CHAPTER 1

GENERAL INTRODUCTION

1.1 Estuary Structure and Function: a Southeast Australian Perspective

Estuaries are unique and highly variable environments that represent the major interface between land-based catchment processes and the marine environment (Day 1981). The interaction of three main factors results in the presence of a range of estuary types (Roy 1984). These: 1) geomorphological factors that determine the size and shape of an estuary, as well as the nature of its sediments; 2) hydrological processes, including tidal currents, waves and river discharge; and 3) human influences on estuary structure and processes. As estuaries are dynamic environments, each with uniquely varied and individual features, making generalisations and categorisations are difficult. Australian estuaries have, however, been classified by a number of characteristics including their geographical location, their geomorphology and the physical, chemical and biological processes that occur within them (Roy 1984; Bucher & Saenger 1994; Edgar et al. 1999; Mondon et al. 2003). Throughout these classifications, the estuarine basin and entrance channel features have remained key factors in differentiating between estuary types. This is because such features may modify currents, restrict mixing and lead to the development of stratification and extreme chemical environments such as anoxia (D'Adamo et al. 1992).

The most recent classification of southeast Australian estuaries (Roy et al. 2001) recognises three broad estuary types: tide-dominated; wave-dominated; and intermittently closed estuaries. Each of these types encompass a range of estuary forms from previous classifications. In brief, the tide-dominated estuaries primarily include drowned river valleys and tidal basins, the wave-dominated type includes barrier estuaries and barrier lagoons, and the intermittently closed estuaries incorporate saline coastal lagoons and small coastal creeks.

Australia is unique in that it is the driest inhabited continent. Australian estuaries experience floods only sporadically and, during droughts, flows in them may become very reduced or cease altogether. In addition, freshwater flows in many systems have been reduced through irrigation and water storage (Hutchings 1999). As a result, the majority of estuaries in southeast Australia have entrances that intermittently close, limiting marine influences. Despite the fact that intermittently closed estuaries are so common, very little is known about their ecology (Arthington & Hadwen 2004) as most research has focused on the permanently open tide- and wave-dominated estuary types. The work presented here focuses on the ecology of intermittently closed estuaries in the Solitary Islands Marine Park (SIMP), and compares this to the ecology of the next most common estuary type, permanently open barrier estuaries.

1.1.1 Intermittently Closed Estuaries

Intermittently closed estuaries are coastal bodies of water that become isolated from the sea, and thus non-tidal, for various periods of time (Roy et al. 2001). They are generally small systems that can experience entrance closure as freshwater input can be irregular and is often not strong enough to keep the entrance open. Under natural conditions, the ocean entrance opens only when a large build-up of catchment runoff breaches the beach berm and gouges a channel to the sea (Adam et al. 1992; Edgar 2001). The entrance may also open when extreme tides breach the sand barrier, or after artificial opening (Edgar et al. 1999). The channel may then remain open for days, months or years before being closed by sand transported by currents and wave action along the shore during the drier months (Edgar 2001). The unpredictability of rainfall in many parts of Australia, especially southeastern Australia, means that the opening behaviour of these estuaries is typically erratic (Roy et al. 2001). Intermittently closed estuaries are widespread around the Australian continent, with particularly high concentrations along the New South Wales (NSW), Victorian and northeastern Tasmanian coasts. In NSW alone, it is estimated that intermittently closed estuaries comprise up to 92 % of all estuaries (Williams et al. 1998).

There are numerous other terms that are commonly used to refer to estuaries of this type. For example, in South Africa they are often called seasonally or temporarily open/closed estuaries, whereas, in Australia, they have been called coastal lakes, coastal lagoons and seasonally closed estuaries. More recently, the most commonly used term in Australia is the acronym ICOLL (intermittently closed and opening lake or lagoon). However, it was decided not to adopt this terminology here when referring to all intermittently closed estuaries, as "lake or lagoon" infers a specific morphology and excludes waterways that are relatively narrow for their entire length. Such features are of particular importance in contributing to, amongst others, the processes of mixing and stratification (Haines 2004). Hence, the term intermittently closed estuary, or simply intermittent estuary, has been used here to refer to all estuaries within this category.

1.1.2 Permanently Open Barrier Estuaries

Barrier estuaries have a narrow inlet to the sea due to presence of a sand barrier that has developed across part of the entrance. The long, narrow entrance channel or sand bar at the mouth of a barrier estuary can restrict tidal currents, which means that wind-driven circulation is primarily responsible for mixing the estuarine waters (Deeley & Paling 1999). The wind-driven circulation, together with the shallow nature of these estuaries, means that they are generally well mixed, although a salt wedge will still be present at the entrance. Barrier estuaries are typically shallow, have variable water clarity and limited outcropping rock along the shore, and show a rapid decrease in salinity from their entrance. The water level does not fluctuate greatly and can be influenced as much by atmospheric pressure changes and river flow as it is by tides (Edgar 2001). These estuaries usually also support extensive areas of seagrass, saltmarsh, mangroves and swampy *Melaleuca* sp. forests (Turner et al. 2004).

Barrier estuaries are the second most abundant estuary type in NSW. Young barrier estuaries usually exist as tidal lakes, whereas more mature forms consist of extensive river systems with relatively high sediment loads (Adam et al. 1992). As such, barrier estuaries are very variable in shape and size. Their catchment sizes range from that of the largest coastal river in NSW, the Clarence River delta at 22400 km^2, to less than 30 km^2: the latter is similar to the catchment sizes of the barrier estuaries examined in this study.

1.1.3 The Importance of Estuaries

Estuaries are one of the most biologically productive ecosystems (Edgar 2001; Kennish 2002) as they provide a sink for nutrients from adjacent freshwater and land habitats, and receive considerable input of plant material from mangroves and seagrasses (Edgar 2001). They also support an array of economically, socially and environmentally important values. Estuaries are of considerable importance to commercial and recreational fisheries, including crustacean and mollusc fisheries. The wholesale value of commercial seafood production in NSW for the 2002/03 financial year was estimated to be around $135 million (ABARE & FRDC 2004), of which two thirds was derived from species that are dependent on estuaries for all, or part of, their life cycle. The NSW oyster industry, alone, was valued at $32 million and is entirely estuarine dependent (DPI 2004a). Similarly, recreational fishing is a substantial industry, with the expenditure of recreational fishers in NSW valued at $550 million dollars in 2001 (DPI 2004b). Whilst it is difficult to gauge how much of this activity relies on estuaries, earlier surveys revealed that the main target species (Yellowfin Bream, Dusky Flathead, Snapper, Tailor and Tarwhine) are dependent on estuarine habitats at some stage of their life cycle (SPCC 1981). Another important economic focus of estuaries is the extraction industry with sand and gravel worth over $100 million per annum being removed from NSW estuaries (Adam et al. 1992).

Throughout coastal regions, estuaries are focal areas for urban development, tourism and many recreational activities, with an estimated 75% of the population of NSW living in the immediate proximity of estuaries (Adam et al. 1992; Saenger 1996). Estuaries and their surrounds are also areas of cultural significance to indigenous Australians, due to the presence of archaeological sites, traditional hunting and fishing grounds, sacred grounds, and sites that are the locations of important cultural or historical events (Brady & Riding 1996; Smyth 1996).

Estuaries are extremely important in maintaining biodiversity (Brady & Riding 1996) as they provide a variety of habitats that support a diverse range of fauna and flora (Adam et al. 1992), including some threatened and endangered species. In particular, an estimated 70% of the coastal fish of NSW are dependent on estuaries (Brady & Riding 1996) as areas for feeding and breeding, or for their importance as nursery areas for juveniles (Adam et al. 1992; Potter & Hyndes 1994). Similarly, estuaries are important feeding, roosting, breeding and recuperation areas for birds, especially migratory waders (Adam et al. 1992).

In view of the widespread occurrence of intermittently closed estuaries in NSW, it is presumed that they are equally, if not more, important than permanently open estuaries in maintaining these economic, social and environmental values. For example, a number of small intermittently closed estuaries in southern NSW provide essential habitat for a diverse assemblage of juvenile fish, many of which are marine species (Pollard 1994; Griffiths 2001). These estuaries were demonstrated to be equally as important fish nursery areas as nearby permanently open estuaries. In addition, some of these estuaries supported greater abundances of individual species and, because these species happened to be commercially important, therefore supported a higher fisheries value than permanently open estuaries in the area (Pollard 1994).

1.1.4 The Ecology of Intermittently Closed Estuaries

1.1.4.1 The Physical and Chemical Environments

The intermittently closed estuaries of southern Australia are similar to many of those on the southern coast of Africa, but differ from those typically found in the temperate regions of the northern hemisphere, where the mouths tend to be wider and deeper, thereby resulting in a greater tidal exchange between the estuary and ocean (Potter & Hyndes 1994). In southeast Australia there are four factors, identified by Moverley and Hirst (1999), which interact to make the entrances of intermittent estuaries susceptible to closing:

1) sand deposition at the entrance may be high as most southeast Australian estuaries discharge into high-energy surf zones, where large volumes of sand are moved along the coast by wind and offshore currents;
2) entrances are shallow and require little sand to fill them in;
3) tidal currents are weak due to a relatively small tidal range and narrow entrance channels, thus, tidal currents alone may not be sufficient to keep the estuary mouth open; and
4) steep catchments and short river lengths mean that rainfall run-off rapidly passes through
   the waterway and, at times of low discharge, scouring of the estuary's entrance channel is reduced allowing sand to build up.

Intermittently closed estuaries can undergo dramatic changes in physical and chemical conditions over short periods owing to their dynamic connection with the sea (Pollard 1994b), though, at times, there may not be a clear demarcation between open and closed entrance states. Physicochemical changes to the estuarine environment start when the mouth is still open but the beach berm partially blocks the entrance channel so that tidal exchange is restricted. Alternatively, when an entrance is closed, marine influence can still occur as a result of rough seas or spring tides overwashing the berm (Moverley & Hirst 1999).

Entrance closure affects mixing processes in estuarine waters as, in closed estuaries, water movement can only be produced by wind and storm action. Further, the shape of such estuaries greatly influences their potential for wind mixing (Haines 2004). For example, Moverley and Hirst (1999) found that narrow, closed estuaries usually possessed a halocline, indicating they were poorly mixed. In contrast, closed estuaries that were wider and more open were well mixed and did not have a halocline.

Entrance closure reduces the capacity of intermittent estuaries to eliminate pollutants and other chemicals (Whitfield 1992). Poor exchange with the ocean particularly exacerbates the effects of nutrient enrichment and salinity changes (McComb 1995). Nutrient enrichment can have a number of effects in estuarine environments (Martins et al. 2001). Firstly, it encourages the rapid growth of aquatic plants and algae (Adam et al. 1992; McComb 1995), which can lead to hypoxic, or anoxic, conditions from the increased bacterial activity associated with processing decaying plant material (Edgar et al. 1999). The generation of large masses of macroalgae resulting from nutrient enrichment can also impede boat progress and decompose in odorous masses on the shoreline (McComb 1995).

Salinity is also affected by the complex hydrodynamic behaviour of intermittently closed estuaries. Although salinity can be relatively stable when entrances are open, during times of closure salinity varies depending on water seepage, freshwater inflow and rates of evaporation (Mackay & Cyrus 2001). This means that salinity is highly variable between estuaries, as well as within individual estuaries and over various time frames (Roy et al. 2001). During times of closure, salinities are often uniform throughout the length of intermittent estuaries, which means that this estuary type often lacks the pronounced salinity gradients that are often thought to typify estuarine ecosystems. Thus, whilst open estuaries only experience low salinities in their upper reaches, closed estuaries can have similarly low salinities further downstream, or even near the entrance. At the other extreme, whilst open estuaries rarely exceed salinities of 35 ppt, closed estuaries can experience hypersaline conditions, particularly during dry summers when evapouration rates are high (Deeley & Paling 1999).

High salinities, particularly when in conjunction with increasing temperatures, have further effects on the physico-chemical characteristics of estuarine waters by reducing the capacity of water to dissolve oxygen. This is particularly relevant to the ecology of intermittent estuaries as it means that, in a hot, dry summer when an estuary is closed, the physico-chemical environment of the water column may be different to that of an open estuary due to a combination of factors (i.e. increased salinity and temperature, reduced dissolved oxygen).

When conditions are not eutrophic, closed estuaries can lack the steady supply of macronutrients generally observed in permanently open estuaries. In permanently open systems, this is provided by the regular input of nutrient-rich freshwater, tidal inputs from the sea and the resuspension of benthic regenerated nutrients. In intermittently closed estuaries, these demands need to be provided by the utilisation of alternative sources such as detritus, phytoplankton, protozoans and microphytobenthos (Perissinotto et al. 2000). Microphytobenthos is particularly important as a source of primary production in intermittent estuaries as these are mostly shallow estuaries with a large proportion of the substrate in the photic zone. This facilitates the growth and accumulation of benthic algae across a relatively large area of the estuary (McComb 1995).

Intermittent estuaries are also generally shallow, dynamic systems, which have a small volume of water per square metre of sediment surface and very shallow sub-tidal areas. These, along with the generally well-mixed nature of shallow systems, are physical conditions that intensify the exchange of matter and energy between the water column and benthic sediments (Deeley & Paling 1999). For example, unlike deeper estuarine benthic habitats, the benthic fauna of shallow estuaries can directly graze on live phytoplankton (Herman et al. 1999).

1.1.4.2 Implications for Biota

Although most biological impacts of entrance closure are indirect, through the physico-chemical environment, there are some direct responses. For example, many estuarine species have marine planktonic life stages, which may occur at various times of the year. If an estuary happens to be closed during these times, then such species will be unable to recruit to that particular estuary. As a result, two estuaries with identical physico-chemical conditions can have different communities due to differences in past entrance histories (Moverley & Hirst 1999). Similarly, the effects of entrance closure on recruitment have been used to explain lower species diversities in the fish communities of intermittently closed estuaries when compared to other estuarine systems (Pollard 1994; Young et al. 1997; Cowley & Whitfield 2001). Marine overwash events can provide an alternative means for the recruitment of juvenile fish into closed estuaries (Bell et al. 2001; Cowley et al. 2001; Young & Potter 2002), resulting in unpredictable and highly variable communities (Cowley & Whitfield 2001).

Faunal communities in intermittently closed estuaries are more vulnerable to environmental degradation when compared to those in permanently open estuaries (Perissinotto et al. 2000). However, as the majority of changes in an estuary's physico-chemical environment resulting from closure are gradual, biotic community changes will also be gradual and will probably occur some time after the entrance closes (Moverley & Hirst 1999). In contrast, the changes following major opening events can occur very quickly. For example, water levels drop rapidly, exposing large areas of substrate and benthic habitat that had previously been submerged (Perissinotto et al. 2000).

During entrance closure, salinities limit the extent to which stenohaline, marine species and oligohaline, freshwater species can survive. Therefore, in addition to the timing of entrance closure, communities will also be influenced by the duration of entrance closure (Young et al. 1997). As such, it is likely that true estuarine organisms will dominate the communities of intermittently closed estuaries as they are independent of the marine environment for reproduction and dispersal, and are more tolerant to changes in the physico-chemical environment (Mackay & Cyrus 2001; Teske & Wooldridge 2003). Estuarine fauna are also affected by nutrient enrichment. The latter stages of eutrophication are often marked by large fish kills due to the oxygen depletion and possibly toxic algae (Edgar et al. 1999). Such events have occurred more frequently in recent years and, as such, eutrophication is recognised as a worldwide problem, which is highly prevalent in intermittently closed estuaries (McComb 1995).

Although the composition and abundance of fish associated with intermittently closed estuaries has been the focus of considerable research, particularly in the temperate regions of South Africa and Australia (Pollard 1994; Young et al. 1997; Cowley et al. 2001; Cowley & Whitfield 2001), there is little information detailing the effects that entrance closures have on other estuarine biota.

1.1.4.3 Artificial Opening of Closed Estuarine Entrances

In the 1970s, commercial fishers on the southern coast of NSW would regularly open many intermittently closed estuaries to facilitate the recruitment of commercially important fish and prawn species into the estuaries, and to maintain water quality at levels favoured by these species (Briggs et al. 1980). Currently, artificial opening is commonly conducted to facilitate flushing to improve water quality, to manage flood risks by lowering water levels and to improve recreational amenity (Gladstone et al. 2002).

The need to conduct such management practices has arisen due to inappropriate past patterns of urban and rural development. For example, many intermittent estuaries have homes situated in their foreshore areas so that, on occasion, residents are exposed to pungent odours (HRCNSW 2002). These odours result from large masses of decaying macroalgae (Dalton et al. 2004) and the consequent production of hydrogen sulfide gas (Murray et al. 2004). At times of closure, communities in areas adjacent to these estuaries are also often concerned about issues associated with reduced water quality, including potential risks to human health. Further, development in some areas has located buildings and infrastructure, as well as agricultural grazing properties, in places that are prone to water inundation when water levels are high (HRCNSW 2002). In such cases, artificial opening of estuary entrances is required to reduce water levels and preventing flooding.

The artificial opening of intermittent entrances can have profound effects on these estuarine ecosystems. This occurs through changes in water levels, salinity regimes and patterns of water inundation in wetlands, as well as by increasing sediment suspension. As such, it alters the natural cycles of flooding, drainage and filling, upon which the ecological processes of these systems depend (Gladstone et al. 2002). Little research, however, has been undertaken to develop an understanding of the ecological consequences of such interventions (HRCNSW 2002). Although the ecological impacts of artificial openings, especially repeated openings, are poorly understood, the practice is regarded as a threatening process by the NSW State government. This is because artificial opening may result in a loss of biodiversity in these estuaries and their associated wetlands (NSWEPA 2000). For example, it is likely that there are species of fauna and flora that rely on the inundation and salinity patterns, as well as the opening and closing regimes, of intermittent estuaries to survive. In terms of benthic fauna, Moverley and Hirst (1999) have suggested that regular, artificial openings appear to have a considerable impact on intermittently closed estuaries. They found that an estuary that was regularly opened artificially had a very different community structure, including a higher species richness, when compared to other intermittent estuaries nearby. This could occur via a number of avenues, such as increased recruitment opportunities and greater chances of survival for marine-spawning species (Gladstone et al. 2002). Over time, repeated openings may, therefore, alter the physico-chemical environment and this, together with an increased persistence of marine species, could affect biodiversity by negatively impacting the natural fauna of such estuaries.

1.2 Estuarine Benthic Ecology: Soft Sediment Benthos

1.2.1 Estuarine Sediments

Subtidal and intertidal estuarine sediments such as shallow mud flats, sand flats, and deeper sedimentary areas represent the majority of substrates in estuaries. They are also one of the major habitats available for plants and animals in estuaries and may be either unvegetated, or vegetated with algae, seagrass, mangroves or saltmarsh. Soft sediments support abundant infaunal communities and are, therefore, used as feeding areas by many fish, birds and motile invertebrates. In shallow areas microscopic algae, known as the microphytobenthos, coat the sediment surface and play important roles in both primary production and providing a food source for higher organisms (Burchmore et al. 1993).

Estuarine sediments consist of mixtures of sand, silt and clay-sized particles, as well as organic detritus. Sediments enter estuaries from a variety of sources, principally the sea and rivers, but are also washed or blown into an estuary from the surrounding land (Morrisey 2000). Most estuaries are characterised by a central region of sedimentation where water currents slacken and drop their sediment loads. This deposition of sediments is primarily controlled by the speed of currents and the particle size of the sediments, whereby small particles generally sink slower than coarse particles (McLusky 1974). To a lesser extent, other factors such as marine incursion also govern the distribution of sediment particles in estuaries (Sly et al. 1982). These processes result in a distinct sorting of the sediment load entering an estuary, such that a gradient exists with fine, muddy sediments in the upper reaches and coarse, sandy sediments in the lower reaches (Deeley & Paling 1999).

The main physical and chemical processes influencing subtidal soft sediments are: a) currents, which erode and deposit sediments, determining the depth and particle size composition of an estuary; and b) the physico-chemical processes that control the rate of diffusion of oxygen and nutrients into the sediments. Such processes are also responsible, at least in part, for the supply of organic matter to the sediments (Morrisey et al. 1998). Soft sediments are also dynamic habitats that are continually structured by both the local physical conditions and by the organisms living on and in the habitat, which bioturbate sediments through burrowing, tube-building and defecation (Woodin 1999).

1.2.2 Estuarine Macrofauna

Benthic organisms are classified, according to their size, as microfauna (< 32 μm), meiofauna (32 μm - 1 mm) and macrofauna (> 1 mm). The macrofauna is usually dominated by molluscs, crustaceans and polychaetes. During the past three decades, several major surveys of Australian estuarine invertebrates have revealed abundant macrofaunal communities, which are also relatively diverse in comparison to reports from similar studies elsewhere. Whilst a large proportion of this fauna is considered to be specialised estuarine biota, much of which is restricted to particular habitats, many animals are also considered to comprise a highly diverse marine community that thrives in the sheltered waters of estuaries and their associated soft sediments (Hutchings 1999).

In addition to the biotic and abiotic conditions already regulating estuarine fauna in general, further factors are specifically relevant to soft-sediment benthos. Of these, the major physico-chemical factors thought to influence the distributions of macro-invertebrates in soft-sediment habitats are the characteristics of the sediments (Gray 1974; Probert 1984), nutrients, food supply and water movement (Pearson & Rosenberg 1987). Recent studies have also highlighted the importance of hypoxia and substratum chemistry in estuarine benthic ecology (Constable 1999). Although these factors are all spatially and temporally variable in permanently open estuaries, they can be further intensified and have very different rates-of-change in intermittently closed estuaries. So, although estuarine species can typically tolerate a high degree of variation in physico-chemical variables, the physical conditions in estuaries can fluctuate widely and are not rigidly predictable (Fairweather 1999). This means that estuarine organisms can be exposed to severe physiological stress.

Physiological stress is a term that covers a range of challenges confronting estuarine organisms. The most conspicuous of these is salinity, as estuarine organisms are exposed to highly variable salinities when compared to the stable salinities that characterise both marine and freshwater environments (Edgar 2001). Many estuarine species can osmoregulate and temporarily tolerate unfavourable salinities by: horizontal or vertical migration; secreting protective substances over sensitive body surfaces; retreating into burrows; withdrawing sensitive body parts; closing shells; or by transitioning into a resting stage (Kinne 1967). However, if such conditions persist for a longer period, these species will become excluded from the effected areas (Teske & Wooldridge 2004). Another physiological stress can be the nature of a substrate, both in terms of the fine particulate matter that can clog delicate organs, and of the virtually anoxic conditions that can occur within muddy sediments (McLusky 1989). Many invertebrates, however, are oxygen conformers (i.e. oxygen consumption is partly regulated by the amount of oxygen available) and, therefore, can tolerate conditions of reduced dissolved oxygen for short periods. If subjected to longer anoxic conditions, some polychaetes are even able to survive on anaerobic respiration for up to 20 days (Ruppert & Barnes 1994).

To adjust to environmental variability, many estuarine animals also have diverse feeding strategies, characterised by a high degree of omnivory (Herman et al. 1999), which enables fauna to feed on different trophic levels. This decreases the probability of local extinctions due to environmental fluctuations. Consequently, although taxonomic diversity of estuarine communities may be low, functional diversity is relatively high (Costanza et al. 1996). Within the macrofauna there are two major feeding types: suspension feeders, which filter their food from the water column; and deposit feeders, which depend on the physical deposition of food particles on to the sediment surface and the incorporation of these particles into the sediment matrix. Some species, such as spionid polychaetes, show varying degrees of generalism and are able to utilise both the suspension and deposit feeding modes (Herman et al. 1999; Beesley et al. 2000).

1.2.3 Generalised Patterns of Community Structure

Most marine and estuarine habitats exhibit natural spatial and temporal variability, as well as natural environmental interactions between spatial and temporal differences (Underwood 2000). This variation in the distribution of organisms and other environmental variables exists at different scales and various complex combinations of a range of biotic and abiotic factors are responsible for short- and long-term variations in estuarine benthic populations (Mackay & Cyrus 2001). Generally, estuarine macrofauna comprises suites of species that occur in the specialised habitats of estuaries, namely soft sediments, seagrass beds, mangroves and saltmarshes (Hutchings 1999). The frequency, intensity and unpredictability of changes in estuarine environments selects for hardy, generalist species that can live and reproduce over a wide range of environmental conditions. This leads to estuarine communities generally having an overall lower species diversity than marine or freshwater environments (Costanza et al. 1996).

Another factor contributing to the relatively low species richness in estuaries may be the comparative youth of these environments. Very few estuaries have existed for longer than 10,000 years, an insufficient period of time for the evolution of most species (Hutchings 1999). Evidence that estuaries have not evolved a full complement of species is provided by the relative ease with which they are colonised by foreign, or exotic, species (Edgar 2001). The low species diversity, diverse feeding strategies of estuarine animals and high productivity of estuaries can result in exceptionally high densities of individuals. Therefore, estuarine benthic assemblages are often dominated by large numbers of just a few species (Costanza et al. 1996).

A gradient of species richness usually extends along an estuary, with the richest biota towards the entrance. This is because marine fauna generally have little tolerance to reduced salinities and are progressively excluded upstream (Schlacher &Wooldridge 1996a; Hutchings 1999; Edgar 2001). Most classifications that have been used to explain the distribution of fauna along an estuary have thus been based entirely on salinity gradients. However, Teske and Wooldridge (2003) have suggested that such systems are usually not suitable to explain the distribution patterns observed in South African estuaries as true estuarine species, which tolerate a wide range of salinities, dominate in these estuaries. As intermittently closed estuaries are the most abundant estuary type in both South Africa and Australia, it is likewise presumed that such classifications may also not suit Australian estuaries, particularly in southeastern Australia. As such, other characteristics that are known to influence the distribution of fauna also need to be considered. For example, sediment type (i.e. mud or sand) and entrance type have also been found to be important in structuring estuarine macrofaunal communities (Day 1964; Boesch 1976; Schlacher & Wooldridge 1996a; Teske & Wooldridge 2003). In relation to sediment type, benthic fauna appear to have a greater species richness in mixed, muddy-sands, when compared to either fine muds or clean sands (Day 1981).

Similar to other coastal environments, estuarine macrofauna also exhibit patterns of zonation from shallower, intertidal areas to deeper, subtidal habitats (Peterson 1991; Edgar 2001). Whilst some fauna can be quite mobile during high tide, during low tides a gradient across the shore is generally characterised by the presence of fauna prone to desiccation in subtidal areas, and fauna with mechanisms to avoid desiccation in intertidal areas. To date, these zonation patterns remain to be investigated in Australian estuaries and the influence they have on the spatial distribution of diversity and abundances are, therefore, unknown.

In addition to physico-chemical processes, the structure of most benthic communities is also determined by biological factors, such as competitive interactions, predation and recruitment (Rainer 1981). For example, benthic macrofauna are an important food source for epibenthic crustaceans, fish and birds. Similarly, humans also harvest many species of molluscs and crustaceans (e.g. oysters and mud crabs) (Herman et al. 1999). The proximity of an area to saltmarsh, mangroves and seagrass has also recently been recognised to contribute to community structure (Long & Ralph 2001) and these 'habitat mosaics' are receiving increasing attention (Jordon 2002; Ross 2006).

In intermittently closed estuaries, the combination of such extreme and unpredictable environmental conditions, along with limited recruitment opportunities for marine spawning species, has resulted in a lower species richness than permanently open estuaries in many cases (Hutchings 1999). In summary, the low species richness of intermittently closed estuaries reflects: (i) the low salinities, which would restrict colonisation by stenohaline marine species; (ii) the prevention of recruitment from the sea by entrance closure during times when many macrobenthic species are producing pelagic larvae; (iii) the very limited tidal movements, and thus mechanisms for transporting larvae from the sea when the estuary mouth is open; and (iv) the lack of a pronounced salinity gradient and reduced variation in sediment composition along the estuary (Platell & Potter 1996). In contrast to this low species richness in intermittent estuaries, abundances may not necessarily be low. This is because the species present may thrive due to both the reduced interspecific competition and their preference for the predominant conditions.

While no long-term studies have been carried out on the benthos of intermittent estuaries in Australia, it has been suggested (Hutchings 1999) that many species living in these systems do not maintain long-term self-sustaining populations. Following extremely unfavourable conditions, such as floods or salinity increases, mass mortality probably occurs and the populations are then renewed by external recruitment when a connection with the sea is re-established.

1.3 Estuarine Macrofauna as Biological Indicators

When examining estuarine health, it is preferable to assess biological components in addition to physico-chemical properties, such as water quality (Mackay & Cyrus 2001). This is especially relevant in intermittently closed estuaries, where dramatic changes in some physico-chemical factors, including salinity and water level, can occur rapidly following entrance openings. Generally, when making environmental assessments, only one part of the biota is examined and it is assumed that the performance of this component is an indication of the general health of the system (Warwick 1993). Benthic macrofauna are one such component that have long been recognised as valuable indicators of water quality and other environmental conditions in estuaries (Barton 1989). As such, measurements of changes in the structure of benthic communities can be used to detect and monitor both natural and anthropogenic perturbations in estuaries (Warwick 1993).

There are a number of advantages in using soft sediment, macrofaunal communities as indicators in estuaries and they have been shown to be particularly effective for baseline studies and impact assessments (Bilyard 1987; Warwick & Clarke 1991; Dauer 1993; Edgar & Barrett 2002). Benthic macrofauna are relatively immobile and, therefore, most are unable to evade changing conditions. It can also take some time for fauna to recolonise an area after a detrimental event. In addition, macrofauna are generally long-lived and represent a range of different feeding types (Boesch et al. 1976). The combination of these factors means that the benthos integrates environmental influences at a particular place over a relatively long time span (Herman et al. 1999; Moverley 2000).

Further, most benthic species can be present in high abundances because, despite the stresses posed by life in an estuary, the great availability of nutrients and the resultant primary production permits many species to achieve very large population densities (Morrisey 2000). Benthic fauna generally live in the top 20 – 30 cm of sediment (Barnes & Hughes 1982), essentially providing a two-dimensional dispersal. This means that designs for sampling benthic fauna are less complicated when compared to plankton or fish communities, where dispersal varies in all three dimensions of the water column (Moverley 2000).

1.3.1 Community Measurements

Community measurements are attempts to summarise the composition of a community and may be as simple as counting the number of species (i.e. species richness) or the abundances of individual species. Other community measures include calculations of diversity and evenness, or multivariate descriptions of a community (Keough & Quinn 1991). Such measures of community structure are widely used in estuarine research and are widely used as indicators of environmental and anthropogenic perturbations to a community (Rainer 1981). In Australian estuaries, species richness and abundance have previously been found to be influenced, at any given point or time, by environmental factors that regulate estuarine fauna. These are primarily salinity, the variety and area of habitats present, and other environmental conditions, including the availability of food, shelter, protection from predators and water movement (Rainer 1981; Jones et al. 1986; Jones 1987; Potter & Hyndes 1994; Worthington et al. 1995; Gray et al. 1996; Edgar & Barrett 2002).

When communities, or populations, respond to environmental changes, they are said to be stressed by whichever particular factors have changed. Therefore, environmental stresses are part of a set of changes that occur in environments (Morrisey 2000). These perturbations can be natural or anthropogenic changes that are either accidental deliberate. Many natural populations have considerable temporal and spatial variance in abundances. This means that the detection of a stress requires not only that the abundance has changed at some place and time, but that the observed change in abundance is larger than can normally be expected to operate at that given place and time (Underwood 1989). This means that consideration must be given to the scale and timing of changes, in relation to the processes that are already stressing a particular population or community.

A direct indication of the consequences of change to the estuarine environment is reflected by either loss or gain of species and positive and negative changes to densities, or a combination of these factors (Bunn 1995). This means that the responses of communities to stress are highly varied. For example, highly stressed estuarine communities can be characterised (Dauer & Alden 1995) by: (1) low levels of species diversity, abundance and biomass; (2) dominance by species that are short-lived (i.e. stress tolerant, opportunistic, *r*-selected species); and (3) a rarity of species that are long-lived (i.e. equilibrium, *k*-selected species). In contrast, abundances and biomass may be very high as a result of an excessive dominance by opportunistic species (Pearson & Rosenburg 1987). A reduction in species richness presumes that there have been local extinctions of some taxa (Keough & Quinn 1991). Stressed benthic assemblages may also undergo a change in size structure to small-sized species (Gray et al. 1990).

1.4 Scope and Aims of Study

Estuaries are vulnerable ecosystems and an understanding of their physical and biological characteristics, and appreciation for their individuality, are essential for their proper use and management (Hodgkin 1994). Potential pressures and threats to estuarine biodiversity and fisheries have come from a variety of human impacts including: catchment clearance for agricultural and urban development; pollution; wetland reclamation; engineering works such as dredging, training walls, marinas, flood mitigation and dams; overfishing; weed infestations and litter (Saenger 1996). Due to the high value, intense use and frequent overuse of estuaries, decision makers and scientists require a directed regional framework and consistently derived, comprehensive datasets to assess and compare estuarine conditions, and develop effective policies to promote the long-term balance between development and conservation (Alexander & Monaco 1994). This need for a better balance between human use and ecological needs is crucial to address selected estuarine problems and ensure the ecological sustainability of our estuaries (Driml 1996).

In regard to intermittently closed estuaries, each is unique in its environmental and human values but the prevailing management approach has been to treat them as if they are all the same, or even as if they are like well-flushed, permanently open estuaries. Such an approach gives little attention to their natural differences to other estuarine systems, or their elevated sensitivity to human interventions (HRCNSW 2002). This situation exists in NSW perhaps because, although intermittently closed estuaries are the most abundant estuary type, most estuarine research has focussed on permanently open systems. This means that there is little scientific information on which to base management guidelines concerning intermittently closed estuaries. For example, there is some empirical evidence suggesting that associations exist between estuary type and ecological function (Roy 1984). There is, therefore, a basis for making predictions concerning species richness and abundance in different estuary types. However, in southeast Australia, there are few datasets available that provide detailed information to support these associations (Roy et al. 2001).

In short, estuaries are environmentally, economically and socially important ecosystems and, despite being the dominant estuary type in southeast Australia, intermittently closed estuaries are, ecologically, very poorly understood. Further, most studies on intermittently closed estuaries in Australia have concentrated on the potentially highly mobile, fish communities, which do not necessarily have the advantage of integrating long-term environmental influences.

Therefore, the overall objective of this work was to develop a greater understanding of the ecology of intermittently closed estuaries by examining the spatial and temporal variation of benthic macrofaunal communities in the intermittently closed estuaries of the Solitary Islands Marine Park (SIMP) in NSW. As the first long-term, benthic study in the region involving a number of estuaries this, essentially baseline, information will contribute to the current knowledge of estuaries in NSW. For intermittently closed estuaries, it is also recognised that a greater understanding of the relative stability of benthic estuarine communities to natural and anthropogenic variations may aid prediction of the effects of cyclical wet and dry periods. This, in combination with a better understanding of population dynamics, would be an advantage in assisting sound coastal management (Mackay & Cyrus 2001).

The advantages of using benthic macrofauna as biological indicators have already been outlined. However, it was specifically the subtidal communities that were the primary focus of this study as intertidal areas are non-existent in intermittent estuaries during closure. Therefore, subtidal sediments provided a habitat that was consistently present and comparable between all estuaries. Similarly, Schlacher and Wooldridge (1996a) also focused their sampling on subtidal fauna as the intertidal area was poorly developed in the estuaries that they studied. As such, most of the benthic habitat was subtidal. They further suggested that short-term environmental disturbances, such as temperature extremes and rainfall, are higher in intertidal habitats, which may affect the distribution of intertidal organisms and confound the results of larger-scale, long-term studies.

The specific aims of the project were to:

1) test for differences in benthic macrofaunal communities between intermittently closed and permanently open estuary types;
2) investigate how the relationship between benthic communities in intermittently closed and permanently open estuary types changed over time, particularly in relation to major climatic events affecting entrance state (i.e. prolonged closure during drought and major opening events following floods);
3) evaluate the spatial variation within each estuary type by comparing benthic macrofaunal communities between individual intermittently closed estuaries and between individual permanently open estuaries; and
4) examine temporal community variation within estuaries that represent not only different estuary types but also a range in the frequency and duration of entrance closures.

These aims were addressed by constructing a broad conceptual model that, initially, consisted of the prediction that, at the time of commencement, the intermittent estuaries had been closed for an extended period and, therefore, their ecology would differ to that of nearby permanently open estuaries. It was further predicted that the ecological differences would be evident in the benthic macrofaunal communities and that the environmental factors directly responsible for such differences would include the entrance history and its effects on the physico-chemical properties of the water column, sediments and the reproductive or recruitment processes of some species. This model was extended and modified as the project progressed.

CHAPTER 2

GENERAL METHODS

2.1 Introduction to Study Location

This study was conducted at a number of estuaries within the Solitary Islands Marine Park (SIMP), which is located on the northern coast of NSW, Australia (Fig. 2.1). The SIMP is the largest marine protected area in New South Wales, covering an area of 71 000 hectares and stretching along 75 km of coastline (SIMPA 2002). The SIMP includes all estuarine systems to their upper tidal limits and to mean high water mark. The region is unique in that the coastal ranges are in close proximity to the ocean and the catchments are, therefore, generally small and characterised by steep slopes. As a result, natural drainage is in the form of numerous small creeks, with limited floodplains. The predominant climate is a humid, sub-tropical one with a high rainfall (1650 mm mean annual rainfall), the majority of which occurs in late summer and early autumn (Carter et al. 2000). Tides are semidiurnal with a maximum range of 2.0 m.

Intermittently closed estuaries comprise 10 out of the 15 main estuaries in the SIMP and range in catchment size from 3.3 to 25.0 km^2. In contrast, the remaining five are permanently open barrier estuaries with catchment sizes between 25 – 190 km^2. Nine of the estuaries in the SIMP were selected as study locations (Fig. 2.1) and an upper, middle and lower site was surveyed in each. The different catchments are subject to various levels of modification for agriculture, and residential or urban development; the degree of development ranges from negligible at Station Creek to extensive urbanisation at Coffs Creek, the catchment of which supports a population of approximately 60 000 people.

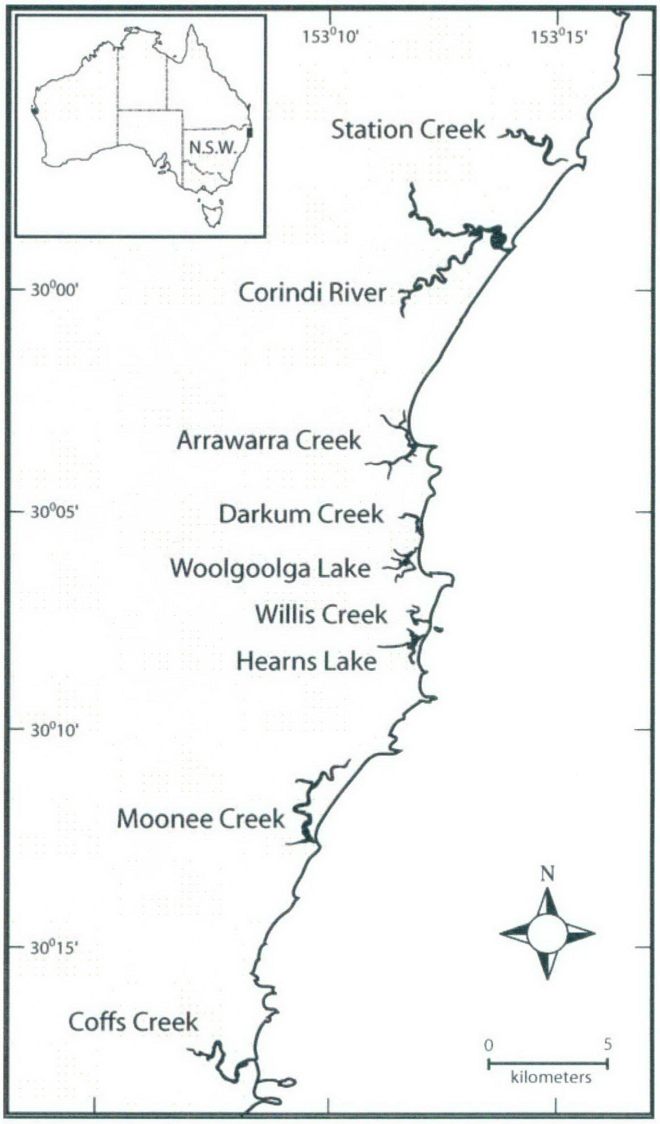

Fig. 2.1. Position of the Solitary Islands Marine Park and the location of study estuaries.

2.2 Descriptions of the Estuaries Studied

This section gives a brief description of each individual estuary and uses information compiled from a number of sources, including: Bell and Edwards (1980); Coffs Harbour City Council (1997); Carter et al. (2000); Geoscience (2001); Department of Infrastructure, Planning and Natural Resources (2004).

2.2.1 *Station Creek*

Station Creek (Figs. 2.2.1a, b) is the most northern of the estuaries surveyed (29˚56'S 153˚14'E) and is also the most pristine, with almost the entire catchment under either National Park or State Forest tenure (Table 2.2). The estuary incorporates extensive saltmarsh areas and tree cover in the catchment is high at 94 %. Mangroves, however, are scarce and only present in the lower reaches. Station Creek is the largest of the intermittently closed estuaries, with a length of 9 km, the majority of which has been designated as a Sanctuary Zone (i.e. no-take area). It is the only intermittently closed estuary in the SIMP that receives this highest level of protection.

Table 2.2 Summary of some the morphological, modification and protection characteristics of the estuaries studied.

	Length (km)	Catchment Size (km^2)	Entrance Status	Entrance modifications	Catchment Tree Cover (%)	Marine Park Zoning
Station Creek	9	24.0	Intermittent	No	94%	S / HP
Corindi River	25	148.0	Open	No	81%	S / HP
Arrawarra Creek	5	20.0	Intermittent	Yes	78%	HP
Darkum Creek	4	7.0	Intermittent	No	-	HP
Woolgoolga Lake	5	25.0	Intermittent	Yes	54%	HP
Willis Creek	2	3.3	Intermittent	No	-	HP
Hearns Lake	4	9.0	Intermittent	No	-	HP
Moonee Creek	13	39.5	Open	No	63%	HP
Coffs Creek	13	25.0	Open	Yes	23%	HP

Marine Park Zoning: S – Sanctuary Zone; HP – Habitat Protection Zone.

Tree Cover: '-' indicates data unavailable.

2.2.2 *Corindi River*

With a catchment area of 148 km^2 and a length of 25 km (Table 2.2), Corindi River (29˚59'S 153˚13'E) was the largest estuary in the study. Its permanently open entrance is unmodified (Fig. 2.2.2a) and the estuary as a whole is, likewise, largely unmodified. This is largely due to approximately half of the catchment being protected by National Park and because the remaining part of the catchment has a relatively small population of less than 400 people. Parts of Corindi River also have a high level of protection within the marine park by being classified as Sanctuary Zones (Fig. 2.2.2b).

Fig. 2.2.1a Aerial view of the entrance of Station Creek. (Photo: A. Davey)

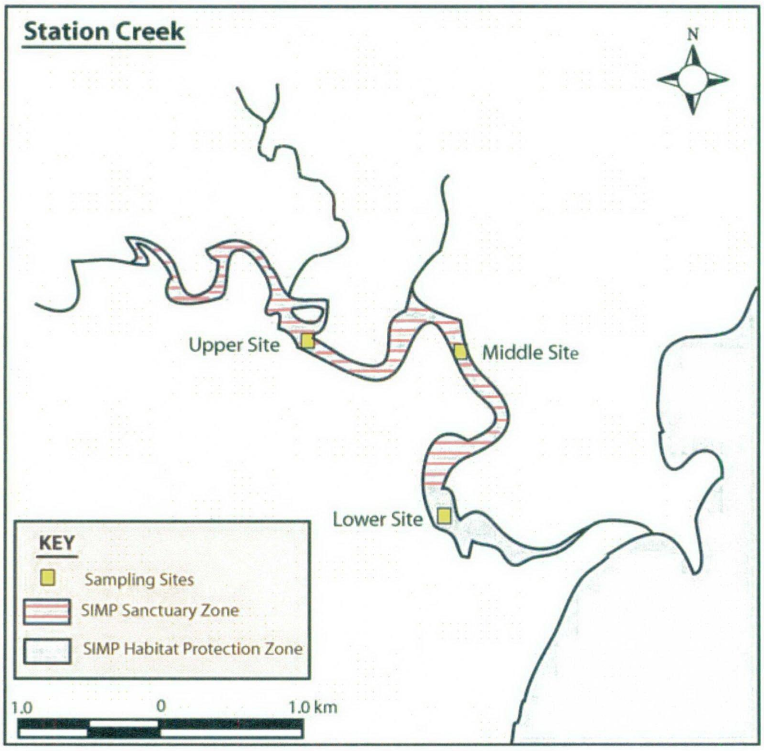

Fig. 2.2.1b Map of Station Creek showing study sites and marine park zoning.

Fig. 2.2.2a Aerial view of Corindi River showing the lower and middle sampling sites. (Photo: NSW DIPNR)

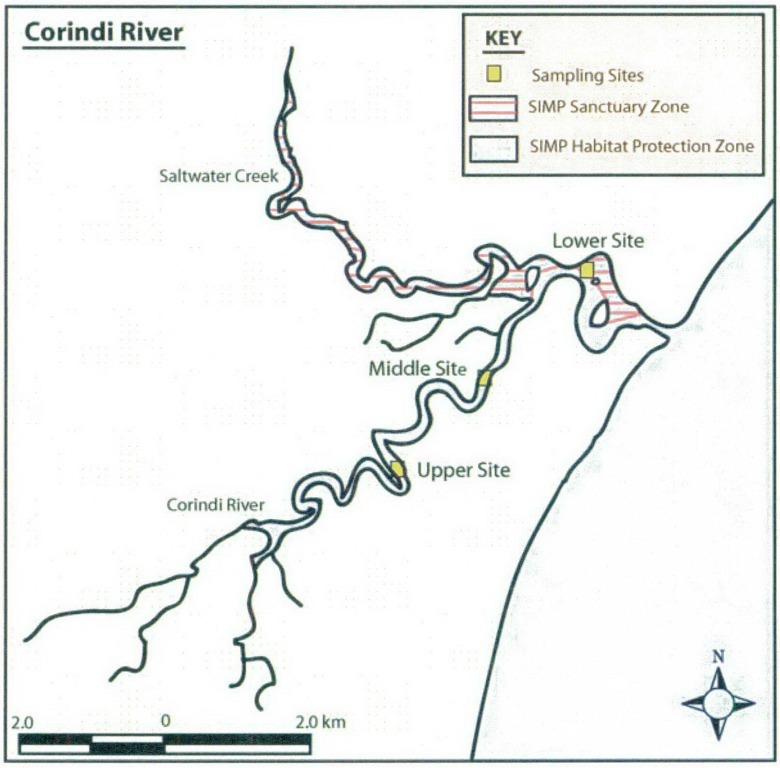

Fig. 2.2.2b Map of Corindi River showing sampling sites and marine park zoning.

2.2.3 *Arrawarra Creek*

Arrawarra Creek (30°03'S 153°11'E) is an intermittently closed estuary with an entrance that has been modified by the construction of a partial training wall (Figs. 2.2.3a, b). The catchment area is relatively small at 20 km^2, though much of this comprises steeply sloping terrain. The catchment is largely unmodified, with almost 60 % under State Forest tenure.

2.2.4 *Darkum Creek*

The intermittently closed Darkum Creek (30°05'S 153°11'E) is the second smallest estuary in the study with a catchment area of 7 km^2 (Figs. 2.2.4a, b). It is a narrow estuary that is largely unmodified, though a golf course and a small residential area are adjacent to its middle reaches.

2.2.5 *Woolgoolga Lake*

Woolgoolga Lake (30°06'S 153°11'E) is an intermittently closed estuary that has been extensively modified by urban development and agriculture, mainly banana plantations (Figs. 2.2.5a, b). It also has partial training walls along most of the lower reaches but not directly at the entrance. Anecdotal evidence suggests that the estuary has infilled considerably over the last 20-30 years and it is now often very shallow (< 1.0 m) throughout most of the waterway area. Woolgoolga Lake also has a history of pollution, with fish kills occurring in the late 1980s due to organochlorine inputs from the banana plantations (Deildrin, DDT, Aldrin) (McDougall & Dettman 1990).

2.2.6 *Willis Creek*

The intermittently closed Willis Creek (30°07'S 153°12'E) (Figs. 2.2.6a, b) is the smallest estuary in the study with a catchment area of only 3.3 km^2 (Table 2.2). It is arguably also the most modified in the study area and is surrounded by three significant developments, including a sewage treatment works, an industrial area and a public tip. Local government monitoring indicates high levels of pollution, especially nutrients and faecal coliforms, contributed by urban and industrialized runoff, septic systems, agriculture and the sewage inputs.

Fig. 2.2.3a Aerial view of Arrawarra Creek showing the lower and middle sampling sites. (Photo: NSW DIPNR)

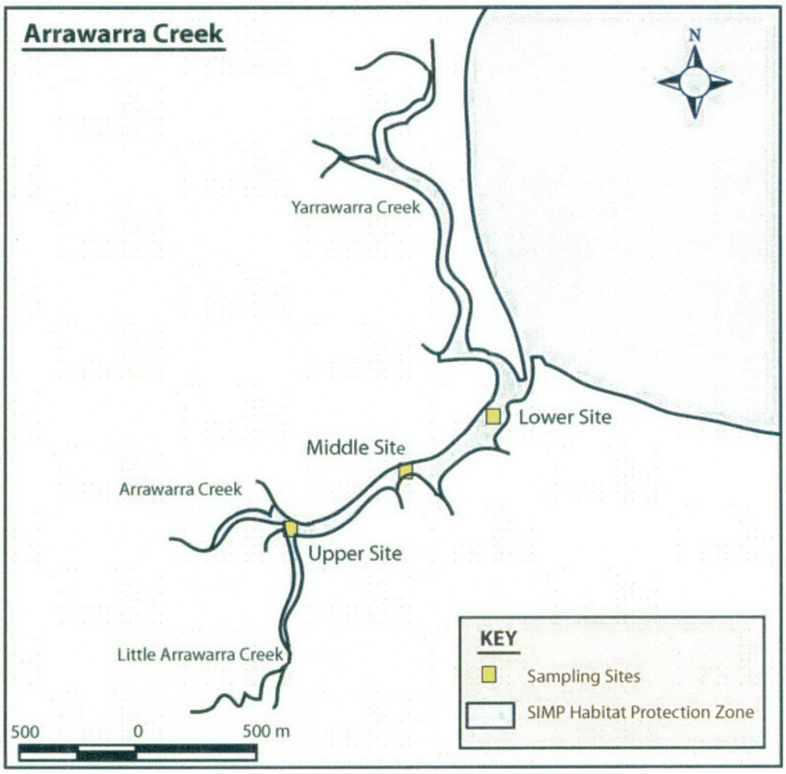

Fig. 2.2.3b Map of Arrawarra Creek showing sampling sites and marine park zoning.

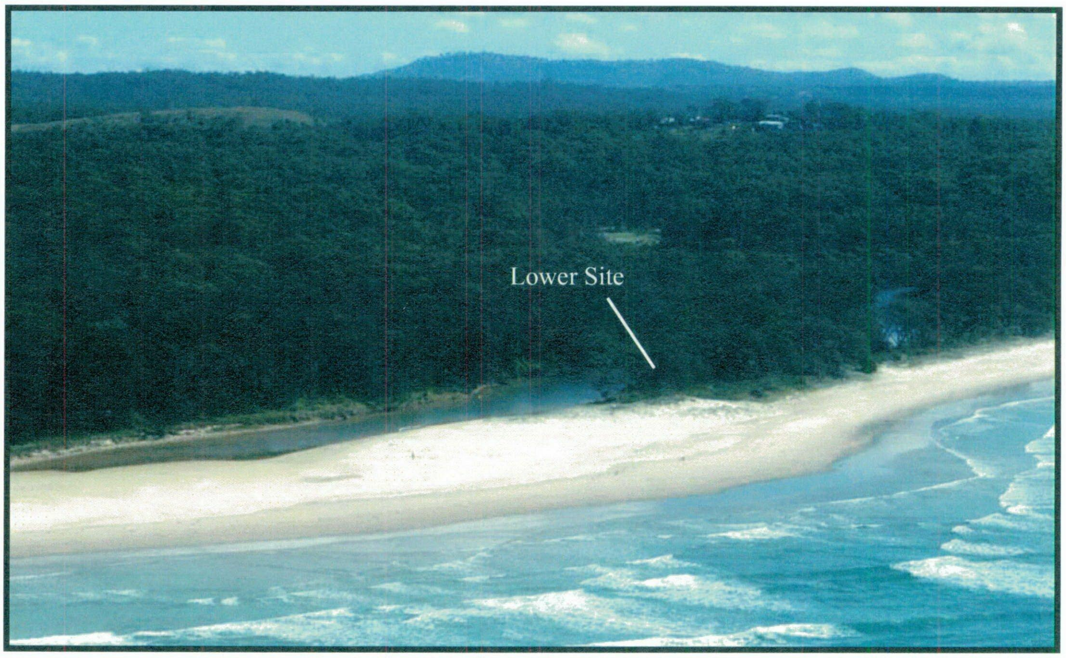

Fig. 2.2.4a The entrance of Darkum Creek during a period of closure. (Photo: A. Davey)

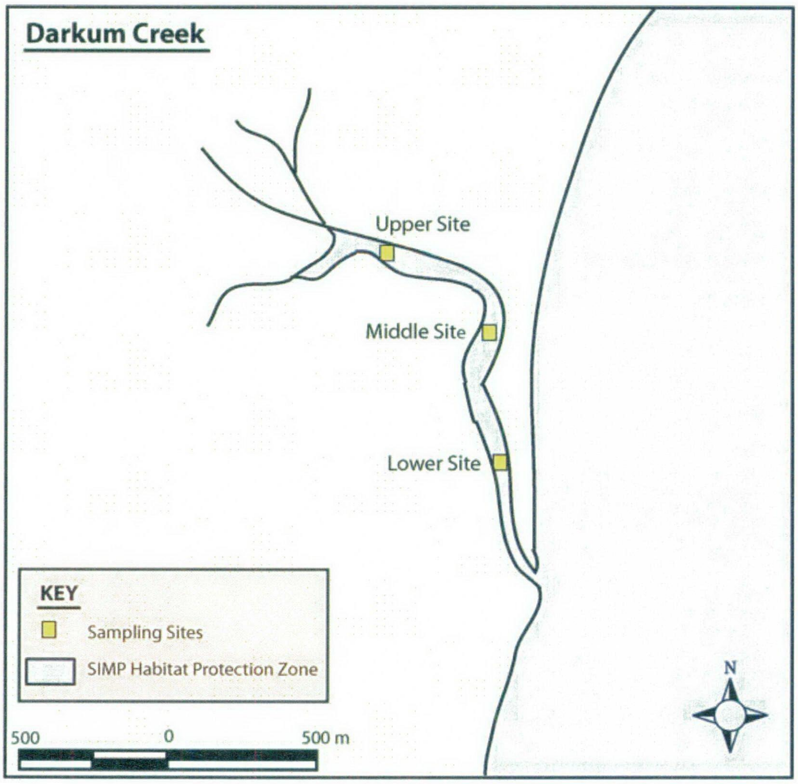

Fig. 2.2.4b Map of Darkum Creek showing sampling sites and marine park zoning.

Fig. 2.2.5a Aerial view of Woolgoolga Lake showing all sampling sites. (Photo: NSW DIPNR)

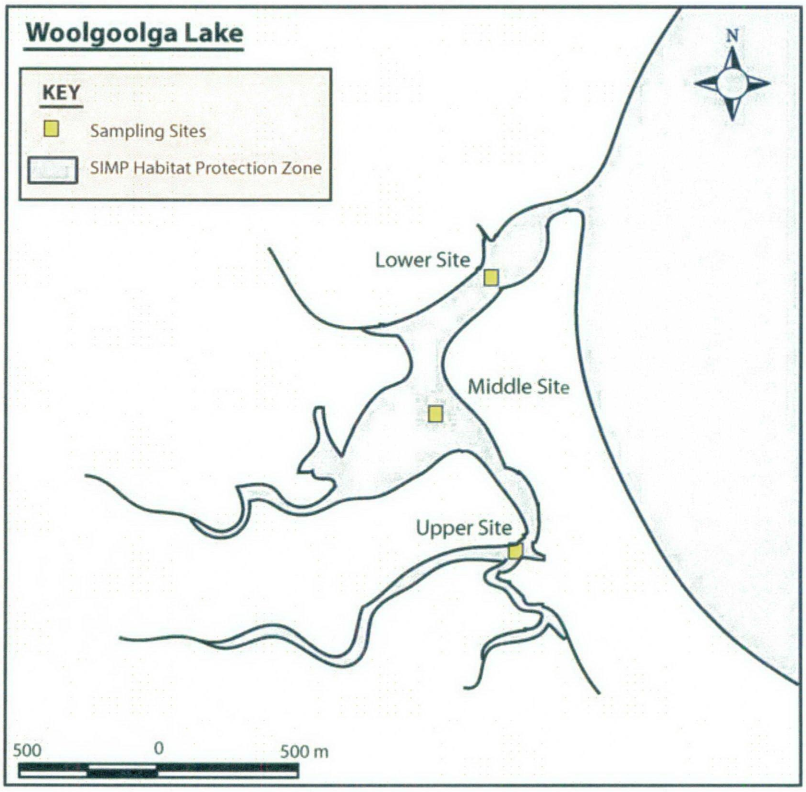

Fig. 2.2.5b Map of Woolgoolga Lake showing sampling sites and marine park zoning.

Fig. 2.2.6a Aerial view of the entrance of Willis Creek during a period of closure. (Photo: A. Davey)

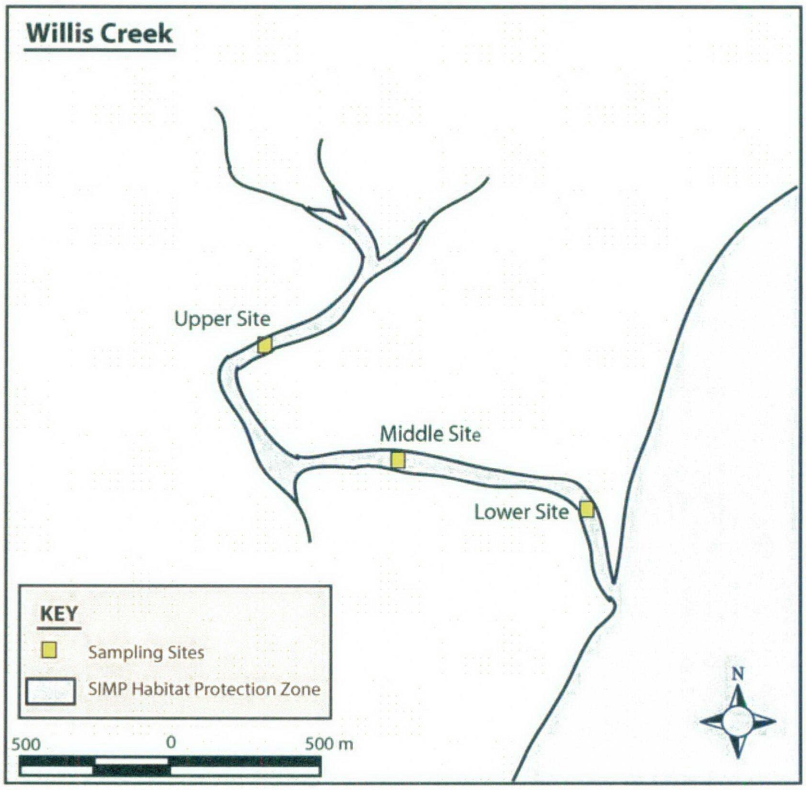

Fig. 2.2.6b Map of Willis Creek showing sampling sites and marine park zoning.

2.2.7 Hearns Lake

Hearns Lake (30°08'S 153°12'E) is another relatively small intermittently closed estuary with a catchment area of approximately 9 km^2. Similar to Woolgoolga Lake, the estuary has a recent history of infilling due to soil erosion from banana plantations on the catchment's steep slopes. Beyond this, the condition of the estuary remains largely unmodified and extensive saltmarsh is present (Figs. 2.2.7a, b).

2.2.8 Moonee Creek

The second largest estuary in the study is Moonee Creek (30°12'S 153°09'E), a permanently open barrier estuary with a catchment area of 39.5 km^2 (Figs. 2.2.8a, b). The majority of the catchment remains uncleared and land uses include State Forest and National Park. There has, however, been an increase in urban development, with recent land releases that will eventually be able to accommodate twice the current population of 1200. The estuary itself supports extensive seagrass areas in addition to considerable mangroves and saltmarsh.

2.2.9 Coffs Creek

The most southern of the estuaries studied, Coffs Creek (30°18'S 153°07'E) is a permanently open barrier estuary with training wall modifications at the lower reaches and entrance (Figs. 2.2.9a, b). It has the smallest catchment area (25 km^2) of the permanently open estuaries and flows through the primary residential area for the region, which supports a population of approximately 60 000. The catchment supports commercial and light industrial developments and much of the upper reaches have been cleared for banana cultivation and dairy farming. Consequently, the estuary has been extensively modified and contains few seagrass or saltmarsh areas. Similar to Woolgoolga Lake, organochlorine inputs from banana plantations in the 1980s have previously caused fish kills and repeatedly resulted in management authorities having to introduce fishing closures.

Fig. 2.2.7a Aerial view of Hearns Lake showing all sampling sites. (Photo: NSW DIPNR)

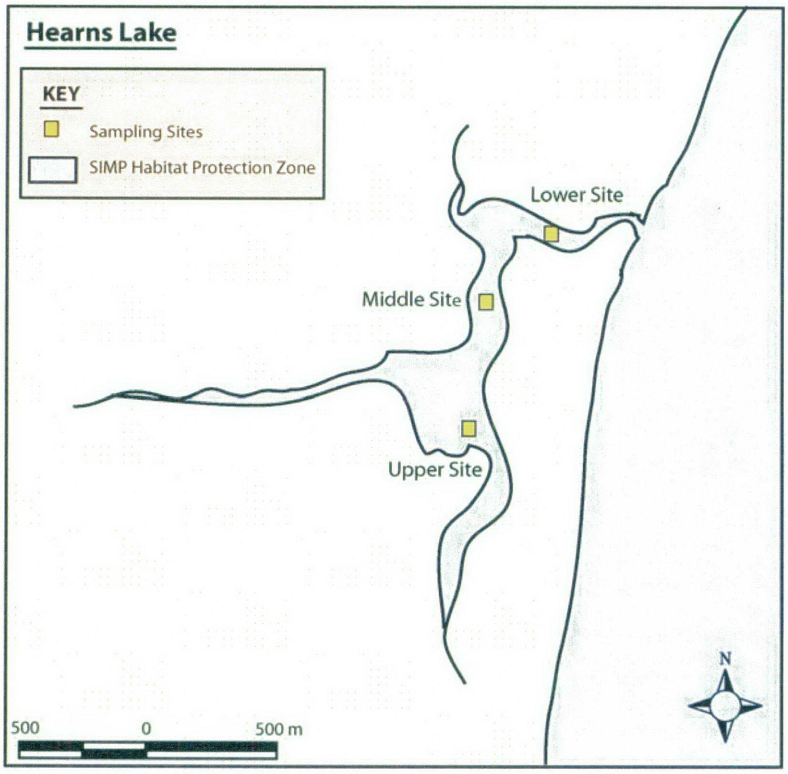

Fig. 2.2.7b Map of Hearns Lake showing sampling sites and marine park zoning.

Fig. 2.2.8a Aerial view of the entrance of Moonee Creek. (Photo: A. Davey)

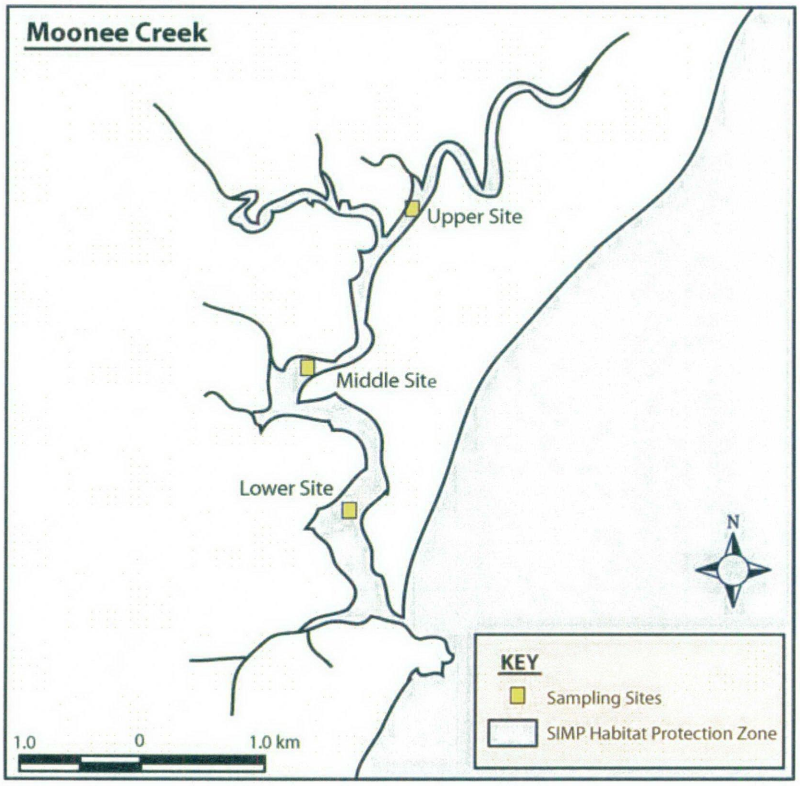

Fig. 2.2.8b Map of Moonee Creek showing sampling sites and marine park zoning.

Fig. 2.2.9a Aerial view of the entrance of Coffs Creek and the lower sampling site. (Photo: A. Davey)

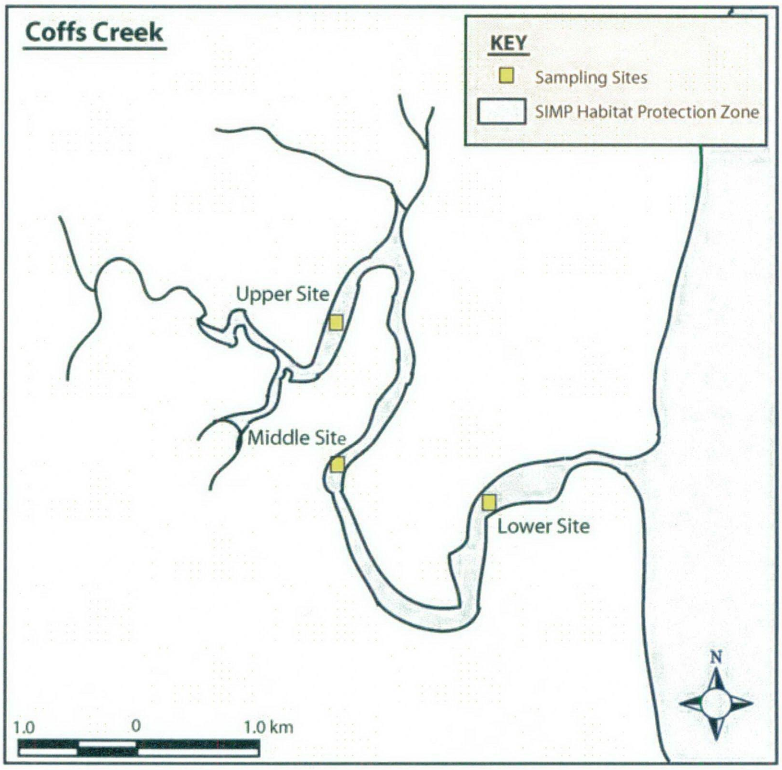

Fig. 2.2.9b Map of Coffs Creek showing sampling sites and marine park zoning.

2.3 General Study Design

2.3.1 Outline of General Study Design

The number of estuaries studied from each estuary type was asymmetrical, with six of the nine selected estuaries being intermittently closed and three being permanently open. This was primarily due to the greater availability of intermittent estuaries as they are so dominant in the area. Edgar and Barrett (2002) have suggested that it is preferable to sample a greater range of estuaries, rather than fewer localities over time, as seasonal and inter-annual temporal variance is generally much lower than spatial variance. Further, as this was the first Australian study to repeatedly examine biological variables in a number of intermittently closed estuaries over a two year temporal scale, it was, therefore, considered necessary to study as many representatives of this estuary type as possible to enable the greatest insight into their ecology over the given timeframe.

Three sites were established along the length of each estuary, one in each of the lower, middle and upper reaches. Previous studies (Sawtell 2002) have indicated that infaunal communities are distinct in each of these parts of local estuaries. Other benthic studies that similarly found community differences between the upper, middle and lower sites of southeast Australian estuaries include Jones (1987), Platell and Potter (1996), Moverley and Hirst (1999), Moverley (2000) and Hirst (2004). Sites measured approximately 10 x 10 m and were situated in the middle of the main estuary channel. The positioning of the sites in the middle of the main estuary channel ensured that only the permanently subtidal benthos was sampled. This was necessary for two reasons. Firstly, for consistency between estuaries, only subtidal areas could be sampled as intermittent estuaries are non-tidal during closure. Secondly, the waterway area of intermittent estuaries can be reduced dramatically following major opening events and the positioning of sites within the middle of the main estuary channel meant that they were in areas that are always inundated, rather than in areas that may later be emerged for months at a time when the entrance is open.

Fringing vegetation (Hutchings 1999) and the relative distance along each estuary were taken into consideration when establishing sites in order to facilitate valid comparisons between equivalent estuary regions. GPS coordinates were recorded for each site to enable accurate relocation. This is particularly important when estuarine sites are sampled periodically as spatial variation can exist at the scale of a few metres (Thrush et al. 1994; Anderson 1998). Consequently, results may be misinterpreted as temporal, rather than spatial change when sites are not accurately located (Edgar & Barrett 2002).

The data comprising the majority of this thesis are derived from seasonal sampling in the upper, middle and lower sites of each estuary over the two-year period from January 2002 through to October 2004. Estuaries were also sampled after major climatic events that opened the entrances of closed estuaries. It must be noted that there is not always a clear demarcation between open and closed entrance states. In this study, a closed estuary is defined as one that has no connection (i.e. is not flowing) to the ocean at low tide. On occasion, such an estuary may still receive some marine influence when waves wash over the beach berm at high tide.

2.3.2 Pilot Study

A pilot study was conducted before the main study commenced, primarily to determine the number of replicate macrofauna samples that would be required to achieve acceptable precision (i.e. ratio of the standard error : mean ≤ 0.2) for the more extensive sampling programme. The precision (D), which refers to the degree of concordance among a number of measurements or estimates of a variable, is paramount in assessing the effectiveness of a sampling method (Sokal & Rolf 1981). Precision is most affected by the size and number of samples collected (Green 1979) and a standard error of 20 % of the mean (i.e. $D = 0.2$) is considered a reasonable error in most benthic samples (Elliot 1971). When preliminary data are available, the minimum level of precision can be set and the optimum number of replicate samples (n_r) required to achieve that level of precision can be calculated using the equation:

$$n_r = S^2 / (x^2 . D^2)$$

where D is the desired precision (0.2), S is the standard deviation and x the mean.

In this case, precision analysis was calculated for estimates of the univariate community variables, species richness and the total number of individuals. Note that, in community-level analyses using multivariate statistics, four replicates are considered to be the minimum to allow a 5 % test of differences using Analysis of Similarity (Clarke & Green 1988; Clarke & Ainsworth 1993). For this reason, the number of replicate samples used throughout the pilot study was four. Four samples were collected from each of the upper, middle and lower sites of Coffs Creek (Fig. 2.2.9b). A 0.068 m^2 van Veen grab was the sampling unit used and this device is detailed further in the next section. Samples were collected from the middle of the main estuary channel. Samples were bagged and later sieved through a 1 mm mesh back at the laboratory before preservation and identification.

This precision analysis revealed that the minimum number of replicate grab samples that would be required to achieve a precision of $D = 0.2$ for both variables at all sites was five. This pilot study also allowed the collection methods to be reassessed in terms of sample manageability and handling time. This led to the development of a piece of custom-made sieving equipment, which enabled later samples to be immediately and easily sieved, aided by a pump-operated hose, whilst aboard a vessel.

2.4 Sample Collection and Processing

2.4.1 Macrofauna Methods

Five macrofaunal samples were collected from each of the upper, middle and lower sites across all estuaries for every sampling occasion. Samples were collected from only non-vegetated substrates, using a 0.068 m^2 van Veen grab. The grab is an efficient tool for quantitative sampling of surface sediments and their associated infauna, obtaining a sample of sediment of a given surface area (Blomqvist 1991). It was necessary to use a grab as the sampling unit in this study as it ensured that standardised, representative samples could be collected from all sites at all estuaries, regardless of varying conditions such as water depth and sediment character. A van Veen grab has long arms attached to the jaws, which enable increased leverage for closing and

Fig. 2.4.1a View of sampling equipment including grab, gantry/winch, sieve and wash tray.

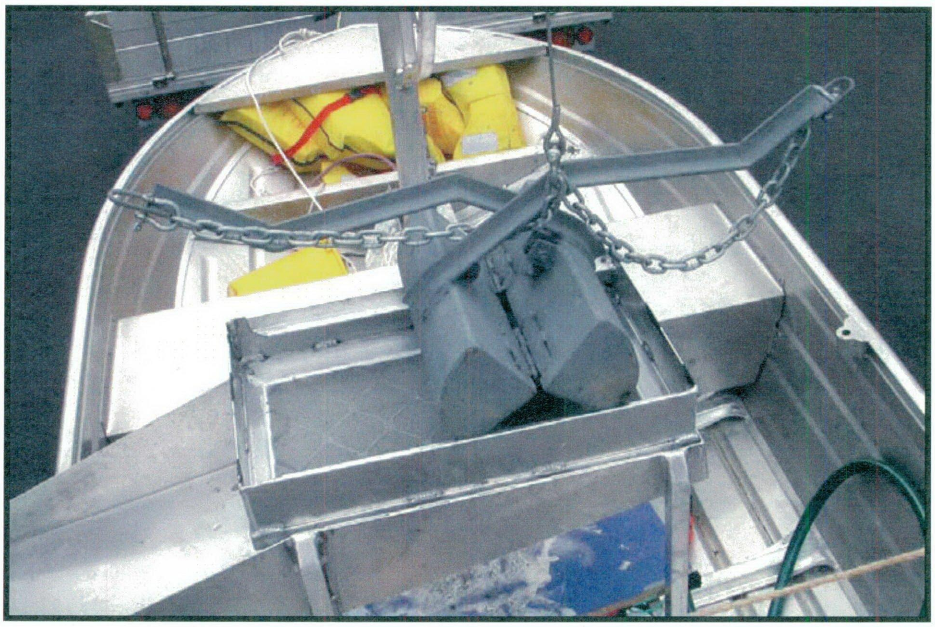

Fig. 2.4.1b Top view of grab, sieve and wash tray set up on board a vessel. Note that here the jaws of the grab are not yet fully extended.

prevent it from being jerked off the bottom if the boat rolls (Eleftheriou & Holme 1984). The grab used here was attached by a stainless steel cable to a winch that was mounted on a revolving gantry (Fig. 2.4.1a). This allowed the grab to be quickly retrieved, as well as easily positioned for deployment and subsequent retrieval.

The position of the samples within each site were haphazard rather than random due to the restrictions imposed by using a remote sampling device and manoeuvring a vessel in a relatively small area. On occasion, the grab did not close properly due to rocks or organic debris becoming wedged in the jaws, resulting in the loss of some or all of a sample. When this occurred any remaining sample was carefully discarded away from the site in a direction that ensured tidal currents would not carry the settling sediments back into the study site.

Once each sample was collected, the grab was positioned on a frame above a rectangular sieve, the jaws were opened and the sample was washed into the sieve using a pump-operated hose (i.e. filtered water from the estuary). The sample continued to be washed until as much sediment as practicable had been removed. To enable the manageable removal of sediments whilst aboard a small vessel, the sieve had been designed to sit over a tray that directed the sediments washed from the sample, and the water from the sieving process, out of the vessel.

The sieve had a stainless steel, square mesh of 1.0 mm. Both 1.0 mm and 0.5 mm mesh sizes are widely used in benthic macrofaunal studies. Whilst the finer mesh can provide more accurate community and population estimates, it also greatly increases the time required to process and sort samples (Schlacher & Wooldridge, 1996b). This, in turn, limits the number of samples that can be taken within a given logistical and financial framework. Therefore, whilst the coarser 1.0 mm mesh will give less accurate data, this can be offset by the time saved being used to collect a larger number of samples (James et al. 1995).

The fauna retained on the sieve were washed into a mesh bag (approx. 0.4 mm mesh size), anesthetized in a magnesium chloride solution and later preserved in 10 % formalin. In the laboratory, each sample was washed thoroughly to remove the formalin and then transferred into a tray of freshwater. The fauna were sorted from any remaining debris and placed in 70 % ethanol prior to identification and enumeration. All fauna were identified to the highest possible taxonomic resolution. Although mostly this was to species level, the taxonomy of a few groups is poorly known for the area and such fauna were left in higher taxonomic categories. These included the nemerteans, nematodes and oligochaetes.

2.4.2 Physico-chemical Variables

At each site a calibrated TPS 90-FLT multi-probe was used to measure the total dissolved salts (TDS), dissolved oxygen (DO), temperature and pH of the lower water column. Each variable was measured five times at each site on all sampling occasions. Three 50 ml sediment samples were also collected at each site for analyses of organic content and physical sediment characteristics, specifically graphic mean grain size and graphic standard deviation as a measure of sediment homogeneity.

Sediment samples were oven-dried at 100˚C for 24 hr and weighed prior to combustion at 550˚C for 6 hr to calculate the percentage content of organic materials (percentage difference after combustion), principally carbon (Buchanan 1984). Organic content provides a measure of the amount of food available to animals that feed on detritus. To measure sediment grain size, samples were initially wet-sieved through a 63 µm mesh to extract the mud fraction, which was dried and weighed. The remaining sand fraction (> 63 µm) was then sieved through a series of nested sieves to enable calculations of grain size and sediment homogeneity from the grain size distribution (Buchanan 1984). These calculations were conducted using the SOILVISION programme (SoilVision Systems Ltd. 2004), whereby grain size was estimated by the graphic mean (M_2) and sediment homogeneity was based on calculations of the graphic standard deviation (σ_G). In SOILVISION, the formulae for these two parameters are taken from Folk (1980) as follows, where each value, Φ16 for example, is read off the Phi (Φ) notation cumulative frequency distribution for each sediment sample.

$$M_2 = (\Phi 16 + \Phi 50 + \Phi 84) / 3$$

$$\sigma_G = (\Phi 84 + \Phi 16) / 2$$

The resultant graphic mean values are categorized into sediment types using the Wentworth Grade Classification (Buchanan 1984) (Table 2.4.2a). Similarly, the graphic standard deviation can also be classified into varying levels of sediment homogeneity, or sorting (Table 2.4.2b).

Table 2.4.2a Wentworth grade classification, modified according to the sieve sizes utilized in the current study (from Buchanan 1984). Note that Φ is equal to –log2 of the sediment grain diameter in millimeters.

Grain Size (mm)	Phi (Φ) Scale	Sediment Type
> 2.000	-2	pebble
2.000	-1	granule
1.000	0	very coarse sand
0.500	1	coarse sand
0.250	2	medium sand
0.125	3	fine sand
0.063	4	very fine sand
< 0.063	5	coarse silt

Table 2.4.2b Sediment homogeneity classification (from Buchanan 1984).

Graphic standard deviation (σ_G)	
< 0.35	Very well sorted
0.35 – 0.50	Well sorted
0.50 – 0.71	Moderately well sorted
0.71 – 1.00	Moderately sorted
1.00 – 2.00	Poorly sorted
2.00 – 4.00	Very poorly sorted
> 4.00	Extremely poorly sorted

2.5 Statistical Methods

2.5.1 Multivariate Analyses

The main objective of this study was to analyse patterns of spatial and temporal change in multi-species assemblages. Such assemblages result in complex arrays of data, which were analysed using multivariate statistical analyses. These are non-parametric analyses that allow one to deal with the simultaneous variation of two or more dependent variables (Underwood & Chapman

1998). Most multivariate analyses were conducted using the PRIMER V.5 (Clarke & Gorley 2001) statistical software. The analytical protocol involves a number of procedures for each analysis. Raw data were initially square-root transformed to increase the weighting of less abundant species (Field et al. 1982; Clarke & Ainsworth 1993). The similarity between pairs of samples was then assessed by constructing a triangular similarity matrix using the Bray-Curtis Similarity coefficient. This similarity matrix was then used to generate non-metric multi-dimensional scaling (nMDS) ordinations. This provides a robust method for graphically representing community structure (Clarke & Ainsworth 1993).

The merits of nMDS techniques for ordination are now well established in ecological applications and comparative studies have consistently demonstrated their reliability, particularly in benthic studies (Gray et al. 1990; Clarke & Ainsworth 1993). A 'stress' statistic in ordinations indicates the success achieved by the nMDS. Stress values < 0.2 are generally considered to indicate a successful plot. If the stress value of a two-dimensional ordination is $>$ 0.2, ordinations can be examined in the less stressful, and therefore more representative, three-dimensional version. In a few instances an nMDS ordination included a sample that contained zero counts (i.e. no fauna), which caused the plot to collaspse. In these cases the offending sample was subsequently excluded from the ordination so that the details of the relationships between the remaining samples could still be presented (Clarke et al. 2006).

Hypothesis testing can be conducted to test for differences between groups of samples, defined *a priori,* using analysis-of-similarity (ANOSIM). Analysis-of-similarity is a non-parametric permutation procedure and the test statistic (R) gives a measure of the degree of separation between groups of samples. The distinction between groups of samples increase as the statistic reaches its upper limit (+1); therefore, large R-values indicate a greater separation than smaller values (Clarke & Warwick 1994). The results provided by the ANOSIM procedure include pairwise *a posteriori* comparisons.

Where ANOSIM revealed significant differences, the species responsible for the differences between groups were identified using the similarity percentages (SIMPER) routine. This analysis gives a hierarchical list of the percentage contributions of individual species to the differences observed, and indicates the test group in which each species was most abundant.

Other multivariate analyses

By comparing patterns of distribution among different variables, insights can be gained into the processes that determine their distributions. These can be tested experimentally. In the case of large-scale patterns, however, experimental testing of derived models is often impossible for practical reasons. In such cases, correlations among patterns for different variables may be as close to demonstrating cause and effect as is feasible (Morrisey et al., 1998). BIOENV is a method for correlating the patterns observed in species assemblages with the patterns in arrays of physico-chemical and other environmental data. This procedure calculates which groups of variables best correlate with the biological data.

In a similar manner, the RELATE routine determines the degree of concordance between two matrices of species abundance data, each from a matching set of sites or times. This particular routine is detailed further in the relevant chapter (Chapter 7).

When required, the MVDISP routine in PRIMER was used to calculate multivariate dispersion indices (IMD values) within and between the sample groups of a similarity matrix. These indicate the relative dispersion between sample groups. Thus, an IMD value close to +1.0 indicates samples are dispersed or dissimilar and, IMD values approaching –1.0 indicate a greater similarity between samples.

The PRIMER V.5 software is limited in that it does not deal comprehensively with complex designs where more than two factors are being investigated; in particular, there is no assessment of the interaction terms between multiple factors. As interaction terms are often of key ecological importance, this may lead to an incomplete interpretation of results. A recent programme that does address these is PERMANOVA (Anderson 2005), which uses a permutational multivariate analysis-of-variance for hypothesis testing and pairwise *a posteriori* comparisons. However, this programme is itself limited in that it is only compatible with symmetrical designs. Therefore, PERMANOVA was only used when the study design permitted. PERMANOVA results are presented with a choice of two *p*-values, the permutational and the Monte Carlo. The permutational *p*-value is the one most commonly used but, when there are very few possible unique permutations, the Monte Carlo should be referred used (Anderson 2005).

2.5.2 Univariate Analyses

Univariate analysis-of-variance (ANOVA) was used throughout the study, primarily to assess differences in the univariate community variables, species richness (*S*) and the total number of individuals (*N*), as well as to test for differences in physico-chemical variables. The design of ANOVAs varied throughout the study according to the design of each experiment. These designs are detailed in the relevant section of each chapter. All univariate analyses were performed using MINITAB v 13.1 (Minitab 1996) statistical software. Levene's test was used to test for homogeneity of variances and, where necessary, data were appropriately transformed to normalise variances (Sokal & Rohlf 1981). Where ANOVA revealed significant results, planned multiple pairwise comparisons among means were conducted using Tukey's test.