may help to further elucidate the structure of the benthic community under investigation.

Pearson and Rosenberg (1978) proposed a model of the response of soft-bottom infaunal invertebrates to a gradient of organic enrichment. Response was assessed by three basic measures of community structure: number of species (S), abundance (A) and biomass (B). SAB curves are expected to occur along a gradient of organic enrichment as shown in Figure 4.1. There is an initial azoic zone where there are no macrofauna. Species encountered after this zone are small and few. Abundances (A) increase very rapidly after this to the peak of opportunists (PO) where the communities are dominated by a few opportunistic species. As distance from the source of organic enrichment increases, the abundances fall to the 'ecotone point' (E), the zone where the community is poor in abundance, species and biomass. Beyond this point, abundances decrease to a level found under normal conditions. The number of species (S) is at a maximum after the 'ecotone point' but decreases to lower levels usually found under normal conditions. Biomass (B) is more variable but the curve generally follows a form that has an initial peak corresponding to the maximum number of opportunistic species (PO) and then a decrease to the 'ecotone point' (E). There is a second maximum for biomass as more species of larger animals are encountered and similar to the A and S curves, the B curve stabilises to a level found under normal conditions. Therefore environmental stress is implied when test SAB curves conform to this model (Table 4.1).

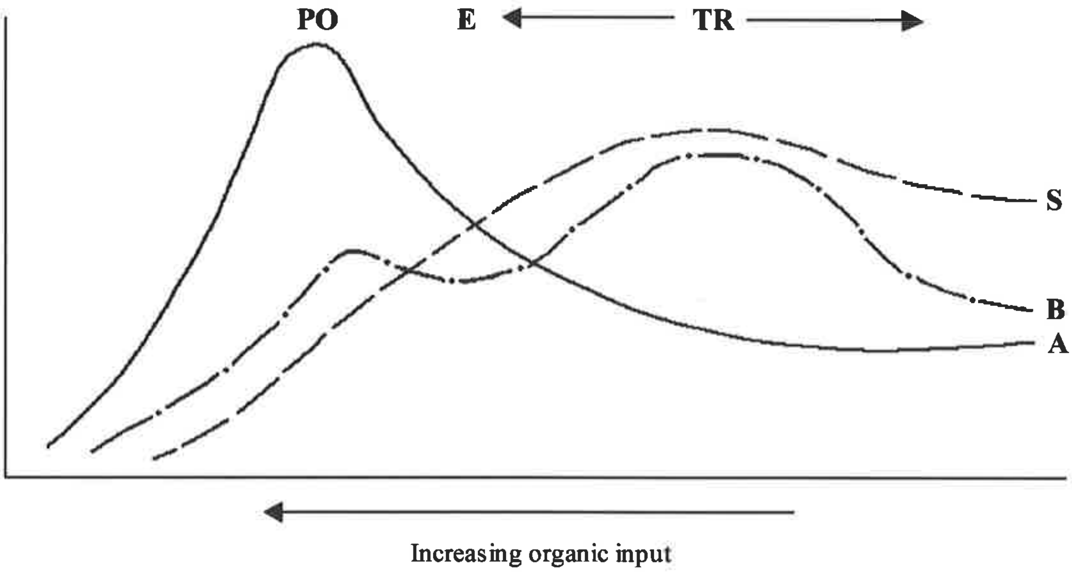

Figure 4.1 **SAB curves along a gradient of organic enrichment. S = Number of species, A = abundance, B = biomass, PO = peak of opportunists, E = 'ecotone point', TR = transition zone (after Pearson and Rosenberg 1978).**

In addition to the three basic community descriptors, derived statistics such as diversity and evenness have also been used to detect community stress. Earlier studies argued that stress acted to decrease diversity (H') and evenness (J) in benthic assemblages (Pearson and Rosenberg 1978, Rapport *et al.* 1985, Schindler 1987, Ferraro and Cole 1990, Weston 1990). However, other works have argued that this is an over-simplification (Gray and Pearson 1982, Clarke and Warwick 1994). Diversity may be reduced when disturbance is minimal and increased when the level of disturbance is higher or when disturbance becomes more frequent. This occurs as a consequence of competitive exclusion between species. At even higher levels (or frequency) of disturbance, diversity is reduced again as species are eliminated by the stress. Therefore diversity tends to be highest when there are intermediate levels of disturbance (Connell 1978, Huston 1979).

Unlike univariate techniques, distributional/graphical techniques do not simplify complex data of species and abundance to a single index. Instead they provide a summary of the data and allow for comparisons between plotted curves or histograms. The method of abundance/biomass comparison (ABC) may be used to compare the distribution of abundances among taxa with the distribution of biomass among taxa between sites (Warwick 1986, Warwick *et al.* 1987). This involves plotting separate k-dominance curves (Lambshead *et al.* 1983) for abundance and biomass on a cumulative scale of percentage dominance (y-axis) against the taxa ranked in order of importance according to these two attributes on a logarithmic scale (x-axis). Theoretically, in undisturbed communities, the presence of large organisms results in the biomass curve lying entirely above the abundance, while in grossly disturbed communities, the abundance curve lies entirely above the biomass curve, being dominated numerically by small r-selected or opportunistic species. In moderately disturbed communities, the curves are closely coincident and may cross over one or more times (Figure 4.2).

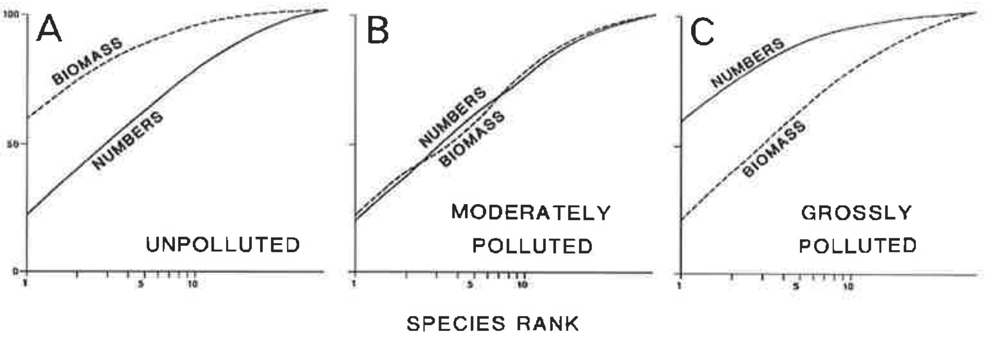

Figure 4.2 **Hypothetical k-dominance curves for species abundance and biomass showing (A) unpolluted, (B) moderately polluted, and (C) grossly polluted conditions (after Warwick 1986).**

To compare the state of disturbance, the W statistic may be calculated. Conceptually the W statistic is calculated as B–A (from ABC plots) and it takes values in the range -1 (severely disturbed) to +1 (undisturbed) (Clarke 1990).

Another technique is based on an assumption of no interactions. The Ewens-Caswell neutral model (Caswell 1976) calculates an expected diversity for a sample (eH') using the number of species and the number of individuals. It also calculates the standard deviations that the observed diversity deviates from the predicted eH' (the V statistic). When the V statistic value is zero, it implies that the samples are derived from a 'neutral' assemblage, while a positive value results from excess equitability (greater diversity than predicted) and a negative value results from excess dominance (lower diversity than predicted) (Caswell 1976). Diversity (e.g. H') has two components, equitability and species richness. Deviations from the neutral model prediction depend only on differences in equitability with fixed species richness; therefore the equitability of diversity may change differently from species richness in response to the same stress. Moreover, diversity may increase or decrease depending on the starting point of a community in relation to existing stress ('intermediate disturbance hypothesis', Connell 1978, Huston 1979).

A better interpretation of the V statistic is that proposed by Platt and Lambshead (1985). These authors presented a hypothetical relationship between diversity (V statistic as calculated by Caswell's neutral model) and disturbance. Depending on the intrinsic disturbance and carrying capacity of the environment, the diversity of a community may be greater or less than that predicted by the neutral model. Assuming a community with low intrinsic diversity, an increase in the frequency or intensity of disturbance will cause diversity to initially increase (V statistic becoming more positive, Figure 4.3, point B to C), but with further disturbance, diversity will decrease because species are eliminated (Figure 4.3, point C to D). When the disturbance abates, there is recovery with increased growth and immigration, causing the V statistic to move in a positive direction (Figure 4.3, point D to E) and eventually attaining a level of competitive exclusion similar to the original value (Figure 4.3, point E to F).

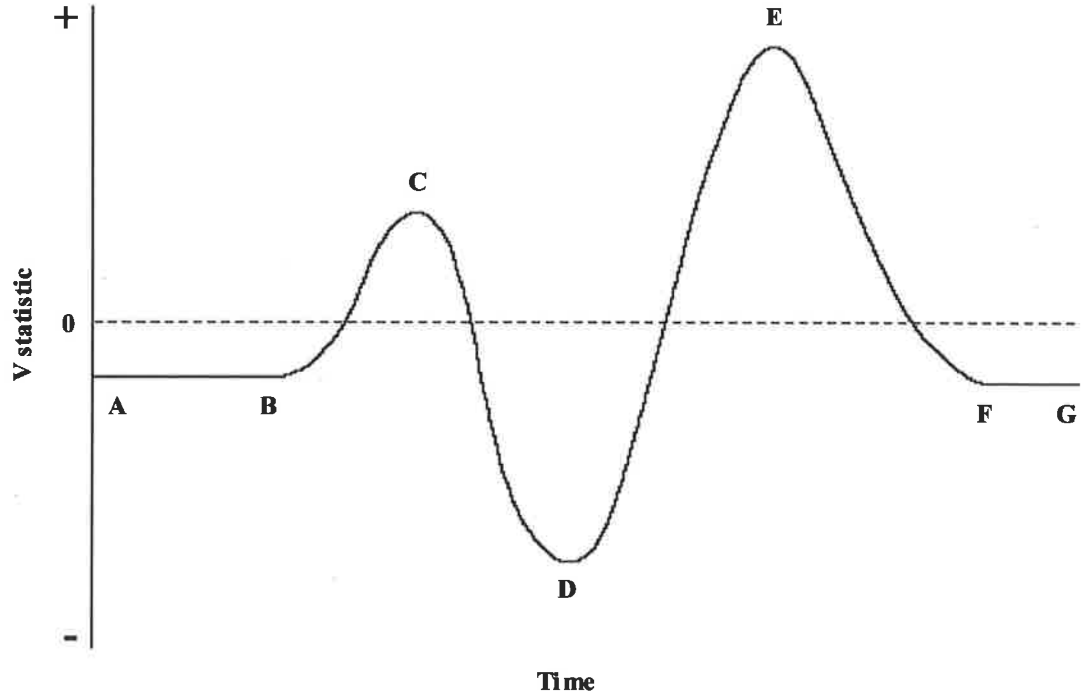

Figure 4.3 **Hypothetical relationship between diversity as measured by the V statistic of Caswell's neutral model and disturbance. Disturbance begins at point B, increases to point D and then stops (adapted from Platt and Lambshead 1985).**

There are several cautions regarding samples to be noted when applying the neutral model analysis (Lambshead and Platt 1988). Sample size should not be too small; otherwise statistical significance will be lost. This is because the number of species decreases with decreasing sample size and species can never be represented by less than one individual. Care must also be taken when pooling non-replicate samples (since one can never be sure if the samples are true replicates), as the results will be severely biased by artificially increasing diversity and especially equitability. However, Karakassis *et al.* (1996) reported that it was reasonable to assume that pooled samples better represented the parent community since the diversity potential, in terms of species richness, is a much closer approximation.

Log-normal plots is another graphical technique and it involves abundance class distributions where the number of taxa on the ordinate is plotted against the number of individuals per taxa in geometric classes on the abscissa. The taxa are grouped into abundance classes based on x2 geometric scale (Class I = 1 individual per taxa, Class II = 2 to 3 individuals per taxa, Class III = 4 to 7 individuals per taxa, Class IV = 8-15 individuals per taxa, etc.) and percentage number of taxa is used for comparable scaling to the figures. This method (Gray and Mirza 1979) and the extended version (Gray and Pearson 1982) have been recommended for possible detection of pollution stress. It is based on the assumption that pollution disrupts the equilibrium of species adapted to prevailing environmental conditions (Gray and Mirza 1979, Gray and Pearson 1982, Ugland and Gray 1982). Rare taxa are more vulnerable to anthropogenic disturbances while taxa of medium abundance are more resilient. Consequently, at undisturbed areas, there will be many rare species giving a smooth curve, with its mode (dominant number of species) to the left. In highly disturbed areas, there are fewer species in the low geometric classes (i.e. rare species) and more abundance groups due to the proliferation of a few opportunistic species. The curves are also irregular with several peaks (Gray and Pearson 1982, Pearson *et al.* 1983). These authors also suggested using species in the intermediate abundance classes, usually Classes V and VI (with 16 to 63 individuals per species) as possible indicators of population change. The basis is that species in these classes are most sensitive to pollution-induced changes and will increase or decrease in numbers rapidly along a spatial or temporal gradient of disturbance.

Essentially the pattern analysis (e.g. cluster analysis or ordination) approach have generally an integrated synthesis of responses but many of these other responses (e.g. change in diversity) do provide significant insights because they are focussed on aspects of the biology of the species rather than on the more complex integrated ecological responses of the community. The application of a whole suite of techniques would therefore help in addressing the question of whether the wastewater effluent has induced changes in the macrobenthic infaunal communities at Christies Beach.

Analysis

The analytical approach for investigating the macrobenthic infaunal communities in this study will encompass the four stages outlined above as (i) analysing the patterns of community structure and discrimination between sites, (ii) measuring community stress, (iii) characterising the communities and (iv) correlating measured environmental variables (Gray *et al.* 1988, Clarke 1993).

With the orthogonal sampling design, multivariate analyses (classification, ordination) were used to elicit the broad patterns of community structure (Clarke 1993). To test a series of hypotheses about the way that selected parameters (e.g. abundance, diversity, richness) would respond to the effluent, univariate analyses were carried out (Warwick 1993). Graphical/distributional representations (abundance/biomass comparisons, k-dominance curves) that are considered an intermediate between univariate and multivariate were also used to extract features of the community response to the effluent (Warwick 1993).

Three stations, Station 1 (adjacent to outfall), Station 60 (60 m from outfall) and Station 300 (300 m from outfall) going both to the north (N) and south (S) of the outfall were included in this analysis. Similar positioning of stations in a north-south direction at the control site of Moana was also used (refer to Chapter 3).

The data were generally aggregated at two levels. For overall spatial patterns of community structure analysed using multivariate techniques, the data used was an aggregation of the temporal dataset, without making distinction between different surveys and pooled from ten replicates of each station from each north-south direction giving a data matrix with 12 samples (e.g. ON_1, CS_{60}, Table 4.1). Temporal patterns were examined using data pooled from ten replicates of each station at each survey time, giving a data matrix with 48 samples (e.g. 1-ON_1, 4-ON_{60}, 7-CN_{300} and 10-CS_{60}, Table 4.1).

In measures of community stress, mean values of the various parameters were calculated from replicate samples at each station from each survey to look at temporal changes. Some parameters were also calculated from data aggregated with disregard for survey times to examine the overall spatial variability (Table 4.1).

Table 4.1 Labelling of samples for different aggregations of data for the testing of effects on spatial and temporal variability (refer to Figure 2.2 for design). (O=Outfall site, C=Control site, N=North, S=South, $_1$=adjacent stations, $_{60}$=stations 60 m from outfall, $_{300}$=stations 300 m from outfall, numbers for temporal analysis indicate survey times in 1997; 1=January, 4=April, 7=July, 10=October).

	Outfall	**Control**
Spatial analysis Aggregated for testing of overall spatial effects across the surveys and pooled from all replicates of each station	ON_1, ON_{60}, ON_{300} OS_1, OS_{60}, OS_{300}	CN_1, CN_{60}, CN_{300} CS_1, CS_{60}, CS_{300}
Temporal analysis Aggregated for testing of changes through time and space and pooled from ten replicates of each station	1-ON_1, 4-ON_1, 7-ON_1, 10-ON_1 1-ON_{60}, 4-ON_{60}, 7-ON_{60}, 10-ON_{60} 1-ON_{300}, 4-ON_{300}, 7-ON_{300}, 10-ON_{300} 1-OS_1, 4-OS_1, 7-OS_1, 10-OS_1 1-OS_{60}, 4-OS_{60}, 7-OS_{60}, 10-OS_{60} 1-OS_{300}, 4-OS_{300}, 7-OS_{300}, 10-OS_{300}	1-CN_1, 4-CN_1, 7-CN_1, 10-CN_1 1-CN_{60}, 4-CN_{60}, 7-CN_{60}, 10-CN_{60} 1-CN_{300}, 4-CN_{300}, 7-CN_{300}, 10-CN_{300} 1-CS_1, 4-CS_1, 7-CS_1, 10-CS_1 1-CS_{60}, 4-CS_{60}, 7-CS_{60}, 10-CS_{60} 1-CS_{300}, 4-CS_{300}, 7-CS_{300}, 10-CS_{300}

Null hypotheses and model responses

In general the null hypotheses in these analyses would be that there are no differences in the macrobenthic infaunal communities across all stations, across all survey times and between the outfall and control sites. However, based on the knowledge of the processes operating in the ecosystem around the outfall and in the Gulf (discussed in Chapter 2), this is not the expected response. Instead, the macrobenthic infaunal community at the outfall site is expected to respond to the wastewater effluent discharge whereas the control site is not and is therefore assumed to represent the natural community in the Gulf. Hence, in an investigation of community structure, it is expected that the general community patterns from the outfall site will differ from those at the control site. In addition, there may be some distinction between the outfall stations with increasing distance from the outfall (Figure 4.4a). For any given environmental status indicator, the response is expected to be similar to the general community patterns with the outfall site having a higher or lower status than the control site and changing with increasing distance from the outfall (Figure

4.4b). For example, Stations ON_1 and OS_1 (closest to the outfall) may have high abundances of given taxa, which decrease with increasing distance from the outfall. Station OS_{300} at 300m south of the outfall may have a response at a similar level as the control site, that is, at background levels. Due to the longshore gradient where there is a net northward flow in the Gulf, the south stations at the outfall site may also be different from the north stations (Figure 4.4b).

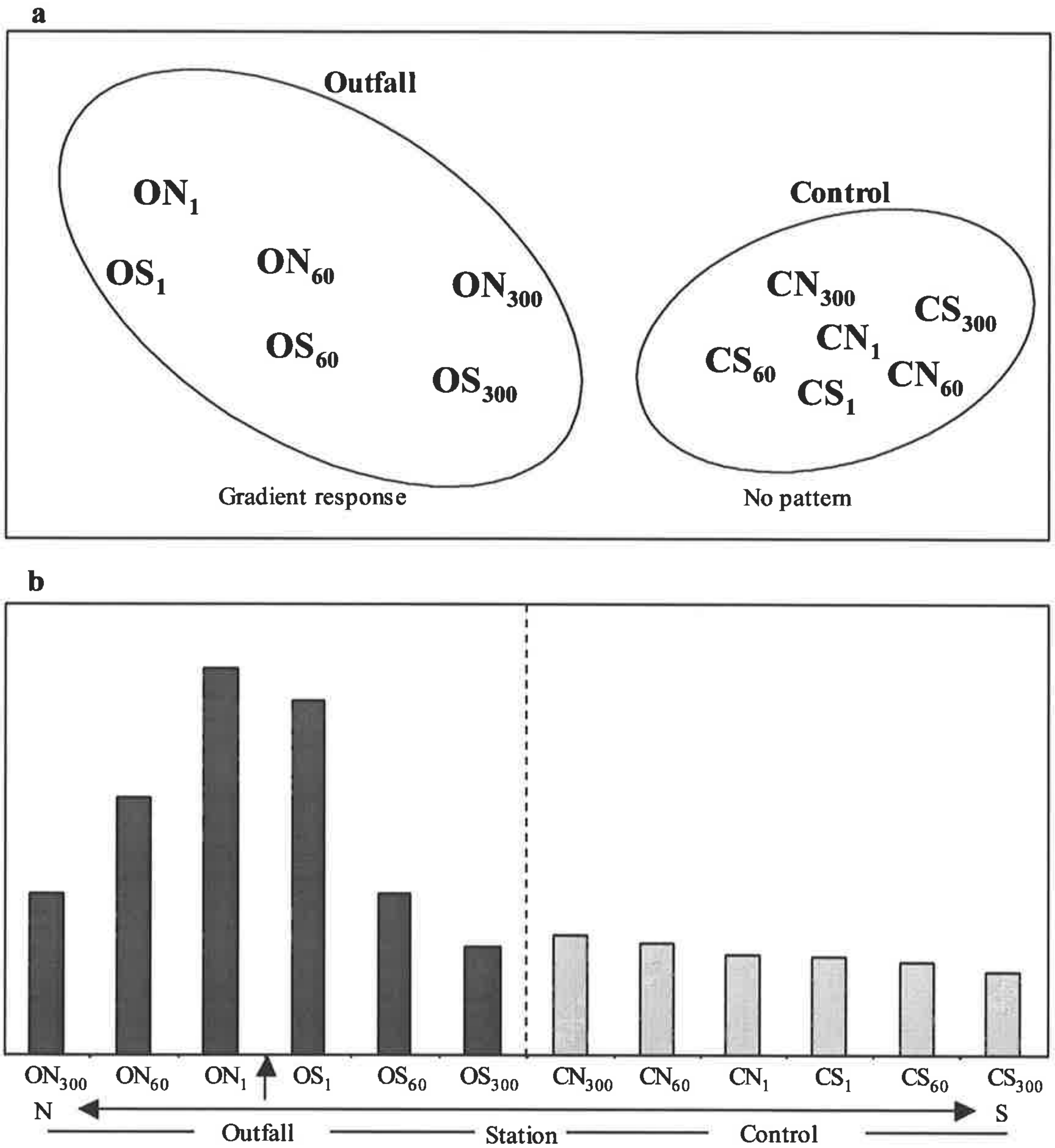

Figure 4.4 **Hypothetical responses for (a) pattern of community structure and (b) environmental status indicator (e.g. abundance or diversity). Stations are arranged in increasing distance from the outfall to the north and south (arrow indicates position of discharge point).**

Furthermore, it is also possible that this overall pattern of change may not be persistent through time. It may be well established during periods when the strongest influence on the community is the wastewater effluent. At other times, the system may be disturbed and driven by other processes such as storms, major recruitment events or increased rates of terrestrial run-off. If the north-south longshore gradient is important, then the change at the control site should be continuous over the whole length, changing from the furthest south to the furthest north.

The multivariate methods used in this study to elucidate patterns in the data on infaunal community structure are essentially descriptive in nature and cannot be used explicitly to test hypotheses about changes or differences in communities. Not withstanding this, it is possible to articulate typical or model responses that are to be expected given the knowledge of the way in which communities generally respond to effluent pollution.

Patterns of community structure

Two multivariate techniques, classification and ordination, were used to analyse the aggregated data. Similarity matrices using Bray-Curtis similarity coefficient (Bray and Curtis 1957) were computed. This similarity matrix was then subjected to hierarchical, agglomerative classification using the flexible beta linkage method (Sneath and Sokal 1973). In general the preferred approach to classification analyses is to use Ward's method, which provides a general-purpose linkage that minimizes distortions in the underlying space (McCune and Mefford 1999). There is however a problem with this approach in that the Bray-Curtis (Sorensen) distance is incompatible with Ward's method of linkage (Wishart 1969). The flexible beta approach provides a solution to this problem in that it allows the user to choose a clustering method that can variously approximate a range of linkage methods from nearest neighbour through to farthest neighbour. A beta value of -0.25 will behave similarly to Ward's method (Sneath and Sokal 1973) and therefore provides a good compromise.

Non-metric multidimensional scaling analyses (NMDS ordination (Kruskal and Wish 1978) were also carried out on the Bray-Curtis similarity matrices. Goodness-of-fit in the MDS ordination plots was measured as stress with Kruskal's stress formula I. Both classification and ordination methods were used to ensure that the underlying community structure was well defined. The results of the classifications were displayed in dendrograms while those of NMDS were in a two or more dimensional plot (dimensionality chosen using a scree plot).

In multivariate analysis of community data, transformations are commonly applied to weight the contributions of common and rare species. Different transformations of increasing severity were carried out on this data set before analysis. Transformations included using; raw abundance data where the between sample similarities were dominated by the common taxa, square root transformation that allowed contributions by intermediate abundance taxa, and a more severe 4th root transformation in which rare taxa were weighted as heavily as more common taxa. Another way of dealing with a data set that has many rare taxa is taxon removal. In the last decade, various works have shown that many taxa are interchangeable in the way they characterise the sample (Gray *et al.* 1988 Warwick 1993). An analysis of a subset of the total number of species may give a similar result to that of an analysis of all species. With this present data set, reducing the number of taxa whereby only taxa having abundance greater than 3% of total number of individuals at any station in any survey were retained, resulted in reducing the number of taxa to 20.

Differences between sites and survey times were determined using the "analysis of similarities" (ANOSIM) randomisation/permutation test (Clarke and Green 1988, Clarke 1993). The ANOSIM procedure is a non-parametric method based on rank similarities among all samples.

The Index of Multivariate Dispersion (IMD) as proposed by Warwick and Clarke (1993b) was also calculated to assess the variability of the community between the outfall and control sites. IMD contrasts the average rank of the similarities among the "polluted" samples with the average rank for each of the control samples. IMD values may range from +1 to -1; a maximum value of +1 indicates that dissimilarities among samples from the polluted location are all greater than any dissimilarity among samples from the control location. That is, the variation among samples from the polluted location is greater than the variation among samples from the control location. A value of -1 indicates that there is greater variability among the samples from the control location than the polluted location.

All multivariate analyses were carried out using the various routines in the software package PRIMER (Plymouth Routines in Multivariate Ecological Research) (version 4ß Clarke and Warwick 1994) or PC-ORD (McCune and Mefford 1999).

Measures of community stress

Abundance/Biomass Comparisons (ABC)

The method of abundance/biomass comparison (ABC) (Warwick 1986, Warwick *et al.* 1987) was used to compare the distribution of abundances among taxa with the distribution of biomass among taxa in each station. The W statistic was also calculated to compare the state of disturbance. The abundance and biomass data were combined across all survey times for each station to enable an overall assessment of putative disturbance. The ABC curves were also plotted for each survey time for each station to examine temporal differences. The ABC plots were constructed and the W statistics were calculated using the routine DOMPLOT in the PRIMER software package (version 4ß Clarke and Warwick 1994).

Neutral model analysis

Using the number of taxa and the number of individuals, the expected diversity (eH') and the standard deviation that the observed diversity deviates from the predicted eH' (V statistic) were calculated as proposed in the Ewens-Caswell neutral model (Caswell 1976).

Due to the cautions regarding samples given by Lambshead and Platt (1988) and Karakassis *et al.* (1996) discussed above and the low mean abundance of each sample in this study (17 individuals), the replicate core samples from each station were pooled for each survey time. The V statistics were then calculated using the routine CASWELL in the PRIMER software package (version 4ß Clarke and Warwick 1994).

Analysis of Variance (ANOVA) was then used on the V statistics calculated. Since replicate core samples were pooled for each survey time, there were three factors considered in the ANOVA, Sites (fixed, orthogonal), Direction (fixed, orthogonal) and Stations (fixed, orthogonal). When a significant interaction was found, higher-order interactions and main effects were not interpreted (Underwood 1981). Tukey-Kramer

HSD (honestly significant difference) test was then used to locate the source of the difference. Before analysis, the data was checked for variance homogeneity. If transformation did not remove heteroscedasticity, the raw data were analysed using Welch's ANOVA (Zar 1996). The traditional alpha value of 0.05 was used to define significance in all analyses.

Log-normal plots

Abundance class distributions were plotted for the number of taxa on the ordinate against the number of individuals per taxa in geometric classes on the abscissa using combined data from the four surveys. The taxa were grouped into abundance classes based on x2 geometric scale (Class I = 1 individual per taxa, Class II = 2 to 3 individuals per taxa, Class III = 4 to 7 individuals per taxa, Class IV = 8-15 individuals per taxa, etc.) and percentage number of taxa was used for comparable scaling to the figures. Taxa occurring in the intermediate abundance classes (Classes V and VI with 16 to 63 individuals per species) were also compared between the outfall and control sites to determine if there were any indications of population change (Gray and Mirza 1979, Gray and Pearson 1982)

Community descriptors

To examine spatial variability, the mean values of descriptive measures were calculated for each station at both the outfall and control sites using data from all surveys, disregarding seasonal variations (refer to Table 4.1).

For temporal variability, the measures were calculated for each station at each survey time (refer to Table 4.1). These measures included number of taxa (S) and abundance of individuals (A). Diversity (H') was also calculated as the Shannon-Wiener index (Shannon and Weaver 1949) using natural logarithm and evenness (J) according to Pielou (1969).

Since there were equal numbers of replicate cores taken at each station at each time, all abundance numbers used were not converted to per m^2 but left as numbers per core (i.e. per 1385 mm^2 and 200 mm deep). In order to determine whether there were differences among sites, directions or stations across survey times, multi-factorial Analysis of Variance (ANOVA) was used on each of these descriptors. There were four factors;

Surveys (fixed, orthogonal), Sites (fixed, orthogonal), Directions (fixed, orthogonal), and Stations (fixed, orthogonal).

When a significant Survey*Site*Direction*Station interaction was found, higher-order interactions and main effects were not interpreted (Underwood 1981). Tukey-Kramer HSD (honestly significant difference) test was then used to locate the source of the difference. Before analysis, the data was checked for homogeneity of variance. Heterogeneous data were transformed using $\log_e(x+1)$ and if transformation did not remove heteroscedasticity, the raw data were analysed using Welch's ANOVA (Zar 1996). The traditional alpha value of 0.05 was used to define significance in all analyses.

Characterising communities

Similarity Percentages (SIMPER)

To determine which taxa were primarily responsible for differences among groups and stations, the similarity percentages routine, SIMPER was utilised (Clarke 1993). This procedure computes the average similarity ($\overline{S_i}$) for all pairs of samples within a group and average dissimilarity ($\overline{\delta_i}$) between all pairs of inter-group samples. The results of the computation give a breakdown of the contributions from each taxa to the average term $\overline{S_i}$ or $\overline{\delta_i}$. The ratio of this average term ($\overline{S_i}$ or $\overline{\delta_i}$) and the standard deviation give a useful measure of how consistently a taxa contributes to the average similarity or dissimilarity. For within group similarities, a high ratio will indicate that the taxa typify that group while for between group dissimilarities, a high ratio will indicate that the taxa is a good discriminating taxa.

Proportions of major taxa

Variables such as nematode-copepod ratio, amphipod-polychaete ratio and trophic groups have been used as methods for assessing the effects of effluent on benthic communities (e.g. Raffaelli and Mason 1981, Warwick 1981, Amjad and Gray 1983, Smith and Simpson 1992, Smith 1994). These comparisons are based on the different sensitivities of the fauna to organic and nutrient enrichment.

Only the amphipod to polychaete ratio was calculated for each sample in this study. Although nematodes and copepods were obtained in the samples, the numbers obtained may be underestimated. Nematodes and copepods are commonly considered meiofauna, retained on 63 µm sieves. In the present study, the macrobenthic infauna was sampled and the smallest mesh size of sieve used was 0.5 mm and therefore too large to sample meiofauna.

Contributions of the major taxa identified in SIMPER were also compared for the various groups and stations identified in the multivariate analyses.

Correlating measured environmental variables

The protocols given by Clarke and Ainsworth (1993) were followed to assess the relation of the measured environmental variables (sediment size and organic carbon content; refer to Chapter 2) to the infauna data. The routine BIOENV in the software PRIMER (version 4ß Clarke and Warwick 1994) was used for this correlation analysis. The rank similarity matrices (Bray-Curtis for infauna and Euclidean distance for environmental variables) are compared using the weighted Spearman rank correlation. The rank correlation coefficient (ρ_w) lies between -1 and +1, where -1 indicates that there is no correlation and +1 indicates that there is complete agreement between the two matrices. Values are typically positive and ρ_w around zero corresponds to no match between the two patterns of biotic data and environmental variables (Clarke and Ainsworth 1993).

Symbols scaled in size to represent the values of environmental variables were also individually superimposed onto the faunistic two-dimensional MDS configurations in order to identify obvious visual concordance (Field *et al.* 1982).

Results

Spatial patterns of community structure

There were differences between the macrobenthic infaunal communities from the outfall site at Christies Beach and the control site at Moana with an apparent gradient with increasing distance from the outfall. From the classification of the untransformed aggregated total abundance of infauna for stations across all survey times, separation into three groups was evident[2] (Figure 4.5). One group (Group I) consisted of stations adjacent to the outfall (ON_1 and OS_1). The second group (Group II) comprised of stations at 60 m (ON_{60} and OS_{60}) and 300 m (ON_{300} and OS_{300}) in both north and south direction. The third group (Group III) had all the stations from the control site at Moana (Figure 4.5).

The two-dimensional MDS ordination plot also showed the distinct separation of these three groups with a very low stress value of 0.06 (Figure 4.6). In general terms, this pattern conformed to the expected result (illustrated in Figure 4.4a) with a clear separation of the impact and control sites and some degree of separation between the impact stations based on distance from the outfall. The control stations (Group III) formed a tight cluster to the right of the configuration while the outfall stations were more spread out with stations closest to the outfall (Group I) grouping to the left of the configuration. The Index of Multivariate Dispersion (IMD), calculated to compare the impact and control sites, gave a strong positive value of 0.911 indicating a much higher variability in the samples from the outfall site.

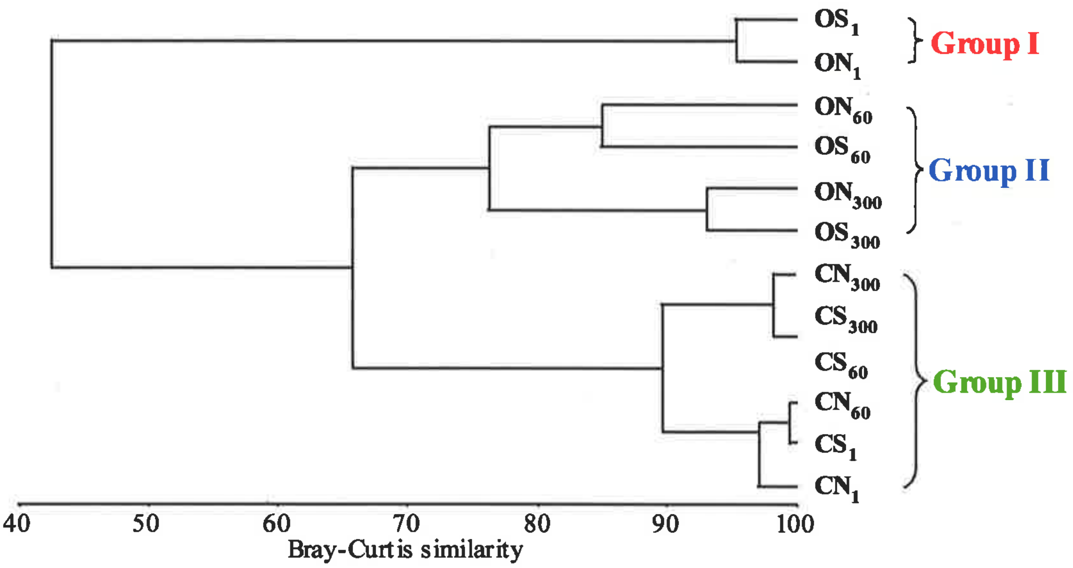

Figure 4.5 **Dendrogram for flexible beta (beta = -0.25) clustering of Bray Curtis similarities of untransformed data from both outfall (ON_1, ON_{60}, ON_{300}, OS_1, OS_{60}, OS_{300}) and control (CN_1, CN_{60}, CN_{300}, CS_1, CS_{60}, CS_{300}) sites. Separation into three groups (Groups I, II and III) was evident.**

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[2] During the analysis a wide range of clustering approaches were tried, all of which gave similar results in terms of the lower level structure in the resultant dendrograms. Importantly however, the higher level clustering was quite different for different clustering strategies. The final cluster (Figure 4.5) is therefore representative but should certainly not be viewed as a unique solution.

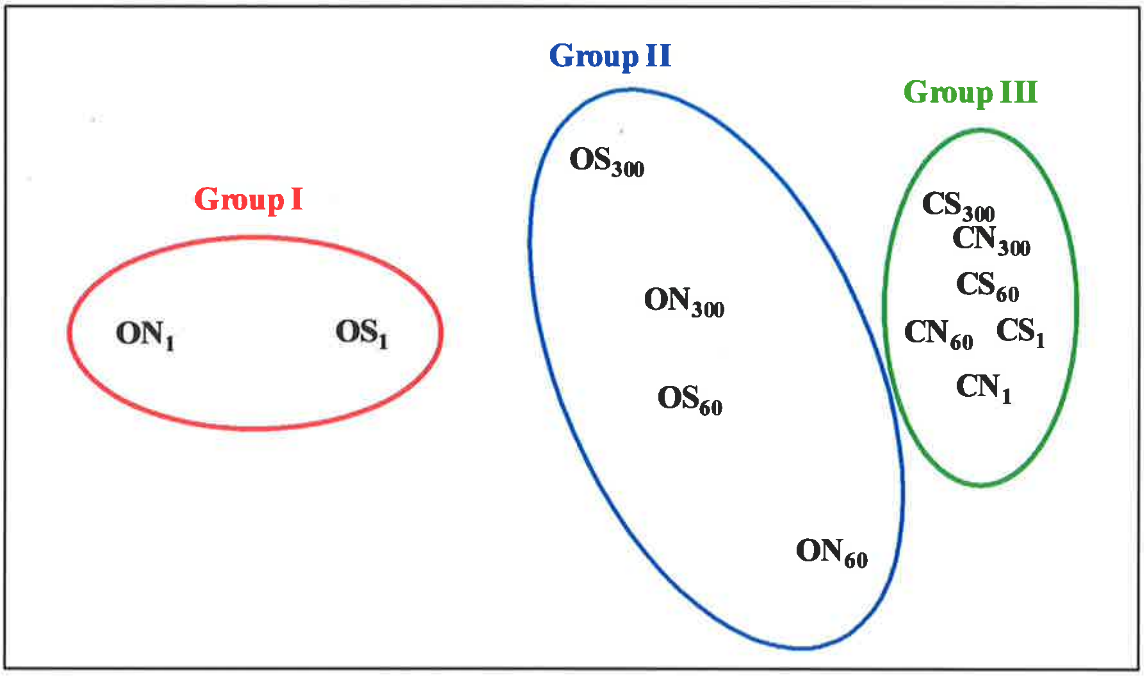


Figure 4.6 MDS ordination plot (2-dimension, stress = 0.06) of untransformed abundance showing separation of the stations into three groups (Groups I, II and III).

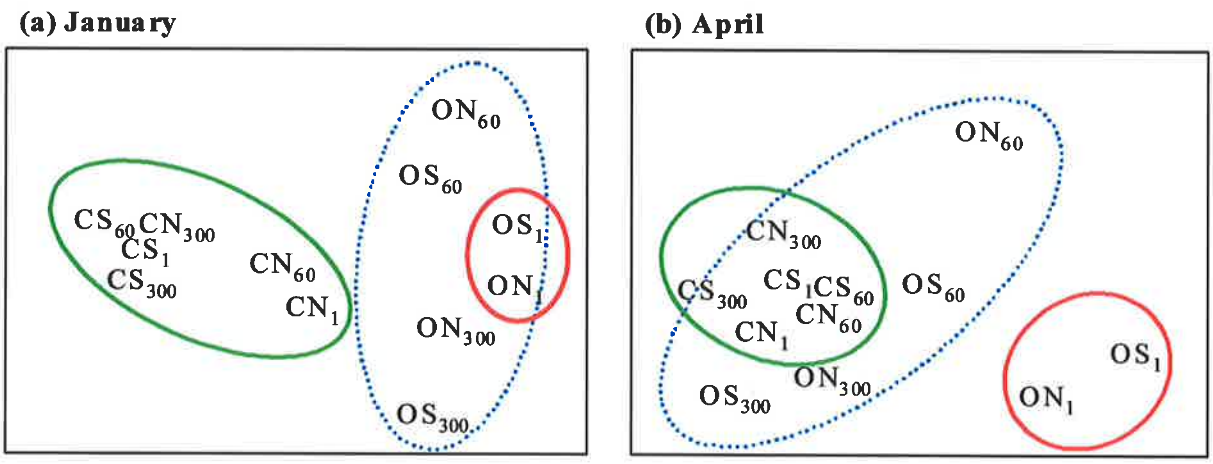
The same general pattern of grouping was obtained even when the data was transformed, either with square root or 4th root transformations. However, the 4th root transformation was found to be too severe as the abundance of any particular taxa ranged from single individual to only a few hundred individuals and not in the thousands. The stress values for the MDS ordination increased with the severity of transformation and the separation of groups were also not as distinct, especially for Group II stations (Appendix III, Figure III.1 to Figure III.4).

The analysis of the data with a reduced number of taxa also produced the same pattern of groupings, indicating a considerable redundancy in the taxa that characterise the community composition (Appendix III, Figure III.5 and Figure III.6).

At different taxonomic resolution, the same general separation of stations into three groups was maintained. When the data were analysed at the class level, the three groups consisting of stations close to the outfall (Group I), mid distance from the outfall (Group II) and control stations (Group III) were still observed. At the phylum level, these three groups were also maintained although the grouping of the mid-distance group (Group II) was not as distinct (Appendix III, Figure III.7 and Figure III.8).

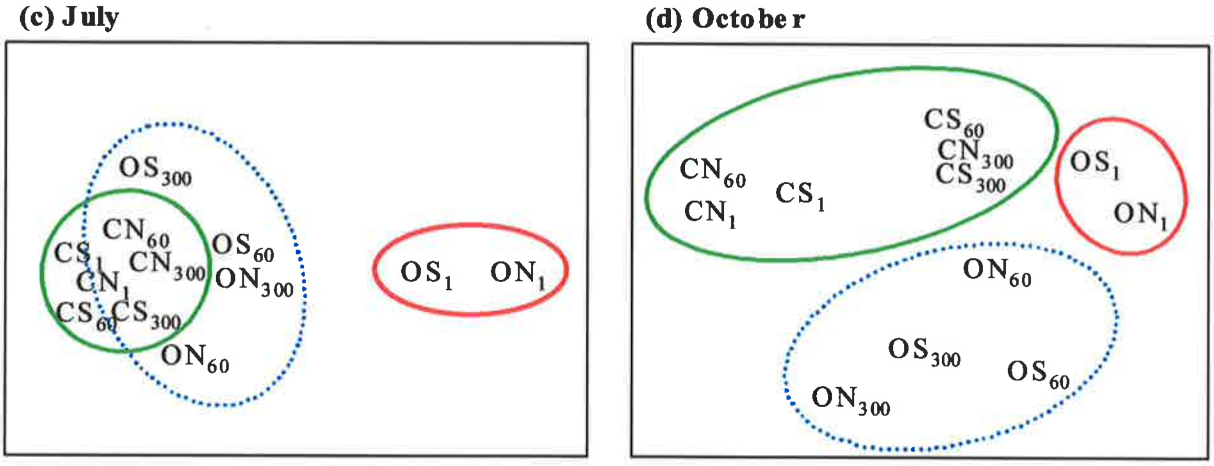
Temporal patterns of community structure

The pattern observed in the overall analysis detailed above was also observed when the data were analysed separately for each survey time. There was a general separation of stations at the outfall site from stations at the control site. The separation of stations into three groups was also evident, although to varying degrees for each survey (Figure 4.7). Station 1 from the outfall site for both north and south transects (ON_1 and OS_1) were clustered together while Stations 60 and 300 from the outfall site were more widely spread but were still generally separated from the stations of the control site (Figure 4.7).



(a) January

(b) April



(c) July

(d) October

Figure 4.7 **MDS ordination plots (2-dimension) of untransformed abundance data for each survey, showing the degree of separation of outfall site from control site and the separation of stations into three groups. (a) January survey (Stress=0.07), (b) April survey (Stress=0.09), (c) July survey (Stress=0.03), (d) October survey (Stress=0.11).**

On the broad spatial scale with data across the surveys analysed together, the general pattern obtained showed a wider spread of the samples for the outfall site as compared to the control site (Figure 4.8 and Figure 4.9). This may be indicative of a greater variability of the samples from the outfall site as distances between samples reflect the degree of similarity between them. There was a general separation of outfall stations from the control stations, although there was some overlap of mid distance stations from the outfall site with the control stations. Consequently, the Index of Multivariate Dispersion was still positive (indicating higher dissimilarities between samples from outfall site) but was a low value of 0.34. A distinct cluster consisting of the stations closest to the outfall from all sampling times (Station 1 from both north and south direction and from all surveys) was obtained and was best represented by a three-dimensional MDS plot (Figure 4.8). Although the stress value was higher (0.16 as compared to 0.11) when the data was subjected to a square root transformation, the ordination plot showed the separation of groups to be more distinct (Figure 4.9).

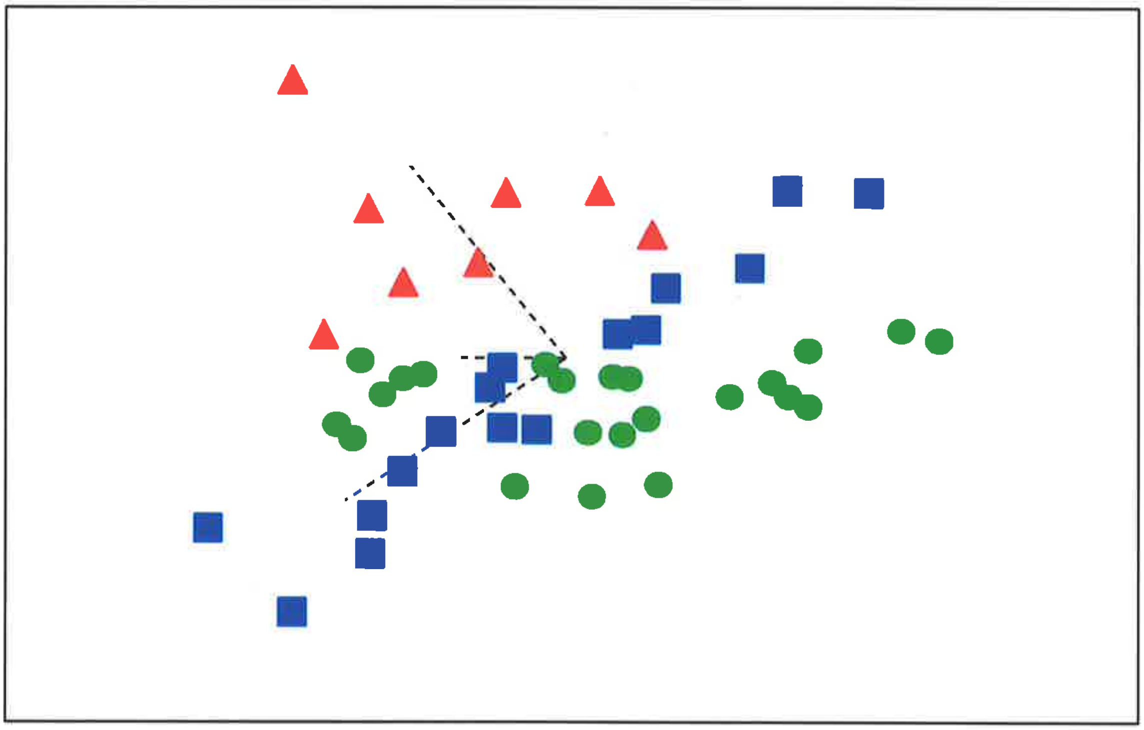


Figure 4.8 MDS ordination plot (3-dimension, stress=0.11) of untransformed abundance data for all stations from all surveys showing the separation of stations closest to the outfall site (▲) from the control site (●) with overlap of mid distance stations (■) from the outfall site with the control stations.

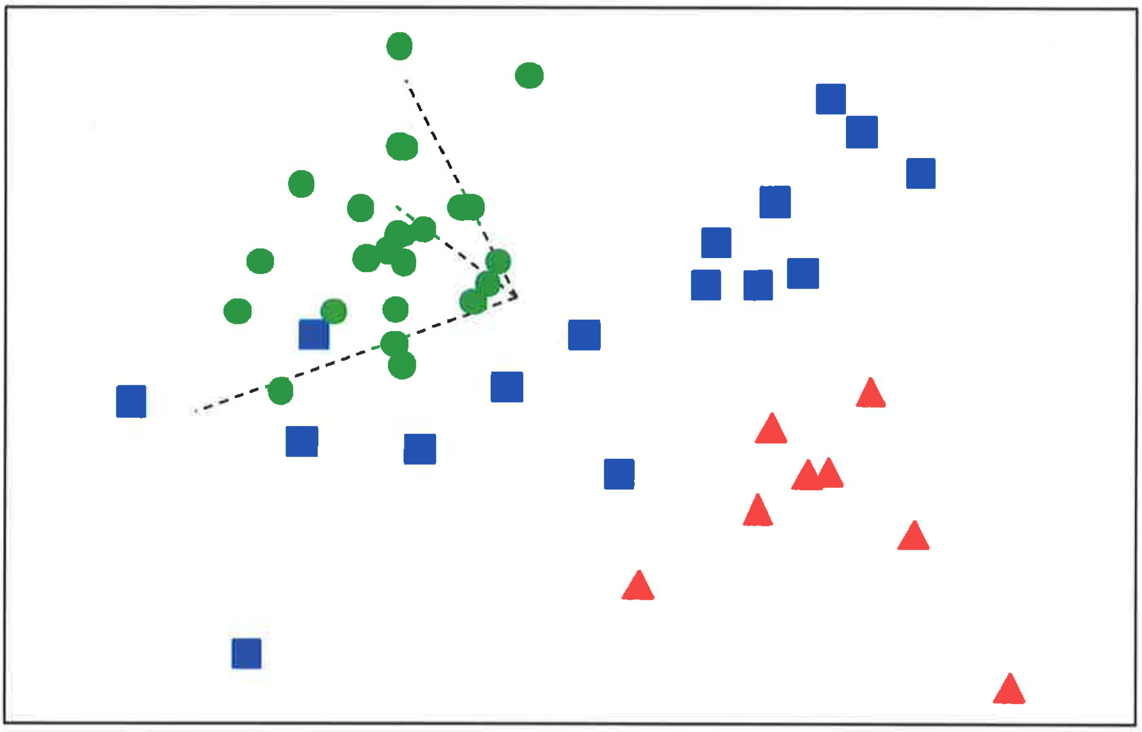


Figure 4.9 MDS ordination plot (3-dimension, stress=0.16) of square root transformed abundance for all stations from all surveys showing the more distinct separation of the outfall site from the control site (▲ Outfall Station 1, ■ Outfall Stations 60 and 300, ● Control stations).

Outfall site

Initial analysis of outfall samples alone (control samples excluded), revealed two distinctly different samples in the two dimensional ordination (stress = 0.19). Samples close to the outfall from the survey carried out in July 1997 (7-ON$_1$ and 7-OS$_1$) were clustered together; separated from all the other samples in both the classification and ordination analyses (Figure 4.10). This was probably due to a much higher abundance of spionids (470 individuals at 7-ON$_1$ and 216 individuals at 7-OS$_1$) as compared to the other samples (<150). However, in the three dimensional ordination (stress = 0.09), these samples did not appear as outliers but were grouped with the other stations (Figure 4.11). The pattern of stations closest to the outfall (Station 1 from both north and south transects) forming a distinct group emerged again. Stations 60 and 300 were also separated from each other regardless of survey times (Figure 4.11).

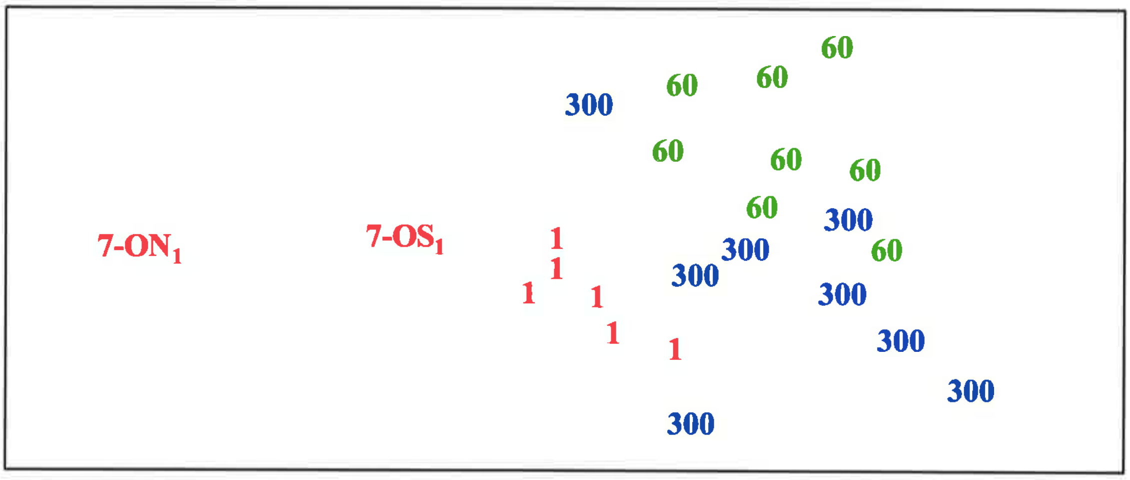


Figure 4.10 **MDS ordination (2-dimension, stress=0.19) of untransformed abundance for outfall site only. Stations 7-ON$_1$ and 7-OS$_1$ (Station 1 of the July survey) clustered together separate from all the other samples (1=Station 1, 60=Station 60, 300=Station 300).**

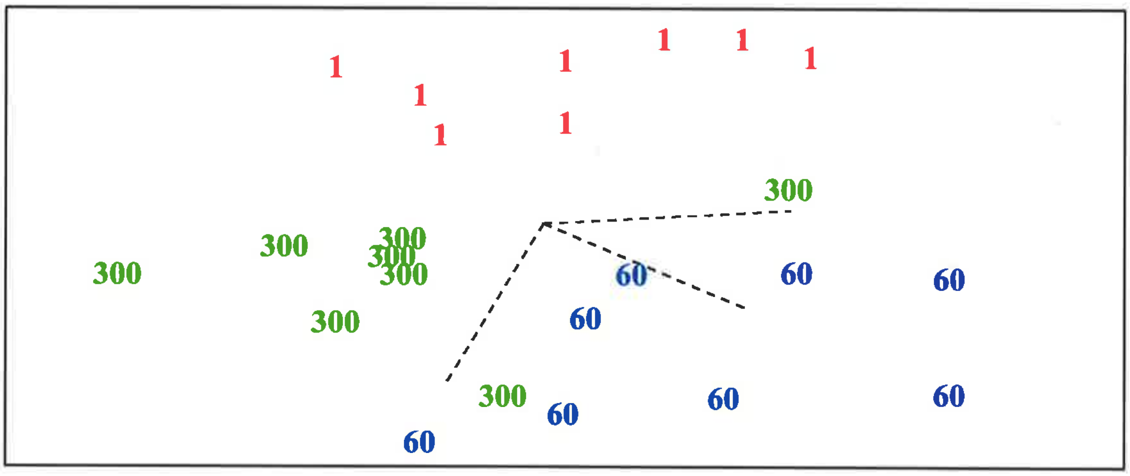


Figure 4.11 **MDS ordination (3-dimentsion, stress=0.09) of untransformed abundance data for outfall site. Stations were grouped according to distance from the outfall (1=Station 1, 60=Station 60, 300=Station 300).**

The group comprising Station 60 from both the north and south transects did not form as tight a cluster as Station 1; neither did the group for Station 300. This apparent gradient in the grouping of the stations appeared to be consistent with increasing distance from the outfall. Any trend associated with survey times appeared to be masked by this gradient of distance from the outfall. The analysis of similarities (ANOSIM) confirmed this difference with increasing distance from the outfall (Table 4.2).

Table 4.2 **Analysis of similarities (ANOSIM) of the three stations at the outfall site. The upper values refer to the R statistic and lower values refer to the significance level. The global R-value was 0.487 at a significant level of 0.0%**

	Station 1	**Station 60**	**Station 300**
Station 1		0.619	0.467
Station 60	0.0%		0.367
Station 300	0.0%	0.3%	

Control site

With the samples from the control site at Moana, variation with survey times was obvious. Samples from the survey conducted in January 1997 (summer) formed a distinct cluster (1-CN$_1$, 1-CN$_{60}$, 1-CN$_{300}$, 1-CN$_1$, 1-CS$_{60}$ and 1-CS$_{300}$) in the top of the ordination plot (Figure 4.12). Those from the survey carried out in April 1997 (autumn) could also be distinguished from the other samples by the cluster in the middle. Samples from surveys carried out in July and October 1997 (winter and spring) were mixed (Figure 4.12) and one-way ANOSIM confirmed this result. Pairwise comparisons showed that all surveys were significantly different (p<0.05) except for the surveys in July and October (Table 4.3).

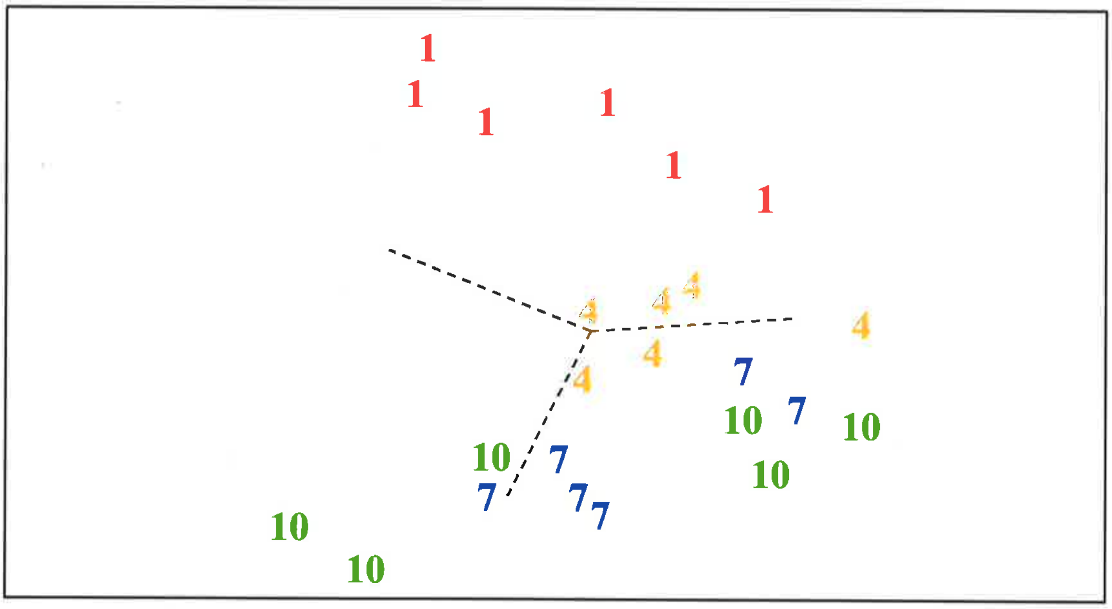


Figure 4.12 **MDS ordination (3 dimension, stress = 0.05) of untransformed abundance data for control sites. Stations were grouped according to survey times (1=January, 4=April, 7=July and 10=October).**

Table 4.3 **Analysis of similarities (ANOSIM) for the surveys at the control site, Moana. The upper values refer to the R statistic and lower values refer to the significance level. The global R-value was 0.55 at a significant level of 0.0 % (* indicate no significant difference).**

	January	April	July	October
January		0.759	0.941	0.893
April	0.2%		0.546	0.455
July	0.2%	0.9%		0.030
October	0.2%	0.2%	*30.1%	

Measures of community stress

Abundance/Biomass Comparisons

There were differences in the ABC plots for the overall spatial assessment of disturbance from the wastewater effluent. However, none of the plots showed the "highly disturbed" condition, where the abundance curve lies above the biomass curve. The ABC plot for Station ON_1 (closest to the outfall in the north) conformed to the configuration expected for a "moderately disturbed" site. The abundance curve starts above the biomass curve, dominating for half its length and crossing it once (Figure 4.13). At Station OS_1 (closest to the outfall in the south), the biomass curve lies just above the abundance curve for its full length and is thus intermediate between the "moderately disturbed" and the "undisturbed" configurations (Figure 4.13). All the other stations (ON_{60}, ON_{300}, OS_{60} and OS_{300}) from the outfall site showed the "undisturbed" configuration (Figure 4.13), similarly for all stations from the control site, Moana (Figure 4.14).

Examination of the graphs for individual surveys indicated that Station ON_1 (adjacent station at the outfall site in the north) showed the "moderately disturbed" condition in the January survey (summer) and the "grossly disturbed" condition in the July survey (winter). Plots of the April survey (autumn) and the October survey (spring) showed undisturbed configurations (Figure 4.15). The ABC plots of Station OS_1 (adjacent station at the outfall site in the south) for each survey were not similar to those of Station ON_1. In the April (autumn) survey, the configuration was that of a "moderately disturbed" site and in the July (winter) survey, it was intermediate between "moderately disturbed" and the "undisturbed" configuration (Figure 4.15).

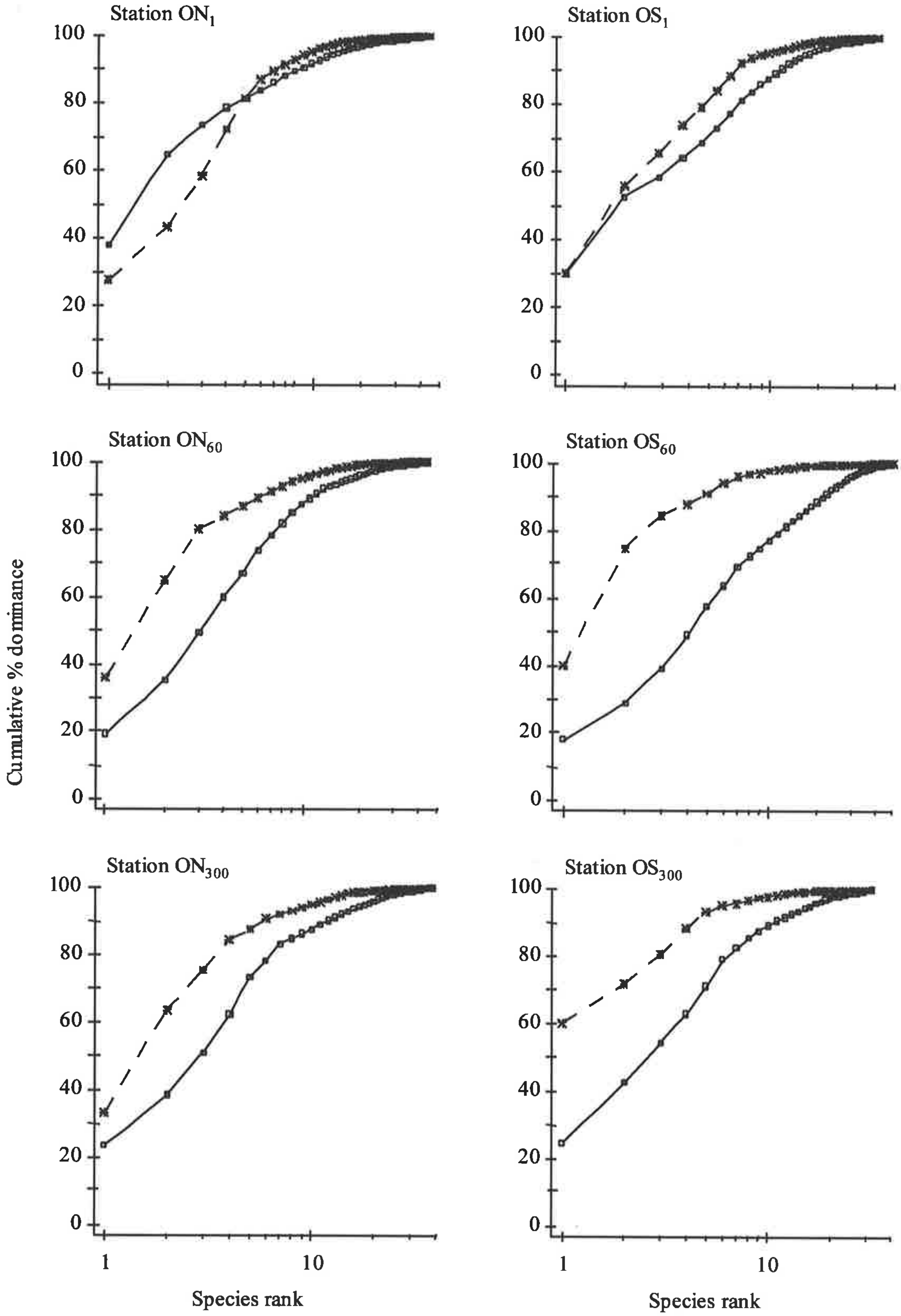


Figure 4.13 ABC plots generated from the combination of all data from the four surveys at the outfall site. Moderately disturbed macrobenthic communities were indicated for the two stations closest to the outfall (ON$_1$ and OS$_1$). (—▫— Abundance, – ✱ – Biomass).

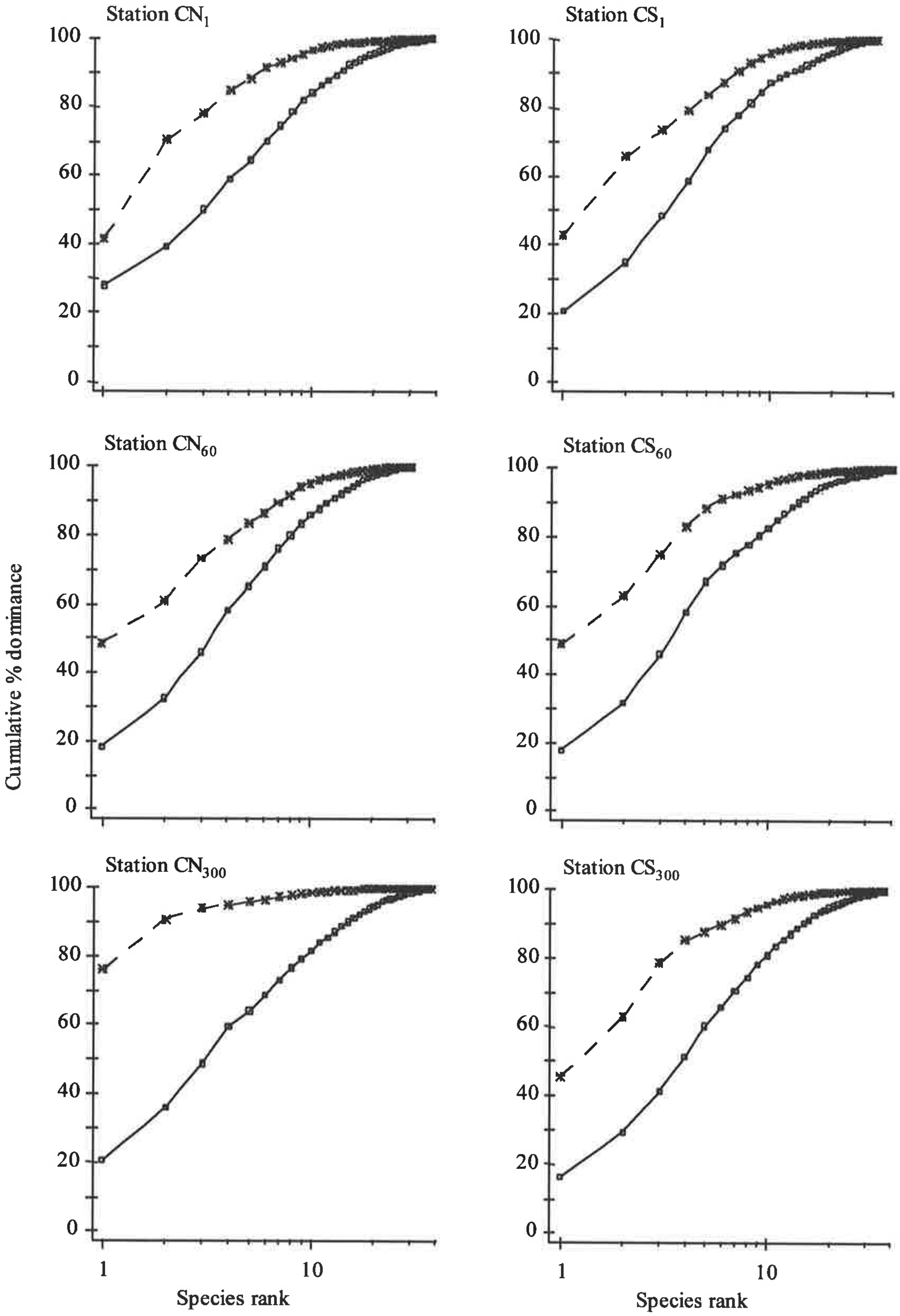


Figure 4.14 ABC plots generated from the combination of all data from the four surveys at the control site. All configurations were indicative of "undisturbed" conditions. (—▫— Abundance, – ✱ – Biomass).

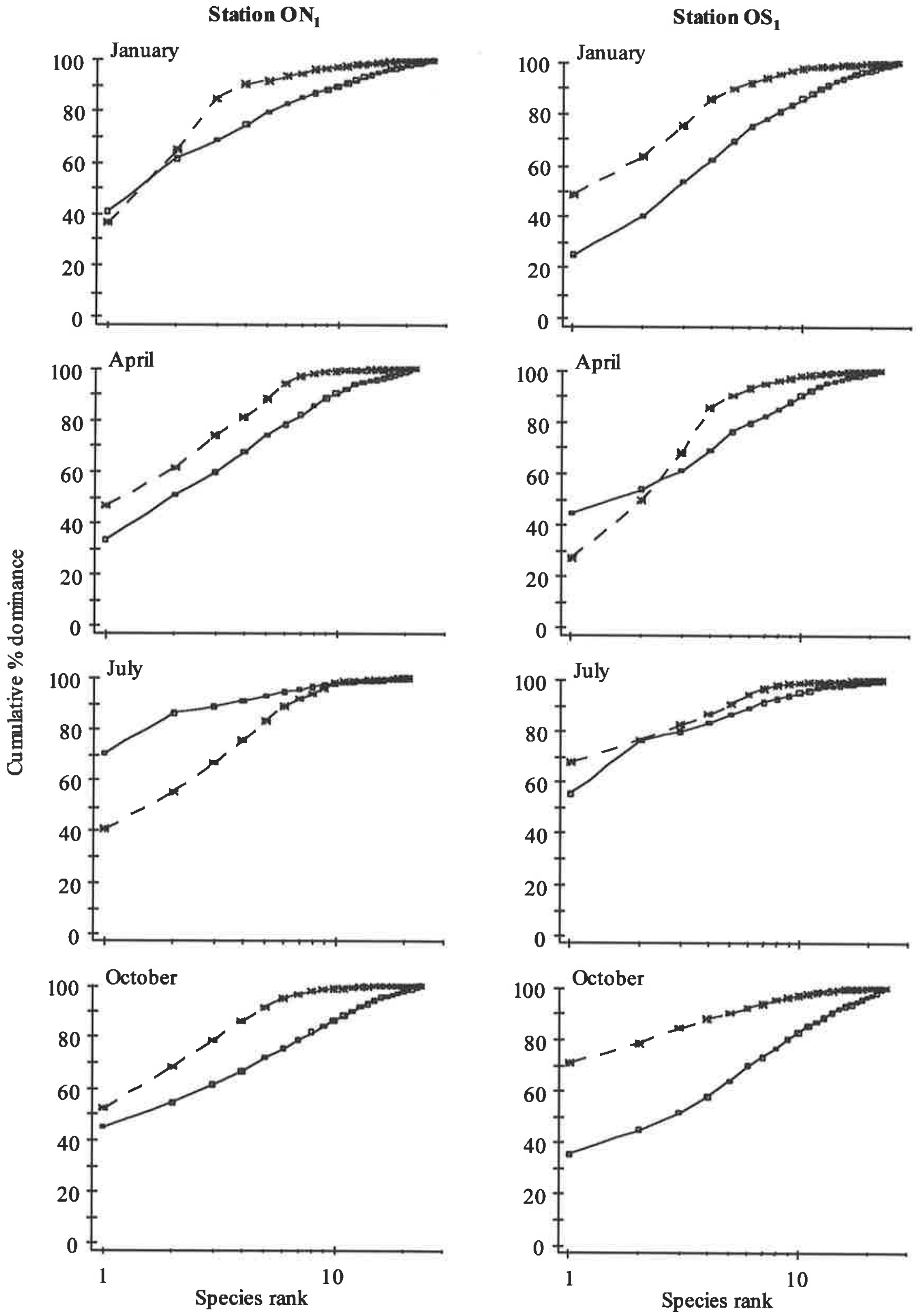


Figure 4.15 **ABC plots generated for stations closest to the outfall (ON_1 and OS_1) for each survey. Disturbed macrobenthic communities were indicated for Station ON_1 in January (summer) and July (winter) while for Station OS_1, it was in April (autumn) and July (winter) (—▫— Abundance, – ✶ – Biomass).**

The crossed configuration for Station ON_1 pooled across surveys occurred at taxa rank five. An examination of the raw abundance data for the samples indicated that the abundant taxa represented a combination of polychaetes, ostracods and nemerteans.

The W statistic indicated that differences between stations were influenced by distance from the outfall. Stations from the outfall site had relatively lower W statistic values although none of the W statistic was below -0.2 (Figure 4.16). This pattern was clearer with the combined data across surveys. Stations closest to the outfall (ON_1 and OS_1) had the lowest W statistic values (0.004 and 0.071 respectively). Stations to the north of the outfall site ($ON_{60} = 0.114$ and $ON_{300} = 0.111$) also had lower values compared to stations to the south of the outfall site ($OS_{60} = 0.245$ and $OS_{300} = 0.167$). The control stations had W statistic values in the range of 0.146 to 0.246 for the combined data across surveys (Figure 4.16).

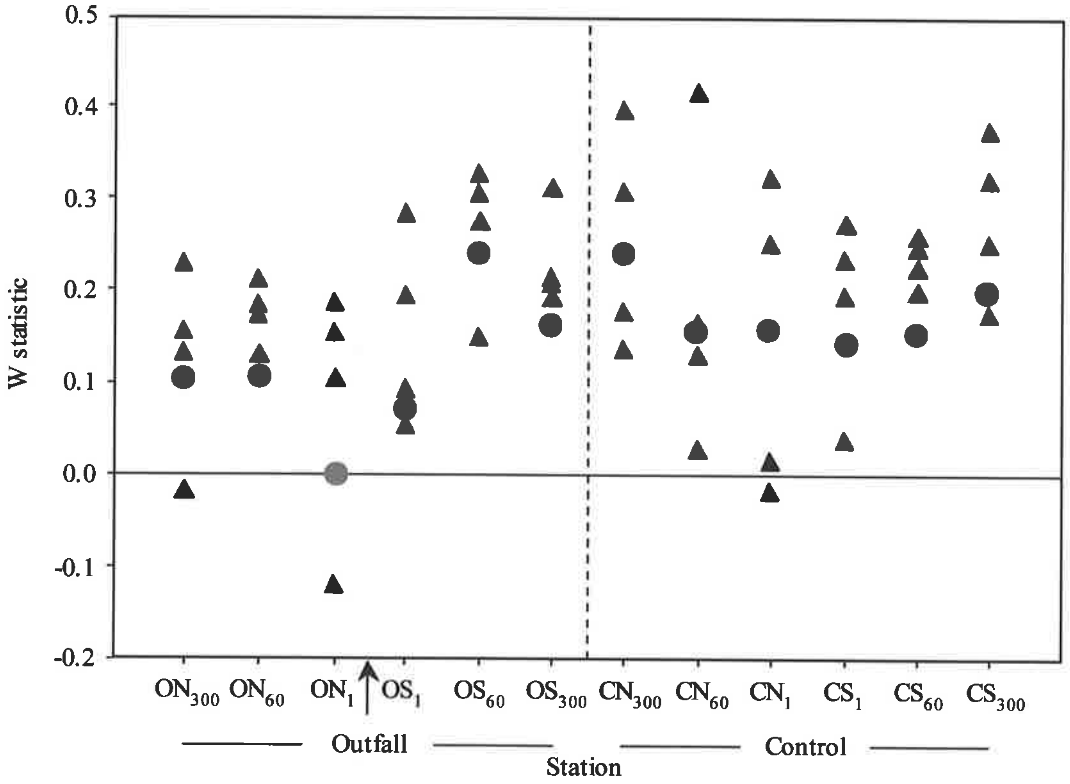


Figure 4.16 Scatter plot of the W statistic against stations at the outfall and control site. Stations are arranged in increasing distance to the north and south of the outfall. Red circles refer to the W statistic for the combined data across all surveys.

Neutral model analysis

Results of the neutral model analysis showed some trends in V statistic values among sites. All mean V statistic values across all surveys were less than zero (Figure 4.17). Generally, stations at the outfall site had more negative V statistics as compared to stations at the control site. The outfall site had mean values ranging from -0.01 to +2.39 (with an overall mean value of -1.08) while those from the control site were in the range of -0.28 to -1.28 (with an overall mean value of -0.58). The mean V statistic for Station ON_1 and OS_1 were more negative than the other stations (-2.39 and -1.57 respectively). All other stations had mean values not less than -1.28 (Figure 4.17).

Results of the ANOVA showed no significant interactions at the Site*Direction*Station level (Table 4.4), but at the Site*Station level, there were significant interactions ($p<0.05$). Testing for differences between surveys was not possible since the data were pooled for each survey. The post-hoc pairwise comparisons at the level of Site-Station indicated that Station 1 of the outfall site was significantly different from Station 60 of the outfall site and Station 300 of the control site.

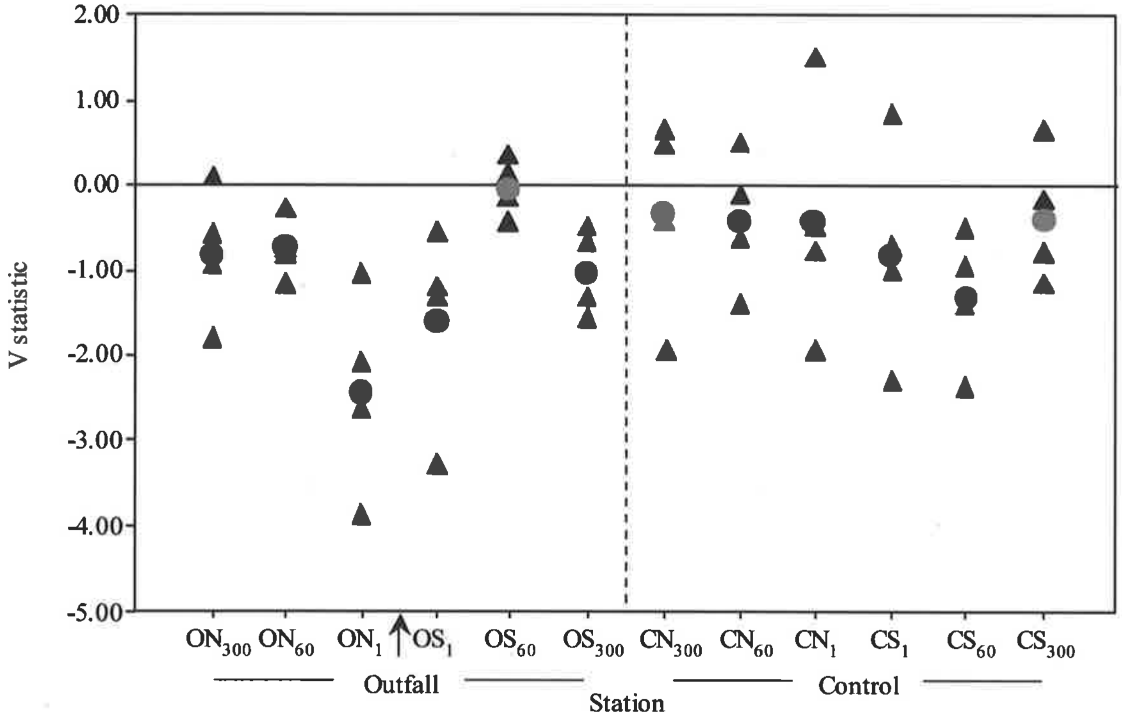


Figure 4.17 **Scatter plot of V statistics against stations at the outfall and control sites. Stations are arranged in increasing distance to the north and south of the outfall. Red circles refer to the V statistics for the combined data across all survey.**

Table 4.4 **ANOVA table for the V statistic comparing site, direction and station ($^+$ indicate significance).**

Source	DF	MS	F	p
Site	1	3.02	3.35	0.08
Direction	1	0.00	0.00	0.99
Station	2	2.44	2.71	0.08
Site*Direction	1	2.39	2.65	0.11
Site*Station	2	3.48	3.85	$^+$0.03
Direction*Station	2	0.14	0.16	0.85
Site*Direction*Station	2	0.84	0.93	0.40
Error	36	0.03		

Log-normal plots

The numerical distribution of taxa and individuals did not show any good fit to log-normal plots characteristic of polluted or unpolluted conditions (Figure 4.18). Rare taxa were not more abundant nor were the curves any smoother at the control site compared to the outfall site. At all stations from both the outfall and control sites, taxa were distributed unevenly with several peaks for most curves. However, Station 1 (closest to the outfall) of the outfall site for both north and south had the most abundance classes (up to Class IX). The other stations (Stations 60 and 300) at the outfall site had seven or eight classes, while at the control site there were eight or less abundance classes.

At the outfall site, taxa occurring in Classes V and VI at each station were variable, while at the control site; similar taxa occurred in these two classes for most stations. Several different gammaridean amphipods were consistently found at the control stations occurring in Classes V and VI. However, no such consistency was found at the outfall stations, where most taxa were variously distributed. The polychaete family Capitellidae was only found at stations closest to the outfall (Station ON_1 and OS_1) belonging to Class V. For all other stations (including control stations), capitellids were either not sampled or occurred in Classes I or II.

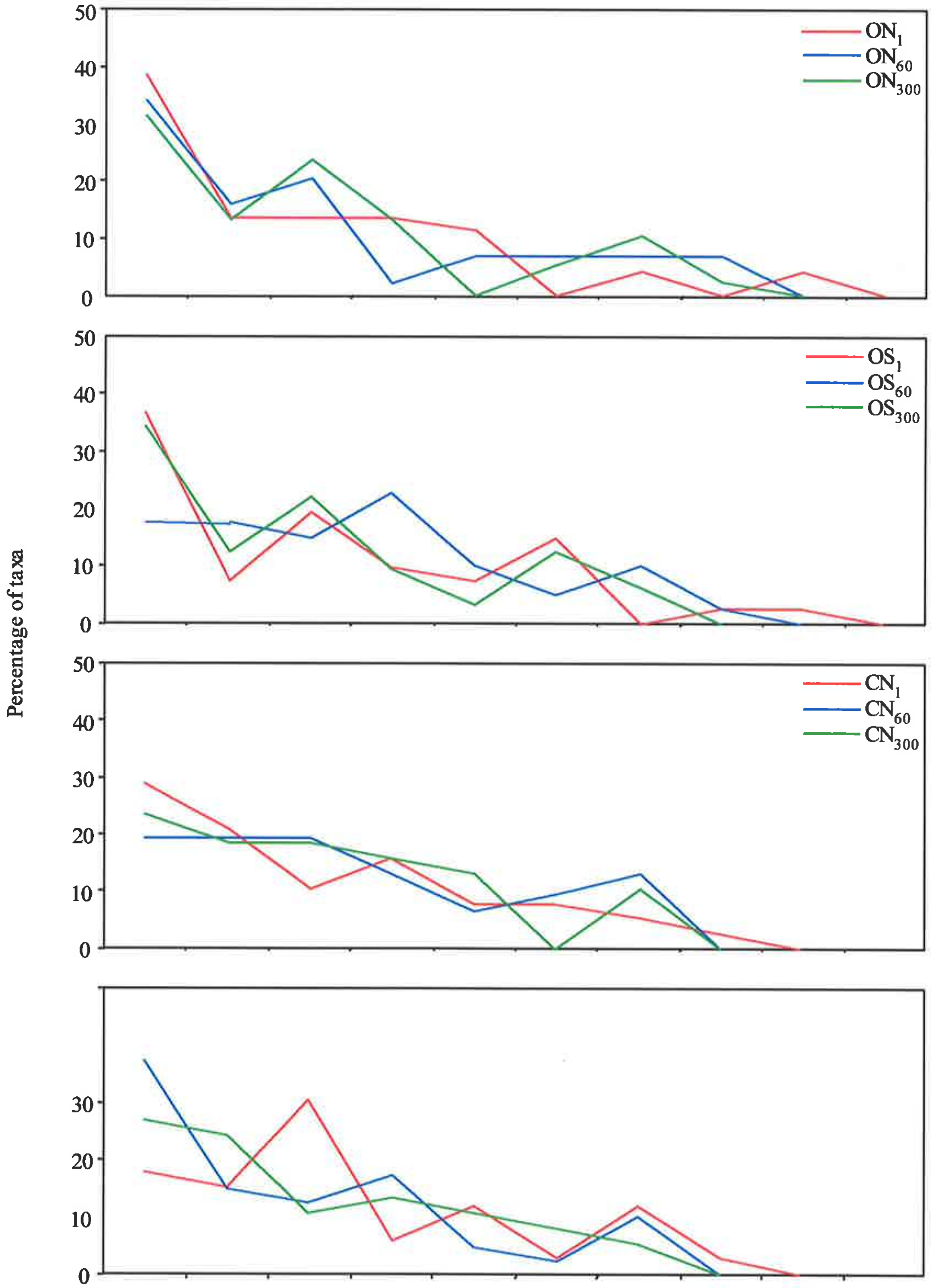


Figure 4.18 **Log-normal distribution of individuals among taxa for stations from both the outfall and control sites. The data are combined from 40 core samples taken over the four surveys, expressed as percentage of taxa. (Abundance classes are I = 1; II = 2-3; III = 4-7; IV = 8-15; V = 16-31; VI = 32-63; VII = 64-127; VIII = 128-255; IX = 256-511; X = 512-1023) (a) Outfall north stations, (b) Outfall south stations, (c) Control north stations, (d) Control south stations.**

Community descriptors

In general, the differences in the various community descriptors between the outfall site and the control site followed the hypothetical model illustrated in Figure 4.4b. Any pattern of change with increasing distance from the outfall was most pronounced at stations closest to the outfall. The values of the community descriptors were either highest or lowest at these stations and change with increasing distance from the outfall. However, no such trends were observed for the control site (Figure 4.19a-e).

The outfall site mostly had higher values for the mean number of taxa (S) across all surveys (greater than the overall mean of 7.5) when compared to the control stations (Figure 4.19a). At the outfall site, Station 60 from both north and south showed a higher mean number of taxa compared to the other stations.

The overall mean abundance had a different pattern with increasing distance from the outfall. Only stations closest to the outfall (ON_1 and OS_1) had elevated numbers of individuals (Figure 4.19b). The control site still had abundances lower than the grand mean value of 18.27 individuals per core (Figure 4.19b).

The mean biomass was spatially very variable and there were no particular trends observed across the stations (Figure 4.19c).

The mean values of the Shannon-Wiener diversity (H') had a similar trend to the number of taxa where values were higher at Station 60 of the outfall site (Figure 4.19d), and mean values of evenness also followed this trend (Figure 4.19e).

The temporal changes in community descriptors at both the outfall and control sites confirmed the patterns of community structure observed in the multivariate analyses. At the outfall site, where the grouping of stations was influenced by distance from the outfall, the change in the community descriptors across the stations (albeit with some variability) was generally similar for each survey (Figure 4.20a-e). The values changed with increasing distance from the outfall, both northwards and southwards. At the control site, where it has been shown that stations were grouping by survey times, there were no observable trends in the community descriptors, varying differently for all stations in each survey (Figure 4.20a-e).

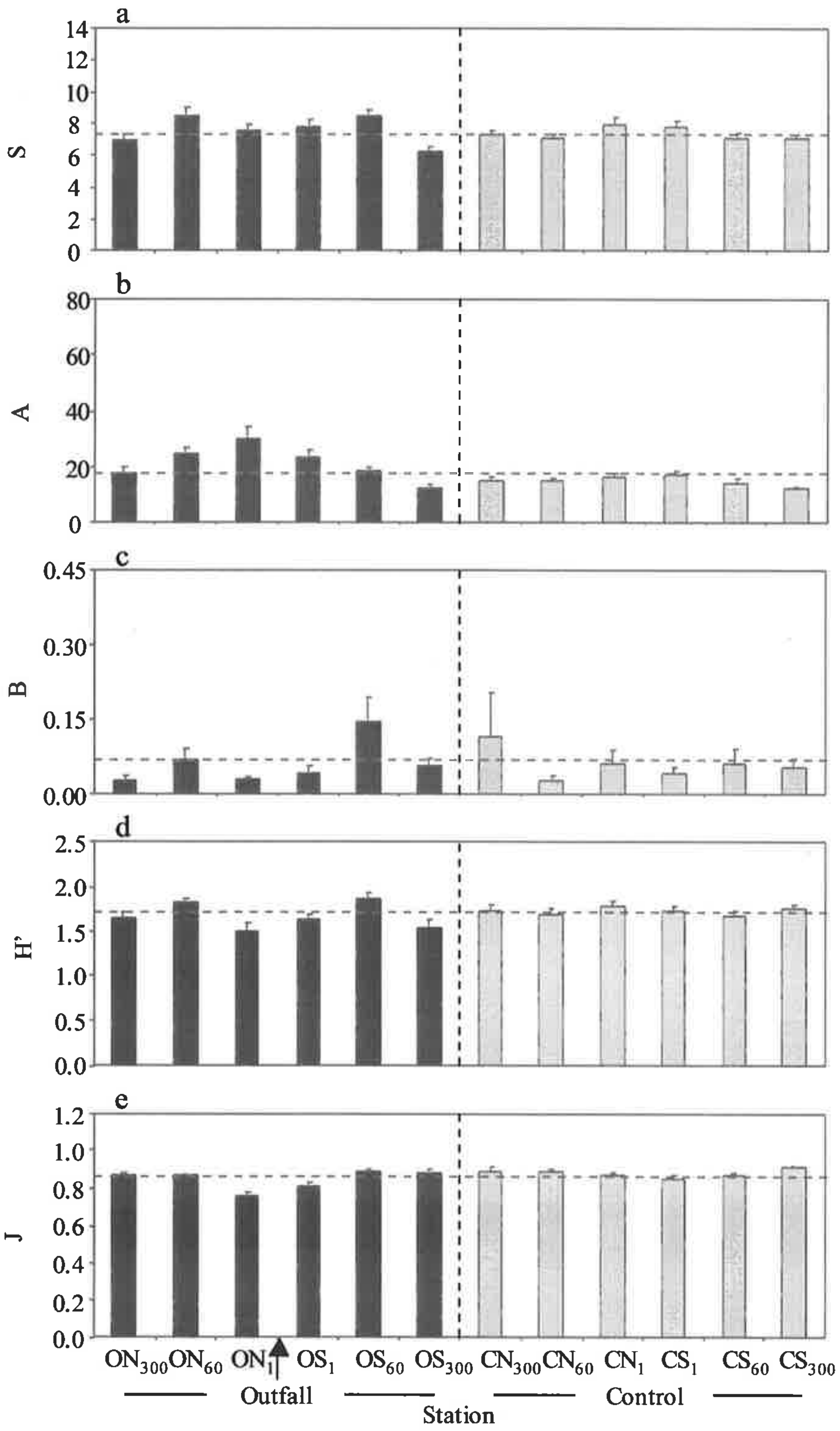


Figure 4.19 **Spatial changes in mean values per core for (a) number of taxa S (b) abundance A, (c) biomass B, (d) diversity H' and (e) evenness J. Stations are arranged in increasing distance from the outfall moving both north and south of the outfall. Arrow indicates discharge point. Horizontal dotted line indicates overall mean.**

There were significant Survey*Site*Direction*Station interactions ($p<0.05$) occurring in all descriptors except for the number of taxa (S) and biomass (B) (Table 4.5). Significant Survey*Site*Station interactions ($p < 0.05$) occurred for the number of taxa (S) while biomass (B) had significant Site*Station interactions ($p < 0.1$).

Table 4.5 ANOVA comparing the community descriptors among stations, transects, surveys and sites for significant interactions (detailed ANOVA presented in Appendix IV).

Source	DF	MS	F	p
a Number of taxa (S)				
Survey*Site*Station	6	21.65	5.18	0.000
b Abundance (A)				
Survey*Site*Direction*Station	6	285.97	4.41	0.000
c Biomass (B)				
Site*Station	2	0.1183	2.78	0.063
d Diversity (H')				
Survey*Site*Direction*Station	6	0.4400	4.34	0.000
e Evenness (J)				
Survey*Site*Direction*Station	6	0.0500	8.06	0.000

Fluctuations in the mean number of taxa (S) over the four surveys were not the same between the outfall and control site. The patternRegardless of direction, the trends observed at the outfall site were generally similar for each survey, changing with increasing distance from the outfall (Figure 4.20). The number of taxa was higher at Station 60 in the January, April and July surveys, but this pattern was not maintained in the October survey. However, at the control site, there were no trends observed for the stations across surveys.

The change in mean abundance at the outfall site with increasing distance from the outfall was more variable and different for both north and south transects (Figure 4.21), hence the significant interaction of Site*Transect*Survey*Station ($p<0.05$) (Table 4.5). On the north transect only the January and April surveys had similar patterns of change while on the south transect, similar changes were observed for the April and July surveys.

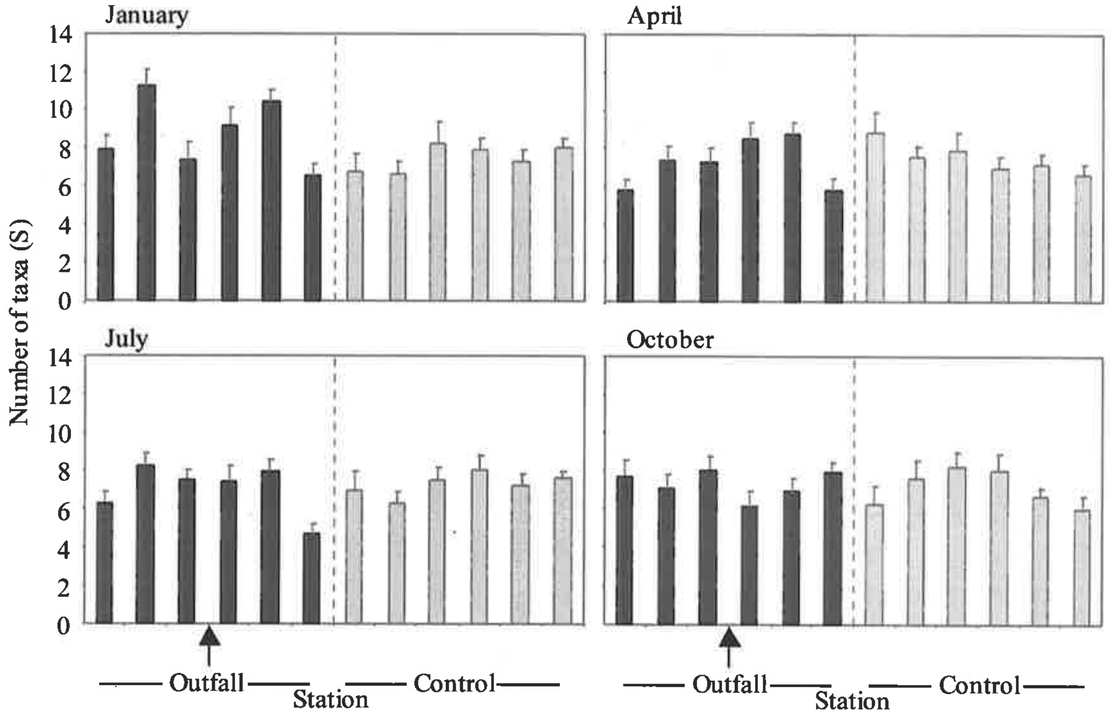


Figure 4.20 Temporal changes in mean number of taxa (S) per core sample (± SE). Stations are arranged with increasing distance from the outfall moving both north and south of the outfall as in Figure 4.19. Arrow indicates discharge point.

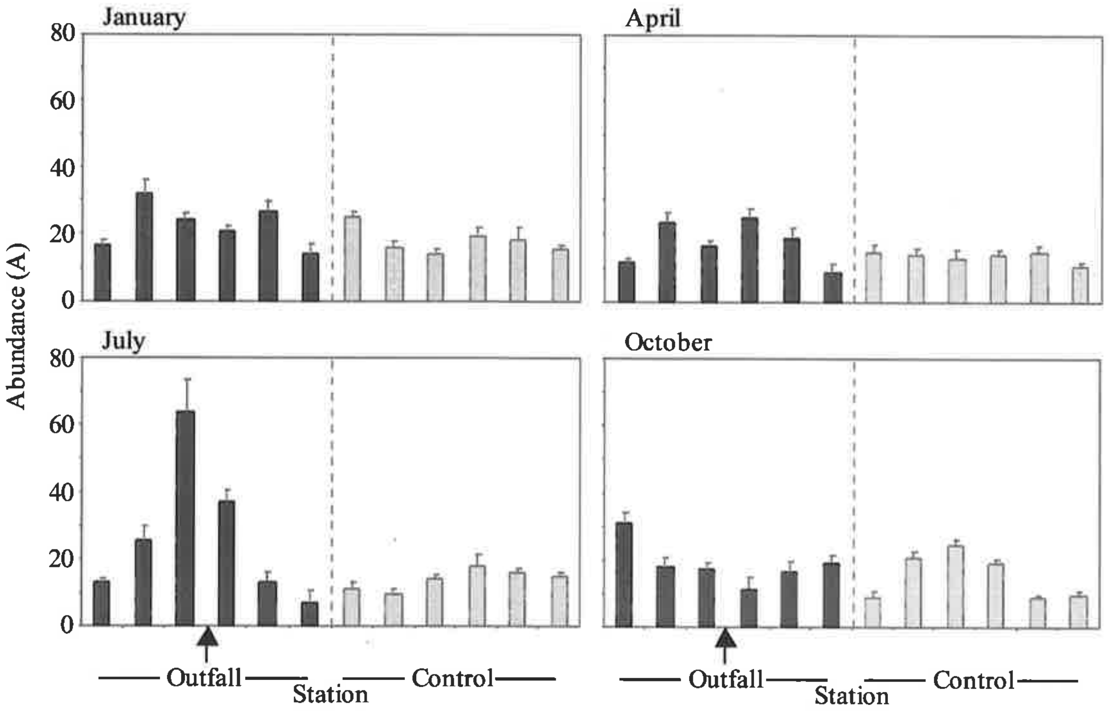


Figure 4.21 Temporal changes in mean abundance (A) for each core sample (± SE). Stations are arranged with increasing distance from the outfall moving both north and south of the outfall as in Figure 4.19. Arrow indicates discharge point.

The trends for the biomass were rather variable, with a significant interaction for Site x Station at a higher alpha value of 0.1. This variability could be attributed to the occasional large ophiuroids and crustaceans (specifically Stomatopoda) sampled at some stations (Figure 4.22).

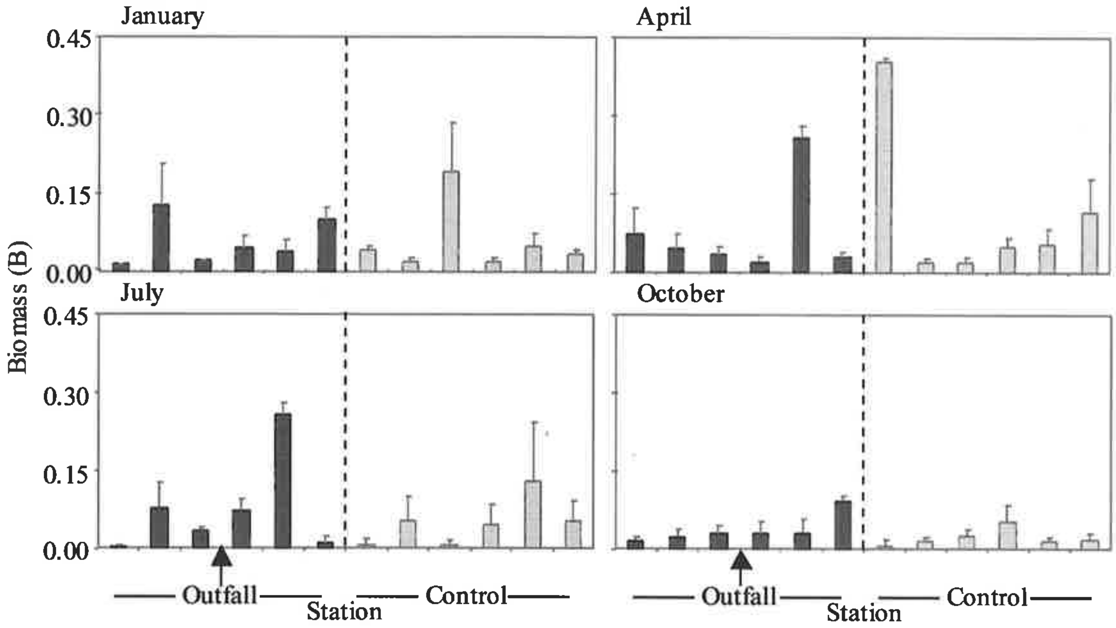


Figure 4.22 Temporal changes in mean biomass (B) in g for each core sample (± SE). Stations are arranged with increasing distance from the outfall moving both north and south of the outfall as in Figure 4.19. Arrow indicates discharge point.

The change in diversity with increasing distance from the outfall followed a similar pattern for all surveys (Figure 4.20d). Station 60 from both the north and south transects of the outfall site was more diverse, with the exception of the south transect in the October survey where Station 300 was more diverse than Station 60 (Figure 4.23). However, no such trends were observed at the control site. This again confirmed the patterns obtained in the multivariate analyses where the communities at the outfall site changed with increasing distance from the outfall while those at the control site varied with survey times.

The mean evenness (J) was generally lower at stations closest to the outfall (ON_1 and OS_1) and followed the temporal trends observed for diversity at the outfall site (Figure 4.24). Similarly for the control site, evenness was variable with no observable trends (Figure 4.24).

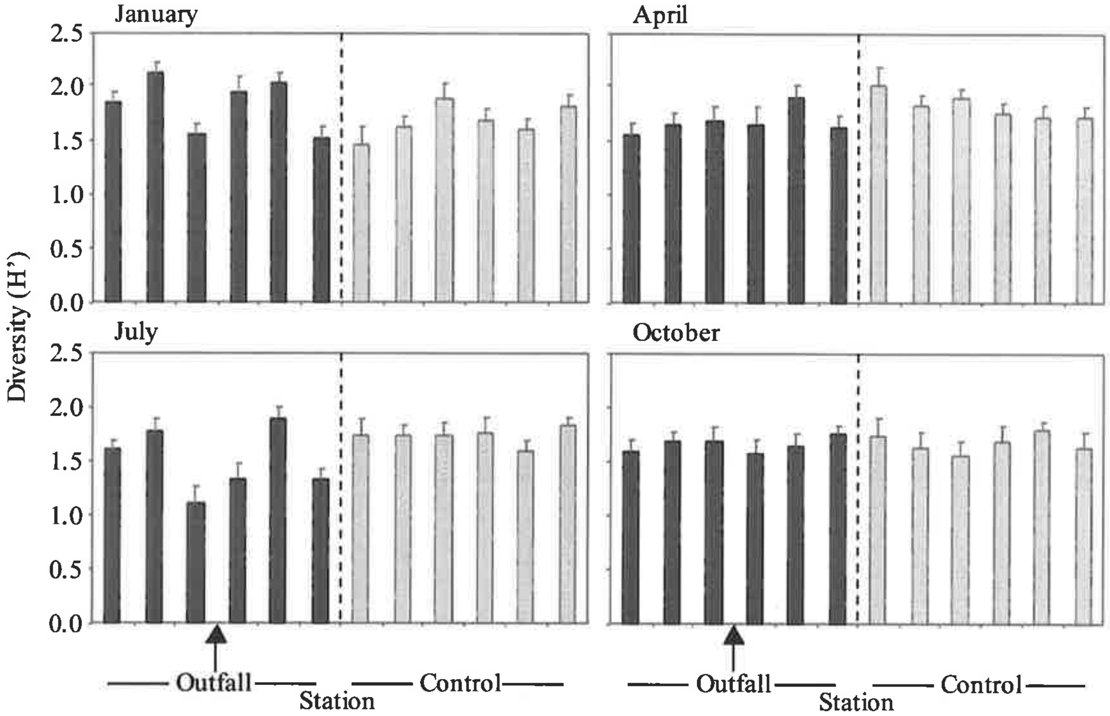


Figure 4.23 **Temporal changes in mean diversity (H') for each core sample (± SE). Stations are arranged with increasing distance from the outfall moving both north and south of the outfall as in Figure 4.19. Arrow indicates discharge point.**

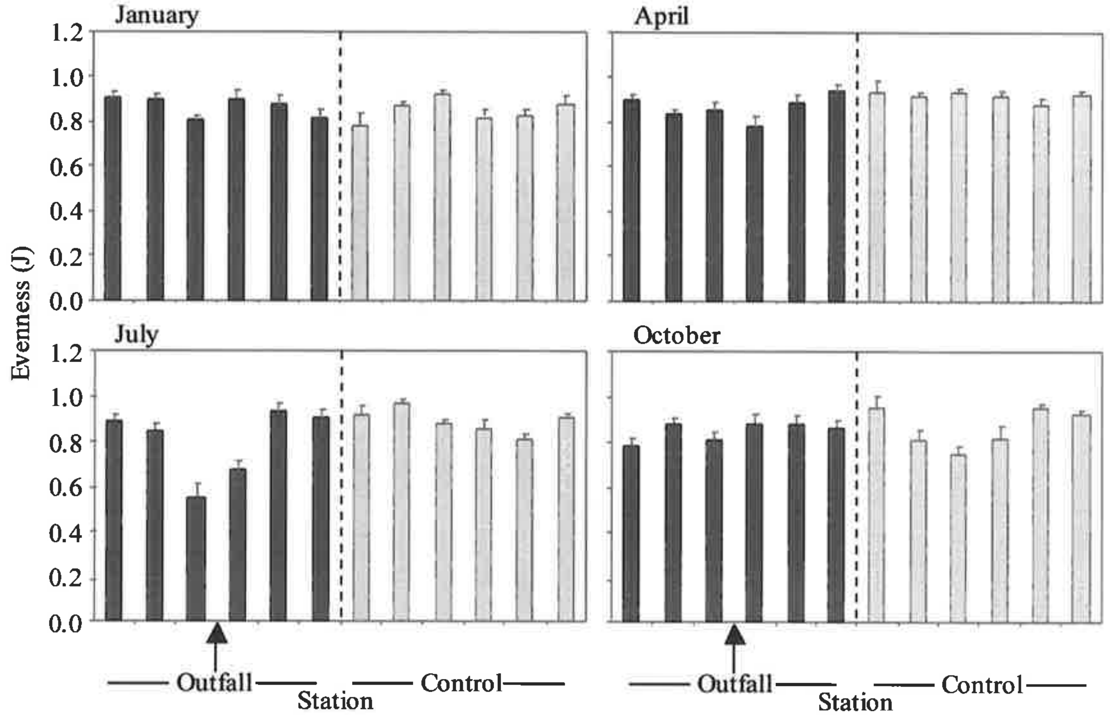


Figure 4.24 **Temporal changes in mean evenness (J) for each core sample (± SE). Stations are arranged with increasing distance from the outfall moving both north and south of the outfall as in Figure 4.19. Arrow indicates discharge point.**

Characterising communities

Overall SIMPER

The group of stations closest to the outfall (Group I) and the control stations (Group III) (defined in the multivariate analysis of spatial community patterns) had high within group similarities (> 79 %). Group II had a lower within group similarity of 63 % (Table 4.6). This separation into the three groups was characterized by the relative abundance of dominant taxa in each group. The stations closest to the outfall (Group I) had an infaunal community dominated by the polychaete Spionidae. Stations away from the outfall were different with gammaridean amphipods dominating the community at the control stations (Group III) while both Spionidae and Gammaridea were equally dominant at the mid-distance group of stations (Group II).

Table 4.6 Average similarities (*italic*) within groups (I-closest to outfall, II-mid distance from outfall, III-control stations) and dissimilarities (bold) between groups. Groups I and III had high within group similarities and the highest dissimilarity was between Groups I and III.

	Group I	Group II	Group III
Group I	*79.28*	**45.43**	**57.76**
Group II		*63.46*	**37.51**
Group III			*80.99*

The apparent gradient observed resulted from spatial dissimilarity between benthic communities in close proximity to the outfall and those farther away. Group I and Group III had the highest dissimilarity (57.76 %) while dissimilarity between Group II and Group III was lowest (37.51 %) and the dissimilarity between Group I and II was intermediate (Table 4.6). Eight out of nine principle taxa (average contribution to dissimilarity, $\bar{\delta}_i > 1$) contributed to the dissimilarities between any two groups.

For Groups I and III, where dissimilarity was highest, five of the eight taxa were more abundant at the stations of Group I (Table 4.7). Spionidae and Ostracoda had high average contribution to the overall dissimilarity. The ratio values were high for all the eight principle taxa of Groups I and III, indicating that they were good discriminating taxa.

Table 4.7 **Principle taxa contributing to differences between Group I (closest to outfall) and Group III (control, furthest from outfall). Average abundance across stations within the clusters of Group I and Group III, also the contribution ($\bar{\delta}_i$) of the *i*th taxa to the average Bray-Curtis untransformed dissimilarity ($\bar{\delta}$) between the two groups.**

Taxa	Average abundance		$\bar{\delta}_i$	Ratio	Cumulative %
	Group I	Group III			
Spionidae	442.50	71.50	21.47	3.06	37.17
Ostracoda	306.50	74.17	13.75	16.34	60.97
Gammaridea	106.50	217.33	6.50	1.84	72.23
Bivalvia	6.50	65.67	3.52	1.48	78.31
Nematoda	35.00	83.33	2.85	4.71	83.25
Nemertea	59.50	18.00	2.46	5.25	87.51
Capitellidae	34.50	0.67	2.04	5.09	91.04
Gastropoda	25.50	7.17	1.17	1.17	93.07

Spionidae and Ostracoda made a high average contribution ($\bar{\delta}_i > 10.00$) to the overall dissimilarity between Groups I and II. Seven of the eight principle taxa consistently contributed to the dissimilarity between the groups. Copepoda was the exception with a low ratio value of 0.85 (Table 4.8).

Table 4.8 **Principle taxa contributing to differences between Group I (closest to outfall) and Group II (mid-distance from outfall). Average abundance across stations within the clusters of Group I and Group II, also the contribution ($\bar{\delta}_i$) of the *i*th taxa to the average Bray-Curtis untransformed dissimilarity ($\bar{\delta}$) between the two groups.**

Taxa	Average abundance		$\bar{\delta}_i$	Ratio	Cumulative %
	Group I	Group II			
Spionidae	442.50	170.50	14.32	1.84	31.53
Ostracoda	306.50	91.00	12.14	3.30	58.24
Nematoda	35.00	123.50	4.65	1.90	68.47
Gammaridea	106.50	160.50	3.38	1.12	75.90
Copepoda	4.00	49.50	2.36	0.85	81.09
Capitellidae	34.50	2.00	1.84	3.97	85.14
Gastropoda	25.50	33.50	1.63	1.26	88.73
Nemertea	59.50	42.25	1.08	2.05	91.11

The dissimilarity between Groups II and III was intermediate (Table 4.9). The average contribution term was not high for any of the taxa. Spionidae and Gammaridea contributed about equally to the dissimilarity between the two groups. The ratios indicative of good discriminating taxa were also generally lower ranging from 0.91 to 1.66.

Table 4.9 Principle taxa contributing to differences between Group II (mid-distance from outfall) and Group III (control, furthest from outfall). Average taxa abundance across stations within the clusters of Group II and Group III, also the contribution ($\bar{\delta}_i$) of the *i*th taxa to the average Bray-Curtis untransformed dissimilarity ($\bar{\delta}$) between the two groups.

Taxa	Average abundance		$\bar{\delta}_i$	Ratio	Cumulative %
	Group II	Group III			
Spionidae	170.50	71.50	7.98	1.40	21.27
Gammaridea	160.50	217.33	6.94	1.38	39.78
Bivalvia	9.00	65.67	4.29	1.49	51.23
Nematoda	123.50	83.33	3.70	1.66	61.10
Copepoda	49.50	3.33	3.12	0.91	69.42
Ostracoda	91.00	74.17	2.41	1.25	75.84
Gastropoda	33.50	7.17	2.11	0.95	81.47
Nemertea	42.25	18.00	1.86	1.65	86.44

SIMPER at outfall site

From the analysis of the patterns of community structure, the separation of the stations from the outfall site was influenced primarily by distance from the outfall. SIMPER analysis was therefore carried out to characterise the communities at Station 1 (closest to the outfall, ON_1 and OS_1), Station 60 (60 m from the outfall, ON_{60} and OS_{60}) and Station 300 (300 m from the outfall, ON_{300} and OS_{300}). Ostracoda, Spionidae, Nematoda and Gammaridea were the four taxa that contributed variously up to 60% at each of the station groups. The separation of the stations appeared to be defined by the relative abundance of these taxa. Similarities within stations were not particularly high for any of the stations, but were still above 50 % (Table 4.10).

Table 4.10 **Average similarities (*italic*) within stations and dissimilarities (bold) between stations (Station 1 is adjacent to outfall, Station 60 is 60 m from outfall, Station 300 is 300m from outfall). Similarities within stations were not high and the lowest dissimilarity was between Station 60 and 300.**

	Station 1	Station 60	Station 300
Station 1	*58.52*	**53.81**	**52.44**
Station 60		*62.93*	**46.46**
Station 300			*59.26*

The dissimilarities between Station 1 and Station 60 or Station 300 were both almost equally high at 53.81 % and 52.44 % respectively (Table 4.10). However, the dissimilarity between Station 60 and 300 was comparatively lower (46.46 %; Table 4.10). These differences could be seen in the composition of contributing taxa at each station. Spionidae and Ostracoda were consistently dominant at Station 1 while Nematoda, Gammaridea, Copepoda and Gastropoda were more dominant at Station 60 (Table 4.11). Nematoda and Gammaridea were dominant at Station 300 when compared to Station 1 but only Spionidae was dominant when compared to Station 60 (Table 4.11).

Table 4.11 **Taxa contributing the most ($\bar{\delta}_i > 1$) to the average dissimilarity between samples from the three stations (data pooled over survey times) as determined by SIMPER breakdowns. The symbol '+' denotes the station at which the taxa had higher dominance. * indicates that Gastropoda was not a good discriminating taxa (ratio < 1). Empty cells in any two-station comparisons indicate that the taxa did not contribute substantially to the dissimilarities.**

Taxa	Station 1	Station 60	Station 1	Station 300	Station 60	Station 300
Spionidae	+		+			+
Ostracoda	+		+		+	
Nematoda		+		+	+	
Gammaridea		+		+	+	
Copepoda		+			+	
Gastropoda*		+	+		+	
Nemertea	+		+		+	
Capitellidae	+		+			
Anthuridae					+	

SIMPER at control site

The analysis of the patterns of community structure revealed that there were variations with survey times for the samples from the control site at Moana. SIMPER analysis carried out to characterise the communities from different survey times showed that the survey groups had high within group similarities ranging from 63 % to 77 % (Table 4.12). There were five major taxa contributing variously to the within group similarities. The January survey had relatively high contributions by Bivalvia, Spionidae, Ostracoda, Gammaridea and Nematoda ($\bar{\delta}_i > 10$). The April survey had high contributions by Gammaridea, Nematoda, Spionidae and Ostracoda while the July survey had Gammaridea and Nematoda. The October survey had 54 % of its within group similarity contributed by Gammaridea.

Table 4.12 Average similarities (*italic)* within survey groups at the control site, Moana and dissimilarities (bold) between groups. Surveys 4 and 5 had high within group similarities. Highest dissimilarity was between Surveys 3 and 6.

	January	**April**	**July**	**October**
January	*69.57*	**40.28**	**51.84**	**56.18**
April		*77.03*	**31.93**	**37.08**
July			*74.79*	**30.92**
October				*63.30*

The highest average dissimilarity was that observed between the surveys in January and October while the lowest was between surveys in July and October (Table 4.12). This was in agreement with the ANOSIM results where no significant difference was found between the July and October surveys. This variation between survey times observed at the control site was defined by the relative abundance of three major taxa. More than 50 % of the dissimilarities between any two surveys were contributed variously by these three taxa. Bivalvia was dominant in the January survey as compared to the other survey times. Gammaridea was dominant for the April survey when compared to the January survey but Spionidae was dominant when compared to the surveys in July and October. The July survey had a high dominance of Gammaridea in comparison to the other surveys except for the October survey where it had higher dominance of Gammaridea (Table 4.13).

Table 4.13 Taxa contributing to the average dissimilarity between samples from the four survey times (data pooled over stations) as determined by SIMPER breakdowns. The symbol '+' denotes the station at which the taxa had higher dominance.

	Jan	Apr	Jan	Jul	Jan	Oct	Apr	Jul	Apr	Oct	Jul	Oct
Bivalvia	+		+		+							
Gammaridea		+		+		+		+		+		+
Spionidae							+		+			

Proportions of major taxa

A total of 8,611 individuals (from 480 cores) were sampled over the study, with Crustacea (28%) and Polychaeta (26%) dominating numerically. Among the crustaceans, the order Amphipoda was the best represented and the most abundant, while the family Spionidae dominated the polychaetes. Ostracoda and Nematoda were also numerous making up 17 % and 12 % respectively of total abundance. All other taxa contributed not more than 5 % to the overall abundance. The outfall site and the control site were differentiated based on the relative abundance of several major taxa. Similarly, the separation of stations at the outfall site was due to the relative abundance of major taxa. The seasonal variation observed at the control site was also a consequence of a few major taxa.

The groups (Groups I, II and III) identified by the spatial multivariate analyses had different macrobenthic infaunal communities. There were nine major taxa and the dominance of these taxa was different for each group. Spionidae, Capitellidae, Nemertea and Ostracoda dominated in Group I (consisting of stations adjacent to the outfall), while Gammaridea and Bivalvia dominated in Group III (all stations from the control site). Group II, which consisted of stations at mid distance from the outfall, had Copepoda, Gastropoda and Nematoda dominating the communities (Figure 4.25).

The differentiation of stations at the outfall site was characterised by nine major taxa. Spionidae, Capitellidae, Nemertea and Ostracoda dominated at Station 1 while Anthuridae, Copepoda, Gammaridea, Gastropoda and Nematoda were dominant at Station 60. Station 300 had from low to moderate abundances of all these taxa (Figure 4.26).

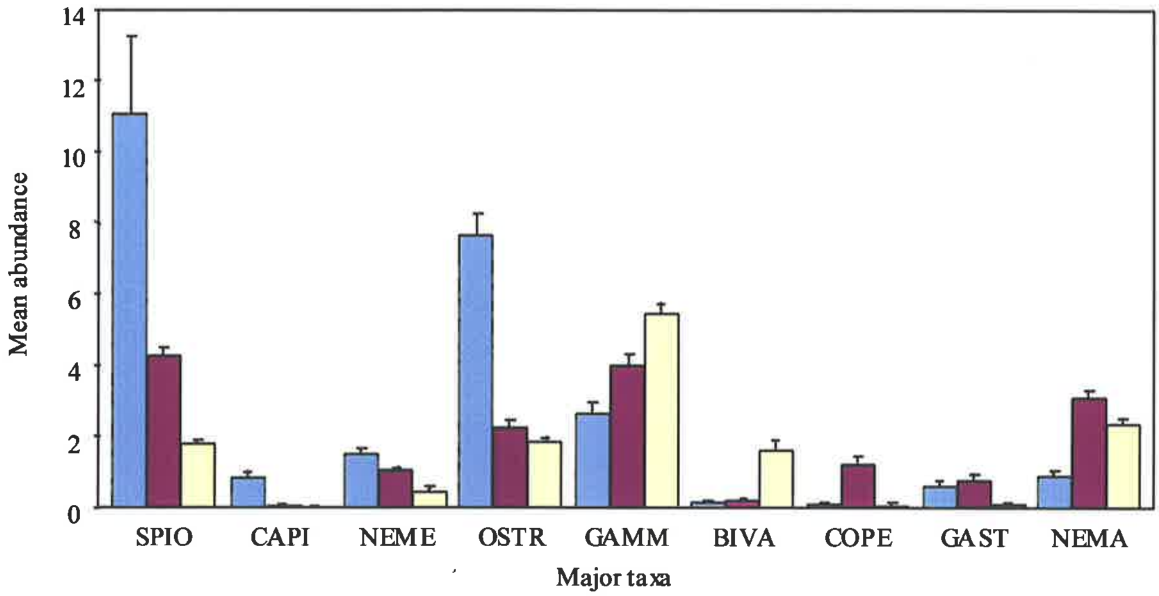


Figure 4.25 **Mean abundance of major taxa characterising the three groups (■ Group I, ■ Group II, □ Group III) as identified by the spatial multivariate analysis (SPIO-Spionidae, CAPI-Capitellidae, NEME-Nemertea, OSTR-Ostracoda, GAMM-Gammaridea, BIVA-Bivalvia, COPE-Copepoda, GAST-Gastropoda, NEMA-Nematoda).**

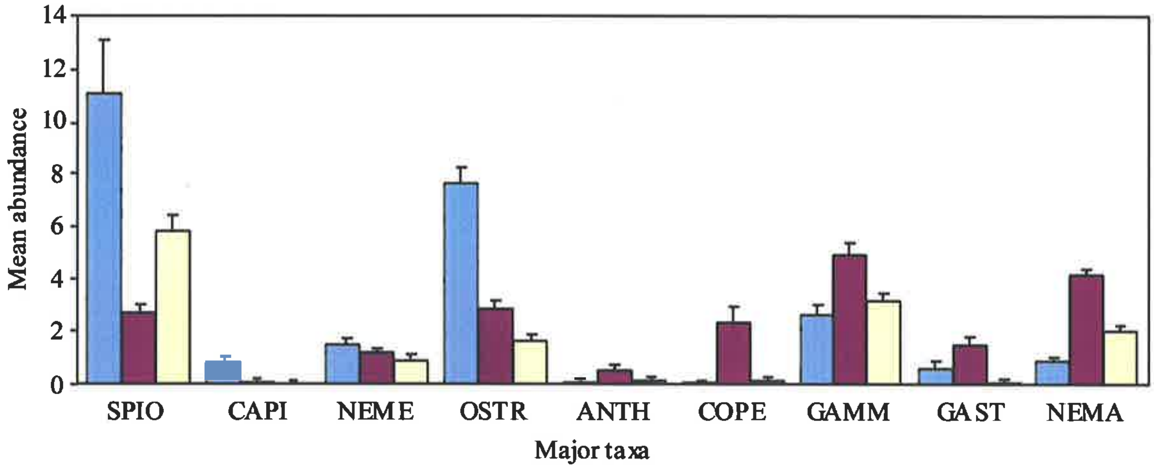


Figure 4.26 **Mean abundance of major taxa characterising the three stations (■ Station 1, ■ Station 60, □ Station 300) at the outfall site (SPIO-Spionidae, CAPI-Capitellidae, NEME-Nemertea, OSTR-Ostracoda, ANTH-Anthuridae, COPE-Copepoda, GAMM-Gammaridea, GAST-Gastropoda, NEMA-Nematoda).**

The three major taxa that characterised the variations with survey times at the control site were Spionidae Gammaridea and Bivalvia. There was a decrease in Spionidae and an increase in Gammaridea across survey times. Bivalvia was dominant only during the January survey and had similar abundances for the other survey times (Figure 4.27).

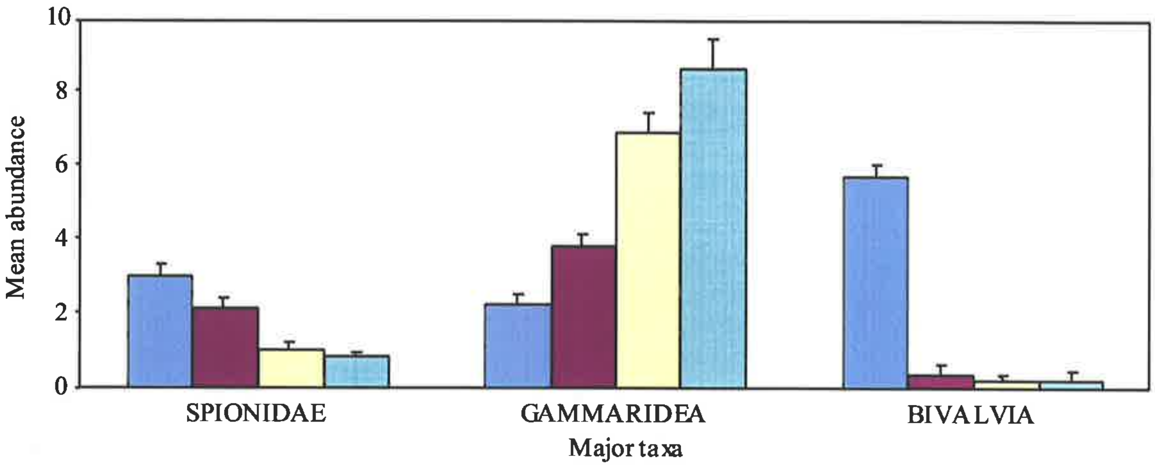


Figure 4.27 **Mean abundance of the three major taxa characterising the variations with survey times at the control site (🟦 January, ■ April, □ July, ▨ October).**

The change in amphipod-polychaete ratio over time differed between the outfall site and the control site. For stations at the control site there was a general increase in the ratio from the January survey through to the October survey (Figure 4.28). Higher amphipod to polychaete ratios were obtained for the July and October surveys (Figure 4.28). However, no such trend was observed at the outfall site. The ratios for the outfall site were consistently lower than the control site and the ratios were not higher during any particular survey (Figure 4.28).

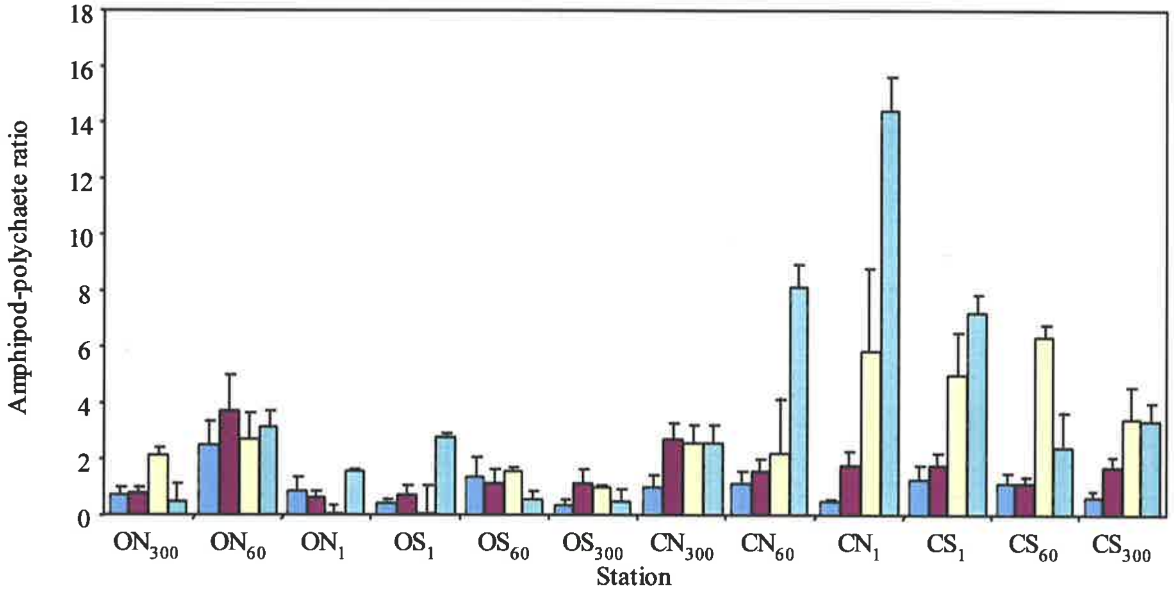


Figure 4.28 **Amphipod-polychaete ratio for the four survey times (🟦 January, ■ April, □ July, ▨ October) at both the outfall and control sites. Ratios at the control site increased from January to October, but no such trend was observed at the outfall site.**

Correlating measured environmental variables

Both measured environmental variables (median grain size and organic carbon content) were not well correlated with the faunal patterns. The weighted Spearman rank correlation coefficients (ρ_w) between the similarity matrix underlying the faunistic MDS and the Euclidean distance matrix underlying the environmental variables were very low for the individual variables ($\rho_w = 0.214$ for median grain size and $\rho_w = 0.051$ for organic carbon content). The two-variable combination also yielded very low ρ_w (0.237), indicating that both environmental variables had a rather weak explanatory power for the faunal patterns.

Superimposing the environmental variables on the faunal abundance ordinations showed that median grain size at the outfall site was generally larger than the control site (Figure 4.29b). For organic carbon content, variability was higher at the outfall site (different sizes of symbols) while at the control site, organic carbon content was generally similar (Figure 4.29c).

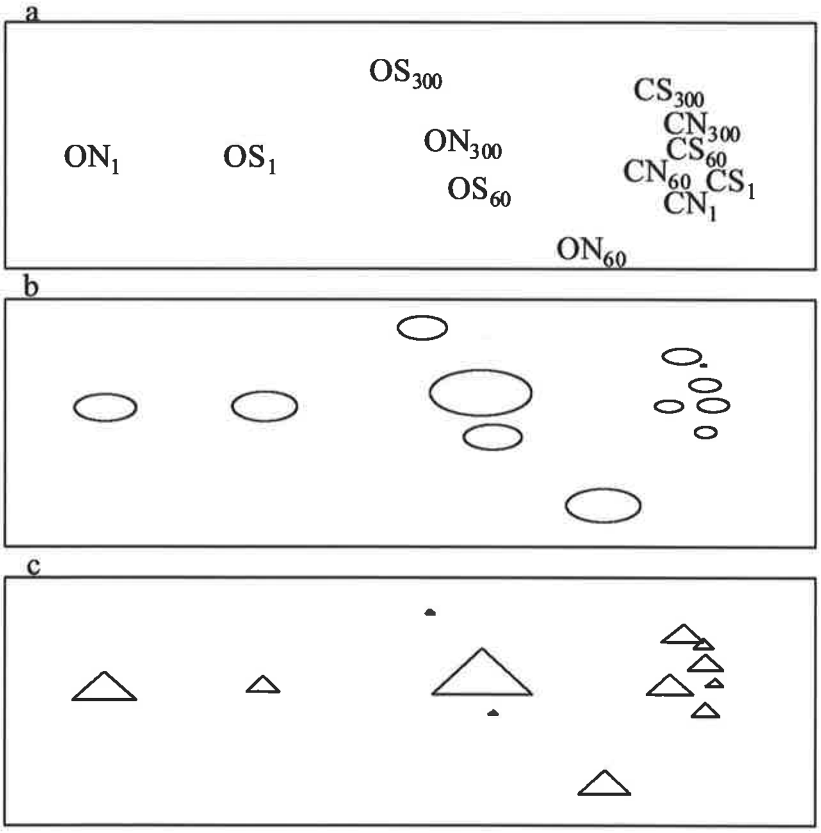


Figure 4.29 **MDS ordination plot (2-dimension, Stress = 0.06) of (a) abundance as in Figure 4.6 with superimposed symbols proportional to the values of (b) median grain size (2.31 – 3.13 phi) and (c) % organic carbon (0.7 - 1.1 %).**

Discussion and Implications

Community structure

The results from all the analyses generally supported the proximate hypothesis that the Christies Beach effluent outfall had induced changes to the macrobenthic infaunal communities. There were significant spatial and temporal variations at both the outfall and control site, which was not surprising given that soft-bottom communities are composed of a mosaic of successional patches resulting from numerous interacting processes (Dayton and Oliver 1980, Morrisey *et al.* 1992a, Morrisey *et al.* 1992b). However, over these variations, differences between the compositions of the macrobenthic infaunal communities at the outfall site and the control site were still evident. Furthermore, the expected gradient response with increasing distance from the outfall was also observed. These differences appeared to occur in a pattern consistent with disturbance on these communities and could be attributed to the increased organic and nutrient enrichment resulting from the Christies Beach Wastewater Treatment Plant's effluent discharge.

The treatment plant has been in operation since 1971 and the region around the outfall has been experiencing chronic levels of organic and nutrient enrichment throughout this period. This form of disturbance constitutes a press disturbance where changes are sustained temporally (Bender *et al.* 1984, Keough and Quinn 1991). In such circumstances the organic and nutrient enrichment will superimpose its own gradient on the environment and the resultant stress will bring about modifications to the distribution of organisms.

Stress is a disturbance that elicits a response in a population (GESAMP 1995). The history and regularity of disturbances will therefore determine the nature of communities and their response to further disturbance (Denslow 1985). Disturbances may result in sensitive taxa becoming locally extinct and a decrease in less sensitive taxa. Conversely, tolerant taxa will usually remain the same whilst opportunistic taxa will increase. In all cases these changes are likely to be associated with changes in spatial and temporal variability (Denslow 1985, Magurran 1988, Morrisey *et al.* 1992a, Morrisey *et al.* 1992b, Connell 1993).

The temporal patterns of community structure indicated that regardless of season, communities at the outfall site were different from communities at the control site. Although there were seasonal differences, communities at the outfall site appeared to be primarily driven by distance from the discharge point. In contrast, no such differences were observed at the control site where the communities varied with seasons.

Measures of community stress

Abundance/Biomass Comparisons

When the k-dominance curve for biomass is above that of abundance, it is an indication of a stable climax community, in undisturbed conditions (Warwick 1986, Warwick *et al.* 1987). If otherwise, there may be indications that the communities are disturbed or it may be explained by seasonal variation (Warwick 1986, Warwick *et al.* 1987, Ibanez and Dauvin 1988). Seasonal variations are characteristic of temperate seas (Ibanez and Dauvin 1988) where macrobenthic populations have maxima at the end of summer and beginning of autumn and minima at the end of winter and beginning of spring. This would result in "disturbed" configurations for summer ABC profiles and "undisturbed" configurations for winter. These seasonal profiles were however not consistently observed at the control site in this study. Stations closest to the outfall (ON_1 and OS_1) showed moderately disturbed conditions or intermediate between moderately disturbed and undisturbed conditions at all times. These stations had communities increasingly dominated numerically by very small r-selected or opportunistic species (especially spionid polychaetes).

Neutral model analysis

At all times the mean value of the V statistic from both the outfall and control site showed departure from eH', with stations at the outfall site having more departure from eH' than the control stations. One of the underlying assumptions about samples used in neutral model analysis is that all taxa must be identified to species level (Lambshead and Platt 1988). In this study, several organisms were assessed on a count for the phylum (e.g. Nemertea and Nematoda). However, each sample was sorted to the same taxonomic levels, which allowed for valid comparisons between samples (Smith and Simpson 1992). Instead of evaluating for significant departure from eH', the V statistic was treated as a single variable and tested for significant differences between sites, transect directions and stations. Departure from eH' may be due to the underestimation of species by identification to higher taxa, but the trend observed for the V statistic still agreed with observations by Platt and Lambshead (1985). Station 1 from the outfall site appeared to be highly disturbed with reduced diversity compared with any of the control stations. Although there was variability in the V statistic for the control stations, which may be due to intrinsic disturbance or the existing local environment, the community at the control site was still more diverse than Station 1 of the outfall site. However, Stations ON_{300} and OS_{300} (300 m from the outfall) appeared to have values in the range of the control site, but this was not observed for Station ON_{60} and OS_{60} (60 m from the outfall). A possible reason for this lesser departure of Station 60 from predicted diversity (eH') was that diversity increased as a consequence of increased suspended; organic material from the outfall, which was coupled with a reduction in species interactions (such as competition) due to the provision of an abundant food supply.

Log-normal plots

The analysis of the abundance of individuals among taxa (log-normal plots) suggested that stations from both the outfall and control sites had uneven distributions of abundance groups. This has been interpreted as being indicative of effects from moderate organic enrichment (Gray and Pearson 1982, Pearson *et al.* 1983). However, only stations closest to the outfall (ON_1 and OS_1) had more abundance classes indicating the possibility of higher organic enrichment at these stations. Moreover, taxa (for example Capitellidae) identified in the abundance groups with individual numbers between 16 and 63 (Classes V and VI) at these two stations were those that have been shown to be responsive to organic enrichment (Pearson and Rosenberg 1978, Pearson *et al.* 1983). Conversely, pollution sensitive taxa such as gammaridean amphipods dominated in the abundance Classes V and VI for the control stations.

This variability in the numerical distribution of taxa and individuals could be due to a number of reasons. To fit a log-normal distribution, the data must be from a large heterogeneous community (Gray and Mirza 1979). Rare species are excluded when the sample size is too small (Gray and Pearson 1982). In addition, the log-normal distribution fits unstructured marine communities that are influenced stochastically and represent communities in equilibrium (May 1975, Pimm and Lawton 1980, Gray 1981a). Communities attain structure when there are disturbances (e.g. pollution or storms) and the log-normal distribution no longer fits. The samples in this present study may not be large enough to give a log-normal distribution. Each sample may also have different dominance patterns, resulting from predation, competition, variable recruitment and differential mortality (Gray and Mirza 1979). Any disturbance from the effluent at Christies Beach is chronic since the treatment plant has been discharging the effluent into the area since 1971. Hence adaptation to the effluent might have occurred and the communities around the outfall have moved to a new equilibrium state, which may respond to stochastic influences, but it is not the same as the original state (Gray 1981a).

Community descriptors

Diversity indices are readily interpreted (where complex data about species and individuals are simplified into a single index) and they are useful in distinguishing gross changes; they are not however, very sensitive and have the tendency to oversimplify complex situations especially in the assessment of pollution effects (Gray and Pearson 1982, Maher and Norris 1990, Gray 1992, Clarke and Warwick 1994). The ability of the sampling procedure to detect rare taxa and the taxonomic proficiency of a researcher causes results to become variable and unreliable (Pearson and Rosenberg 1978, Keough and Quinn 1991). In addition, it is also difficult to ascertain where an effect of pollution has occurred in a naturally variable environment if there are no changes in the indices pre- and post-pollution or when there are no pre-pollution data (Warwick 1993).

Nevertheless, over the spatial scale measured in this study, the changes in the abundance and the number of taxa along a gradient of organic enrichment, as predicted by Pearson and Rosenberg (1978), were generally observed. There was no azoic zone close to the outfall, instead depressed numbers of taxa characteristic of organic enrichment occurred at stations within 5 m of the Christies Beach outfall, together with elevated number of individuals, representing a community dominated by opportunistic species. This was followed by a decline in abundance at 300 m from the outfall to a level similar to those of the control site that may be considered characteristic of undisturbed communities (if the control site represented background levels). There was however no corresponding response for increased biomass with this increased abundance near the discharge point.

In addition to changes in the taxa richness and relative abundance, the effect of organic enrichment may also be apparent at the community level (Pearson and Rosenberg 1978, Ferraro and Cole 1990, Weston 1990). Earlier studies argued that stress acted to decrease diversity (H') and evenness (J) in benthic assemblages (Pearson and Rosenberg 1978, Rapport *et al.* 1985, Schindler 1987, Ferraro and Cole 1990, Weston 1990). Other works have argued that this is an over-simplification (Gray and Pearson 1982, Clarke and Warwick 1994); diversity may be reduced when disturbance is minimal and increased when the level of disturbance is higher or when disturbance becomes more frequent. This occurs as a consequence of competitive exclusion between species. At even higher levels (or frequency) of disturbance, diversity is reduced again as species are eliminated by the stress. Therefore diversity tends to be highest when there are intermediate levels of disturbance (Connell 1978, Huston 1979). The change in diversity and evenness with increasing distance from the outfall appeared to be variously in agreement with models proposed in previous studies. The stations adjacent to the outfall had lower diversity and evenness but stations at 60 m from the discharge point had higher values, and further away (up to 300m to the north and south) the communities became less diverse again. This again may be a consequence of increased suspended organic material from the outfall, with reduction in species interactions by the provision of an abundant food supply, thus increasing diversity at Station 60.

Characteristics of communities

Communities at the two sites (Christies Beach and Moana) illustrated differences in community structure that are broadly explicable in terms of the changing stresses associated with the pollution gradient. The continual deposition of organic particulates around the outfall provides a reliable food source, which would artificially increase the carrying capacity of the habitat for species able to use the material. Increases in abundances of organisms at lower trophic levels could then drive many other structural and functional changes in a community (Smith *et al.* 1973, Pearson and Rosenberg 1978, Camargo 1992). Although the communities in this study overlapped in many of their constituent taxa, they differed in the relative abundances of these taxa, which also differed with increasing distance from the outfall. At the outfall site, there were elevated numbers of capitellid and spionid polychaetes, nemerteans and ostracods and reduced number of gammaridean amphipods and bivalves (see Appendix V for a summary of the major taxa and their responses to organic enrichment).

Polychaetes tend to be the initial macrobenthic colonists after a disturbance and the major taxon inhabiting the most stressed regions of a pollution gradient (see e.g. Christie and Moldan 1977, Pearson and Rosenberg 1978). Capitellid and spionid polychaetes are generally indicative of organically enriched sediments (Pearson and Rosenberg 1978) and accordingly they have a higher abundance on the outfall transects relative to the control transects. Several species of capitellids (in particular the *Capitella 'capitata'* complex) are considered to be indicators of organic pollution and are capable of invading areas where significant defaunation has occurred either through natural causes or anthropogenic effects (Boesch 1973, Grassle and Grassle 1974, Pearson and Rosenberg 1978). No attempt was made to identify this family to species as the distribution of various morphological features is related to size and may change ontogenetically (Glasby *et al.* 2000). However, spionids do have different responses to organic enrichment depending on the species. Some species can act as primary colonizers of denuded areas (Pearson and Rosenberg 1978) whilst other species may only appear after the decline of early opportunists (such as *Capitella;* Pearson and Rosenberg 1978). Spionids may also be present in large numbers on the edges of grossly polluted areas (Pearson and Rosenberg 1978). This variable response by different species of spionids could account for the different abundances at stations close to the outfall relative to those further away along the outfall transect and also to the control site. Although further identification was not carried out on the spionids due to lack of taxonomic expertise, they were separated into pseudo-species based on general morphological characteristics. Three pseudo-species dominated the spionid family sampled in this study. Two of which appeared to be common at both the outfall and control sites, although the abundances were lower at the control site. However, one of the spionids was only found at stations closest to the outfall discharge point. Therefore more work is required on this group of polychaetes to clarify the response of the major species present at these sites.

Gastropods are mainly deposit feeders (Pearson and Rosenberg 1978, Ruppert and Barnes 1994) and the organically enriched sediments near the outfall would favour them with increased food. This is consistent with the elevated numbers of gastropods found at stations on the outfall transects.

The responses of some infaunal taxa may not be directly related to the increased availability of organic carbon. Nemerteans are carnivores or scavenging macrophages, feeding primarily on annelids and crustaceans (Ruppert and Barnes 1994, Gibson 1997). They were more abundant at the outfall site than the control site and were also most abundant at the stations closest to the outfall, which is likely to be related to increased abundance of prey associated with higher abundance of infauna near the outfall.

The reduced abundance of bivalves along the outfall transect (relative to the control site) may relate to the problems filter feeders encounter in systems with high amounts of suspended organic matter rather than availability of organic matter as food. Suspension feeders, such as bivalves, are often disadvantaged by increased organic enrichment as the organic matter can clog cilia and siphons (Pearson and Rosenberg 1978, Ruppert and Barnes 1994).

Nematodes include some of the most wide spread and numerous of all invertebrate animals (Ruppert and Barnes 1994). In organically enriched environments, the reducing nature of sediments may affect infaunal nematodes (Gee *et al.* 1992), which would account for the reduced abundance of nematodes at stations closest to the outfall. At stations mid distance from the outfall, nematodes were most abundant and this could be a consequence of the fact that species associated with organic enrichment tend to be very large in comparison with their normal meiofaunal counterparts and are therefore retained on the larger meshed sieves (Warwick and Clarke 1993a). However, it is important to note that nematodes are generally considered to be meiofauna, (animals passing through a 500 μm sieve but retained on a 63 μm sieve) and it could also be that those encountered in this study represent only a small fraction of the species in these communities. Copepoda are also classed as meiofauna and may similarly be underestimated. However, the higher abundance found at mid distance from the outfall could be a consequence of the copepods flourishing in more oxygenated conditions where food (bacteria) levels are enhanced (Moore *et al.* 1987).

Crustaceans and echinoderms are less tolerant of pollution, especially of organic enrichment (Pearson and Rosenberg 1978), and this may account for the higher overall abundance sampled at the control site. The most abundant gammaridean amphipods at all stations were the phoxocephalids, which are known to be important in structuring soft-bottom benthic communities through predation on soft-bodied invertebrate larvae (Oliver *et al.* 1982). In addition, they are motile and brood the young rather than disperse the larvae in the plankton (Boesch and Rosenberg 1981). Consequently they are adapted to be quite resilient to small-scale disturbance. It is therefore likely that the relatively high numbers of phoxocephalids found at the outfall site is related to this ability to adapt.

Ostracods are usually abundant in areas under moderate influence of wastewater discharges (Swartz *et al.* 1986). As such, there was a relatively higher abundance of ostracods found at the outfall site and also at stations closest to the discharge point.

Amphipod to polychaete ratios showed a consistent trend with lower values at the outfall site. The reason for lower number of amphipods is presumably their lower tolerance of polluted conditions (Pearson and Rosenberg 1978). In contrast, polychaete worms often form the most abundant taxa near effluent outfalls (Pearson and Rosenberg 1978). The amphipod to polychaete ratio appeared to be useful for environmental monitoring where laborious taxonomic sorting is obviated. However, further studies are required before it may be proven to be a useful ratio.

Mortality is generally high during autumn and winter, hence maximum abundance is attained in summer and autumn, a consequence of increased recruitment in spring and summer (Buchanan *et al.* 1978, Ibanez and Dauvin 1988). This was not consistently observed for the control site at Moana where analyses of infauna indicated that the patterns of community structure changed between survey times (seasons). This could be due to the different local environment at Moana where dominance patterns resulting from predation, competition and grazing affect the recruitment and survival of populations (Gray and Mirza 1979, Barry and Dayton 1991).

Correlation of measured environmental variables

The lack of concordance between the environmental variables (median sediment grain size and organic carbon content) and the faunal patterns indicates that the differences in the community structure at the outfall site relative to the control site may be a consequence of more than these two variables. In addition, the environmental data was not matched to the fauna data sample for sample, as cautioned by Clarke and Ainsworth (1993), in that they were not sub-samples of the same core sample for the infauna, but were collected separately at the same time. Therefore, with no other environmental variables measured, it was not possible to confirm a link between the community structure and any other environmental variables.

This study has essentially demonstrated that the composition and structure of the macrobenthic infaunal communities at the outfall site is different from that at the control site. However the organic gradient and/or environmental stress related to this discharge did not produce a degraded community. The differences observed at the outfall site are broadly consistent with what would be expected in an area that is impacted by increased levels of organic and nutrient enrichment. Therefore, the wastewater effluent discharged from the Christies Beach Wastewater Treatment Plant appears to have induced significant and presumably persistent changes to the macrobenthic infaunal communities.

Chapter 5 Addressing spatial and temporal variability

This chapter aims to address some fundamental issues, which were incumbent on the interpretation of the information presented in Chapter 4. The first issue is addressing the question whether Moana is an appropriate control site for the infaunal study and the second issue is the need of a more detailed analysis of the infauna data from the outfall site to better understand the differences in the macrobenthic infaunal community with increasing distance from the discharge point and the inter-annual variability if any. The following discussion will therefore be divided into two sections: (Part A) comparisons with other communities in the Gulf, (Part B) comparisons within the outfall site to address the spatial and temporal scale of the data.

Part A - Comparison with other communities in the Gulf

The problem of finding appropriate control sites was highlighted in Chapter 3. Moana was the only control site identified as appropriate for the infaunal study and it has been shown to have different macrobenthic infaunal communities from stations at the outfall site at Christies Beach (see Chapter 4). There is however the question of whether Moana is indeed an appropriate control site, and whether having different macrobenthic infaunal communities from the outfall site at Christies Beach is not purely due to spatial heterogeneity. In order to address this question, data from another study was used together with the present study to assess if Moana has comparable macrobenthic infaunal communities with other communities in the Gulf.

The University of Adelaide had been engaged as a specialist consultant to examine the effects of sand dredging for beach replenishment at Port Stanvac over several years (Cheshire and Kildea 1992, Cheshire and Kildea 1993, Cheshire *et al.* 1996, Cheshire and Miller 1999). An extension to this work was a study in 1996 on the potential impact of beach sand replenishment dredging on the Adelaide northern metropolitan beaches (Cheshire and Miller 1996). The infaunal study was carried out using the same methods as the present study on the effects of effluent at Christies Beach. With access to this data from the northern beaches, it was possible to compare the benthic infaunal communities at Christies Beach and at Moana with other infaunal communities in the Gulf.

Seven transects based on existing survey lines (rodlines) used by the Coast and Marine Section of the South Australian Environment Protection Authority (formerly Coast Protection Branch) were used in the northern beaches study. These transects span the Adelaide metropolitan coastline from the north in New Haven and Semaphore, the central region in Tennyson, Grange and Torrens and to the south in Somerton Park and Brighton (Figure 5.1). There were four to eight stations sampled along each of the seven transects. Five core samples were taken at each station and counts of infauna were pooled for the five samples. The sampling stations ranged from deeper areas of 7-8 m to shallow areas of 3-4 m. Since the present study consisted of stations with depths of 6-8 m, only stations sampled at depths greater than 6m for the northern beaches were considered. This reduced the number of stations from 43 to 21. The labelling of all the sampling stations used is as shown in Table 5.1.

Table 5.1 Sampling stations for northern beaches study (Rodline) and stations of present study at Christies Beach (Outfall) and Moana (Control)

Rodline stations	Outfall stations	Control stations
01A (North Haven)	ON_1, OS_1	CN_1, CS_1
04A (Semaphore)	ON_{60}, OS_{60}	CN_{60}, CS_{60}
12A, 12B (Tennyson)	ON_{300} OS_{300}	CN_{300}, CS_{300}
14A, 14B (Grange)		
19A, 19B, 19C, 19D, 19E, 19F, 19G, 19H (Torrens)		
30A, 30B, 30C, 30E, 30F (Somerton Park)		
34A, 34B (Brighton)		

Analysis

For the comparison between the present study and those of the northern beaches study, the two sets of data had to be standardised in terms of the number of replicates and the taxonomic resolution before any analyses could be carried out. Since data from the northern beaches study were pooled from five core samples while those from the present study were from ten core samples, therefore each of the abundance values, from the northern beaches samples, was multiplied by two. The taxonomic resolution for the northern beaches study was also lower for all taxa; hence the data from the Christies Beach study were re-aggregated to higher taxonomic levels before analysis.

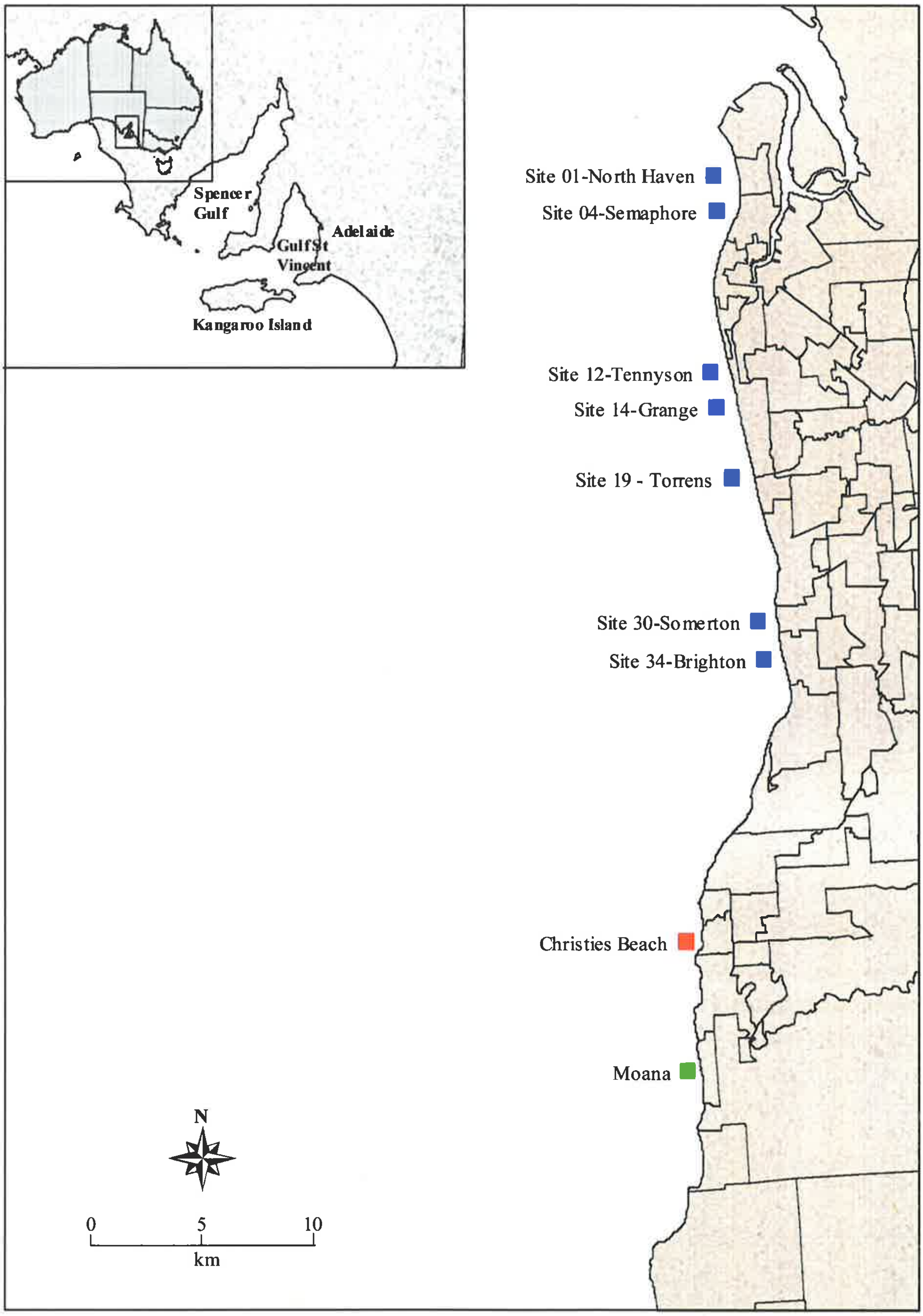


Figure 5.1 Map showing the location of sampling stations in the northern beaches study (🟦 Rodline) and stations of the present study at Christies Beach (🟥 outfall) and Moana (🟩 control).

Raw abundance data were used for the computation of similarities using the Bray-Curtis measure. Non-metric Multidimensional Scaling (MDS) analyses were then carried out on the resultant similarity matrix to generate ordination plots. Dimensionality for the MDS analyses was chosen using a scree plot and a three dimensional ordination was defined as being optimal.

Results

There were obvious gradients in the ordination plots indicating differences in the communities from all sites. The three-dimensional ordination plot was used since the gradients obtained with the data from both the present study and the northern beaches study appeared to be defined by more than two dimensions.

The stations from the northern beaches study were spread across the entire ordination plot, showing the same groupings of a general north-south gradient in the infaunal communities as reported in Cheshire and Miller (1996; Figure 5.2). The northernmost sites 01 and 04 (North Haven and Semaphore) were on the periphery of the ordination plot. The central rodline sites 12 and 14 located at Tennyson and Grange were grouped separately to the left of the ordination while the southern sites 30 and 34 (Sommerton and Brighton) occupied the central region of the plot. The stations from rodline site 19 located at Torrens were not clustered together, but were separated into two groups. Stations 19A to 19E were grouped with the southern sites 30 and 34 while Stations 19F to 19H were grouped with the central sites 12 and 14 (Figure 5.2).

The separation of the stations closest to the outfall (Station 1) from the others was still evident when the data from this study was combined with data from the northern beaches study. Regardless of surveys, Station 1 formed a distinct cluster. Stations 60 and 300 from the outfall were not as clearly separated, being grouped with rodline Stations 30 and 34 in the central region of the ordination plot. Similarly stations from the control site at Moana were grouped in the centre of the ordination with the southern Stations 30 and 34 (Figure 5.2).

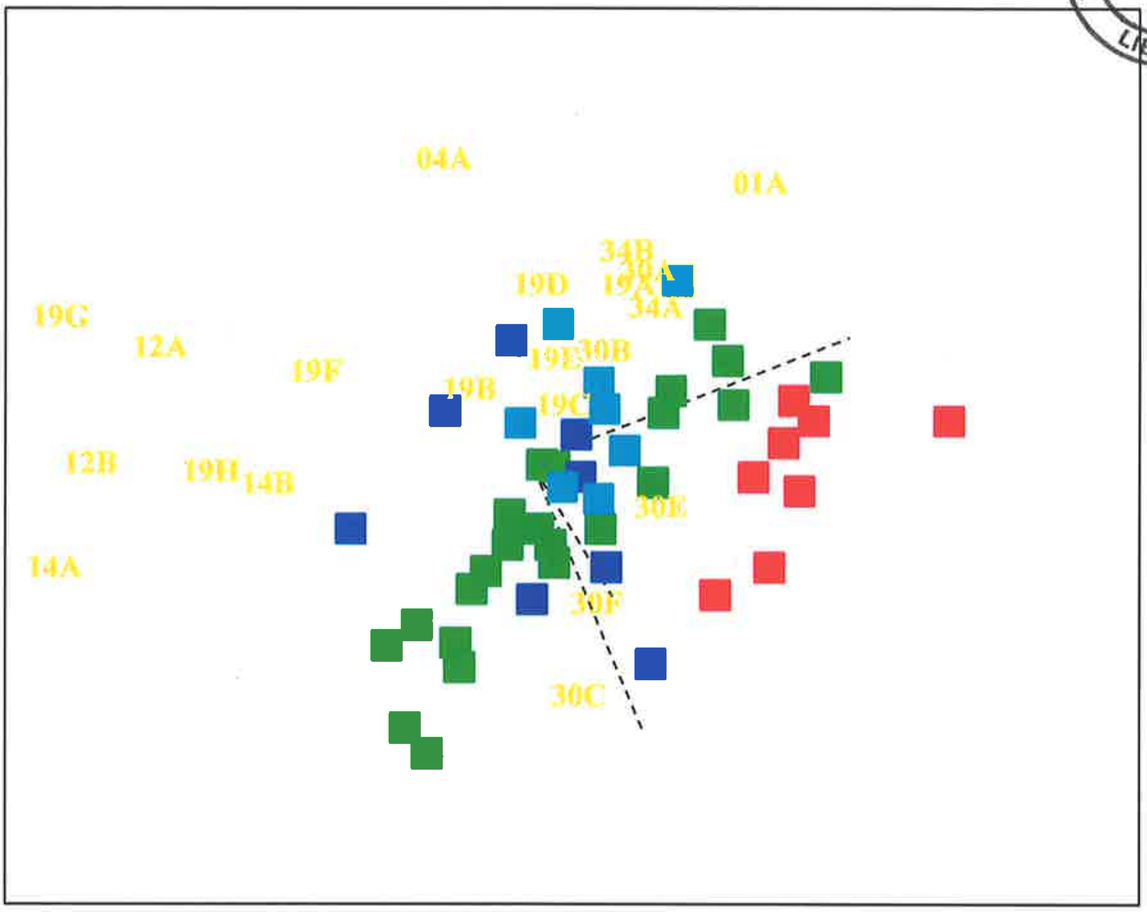


Figure 5.2 **Three-dimensional MDS plot (stress = 0.11) of untransformed abundance data for combined northern beaches study and Christies Beach study showing obvious gradients in community structure (🟥 Outfall Station 1, 🟦 Outfall Station 60, 🟦 Outfall Station 300, 🟩 Control stations, alphanumeric labels refer to Rodline stations).**

Discussion

The results of the combined data from the northern beaches study and the present study indicated that Moana was an appropriate control site for the present study even though there were several differences between the two studies. The two studies were carried out at separate times with the northern beaches study being carried out in June 1996 while the present study was in 1997. In addition, some of the sites from the northern beaches study had extensive seagrass patches (Cheshire and Miller 1996), which might be expected to affect the distribution of infauna communities. However, this did not appear to be the case. The macrobenthic infauna communities obtained in the present study were comparable with the other communities in the Gulf. The north-south gradient obtained for the communities in the Gulf was maintained with Stations 60 and 300 of the outfall site and the control site at Moana grouping with the southern sites (Somerton and Brighton) from the northern beaches study.

This indicated that Moana has comparable macrobenthic infaunal communities with the other communities in the Gulf, and is appropriate to be used as a control site for the present study. Therefore the differences in the macrobenthic infaunal communities between Moana and the outfall site at Christies Beach may not be a consequence of spatial heterogeneity alone. This indicated that the communities in the vicinity of the outfall discharge point might have changed.

Part B - Comparison within outfall site

A more detailed analysis of the infauna data was also carried out to address the spatial (resolution of the gradient response) and temporal (inter-annual differences) variability at the outfall site. Only data from three stations were analysed although samples were collected from nine stations for each of two transects at both the outfall and control sites (see Chapter 3). Stations located at 1 m, 60 m and 300 m from the outfall on both the north and south transects were selected for processing and this was mirrored at the control site, Moana. It has been shown that there were differences in the macrobenthic infaunal communities with increasing distance from the outfall (see Chapter 4). However, there is the question of whether these stations were representative of a gradient response to the effects of effluent or was it merely due to spatial heterogeneity. Therefore, samples from the preliminary surveys in 1996 and the January survey in 1997 where samples from all stations were processed, were analysed to assess the resolution of this gradient response. Analyses have also shown that there were differences in the macrobenthic infaunal communities over survey times, especially for the control site at Moana (see Chapter 4). Although patterns of community structure at the outfall site appeared to change with distance from the outfall, there was still the question of inter-annual variability. To address this, data collected in March and August of 1996 (autumn and winter) during the preliminary surveys, were compared with data collected in April and July 1997 (autumn and winter) during the actual surveys.

Analysis

For addressing the resolution of the gradient response at the outfall site, data from March 1996, August 1996 and January 1997 surveys, where there were samples for all nine stations, were analysed. Comparisons were also made using data from all surveys of the outfall site. In the March 1996 survey, only the north transect of the outfall site were sampled and in August 1996, both the north and south transect of the outfall site were sampled. The January 1997 survey also had samples of all stations from both the north and south transects. For inter-annual comparisons, data from March and August 1996 were analysed together with data from April and July 1997.

Raw abundance data were used for the computation of similarities using the Bray-Curtis measure. Non-metric Multidimensional Scaling (MDS) analyses were then carried out on the resultant similarity matrix to generate ordination plots. Dimensionality for the MDS analyses was chosen using a scree plot and in all cases a three dimensional ordination was defined as being optimal.

Results

The gradient response at the outfall site was different for each of the surveys (March 1996, August 1996 and January 1997) examined. For the survey in March 1996, only stations of the north transect at the outfall site were sampled and initial analysis indicated that Station 500 had a very different community from all other stations, therefore in subsequent ordination, Station 500 was removed as an outlier. The ordination of the remaining stations showed that Stations 1 and 300 were separated from the other stations with a general clustering for stations between 5 and 120 m from the outfall (Figure 5.3).

In the August 1996 survey, Stations 1 and 500 from both the north and south transects were distinctly separated from all the other stations (Figure 5.4). The other stations appeared to follow a north-south separation, except for Stations 5 and 60 (Figure 5.4).

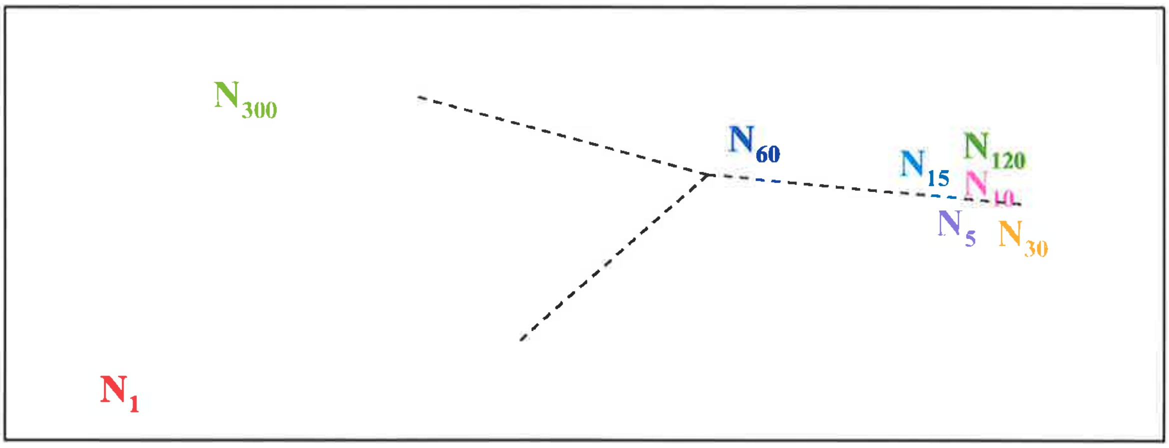


Figure 5.3 MDS ordination plot (3-dimension, stress = 0.01) of raw abundance data from the survey in March 1996, showing separation of Stations 1 and 300 from the other stations (N=North, subscript numbers refer to stations).

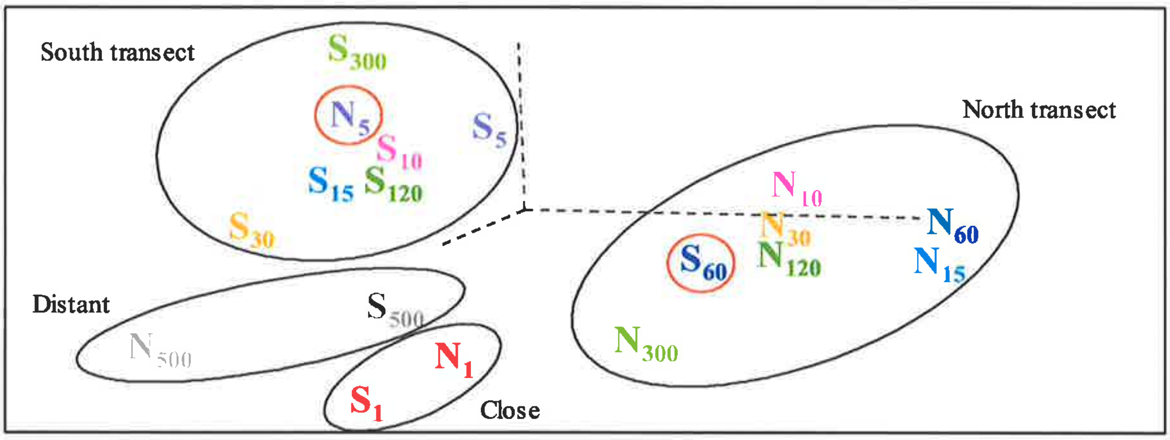


Figure 5.4 MDS ordination plot (3-dimentsion, stress = 0.07) of raw abundance data from the survey in August 1996, showing a north-south separation of stations except Stations N_5 and S_{60} and a distinct separation for Stations 1 (Close) and 500 (Distant) from the other stations (N=North, S=South, subscript numbers refer to stations).

However this north-south separation of stations was not obtained for the survey in January 1997. Stations were separated into three groups, corresponding to increasing distance from the outfall (Figure 5.5). Station 1 (N_1 and S_1) formed one group while Stations 120, 300 and 500 from both the north and south transects formed another group. Mid distance stations (from 5 to 60 m from the outfall) formed the third group in the middle of the ordination plot (Figure 5.5).

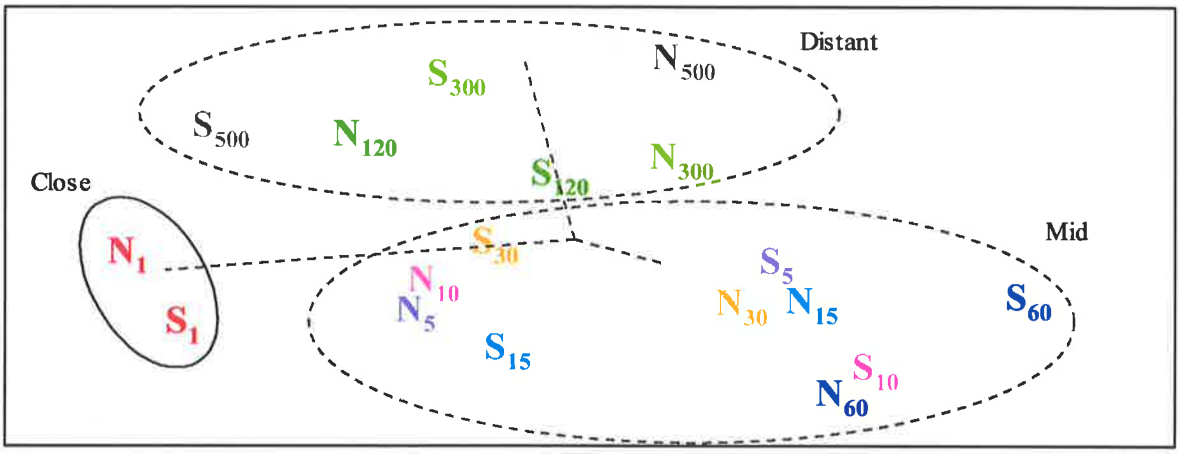


Figure 5.5 MDS ordination plot (3-dimension, stress = 0.07) of raw abundance data from the survey in January 1997, showing separation of stations corresponding to increasing distance from the outfall (N=North, S=South, subscript refer to station numbers).

When data from all surveys (both 1996 and 1997) were analysed together, Station 1 (closest to the outfall) and Station 500 (furthest from the outfall) were separated from all other stations (Figure 5.6). There were no distinct groupings for all the other stations, except for Station 60, which appeared to be clustered closer together (Figure 5.6).

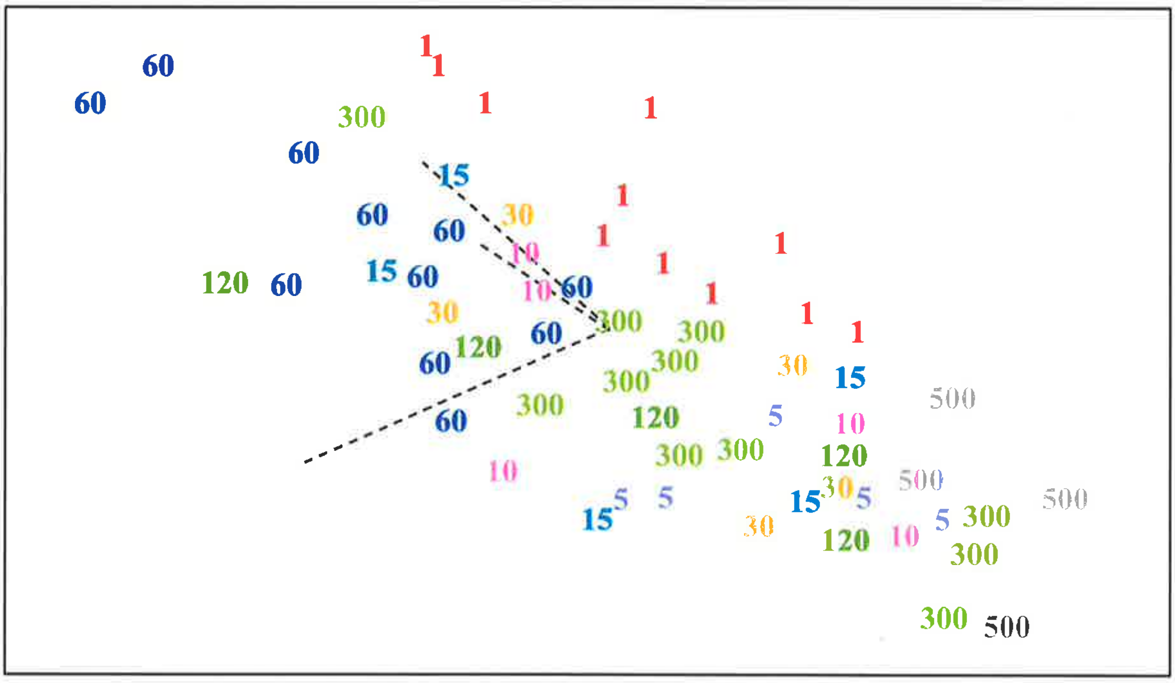


Figure 5.6 MDS ordination plot (3 dimension, stress = 0.11) of raw abundance data from surveys in March 1996, August 1996 and January 1997, showing separation of Station 1 (closest to outfall) and Station 500 (furthest from outfall) from the other stations (numbers refer to stations).

In the inter-annual comparison, there was separation of stations according to survey times. The March 1996 and August 1996 surveys formed obvious groups in the three dimensional MDS ordination (Figure 5.7). However, there appeared to be a grouping of similar stations regardless of sampling time, especially for the surveys in 1997. Station 1 (closest to outfall) formed a group on the lower left of the ordination plot while most of Station 60 were in the upper left and Station 300 were lined up on a similar axis (Figure 5.8).

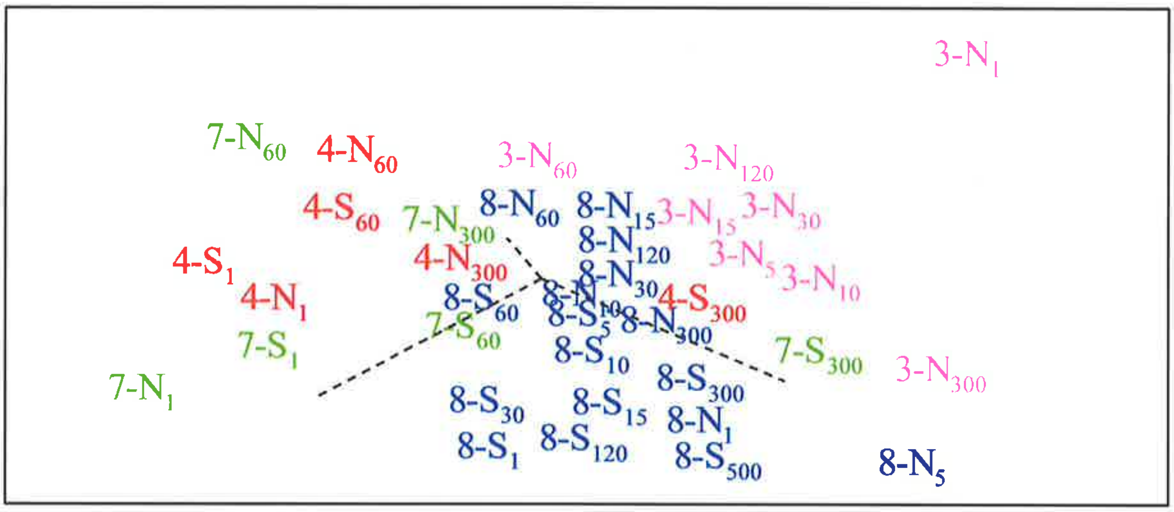


Figure 5.7 MDS ordination plot (3 dimension, stress = 0.11) of raw abundance data from surveys in March 1996 (3-), August 1996 (8-), April 1997 (4-) and July 1997 (7-), showing general separation according to survey times.

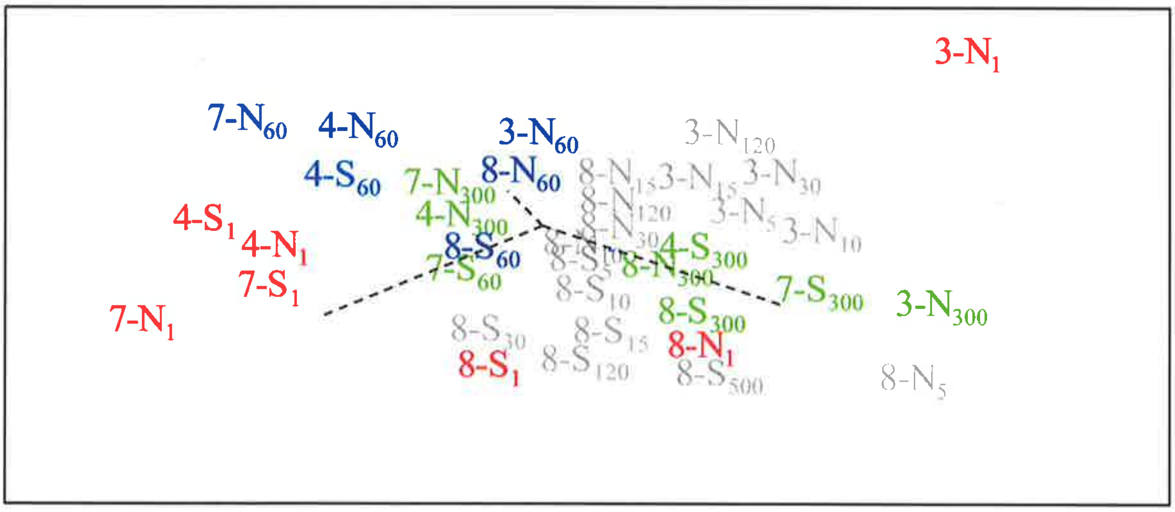


Figure 5.8 MDS ordination plot similar to Figure 5.7 but coloured differently to highlight the grouping of stations rather than survey times, especially for Stations 1, 60 and 300.

Discussion

The comparison within the outfall site in terms of spatial resolution of the gradient response indicated that stations closes to the outfall (Station 1) appeared to be most affected by the effluent discharge. The longshore gradient (difference between the north and south transects) was only observed in the August 1996 survey. Analysis of data to address inter-annual differences showed that there was variability at the outfall site with survey times. However, over this temporal variability, macrobenthic infaunal communities appeared to change with increasing distance from the outfall. Therefore, the selection of Stations 1, 60 and 300, to represent the changing communities along the gradient of nutrient and organic enrichment resulting from wastewater effluent discharged at Christies Beach, appeared to be appropriate.

Chapter 6 Benthic Respirometry

The structure of macrobenthic infaunal communities at the outfall and control sites has been shown to be different both spatially and temporally in a pattern consistent with disturbance on these communities attributable to increased nutrient and organic enrichment from the effluent outfall (Chapter 4). However, there was no corresponding increase in organic carbon content in the sediments as compared to the control site at Moana even though effluent is discharged at a rate of 27 ML per day at the outfall site. These findings indicate that different processes may be operating at the two sites. This chapter therefore provides results from work carried out to look at differences in sediment metabolism via an assessment of oxygen consumption at the outfall and control sites.

Introduction

Benthic oxygen uptake is an integrated measure of the numerous microbial reactions and processes responsible for the decomposition of organic matter in marine sediments. Sediment oxygen consumption encompasses both biological and chemical oxygen uptake (Figure 6.1). The biological component consists of aerobic oxygen consumption by organisms (aerobic microbes, macrofauna, meiofauna and microfauna) and the chemical component is a result of oxidation of reduced compounds (reduced nitrogen, manganese, iron and sulfur compounds) (Jørgensen 1977, Sorensen *et al.* 1979, Sorensen 1982, Sampou and Oviatt 1991).

The measurement of sediment oxygen consumption constitutes an important part of a large number of studies concerning benthic metabolic processes and the turnover of organic matter in sediments (e.g. Pamatmat 1968, Jørgensen 1977, Hargrave and Phillips 1981, Es 1982, Andersen and Hargrave 1984, Wilde *et al.* 1984). Most studies on community metabolism are coupled with looking at nutrient dynamics in the sediments (e.g. Boynton and Kemp 1985, Boucher and Clavier 1990, Boucher *et al.* 1994, Giblin *et al.* 1997).

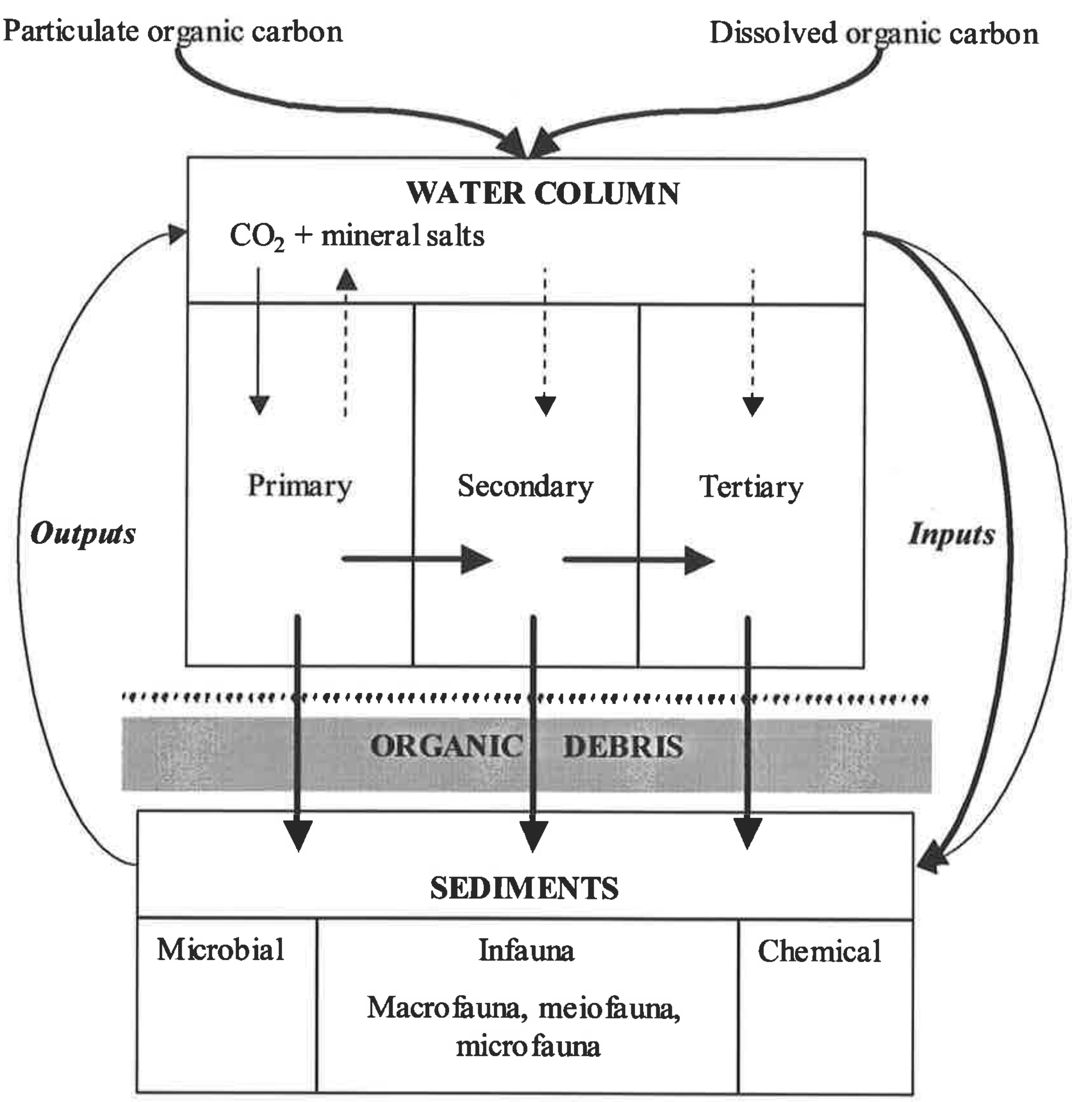


Figure 6.1 **Both biological (microbial and infaunal) and chemical components in the sediment contributing to the total sediment oxygen consumption measured in terms of the movements of oxygen (- - -►), organic carbon (──►) and CO_2 and mineral salts (──►) in the system (adapted from Parsons *et al.* 1984).**

Furthermore, such studies usually concentrate on nutrient fluxes as a consequence of natural upwelling and seasonal dynamics (Smith *et al.* 1972, Banta *et al.* 1995). However, because eutrophication, especially as a result of organic enrichment from anthropogenic sources, may significantly increase organic sedimentation and seasonal storage of organic matter in surface sediment (GESAMP 1990a, Sampou and Oviatt 1991), knowledge of processes such as oxygen consumption and nutrient regeneration in sediments has become increasingly important (Jørgensen 1980, Seliger *et al.* 1985, Westernhagen *et al.* 1986, Giblin *et al.* 1997).

Several factors affect the decomposition of organic matter within sediments including temperature, water circulation and sediment composition (Bonyton *et al.* 1981, Shaffer and Onuf 1983, Grant 1986, Banta *et al.* 1995). In addition, the rate of decomposition can be influenced by benthic macrofaunal community structure (Rhoads 1974, Aller 1982, Banta *et al.* 1995). Benthic organisms also have an important role in mediating the exchange of oxygen, carbon dioxide and nutrients across the sediment-water interface (Andersen and Kristensen 1988). Bioturbation of sediments by infauna results in irrigation and the redistribution of organic matter in sediments (Aller 1982, Kristensen 1988). These activities stimulate aerobic and anaerobic microbial decomposition processes and solute fluxes in the bioturbated zone of sediments and thereby affect the overall dynamics of community metabolism (Kristensen 1985, Andersen and Helder 1987, Kristensen and Blackburn 1987, Aller 1988).

Various techniques have been used to measure community metabolism at the sediment-water interface. The most frequently used technique to measure oxygen uptake rates of sediments consists of incubating sediments and following the decrease in the oxygen concentration of overlying water as a function of time (e.g. Pamatmat and Banse 1969, Hargrave *et al.* 1993, Hatcher *et al.* 1994, Banta *et al.* 1995). Alternative methods include estimating oxygen concentration gradients using oxygen microelectrodes or minielectrodes at the sediment surface (Helder and Bakker 1985, Jørgensen and Revsbech 1985, Lindeboom *et al.* 1985, Reimers and Smith 1986). Another approach for estimating community metabolism is to measure biochemical activity. This technique involves measuring electron transport system activity (ETSA) in marine sediments where the ETSA gives an estimate of respiratory enzyme activity and therefore includes both aerobic and anaerobic respiration processes (Christensen and Packard 1977, Vosjan and Olanczuk-Neyman 1977, Pamatmat *et al.* 1981).

There are as many studies on oxygen uptake rates by sediments carried out using sediment cores both in the laboratory (e.g. Smith 1973, Banta *et al.* 1995, Bartoli *et al.* 1996, Hansen and Kristensen 1997) and *in situ* (e.g. Pamatmat and Banse 1969, Smith *et al.* 1972, Smith *et al.* 1973, Hargrave and Phillips 1981, Mazouni *et al.* 1996), however, a better estimate of naturally occurring rates would be to measure oxygen uptake *in situ* under conditions of minimal disturbance. Benthic chambers do interfere with some processes at the sediment-water interface by temporarily restricting the supply of organic matter to the sediment and by changing the flow regime. In spite of this drawback, benthic chambers are the best technique available for the study of certain exchange processes, but it should be kept in mind that absolute values of the fluxes may be affected (Bott *et al.* 1978, Sundby *et al.* 1986).

The present study aimed to understand the processes that are operating in the system to help explain the differences observed in the community structure at the outfall and control sites with the specific objective of determining whether the argument could be made that the increased levels of organic carbon either in the form of particulate or dissolved material would lead to the macrobenthic infaunal communities in close association with the outfall having higher overall benthic activity which would be defined as higher overall benthic respiration. Therefore, oxygen uptake rates were measured by incubating the sediments and measuring the decrease of oxygen concentration of overlying water.

Materials and Methods

Equipment

In situ measurements of total sediment oxygen consumption (include both chemical and biological oxygen consumption) were made with an automated respirometer (Plate 6.1). The respirometer system consists of oxygen electrodes, stirrers and a temperature probe. The sensors are connected to a data logger in a watertight housing. The housing also contains the battery power supply for both the data logger and stirrers. Acrylic chambers with no steel fittings were used in order to avoid any possible oxygen consumption due to corrosion (Cramer 1989). These chambers consist of a cylinder 900 mm in diameter and a height of 150 mm. Moulded to this cylinder is a hemispherical dome of the same diameter. The chambers are mounted on a square flange (1.15 m x 1.15 m), which provided a base to rest on the benthic surface (Plate 6.1; inset). The chambers are made of opaque acrylic to exclude light.

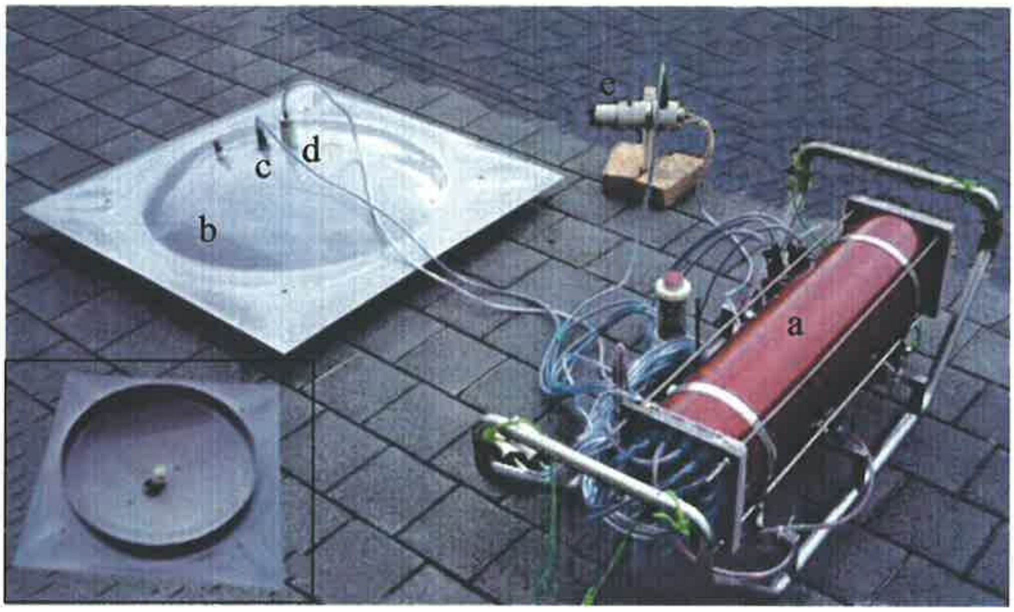


Plate 6.1 **The respirometer system showing (a) respirometer, (b) chamber, (c) stirrer, (d) oxygen electrode and (e) ambient set-up; (inset – inside view of chamber).**

Deployment

For each deployment, two SCUBA divers would carefully place the two chambers on the bottom; there is a 30 mm aperture at the top of each chamber that allowed water to freely escape to avoid disruption of surficial sediments and pore water beneath the chambers. Drift macrophytes if present were cleared before the chambers were inserted into the sediment. Each chamber was inserted 150 mm in the sediment until the flange of the chamber was flush with the sediment surface. The chamber would thus enclose a volume of 90 L of water and an area of 0.64 m^2 of the seabed. The oxygen electrodes and stirrers from the respirometer were then connected to the chambers. Three other oxygen electrodes were also used to record the ambient oxygen levels in the water column near the sediment-water interface. The oxygen electrodes coupled to a data logger, provided a record of oxygen tension within the overlying water enclosed in each chamber every 20 seconds. The stirrer circulates water within each chamber, preventing stratification and maintaining a constant flow over the oxygen sensor. Water temperature was also recorded at the same time. An incubation period of four hours was used for each deployment, a duration producing no critical oxygen depletion (< 80 % saturation), which might affect the sediment oxygen demand.

The *in situ* measurements were carried out at similar stations to the infaunal study at both the outfall site and the control site at Moana. Stations 1, 60 and 300 to the north of the outfall site and Stations 1 and 60 to the north of the control site at Moana were used. Measurements were also carried out at two other stations for a northern control site at Hallett Cove (see Chapter 3; Figure 3.1).

Calibration and rate calculation

The zero point of electrodes was calibrated in the laboratory before use in the field. Zero readings were obtained by deoxygenating seawater with sodium thiosulphate. Saturated calibrations were obtained *in situ* by bubbling air through a calibration chamber. The saturated values were corrected for salinity, temperature and depth. The calibration data was used to compute dissolved oxygen concentrations in µmols L^{-1}. The rate of oxygen consumption decreased with time and a best fit was obtained using an exponential function ($r^2 > 0.9$; although a reasonable fit could also be obtained using a linear equation). This function was then used to evaluate the rate of oxygen consumption under ambient conditions by calculation of the slope of the tangent to the fitted curve at $t = 0$. This slope is essentially an estimate of the rate of oxygen consumption in the chamber under conditions when the internal water chemistry was as close to ambient as possible. It has been assumed that this rate is most representative of the actual rates under ambient conditions. An alternative model (fitting a linear equation to the first 4 hours of the deployment) was trialled and the trend in the results was essentially the same (although absolute rates were uniformly lower at around 87 % of the rate calculated using the exponential fit).

Initial problems encountered

Initial attempts to measure oxygen consumption rates of the benthos were undertaken at the same time as the infauna sampling in 1997. This was done using smaller chambers (internal volume 11.5 L; surface area 0.05 m^2) a number of problems were encountered mainly due to the insensitivity of the electrodes (given the surface area to volume ratio of the chambers) and this did not provide sufficient power to resolve changes in oxygen concentration. Chambers were modified in various ways before the larger chambers were developed in early 1999. The data presented here were therefore obtained during March/April 1999.

Data analysis

Analysis of sediment oxygen consumption rates

Analysis of Variance (ANOVA) was used to test for significant site differences. If differences were significant, then the Tukey-Kramer HSD (honestly significant difference) test was used to locate the source of the difference. On checking the data for homogeneity of variance, variances were found to be significantly heteroscedastic even after $\log_e(x+1)$ transformation, therefore the raw data were analysed using Welch's ANOVA (Zar 1996). The traditional alpha value of 0.05 was used to define significance in all analyses.

Estimation of metabolic rates of macrobenthic infauna

The oxygen consumption of macrobenthic infauna was estimated based on mass-specific oxygen consumption rates. The estimated values were then used to provide an indication of the proportion of oxygen consumption that could be attributed directly to the macrobenthic infaunal communities as distinct from all other contributing processes. This would provide an understanding of the importance of macrobenthic infaunal communities in the system.

Banse (1982) provided a range of values that could be used to estimate respiration rates based on body size via a log-linear relationship between the oxygen consumption rate per animal and the mean individual dry biomass. This equation takes the form

$$\log q = \log a + b \log M$$

where q is oxygen consumption rate (nl O_2 animal^{-1} h^{-1} at 20°C) and M is mean individual dry biomass (µg); *a* and *b* are coefficients which are specific to different types of animals (Banse 1982).

The proportionality coefficient *a* may vary depending on the group of animals concerned, with values ranging from 0.34 for unicells to 1.29 for poikilotherms (Table 1 in Banse 1982). The coefficient *b* is less variable with the lowest value of 0.52 for rotifers and the highest of 0.85 for enchytraeids. The calculated oxygen uptake rate (q) will therefore vary depending on the selection of these parameter values.

The infauna sampled in this present study were mostly very small macrofauna. In addition, nematodes and copepods were also retained on the 500 µm sieve. Therefore estimates provided in Table 6.1 were obtained using mean values for the coefficients *a* and *b* (mean of nematode and poikilotherms, log $a = 1.01$ and $b = 0.75$); an indication of the potential error can be obtained by calculating the rates for nematodes and poikilotherms in order to estimate the upper and lower bounds for this calculated q value (Table 6.1).

Table 6.1 Calculated rates of oxygen consumption q (nl O_2 animal^{-1} h^{-1} at 20°C) using different coefficients *a* and *b* derived for nematodes, poikilotherms and the mean of nematodes and poikilotherms (coefficients are based on Banse 1982).

Site	Calculated oxygen consumption rate (q) for infauna (nl O_2 animal^{-1} h^{-1})		
	Minimum rate (nematodes) **log a = 0.73** **b = 0.76**	**Mean rate (nematodes and poikilotherms)** **log a = 1.01** **b = 0.75**	**Maximum rate (poikilotherms)** **log a = 1.29** **b = 0.74**
Outfall Station 1	410	737	1,326
Outfall Station 60	403	726	1,307
Outfall Station 300	987	1,755	3,123
Moana	433	779	1,401

This approach is comparable to the method used by Dewey (1976) cited in Banse (1982) who recalculated literature data for small invertebrates. She observed that values for the coefficient *a* were intermediate between 0.43 and 1.29 and values for the coefficient *b* did not differ significantly from 0.75.

Estimates for the oxygen consumption rates of the macrobenthic infauna were calculated using the biomass and abundance data for infauna sampled in April 1997 (values were then converted to mmol O_2 m^{-2} d^{-1}) and used to estimate the contribution of infaunal respiration to total sediment respiration. This was based on the assumption that at similar time of the year, the infaunal communities were "typical" in terms of body sizes but not in actual species composition, and recognising that there are inter-annual variability (see Chapter 5).

Results

Sediment oxygen consumption

The mean rates for total oxygen consumption of sediments at all the three outfall stations measured were significantly higher (101 - 102 mmol O_2 m^{-2} d^{-1}; $p < 0.05$) than that of the southern control site at Moana (51 mmol O_2 m^{-2} d^{-1}). However, the rates were not significantly different to those for the northern control site at Hallett Cove (Table 6.2). The mean rate at Hallett Cove (101 mmol O_2 m^{-2} d^{-1}) was significantly higher than the mean rate at Moana. There were no within site differences in oxygen consumption rates for the three stations at the outfall site (Table 6.2).

Table 6.2 Mean rates of total sediment oxygen consumption (mmol O_2 m^{-2} d^{-1} ± SE) with the number of deployments at each site and the mean temperature (°C ± SE) during incubation (* indicates significant difference).

Site	Number of deployments	Total sediment oxygen consumption (mmol m^{-2} d^{-1})	Temperature (°C)
Hallett Cove	4	101 ± 11	19.1 ± 0.0
Outfall Station 1	6	102 ± 5	20.1 ± 0.4
Outfall Station 60	6	102 ± 7	19.7 ± 0.0
Outfall Station 300	4	101 ± 10	20.5 ± 0.3
Moana	4	*51 ± 5	19.1 ± 0.1

The rates of total oxygen consumption by the sediment were most variable at the station 300 m north of the outfall site (Station 300) and at Hallett Cove (high SE; Table 6.2). However, the mean rates at Station 1 and 60 of the outfall site were no different from Station 300 of the outfall site and Hallett Cove. The total sediment oxygen consumption recorded at the southern control site of Moana was half the rate of the other sites.

The change in ambient oxygen concentration in the water column did not exceed 0.08 mmol L^{-1} d^{-1} for any of the deployments. By contrast, oxygen consumption within the chambers was around an order of magnitude higher (averaging 0.63 mmol L^{-1} d^{-1} across all deployments). In addition, oxygen uptake by the water column within the chamber did not exceed 5 mmol O_2 m^{-2} d^{-1}, indicating that the total oxygen consumption measured within the chamber was a consequence of uptake by the sediment.

Infauna abundance and biomass

Due to time constraints, core samples of the infauna were not obtained when the respirometry measurements were carried out. However, in order to have some indication of the contribution by macrobenthic infauna to the sediment oxygen uptake, the mean abundance and biomass (wet weight) of infauna from the 1997 sampling at similar stations were used. In order to account for the seasonal variations in the infaunal community (see Chapters 4 and 5), only the April 1997 data were analysed. Since no infauna samples were collected from the northern control site at Hallett Cove in 1997, comparisons were only made between the outfall site and the southern control site at Moana.

The mean numbers of animals in the outfall sediments at two stations (Stations 1 and 60) were higher than the southern control site at Moana and were also more variable with higher standard errors (Table 6.3). Station 300 of the outfall site had the highest mean abundance and also high variability when compared to the control site at Moana. The mean abundance at the outfall stations ranged from 8,445 to 17,034 individuals per m^2 and the control site, Moana, had 9,937 individuals per m^2 (Table 6.3).

Table 6.3 Mean abundance (individuals m^{-2} ± SE) and biomass (g m^{-2} ± SE) of the macrobenthic infauna from the outfall site and the southern control site at Moana sampled in April 1997.

Site	Abundance (individuals m^{-2})	Biomass (g m^2)
Outfall Station 1	12,054 ± 1,395	24 ± 12
Outfall Station 60	17,034 ± 2,125	33 ± 18
Outfall Station 300	8,445 ± 1,004	54 ± 35
Moana	9,937 ± 673	21 ± 4

In the outfall sediments, spionid polychaetes and ostracods dominated at Station 1 (closest to the outfall) while gammaridean amphipods, ostracods and nematodes dominated at Station 60 (60 m from the outfall). Station 300 had either intermediate or low numbers of these taxa. In comparison, gammaridean amphipods dominated the control site at Moana. This could account for the differences in the biomass at each site (Table 6.3). The mean biomass was highest (54 g m^{-2}) at Station 300 (300 m from the outfall) while the other outfall stations, Station 1 and 60 had mean biomasses of 24 and 33 g m^{-2} respectively

(Table 6.3). The control site, Moana, had the lowest biomass of 21 g m^{-2} (Table 6.3). This variability could also be due to the occasional sampling of large animals at some stations (ophiuroids and bigger bivalves where wet weights were greater than 0.1 g per individual). Station 300 of the outfall site in particular, had several large bivalves and a large ophiuroid, but these animals were not consistently sampled in all core samples taken, consequently there is a higher mean wet biomass at this station.

Sedimentary organic carbon content

Sedimentary organic carbon content from the April 1997 sampling (details in Chapter 2) was also used to give an indication of possible effects of carbon deposition from wastewater effluent on sediment oxygen consumption. Comparisons were only made for stations where oxygen uptake rates were measured. The organic carbon contents were almost similar at Stations 1 and 60 of the outfall site (0.99 and 1.10 % dry weight of sediment respectively) and decreased to a low of 0.69 % at Station 300 of the outfall site (Table 6.4). The control site at Moana had organic carbon content (0.97 %) that was comparable to the outfall Stations 1 and 60 (Table 6.4).

Table 6.4 **Mean sedimentary organic carbon content (± SE) in % dry weight of sediment from the outfall site and the southern control site at Moana sampled in April 1997.**

Site	Sedimentary organic carbon content (%)
Outfall Station 1	0.99 ± 0.03
Outfall Station 60	1.10 ± 0.06
Outfall Station 300	0.69 ± 0.04
Moana	0.97 ± 0.05

Estimated metabolic rates of macrobenthic infauna

Based on the model of Banse (1982) and using mean values for coefficients *a* and *b* (see above), the infauna were estimated to contribute between 9.4 to 16.3 % of the total sediment oxygen consumption (Table 6.5). Contribution was highest at the control site Moana (16.3 %) and lowest at Station 1 of the outfall site (9.4 %; Table 6.5). The contribution by the macrobenthic infauna to total sediment oxygen consumption was still highest at the control site when using the coefficients for nematodes or poikilotherms, although the absolute values for oxygen consumption rates were different.

Table 6.5 Different rates of oxygen consumption (mmol m^{-2} d^{-1}) using different coefficients *a* and *b* for the log-transformed equation $q = a M^b$ and the % contribution by infauna to total sediment oxygen consumption (coefficients based on Banse 1982).

Site	Calculated oxygen consumption rate for infauna (mmol m^{-2} d^{-1})	Contribution by infauna to total sediment oxygen consumption (%)
log *a* = 0.73 and *b* = 0.76 (nematodes)		
Outfall Station 1	5.29	5.2
Outfall Station 60	7.36	7.3
Outfall Station 300	8.93	8.8
Moana	4.61	9.1
log *a* = 1.29 and *b* = 0.74 (poikilotherms)		
Outfall Station 1	17.13	16.8
Outfall Station 60	23.85	23.5
Outfall Station 300	28.26	27.9
Moana	14.92	29.3
log *a* = 1.01 and *b* = 0.75 (mean of nematodes and poikilotherms)		
Outfall Station 1	9.52	9.4
Outfall Station 60	13.25	13.1
Outfall Station 300	15.88	15.7
Moana	8.30	16.3

Discussion

Comparison between impact and control sites

Differences in the oxygen consumption rates between the outfall site and the southern control site at Moana may be attributed to differences in the supply of organic carbon to the sediments. At the outfall site, 27 ML per day of secondarily treated wastewater effluent are discharged and at the northern control site, there is discharge from a storm water drain, which may be irregular but occasional flows into the area are substantial (there was discharge during the field experiment because of rain). In contrast, the southern control site has no such discharges in the immediate vicinity. Studies have shown that increased organic loading to the sediments can stimulate sediment metabolism (Hargrave

1973, Graf *et al.* 1982, Kelly and Nixon 1984, Jensen *et al.* 1990). However, inputs of organic carbon may be out of phase with other processes controlling sediment respiration rates (e.g. temperature, water circulation, cycles in faunal populations).

In the present study, measurements of oxygen consumption were carried out over a period of two weeks in March/April (autumn) and temperatures for all deployments were in the narrow range of 19.1°C to 20.5°C. Seasonal variations in temperature affect respiration rates (Smith 1973, Hatcher *et al.* 1994) and work by Grant (1986) suggested that benthic communities responded to an average temperature and not to its variation over a span of a few hours, therefore indicating that differences in oxygen uptake rates at the outfall and control sites were probably not a consequence of temperature fluctuations over the small time scale of this study.

Sediments at both the outfall and control site (Moana) were essentially similar, classified as fine sand according to the Wentworth grade classification (Appendix II). Therefore although muddy or fine-grained sediments are known to increase respiration rates (Shaffer and Onuf 1983, Grant *et al.* 1991), the higher rates of sediment oxygen consumption at the outfall site were probably not due to differences in sediment size. The sediment type at the northern control site (Hallett Cove) was mostly coarse sand with scattered rocks; again indicating that the higher oxygen uptake rates at this site were probably not due to sediment size.

Benthic animals have an important impact on the exchange of oxygen, carbon dioxide and nutrients across the sediment-water interface (Andersen and Kristensen 1988). Through their irrigational activities, macrofauna can have a major influence on respiratory pathways by increasing oxygen flux across this interface (Aller 1982, Fisher 1982) and thereby have a significant role in the organic carbon cycle of the sediments (Archer and Devol 1992).

The number of infauna sampled at the outfall site was higher than the control site, being most abundant at the station closest to the outfall, decreasing with distance from the outfall. However, the biomass did not reflect this decreasing trend away from the outfall, implying that the macrofauna may not be driving the sediment oxygen uptake in the present study. The oxygen consumption is almost certainly also driven by other processes such as meiofauna, microfauna and microbial respiration. In addition, chemical oxidation is likely to contribute to the oxygen uptake.

A high proportion of sediment metabolism proceeds through pathways of nitrate, manganese, iron, and sulphate respiration (Jørgensen 1977, Sorensen *et al.* 1979, Sampou and Oviatt 1991). This anaerobic metabolism (chemical oxygen consumption) may be in excess of 50 % of the total oxygen demand in some coastal areas (Pamatmat and Banse 1969, Jørgensen 1977, Dale 1978, Grant 1986).

Profiles of carbon concentration over depth were not assessed but a study by Banta *et al.* (1995) showed that particulate organic carbon decreased with depth into the sediment. The sediment used in the present study for the determination of organic carbon content was from a mixed dried core sample taken to a depth of 200 mm. Consequently, the concentrations of sedimentary carbon measured may not be indicative of the accumulation or remineralisation of organic matter in surficial layers.

Comparison with other studies

Some perspective on these results can be developed from an analysis of data from the literature (Smith *et al.* 1973, Hatcher *et al.* 1994, Mazouni *et al.* 1996, Giblin *et al.* 1997). Oxygen consumption is temperature dependent; therefore the comparative data used were limited to published reports for temperatures between 17 and 22°C and values in other units were converted to mmol O_2 m^{-2} d^{-1}. In addition, the literature data were restricted to studies where there were 'Impact' and 'Control' data. The 'Impact' data referred to those sites that were collected near sources of organic and nutrient pollution.

Total sediment oxygen consumption

For comparisons of total sediment oxygen consumption rates, 'Impact' data were plotted against 'Control' data for each individual study taken from the literature. Data from the present study were also plotted similarly. Pairwise comparisons were then carried out to determine the mean response of each set of data using paired t-test (Figure 6.2).

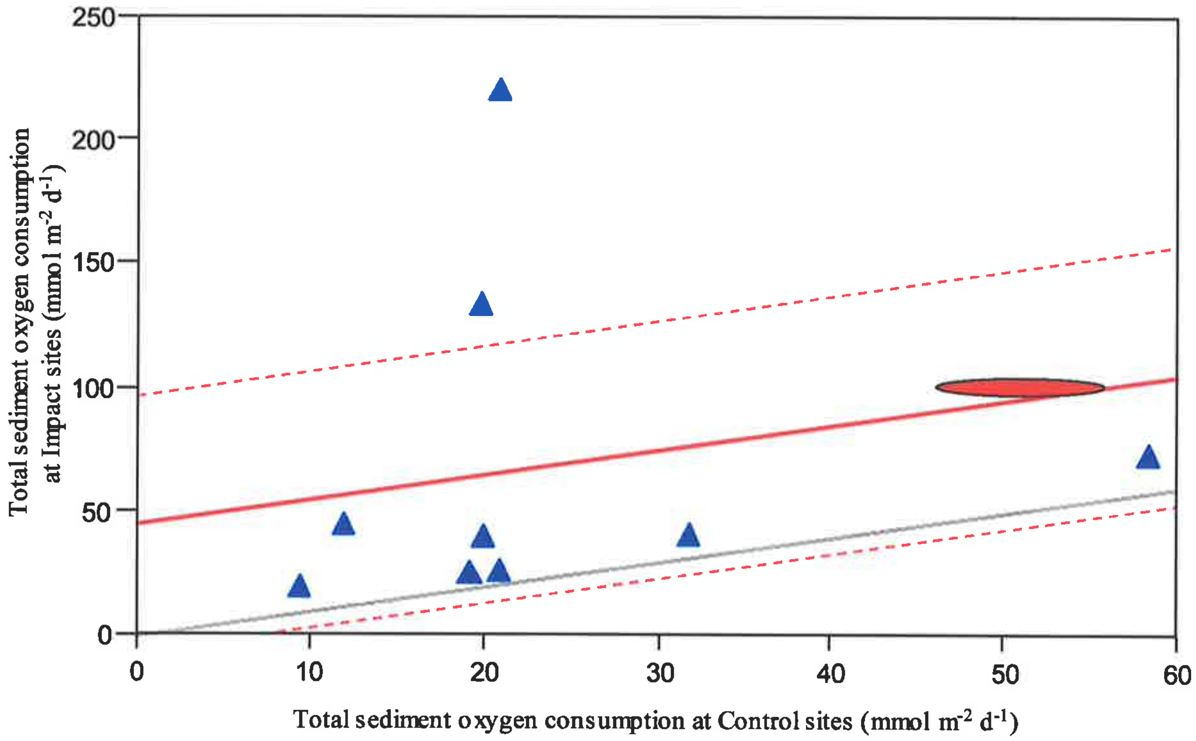


Figure 6.2 **Total sediment oxygen consumption rates (mmol m^{-2} d^{-1}) for paired comparisons of Impact sites plotted against Control sites using comparative literature data and present study. The coloured area (⬬) refers to the mean rate (± SE) for the present study, ▲ refers to literature data with 'Impact' and 'Control' sites. —— refers to the overall mean response of literature data, ----- refers to 95% confidence limits of the mean response and —— is the hypothetical case where there is no difference between impact and control sites.**

A wide range of values has been reported in the literature for the rate of oxygen consumption in sediments. Data for sites defined as 'Control' sites range from 9.6 to 58.5 mmol O_2 m^{-2} d^{-1} and sites designated as 'Impact' sites from 18 to 220 mmol O_2 m^{-2} d^{-1}. Whereas the huge variation in absolute oxygen consumption rates across these studies precludes a comparison based on a simple comparison of the means, it is possible to compare the relative response of control versus impact for each study. In all cases the reported rates of oxygen consumption were higher for impact sites when compared with control sites. The mean response is for impact sites to have an oxygen consumption rate that is around 65 % higher than that of the corresponding control sites. An analysis of these data using a pairwise comparison (Figure 6.2) demonstrates that the mean response for impact sites is significantly different to that for control sites ($p < 0.05$). This pattern holds even though two data points (from the study by Giblin *et al.* 1997) differed substantially from the overall mean response with very much higher rates of oxygen consumption at the impact sites relative to that found for most other studies.

The large variation in total sediment oxygen consumption rates recorded in the literature is attributable to a number of reasons. Each of the studies was carried out at different sites and likely to have significant difference in community structure, which in turn would translate to substantial differences in sediment oxygen consumption. In addition, the method used to measure oxygen consumption rates in each study was different (e.g. *in situ* versus laboratory incubation), resulting in different absolute rates. However, the relative rates of control versus impact were still maintained for all the studies that were compared.

Data from the present study fitted very closely to the overall pattern obtained from these other studies. The mean response for the impact sites at Christies Beach was close to double that for the control site at Moana (which was just slightly higher than the average obtained from the analysis of the data from the literature; Figure 6.2).

Oxygen consumption attributed to macrofauna

The calculated oxygen consumption rates of the macrofaunal component of the community from this present study could only be compared to the study by Smith *et al.* (1973) since it was the only work available that had data on macrofaunal respiration for 'Impact' and 'Control' sites. The calculated rates from the present study were within the range reported, however, they do not follow the trend reported by Smith *et al.* (1973) where the macrofauna respiration rates at the 'Impact' site was lower (11.4 mmol O_2 m^{-2} d^{-1}) than the 'Control' site (17.0 mmol O_2 m^{-2} d^{-1}; Table 6.6). For the present study, all the three outfall stations (Stations 1, 60 and 300) had higher estimated oxygen consumption rates for macrofauna than the control site at Moana (Table 6.6).

Table 6.6 **Comparison of macrofauna respiration rates (mmol O_2 m^{-2} d^{-1}) and its contribution to total sediment oxygen consumption (%) between the present study and the study by Smith *et al.* (1973).**

Sites	Respiration rates of macrofauna (mmol O_2 m^{-2} d^{-1})	Contribution by macrofauna to total sediment oxygen consumption (%)
Present study		
Outfall Station 1	9.5	9.4
Outfall Station 60	13.3	13.1
Outfall Station 300	15.9	15.7
Moana	8.3	16.3
Study by Smith *et al.* (1973)		
Impact	11.4	20.1
Control	17.0	34.0

The differences in absolute values of macrofauna respiration rates between the two studies could be due to different methods of computation. Smith *et al.* 1973 estimated macrofaunal respiration rates from an earlier study (Smith 1971; cited in Smith *et al.* 1973) in which the respiration rates for individual animals were measured and the community rates were calculated as an aggregated estimate while rates for the present study were estimated from the equation of Banse (1982). In addition, the equation of Banse (1982) may need to be applied differentially in terms of the parameters *a* and *b* for the outfall and control sites because of substantial differences in community composition between the sites.

In terms of contribution of macrofaunal oxygen consumption to total sediment oxygen consumption, the present study does follow a similar trend as the study by Smith *et al.* 1973 where the rates were higher at the control site compared to the impact site (Table 6.6).

For biomass comparisons, all values were converted to ash-free dry weight (AFDW) from wet weight or dry weight according to the conversion used by Cammen (1980). In all other studies used for comparisons, the ash-free dry weights of macrofauna were higher at 'Control' sites than 'Impact' sites (Table 6.7). However, this trend was not observed in the present study where biomass values were generally lower. At the outfall site, biomass of macrofauna did increase with increasing distance from the outfall, but at the control site Moana, the biomass was the lowest (Table 6.7).

Table 6.7 Ash-free dry weight (g m^{-2}) of the macrofauna component for the benthic community and sedimentary organic carbon content (%) from comparative literature data and present study. Blank cells refer to no data.

Site	Ash-free dry weight (AFDW) of macrofauna (g m^{-2})	Sedimentary organic carbon content (%)
Present study		
Outfall Station 1	3.18	0.99
Outfall Station 60	4.40	1.10
Outfall Station 300	7.08	0.69
Moana (control)	2.82	0.97
Study by Smith *et al.* (1973)		
Impact	2.05	13.70
Control	15.29	3.00
Study by Mazouni *et al.* (1996)		
Impact	34.26	
Control	143.56	
Study by Hatcher *et al.* (1994)		
Impact	23.58	7.29
Control	40.66	6.93
Study by Giblin *et al.* (1997)		
Impact		4.46
Control		3.42

A possible explanation for this difference could be attributed to the movement of organic carbon. Effluent outfalls contribute to enhanced rates of sedimentation of organic materials in their vicinity (McIntyre 1990), consequently sedimentary organic carbon contents are expected to be high in areas where there is organic and nutrient enrichment. This was seen in the various studies used for comparison with the present study. The organic carbon concentrations in the sediments were higher at 'Impact' sites compared to 'Control' sites (Table 6.7). This trend appeared to be evident for the outfall stations where the organic carbon concentration was lowest at the station located 300 m from the discharge point (Table 6.7). However, the organic carbon content at the control site, Moana, was at a similar level as Stations 1 and 60 of the outfall site. This discrepancy may arise because most of the particulate material from the effluent did not make it to the benthos or is quickly remobilised at the outfall site. This loss through remobilisation may be as dissolved organic carbon, macrofaunal biomass or export as resuspended particulates (Hatcher *et al.* 1994, Giblin *et al.* 1997).

Implications

The benthic respirometry work was carried out to determine whether the argument could be made that the increased levels of organic carbon emanating from the effluent outfall would lead to higher rates of benthic respiration in close proximity to the outfall. The results appeared to confirm this with higher rates of respiration being recorded at the three outfall stations relative to the control stations at Moana. However, this pattern was not consistent with the other control site (Hallett Cove) having oxygen consumption rates that were comparable to those measured at the outfall site.

There are numerous other sources of organic carbon entering the sediments (other than from the outfall) that would be providing nutrition for benthic infauna. These would include detrital material from nearby reefs and seagrass beds as well as organic detritus from the water column (planktonic production). At a site such as Moana where there are no effluent inputs or storm water inflows these are the only sources of carbon to the benthos. At the outfall site these inputs are still present but are augmented by inputs from the effluent outfall. The high rates of benthic respiration at Hallett Cove can also be explained in this way. This site is relatively close to reefs and seagrass beds that occupy substantial areas in the nearshore region (thereby providing a plentiful supply of detritus). In addition, there is a number of storm water inputs associated with relatively recent residential developments along this section of coast. One such input was present and was discharging during the period when the measurements were being made at Hallett Cove.

The next question was whether there was a direct correlation between benthic metabolism and the abundance and biomass of the macrofaunal community. The results did not show a correlation in terms of the absolute values of macrofaunal metabolism. This result is not surprising given that in most other studies the respiration rates of benthic macrofauna generally can vary widely and comprises a minor component (from 5 % to 35 %; Smith *et al.* 1972, Smith *et al.* 1973) of the overall rates of oxygen consumption. The values reported here for the present study (9 % - 16 %; Table 6.6) were in agreement with this.

The macrofauna therefore, may provide an index of community response to stress, but are not as useful in providing a quantitative measure that is related to changes in the tropho-dynamics of the system.

What then is happening in this system? Future work is needed to resolve the question of whether these higher values should be attributed to chemical or biological oxygen demand and in particular the relative importance of microbial, microfaunal and meiofaunal communities.

Chapter 7 Synthesis

Gulf St Vincent is an inverse estuary and thereby represents a unique environment for both researchers and environmental managers. Importantly, there are no data available[3] for such systems from elsewhere in the world that can be used as a basis for comparisons or on which to base environmental management decisions. This lack of knowledge is problematical in that it creates major uncertainties about the efficacy of alternative management arrangements, particularly as they relate to issues such as effluent or storm water disposal. Plans by the management agencies (South Australian Water Corporation, Environment Protection Authority) to expend upwards of $280 million to upgrade effluent outfalls are being currently evaluated. These proposals variously argue for achieving substantial (>75%) reductions in inorganic nutrient outputs (with a particular focus on nitrogen) but there has been little consideration of the options for reduction of organic carbon loads to the system. The work presented in this thesis is therefore central to informing this process about the relative importance benthic systems and the impact that effluent outfalls are likely to have on these systems along the Adelaide metropolitan coast.

The ability to distinguish natural from pollution-related environmental changes is a major challenge in pollution ecology overall (McIntyre 1977, Miller 1984, Boesch *et al.* 1990). In this context, the assessment of environmental impacts associated with the wastewater effluent outfall at Christies Beach, needs to acknowledge the potential for temporal and spatial variability in communities, which will otherwise confound attempts to identify differences in communities caused by anthropogenic impacts. Benthic communities in the Gulf vary in relation to their location (position on the long shore gradient and depth) as well as through time both seasonally and inter-annually (Shepherd and Sprigg 1976, Cheshire and Miller 1996, Cheshire *et al.* 1998, Cheshire and Miller 1999). Furthermore, other factors such as time lags in the response of biota to disturbances (Westman 1978) will also make the identification of impacts more difficult.

These issues are particularly pertinent in respect of the Christies Beach outfall; historical reports by the management agency (formerly Engineering and Water Supply Department,

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[3] The author has not been able to identify any published accounts for inverse estuaries from elsewhere in the world.

now United Water International/South Australian Water Corporation; Steffensen 1985) suggested that there was only a very localised effect on water chemistry and that biological communities were largely unaffected and generally similar to comparable unpolluted areas. This finding, based on a single day of surveys in 1978, would appear to conflict with the results from the present study which has shown broad scale impacts with substantial modifications to infaunal community structure and metabolism. In turn, this underscores the need for comprehensive environmental assessments as a basis for evaluating impacts.

Marine benthic systems are complex and typically heterogeneous with a diverse array of taxa that may vary both in their absolute and relative abundances (Gray 1974). This biota responds to the range of physical and chemical conditions of the environment and through complex interactions with other biotic elements in the ecosystem (Snelgrove and Butman 1994). In order to elucidate the relative importance of natural versus anthropogenic environmental forces, field studies aimed at assessing pollution impacts require observations at appropriate scales in time and space and across periods of sufficient duration (Green 1979, Gray 1981b, Stewart Oaten and Murdoch 1986).

The present study aimed to assess the effects of wastewater effluent on macrobenthic infaunal communities; to achieve this, the study addressed four main objectives:

1. To characterise the physical, chemical and biological environment of the Gulf and the region around the outfall and control sites (Chapter 2).

2. To compare and contrast the macrobenthic infaunal communities between the outfall site and the control site (Chapter 4).

3. To compare communities from the outfall and control sites with other communities in the Gulf, addressing spatial and temporal variability (Chapter 5).

4. To quantify the physiological responses of the sediment communities (in terms of oxygen exchange rates) at both the outfall and control sites (Chapter 6).

Collectively these objectives allowed for the assessment of impacts from the wastewater effluent whilst accounting for the natural variability in the ecosystem associated with the varying biotic and abiotic environment of Gulf St Vincent, including differences in location (particularly longshore position), time of year and differences at inter-annual scales.

Summary of findings

Gulf St Vincent presents a unique environment as an inverse estuary where salinity is higher than waters outside the Gulf. This is a consequence of the high rate of evaporation with low freshwater input either from rainfall or terrestrial runoff into the marine environment (Tippins 1996). Its circulation appears to be predominantly driven by tides, winds and density differences caused by the high rates of evaporation, heating and intermittent fresh water inputs. The resulting flow is north south along the metropolitan coast; being predominantly north in summer and south in winter (Bye 1976) and a complete renewal of Gulf waters takes approximately half a year (Tippins 1996). The Gulf also has an unusual tidal regime, which results in a 'dodge' tide (no net movement of water for a whole day) occurring every fortnight (Bye 1976).

Waters in the Gulf are generally regarded as oligotrophic, having low natural levels of nitrate, phosphate and ammonia (Butler *et al.* 1997). Nutrients such as nitrogen and phosphorus entering the Gulf are mainly of terrestrial origin, from sewage treatment works, urban storm water containing soil and fertilisers and industrial discharges rather than upwelling and advection on-shore.

The results from the analysis of benthic community structure generally supported the hypothesis that the Christies Beach effluent outfall has induced significant and persistent changes to the structure of the macrobenthic infaunal communities, which increases in severity in relation to the proximity to the outfall. The temporal patterns of community structure also indicated that, over and above the seasonal variations, communities at the outfall site were still different from communities at the control site and this appeared to relate primarily to distance from the discharge point.

The differences in community structure between the control and outfall site appeared to be consistent with the sort of disturbance expected from the outfall and may be attributed to the increased organic and nutrient enrichment resulting from the Christies Beach

Wastewater Treatment Plant's effluent discharge. Furthermore, the results from analyses to determine community stress generally showed that the stations in close proximity to the outfall had macrobenthic infaunal communities that were characteristic of moderately disturbed to highly disturbed systems.

Stations closest to the discharge point had an elevated abundance and depressed biomass of macrobenthic infauna, being dominated numerically by small r-selected/ opportunistic species. This difference (assessed using abundance/biomass comparison plots; Warwick 1986, Warwick *et al.* 1987) indicated that the outfall site was moderately disturbed. The neutral model analysis also showed that these stations appeared to be disturbed with reduced diversity and higher deviations from the predicted diversity (Caswell 1976). The stations at mid distance from the outfall were however at intermediate disturbance with higher diversity and lesser deviations from the predicted diversity of the neutral model.

The uneven distributions of abundance groups in log-normal plots (Gray and Pearson 1982, Pearson *et al.* 1983), for the stations closest to the outfall, also indicated that the communities were experiencing effects of moderate organic enrichment. This result was further reinforced by taxa such as capitellid polychaetes occurring in intermediate abundance groups (considered to be most sensitive to pollution-induced changes) for the outfall stations as compared to the occurrence of pollution sensitive taxa such as gammaridean amphipods at the control stations.

The macrobenthic infaunal communities at the outfall and control sites overlapped in many of their constituent taxa, but were characterised by differences in the relative abundance of several key taxa. The outfall site was characterised by elevated numbers of capitellid and spionid polychaetes, nemerteans and ostracods and reduced numbers of gammaridean amphipods and bivalves relative to the control site. These differences in the overall structure of macrobenthic infaunal communities are consistent with enhanced food availability and the physical effects from increased suspended matter, close to the outfall site.

The respirometry work demonstrated that benthic respiration rates were higher at the outfall stations relative to the control stations at Moana. These results appeared to confirm the proposal that increased levels of organic carbon, emanating from the effluent outfall, led to higher rates of benthic metabolism in close proximity to the outfall. These results need to be qualified however, in the context of the high rates of benthic respiration observed at the other control site (Hallett Cove) that was probably influenced by storm water inputs.

Collectively the results from this study suggest that the Christies Beach effluent outfall has induced changes to the macrobenthic infaunal communities of stations in close proximity to the outfall (extending over a 1 km stretch of coastline, 500 m to the North and South) relative to the control stations. Some results, however, did not conform to this general conclusion. For example, there were no particular trends observed for biomass of infauna across the outfall stations, which would correspond to disturbance by organic enrichment. These discrepancies could be attributed to the fact that the discharge of wastewater effluent at Christies Beach is a chronic disturbance as the treatment plant has been discharging the effluent into the area since 1971. Adaptation to this effluent discharge has probably occurred and the macrobenthic infaunal communities around the outfall are likely to have moved to a new equilibrium state where deposit feeders dominate. This state may respond to stochastic influences (e.g. storm related disturbances), but is not the same as the original state (Gray 1981a). In addition, biotic interactions and in particular biogenic environmental changes may be responsible for the micro-scale horizontal patchiness in these benthic communities (Barry and Dayton 1991).

Implications of findings

Gulf St Vincent is an inverse estuary and as such represents an environment that is unique. There is little quantitative baseline data on these systems from elsewhere in the world and, to date, very few studies on these systems in South Australia. Prior to this study there was very little knowledge about how this system would respond to environmental impacts of any type. Not withstanding this paucity of data, there have been a number of studies, which demonstrated the fragility of this environment to impacts with particular reference to the effects of effluent on epibenthic communities. Since the early 1960's there has been a substantial and progressive loss of seagrasses in nearshore systems of Gulf St Vincent that has been linked to increased levels of effluent being discharged into the Gulf (Neverauskas 1987, Burns 1992). Furthermore, recent studies by Cheshire and his colleagues (Cheshire *et al.* 1998) have shown ongoing degradation of reefal systems in proximity to the Christies Beach outfall. The degradation of these epibenthic and reefal communities is visually obvious but it is likely to represent only one aspect of the problem facing environmental managers of the Gulf.

The present study has played an important role in expanding our understanding of the susceptibility of South Australian gulf systems to anthropogenic impacts. The results provide the first data in Australia for the response of macrobenthic infaunal communities in an inverse estuary to the effects of organic enrichment and elevated inorganic nutrients associated with effluent discharge. Such information is critical to the effective management of these systems, in particular in defining discharge limits, and the scope of environmental improvement that is necessary if we aim to limit or mitigate these impacts. Importantly however this work also provides us with an understanding of the extent to which previous work on effluent impacts in estuaries can be generalised to systems which are structurally quite dissimilar (i.e. inverse estuaries) when compared to those studied elsewhere.

The results from this study demonstrated that communities close to the Christies Beach effluent outfall were substantially different in composition and that these differences were consistent with the environmental effects one would expect from an effluent discharge. In fact, all the results from this study are in close agreement with one another and together they define a clear indication of the magnitude and nature of the disturbance gradient associated with the outfall. Furthermore, these results were comparable to other studies on macrobenthic infaunal community structure in estuarine and marine systems subjected to organic enrichment (e.g. Stull *et al.* 1986, Aschan and Skullerud 1990, Ferraro *et al.* 1991, Grizzle and Penniman 1991, Anderlini and Wear 1992, Ismail 1992).

Future research

There is clearly a need for additional research to answer some of the questions that arose during the course of this study. The variability observed in the present study indicated that continuing studies with spatially and temporally replicated sampling over longer periods and across a wider area of the macrobenthic infaunal communities are needed. This will enable a better interpretation of data to determine the extent to which the environment around the outfall is perturbed relative to areas more remote.

The lack of correlation between the infaunal data with the environmental variables measured (sediment grain size and organic carbon content) means that there is a need to establish whether the differences in the macrobenthic infaunal communities between sites are correlated with levels of other pollutants (e.g. nutrients and heavy metals) or whether other differences in the natural environmental (e.g. salinity and temperature) are important in structuring these systems. A cause-and-effect relationship between environmental variables and benthic faunal communities can be achieved by using experimental manipulations of pollutant levels under controlled conditions either in the field, or in laboratory mesocosms.

A wider series of assessments of benthic metabolism is also needed to provide a better understanding of how metabolism varies both temporally and spatially. Results were not conclusive due to confounding influences such as storm water input at one of the control sites. The uncertainty of whether the differences obtained between the outfall and control sites are a result of differences in community structure means that measurements of benthic metabolism must match the faunal study in space and time. In addition, studies that more fully elucidate the partitioning of sediment oxygen consumption into its various components (chemical and biological) may help to explain the movement of this increased organic input from the effluent outfall in terms of carbon mobilisation and utilisation in the system.

Conclusion

In the preface to this thesis it was argued that "The oceans have always been viewed as boundless in size, able to absorb all wastes and still be able to feed a hungry world [however] … chemical contamination and litter can be observed from the poles to the tropics and from beaches to abyssal depths … [If nothing is done, pollution will lead to] … global deterioration in the quality and productivity of the marine environment …".

This study has shown that ecosystems do respond to impacts and all too often the ecological importance of a particular impact is lost to economic developments. If we are to continue a lifestyle we have grown to appreciate, we need to make meaningful judgements about the relative costs and benefits of our impacts on the environment.

This thesis has tried to illustrate how the application of ecological science carried out effectively can provide the general public, managers and politicians with the tool and scientific knowledge on which to make decisions for the benefit of all living things on earth.

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Appendix I Water Quality

Table I.1 Seasonal means of water quality parameters for effluent from Christies Beach Wastewater Treatment Plant and background levels for Gulf St Vincent. All values exceed the chronic levels (criteria) set out in the Guidelines for Licensing Discharges to the Marine Environment (Environment Protection Authority 1993) except for "*" which indicates a level below detectable limits of the analysis. The Gulf St Vincent values are mean minimum and maximum of a range of water quality parameters determined from various sites along the metropolitan coast of Adelaide.

			Gulf St Vincent[2]	**Effluent[3]**			
Parameters	**Units**	**Criteria[1]**	**Mean Minimum-maximum**	**Dec ‘96 to Feb ‘97**	**Mar ‘97 to May ‘97**	**Jun ‘97 to Aug ‘97**	**Sep ‘97 to Nov ‘97**
Effluent Flow	ML/month			770	773	821	834
Turbidity	NTU		1.1-11.3	5.7	3.9	3.8	4.0
Colour	HU			39.5	41.5	34.8	40.4
Microbiological							
Faecal coliform	per 100ml		Geometric mean 1.4-4.8	23.9	8.5	7.2	8.2
Nutrients							
Total Phosphorus	mg/L	< 0.005	0.025-0.070	8.833	8.588	8.500	8.500
Oxidised nitrogen	mg/L	0.010 - 0.060	0.014-0.039	13.383	16.758	22.090	16.523
Ammonia	mg/L	0.001 - 0.010	0.035-0.068	18.233	16.119	19.985	22.077
TKN	mg/L		0.325-0.484	20.600	18.829	20.417	24.638
Metals							
Copper	mg/L	< 0.0029	0.003-0.004	0.029	0.026	0.022	0.020
Mercury	mg/L	< 0.000025		*	0.0001	0.0001	0.0001
Nickel	mg/L	< 0.0083	0.002-0.002	*	*	0.009	0.021
Zinc	mg/L	< 0.086	0.018-0.031	0.084	0.091	0.123	0.099

Source: 1 - Environment Protection Authority 1993, 2 - Environment Protection Authority 1997, 3 - United Water 1997, United Water 1998

Table I.2 Seasonal means of water quality parameters for receiving waters 50m north and south of the outfall at Christies Beach Wastewater Treatment Plant and background levels for Gulf St Vincent. Values in **bold** exceed the chronic levels (criteria) set out in Schedule 7, Tables 1 and 4 in the Guidelines for Licensing Discharges to the Marine Environment (Environment Protection Authority 1993). "*" indicates levels below detectable limits of the analysis and "nd" indicates no data. The Gulf St Vincent values are mean minimum and maximum of a range of water quality parameters determined from various sites along the metropolitan coast of Adelaide.

			Gulf St Vincent[2]	**Receiving waters 50m north[3]**				**Receiving waters 50m south[3]**			
Parameters	**Units**	**Criteria[1]**	**Mean minimum-maximum**	**Dec '96 to Feb '97**	**Mar '97 to May '97**	**Jun '97 to Aug '97**	**Sep '97 to Nov '97**	**Dec '96 to Feb '97**	**Mar '97 to May '97**	**Jun '97 to Aug '97**	**Sep '97 to Nov '97**
Turbidity	NTU		1.1-11.3	0.50	0.31	0.47	2.53	0.39	0.52	0.50	2.13
Colour	HU			3	4	4	4	4	4	2	7
Microbiological											
Faecal coliform	per 100ml		geometric mean 1.4-4.8	2	0.5	0	nd	5	0.5	0	nd
Nutrients											
Total Phosphorus	mg/L	< 0.005	0.025-0.070	**0.157**	**0.024**	**0.253**	**0.023**	**0.290**	**0.234**	**0.112**	**0.112**
Oxidised nitrogen	mg/L	0.010 –	0.014-0.039	**0.187**	**0.113**	**0.239**	0.023	**0.305**	**0.316**	**0.237**	**0.092**
Ammonia	mg/L	0.001 –	0.035-0.068	**0.140**	**0.030**	**0.137**	**0.018**	**0.130**	**0.148**	**0.115**	**0.060**
TKN	mg/L		0.325-0.484	**0.800**	**0.600**	**0.480**	nd	**0.750**	**0.950**	**0.580**	nd
Metals											
Copper	mg/L	< 0.0029	0.003-0.004	0.00200	0.00200	0.00233	0.00213	0.00100	**0.00300**	0.00233	0.00153
Mercury	mg/L	< 0.000025		*	*	**0.00010**	**0.00010**	*	**0.00020**	**0.00010**	**0.00010**
Nickel	mg/L	< 0.0083	0.002-0.002	0.00100	*	0.00233	0.00180	*	0.00100	0.00233	0.00183
Zinc	mg/L	< 0.086	0.018-0.031	0.01450	0.00700	0.05780	0.02040	0.01400	0.00700	0.03540	0.02700

Source: 1 - Environment Protection Authority 1993, 2 - Environment Protection Authority 1997, 3 - United Water 1997, United Water 1998

Appendix II Sediment Size Analysis

Introduction

The relationship between animals and sediment is complex. Although community structure is loosely related to sediment composition, the exact makeup may be changed by interactions between organisms and sediment. Many studies have shown that the distribution of infaunal invertebrate species is associated with various properties of the sediment in which they reside. These properties include grain size, organic content, microorganisms and sediment stability (see reviews by Rhoads and Young 1970, Gray 1974, Rhoads 1974, Gray 1981b, Peres 1982, Probert 1984). The various reviews had identified many different aspects of the relationships of organisms to sediment. Some aspects are being highlighted here.

Gray (1974) suggested that many species are restricted to certain types of sediment as a consequence of the selection of substratum for the establishment of a population by both larvae and mobile adults. This selection involved complex behavioural responses to bring the organism to the substrate. The organism would establish at a chosen site only if there were favourable stimuli from the substrate. The factors determining the site of settlement may be physical, which involve the structure and contours of the sediment surface and grain size. There may also be chemical or biological factors, which include the amount of inorganic and organic compounds, presence of a living microorganisms film on the substrata, presence of populations of the same species and other factors like reproductive patterns. However, the interactions of populations and sediment altering the properties of the sediment may result in the exclusion of other organisms. Hence there is an existing balance between suspension- and deposit-feeders.

In general, suspension-feeders have been shown to be abundant in well-sorted fine grain deposits and their numbers decrease as the silt-clay content increases (Gray 1974). This is attributed to coarse-grained sediments being more stable and able to support higher density of suspension-feeders. It could also be due to reduced amount of suspended food in silt-clay giving deposit-feeders a competitive advantage. However, Rhoads and Young (1970) and Rhoads and Young (1971) gave an alternative hypothesis that the reworking of sediments by deposit-feeders inhibited suspension-feeders. They suggested that the reworking led to physical instability of the mud which would cause the filtering structures of suspension-feeders to get clogged or preventing larvae from settling because of re-suspension and being buried. This phenomenon called “trophic group amensalism”, allowed for the prediction of fauna based on the stability of the substrate.

Another review by Snelgrove and Butman (1994) purported that organism distributions should be evaluated relative to the hydrodynamic and sediment-transport processes that were responsible for sediment distributions. He critically reviewed past works on animal-sediment relationships and argued that there was too many inconsistencies in species distributions based on grain-size and that grain size may be a correlate of the actual causes. His comment on organic content in sediment was that there were to many unresolved issues presently to allow the development of principles relating organic content as a cause for determining infaunal distribution patterns. He recognised that the microbial community was a very important aspect of bottom sediments, particularly as a food supply to deposit-feeders in mud, and influenced infaunal distributions. However, he considered microbial activity as only a crude correlate of sediment type and cited recent studies which suggested that the factors controlling the distribution, growth rates and biomass of sediment microorganisms were complex and needed more work. His criticism of “stability” was that the terms “stable” and “unstable” as used by benthic ecologists were lacking meaningful and consistent definitions. As for “trophic group amensalism”, the hypothesis had little support as a rigorous explanation for animal-sediment relations since the designation of functional groups were based on still-water observations and there were also too many “special” cases.

As much as sediment type determines benthic infaunal distribution, the effects of these organisms on the physical properties of sediment cannot be ignored. All the works and reviews recognised that the correlation between animal and sediment distributions may be a result of physical processes giving rise to the sediment environment rather than particular aspect of the sediment itself and the establishment of various organism communities and their interactions with individuals and other populations. Whether it is correlative, experimental or causative in drawing conclusions, existing explanations for relationships between sediment and benthic community distributions are still inadequate.

In view of the importance of animal-sediment relations and recognising the complexities of such relationships, the aim of this work presented here was to investigate the extent to which sediment size distribution might be a confounding influence in the present study in assessing the effects of wastewater effluent on macrobenthic infaunal communities.

Field method

In the field, sediment samples were collected using a PVC corer (length of 400 mm and a diameter of 42 mm). Each corer was pushed into the sediment until the depth of penetration was 200 mm. A rubber stopper was pushed into the open end at the top of the corer. The corer was then gently extracted and the bottom end sealed with another rubber stopper to prevent any loss of sediment. At each station, four samples were taken. These samples were then emptied into jars and labelled on site.

Laboratory method

On return to the laboratory, the samples were kept refrigerated until further processing. For longer storage before processing, the samples were oven dried at 100°C.

The equipment used for particle size analysis included a graded series of standard sieves according to the intervals of the Wentworth scale and a mechanical sieve shaker. All other equipment used was standard laboratory apparatus. The Wentworth scale is a geometric scale, based on 1 mm and ratio of 2 (Table II.1).

Table II.1 Wentworth grade classification

Name	Grade Limits	∅ (phi) Notation
Boulder	> 256 mm	-5
Cobble	256 - 64 mm	-4
Pebble	64 - 4 mm	-3
Granule	4 – 2 mm	-2
Very coarse sand	2000 – 1000 µm	-1
Coarse sand	1000 - 500 µm	0
Medium sand	500 - 250 µm	+1
Fine sand	250-125 µm	+2
Very fine sand	125 - 62 µm	+3
Silt	62 - 4 µm	+4 to +8
Clay	< 4 µm	>+8

The procedure outlined below for the analysis of sediment particle size was adapted from Buchanan 1984.

1. Pre-treatment

a) 50 g of oven-dried sediment was placed in a litre beaker with 100 ml of 6% hydrogen peroxide and stood overnight.

b) While the beaker of sediment and peroxide was being heated gently in a water bath, small quantities of peroxide were added until there were no further reactions. This process removed the organic matter.

c) The contents of the beaker were then washed onto a filter paper (Whatman No. 50) in a funnel. The sediment was washed thoroughly under gentle suction with distilled water to remove any electrolytes.

d) The sediment from the filter paper was washed into a beaker using a jet of distilled water and a camel hairbrush, using only 200-300 ml of water.

e) 10 ml of sodium hexametaphosphate solution (6.2 g L^{-1} aqueous) was then added and the mixture was mechanically stirred for 10-15 minutes. Whereat the sediment was left to soak overnight.

2. Initial splitting of silt-clay fraction

a) The sediment was again stirred for 10 - 15 minutes and transferred to a clean 63 μm sieve placed in a flat-bottomed white basin. Sufficient amount (300 - 400 ml and not exceeding 1 L) of distilled water to flood the sieve surface was added.

b) The sediment was wet-sieved by agitating and puddling in the basin of water until most of the fine fraction had passed. The sieve was then lifted and allowed to drain over the basin.

c) The contents of the sieve (sand fraction) were then transferred to an ovenproof dish and dried at 100°C. The suspension left in the basin constituted the silt-clay fraction.

3. Dry sieving of the sand fraction

a) A stacked series of graded sieves were used for dry sieving, which comprised of 2 mm, 1 mm, 500 µm, 250 µm, 125 µm and 63 µm mesh size. The oven-dried sediment was carefully transferred to the uppermost (2 mm) of the stacked series of graded sand sieves. A pan below the finest sieve (63 µm) was used to catch any remnant of the fine material.

b) The stacked column of sieves was then placed on an automatic sieve shaker for a period of 10 to 15 minutes.

c) When the finality of the sieving had been checked, the material on each of the sieve was emptied onto separate sheets of glazed paper, including any grains lodged in the sieve, which were removed with a sieve brush. Each fraction was then weighed.

d) Any material passing through the last sieve (63 µm) into the pan was transferred to the suspension of silt and clay in the basin.

4. Grading of the silt-clay fraction

a) The fine material in the basin was washed into a 1 L cylinder using a large filter funnel and a wash bottle of distilled water. The volume was made up to exactly 1 litre with distilled water.

b) The cylinder was then placed in a thermostatic water bath until the temperature had equilibrated at 20°C.

c) The cylinder was removed from the water bath, shaken and turned to suspend the sediment evenly throughout the water column.

d) Upon placing the cylinder upright, 20 ml pipette samples was immediately withdrawn from a depth of 200 mm. This pipette sample was then transferred to a tarred crystallizing dish and dried in the oven at 100°C. The weight of this material represented the total amount of sediment < 63 µm in the suspension.

e) The second pipette sample was withdrawn from a depth of 100 mm below the surface of the suspension at 7 minutes 44 seconds after the cylinder was placed upright. The third pipette sample was taken at 2 hours 3 minutes. Each of these samples was then oven dried at 100°C.

f) The weight of the second pipette sample represented the amount of sediment < 15.6 µm in the suspension. The difference in weight between the first and second pipette samples would therefore give the weight of silt fraction in the 63 - 15.6 µm grade. Similarly, the difference between the second and third pipette samples gave the silt fraction in the 15.6 - 3.9 µm grade. The weight of material from the third pipette sample also represented sediment < 3.9 µm, the clay content.

A rapid partial analysis by wet sieving which involved stages 1 and 2 of the procedure was carried out to determine an appropriate amount of sediment required. A starting amount of 25 g and 50 g of oven-dried sediment were used. The sediment was initially split into a sand fraction (particles > 62 µm) and a silt-clay fraction (particles < 62 µm) by wet sieving. A 63 µm sieve was used instead because that was the standard sieve size available commercially. From this initial analysis, 25 g of sediment was found to be insufficient for both sand fraction and silt-clay fraction analysis. On the other hand, with a starting amount of 50 g of sediment, there was sufficient sand fraction but only 1 - 2 g of silt-clay fraction was obtained. Buchanan 1984 recommended a suspension of silt and clay weighing approximately 15 g for a full silt-clay fraction analysis. The starting amount of sediment required for this study would then be in the order of 1 kg. Consequently, with such low silt-clay content of the sediment, only analysis of the sand fraction was undertaken on subsequent samples. Only two replicate core samples were used after initial analysis showed that there was little variation in the sediment size composition.

Data analysis

The weights of sediment particles in each grade were expressed as a percentage of the dry weight of the total sample. The median diameter (in ∅ or phi notation) was calculated for each replicate sample. ANOVA and post-hoc Tukey-Kramer test was carried out to test for significant differences in the median grain size between sites and surveys.

Results

There was a significant difference ($p < 0.05$) between the median particle size (MPS) of the outfall site (2.61) and the control site (2.88). However, there were also differences between survey times. The median particle sizes of the January survey were consistently lower than those of the April, July and October survey at the control site (Figure II.1). A different researcher to those from the later surveys carried out the analyses for this set of samples from the January survey of the control site, which could account for the difference in the data obtained.

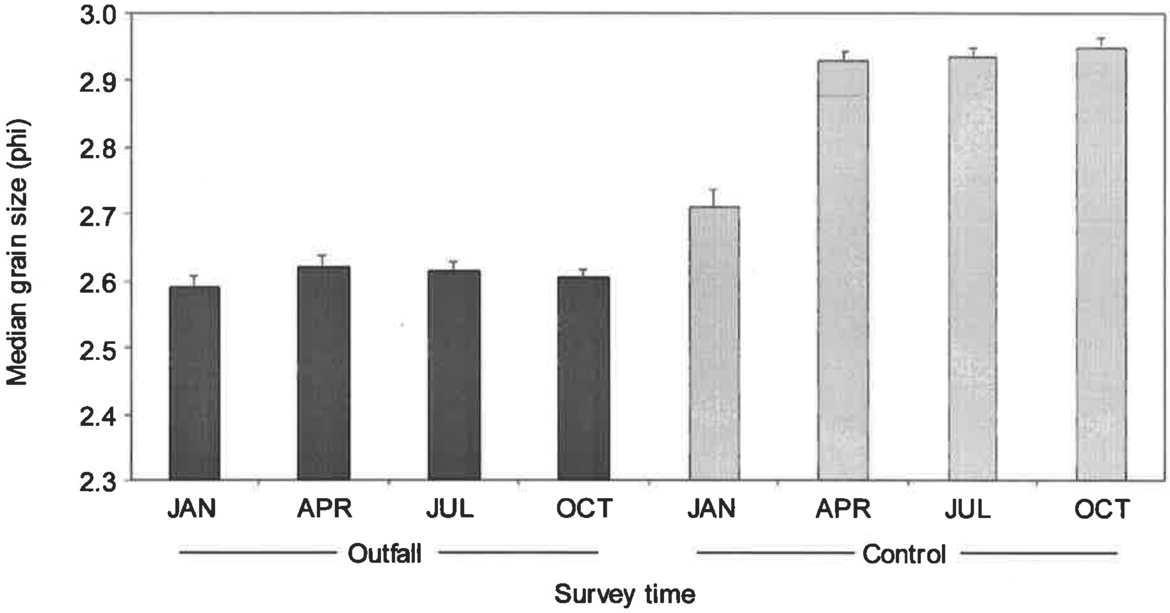

Figure II.1 **Mean median particle size (± SE) according to the Wentworth classification scale for the outfall and control sites over the four surveys in 1997.**

The median particle size at the nine stations from both the outfall and control sites showed little variations (SE = 0.01 for both). Stations to the north of the outfall site had median particle size ranging from 2.31 to 2.91 phi while stations to the south ranged from 2.50 to 2.77 phi (Figure II.2). At the control site, the median particle sizes were slightly higher with a range of 2.44 to 3.13 phi (Figure II.3).

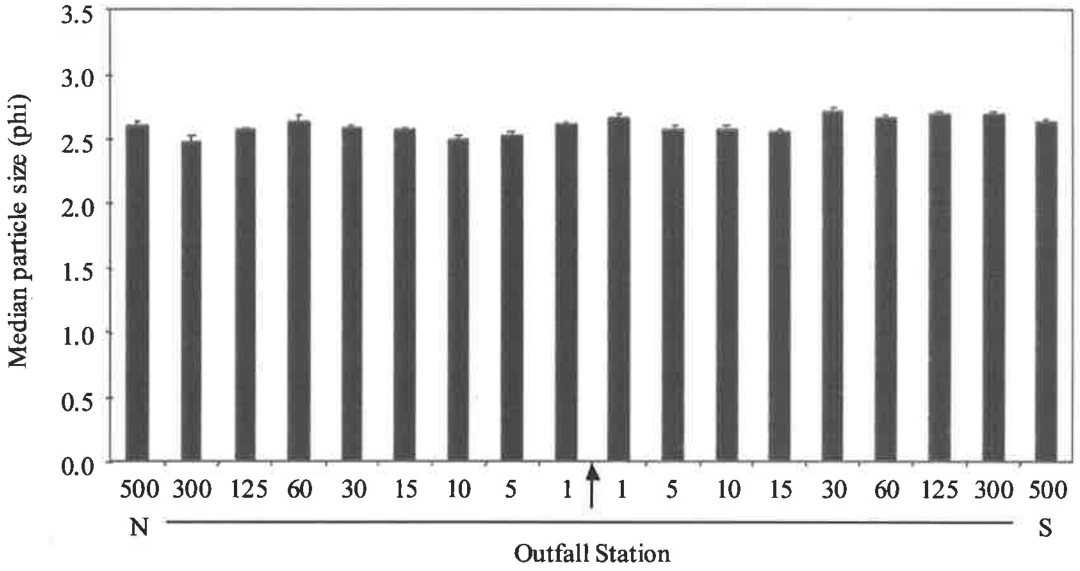

Figure II.2 Mean median particle size (± SE) according to the Wentworth classification scale for stations at the outfall site. Stations are arranged to the north and south of the discharge point (arrow indicates discharge point).

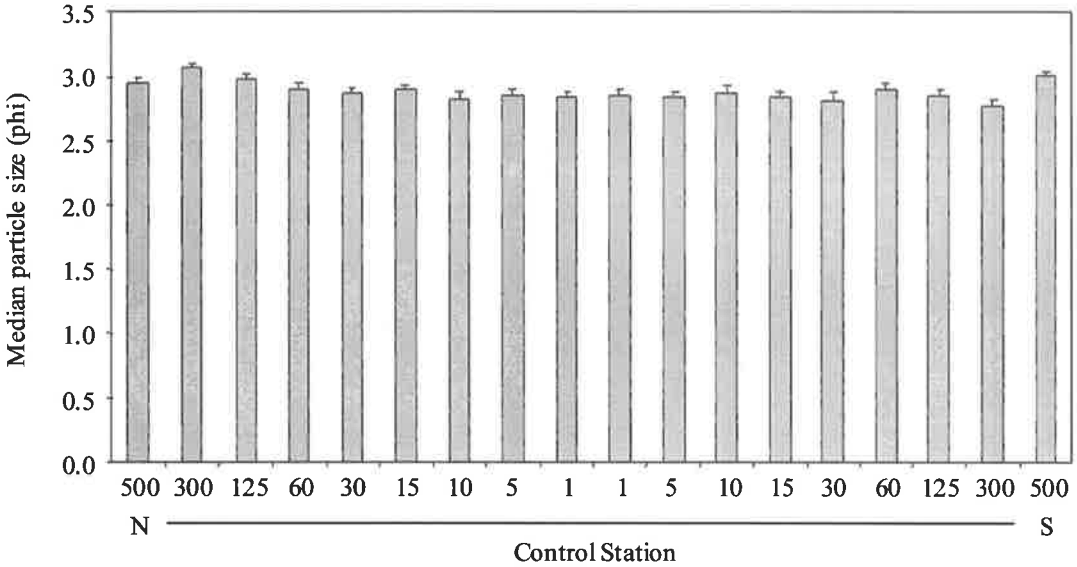

Figure II.3 Mean median particle size (± SE) according to the Wentworth classification scale for stations at the control site. Stations are arranged to the north and south of a starting point, mirroring the station layout at the outfall site.

Discussion

The detailed relationships of animal associations to environmental parameters, such as sediment type, require experimental verification. However, this is not the aim of this work here. The purpose of sediment size analysis in this study was to determine if sediment distribution would be a confounding influence on the benthic community structure found at both the outfall and control site.

Although there was a significant difference in the median grain size between the outfall site and control site, on the Wentworth classification scale, particle size between +2 and +3 phi represented the fine sand class, in the range of 0.125 mm to 0.250 mm. This meant that both the outfall site (mean MPS = 2.61 phi) and the control site (mean MPS = 2.88 phi) had median particle size in the same class of fine sand, although those from the control site Moana were slightly finer. This indicated that whereas there was a significant difference in sediment distribution, this difference was unlikely to have profound biological differences.

Appendix III

Transformation

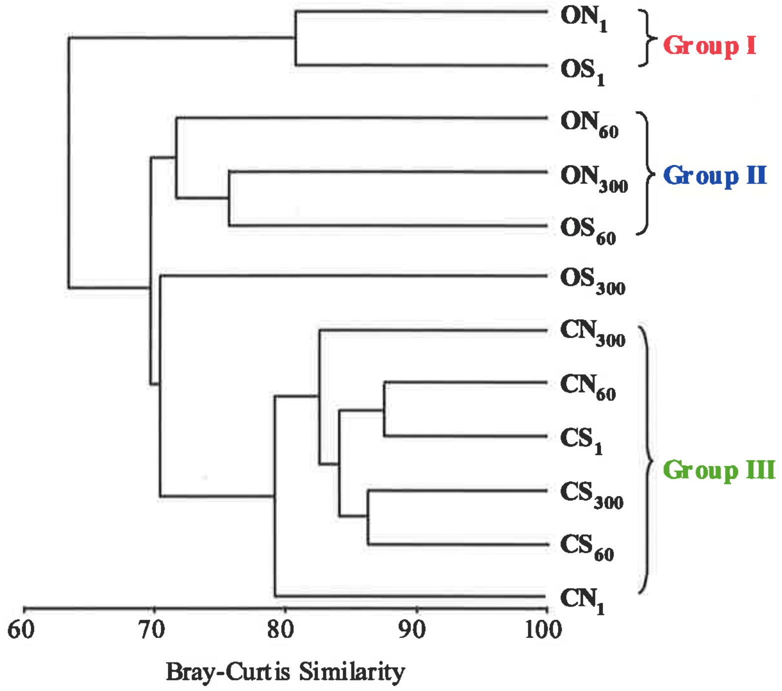

Figure III.1 Dendrogram for group average clustering of Bray-Curtis similarities (x-axis) of square root transformed data from both outfall and control sites. Separation into three groups (Groups I, II and III) was evident, except for station OS_{300}, which was grouped with the control stations.

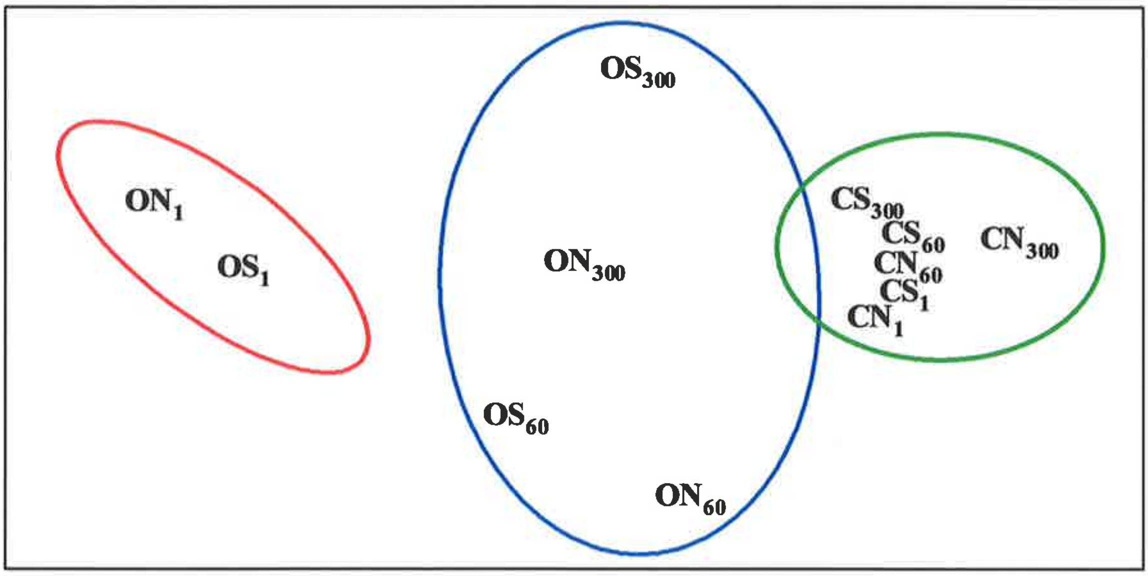

Figure III.2 MDS ordination plot (2-dimension, stress = 0.08) of square root transformed data showing separation into three groups (Groups I, II and III).

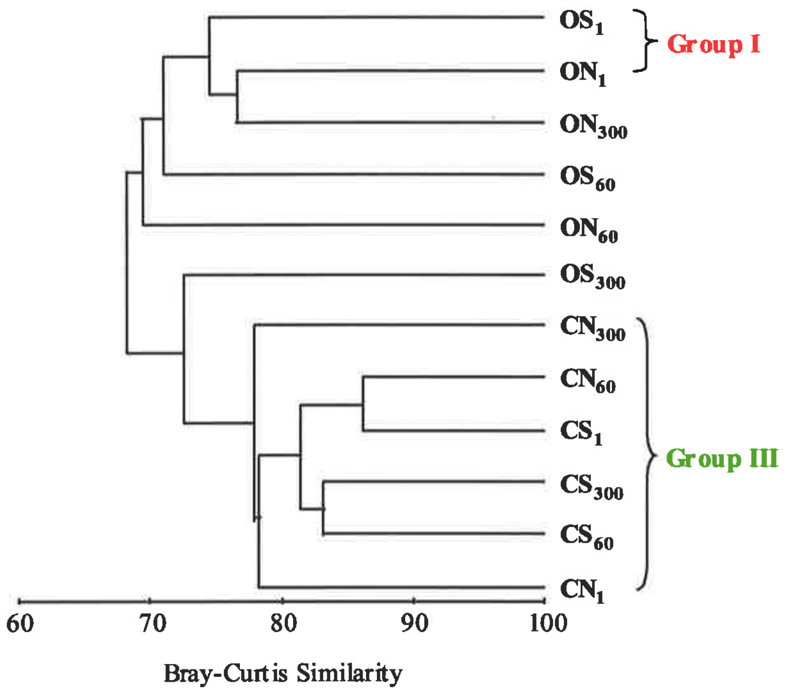

Figure III.3 Dendrogram for group average clustering of Bray-Curtis similarities (x-axis) of 4th root transformed data from both outfall and control sites. Separation into Group I (ON_1 and OS_1) and Group III (control stations) was still evident. There was no distinct clustering of Group II stations. Station OS_{300} grouped with the control stations (Group III) and Station ON_{300} grouped with Group I stations.

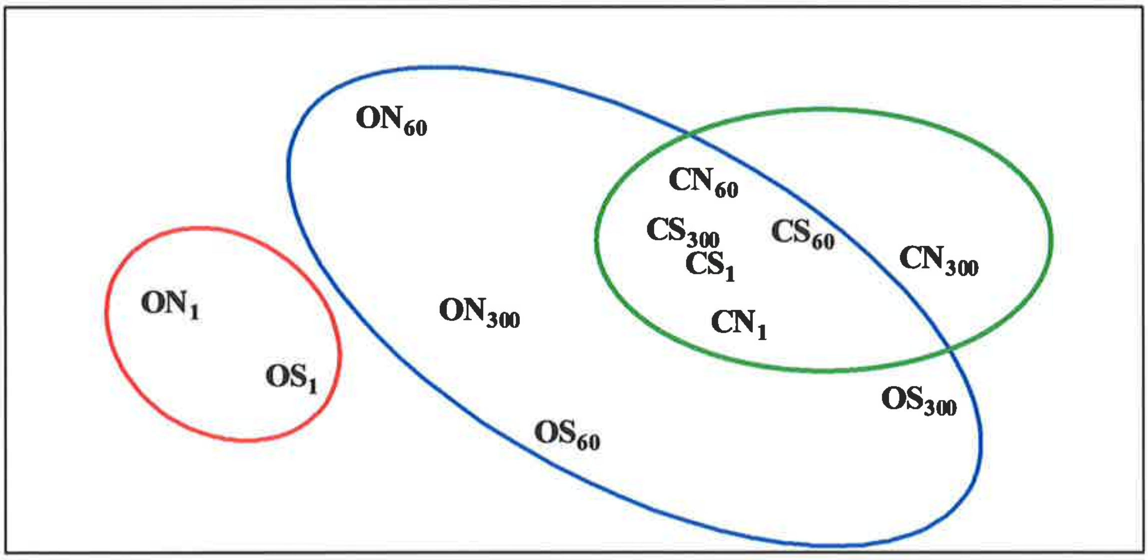

Figure III.4 MDS ordination plot (2-dimension, stress = 0.12) of 4th root transformed data showing general separation of stations into three groups (Groups I, II and III) although clustering of Group II stations were not as distinct.

Taxa removal

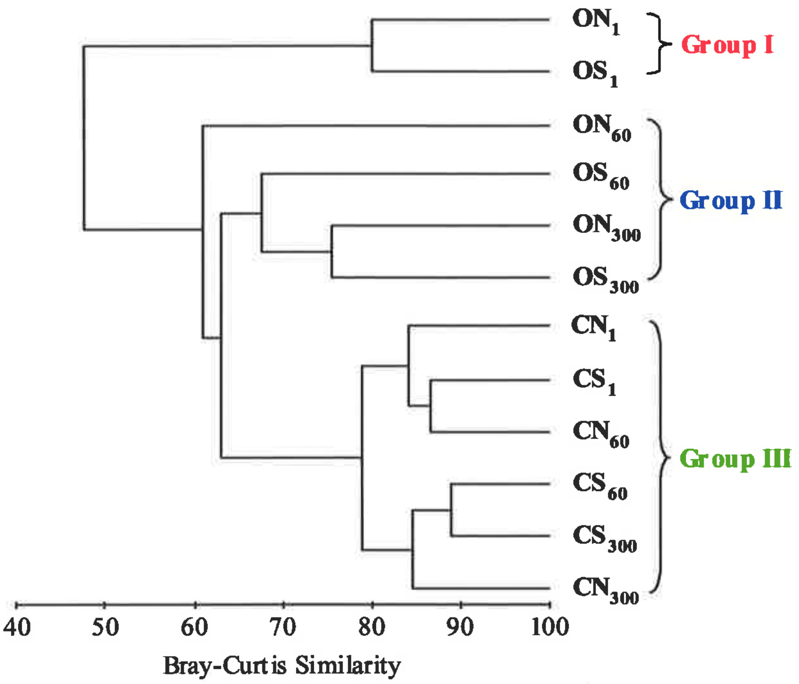

Figure III.5 Dendrogram for group average clustering of Bray-Curtis similarities (x-axis) of abundance data with reduced number of taxa from both the outfall and control sites. Separation into three groups (Groups I, II and III) was evident.

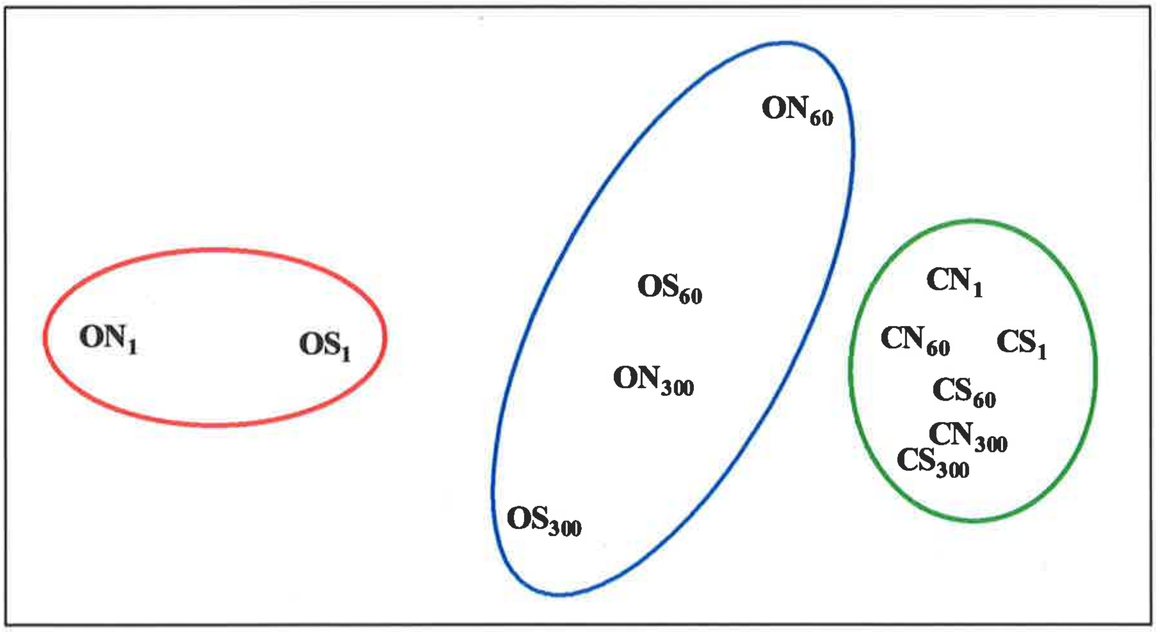

Figure III.6 MDS ordination plot (2-dimension, stress = 0.06) of abundance data with reduced number of taxa showing general separation of stations into three groups (Groups I, II and III).

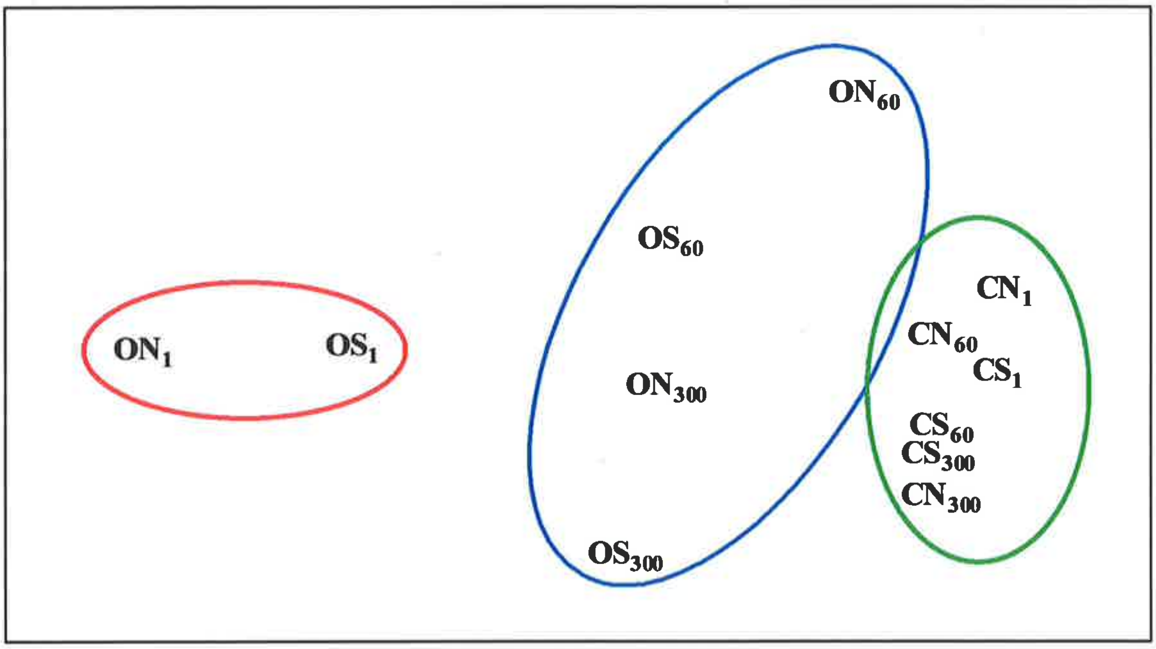

Figure III.7 MDS ordination plot (2-dimension, stress = 0.05) of abundance data with taxa aggregated to the class level, showing general separation of stations into three groups (Groups I, II and III).

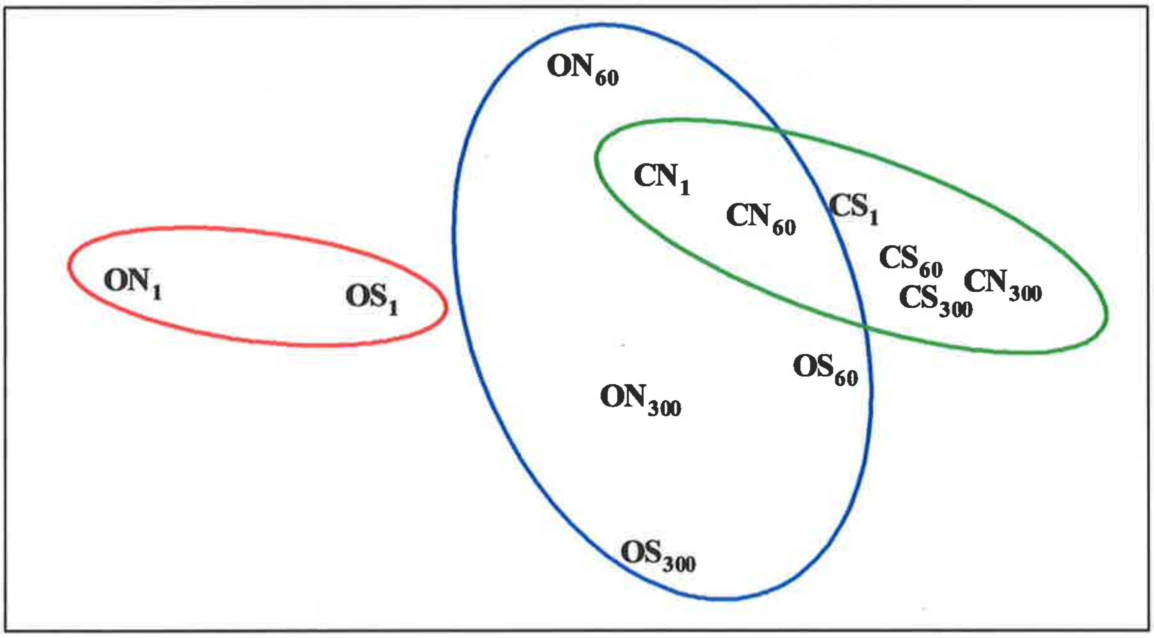

Figure III.8 MDS ordination plot (2-dimension, stress = 0.06) of abundance data with taxa aggregated to the phylum level, showing general separation of stations into three groups although grouping of mid distance stations (Group II) was not as distinct.

Appendix IV Detailed ANOVA for community descriptors

Source	DF	MS	F	p	MS	F	p	MS	F	p
		1. Number of taxa (S)			**2. Abundance (A)**			**3. Biomass (B)**		
Survey	3	23.43	5.60	0.000	692.49	10.68	0.000	0.0844	1.98	0.116
Site	1	6.66	1.59	0.208	4514.77	69.62	0.000	0.0005	0.01	0.911
Direction	1	1.70	0.41	0.524	1473.38	22.72	0.000	0.0216	0.51	0.477
Station	2	44.26	10.59	0.000	2317.08	35.73	0.000	0.0432	1.02	0.363
Survey*Site	3	17.67	4.23	0.006	564.22	8.70	0.000	0.0199	0.47	0.705
Survey*Direction	3	2.63	0.63	0.596	197.77	3.05	0.028	0.0434	1.02	0.383
Survey*Station	6	4.91	1.17	0.319	1260.03	19.43	0.000	0.0854	2.01	$^{++}$0.063
Site*Direction	1	0.15	0.04	0.849	952.80	14.69	0.000	0.0853	2.01	0.157
Site*Station	2	43.75	10.47	0.000	764.07	11.78	0.000	0.1183	2.78	0.063
Direction*Station	2	3.04	0.73	0.484	8.32	0.13	0.880	0.0573	1.35	0.261
Survey*Site*Direction	3	16.47	3.94	0.009	662.55	10.22	0.000	0.0199	0.47	0.704
Survey*Direction*Station	6	3.74	0.90	0.498	207.48	3.20	0.004	0.0725	1.71	0.118
Survey*Site*Station	6	21.65	5.18	$^{+}$0.000	1824.36	28.13	0.000	0.0696	1.64	0.135
Site*Direction*Station	2	1.87	0.45	0.640	50.33	0.78	0.461	0.0110	0.26	0.772
Survey*Site*Direction*Station	6	5.76	1.38	0.222	285.97	4.41	$^{+}$0.000	0.0408	0.96	0.453
Error	432	4.18			64.85			0.0425		
		4. Diversity (H')			**5. Evenness (J)**					
Survey	3	0.49	4.89	0.002	0.05	7.43	0.000			
Site	1	0.38	3.81	0.052	0.14	23.56	0.000			
Direction	1	0.003	0.04	0.845	0.01	2.41	0.121			
Station	2	0.56	5.59	0.004	0.22	35.44	0.000			
Survey*Site	3	0.87	8.67	0.000	0.06	9.70	0.000			
Survey*Direction	3	0.03	0.31	0.820	0.03	4.37	0.005			
Survey*Station	6	0.18	1.81	0.095	0.07	12.00	0.000			
Site*Direction	1	0.07	0.65	0.422	0.05	8.73	0.003			
Site*Station	2	1.31	13.02	0.000	0.07	10.67	0.000			
Direction*Station	2	0.07	0.69	0.502	0.003	0.40	0.670			
Survey*Site*Direction	3	0.21	2.06	0.104	0.03	5.44	0.001			
Survey*Site*Station	6	0.31	3.06	0.006	0.09	14.34	0.000			
Survey*Direction*Station	6	0.08	0.78	0.587	0.01	2.10	0.052			
Site*Direction*Station	2	0.18	1.79	0.169	0.01	1.81	0.165			
Survey*Site*Direction*Station	6	0.44	4.34	$^{+}$0.000	0.05	8.06	$^{+}$0.000			
Error	432	0.10			0.006					

Note: Variances were homogeneous for all ($p < 0.05$) except (a) where variances were heterogeneous and untransformable. $^{+}$ significant at $p<0.05$ $^{++}$ at $p<0.1$

Appendix V Feeding and reproductive biology of major taxa and their responses to organic enrichment

Major Taxa	Feeding and reproductive biology	Higher abundance at outfall site	Higher abundance at control site
Spionidae	Deposit and suspension feeders with some species alternating between the two modes Planktonic larvae	Opportunistic species, with varying responses depending on species	
Capitellidae	Non-selective deposit feeders Planktotrophic and demersal larvae with wide dispersal, reproduction the whole year round and short life cycle	Opportunistic species	
Gastropoda	Deposit feeders	Increased organic enrichment leading to increased food availability	
Nemertea	Carnivores, scavenging macrophages	Increased organic enrichment leading to increased food availability	
Ostracoda	Carnivores, herbivores, scavengers, filter feeders	Abundant under moderate influence of wastewater discharges	
Bivalvia	Suspension feeders		Increased organic matter may cause clogging of cilia and siphons.
Echinodermata	Mostly bottom detritus feeders, browser or scavengers, but some do filter feed		Sensitive to organic pollution
Amphipoda (Gammaridea)	Mostly detritus feeders or scavengers but some do filter feed Motile and brood their young rather than disperse their larvae		Able to withstand small-scale disturbance but are less tolerant of organic pollution