Coastal Responses to Estuarine Discharge from Intermittently Open Systems

by

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Abstract

Estuarine discharge from permanently open systems can make a significant contribution to the productivity of nearshore marine environments, through the delivery of nutrients and organic matter into offshore waters. In southern Australia, small intermittently open estuaries are very common; however we know very little about their ecology or the importance of links between intermittent systems and nearshore marine ecosystems. This PhD thesis is the first study to report on coastal responses to estuarine discharge from intermittent systems. The aim of this study was to detect the influence of estuarine discharge from intermittently open estuaries on a subset of coastal responses of nearshore coastal environments.

In Chapter 2, I examined the physico-chemical environment within and outside estuary mouths (i.e. estuary swash), the frequency of mouth closure and changes in estuary water height in four intermittently open estuaries in south-west Victoria (Curdies, Barham, Skene and Kennett River estuaries); observations were made over a 12 month period. Additionally, this chapter examined the response of coastal sediments to estuarine discharge by comparing microphytobenthic chlorophyll a concentrations and sediment organic matter within and outside each estuary over a the 12 month period and specifically tested the effect of artificial mouth openings and natural mouth closures. I compared stable isotope signatures (δ¹³C and δ¹⁵N) of the sediment within and outside an estuary before and after an artificial mouth opening, to identify whether terrestrial or estuarine sources of organic matter are being deposited into coastal sediments. I also quantified water column nutrients within and outside a single estuary to determine whether increased nutrient concentrations are being discharged into coastal waters at Curdies estuary.
Chapter 2 provided evidence that discharge of estuarine waters caused significantly changes to physico-chemical variables of adjacent coastal waters, with water temperature and salinity declining, while dissolved oxygen levels increased in the estuary swash following the artificial estuary mouth opening. The artificial estuary mouth opening also delivered nutrient rich estuarine water to the coastal zone, with concentrations increasing several fold in the adjacent coastal waters. Additionally the artificial opening of the estuary mouth caused a decline in microphytobenthic chlorophyll \( a \) concentration at the estuary swash, while sediment organic matter showed no change in concentration. The natural closure of estuary mouths resulted in no change in microphytobenthic chlorophyll \( a \) or organic matter concentration at the estuary swash. Furthermore, stable isotope analyses of Chapter 2 could not identify a change in terrestrial/estuarine sources of sediment organic matter throughout the catchment following the artificial mouth opening of the Curdies estuary, due to concentrations of sediment organic matter being well below the detection limit of the instrument for most of the sampling locations.

Chapter 3 expanded on the results from Chapter 2, examining the influence of estuarine discharge on coastal sediment productivity in greater detail, by assessing microbial utilisation of carbon sources as well microphytobenthic chlorophyll \( a \) and sediment organic matter during two separate mouth events: an artificial mouth opening and a separate natural flood both occurring at two intermittently open estuaries, Curdies and Anglesea. Sampling occurred one week before and at one and nine weeks after both mouth events. Significant temporal changes were detected in coastal responses to the artificial mouth opening, with microphytobenthic chlorophyll \( a \) concentrations decreasing at the estuary swash of Curdies estuary, while at Anglesea chlorophyll \( a \) concentrations increased after the artificial mouth opening. Additionally, there was no change in organic matter content of the estuary swash following the artificial mouth opening. The flood event resulted in an increase in microphytobenthic chlorophyll \( a \) at the estuary swash at both Curdies and Anglesea estuaries, one week post flood. Only Anglesea showed an increase in
organic matter in the estuary swash after the flood. At Curdies, the microbial utilisation of different carbon sources changed after both mouth events; with bacteria at the estuary mouth and estuary swash using similar carbon sources at one and nine weeks post mouth events. At Anglesea, bacteria utilised different carbon sources between locations, but not across time during the artificial mouth opening. During the flood at Anglesea, the greatest difference in carbon source utilisation was seen at the estuary swash before and after the flood, however this difference had disappeared nine weeks post flood. Results of this chapter showed that changes in microbial communities in the estuary swash was detected following the release of estuarine discharge.

Chapter 4 tested coastal response to estuarine discharge further, by comparing macroalgal assemblage structure and recruitment and stable isotope signatures in intertidal mussels *Austromytilus (Brachidontes) rostratus*, between rocky shores exposed to estuarine discharge and remote rocky shores distant from any estuarine influence. Results of Chapter 4 identified that estuarine discharge from intermittently open estuaries influenced macroalgal assemblage structure and recruitment of algal species living on rocky shores adjacent to estuary mouths, but the effects were spatially and temporally variable. The rocky shore exposed to discharge from the Curdies estuary had higher algal cover of established assemblages, while the other estuary shores of Barham, Skene and Kennett showed no difference from controls. Similar spatial variability was shown for algal cover of recruits, where rocky shores exposed to Barham and Skene estuarine discharge exhibited higher recruitment of macroalgae cover compared to their respective control shores during the months of June-August, whilst Curdies estuary shore showed no difference and Kennett estuary shore exhibited lower recruitment compared to their control shores. Species richness of established and recruited macroalgae was the same or greater at rocky shores exposed to estuarine discharge compared to control shores. The abundance of opportunistic ephemeral taxa such as *Ulva* spp. was greatest at rocky shores exposed to estuaries, presumably benefiting from increased nutrient loads, however *Ulva* spp. did not recruit onto artificial
substrata. Additionally, chlorophyll $a$ and organic matter content of recruited algae were greater at estuary rocky shores, with concentrations increasing over time (8 weeks). For most estuaries, intertidal mussels exposed to estuarine discharge were significantly depleted in $\delta^{13}$C and more enriched in $\delta^{15}$N compared to control shores, suggesting that $A. rostratus$ in areas adjacent to estuaries are assimilating terrestrial/estuarine organic material that is being supplied by estuarine discharge.

All field sampling and experiments were conducted between 2006 and 2008, which coincided with a period of drought that was experienced by most of southern Australia since 1997, with significantly reduced river flows. The ability to detect an influence of estuarine discharge for several variables is significant, given that a greater effect is likely to be observed when river discharges follow the longer term averages. Together, the results of the present study suggest that just like river discharge from permanently open systems, estuarine discharge of small intermittently open systems is an important driver for the productivity of nearshore marine environments. Furthermore, intermittent systems are likely to be making a significant contribution to coastal productivity in a variety of other coastal habitats, including the pelagic zone, soft-sediments and rocky shores.
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CHAPTER 1

1. General Introduction

1.1. Estuaries

Estuaries are dynamic environments and provide important nursery habitats for several fish species as well as feeding grounds for many birds (Grimes & Kingsford 1996, Dagg & Breed 2003, Smith & Hindell 2005, Becker 2007, Botto et al. 2011). They are highly productive ecosystems and act as the transition zone between marine and riverine environments (Levin et al. 2001, Gillanders 2007). There are numerous formal definitions for an estuary (Pritchard 1967, Day 1980, Fairbridge 1980, Elliott & McClusky 2002, McClusky & Elliott 2007, Tagliapietra et al. 2009, Potter et al. 2010). The definition which best describes estuaries of southern Australia is by Potter et al. (2010, page 499), who defines an estuary as “a partially enclosed coastal body of water that is either permanently or periodically open to the sea and which receives at least periodic discharge from a river(s), and thus, while its salinity is typically less than that of natural sea water and varies temporally and along its length, it can become hypersaline in regions when evaporative water loss is high and freshwater and tidal inputs are negligible”. Estuaries can be permanently or intermittently open to the ocean, depending on wave action, movement of tides and river flow (Day 1980, Fairbridge 1980, Whitfield 1992, Perillo 1995, Kench 1999, Roy et al. 2001). Intermittently open estuaries are common in the southern hemisphere particularly in southern Australia (Kench 1999, Roy et al. 2001, Sherwood et al. 2008), but also in southern Africa (Whitfield 1992, Cooper et al. 1999, Kench 1999, Perissinotto et al. 2000, Snow & Adams 2007, Froneman & Henninger 2009) and South America (Smith & Demaster 1996, Kench 1999).

Tidal exchange within intermittently open estuaries and the ocean is possible when the mouth is open or when marine water washes over the sandbar during
overtopping events, usually during spring high tides or winter storms (Kemp & Froneman 2004). Periods of prolonged mouth closure limit the exchange of nutrients and organic material between the ocean and the estuary, and can lead to the alteration of the physico-chemical environment, as well as increased water levels, inside the estuary. In addition, flora and fauna living inside the estuary have to cope with a stressful environment, and potentially deal with lower salinities and low dissolved oxygen concentrations, which can develop during extended periods of mouth closure (McComb & Humphries 1992, Sherwood & Rouse 1997, Whitfield & Bate 2007, Sherwood et al. 2008).

Estuaries have a number of sources of photosynthetic primary production including phytoplankton, benthic diatoms, seagrasses, various algal mats and surrounding mangroves and saltmarshes (Teal 1962, Paterson & Whitfield 1997, Adams et al. 1999, Cahoon 1999, Froneman 2002, 2006). However, these producers are not the only sources of organic material within the estuary. Estuaries act as sinks for organic material, nutrients and sediment and receive inputs from rivers (from upper catchment and agricultural run-off), groundwater inflow, direct precipitation and runoff (Dame 1993, Schlacher & Wooldridge 1996, Bate & Adams 2000, Gillanders & Kingsford 2002, Gillanders 2007). They also receive marine inputs (e.g. wrack) which are driven into estuaries predominantly by tides, waves and wind (Dame 1993, Levin et al. 2001) and some from overtopping of the sandbar during periods of mouth closure (Froneman 2002, Froneman 2004, Kemp & Froneman 2004, Riddin & Adams 2010). Additionally, microbial primary producers such as bacteria play a significant role in primary production and in the recycling of nutrients in all aquatic ecosystems (Cho & Azam 1990, Smith & Hall 1997), including estuaries (Pace & Cole 1994, Calbet & Landry 1999, Dagg & Breed 2003, Dagg et al. 2004, Allan & Froneman 2008). The recycling of organic material provides an important link between dissolved organic matter and higher trophic levels (Pace & Cole 1994, Allan & Froneman 2008), where available nutrients are remineralised and bio-available for the consumption by higher trophic levels (Pace & Cole 1994, Dagg & Breed 2003).
Primary productivity of estuaries is very high, mainly due to these freshwater inputs of nutrients and particulate organic matter of terrestrial origin (Schlacher & Wooldridge 1996, Bate & Adams 2000, Gillanders & Kingsford 2002), which promote the growth of phytoplankton and recycling of nutrients (Cho & Azam 1990, Mallin et al. 1993, Smith & Hall 1997, Adams et al. 1999, Calbet & Landry 1999, Schlacher et al. 2008, Botto et al. 2011). Freshwater inputs eventually leave estuaries (Levin et al. 2001, Gillanders 2007), and enter nearshore marine environments through estuary and river discharge that is released into offshore waters, when the mouth is open, in the form of a plume. The plume delivers sediments, nutrients and organic material to surrounding coastal waters and is the critical link between estuarine and marine environments (Grimes & Kingsford 1996, Schlacher et al. 2008). The size and water composition of the plume can change within a few hours and varies greatly, depending upon river discharge and marine currents (Fairbridge 1980, Harding 1994, Devlin et al. 2001, Roy et al. 2001, Dagg & Breed 2003, Darnaude et al. 2004).


Interestingly, bacterial carbon biomass represents an important carbon source for planktonic food webs, due to bacterial biomass being two to three times greater than phytoplankton biomass within estuaries (Allan & Froneman 2008) and in low productivity areas of the world’s oceans (Cho & Azam 1990, Smith

1.2. Drivers of primary productivity in coastal ecosystems

Light, nutrients, water movement and vertical mixing are all critical factors that drive primary production in estuary ecosystems, and in many cases water movement can influence the amount of light available for primary production (Adams et al. 1999, Froneman 2002, Nybakken & Bertness 2005, Dittmann 2007, Kyewalyanga et al. 2007, Snow & Adams 2007, Anandraj et al. 2008, Storm et al. 2010). Phytoplankton and microphytobenthos require light energy to carry out photosynthesis, which in turn allows these algae to grow and reproduce (Kiefer & Strickland 1970, MacIntyre & Cullen 1996, Waite & Suthers 2007). Productivity increases in areas experiencing high light conditions (Alpine & Cloern 1988, Barranguet et al. 1998, Adams et al. 1999, Perkins et al. 2001, Carmack et al. 2004, Anandraj et al. 2008), in particular in the surface layer of the water column or top few millimetres of the sediment (MacIntyre et al. 1996, Dittmann 2007), where light penetration is at its highest (Barranguet et al. 1998), while in areas of limited light, productivity declines (Cadee 1978, MacIntyre et al. 1996, Barranguet et al. 1998, Adams et al. 1999, Perkins et al. 2001, Anandraj et al. 2008, Storm et al. 2010). Light variability experienced by primary producers is caused by external forces such as daily and seasonal variations in light intensity, or through physical characteristics of the water column such as water mixing. For example, when tidal and wind mixing occurs or in areas where river and estuarine inputs are released, benthic microalgae and sediment are resuspended into the water column, not only increasing productivity of pelagic waters (MacIntyre & Cullen 1996), but it also increases turbidity, which reduces light penetration and therefore limits

Temperature is an important factor that influences primary productivity of microphytobenthos and bacterial growth (Blanchard et al. 1996, Guarini et al. 1997, Perkins et al. 2001, Allan & Froneman 2008, Anandraj et al. 2008). Similar to light, at low temperatures productivity is at its lowest, however soft sediment studies have shown that there is an optimal sediment temperature at which primary productivity of microphytobenthos is at its greatest, and declines above this temperature (Blanchard et al. 1996, Guarini et al. 1997, Perkins et al. 2001, Morris & Kromkamp 2003, Anandraj et al. 2008). The optimum temperature depends on a number of factors such as season, algal community structure and the overlying water temperature (Blanchard et al. 1996, Guarini et al. 1997, Perkins et al. 2001, Morris & Kromkamp 2003). Bacterial growth shows similar effects to temperature: lower temperatures during cold winter months have been shown to limit bacterial growth; however with elevated temperatures (Allan & Froneman 2008) reaching temperatures greater than 14°C, bacterial growth appears to be dependent on factors such as carbon substrate and nutrient availability and bacteria’s interaction with other microorganisms (e.g. bacterivores) (Scavia & Laird 1987, Ameryk et al. 2005, Allan & Froneman 2008).

1.3. Sources of nutrients to coastal waters

Globally, coastal upwelling has been shown to be an important source of nutrients to the nearshore marine environment (Middleton & Platov 2003, Middleton & Bye 2007, Patti et al. 2008, van Ruth et al. 2010). Upwelling brings cold nutrient-rich water from great depths into surface waters, resulting in high rates of primary productivity (Kingsford 1995, Middleton & Platov 2003, Ribeiro et al. 2005, Middleton & Bye 2007, Patti et al. 2008, Patti et al. 2010, van Ruth et al. 2010). Sewage outfalls also act as a source of nutrients to coastal waters, by releasing nutrient-rich effluent into coastal waters (Smith 1996, Bellgrove et al. 1997). Through the release of nutrient-rich effluent, primary productivity rates are not only increased (Firstater et al. 2010), but the dynamics and structure of intertidal macrofauna and algal assemblages of rocky shore communities can also change (Borowitzka 1972, Brown et al. 1990, Bellgrove et al. 1997, Hindell & Quinn 2000, Wear & Tanner 2007, Bellgrove et al. 2010). For example, rocky shores exposed to outfalls often exhibit algal assemblages that are dominated by just a few, opportunistic ephemeral taxa, and have reduced species richness compared to rocky shores.
1.4. The contribution made by permanently open estuaries and intermittently open estuaries as sources of nutrients for coastal waters

The coastal response to discharge of estuarine plumes has been studied for large permanently open river systems such as the Amazon and Mississippi (Smith & Demaster 1996, Lohrenz et al. 1999, Dagg & Breed 2003, Dagg et al. 2004, Lane et al. 2007, Lu et al. 2010). These large river systems can discharge high concentrations of nutrients and organic matter into the receiving waters, stimulating high levels of phytoplankton biomass and production within the plume (Harding 1994, Smith & Demaster 1996, Lohrenz et al. 1999, Rabalais et al. 2000, Dagg & Breed 2003, Liu & Dagg 2003, Dagg et al. 2004, Varela et al. 2005, Banaru et al. 2007, Lane et al. 2007, Lu et al. 2010). These high levels of phytoplankton production in the plume can in turn result in elevated zooplankton densities which are greater than in surrounding coastal waters (Govoni et al. 1989, Dagg & Govoni 1996, Grimes & Kingsford 1996, Kingsford & Suthers 1996, Dagg & Breed 2003, Dagg et al. 2004). Plume regions and nearshore water are dominated by zooplankton and larval fish grazing (Grimes & Kingsford 1996, Dagg & Breed 2003) and as such, these discharge areas are not only providing valuable food sources for higher trophic levels, but they are also enhancing fish recruitment in estuarine and marine habitats (Govoni et al. 1989, Grimes & Kingsford 1996). Additionally, studies on large plumes have also shown that the organic material being released from rivers is being assimilated through coastal food webs. For example, the diet of benthic invertebrates such as polychaetes, bivalves, brachyurans and shrimps living in the waters of the Mediterranean Sea reflect terrestrial particulate organic matter from the Rhone River (Darnaude et al. 2004). However, patterns for benthic fish were less clear, with depth distribution and diet composition playing a key role in determining their sensitivity to Rhone River floods (Darnaude 2005).
In Australia, there are many permanently open estuaries discharging into open coastlines (McComb & Humphries 1992, Kench 1999, Mondon et al. 2003, Radke et al. 2004, Gaston et al. 2006, Schlacher et al. 2008, Chuwen et al. 2009b, Connolly et al. 2009, Schlacher & Connolly 2009, Schlacher et al. 2009), however these estuaries and their respective plumes are significantly smaller than those studied elsewhere (Lohrenz et al. 1999, Dagg & Breed 2003, Dagg et al. 2004). For example, an annual discharge of the Amazon River, Brazil, is $6300 \times 10^6$ ML/yr (Dagg et al. 2004) and is several orders of magnitude greater than a single peak discharge event that occurred at a permanently open estuary in south-eastern Queensland (Mooloolah estuary) during the 2002/2003 season, discharging 1500 ML/day (Gaston et al. 2006).

Few studies have actually investigated the link between estuarine discharge and nearshore marine environments in these smaller systems, with most research being conducted on estuaries in northern Australia (Kingsford & Suthers 1994, Gaston et al. 2006, Ostrander et al. 2008, Schlacher et al. 2008, Schlacher & Connolly 2009, Schlacher et al. 2009). In south-eastern Queensland, research investigated plume traits and their influence on coastal productivity for two small permanently open estuaries, the Mooloolah and Maroochy Rivers, suggesting that the patterns of large river systems scale down to smaller systems in Australia (Gaston et al. 2006, Schlacher et al. 2008, Connolly et al. 2009, Schlacher & Connolly 2009, Schlacher et al. 2009). These studies have found that these estuaries are releasing nutrient rich plumes into adjacent coastal waters, with concentrations being several-fold higher than areas outside the plume (Gaston et al. 2006, Schlacher et al. 2008, Schlacher et al. 2009).

Similar patterns to those from larger river systems are evident for these smaller estuaries, with increased nutrient levels enhancing phytoplankton and zooplankton biomass production in the estuary plume (Schlacher et al. 2008, Schlacher et al. 2009). Additionally, these estuary plumes supply food sources for coastal benthic fisheries, with marine species such as bivalves, crustacean and at least one species of fish (flounder - *Pseudorhombus arsius*) assimilating terrestrial organic matter that is delivered by the nearby estuary (Connolly et al. 2009, Schlacher & Connolly 2009).
Intermittently open estuaries are common in southern Australia (Kingsford & Suthers 1994, Mondon et al. 2003, Haines et al. 2006, Sherwood et al. 2008), and South Africa (Froneman 2002, Perissinotto et al. 2002, Snow & Adams 2007) and are very different to permanently open systems, with much smaller catchments and hence, smaller river discharges. Furthermore, the river’s connections to the ocean are often disrupted during summer when low flows occur, contributing to the closure of the estuary mouths. These estuaries exhibit several physical, chemical and biological differences to their permanently-open counterparts in both northern Australia and the northern hemisphere (Potter & Hyndes 1994, Smith & Demaster 1996, Lohrenz et al. 1999, Potter & Hyndes 1999, Dagg & Breed 2003, Dagg et al. 2004). The major differences are: a) they are smaller, e.g. annual discharge of Amazon River, Brazil is $6300 \times 10^6$ ML/yr (Dagg et al. 2004), whilst that of Curdies River estuary, south-eastern Australia is $119 \times 10^3$ ML/yr; (Mondon et al. 2003); and b) intermittently open estuaries often become temporarily disconnected from the sea by the presence of a sandbar at the mouth, particularly during summer and autumn periods of reduced river flow (Eyre & France 1997, Cowley et al. 2001, Harrison 2004, Anandraj et al. 2008, Sherwood et al. 2008). Along the Victorian coastline in southern Australia, there are more than 100 estuaries, most of which are only intermittently connected to the ocean (Mondon et al. 2003). Despite the high numbers of intermittently open estuaries in Victoria, very little is known about their ecology and the contribution of estuarine waters to primary productivity of surrounding coastal ecosystems, or the extent of the potential effect of estuarine discharge on coastal soft sediment or rocky shore communities. This represents a significant gap in the knowledge required for understanding and managing intermittently open estuaries in southern Australia and the productivity of the receiving marine environment.
The present study measured a series of biological variables to detect the influence of discharge from intermittently open estuaries on nearshore marine environments. One variable used in this research were microphytobenthic chlorophyll $a$ as a surrogate for primary productivity. The research focused on the response of microphytobenthos rather than phytoplankton, due to previous estuarine studies reporting benthic chlorophyll $a$ levels to be one to three orders of magnitude higher than in the water column (de Jonge 1995, Perissinotto et al. 2000, Nozais et al. 2001, Perissinotto et al. 2002, Anandraj et al. 2008).

Sampling of phytoplankton from coastal waters was also impractical because of the wave-exposed nature of these shores. Secondly, sedimentary organic matter was measured as an indicator of organic matter exchange between the estuary and nearshore marine environment. Other variables measured during this study included microbial bacteria in sediments, macroalgal assemblage structure and recruitment and intertidal mussels of adjacent rocky shores. Bacteria and mussels were included to identify whether the influence of intermittent estuarine outflows could be detected in trophic levels higher than primary producers of the open coast.

1.5. Thesis aim and structure

The aim of this study was to determine the influence of estuarine discharge from intermittently open estuaries on a number of biological variables of nearshore marine environments. This was based on a test of the model that estuarine discharge provides an important source of nutrients and organic matter that contributes to coastal productivity. The hypotheses tested were that there would be a greater response to estuarine discharge of all biological variables at locations associated with estuarine discharge such as the estuary swash and estuary mouth compared to locations not influenced by estuaries. These hypotheses were tested using a sampling design that included samples taken before and after artificial mouth openings, natural flood events and mouth closures at multiple estuaries and associated control beaches in south west Victoria, Australia.
This PhD thesis consists of three data chapters. Chapter 2 describes the temporal and spatial variability in the physico-chemical environment within and outside estuary mouths, the frequency of mouth closure and changes in estuary water heights in four intermittently open estuaries in south-west Victoria. Observations were recorded monthly over a 12 month period. These observations provided background information on the dynamics of each estuary, which were used in later chapters to develop research questions to examine the response of coastal sediments to estuarine discharge by:

1) comparing microphytobenthic chlorophyll $a$ concentrations and sediment organic matter within and outside each estuary over the 12 month period, with particular focus on changes associated with artificial mouth openings and natural closures; and

2) comparing stable isotope signatures ($\delta^{13}C$ and $\delta^{15}N$) of the sediment within and outside an estuary before and after an artificial mouth opening event.

Chapter 2 also quantifies water column nutrients within and outside a single estuary to determine whether increased nutrient concentrations are being discharged into coastal waters.

Chapter 3 examines the influence of estuarine discharge on coastal sediment primary productivity in greater detail, by sampling microbial bacteria during two separate mouth events, an artificial mouth opening and a separate natural flood both occurring at two intermittent estuaries.

The final data chapter compared macroalgal assemblage structure and recruitment and stable isotope signatures in intertidal mussels between rocky shores exposed to estuarine discharge compared with associated control shores (= no estuarine discharge) (Chapter 4). Stable isotope analysis was used to determine whether intertidal mussels are assimilating terrestrial/estuarine nutrients.
This thesis is structured as a series of stand-alone research chapters in manuscript style, bound by this general introduction (Chapter 1) and a general discussion (Chapter 5). Consequently there is some repetition between research chapters. Chapter 3 has been published in Estuarine Coastal and Shelf Science (McKenzie et al. 2011) (Appendix 1).
CHAPTER 2

2. Influence of mouth status on the physico-chemical environment, water column nutrients and soft-sediment microflora within and outside four intermittently open estuaries

2.1. Introduction

Most estuaries in south-west Victoria, including those used in the present study, are classified as ‘seasonal’ or ‘intermittently open’ estuaries, which are common in temperate coastal regions of the southern hemisphere (Whitfield 1992, Cooper et al. 1999, Perissinotto et al. 2002, Mondon et al. 2003, Snow & Adams 2007, Sherwood et al. 2008, Froneman & Henninger 2009). The mouths of these estuaries close during periods of low summer flow, thereby cutting off connectivity between the estuary and the ocean (Eyre & France 1997, Perissinotto et al. 2000, Cowley et al. 2001, Froneman 2002, Barton & Sherwood 2004, Harrison 2004, Gladstone et al. 2006, Haines et al. 2006, Hastie & Smith 2006, Matthews 2006, Pope 2006, Allan & Froneman 2008, Anandraj et al. 2008). The timing and duration of mouth closure, as in other temperate regions of the southern hemisphere, are variable between and within estuaries and depend on rainfall river flow, tidal movement and the level of ocean wave exposure at the estuary mouth (Kench 1999). Mouth closure is caused by a process known as long-shore drift, which is common on high wave energy coastlines with low tidal movement (Kench 1999). Long-shore drift causes alongshore movement of large volumes of sand parallel to the coastline. Estuaries with low river discharge lack the ability to remove accumulating sand from the mouth, and over time, the deposited sand builds up and eventually blocks the mouth (Kench 1999, Froneman 2002). It is common for these types of estuaries to experience prolonged periods of mouth closure (Matthews 2006). Mouth closure can lead to local flooding of adjacent farmland, roads and public amenities, and therefore, these estuaries are sometimes artificially opened. Artificial opening of the mouth usually involves a trench being dug by
hand or machinery through the sandbar to release water into the ocean (Figure 2.1). This quickly reduces water levels and re-establishes connection between estuary and ocean (Barton & Sherwood 2004). Alternatively, mouths of seasonal and intermittently open estuaries can remain open if the erosion of the mouth caused by river flow, is greater than the amount of onshore deposition of sand (Schumann et al. 1999).

Figure 2.1 Artificial opening of the estuary mouth at Hopkins River estuary, south-west Victoria.
Discharge of these intermittent estuaries in south-west Victoria follows patterns associated with a Mediterranean climate, having cold wet winters and hot dry summers. River flow in the south-west region, has been well below the average annual flow during the last 14 years, due to a prolonged period of drought (Figure 2.2) (Matthews 2006, DSE 2010). A gauged river system nearby to the estuaries used in this study, the Hopkins River, recorded 210,000 ML between 2007/2008, making up only 33% of the average annual flow of 635,000 ML (DSE 2010). Most of the annual discharge in the south-west rivers occurs between July and November (Figure 2.2, 2.3a,b) (Newton 1996, Arundel 2003, Barton & Sherwood 2004, Matthews 2006). As a result, it is common for estuaries in this region to close during the summer and autumn months (January - May) and open during the wetter months of winter and spring (June - October). Along this stretch of coast, the movement of sediment is predominantly eastward during winter and spring, driven by prevailing south-westerly winds, currents and waves, causing the estuaries to be open (Bird 1993). However, during summer, movement of sediment is towards the shore due to south-easterly winds (Bird 1993) and, together with reduced river flows, depositing sediment at the estuary mouth commonly causes mouth closure during summer and autumn.
Figure 2.2 Average daily flow (ML/Day) of Barham River between (a) 1980-1996 (b) Low flow/drought years 1997-2009.
Figure 2.3 Daily flow (ML/Day) of (a) Curdies River measured at ‘Curdie’ station (Site code: 235203); (b) Barham River measured at East Branch (Site code: 235233) during the study period April 2007 – March 2008.
The ecology of estuaries in south-west Victoria is poorly understood, although the catchment characteristics for the Curdies, Barham, Skenes and Kennett river estuaries have been documented, together with some limited temporal scale physico-chemical and mouth status data (Arundel 2003, Mondon et al. 2003, Barton & Sherwood 2004, Barton 2006, Sherwood et al. 2008). There are however, no published studies that have examined the influence of estuarine discharge on nearby coastal areas for any intermittently open estuaries of southern Australia, which is surprising given the considerable contribution that estuaries are suggested to make to productivity of adjacent coastal waters (Gillanders & Kingsford 2002). The status of estuary mouths (i.e. open or closed) may influence the benthic communities directly by preventing exchange of freshwater/estuarine material with the marine environment, particularly during periods of low river flow that are associated with drought.

It is also important to identify whether terrestrial or estuarine organic material is being deposited throughout the catchment, particularly during periods of flood or during an artificial mouth opening event. An aspect of this study used stable isotope analysis, which is used to trace organic matter and nutrients of different origins through aquatic ecosystems (Peterson & Fry 1987, Wissel & Fry 2005, Perdue & Koprivnjak 2007, Bannon & Roman 2008). Stable isotopes can provide a measure of the contribution of terrestrial and marine material to the aquatic environment, because different organic matter sources display distinct isotope ratios according to the site of production (Peterson & Fry 1987). Nitrogen stable isotopes are often used to investigate anthropogenic influences on aquatic ecosystems due to elevated signatures caused by sewage and agricultural run-off (Cabana & Rasmussen 1996). Many catchments in south-west Victoria are used extensively for agriculture, which can potentially lead to estuaries receiving significant volumes of surplus fertilizer and animal excrement. Furthermore, estuaries often receive human effluent discharge, and as such, can exhibit elevated nitrogen signatures (Cabana & Rasmussen 1996, Savage & Elmgren 2004, Cole et al. 2006, Fair & Heikkoop 2006, Kroeger et al.)
Isotopic signatures of nitrogen vary as a result of the effects of changes in catchment land practices, particularly urbanization with high population densities being drawn to coastal areas and through agricultural practices (McClelland et al. 1997, Valiela et al. 1997, Schlacher et al. 2005). For carbon isotope signatures, distinct terrestrial and marine signatures arise from differences in photosynthetic pathways or by plants obtaining carbon from air rather than water (Peterson & Fry 1987, Schlacher & Connolly 2009). By distinguishing the different sources of organic matter in the sediment throughout the catchment and adjacent coasts, the study can identify whether terrestrial or estuarine organic material is being deposited downstream and released into coastal areas, and therefore available as a potential food source for consumers.

As described in Chapter 1, the aim of this thesis is to explore coastal response to estuarine discharge by testing the model that estuarine discharge provides an important source of nutrients and organic matter for soft sediment assemblages in the adjacent receiving environment. Soft sediment assemblages are especially difficult to examine on these high energy coastlines particularly for macroinvertebrate fauna within the swash, which were rare or absent in the study area (J. McKenzie unpublished data). Therefore, this study focused on floral assemblages such as microphytobenthic chlorophyll $a$ and organic matter being measured. This chapter describes the first series of sampling used to test this model using four intermittently open estuaries in south-west Victoria including the Curdies, Barham, Skenes and Kennett River estuaries.

Specifically, this chapter consists of the following components: 1) description of the temporal and spatial variability in the physico-chemical environment within and outside each estuary mouth, the frequency of mouth closure and changes in estuary water heights during monthly observations over a 12 month period; 2) comparison of organic matter and microphytobenthic chlorophyll $a$ concentrations in sediments within and outside each of the four estuaries over a 12 month period and a test of the effect of artificial mouth openings and natural closures on the coastal environment; and 3) comparison of water column
nutrients and stable isotope signatures (δ¹³C and δ¹⁵N) of the sediment within and outside the largest of these four estuaries (i.e. Curdies) before and after an artificial mouth opening event.

2.2. Materials & Methods

2.2.1. Study Sites

The influence of estuarine discharge on surrounding sandy beaches was examined at four estuaries in south-west Victoria, and each estuary had an accompanying control beach. The four estuaries were the Curdies, Barham, Skenes and Kennett and four associated controls consisted of an open beach at the Bay of Islands, Shelly, Pools and Sugar Loaf, respectively (Figure 2.4). Estuaries were selected from a larger number of intermittent estuaries along the south-west Victorian coast, on the basis of the presence of an extensive sandy beach on either side of the estuary mouth and reasonably short daily travel time between sites (to avoid confounding differences between estuary and controls with time).

The Curdies estuary (142°52'46"E, 38°36'36"S) is part of a catchment covering an area of ~1100 km² (Figure 2.5) and is the third largest in Western Victoria, extending ~16 km (Table 2.1) (Sherwood et al. 2008). The estuary differs from the other three estuaries in this study by terminating into a shallow inlet, rather than discharging via a narrow channel (Figure 2.5). The widest section of the estuary is approximately 300 m. The second largest estuary in this study was the Barham (143°40'28"E, 38°45'52"S), which is part of a catchment covering an area of ~79 km² (Figure 2.6) (Sherwood et al. 2008). The other two estuaries, Skenes (143°42'35"E, 38°43'37"S) (Figure 2.7) and Kennett (143°51'43"E, 38°40'05"S) (Figure 2.8) are much smaller, with catchment areas of ~18 km² and ~21 km², respectively (Sherwood et al. 2008).
Control beaches were chosen so that they had similar wave exposures and sediment types to their associated estuary and were all situated at least 1 km west of the mouth away from any accompanying estuarine discharge (Figure 2.4). The Skenes control beach is actually located to the east of the estuary, due to limited beach access (high cliffs) and numerous freshwater drains discharging onto beaches to the west of the estuary (Figure 2.4). The control beaches were the Bay of Islands (142°52'10"E, 38°36'13"S Figure 2.6: Curdies control), Shelly (143°37'06"E, 38°47'39"S Figure 2.7: Barham control), Pools (143°45'20"E, 38°42'28"S Figure 2.4: Skenes control) and Sugar Loaf (143°47'15"E, 38°41'58"S Figure 2.4: Kennett control).
Figure 2.4 Map of the location of the four estuaries and their associate controls studied in south-west Victoria. To date, the fluvial and estuary catchment of Skenes has not been mapped.
Figure 2.5 Aerial photograph of the Curdies estuary; red symbols denote sampling locations, triangle is the estuary mouth; star is the estuary swash and circle is the control swash.
Figure 2.6 Aerial photograph of the Barham estuary; red symbols denote sampling locations, triangle is the estuary mouth; star is the estuary swash and circle is the control swash.
Figure 2.7 Aerial photograph of the Skenes estuary; red symbols denote sampling locations, triangle is the estuary mouth and star is the estuary swash. An aerial photograph of the control swash location is not available.
The four estuary catchments all have different levels of urbanization. The township of Apollo Bay surrounding the Barham catchment has the greatest population density of estuaries used in this study (Table 2.1). The Curdies has the biggest catchment area and is dominated by dairy farming (98 %) and has the second largest human population of the four estuaries (Table 2.1). Skenes and Kennett have the lowest number of permanent residences (Table 2.1), however tourist populations increase at all four estuary locations over holiday periods, particularly during the summer months. Caravan parks and camping grounds are located on all four estuaries. The control beaches have minimal urbanization compared to the estuaries.
Tides along the coastline of south-west Victoria are semi-diurnal and microtidal ranging by approximately 0.8 m during spring tides and 0.6 m during neap tides. For summer spring tides, the greatest tidal difference occurs at night, with the opposite occurring in winter with the greater difference occurring during the day. When present, the sand bar at each estuary mouth further reduces the tidal influence in the estuary.

Table 2.1 Characteristics of the four estuary catchments used during the present study

<table>
<thead>
<tr>
<th>Estuary</th>
<th>Catchment Area (km²)*</th>
<th>Estuary Length (km)*</th>
<th>Population Density (km²)*</th>
<th>Catchment Use* (%)</th>
<th>Beach Type**</th>
<th>Sediment size at mouth (phi units)***</th>
</tr>
</thead>
<tbody>
<tr>
<td>Curdies</td>
<td>1,124</td>
<td>16</td>
<td>4.39</td>
<td>Agriculture 93.1, Native Veg. 3.3, Forest 2.0, Urban 0.3, Other 1.2</td>
<td>R/LTT</td>
<td>1.5</td>
</tr>
<tr>
<td>Barham</td>
<td>79</td>
<td>2.2</td>
<td>5.12</td>
<td>Forest 56.2, Agriculture 35.7, Native Veg. 3.6, Other 4.5</td>
<td>I/LTT/TBR</td>
<td>1.5</td>
</tr>
<tr>
<td>Skenes</td>
<td>18</td>
<td>0.25</td>
<td>2.97</td>
<td>Agriculture 91.3, Forest 8.7</td>
<td>I/RBR</td>
<td>1.5</td>
</tr>
<tr>
<td>Kennett</td>
<td>21</td>
<td>1.2</td>
<td>1.31</td>
<td>Forest 72.0, Native Veg. 25.9, Other 2.0</td>
<td>I/TBR</td>
<td>1.5</td>
</tr>
</tbody>
</table>

2.2.2.  Spatial and temporal variability in estuary mouth status, water surface level, estuary flow and physico-chemical environment of estuarine discharge of four intermittent estuaries in south-west Victoria

To characterise the spatial and temporal variability in the physico-chemical environment within and outside each estuary mouth, the frequency of mouth closure and changes in estuary water heights, the four intermittent estuaries were sampled monthly from April 2007 - March 2008. The status of the estuary mouth and water surface levels were recorded, along with physico-chemical profiles being measured monthly from three sampling locations for each estuary: 1) estuary mouth; 2) the estuary outflow/ocean swash interface, hereafter referred to as estuary swash and 3) a single “control” beach (control swash) (Figure 2.4).

2.2.2.1.  Mouth status, water surface level and estuary flow of four estuaries

Mouth status (i.e. open versus closed) was recorded during each sampling period and additional observations were provided by local landowners or members of the Apollo Bay Kennett River Public Reserves Committee of Management: Curdies (H. Arundel), Barham (G. McPike), Skene and Kennett (G. Bolger).

Estuary water surface level is closely associated with the status of the estuary mouth. A closed estuary effectively acts as a dam leading to increased water volumes and increased surface areas. Rising waters eventually overtop the banks of the main channel and flood surrounding low lying ground. Water surface levels within the four estuaries were measured monthly from gauge boards calibrated to Australian Height Datum (AHD), located inside the estuary approximately 300 m from the estuary mouth.
Daily river flows for the Curdies (Site code: 235203) and Barham (Site code: 235233) estuaries were sourced from Victoria data warehouse website (www.vicwaterdata.net). There are currently no gauging stations throughout smaller catchments including Skenes and Kennett. Therefore, flow data was not available for these two smaller estuaries.

2.2.2.2. Physico-chemical parameters of four estuary mouths and receiving waters

Physico-chemical profiles were collected monthly at three randomly chosen sites within the estuary mouth, estuary swash and control swash locations. A Yeokal Intelligent Water Quality Analyser (Yeokal 611 Intelligent water quality analyser) was used to measure temperature (°C), salinity (psu) and dissolved oxygen (mg/L) at the surface and bottom water at the estuary mouth location and mid depth water was measured at the estuary and control swash locations. Physico-chemical profiles were conducted at water depths of 0.1 m at the surface and at 0.5 m for the bottom and mid depth (swash locations) profiles.

2.2.3. Temporal and spatial patterns of the influence of estuarine discharge on adjacent coastal sediment of four intermittent estuaries

To assess temporal and spatial variability in the influence of estuarine discharge on adjacent coastal sediments, the four estuaries were sampled monthly between April 2007 - March 2008. Sediment samples were collected for microphytobenthic chlorophyll a and sediment organic matter analyses. Sampling occurred at three sampling locations for each estuary: 1) estuary mouth; 2) estuary swash and 3) control swash (for example Figure 2.6). For the estuary mouth, the area sampled was on the edge of the main channel, where the channel begins to level out. A similar water depth of 0.5 m was sampled for both the estuary swash and control swash sites. For each of the three locations, a 10 x 2-m sampling area was haphazardly sampled. Sampling
of all locations for each estuary was conducted at low tide, within 4 hrs of each other.

The hypothesis tested for the temporal component of this study was that locations associated with estuarine discharge (e.g. estuary mouth and estuary swash) would have greater amounts of microphytobenthic chlorophyll a and sediment organic matter compared to the control swash and that this pattern would be consist over months and amongst estuaries.

2.2.3.1. Relationship between locations of estuarine discharge and microphytobenthos

Water content of benthic samples is an important factor influencing the effectiveness of the extraction of chlorophyll a from the sample (Snow et al. 2000). For the present study, an optimal sample size determined by Rodriguez (1993) was used, as it has been shown to be least affected by water content (Rodriguez 1993, Snow et al. 2000). Ten replicate benthic microalgal sediment cores, 1-cm long (30-mm diameter, 7.07-cm² area), were haphazardly collected at each location. Samples were stored in the dark on ice and frozen within 6 hrs of collection. Pigment extraction occurred within one month of sample collection. Chlorophyll a was extracted using acetone, following a modified method of Light & Beardall (1998). Technical details followed USEPA Method 446.0 (Arar 1997) for determining chlorophyll a by visible spectrophotometry. With this method, all samples were corrected for phaeopigments and thus measurements of chlorophyll a actually represent concentrations of all magnesium-containing pigments (Carlson & Simpson 1996).

Each sample was put into 35 mL of 100 % acetone, and mechanically mixed before being refrigerated at 4 °C for 24 hrs. Prior to reading, samples were shaken and then spun in a centrifuge at 1,000 rpm for 5 minutes. A sub-sample of 2.7-mL supernatant was diluted with 0.3 mL of distilled water to a final concentration of 90 % acetone in a 1-cm glass cuvette. Absorbance was read
on a Shimadzu UV-Visible Recording Spectrophotometer (UV-265, Shimadzu Corporation, Kyoto, Japan) at 750 nm and 664 nm prior to acidification, and 750 nm and 665 nm post acidification. Acidification to determine phaeophytin a was achieved by the addition of 0.1 N HCl (Arar 1997). Chlorophyll a concentrations were calculated following the equation of Lorenzen (1967).

2.2.3.2. **Effect of estuarine discharge on sediment organic matter**

There was no visual difference in sediment particle size between estuaries, but within locations sediment from estuary mouths appeared slightly finer compared to the estuary and control swash locations (pers. observ.). The same corer used to sample microphytobenthos was used to collect five replicate sediment cores 3-cm long, for organic matter content. Barham, Skenes and Kennett estuaries and their control shores were sampled on the same day, within 4 hours of each other at low tide. The large distance between estuaries meant that the Curdies and the control beach (Bay of Islands) were sampled on the following low tide. Organic matter was determined following a modified method from Light and Beardall (1998) where samples were initially dried at 60 °C for 24 hrs. After stabilising in a desiccator, 10 g of sediment was weighed to the nearest 0.0001 g then ashed in a muffle furnace at 550 °C for 3 h and loss of mass recorded. Total organic matter was determined from loss on ignition.
2.2.4. *Effect of artificial mouth openings and natural closures on coastal nutrients and concentrations of microphytobenthic chlorophyll a and sedimentary organic matter*

This aspect of the study tested the impact of estuarine discharge following an artificial mouth opening and a natural closure on the productivity of sandy beaches, focusing on surrogates of primary productivity which included water column nutrients (total nitrogen, total phosphorus, soluble phosphate, oxidised nitrogen and total organic carbon) and sediment characteristics (microphytobenthic chlorophyll a, organic matter content and stable isotope signatures). This study could not reliably assess the effects of estuarine discharge on secondary productivity due to preliminary sampling surveys showing that macroinvertebrate infauna were either rare or absent in the shallow subtidal zones of the high energy beaches onto which the estuaries discharged (J. McKenzie unpublished data). During April 2007 – March 2008, Curdies and Barham estuaries were artificially opened once and both naturally closed over once. In contrast, Skenes and Kennett remained open for the entire sampling period, and are therefore not examined here.

The following hypotheses were tested: 1) Microphytobenthic chlorophyll a concentrations and sediment organic matter within the microphytobenthos and water column nutrients of the adjacent estuary swash would increase in the adjacent coastal zone following an artificial mouth opening and exhibit similar concentrations to the estuary mouth; 2) Microphytobenthic chlorophyll a and sediment organic matter would progressively decrease over time in the adjacent coastal zone at periods subsequent to a natural closure of the estuary mouth; 3) Isotopic carbon and nitrogen sediment signatures would change throughout the catchment following an artificial mouth opening, resulting in freshwater and estuarine sources being deposited downstream.

To test for the effect of an artificial mouth opening and a natural mouth closure on coastal sediment of surrounding sandy beaches, data were collected during two separate mouth status events at Curdies and Barham estuaries: (1) *artificial*
mouth opening and (2) natural closure event. Samples were collected on three occasions associated with two mouth status events: (a) one month prior to mouth event (before mouth event), (b) one month after the mouth event (one month post) and (c) two months after the mouth event (two months post). The Curdies was artificially opened on the 14th July 2007; Barham 7th May 2007; natural closure of the mouths occurred on 18th January and 13th February 2008 for Curdies and Barham, respectively.

2.2.4.1. **Effect of artificial mouth openings and natural closures on concentrations of microphytobenthic chlorophyll a and sedimentary organic matter**

Sediment samples were collected for microphytobenthic chlorophyll a and organic matter at the same sampling locations, using the same sampling method as the temporal sampling described above (see Section 2.2.3 for sampling locations; 2.2.3.1 and 2.2.3.2 for sampling methods and analysis).

2.2.4.2. **Effect of estuarine discharge on nutrients of coastal waters**

The largest of the four estuaries (Curdies) and its control (Bay of Islands) were chosen to compare nutrient concentrations before and after an artificial mouth opening in July 2007 and to identify whether nutrient rich estuarine water is being discharged into adjacent coastal waters and available for uptake by microphytobenthos. Water samples were taken on three separate occasions (a) one month prior to artificial mouth opening in June 2007 (b) one week post opening and (c) two months post opening. The Curdies estuary was sampled because it has the largest catchment area and estuary length (Table 2.1), and is most likely to exhibit differences in nutrient concentrations between sampling periods, even during periods of low flow.

At the estuary mouth, three replicates of top and bottom water were collected. Three replicates of mid-depth (0.5m) water were collected from the estuary swash and control swash locations. Samples were transported on ice, filtered
within 48 h of collection and frozen until analysed. Samples were tested for total nitrogen (TN), total phosphorus (TP), soluble phosphate (SRT), oxidised nitrogen (NOX) and total organic carbon (TOC) within three weeks of collection. Samples were processed by the NATA Accredited (No: 2457) Water Quality Laboratory, Deakin University, Warrnambool using in-house methods (TN: WQL-05; TP: WQL-07; SRT: WQL-06; NOx: WQL-04; TOC: EP005).

2.2.4.3. Inputs of terrestrial/estuarine organic matter to coastal sediment

The Curdies estuary was also targeted for a subsequent study during winter 2008 (June-August), to test whether the upper catchment (freshwater) was supplying the lower catchment and estuary with organic matter and in turn making it available for the coastal zone. During winter 2008, water surface levels increased which caused local flooding that inundated farmland and public roads, resulting in artificial opening of the mouth. This provided an opportunity to further investigate the influence of estuarine discharge on coastal sediments by identifying whether there is a change in the source of organic matter in the sediment from the upper catchment into the estuary, and whether these terrestrial or estuarine sources of organic matter are being deposited into the coastal sediment. I hypothesised that after an artificial mouth opening, carbon and nitrogen isotope signatures in coastal sediments would change to reflect fresh estuarine or terrestrial sources. Carbon and nitrogen signatures were measured in the sediment, before (one day: 29th July 2008) and after (three weeks: 22 August 2008) an artificial mouth opening (30th July 2008). Sampling occurred at seven locations within the Curdies catchment (Figure 2.10).

Five surface sediment (5-cm deep) samples were collected with a PVC corer (63-mm diameter) at the seven locations throughout the Curdies catchment: (1) Freshwater (upstream of bridge at Timboon), (2) upper estuary (upstream north-east of Boggy Creek bridge), (3) lower estuary (inside estuary mouth), (4) estuary swash east, (5) estuary swash west, (6) control swash east, (7)
control swash west (Figure 2.9). The samples were stored frozen until they were thawed and dried in oven for 24-48 hrs at 50-60 °C. Once dried, samples were put through a series of sieves (1 mm, 500 μm, 250 μm, 125 μm and 63 μm) to remove large debris and animals. Sediment <125 μm in size was ground to a fine powder, with half of the sample being placed in vials for nitrogen analysis, whilst the remainder was prepared for carbon analysis with sediment being treated with 1 M HCl to remove carbonate material (Meksumpun et al. 2005, Ogrinc et al. 2005). Carbon samples were rinsed with deionised water, dried, ground and put into vials. All samples were transported to an external laboratory at Isotope Analytical Facilities of Flinders University, Adelaide. Stable isotope analysis was conducted on all samples on an automated Isoprime (GV Instruments, Manchester, UK) isotope-ratio mass spectrometer. Stable isotope ratios are expressed in parts per thousand (‰) using the standard delta (δ) notation: δX (‰) = [(Rsample/Rstandard) -1] x1000; where X is δ13C or δ15N, and R is the 13C/12C (carbon) 15N/14N (nitrogen) ratio in the sample and standards respectively. Isotopic standards used are referenced to Pee Dee Belemnite equivalent for carbon and the IAEA international standard of atmospheric N2 for nitrogen.
Figure 2.9  Map of the Curdies estuary, location of sites throughout catchment sampled for sediment stable isotope analysis (1) Freshwater; (2) Upper estuary; (3) Lower estuary (inside estuary mouth); (4) Estuary swash east; (5) Estuary swash west; (6) Control swash east and (7) Control swash west.
2.2.5.  Statistical Analysis

2.2.5.1.  Temporal and spatial patterns of the influence of estuarine discharge on adjacent coastal sediment of four intermittent estuaries

Assumptions of normality and homogeneity of variance of the data were checked using histograms and residual plots (Quinn & Keough 2002) before all univariate Analysis of Variance (ANOVA). Sediment chlorophyll $a$ were log $(x+1)$ transformed to meet these assumptions. All four estuaries were tested for differences in chlorophyll $a$ and organic matter throughout the 12 month sampling period; three-way ANOVAs were used in which Time (twelve levels: months April 2007-March 2008), Estuary (four levels: Curdies, Barham, Skenes and Kennett) and Location (three levels: estuary mouth, estuary swash and control swash) were treated as fixed factors. Where results showed significant interactions, two-way ANOVAs with simple main effects were used for each estuary to test for differences between months and sites (Quinn & Keough 2002). Statistical analyses were performed using SYSTAT version 11. All hypothesis tests were conducted at the 0.05 significance level.

2.2.5.2.  Effect of artificial mouth openings and natural closures on coastal nutrients and concentrations of chlorophyll $a$ and sedimentary organic matter

Assumptions of normality and homogeneity of variance of the data were checked using histograms, and residual plots (Quinn & Keough 2002) before univariate Analysis of Variance (ANOVA). Sediment chlorophyll $a$ and organic matter data were log $(x+1)$ transformed to meet these assumptions of ANOVA. The two estuaries Curdies and Barham were analysed separately to test for differences in chlorophyll $a$ and organic matter; two-way ANOVAs were used in which Time (three levels: one month before mouth opening/closing event, one month post event and two months post event) and Location (three levels: estuary mouth, estuary swash and control swash) were treated as fixed factors. Two planned contrasts (Quinn & Keough 2002) were
used to test whether the magnitude of the difference between estuary mouth and estuary swash was consistent: (1) before event (T1) compared to one month after event (T2); and (2) before event (T1) compared to two months after event (T3). Similar planned contrasts were tested comparing estuary swash and control swash.

For Curdies nutrient data, each nutrient was analysed separately to test for differences in water column nutrients between sampling times and locations: Two-way analyses of variance (ANOVA) were used in which Time (three levels: one month before mouth opening, one week post mouth opening and two months post mouth opening) and Location (three levels: estuary mouth, estuary swash and control swash) were treated as fixed factors. Two planned contrasts (Quinn & Keough 2002) were also included to test whether the magnitude of the difference between estuary mouth and estuary swash was consistent: (1) before event (T1) compared to one month after event (T2); and (2) before event compared to two months after event (T3). Similar planned contrasts were tested comparing estuary swash and control swash.

Carbon and nitrogen stable isotope signatures were below the detection limit of the instrument for sediment samples from five of the seven locations: lower estuary (post opening only), the estuary swash (east and west) and the control swash (east and west), therefore no statistical analysis was conducted on these locations. The freshwater and upper estuary locations were analysed for differences in carbon and nitrogen stable isotope signatures between sampling times and locations: Two-way ANOVA compared Time (two levels: before opening, three weeks post opening) and Location (two levels: freshwater and upper estuary), which were both treated as fixed factors. Where results showed significant interactions, simple main effects were used for each location to test for differences between sampling times. Statistical analyses were performed using SYSTAT version 11. All hypothesis tests were conducted at the 0.05 significance level.
2.3. Results

2.3.1. Spatial and temporal variability in estuary mouth status, water surface level, estuary flow and physico-chemical environment of estuarine discharge of four intermittent estuaries in south-west Victoria

2.3.1.1. Mouth status, water surface level and estuary flow of four estuaries

The patterns of mouth opening and closure were very different for the four estuaries in this study (Figure 2.10). Curdies and Barham estuaries showed similar patterns, with both mouths being closed at the start of the sampling period during early winter and late autumn (2007), respectively. Closure of the mouths resulted in water levels rising, reaching a peak during June (Curdies) and May 2007 (Barham) (Figure 2.10a, b). This rise in water level resulted in both estuary mouths being artificially opened (Curdies 14th July 2007, Barham 7th May 2007). Water levels decreased following the openings and then fluctuated during late winter and spring (Figure 2.10a, b). During summer similar patterns were seen at Curdies and Barham with both estuary mouths closing over, resulting in water levels increasing again (Figure 2.10a, b). Different patterns were seen at the smaller estuaries, Skenes and Kennett, with their mouths remaining open for the entire sampling period (Figure 2.10c, d). Temporal patterns in water levels were similar at Skenes and Kennett estuaries, with levels peaking in late autumn and early winter and declining during late spring and summer (Figure 2.10c, d).

The present study coincided with a prolonged period of drought in southeastern Australia that began in 1997 (Figure 2.2). Since that time river flow has been well below the long term average (Figure 2.2) (Matthews 2006). Flow for the Curdies River was low over autumn and peaked during winter and spring (Figure 2.3a). During the study period, two large flood events occurred in August 2007 and November 2007, recording a flow similar to those previous to 1997, greater than 1400 ML/Day (Figure 2.2 and 2.3). Barham River had
lower flow compared to Curdies (Figure 2.3b), however Barham showed a
similar large flow event, peaking in November 2007. On the 3rd November
2007 there was a large rainfall event compared to what has previously been
experienced throughout the sampling period, resulting in extensive flooding at
all four estuaries. The extent of sand movement and flooding was evident with
the banks of the main channels being eroded and large volumes of woody
debris being washed down the catchment and deposited on estuary banks
(Figure 2.11).
Figure 2.10 Water level of the four estuaries during the sampling period of April 2007 – March 2008, (a) Curdies, (b) Barham, (c) Skenes and (d) Kennett. Solid black bars denote periods of mouth closure.
Figure 2.11 Aftermath of the November flood 2007 at Skenes estuary. (Top) Red arrow pointing to large debris spread along beach on estuary bank. (Bottom) Note erosion of estuary mouth and extent of debris.
2.3.1.2. **Physico-chemical parameters of four estuary mouths and receiving waters**

The estuary mouths, estuary swash and control swash showed similar patterns in temperature across all four estuaries. All locations followed seasonal trends with cold water temperature recorded in winter months, increasing during the summer months (Figure 2.12). Inside all estuary mouths, bottom waters were warmer than surface waters in summer and autumn whereas during winter and spring all estuaries except Kennett were well mixed (Figure 2.12a). After the opening of the Curdies and Barham estuary mouths, estuary water temperatures decreased and the estuaries became well mixed. Temperatures outside the estuaries also decreased and were similar to inside the estuary (Figure 2.12a, b).

The Curdies estuary was closed during the first few months of sampling and therefore salinity declined inside the estuary mouth with bottom water having slightly higher salinity compared to surface water (Figure 2.13a). When the estuary mouth was artificially opened during July 2007, salinity peaked and remained consistently high in the following months August to September 2007 (Figure 2.13a). During January 2008 the mouth closed over; however salinity levels remained relatively high during the following months (Figure 2.13a). Similar patterns in salinity were seen at Barham estuary with bottom water having higher salinity levels compared to surface water (Figure 2.13a). The highest salinity in the estuary mouth was recorded at the end of winter August 2007 (Figure 2.13a). Once the mouth of the Barham estuary was opened (May 2007), there were several spikes in salinity in the months following. Similar to Curdies, salinity increased inside the estuary mouth of Barham once the mouth was artificially opened, with marine water flushing into the mouth (Figure 2.13a). In late August and early September 2007 salinity declined at the Barham estuary mouth (Figure 2.13a, b). Both estuary mouths of Skene's and Kennett were heavily stratified, with the lowest salinity occurring during July 2007 and the highest in May 2007 for Skene's and during May 2007 and February 2008 at Kennett (Figure 2.13a).
Salinity in the Curdies estuary swash ranged between 30 – 40 psu for the months of April to June 2007 (Figure 2.13b). There was a sudden drop in salinity of 15 psu in July, which coincided with the artificial opening of the estuary mouth, where large amounts of freshwater discharged into adjacent marine waters (plume evident, pers. obs.). Two months after this large discharge event, salinity began to rise again (Figure 2.13b). Over spring there was strong mixing in the estuary swash at Barham, evident from low salinities recorded in September 2007 and then increasing to high salinities during summer when the mouth closed over (Figure 2.13b). Salinity in Skenes and Kennett estuary swash remained high throughout the sampling period between 32 - 38 psu, except for a decline in September and October 2007 following increased freshwater discharge into the coastal zone (Figure 2.13b). As expected the salinity of the control swash locations for all four estuaries remained relatively consistent throughout the sampling period, recording levels between 35 - 38 psu (Figure 2.13c).

Dissolved oxygen within the Curdies and Barham estuaries varied considerably throughout the study period and, like salinity, followed similar seasonal trends. Upon opening of both estuary mouths, dissolved oxygen concentrations within the estuary mouths and estuary swash locations increased (Figure 2.14a, b). Following this initial increase, dissolved oxygen levels slowly declined over winter, before concentrations peaked again during spring and summer (Figure 2.14a, b). Dissolved oxygen concentrations within the estuary mouth began to decline following the mouth closures of Barham and Curdies during January and February (Figure 2.14a, b). Both Curdies and Barham estuary swash increased in dissolved oxygen after the opening of the estuary mouths and during summer months (Figure 2.14b). The estuary mouth water columns of Skenes and Kennett were stratified with high levels of dissolved oxygen at the surface and low levels on the bottom (Figure 2.14a). Dissolved oxygen levels peaked inside both Skenes and Kennett estuary mouths during winter and summer (Figure 2.14a). The estuary swash and control swash locations of Skenes and Kennett estuaries showed similar trends, peaking in dissolved
oxygen during summer (Figure 2.14b, c). Similarly, dissolved oxygen concentrations of Curdies control swash remained consistent for most of the year, increasing during the summer months (Figure 2.14c), however Barham control swash had large fluctuations between winter and spring peaking in August and December (Figure 2.14c).
Figure 2.12  Temperature (°C) measured over the 12 month monitoring period of all four estuaries, columns represent different estuaries and rows represent locations (a) estuary mouth (b) estuary swash and (c) control swash. Bar under row (a) represents mouth status, solid black bar denotes mouth closure.
Figure 2.13 Salinity (psu) measured over the 12 month monitoring period of all four estuaries, columns represent different estuaries and rows represent different locations (a) estuary mouth, (b) estuary swash and (c) control swash. Bar under row (a) represents mouth status, solid black bar denotes mouth closure.
Figure 2.14 Dissolved oxygen (mg/L) measured over the 12 month monitoring period of all four estuaries, columns represent different estuaries and rows represent different locations (a) estuary mouth, (b) estuary swash and (c) control swash. Bar under row (a) represents mouth status, solid black bar denotes mouth closure.
2.3.2. Temporal and spatial patterns of the influence of estuarine discharge on adjacent coastal sediment of four intermittent estuaries

2.3.2.1. Relationship between locations of estuarine discharge and microphytobenthos

Differences in microphytobenthic chlorophyll $a$ between locations were not consistent amongst estuaries or times during the twelve month sampling period (estuary x location x time interaction: 3-way ANOVA, $F_{66, 1294} = 9.034, P < 0.001$). Additionally, for each estuary the differences in chlorophyll $a$ between locations were not consistent through time (location x time interaction) (Curdies 2-way ANOVA $F_{22, 1294} = 11.099, P < 0.001$; Barham 2-way ANOVA $F_{22, 1294} = 16.140, P < 0.001$; Skenes 2-way ANOVA $F_{22, 1294} = 9.319, P < 0.001$ and Kennett 2-way ANOVA $F_{22, 1294} = 9.106, P < 0.001$). For most months however, chlorophyll $a$ concentrations were higher at the estuary mouth locations compared to estuary swash and control swash for each estuary (Figure 2.15). Estuary mouths showed peaks in microphytobenthic chlorophyll $a$ during autumn and summer at Curdies and Barham, particularly during periods when the estuary mouth was closed (Figure 2.15a, b). While at Skenes and Kennett microphytobenthic chlorophyll $a$ concentrations were also high at the estuary mouth during autumn and summer, and concentrations remained high during early winter (Figure 2.15c, d). Patterns of temporal variability between the estuary- and control- swash locations across estuaries were inconsistent for all estuaries. For example, microphytobenthic chlorophyll $a$ concentrations were not consistently greater at the estuary swash compared with the controls (Figure 2.15).
Figure 2.15 Monthly mean ± SE chlorophyll $a$ of benthic samples collected at (a) Curdies, (b) Barham, (c) Skenes and (d) Kennett estuaries over twelve months from April 2007 – March 2008. Estuary mouth is white, Estuary swash is grey and control swash is black. Horizontal bar under figures represents estuary mouth status at point of sampling: black is closed, white is open. Months underlined on figures (a) and (b), highlights the months analysed.
2.3.2.2. Effect of estuarine discharge on sediment organic matter

Similar patterns to microphytobenthic chlorophyll $a$ were shown for sediment organic matter with differences being inconsistent amongst estuaries and times (estuary x location x time interaction: 3-way ANOVA $F_{66,568} = 7.448, P < 0.001$). For each estuary the differences in organic matter between locations were not consistent through time (location x time interaction) (Curdies 2-way ANOVA $F_{22,568} = 5.430, P < 0.001$; Barham 2-way ANOVA $F_{22,568} = 6.784, P < 0.001$; Skenes 2-way ANOVA $F_{22,568} = 7.194, P < 0.001$ and Kennett 2-way ANOVA $F_{22,568} = 3.305, P < 0.001$). For Curdies estuary, the amount of sediment organic matter was generally higher at the estuary mouth and estuary swash locations compared to the control swash, with a peak concentration occurring in October 2007 (Figure 2.16a). However, the patterns were less clear at the other estuaries. There were periods during summer at Barham and during autumn and spring at Skenes, where the control swash showed the highest concentration of organic matter compared to the other locations. Outside these times there was little variability between locations (Figure 2.16b, c). Similarly at Kennett, organic matter at the control swash showed similar concentrations to the estuary mouth and estuary swash throughout most of the sampling period (Figure 2.16d).
Figure 2.16 Monthly mean SE organic matter of benthic samples collected at (a) Curdies, (b) Barham, (c) Skenes and (d) Kennett estuaries over twelve months from April 2007 – March 2008. Estuary mouth is white, Estuary swash is grey and control swash is black. Horizontal bar under figures represents estuary mouth status at point of sampling: black is closed, white is open. Months underlined on figures (a) and (b), highlights the months analysed.
2.3.3. Effect of artificial mouth openings and natural closures on coastal nutrients and concentrations of microphytobenthic chlorophyll a and sedimentary organic matter

2.3.3.1. Effect of artificial mouth opening and natural closure on microphytobenthos

Following the artificial mouth opening at both Curdies and Barham estuaries, differences in microphytobenthic chlorophyll a concentrations between locations were not consistent through time (time x location interaction, Table 2.2a, b; Figure 2.17a, b). At both estuaries there were significant differences between estuary mouth and estuary swash before, compared to one month post opening and also compared to two months post opening at Curdies only (Table 2.2a, b; Figure 2.17a, b). However, in contrast to what was predicted, concentrations decreased at the estuary mouth and swash one month after the opening at both estuaries (Figure 2.17a, b), but increased two months post opening at Curdies (Figure 2.17a). Despite what was expected, there were no significant differences in chlorophyll a concentrations between estuary swash and control swash from before to one or two months post opening, with both locations having similar concentrations through time for Curdies estuary (Table 2.2). However, at Barham estuary, there was a significant difference between estuary swash and control swash from before to one month post opening but this was due to a decrease in chlorophyll a in the estuary swash post opening rather than the predicted increase at estuary swash (contrast 3, Table 2.2b; Figure 2.17b).

Following the natural mouth closure of both the Curdies and Barham estuaries, the differences in microphytobenthic chlorophyll a concentrations between locations were not consistent through time (time x location interactions; Table 2.2c, d; Figure 2.17c, d). The concentration of microphytobenthic chlorophyll a in the estuary mouth increased one month post closure at Barham (contrast 1, Table 2.2d; Figure 2.17d), but not until two months post closure at Curdies (contrast 2, Table 2.2d; Figure 2.17d). In contrast to predictions, there were no
changes in microphytobenthic chlorophyll a at the estuary swash following the
closure of the estuary mouth at both estuaries (contrast 3, $P > 0.05$, Table 2.2c, d; Figure 2.17c, d). However, there was an increase in microphytobenthic chlorophyll a at the Barham estuary swash two months post mouth closure (contrast 4, Table 2.2d; Figure 2.17d).
Table 2.2 Two-way ANOVAs comparing sediment chlorophyll \(a\) in the Curdies and Barham estuaries during an artificial mouth opening and a natural closure of the mouth. Planned contrasts were (1) Estuary mouth versus estuary swash before mouth event X estuary mouth versus estuary swash one month post mouth event, (2) Estuary mouth versus estuary swash before mouth event X estuary mouth versus estuary swash two months post mouth event, (3) Estuary swash versus control swash before mouth event X estuary swash versus control swash one month post mouth event, (4) Estuary swash versus control swash before mouth event X estuary swash versus control swash two months post mouth event. Data log(x+1) transformed.

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Figure 2.17 Monthly mean ± SE chlorophyll $a$ of benthic samples collected at (a) Curdies and (b) Barham estuaries during the artificial mouth opening and (c) Curdies and (d) Barham during the natural mouth closure. Estuary mouth is white, Estuary swash is grey and control swash is black. Horizontal bar under figures represents estuary mouth status at point of sampling: black is closed, white is open.
2.3.3.2. Effect of artificial mouth opening and natural closure on sediment organic matter

Organic matter content ranged between 0.19 – 0.25 g in the estuary mouth, 0.2 – 0.28 g in the estuary swash locations of Curdies, and 0.11 – 0.20 g in the estuary mouth and 0.11 – 0.40 g in the estuary swash of Barham during the artificial mouth opening and closure.

Following the artificial mouth opening of the Curdies estuary, the differences in organic matter between locations were consistent through time (Table 2.3a; Figure 2.18a). Despite what was expected, there was no increase in organic matter at the estuary swash following the opening of the estuary mouth (Table 2.3a; Figure 2.18a). However, at Barham estuary, the differences in organic matter between locations were not consistent through time (time x location interaction; Table 2.3b; Figure 2.18b). Organic matter in the estuary swash did not differ as predicted one month after the artificial mouth opening, however the lack of difference between estuary swash and control swash was not sustained through time, with organic matter significantly increasing at the estuary swash two months after the opening (Table 2.3b; Figure 2.18b).

Interestingly, and in contrast to what was predicted, there was significantly greater organic matter in the estuary mouth compared to the estuary swash, one month after the opening of the estuary mouth. This magnitude of change was not sustained over time, with no difference identified between estuary locations two month after the mouth opening (Table 2.3b; Figure 2.18b).

Following the closure of both estuary mouths at Curdies and Barham, the differences in organic matter between locations were consistent through time (Table 2.3c, d; Figure 2.18c, d), which was not what was expected. At the Curdies estuary, the greatest amount of organic matter was shown at both the estuary mouth and swash locations, with both locations having similar amounts of organic matter between times, while the control swash had the lowest amount of organic matter (Figure 2.18c). Similar results were shown at Barham estuary with the estuary swash and mouth locations having similar
amounts of organic matter, however control swash had the greatest organic matter across all sampling times (Table 2.3d; Figure 2.18d).
Table 2.3 Two-way ANOVAs comparing sediment organic matter (g) in the Curdies and Barham estuaries during an artificial mouth opening and a natural closure of the mouth. Planned contrasts were (1) Estuary mouth versus estuary swash before mouth event X estuary mouth versus estuary swash one month post mouth event. (2) Estuary mouth versus estuary swash before mouth event X estuary mouth versus estuary swash two months post mouth event. (3) Estuary swash versus control swash before mouth event X estuary swash versus control swash one month post mouth event. (4) Estuary swash versus control swash before mouth event X estuary swash versus control swash two months post mouth event. Data is log(x+1) transformed.

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60
Figure 2.18 Monthly mean (± SE) organic matter of benthic samples collected at (a) Curdies and (b) Barham estuaries during the artificial mouth opening and (c) Curdies and (d) Barham during the natural mouth closure. Estuary mouth is white, Estuary swash is grey and control swash is black. Horizontal bar under figures represents estuary mouth status at point of sampling; black is closed, white is open.
2.3.3.3. Effect of estuarine discharge on nutrients of coastal waters

At Curdies estuary, the concentration of soluble phosphate from all locations was very low and was below detectable limits (0.010 mg/L), and therefore this data is not presented. For all other nutrients, there were no significant differences in concentrations in top and bottom water samples from inside the estuary mouth, so concentrations were averaged across water depths. These samples are hereafter referred to as 'estuary mouth'.

Artificial mouth opening resulted in a pulse of nutrients to receiving water, however this was not sustained over time. Concentrations of total nitrogen, oxidised nitrogen and total organic carbon significantly increased in the estuary swash one week post mouth opening and concentrations were similar to the estuary mouth (Table 2.4; Figure 2.19). However, by nine weeks post opening, all estuary swash nutrients had depleted, and did not differ from the before mouth opening sampling period (Table 2.4; Figure 2.20). There was no change in total phosphorus concentration at the estuary swash at any time and concentrations were similar to the control swash (Table 2.4; Figure 2.19). Phosphorous concentrations at the estuary mouth did decline significantly after the opening of the estuary mouth (Table 2.4; Figure 2.19). The control swash showed little change through time for all nutrients (Figure 2.19). Similar patterns in nutrient concentrations were shown across all three locations through time, with all except total phosphorus having the highest concentration inside the estuary mouth, with the lowest concentrations occurring at the estuary swash and control swash, respectively (Figure 2.19).
Table 2.4 Two-way ANOVAs comparing water column nutrients at Curdies estuary during the artificial opening. Planned contrasts were (1) Estuary mouth versus estuary swash before mouth event X estuary mouth versus estuary swash one week post mouth event. (2) Estuary mouth versus estuary swash before mouth event X estuary mouth versus estuary swash nine weeks post mouth event. (3) Estuary swash versus control swash before mouth event X estuary swash versus control swash one week post mouth event. (4) Estuary swash versus control swash before mouth event X estuary swash versus control swash nine weeks post mouth event. Estuary mouth is the average of top and bottom water.

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Figure 2.19 Mean ± SE concentration of water column nutrients at Curdies estuary during the artificial mouth opening where (a) Total Nitrogen; (b) Total Phosphorus; (c) Oxidised Nitrogen and (d) Total Organic Carbon. Estuary mouth is white, Estuary swash is grey and control swash is black. Estuary mouth is the average of individual top and bottom replicates. Concentration of soluble phosphate at all sites was below detection limit, hence data has not been presented. Figures present untransformed data. Horizontal bar under figures represents estuary mouth status at point of sampling: black is closed, white is open.
2.3.3.4. Inputs of terrestrial/estuarine organic matter to coastal sediment

Following the opening of the Curdies estuary mouth the differences in carbon isotopes ($\delta^{13}C$) between locations (freshwater and upper estuary) were consistent through time (time x location interaction: 2-way ANOVA $F_{1,16} = 1.150, P = 0.299$). In contrast, the differences in nitrogen isotopes between the freshwater and upper estuary locations were not consistent between times (time x location interaction: 2-way ANOVA $F_{1,16} = 11.668, P = 0.004$), with sediments at the freshwater location being significantly more enriched in nitrogen prior to the artificial mouth opening compared to after mouth opening ($F_{1,16} = 32.562, P = 0.000$; Figure 2.20). However, this was not reflected in the upper estuary with no difference in sediment $\delta^{15}N$ occurring between times ($F_{1,16} = 0.767, P = 0.394$; Figure 2.20).
Figure 2.20 Mean ±SE (a) Carbon isotope signature ($\delta^{13}C$) and (b) Nitrogen isotope signature ($\delta^{15}N$) of benthic sediment from different locations along the Curdies catchment before (Δ) an artificial mouth opening and one month post opening (○). ND = not detectable. Signatures were not detectable at estuary swash east and west, and control swash east and west locations.
2.4. Discussion

The results of this study detected an influence of estuarine waters on coastal environments, despite the estuaries experiencing periods of low flow. Estuarine waters of intermittent estuaries change the physico-chemical environment and provide nutrients to receiving waters of adjacent coasts. Mouth status also influenced the sediment productivity (microphytobenthic chlorophyll a and organic matter) of adjacent ocean beaches.

Highly variable flows are typical for Australian rivers (Finlayson & McMahon 1988). The sampling period of the present study (April 2007 – March 2008) was conducted during a period of extended drought, with reduced flow events until early November 2007. The four estuaries in this study have shown different patterns of mouth closure, however all estuaries have shown similar physico-chemical regimes. Greater similarities in these patterns were evident for the two larger estuaries (Curdies and Barham), and for the two smaller estuaries (Skenes and Kennett). The Curdies and Barham estuaries are part of large catchments and discharge relatively high volumes of freshwater into the adjacent coast compared to many of the other small estuaries in this region. The Curdies and Barham estuary mouths closed during late autumn/early winter and summer due to declining river flows, resulting in water levels rising inside the estuary. Similar mouth closure patterns have been seen at other intermittent estuaries in the area such as Gellibrand, Hopkins and Surrey, where mouth closure has occurred during autumn and summer with high flow occurring during July and October causing the mouth to remain open (Matthews 2000, Arundel 2003, Becker 2007). In comparison, Skenes and Kennett River estuaries have smaller catchments than the Curdies and Barham and discharge into more protected beaches, where wave action and long shore sand movement is likely to be less than those for the two larger estuaries. This may explain why the estuary mouth of the two smaller estuaries remained open throughout the entire sampling period. Even during periods of extremely low
discharge, there was always a shallow discharge across the sand bar at both the Skene's and Kennett estuaries.

At Curdies and Barham estuaries, water temperatures at the estuary mouth and estuary swash locations declined after the mouth was opened, due to the mixing of estuarine and marine waters. Temporal patterns in water temperatures were similar to those of other estuaries in the region (Hopkins, Gellibrand, Merri and Surrey) (Matthews 2000, Arundel 2003, Becker 2007), with colder temperatures occurring during the winter, then increasing during spring and summer.

During periods of mouth closure, salinity levels inside the estuary mouth of Curdies and Barham were very low and virtually completely fresh. This pattern of dilution also occurs in South Africa (Mackay & Cyrus 2001) and other southern Australian estuaries (Arundel 2003, Becker 2007, Sherwood et al. 2008). In contrast, estuaries in very arid regions of South Africa and southern Australia can exhibit hypersaline conditions (Whitfield & Bate 2007, Sherwood et al. 2008, Froneman & Henninger 2009). Upon opening, inputs of marine water caused salinity to increase, and by two months after the opening, salinity levels inside the estuary mouths reflected marine levels of 35 psu, due to a combination of both consistent tidal exchange and reduced freshwater inflow. When the estuary mouths of Curdies and Barham were artificially opened (July and May respectively), the salinity of the estuary swash shifted to almost completely fresh through the mixing of estuarine and marine water in the coastal zone. However this initial drop in salinity was not sustained, with salinity levels gradually increasing, due to estuarine discharge decreasing and gradually dispersing, resulting in salinities of 35 psu by 3 - 4 months after the opening. Compared to Curdies and Barham, waters at the estuary mouths of Skene's and Kennett were heavily stratified, with salinity peaking in summer and autumn. The salinity of the estuary swash of Skene's and Kennett remained fairly consistent, except during late winter/early spring where a discharge event caused a decrease in salinity. These large temporal changes in salinity
observed inside the estuary mouths and swash locations are typical of estuarine/marine environments, with similar patterns occurring at other estuaries along the south-west coastline of Victoria (Matthews 2000, Arundel 2003, Becker 2007). As it would be expected, all four control beaches showed consistent salinity (35 - 40 psu) throughout the entire sampling period.

The artificial mouth opening of the Curdies and Barham estuary mouths contributed to an increase in dissolved oxygen levels at the estuary mouth and swash locations. An artificial mouth opening can add oxygen rich water to the estuary by three main mechanisms; oxygenated freshwater flow from the catchment, marine waters entering the estuary via tidal movement and increased flows may lead to greater turbulence and exchange of oxygen with air (Becker 2007, Whitfield et al. 2008). Vertical mixing and photosynthesis of surface waters remain mechanisms for oxygenating deeper waters (Whitfield et al. 2008), however high turbidity and stratification can reduce the effectiveness of these processes and can result in a decrease in dissolved oxygen months following an opening (Becker 2007), as seen two months after the openings of Curdies and Barham. Intermittent estuaries can also experience periods of low oxygen or ‘hypoxia’ (< 2-3 mg/L), which is caused by bacteria consuming the available oxygen for the degradation of organic material (Whitfield & Bate 2007). Dissolved oxygen concentrations at all locations across all estuaries, peaked during spring and summer months. During spring and summer periods, there is an increase in daylight hours and sunlight, potentially stimulating phytoplankton photosynthesis contributing to this increase in oxygen production in these waters.

As expected, increased nutrient concentrations were identified in the coastal zone following mouth openings. All nutrients were 5 – 8 times greater in concentrations in the estuary swash of Curdies one week after the mouth was artificially opened. Similar results were found at the Mooloolah estuary in south-eastern Queensland, where concentrations of nitrogen and phosphorus were higher in the estuary plume compared to outside the plume (Gaston et al.
Such nutrient rich waters discharging out of the Curdies estuary and into the coastal zone may originate from intense agricultural runoff (93% of catchment land use), which is conducted on the lower and upper catchment, draining directly into the estuary (Eyre & France 1997, Mondon et al. 2003). Nine weeks after the artificial opening, nutrient concentrations had decreased to levels similar to those recorded before the mouth was opened. This shows that while these relatively small estuaries are delivering nutrient rich water to coastal zones, nutrient delivery may not be sustained over long periods during periods of low flow. Nutrients are an important driver of coastal productivity, via stimulation of microphytobenthic chlorophyll a and in turn potentially result in an increase in organic matter depending upon mouth conditions. The duration of time that these nutrients remain in the coastal waters would depend upon the influence of dispersal agents such as ocean currents and the strength of the wind (Grimes & Kingsford 1996, Devlin et al. 2001). This is the only record of nutrients of coastal waters in the study area, therefore it is not understood how far these nutrients disperse, or whether nutrients are being taken up by intertidal or pelagic organisms in coastal waters. Nutrient records for estuary mouths and adjacent coastal waters of other estuaries in south-west Victoria are very important to understand the link between estuarine and marine systems and, as such, nutrient analyses should be conducted in future studies.

Similar patterns were shown for the artificial opening of both Curdies and Barham estuary mouths, with microphytobenthic chlorophyll a concentration at the estuary swash decreasing in concentration one month after the mouth opening. Although these results were not in agreement with what was predicted one month post opening, concentrations did show what was expected at two month post opening, with the estuary swash increasing in microphytobenthic chlorophyll a concentration at Curdies estuary only. The estuary swash at Curdies actually had greater concentrations than the estuary mouth two month post opening. Similar variables patterns were shown for sediment organic matter following the artificial opening of the estuary mouths.
The estuary swash at both Curdies and Barham estuaries exhibited no change in organic matter one month post opening, however two months after the opening Barham corresponded with what was predicted, with organic matter increasing at the estuary swash, and had similar content to the estuary mouth. The opening of an estuary mouth creates an increase in water movement and strong mixing between freshwater and the ocean. This is likely to have caused scouring of sediment at the estuary mouth and estuary swash increasing turbidity and resuspending benthic microalgae and organic matter (Perissinotto et al. 2000, Nozais et al. 2001, Froneman 2002, Perissinotto et al. 2002, Snow & Adams 2007, Anandraj et al. 2008). This disturbance may have prevented microphytobenthic chlorophyll a and organic matter to initially accumulate in the sediment of the estuary mouth and swash one month after the artificial mouth opening and instead organic material was resuspended in the water column. The initial increase in flow and scouring of sediment that occurred upon the mouth opening may have reduced by two months post opening, causing an increase in light penetration and enabled dislodged organic material and nutrients to settle out of the water column and deposit onto the bottom of the estuary. This settlement of organic material and nutrients would stimulate microphytobenthic growth and increase the accumulation of organic matter as evident at Curdies and Barham, respectively (Mallin et al. 1993, Adams & Bate 1999, Adams et al. 1999, Nozais et al. 2001, Froneman 2002, Allan & Froneman 2008, Anandraj et al. 2008).

In contrast to what was predicted, both estuary swash locations at Curdies and Barham estuaries showed no change in microphytobenthic chlorophyll a concentration or organic matter after the natural closure of the estuary mouth. With the closure of an estuary mouth, there is a reduction in tidal exchange between the estuarine and marine environment, preventing organic material and nutrients from depositing in the estuary swash, which is important for stimulating microphytobenthos production (Mallin et al. 1993, Adams & Bate 1999, Adams et al. 1999, Lohrenz et al. 1999, Nozais et al. 2001, Froneman 2002, Allan & Froneman 2008, Anandraj et al. 2008). It is surprising that with
these reduced inputs that there was not a decrease in microphytobenthos or organic matter at the estuary swash following the closure of the estuary mouth. Interestingly, microphytobenthic chlorophyll a concentration at both estuary mouths and the Barham estuary swash showed a significant increase in concentration two months after the closure. During a period of mouth closure there is less resuspension of organic material due to reduced flow, resulting in an increase in light penetration, which is a critical factor for microphytobenthic growth (Nozais et al. 2001, Anandraj et al. 2008). Similarly, scouring and mixing of coastal waters adjacent to the estuary mouth may have subsided two months post-closure, allowing an increase of light penetration to occur at the estuary swash location resulting in this peak in microphytobenthic chlorophyll a. Seepage of water carrying nutrients through the sandbar may have occurred at the Barham estuary swash, causing this increase in microphytobenthic chlorophyll a two month post closure.

In the present study, the highest concentration of microphytobenthic chlorophyll a at Curdies and Barham estuary mouths occurred during the closed phase with concentrations ranging between 1.0 to 59.2 mg/m² and 5.1 to 226.8 mg/m² at Curdies and Barham, respectively (Figure 2.16, 2.17). These values are lower than microphytobenthic concentrations reported by Anandraj et al. (2008), Nozais et al. (2001) and Froneman (2002) for intermittently open estuaries in South Africa (75 to 480 mg/m²). Despite concentrations being lower, comparable patterns have been shown for both local and South African estuaries, where the greatest microphytobenthic concentration occurred when the estuary mouth was closed (Nozais et al. 2001, Froneman 2002, Anandraj et al. 2008, Whitfield et al. 2008). This particular study focused on microphytobenthos opposed to phytoplankton, as many studies have reported benthic chlorophyll a levels to be one to three orders of magnitude higher than in the water column (Perissinotto et al. 2000, Nozais et al. 2001, Anandraj et al. 2008). Therefore for this study to detect a response to estuarine discharge it was assumed that it would be easier to detect a change in the benthos opposed to the water column.
Numerous studies have used stable isotope analysis to identify sources of organic matter in terrestrial, estuarine and coastal environments (Hedges & Parker 1976, Hu et al. 2006, Kuwae et al. 2007, Harmelin-Vivien et al. 2008, Ramaswamy et al. 2008, Yu et al. 2010). Sediment stable isotope results exhibited similar patterns to benthic organic matter, by identifying low concentrations of organic matter in the sediment at a number of locations throughout the Curdies estuary on different sampling periods. The amount of organic matter in the sediment was so low at the lower estuary, estuary swash and control swash locations this prevented a stable isotope signature to be detected by the automated Isoprime (GV Instruments, Manchester, UK) isotope-ratio mass spectrometer and therefore no isotope data were available for these locations. Coarse sediment particle size may have been a contributor to these limited results. The lower estuary and coastal locations of the Curdies estuary are very coarse (1.5 Phi (Mondon et al. 2003)), which suggests that settlement and accumulation of organic material may be minimal in these areas. Similar results have been found in previous studies, where organic matter and microalgal biomass was shown to be higher in muddy sediments than sandy sediments (Adams et al. 1999, Dittmann 2000, Due et al. 2009). Therefore, larger samples (i.e. sediment volume greater than corer used in the present study (5-cm deep, 63-mm diameter)) would be required to obtain sufficient organic matter for future sampling protocols.

In contrast to what was predicted, there was no change in carbon isotope ratios at the upper estuary, or freshwater location after the mouth of the estuary was opened, with both locations reflecting terrestrial sources of organic matter. Similarly, there was no change in nitrogen isotope ratios at the upper estuary location after the opening of the estuary mouth. However, there was a significant change in nitrogen isotope ratios at the freshwater location, with sediment having similar $\delta^{15}$N signatures to the upper estuary location (pre- and post-artificial mouth opening sampling times), reflecting an estuarine signal. These unexpected results imply that organic matter would have had to move
upstream against the prevailing current. It is important to note that post-opening sampling occurred three weeks after the opening, and by this time, the volume of water and strength of the currents may have declined, enabling wind and tidal mixing to carry organic matter from the upper estuary upstream into freshwater reaches of the catchment. These findings demonstrate that while estuaries may influence the food webs of coastal environments, the incoming marine water is also potentially playing a significant role in productivity of the estuary and river. For example, marine macroalgae deposits are often seen to penetrate long distances into estuaries (pers. observ.).

This study has contributed to our understanding of coastal responses to estuarine discharge from intermittent systems, by showing that estuarine discharge provides an important source of nutrients to coastal waters. Intermittent estuaries of south-west Victoria experienced periods of low flow during the present study. However despite this, estuarine waters do appear to be contributing to primary productivity of receiving sandy sediment environments by providing an important source of nutrients and organic material, which is potentially facilitating secondary production.
CHAPTER 3

3. Influence of intermittent estuarine discharge on coastal sediments of adjacent sandy beaches

A peer reviewed publication has been derived from this chapter:


3.1. Introduction

In temperate Australia (Kench 1999, Roy et al. 2001, Mondon et al. 2003, Sherwood et al. 2008) and South Africa (Whitfield 1992, Adams et al. 1999, Cooper et al. 1999, Kench 1999, Perissinotto et al. 2000, Froneman 2002, Froneman 2004) small seasonal or intermittently open estuaries discharging onto open coastlines are very common, however few studies have investigated plume traits and their influence on adjacent coasts for these smaller systems (Kingsford & Suthers 1994, Gaston et al. 2006, Ostrander et al. 2008, Connolly et al. 2009, Schlacher et al. 2009). Most studies that have investigated the effect of estuary plumes of larger rivers on coastal waters have focused on fish recruitment, water quality and macroinvertebrate assemblages (Grimes & Finucane 1991, Kingsford & Suthers 1994, Grimes & Kingsford 1996, Eyre & France 1997, Lohrenz et al. 1999, Cowley et al. 2001, Froneman 2002, Gladstone et al. 2006, Hermand et al. 2008, Chuwen et al. 2009a). This study is one of the first to be conducted on intermittent estuaries in Australia, as previous studies have examined plume traits of small permanently open estuaries (Gaston et al. 2006, Schlacher et al. 2008, Connolly et al. 2009, Schlacher & Connolly 2009, Schlacher et al. 2009). Preliminary data showed that macroinvertebrates were not suitable for use in the present study because they were either rare or absent in the shallow subtidal zones of the highly exposed sandy beaches onto which the estuaries opened (J. McKenzie unpublished data (Chapter 2: section 2.2.4)). Sampling of fish was impractical because of the exposed nature of the shores and there was no data on nearby commercial or recreational fish catches. Instead, this study specifically investigated the influence of estuarine discharge on different aspects of coastal sediments. Previous studies (Barton 2006, Pope 2006, Sherwood et al. 2008) have shown that the estuaries investigated in this study and others along the Victorian coastline are nutrient rich, and therefore this study focused on the influence of estuarine discharge on sediment organic matter, microphytobenthos and microbial diversity. Many studies have reported benthic chlorophyll $a$ levels to be one to three orders of magnitude higher than in the water column (Perissinotto et al. 2000, Nozais et al. 2001, Perissinotto et al. 2002, Anandraj et al. 2008), and therefore microphytobenthos was the focus
as opposed to phytoplankton. The present study compared these variables for two intermittent estuaries in south-eastern Australia, during an artificial mouth opening and a separate natural flood. Variables were measured inside the estuary mouth, at the estuary outflow/ocean swash interface and from the swash zone of a nearby “control” beach with no estuary input. The objective of this study was to assess the influence of estuarine discharge on microphytobenthos, sediment organic matter and microbiota. The present study hypothesised that estuary discharge following an artificial mouth opening would increase organic matter, microphytobenthos and microbiota of the adjacent estuary swash, potentially showing similar results to the estuary mouth. The study also hypothesised that in response to continued discharge and increased flow from a natural flood, microphytobenthos, sediment organic matter and microbiota would continue to increase in the estuary swash. Results from this study provide a unique assessment of the impact of the discharge of small intermittently open estuaries on sediment productivity, providing valuable tools for estimating future effects of climate change and drought.

The present chapter builds on previous work conducted in Chapter 2 in the following ways: 1) microbial diversity sampling uses BIOLOG EcoPlates: this is a relatively novel technique for estuaries, with the present study being one of the first to use these plates in the marine environment; 2) tests the impact of increased river discharge (natural flood) in addition to artificial mouth opening events. Sampling occurred at various stages during a continuous flow event rather than before and after an abrupt artificial mouth opening; and 3) sampling is conducted at an additional estuary (Anglesea) helping further generalise results, by covering a greater number of systems.
3.2. Materials & Methods

3.2.1. Study Sites

Intermittent estuaries in south-eastern Australia such as Aire, Anglesea, Barham, Curdies, Gellibrand, Hopkins, Merri and Painkalac are often nutrient enriched (EPA 2000, Sherwood et al. 2008). These estuaries have demonstrated high nutrient levels in the water column particularly during autumn and winter (EPA 2000), with nitrogen and phosphorus exceeding ANZECC and ARMCANZ (2000) guidelines of 0.08 mg/L and 0.035 mg/L respectively (ANZECC 2000, ANZECC & ARMCANZ 2000, Sherwood et al. 2008). These data suggest that estuaries are releasing high nutrient loads into coastal waters, providing the opportunity to potentially detect an influence on organic matter, microphytobenthos and bacteria communities of coastal sediments on adjacent sandy beaches.

The influences of artificial estuary mouth openings and natural floods on sediment organic matter, microphytobenthos and microbiota were examined at two intermittent estuaries, the Curdies and Anglesea, located in south-west Victoria, Australia (Figure 3.1). Along this coast, strong south-easterly winds are common during the winter and spring when estuaries are likely to be open, meaning inshore currents and potential estuary plumes travel to the east. To avoid the influence of the plume from south-easterly prevailing currents, control sites were selected at least 1 km west of the estuary mouths, with no freshwater discharging onto the beaches. The Curdies estuary (142°52'46"E, 38°36'36"S) is part of a catchment covering an area of ~1100 km², and is one of the largest estuaries in Victoria, extending ~16 km, (Sherwood et al. 2008), discharging onto a high energy coast at Peterborough. The widest section of the estuary is approximately 300 m. Most of the catchment is used for agriculture (90 %), with only a small portion of native vegetation and forest remaining (Mondon et al. 2003, Barton & Sherwood 2004). Generally, the mouth closes over in the drier months of December to June (Barton & Sherwood 2004), and over the past ten years, the estuary has been artificially
opened between June and August due to public pressure from the rising river inundating adjoining farmland and other public amenities (Arundel 2003, Barton & Sherwood 2004). The Anglesea estuary (144°11'28"E, 38°24'59"S) catchment covers an area of ~125 km² and is much shorter than the Curdies estuary (length approximately 3.5 km) (Sherwood et al. 2008). Near the mouth, the estuary is ~110 m wide (Pope 2006) and flows out into a beach which is more protected compared to Curdies. The Anglesea catchment mostly consists of native vegetation (80 %), including heathland and open forest (Mondon et al. 2003, Pope 2006). The estuary is open much of the year, but during the past five years the estuary has closed in autumn and during the middle of winter, which has triggered artificial openings of the mouth (pers. observation). The present study coincided with a prolonged period of drought in south-eastern Australia that began in 1997 (See Chapter 2). Since that time river discharge has been well below the long term average (Matthews 2006, Lester & Fairweather 2009, DSE 2010). Curdies discharge shows a seasonal pattern of flow with very low discharge over late summer and autumn and with peaks over winter and early spring. During this study Curdies recorded two flood events during July (causing an artificial mouth opening) and late October 2007, recording a discharge of >1400 mega litres per day (ML/Day). Previous studies have shown Anglesea to have similar seasonal flow to Curdies, with little flow from mid-summer to autumn and high flow across winter and spring (Pope 2006). Daily discharge between the years August 1999 – March 2002 recorded 0.01 – 6 m³/s (Pope 2006).
Figure 3.1 Location of study sites along the south-western Victoria coastline. Black circles denote control beaches 1-4, squares is the estuary mouths and triangles are estuary swash locations that were sampled. Insert: Location of Curdies and Anglesea estuaries within Victoria and south-eastern Australia.
Samples of microphytobenthos, sediment organic matter and microbiota occurred during two events at each estuary: (1) artificial mouth opening where the adjacent coast received discharge from the previously closed estuary; and (2) natural flood event where there was a marked increase in discharge volume through the open estuary. Sampling was conducted one week prior to the events (before event), then one and nine weeks after the events (one week post and nine weeks post, respectively). The Curdies was artificially opened on 14th July 2007 (hence, sampled during June, July and August 2007); and the Anglesea on 13th August 2007 (sampled July, August, September 2007). Both estuaries remained open up until a natural flood that occurred on 3rd November 2007. The Anglesea estuary mouth remained open for the entire sampling period after the flood event; however Curdies closed on 31st Dec 2007, between the second and third sampling period (late November and January respectively). For the artificial mouth openings, sampling occurred at three sampling locations for each estuary: 1) estuary mouth; 2) the estuary outflow/ocean swash interface, hereafter referred to as estuary swash; and 3) a single “control” beach (control swash) (Figure 3.1 C1). For the estuary mouth the area sampled was on the edge of the main channel, where the lip of the channel begins to level out. A similar water depth was sampled for both swash sites. The control beaches have similar wave exposure to the estuary beaches, with no freshwater discharging onto the beach. A 10 x 2 m area was used for sampling at each of the three locations. The same sampling method was used for the natural flood, however three additional control beaches were used to better characterize the degree of natural spatial variation in the measured responses (represented in Figure 3.1. C2-C4). Sampling of all locations for each estuary was conducted at low tide, within 4 hrs of each other.

Standard techniques were used for microphytobenthos and organic matter analysis (Lorenzen 1967, Winter et al. 1996, Arar 1997, Light & Beardall 1998, Perissinotto et al. 2000, Froneman 2006); however a relatively new technique, BIOLOG EcoPlates, were used to assess changes in microbial diversity based on carbon source utilisation (Barton 2006). To date,
microplates such as these and others (BIOLOG GN®) have primarily been used to investigate changes in microbial communities in terrestrial soils, fertilizers, fungal communities, waste water and freshwater systems (Garland & Mills 1991, Preston-Mafham et al. 2002, Winding & Hendriksen 2007, Chen et al. 2008, Weber et al. 2008, Kunito & Nagaoka 2009); few studies have used this technique in marine environments (Garland & Mills 1991, Tam et al. 2003, Sala et al. 2005, Barton 2006, Sala et al. 2006). While literature has addressed limitations to using BIOLOG plates (Konopka et al. 1998, Preston-Mafham et al. 2002, Christian & Lind 2006, Weber et al. 2008), I have continued to use this technique as the plates are tailored to ecological applications, they are simple to use and provide rapid and reproducible results, and are also relatively inexpensive compared to molecular techniques, which require specialized expertise (Urakawa et al. 2001, Bouvier & del Giorgio 2002, Henriques et al. 2004, Hawkins & Purdy 2007, Sorensen et al. 2007, Zhang et al. 2007, Chiu et al. 2008).

3.2.2. Microphytobenthos and benthic organic matter

The microphytobenthos and organic matter sediment cores were collected and analysed using the same method as described in Chapter 2 (see section 2.2.3.1 and 2.2.3.2).

3.2.3. Benthic microbial bacteria

The benthic microbial sampling, extraction and plating followed the methods of Barton (2006). Briefly, at each location, five replicate, subtidal (estuary mouth) and intertidal (estuary swash and control swash) sediment cores (5-cm diameter x 15-cm deep), were collected from unvegetated sediment. A subsample (22-mm diameter x 3-cm deep) was collected from the centre of the core, and stored in sterile bags in the dark on ice until microbial extraction. The sub-sampler corer was washed in water and rinsed in 100 % ethanol between replicates. A field procedural control was conducted at each location.
The microbial samples were extracted and plated on the same day as they were collected.

Microbial extraction followed aseptic techniques (Barton 2006) and involved the addition of 100 mL of 2% sterile saline (NaCl) containing 6 glass beads (4-mm diameter). Samples were shaken vigorously by hand for 1 min, and then put on ice for 15 min to allow sediment to settle. A 20 mL extract of the sediment was then syringe filtered (5-μm pore size) before plating onto BIOLOG EcoPlates (Oxoid Australia Pty Ltd, Adelaide S.A). The BIOLOG EcoPlates have three replicates of 31 carbon substrates and one control (non-carbon) per replicate. Each well received 100 μl of the microbial sample using an eight-channel pipette. EcoPlates were incubated in the dark at 14 °C in a constant temperature cabinet for five days. As the carbon source is utilized, the tetrazolium violet dye in the well is reduced, precipitating a purple colour. Colour development occurred faster in samples from Anglesea, and therefore, plates were read on the fourth day, compared to Curdies which were read on day five. Plates were read by spectrophotometric light absorption at 620 nm and recorded in absorption units with an Expert Plus Microplate Reader (Model: Expert Plus, ASYS Hitech GmbH, Austria). Absorbance in the control well for each replicate was subtracted from all other wells to account for the background absorbance (Sala et al. 2006). The colour development of each well was used as a measure of carbon source utilization, assessing the functional diversity of microbial bacteria.

3.2.4. Statistical Analysis

Assumptions of normality and homogeneity of variance of the data were checked before univariate Analysis of Variance (ANOVA) using histograms, and plots of residuals (Quinn & Keough 2002); subsequently, chlorophyll a and organic matter data were log (x+1) transformed to meet these assumptions. The two estuaries were analysed separately to test for differences in chlorophyll a and organic matter; two-way analyses of variance (ANOVA) were used in which both Time (three levels: before event, one week post event
and nine weeks post event) and Location (three levels for artificial opening: estuary mouth, estuary swash, control swash; six levels for natural flood: estuary mouth, estuary swash, control swash 1-4) were treated as fixed factors. Two planned contrasts (Quinn & Keough 2002) were also included to test whether the magnitude of the difference between estuary mouth and estuary swash was consistent: (1) before event (T1) compared to one week after event (T2); and (2) before event compared to nine weeks after event (T3). Similar planned contrasts were tested comparing estuary swash and control swash (or average of the four controls for natural flood mouth event). Statistical analyses used SYSTAT v 11. All hypothesis tests were conducted at the 0.05 significance level. The procedural controls for microbial samples had no colour development indicating no microbial contamination and therefore were excluded from further analysis.

Two-way Permutational Multivariate Analysis of Variance (PERMANOVA), based on Bray-Curtis dissimilarities, was used to test carbon utilisation (based on 31 carbon substrates) across ‘Location’ and between ‘Times’. The Bray-Curtis distance between group centroids for each location was calculated for the contrasts of (1) before event compared to one week after event; and (2) before event compared to nine weeks after event.
3.3. Results

3.3.1. Microphytobenthos

The Curdies estuary showed temporal variability in microphytobenthic chlorophyll $a$ at different locations following the artificial opening of the estuary mouth (time x location interaction; Table 3.1; Figure 3.2a), with three of the four contrasts being significant. The estuary swash decreased in microphytobenthic chlorophyll $a$ one week post opening; however there was no significant difference between estuary swash and control swash from before to one week after opening (Figure 3.2a). It is clear from Figure 3a that chlorophyll $a$ was present at all locations before the opening (highest at estuary mouth, lowest at control swash). One week after the artificial mouth opening, chlorophyll $a$ was below the detection limit in the estuary swash and had significantly declined at the estuary mouth and control swash. However after nine weeks chlorophyll $a$ was detectable at the control swash only.

At the Anglesea estuary, there was a significant difference in microphytobenthic chlorophyll $a$ between locations (Table 3.1) following the artificial mouth opening, but no interaction between times. There was a significant increase in chlorophyll $a$ for the estuary swash compared to control swash from before to one week after the opening (contrast 3; Table 3.1), but the other contrasts were not significant. Chlorophyll $a$ concentrations were much lower at estuary and control swash locations compared to the estuary mouth one week after the opening, while concentrations slightly decreased at the estuary swash nine weeks after the opening (Figure 3.2b).

Curdies estuary showed temporal variability in microphytobenthic chlorophyll $a$ concentrations at the different locations following the flood event (time x location interaction; Table 3.1; Figure 3.2c). Three of the planned contrasts were significant. The estuary swash and mouth showed significant increases in chlorophyll $a$ concentrations one week post flood, at nine weeks post flood concentrations remained the same at the estuary swash, while the estuary
mouth decreased (Table 3.1; Figure 3.2c). The control swash showed little change in chlorophyll $a$ from before to one or nine weeks post flood (Table 3.1; Figure 3.2c). Anglesea showed similar temporal variability for chlorophyll $a$ following the flood, but only the planned contrast between estuary mouth and estuary swash before the flood compared to one week after the flood was significant (Table 3.1; Figure 3.2d). The estuary and control swash locations showed little change through time, whereas chlorophyll $a$ increased at the estuary mouth one week after compared to before and at nine weeks concentrations decreased (Figure 3.2d).
Table 3.1 Two-way ANOVAs comparing sediment chlorophyll \( a \) in the Curdies and Anglesea estuaries during an artificial mouth opening and a natural flood event. Planned contrasts were (1) estuary mouth versus estuary swash before mouth event X estuary mouth versus estuary swash one week post mouth event. (2) estuary mouth versus estuary swash before mouth event X estuary mouth versus estuary swash nine weeks post mouth event. (3) estuary swash versus control swash before mouth event X estuary swash versus control swash one week post mouth event. (4) estuary swash versus control swash before mouth event X estuary swash versus control swash nine weeks post mouth event.

<table>
<thead>
<tr>
<th>Source</th>
<th>Curdies Artificial Opening (log(x+1) transformed)</th>
<th>Anglesea Artificial Opening (log(x+1) transformed)</th>
<th>Curdies Natural Flood Opening (log(x+1) transformed)</th>
<th>Anglesea Natural Flood Opening (log(x+1) transformed)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( df )</td>
<td>MS</td>
<td>( F )</td>
<td>( P )</td>
</tr>
<tr>
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<td>2</td>
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<td>121.284</td>
<td>(&lt;0.001)</td>
</tr>
<tr>
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<td>7.099</td>
<td>0.001</td>
</tr>
<tr>
<td>Time X Location</td>
<td>4</td>
<td>0.811</td>
<td>10.994</td>
<td>(&lt;0.001)</td>
</tr>
</tbody>
</table>

Contrasts

| (1)              | 1        | 0.977    | 13.254  | \(<0.001\) | 1        | 0.134    | 1.787   | 0.185    | 1        | 0.891    | 5.719   | \(0.018\)  | 1        | 0.445    | 5.751   | \(0.018\) |
| (2)              | 1        | 1.104    | 14.971  | \(<0.001\) | 1        | 0.086    | 1.157   | 0.285    | 1        | 0.431    | 2.749   | 0.099    | 1        | 0.013    | 0.169   | 0.682    |
| (3)              | 1        | 0.001    | 0.013   | 0.908    | 1        | 0.311    | 4.164   | \(0.045\) | 1        | 2.918    | 18.593  | \(<0.001\) | 1        | 0.109    | 1.413   | 0.236    |
| (4)              | 1        | 0.416    | 5.641   | \(0.020\) | 1        | 0.210    | 2.803   | 0.098    | 1        | 2.593    | 16.520  | \(<0.001\) | 1        | 0.076    | 0.986   | 0.322    |
| Error            | 81       | 0.074    | 81      | 0.075    | 162      | 0.157    | 162     | 0.077    |
Figure 3.2 Mean ± SE Chlorophyll a of benthic samples collected at a) Curdies and b) Anglesea estuaries during the artificial mouth opening and c) Curdies and d) Anglesea estuaries during the natural flood event. Estuary mouth is white, Estuary swash is grey and control swash or average of the four control swash locations for the natural flood event is black. Vertical line defines mouth event.
3.3.2. *Benthic organic matter*

There was temporal variability in sediment organic matter at the different locations following the artificial mouth opening of the Curdies estuary (time x location interaction; Table 3.2; Figure 3.3a). There was no change in organic matter at the estuary swash one week after the opening; however there was significant difference between estuary swash and control swash before compared to nine weeks, with both locations increasing (Table 3.2; Figure 3.3a). None of the other contrasts were significantly different. There was less sediment organic matter at the control swash location compared to both estuary locations before opening, but it gradually increased through time so that by week nine, all three locations had similar organic matter (Figure 3.3a). At the Anglesea estuary, only the effect of time on organic matter was significant for the artificial opening (Table 3.2; Figure 3.3b).

One week after the natural flood event at Curdies estuary, sediment organic matter increased at the estuary swash and estuary mouth, however there were no significant differences (Table 3.2; Figure 3.3c). There was temporal variability in sediment organic matter at the different locations after the natural flood at Anglesea estuary, with three of the four planned contrasts being significant (Table 3.2). There was a significant difference in sediment organic matter between estuary mouth and estuary swash after the natural flood; one week post flood saw an increase at the estuary mouth and a decrease at the estuary swash in organic matter; however at nine weeks post both locations showed similar organic matter, with the estuary swash increasing slightly, recording similar organic matter to before the flood (Table 3.2; Figure 3.3d).
Table 3.2 Two-way ANOVAs comparing sediment organic matter (g) in the Curdies and Anglesea estuaries during an artificial mouth opening and a natural flood event. Planned contrasts were (1) estuary mouth versus estuary swash before mouth event X estuary mouth versus estuary swash one week post mouth event. (2) estuary mouth versus estuary swash before mouth event X estuary mouth versus estuary swash nine weeks post mouth event. (3) estuary swash versus control swash before mouth event X estuary swash versus control swash one week post mouth event. (4) estuary swash versus control swash before mouth event X estuary swash versus control swash nine weeks post mouth event.

<table>
<thead>
<tr>
<th>Source</th>
<th>Curdies Artificial Opening (log(x+1) transformed)</th>
<th>Anglesea Artificial Opening (log(x+1) transformed)</th>
<th>Curdies Natural Flood Opening (log(x+1) transformed) (Control = Average of 4 controls)</th>
<th>Anglesea Natural Flood Opening (log(x+1) transformed) (Control = Average of 4 controls)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>df</td>
<td>MS</td>
<td>F</td>
<td>P</td>
</tr>
<tr>
<td>Time</td>
<td>2</td>
<td>0.002</td>
<td>12.614</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Location</td>
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<td>0.004</td>
<td>31.108</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time X Location</td>
<td>4</td>
<td>0.001</td>
<td>6.355</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Contrasts

(1) 1 0.000 0.953 0.336 1 0.000 2.518 0.121 1 0.000 0.001 0.973 1 0.000 28.758 <0.001
(2) 1 0.000 0.088 0.769 1 0.000 0.579 0.452 1 0.028 2.026 0.159 1 0.000 4.567 0.036
(3) 1 0.000 1.378 0.248 1 0.000 0.427 0.518 1 0.000 0.006 0.940 1 0.000 0.848 0.360
(4) 1 0.002 16.792 <0.001 1 0.000 0.035 0.853 1 0.001 0.066 0.798 1 0.000 21.971 0.000
Error 36 0.000 36 0.000 72 0.014 72 0.000
Figure 3.3  Mean ± SE organic matter (grams) of benthic samples collected at a) Curdies and b) Anglesea estuaries during the artificial mouth opening and c) Curdies and d) Anglesea estuaries during the natural flood event. Estuary mouth is white, Estuary swash is grey and control swash or average of the four control swash locations for the natural flood event is black. Vertical line defines mouth event.
3.3.3.  

**Benthic microbial bacteria**

There was temporal variability in carbon utilisation patterns at the different locations following the artificial opening of the Curdies estuary mouth (time x location interaction; Table 3.3). The estuary mouth (PERMANOVA $F_{2,12} = 3.09, P < 0.001$) and estuary swash (PERMANOVA $F_{2,12} = 12.83, P < 0.001$) showed significant differences in carbon utilisation across times, however the control swash showed no differences (PERMANOVA $F_{2,12} = 1.21, P = 0.252$). The multivariate differences for the comparison of before to after opening were much greater for estuary swash (distances between centroids T1 vs T2 = 71.70, T1 vs T3 = 78.15) than for control swash (26.37, 22.63) with estuary mouth intermediate (38.31, 41.76). The Anglesea estuary showed a significant difference in carbon utilisation patterns between locations after the artificial mouth opening, but no interaction through time (Table 3.3). The multivariate differences in carbon utilisation for the comparison of before to after opening were greater for control swash (T1 vs T2 = 33.86, T1 vs T3 = 28.92) than for estuary mouth (15.91, 27.38) and estuary swash (20.74, 20.84).

Similar patterns to those shown during the artificial opening were seen following the natural flood at Curdies, where there was temporal variability in carbon utilisation (time x location interaction; Table 3.3). The estuary mouth (PERMANOVA $F_{2,12} = 4.21, P < 0.001$), estuary swash (PERMANOVA $F_{2,12} = 2.62, P < 0.001$) and all control swash sites (Control 1- $F_{2,12} = 3.20, P < 0.001$; Control 2- $F_{2,12} = 2.33, P < 0.001$; Control 3- $F_{2,12} = 1.74, P < 0.001$) except control 4 (PERMANOVA $F_{2,12} = 1.35, P > 0.001$) showed significant differences in carbon utilisation across times. The multivariate differences for the comparison of before to after the flood for estuary mouth (T1 vs T2 = 17.32, T1 vs T3 = 24.17) and estuary swash (25.39, 32.94) were within the 95% CIs for the four control swash sites (14.33 – 32.31, 20.76 – 34.27). Carbon utilisation at the estuary mouth was most similar between Times 1 and 2 (distance between centroids = 17.32), compared to Times 1 and 3 (distance between centroids = 24.17). Estuary swash showed the same pattern, with
Times 1 and 2 (T1 vs T2 = 25.39) showing more similar carbon sources utilisation compared to Times 1 and 3 (T1 vs T3 = 32.94).

Similarly to Curdies, the Anglesea estuary showed temporal variability in carbon utilisation at different locations after the natural flood event (Table 3.3). Estuary mouth (PERMANOVA $F_{2,12} = 6.85, P < 0.001$), estuary swash (PERMANOVA $F_{2,12} = 6.05, P < 0.001$) and all control swash sites (Control 1- $F_{2,12} = 3.32, P < 0.001$; Control 3- $F_{2,12} = 5.15, P < 0.001$; Control 4- $F_{2,12} = 3.23, P < 0.001$) except control 2 (PERMANOVA $F_{2,12} = 1.95, P > 0.001$) showed significant differences across times. The multivariate differences for the comparison of before to after the flood for estuary mouth (T1 vs T2 = 42.05, T1 vs T3 = 42.83) and estuary swash (47.27, 28.99) were within the 95% CIs for the four control swash sites (19.65 – 47.72, 5.40 – 51.21). Like the Curdies, there was a lot of variability in temporal differences between control swash locations (95% CIs T1 vs T2 = 1965 – 47.72; T1 vs T3 = 5.40 – 51.21), and estuary mouth and swash again fall within this range.
Table 3.3  Two way PERMANOVA comparing carbon utilisation during (a) artificial opening (b) natural flood event.

<table>
<thead>
<tr>
<th>Source</th>
<th>(a) Artificial Mouth Opening</th>
<th></th>
<th></th>
<th>(b) Natural Flood Event</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Curdies estuary</td>
<td>Anglesea estuary</td>
<td>Curdies estuary</td>
<td>Anglesea estuary</td>
<td>Curdies estuary</td>
<td>Anglesea estuary</td>
</tr>
<tr>
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<td>MS</td>
<td>F</td>
<td>P (perm)</td>
<td>df</td>
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<td>F</td>
</tr>
<tr>
<td>Time</td>
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<td>6.31</td>
<td>0.0001</td>
<td>2</td>
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</tr>
<tr>
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<td>4.66</td>
<td>0.0003</td>
<td>2</td>
<td>3556.83</td>
</tr>
<tr>
<td>Time x Location</td>
<td>4</td>
<td>3912.21</td>
<td>3.49</td>
<td><strong>0.0001</strong></td>
<td>4</td>
<td>1638.34</td>
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<tr>
<td>Residual</td>
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<td>1117.96</td>
<td></td>
<td></td>
<td>36</td>
<td>1232.51</td>
</tr>
<tr>
<td>Total</td>
<td>44</td>
<td>1117.96</td>
<td></td>
<td></td>
<td>44</td>
<td>1232.51</td>
</tr>
</tbody>
</table>
3.4. Discussion

The characteristics of estuary plumes from large systems are relatively well known; previous studies have shown plumes to deliver nutrients and deposit organic material from the catchment to the coastal waters (Smith & Demaster 1996, Lohrenz et al. 1999, Rabalais et al. 2000, Dagg et al. 2004). However, few studies have investigated the plumes of small systems and their influence on coastal sediment (Gaston et al. 2006, Haines et al. 2006, Ostrander et al. 2008, Connolly et al. 2009, Schlacher et al. 2009). The methods used during this chapter were sufficient to detect the influence of estuarine discharge on adjacent coastal ecosystems for two small intermittent estuaries, both before and after an artificial mouth opening and during a separate natural flood. Freshwater discharge from artificial mouth openings and an increase in flow from floods did correspond with a change in concentrations of sediment chlorophyll $a$, sediment organic matter and microbial diversity of adjacent coasts. However, the responses of chlorophyll $a$, organic matter and microbial diversity at both estuaries were not consistent for the artificial mouth openings and natural flood events. This highlights the difficulties of detecting whether estuarine discharge from relatively small intermittent estuaries makes a significant contribution to the microphytobenthos, organic matter and microbiota of adjacent coastal sediments, particularly during periods of prolonged drought.

Microphytobenthic chlorophyll $a$ concentrations at the estuary swash and mouth during the artificial mouth opening at Curdies estuary, did not follow expected patterns, with the highest concentration occurring during the closed phase, and concentrations decreasing at one and nine weeks after the opening. Similar results were shown by Froneman (2002) and Nozais et al. (2001) in temporarily open/closed estuaries in South Africa, where microphytobenthic biomass decreased by 60-98 % when the estuary mouth was opened. The decrease in microphytobenthic concentration during the mouth opening is likely to be associated with strong mixing between freshwater and tidal flow,
causing scouring of the sediment, increasing turbidity and resuspending benthic microalgae (Adams et al. 1999, Nozais et al. 2001, Froneman 2002, Perissinotto et al. 2002, Snow & Adams 2007, Anandraj et al. 2008). Such mixing may also cause a reduction in light penetration, decreasing light availability to the sediments and leading to limited microphytobenthic growth (Nozais et al. 2001, Froneman 2002, Perissinotto et al. 2002). In contrast, as predicted at Anglesea, microphytobenthic concentrations increased at the estuary swash and mouth one week post opening; however concentrations at both locations decreased at nine weeks post opening. The scouring of sediment when the mouth was initially opened may have potentially dislodged microphytobenthos from inside the estuary mouth, resuspending and depositing them into the estuary swash, resulting in this initial increase in concentration (Adams et al. 1999, Perissinotto et al. 2002). Additionally, the Anglesea estuary flows out into a protected beach compared to the Curdies. These calmer waters at Anglesea may allow the accumulation of microphytobenthos at the estuary swash to occur one week post opening. The increase in microphytobenthos that was shown at Anglesea may be a pulse response and therefore the sediment is not able to sustain such high concentrations nine weeks post opening.

The results of the natural flood at the Curdies corresponded with what was predicted for one week post flood, with estuary swash increasing in microphytobenthic chlorophyll a, however concentrations remained the same at nine weeks later. A number of studies in South Africa have reported similar patterns, where increased freshwater inflows were associated with an increase in chlorophyll a inside the estuary (Adams et al. 1999, Froneman 2002, Allan & Froneman 2008). Flood events can potentially introduce high concentrations of nutrients, sediment, dissolved and suspended organic matter into the estuary. When river flow slows, these organic materials, nutrients and fine sediments settle out and are deposited on the bottom of the estuary, where mineralization of nutrients occurs stimulating microphytobenthic growth (Mallin et al. 1993, Adams & Bate 1999, Adams et al. 1999, Lohrenz et al. 1999, Froneman 2002,
Anglesea showed the opposite pattern with the estuary swash showing no change in microphytobenthic chlorophyll a after the flood event, this highlights how variable the influence of estuarine discharge can be on sediment microphytobenthos between estuaries.

Results for sediment organic matter at both estuaries showed different patterns to what was predicted, with no change at the estuary swash and mouth one week after the artificial mouth opening. There were differences between estuary swash and control swash nine weeks post opening at Curdies, however this was due to the control swash increasing in organic matter rather than organic matter increasing at the estuary swash. Similar results were seen during the flood event at Curdies, with no differences in organic matter at any location or across time. At Anglesea, there was an increase in organic matter in the estuary mouth at one and nine weeks post flood, however variable results were seen at the estuary swash with an increase only occurring at nine weeks post flood. It is possible that sediment mixing and scouring (Froneman 2002, Perissinotto et al. 2002, Lane et al. 2007, Anandraj et al. 2008), may explain these anomalies in sediment organic matter, compared with the microphytobenthos results. For example, sediment cores for organic matter analysis were 3 cm long, compared to shorter cores for microphytobenthos (1 cm long); so much of the organic matter may have been washed out of the top sediment.

As well as nutrients, other factors such as sediment characteristics (e.g. particle size) and light have been shown to regulate microphytobenthic biomass (Cahoon 1999, Nozais et al. 2001, Snow & Adams 2007, Brito et al. 2009). This study clearly shows that the Anglesea estuary has higher concentrations of chlorophyll a inside the estuary mouth and swash during both mouth events compared to Curdies. The type of sediment at Anglesea is fine sand (Sherwood et al. 2008), compared to Curdies which is medium sand (Sherwood et al. 2008). Microalgal biomass and organic matter has been
shown to be higher in fine muddy sediments, and much lower in sandy sediments (Adams et al. 1999, Dittmann 2000, Du et al. 2006, Snow & Adams 2007, Brito et al. 2009). There have been variable results on whether light intensity is a critical factor for benthic microalgal production. Some studies report that benthic microalgae are capable of growth at very low light intensities (Cahoon 1999), others suggest there is a minimum light intensity required to support growth (Falkowski 1988, Nozais et al. 2001, Perissinotto et al. 2002) or no correlation at all (Brito et al. 2009). Light intensity and water column nutrients were not measured during this study, but such measurements would be useful in future studies.

For both the artificial opening and flood at Curdies estuary, results corresponded with what was predicted, with the biggest change in microbial utilisation of different carbon sources occurring after the events. At both the estuary swash and mouth locations bacteria used similar carbon sources at one and nine weeks post mouth events. This suggests that estuarine discharge may be contributing to microbial diversity in the coastal environment because similar bacterial communities are occurring at the estuary mouth and swash after artificial mouth openings and flood events, but not before. Alternatively, there may be similar organic sources becoming available after the mouth events for bacteria to use; through the input of ‘fresh’ material (e.g. nutrients and organic matter) being delivered by freshwater inputs (Lohrenz et al. 1999, Froneman 2002, Schlacher et al. 2009) causing this change at the estuary swash after the events. Bacterial production is regulated by organic carbon and nutrients (Button 1994, Pace & Cole 1994, Froneman 2006); an increase in estuarine discharge into the coastal zone during the mouth opening and natural flood would not only cause the resuspension of organic material and nutrients into the water column (Hopkinson 1987, Wainright 1987, Matthews & Constable 2004, Seymour et al. 2007, Celussi 2008), but it would deliver ‘fresh’ nutrient loads into the mouth and surrounding coastal waters (Lohrenz et al. 1999, Froneman 2002, Schlacher et al. 2009). As a result of this, it would stimulate bacteria growth and production (Wainright 1987, Ritzrau &
Graf 1992), increasing the bacteria available to utilise more carbon sources post event, as evident at Curdies.

In contrast, the artificial mouth opening at Anglesea did not result in predicted effects on sediment microbes, with results showing a difference in carbon source utilisation between locations but not across time. Importantly, bacteria in the estuary swash used similar carbon sources to those used at the mouth, suggesting similar dominant sources of carbon in the system or similar bacterial communities living at the estuary mouth and swash (Hopkinson 1987, Wainright 1987, Seymour et al. 2007, Celussi 2008), but there were no differences between times. The width of the sand bar at the mouth of the Anglesea estuary is much narrower than the Curdies, suggesting that groundwater seepage may be occurring into nearshore marine environments, contributing to these similarities in carbon source utilisation at the estuary mouth and swash before the opening of the estuary mouth. During the flood at Anglesea, the greatest difference in carbon sources at the estuary swash was seen as expected, between before and at one week post flood; however this difference had disappeared by nine weeks. This suggests that by nine weeks, any ‘fresh’ nutrients may have been used up or dispersed out to sea particularly from the estuary swash, causing carbon utilisation to show similar diversity to before the flood.

The size of the catchment, type of land use and freshwater inflow are all important factors influencing primary productivity of estuaries and their associated plumes (Howarth et al. 2000, Schlacher et al. 2008). Therefore the variability observed between the two estuaries is not surprising, given the considerable difference between the Curdies and Anglesea catchments, where Curdies has high intensity agriculture (>90 %), and Anglesea has a much higher percentage of remnant vegetation (>80 %) (Sherwood et al. 2008); this differing land use may explain a smaller response in the coastal areas adjacent to Anglesea. The size of the catchments is also different with Anglesea (125 km$^2$) being much smaller than the Curdies (1100 km$^2$), this would contribute to
a greater volume of water being discharged out of Curdies compared to Anglesea (Sherwood et al. 2008).

Previous studies have described estuaries as highly productive environments (Robertson & Duke 1987, Laegdsgaard & Johnson 1995, Nagelkerken et al. 2001, Mcclusky & Elliott 2004) along with local intermittent estuaries, which have been shown to be nutrient enriched (EPA 2000, Sherwood et al. 2008). Therefore, it seems logical that the discharge of estuary water should contribute to the productivity of adjacent coastal sediments, particularly during periods of high flow (Grimes & Kingsford 1996, Dagg & Breed 2003). Tracing the spatial and temporal extent of the influence of these nutrient-carrying plumes (Kingsford & Suthers 1994, Gaston et al. 2006, Ostrander et al. 2008) appears to be difficult particularly when estuaries are discharging onto high energy coastlines and the plume disperses so quickly. Therefore their influence remains largely untested for the majority of the world’s estuaries.

Of the few studies that do exist, the contribution by plumes to productivity in coastal waters has been reported for large rivers such as the Amazon and Mississippi (Calef & Grice 1967, Trefry et al. 1994, Smith 1996, Lohrenz et al. 1999, Rabalais et al. 2000, Liu & Dagg 2003, Dagg et al. 2004, Green et al. 2006). These large estuaries are permanently open to the ocean, and they also have a much greater catchment area and river discharge compared to these smaller system that are common in southern Australia. For example, the annual river discharge for the largest estuary in south-west Victoria, the Glenelg River, is 725,000 ML (Glenelg Hopkins Catchment Management Authority 2004), which is orders of magnitude less than that of the Amazon (6300 x10^6 ML) (Dagg et al. 2004). Current predictions of climate change foresee a substantial reduction in rainfall across temperate regions such as southern Australia and Africa (Schulze et al. 2001, Hughes 2003). This will lead to reduced freshwater flows into estuaries, resulting in seasonal and intermittently open estuaries becoming separated from the marine environment more regularly and potentially reducing the impact of estuary plumes on
coastal waters (Sherwood 1988). Given that this study was conducted during periods of low flow and extensive drought, it was difficult to detect a consistent response in some aspects of coastal sediment, suggesting that the contribution of estuaries for sediment productivity is variable between estuaries and flood types, and isn’t as clear as once thought. The overall impact of prolonged periods of drought on coastal flora and fauna remains largely unknown, particularly south-west Victoria, because very little ongoing monitoring is occurring.

Assessing the influence of estuarine discharge on coastal sediment is complex because of the interactions and processes involved in this environment. Therefore, longer-term studies are crucial to establish ranges of spatial and temporal variability in responses and to assess whether changes in river flows and estuary management might affect nearshore coastal ecosystems. Ideally, similar future studies need to be repeated during periods of higher rainfall as well as potentially measuring variables such as nutrients and light, to identify whether these plume characteristics are more consistent across estuaries and more easily detected during large flood events. However, predicted reductions in rainfall for this region, together with present drought, suggest that flood events are likely to become rarer over the next 50 yrs (Sherwood 1988, Whetton et al. 2002, Jones & Durack 2005). The consequences of reduced estuary outflows for adjacent coastal waters of south-eastern Australia (e.g. reduced fisheries production) remain largely unknown.
CHAPTER 4

4. Influence of estuarine discharge on intertidal algal assemblage structure and assimilation of carbon and nitrogen by the bivalve Austromytilus (Brachidontes) rostratus on adjacent coastal rocky shores

4.1. Introduction

The extent to which estuary plumes have been detected within adjacent coastal areas is extremely limited for many of Australian estuaries, especially for intermittent estuaries (Gaston et al. 2006, Schlacher et al. 2008, Schlacher et al. 2009). Large permanently open estuaries in south-eastern Queensland have been shown to release nutrient-rich plumes into adjacent coastal waters, enhancing biological production (Loneragan & Bunn 1999, Gillanders & Kingsford 2002, Dagg et al. 2004, Gaston et al. 2006, Schlacher et al. 2008, Schlacher et al. 2009). Intermittent estuaries in southern Australia exhibit several physical, chemical and ecological differences to their permanent counterparts in both northern Australia and the northern hemisphere. For example, they are usually much smaller and often become temporarily disconnected from the sea, during summer and autumn periods of reduced river discharge. Despite these differences in size, smaller plumes can contribute to nearshore coastal productivity of soft-sediment habitats, even during periods of prolonged drought (McKenzie et al. 2011).

Estuaries are not the only contributors of nutrients to coastal environments. The impact of sewage outfalls on intertidal rocky shore communities has been well studied (Borowitza 1972, May 1985, Fairweather 1990, Lopez Gappa et al. 1990, Chapman 1995, Bellgrove et al. 1997, Bellgrove et al. 2010, Firstater et al. 2010). Sewage-effluent outfalls tend to be nutrient-rich areas, which alter the structure of nearby algal assemblages (Borowitza 1972, Brown et al.)
Rocky shores surrounding outfalls often exhibit algal assemblages that are dominated by just a few, opportunistic ephemeral taxa such as *Ulva* spp., and also have reduced species richness compared to rocky shores that do not receive effluent discharge (Borowitzka 1972, May 1985, Brown *et al*. 1990, Fairweather 1990, Lopez Gappa *et al*. 1990, Chapman 1995, Bellgrove *et al*. 1997, Valiela *et al*. 1997). Similar observations have been seen at the mouths of intermittent estuaries in south-west Victoria; where, for example, the ephemeral green algae *Ulva* spp. dominate algal assemblages of rocky shores (personal observation). The similarity in algal assemblages of nutrient-enriched shores adjacent to effluent outfalls and rocky shores at estuary mouths provided an opportunity to investigate whether estuarine discharge influences algal recruitment and composition of algal communities.

The second aspect of this study aimed to identify whether the uptake of terrestrial/estuarine nutrient loads can be detected in fauna living in coastal areas adjacent to estuaries, by using stable isotope analysis (for detailed description of stable isotope analysis see Chapter 2, section 2.1). Stable isotope analysis is an effective technique for tracing organic matter and nutrients of different origins through aquatic ecosystems (Peterson & Fry 1987, Wissel & Fry 2005, Perdue & Koprivnjak 2007, Bannon & Roman 2008). The stable isotope ratio of an organism’s tissue provides a measure of the assimilated (not just ingested), time-integrated diet of an animal. The contribution of food sources to the organism’s diet such as terrestrial and marine organic material can be identified, because different organic matter sources display distinct isotope ratios according to the site of production (Peterson & Fry 1987). Therefore, when the isotope ratio of the animal’s tissue is compared to the ratio of a dominant producer in the system or to similar animals from different areas, the source of the animal’s diet can be inferred. Filter-feeding organisms, such as intertidal mussels, are ideal model species for detecting whether terrestrial/estuarine food sources are being consumed in the marine environment, since mussel tissue provides a time-integrated measure of
the presence of terrestrial/estuarine organic material and phytoplankton in the water column, which is delivered to the nearshore coast through estuarine discharge. Additionally, it demonstrates whether terrestrial/estuarine organic material is taken up by higher trophic levels of the food chain. The intertidal mussel *Austromytilus (Brachidontes) rostratus* was chosen for this study because it is one of the most common consumers of suspended organic matter on rocky shores and forms extensive dense mats and clumps on top of rocks and in crevices on intertidal shores from southern WA to southern NSW and Tasmania (*Quinn et al.* 1992, Edgar 2000). *A. rostratus* are efficient filter feeders, and play an important role in mineralizing organic material (*Quinn et al.* 1992, Peake & Quinn 1993), which provides an important food source for higher trophic levels (*Dame* 1993).

Many of the high energy beaches adjacent to estuaries in south-west Victoria are intermediate beaches with coarse sands (*Short* 1996). Severe hydrodynamic conditions probably account for the paucity of large, infaunal macroinvertebrates in these beach sediments (*J. L. McKenzie*, unpublished data (Chapter 2)). Thus, previous chapters focused on changes in sediment and water column parameters (e.g. microphytobenthic chlorophyll *a*, organic matter, nutrients) rather than infauna to test the influence of estuarine discharge on coastal productivity. Furthermore, carbon and nitrogen signatures in the sediment were below detectable limits (Chapter 2), so the current chapter describes two additional approaches involving algal assemblages (i.e. recruitment and established assemblage composition) and stable isotope analysis using an intertidal mussel.

The present chapter continues to test the model that intermittent plumes from small, intermittently open estuaries provide an important source of nutrients and organic matter for adjacent coastal ecosystems. The present study adds to previous work (*McKenzie et al.* 2011) and our knowledge of the ecological responses to estuarine outflows in two ways: 1) by comparing differences in macroalgal assemblage structure and recruitment and 2) by using stable isotope
analyses, to detect differences in δ¹³C and δ¹⁵N signatures in a common intertidal bivalve, *Austromytilus rostratus* between rocky shores adjacent to, and distant from, estuaries. Specifically, this chapter provides a test of the following hypotheses: 1) intertidal reefs at the mouths of estuaries will have greater algal cover and recruitment (as indicated by increased algal biomass and increased chlorophyll *a*), but lower species diversity, compared with control rocky shores; 2) algal assemblages adjacent to estuary mouths will be dominated by ephemeral species such as *Ulva* spp. relative to controls; 3) populations of the mussel *A. rostratus* adjacent to estuary mouths will assimilate different carbon and nitrogen sources that reflect sources supplied by estuarine discharge compared to those on control rocky shores.

### 4.2. Materials & Methods

#### 4.2.1. Study Sites

The influence of estuarine discharge on algal assemblages was assessed at four rocky shores located adjacent to mouths of intermittent estuaries: Curdies, Barham, Skenes, Kennett in south-west Victoria, and at four control rocky shores that were located at least 1 km from the nearest estuary mouth. The largest estuary sampled in this study was the Curdies estuary see Chapter 3 (Section 3.2.1). At Curdies estuary, algal assemblage surveys and recruitment sampling occurred at a calcarenite rocky shore that is located on the western headland of the estuary mouth. The second largest estuary in this study is the Barham (143°40'28"E, 38°45'52"S), which is part of a catchment covering an area of ~79 km² (Figure 4.1). Most of the land use surrounding the catchment is for forestry and agriculture (Sherwood *et al.* 2008). Sampling took place on an extensive basalt rock platform, which is located on the eastern bank of the Barham estuary. The other two estuaries, Skenes (143°42'35"E, 38°43'37"S) and Kennett (143°51'43"E, 38°40'05"S) (Figure. 4.1), are much smaller, with catchment areas of ~ 19 km² and ~ 21 km² respectively (Sherwood *et al.* 2008). Most of the Skenes catchment is used for agriculture with only a small portion of forest; whilst the land surrounding the Kennett catchment is dominated by
forest and native vegetation (Sherwood et al. 2008). For the majority of the year, the Kennett estuary mouth discharges across the beach between two sandstone rock platforms. During this study, sampling occurred on the rocky shore located on the western bank of the estuary. Rock platforms at Skenes estuary are located on both headlands; sampling took place on the eastern basalt rocky shore. Control rocky shores were paired with each estuary and were chosen to closely match wave exposure and rock type found at the estuary sites. These control rocky shores are referred to as Bay of Islands (142°52'10"E, 38°36'13"S Curdies control), Shelly beach (143°37'06"E, 38°47'39"S Barham control), Pools (143°45'20"E, 38°42'28"S Skenes control) and Sugar Loaf beach (143°47'15"E, 38°41'58"S Kennett control) (Figure 4.1).

To test whether terrestrial/estuarine nutrients are being assimilated by filter feeders on adjacent rocky shores, intertidal mussels, Austromytilus rostratus, were collected from eight rocky shores (i.e. four adjacent to four separate estuaries and four separate controls). These sites differed slightly from those used for the algal assemblage sampling due to the inconsistent distribution of mussels. Therefore, the four suitable estuary rocky shores included Barham, Kennett, St. George (143°58'31"E, 38°33'24"S) and Anglesea (144°11'28"E, 38°24'59"S, Figure 4.1) (see Chapter 3 section 3.2.1 for Anglesea estuary description). The control rocky shores were Shelly, Sugar (see Chapter 2 section 2.2.1 for control beach descriptions), Point Grey Beach (Lorne) and Point Roadknight, respectively. The St. George estuary is part of a catchment covering an area of 33.9 km² (Sherwood et al. 2008). The estuary is 1.5 km in length, and discharges out into a rocky headland approximately 1 km west of the township of Lorne. Most of the land surrounding the catchment is dense forest with less than 1% used for agriculture (Sherwood et al. 2008). Mussels were collected from a basalt rocky shore located on the western headland at the St. George estuary mouth. An extensive rocky shore platform at Point Grey Beach acted as a control for St. George estuary, and is located on the western side of the Lorne pier (143°59'10"E, 38°33'02"S). The calcarenite rocky shore on the western headland of the Anglesea main beach was the location of the
estuary rocky shore for mussel sampling. A rocky shore at Pt. Roadknight acted as a control shore for the Anglesea estuary (144°11'04"E, 38°25'42"S). Similarly to Anglesea, Pt. Roadknight is a bay and therefore mussels were collected from on the rocky shore on the western headland of Pt. Roadknight beach.
Figure 4.1 Location of all study locations along the south-western Victorian coastline. Insert: Location of estuaries within Victoria and south-eastern Australia.
4.2.2. **Effect of estuarine discharge on established algal assemblages**

Surveys of established algal assemblages were conducted during January 2007. Sites were surveyed in daylight hours when low tide was below 0.4 m. Thirty random point-quadrats (100 points, 50 cm x 50 cm) were used to survey each site. Percent covers of all algal taxa within a 1-cm radius of each point for multiple strata (e.g. canopy and understorey) were scored.

4.2.3. **Effect of estuarine discharge on algal recruit assemblages**

Recruitment panels were attached to the same eight rocky shores used during the algal assemblage surveys on two separate occasions between June-October 2007 (expected periods of peak recruitment; Bellgrove *et al* 2004) and left for a 6-9 week period. Experiment 1 ran between June-August and experiment 2 ran between August-Oct. At each site, 10 Hardiflex square panels (Gyprock, 5 x 5 cm, 7-mm thick) were randomly attached to the intertidal rock platform with one central, stainless-steel, self-tapping countersunk screw inserted into a wall plug (25-mm long). At Curdies and Bay of Islands, the rock is extremely soft, therefore stainless-steel Dyna-bolts (Warrnambool Bolts & Nuts, Victoria Australia; 65-mm long) were used to firmly attach the artificial panels. Each individual panel sat in an 80-mm PVC pipe end cap painted with copper-based antifouling paint (Paine 1980, Cubit 1984, Farrell 1988) (no leaching occurred), acting as a herbivore exclusion barrier. Holes (7-mm diam.) were drilled into the sides of the PVC caps to ensure drainage. A 2-mm thick (10 x 10 cm) neoprene-sponge gasket was placed in-between the bottom of the PVC end cap and the rock platform to allow the cap to fit flush despite slight rock-surface irregularities (Bellgrove 1998). At the end of each experiment, panels were collected in individual containers without water to prevent disturbance when transported on ice to the laboratory, where filtered seawater was added to each container, and bubbled with carbon dioxide to kill any copepods that may feed on the algae (Bellgrove *et al.* 2004). Panels were stored in constant temperature cabinets at 15°C. Photographs of panels were taken from the same height within 72 hrs to record percent cover of algal recruits, before being
prepared for chlorophyll a and algal biomass analysis. Individual panels were divided in half and algae were removed from each half of the panel and kept for the two different analyses.

To estimate percentage cover of each algal taxon that recruited to the panels, a quadrat (100 points, size 6.5 cm x 6.5 cm) was overlayed on photographs; the outer 0.5 cm was excluded to avoid edge effects, and species were recorded at the point of intersection of the grid lines. Percentage cover of different algal taxa was used as a measure of abundance of recruited species, and the variables of total algal cover (%), taxon richness and percentage cover of Ulva spp. were recorded.

To prevent degradation of chlorophyll a pigments, algae were removed from each panel within 48 hrs of returning to the laboratory. Chlorophyll a was extracted using acetone, following the method of Light & Beardall (1998). Technical details followed USEPA Method 446.0 (Arar 1997) for determining chlorophyll a by visible spectrophotometry. With this method, all samples were corrected for phaeo-pigments and thus measurements of chlorophyll a actually represent concentrations of all magnesium-containing pigments (Carlson & Simpson 1996).

Each sample was put into 35 mL of 100 % acetone, and mechanically mixed before being refrigerated at 4 °C for 24 hrs. Prior to reading, samples were shaken and then spun at 1000 rpm for 5 min. A sub-sample of 2.7-mL supernatant was diluted with 0.3 mL of distilled water to a final concentration of 90 % acetone in a 1-cm glass cuvette. Absorbance was read on a Shimadzu UV-Visible Recording Spectrophotometer (UV-265, Shimadzu Corporation, Kyoto, Japan) at 750 nm and 664 nm prior to acidification, and 750 nm and 665 nm post acidification. Acidification to determine phaeophytin a was achieved by the addition of 0.1 N HCl (Arar 1997). Chlorophyll a concentrations were calculated following the equation of Lorenzen (1967). Algae were removed from the panel and initially dried at 60 °C for 24 hrs.
After stabilising in a desiccator, samples were weighed to the nearest 0.0001 g then ashed in a muffle furnace at 550 °C for 3 hrs and loss of mass recorded. Biomass of algae was determined after loss on ignition (Light & Beardall 1998).

4.2.4. Assimilation of terrestrial/estuarine food sources by intertidal mussels

Previous studies have shown that suspended particulate organic matter (SPOM) can exhibit high spatial and temporal variability, and only provides a snap shot of what is present in the water column at any one particular time (Owens 1985, Kling et al. 1992, Cabana & Rasmussen 1996). The advantage of using tissue from filter-feeding bivalves is that it provides an integrated measure of carbon and nitrogen assimilation in their diet over time (Cabana & Rasmussen 1996). Accordingly, mussel tissue was expected to reflect all food sources that had been digested over the past few weeks to months (Hawkins 1985, Raikow & Hamilton 2001), incorporating any flood or discharge events that occurred during this period.

Fifty mussels were collected from each rocky shore adjacent to the estuary mouths of Barham, Kennett, St. George, Anglesea and their respective control rocky shores during September 2009. Large individuals (min. length = 1 cm) were targeted and assumed to be of a similar age across all locations. The mouths of all estuaries were consistently open during the five months prior to the collection of mussels. Mussels were rinsed and purged for 24 hrs, and then frozen until they were prepared for analysis. Once defrosted, 10 individuals were pooled for each replicate \( n = 5 \) to ensure there was enough tissue for analysis. The small size of the mussel hindered the use of tissue specific analyses, and therefore whole mussels of \( A. \ rostratus \) were analysed for carbon and nitrogen signatures. Mussel tissue was removed from the shell, rinsed twice in deionised water and dried at 60°C for 24 hrs. Samples were then ground and placed into vials for transport to the Isotope Analytical Facilities of Griffith University, Gold Coast Queensland. Samples were analysed using an
automated Isoprime (Micromass, UK) isotope-ratio mass spectrometer. Stable isotope ratios are expressed in parts per thousand (‰) using the standard delta (δ) notation: δX (‰) = [(Rsample/Rstandard) -1] x 1000; where X is δ13C or δ15N, and R is the 13C/12C (carbon) or 15N/14N (nitrogen) ratio in the sample and standards. Isotopic standards used are referenced to Pee Dee Belemnite equivalent for carbon and the IAEA international standard of atmospheric N2 for Nitrogen.

4.2.5. **Statistical Analysis**

4.2.5.1. *Univariate analysis*

Assumptions of normality and homogeneity of variance were checked using boxplots and residual plots (Quinn & Keough 2002) before univariate Analysis of Variance (ANOVA). Subsequently, chlorophyll a and algal biomass were log10(x) transformed to meet these assumptions. All dependent variables were analysed using two-way analyses of variance (ANOVA). The two factors were Estuary (four levels: established algal assemblages and recruitment data - Curdies, Barham, Skenes and Kennett; mussel data - Barham, Kennett, St. George and Anglesea) and Rocky Shore Type (two levels: estuary rocky shore, control rocky shore). Both factors were considered fixed factors. Where results showed significant interactions, simple main effects were used for each estuary to test for differences between the rocky shore types (Quinn & Keough 2002). Dependent variables analysed were total algal cover, taxon richness, percent cover of opportunistic algae (*Ulva* spp.), chlorophyll a and algal biomass for established algal assemblage and recruitment data. For the mussel study, dependent variables were carbon and nitrogen signatures. At some rocky shores the algal percentage cover sometimes exceeded 100% because multiple canopy strata were sampled together. Each of the recruitment experiments were analysed using separate ANOVAs. All statistical analyses were performed using SPSS v 17 and hypothesis tests were conducted at α = 0.05.
4.2.5.2. **Multivariate analysis**

Multivariate patterns in species composition of both established and recruit assemblages were examined with nMDS ordination using Bray-Curtis dissimilarity measure on untransformed data. Two-way Permutational Multivariate Analysis of Variance (PERMANOVA) was used to test species composition across 'Estuary' and between 'Rocky Shore Types'. All tests showed a significant estuary by rocky shore type interaction and therefore a one-way PERMANOVA was conducted between rocky shore types for each estuary (Quinn & Keough 2002). Where significant differences occurred, similarity percentages (SIMPER) were used to identify which algal species were primarily responsible for differences between rocky shore types. Only algal species that contributed to 80% of the overall dissimilarity between rocky shore types were reported. All multivariate analyses were conducted using Primer (version 6).

4.3. **Results**

4.3.1. **Relationship between sites of estuarine discharge and established algal assemblages**

Differences in total algal cover of established assemblages between rocky shore types were not consistent for each estuary (Table 4.1a, 4.2a; Figure 4.2a). The Curdies estuary rocky shore had higher algal cover compared to the control shore, but; there were no differences at Barham, Skenes and Kennett (Table 4.2a; Figure 4.2a). There were no significant differences in taxon richness of established assemblages at any of the four estuaries and their controls (Table 4.1b; Figure 4.2b).

The differences in percentage cover of *Ulva* spp. between rocky shore types were not consistent amongst estuaries (Table 4.1c). The estuary rocky shores of Barham, Skenes and Kennett showed higher percentage covers of *Ulva* spp. compared to the control shores; however these differences were only
statistically significant at Kennett (Table 4.2b; Figure 4.2c). *Ulva* spp. were not found at the Curdies estuary rocky shore and were sparse at the control shore (Figure 4.2).

Table 4.1 Survey data: Two-way ANOVAs of established algae comparing (a) Total Algal Cover (%); (b) Taxon Richness; (c) *Ulva* spp. Cover (%) between estuaries and rocky shore types.

<table>
<thead>
<tr>
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<th>df</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
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<td><em>(a) Total Algal Cover (%)</em></td>
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<td></td>
<td></td>
<td></td>
</tr>
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<td>Estuary</td>
<td>3</td>
<td>126886.00</td>
<td>108.062</td>
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<td>3117.60</td>
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<td>Residual</td>
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<td></td>
</tr>
<tr>
<td><em>(b) Taxon Richness</em></td>
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<td></td>
<td></td>
<td></td>
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<td>1.24</td>
<td>0.296</td>
</tr>
<tr>
<td>Residual</td>
<td>232</td>
<td>1.53</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>(c) Ulva spp. Cover (%)</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estuary</td>
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<td>3570.10</td>
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</tr>
<tr>
<td>Residual</td>
<td>232</td>
<td>164.68</td>
<td></td>
<td></td>
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</table>
Table 4.2 Survey data: One-way ANOVAs of established algae comparing (a) Total Algal Cover (%); (b) *Ulva* spp. cover (%) between rocky shores types for each estuary. Simple main effects used for this analysis from Table 4.1, where there was a significant estuary by rocky shore type interaction.

<table>
<thead>
<tr>
<th>Estuary</th>
<th>df</th>
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<th>F</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td>Curdies Estuary</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>(a) Total Algal Cover (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between rocky shore types</td>
<td>1</td>
<td>19046.00</td>
<td>16.22</td>
<td>0.0001</td>
</tr>
<tr>
<td>Residual</td>
<td>232</td>
<td>1174.20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(b) Ulva spp. cover (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between rocky shore types</td>
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<td>8.07</td>
<td>0.049</td>
<td>0.825</td>
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<tr>
<td>Residual</td>
<td>232</td>
<td>164.68</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Barham Estuary</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>(a) Total Algal Cover (%)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between rocky shore types</td>
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<tr>
<td>(b) Ulva spp. cover (%)</td>
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<td></td>
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<td>Skene Estuary</td>
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<td>(a) Total Algal Cover (%)</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Between rocky shore types</td>
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<td>72.60</td>
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<tr>
<td>(b) Ulva spp. cover (%)</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Between rocky shore types</td>
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<td>1.658</td>
<td>0.199</td>
</tr>
<tr>
<td>Residual</td>
<td>232</td>
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<td>Kennett Estuary</td>
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<td></td>
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<tr>
<td>(a) Total Algal Cover (%)</td>
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<tr>
<td>Between rocky shore types</td>
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<tr>
<td>(b) Ulva spp. cover (%)</td>
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<td>6510.42</td>
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<td>Residual</td>
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Figure 4.2 Means ±SE of established algae on rocky shores (survey data): (a) Total algal cover (%) (b) Taxon richness; (c) Total *Ulva* spp. cover (%). Grey bars represent rocky shores at estuary mouths, white bars represent rocky shores at control beaches.
A total of twenty-six species of macroalgae and one species of cyanobacteria were recorded in the established algal assemblages across all estuary and control rocky shores, with nineteen on estuary shores and twenty-three on control shores (Table 4.3). Differences in the composition of the established algal assemblages between rocky shore types were not consistent among estuaries (estuary x rocky-shore-type interaction PERMANOVA $F_{3,232} = 15.49$, $P = 0.001$). Although it is difficult to see groupings of rocky shore types in the nMDS plot (Figure 4.3), all estuary rocky shores differed from their respective controls (PERMANOVA Curdies: $F_{1,58} = 34.71$, $P = 0.001$; Barham: $F_{1,58} = 7.05$, $P = 0.001$; Skenes: $F_{1,58} = 3.91$, $P = 0.005$ and Kennett: $F_{1,58} = 23.79$, $P = 0.001$). However, SIMPER analyses showed that the combination of algal species that were driving differences between rocky shore types were inconsistent across estuaries. For example, *Corallina officinalis* contributed 18 - 36% of the dissimilarity in established assemblages between rocky shore types. *C. officinalis* was more abundant at the Curdies and Barham than their controls, but in contrast, was more abundant at the control than estuary shore for Skenes. Similarly, *Hormosira banksii* contributed 28 - 45% of dissimilarity but differed in relative abundance between estuaries. *H. banksii* cover was greater at the Curdies and Skenes compared with controls, but the opposite trend occurred at Barham and Kennett. Ephemeral species such as *Ulva* spp. and *Ectocarpus* sp. were consistently more abundant at estuary shores than control shores, but were only contributing 15 - 18% and 24% of the dissimilarity between assemblages at Barham (*Ulva* spp.) and Kennett (*Ulva* spp. and *Ectocarpus* sp.), respectively. *Scytosiphon lomentaria* accounted for 11 - 13% of the dissimilarity in established assemblages and was more abundant at Barham than the control, but vice versa for Skenes.
Table 4.3 Macroalgae species list of established algae (E) and recruited algae (R1 = recruitment experiment one; R2 = recruitment experiment two) present at estuary and control rock shores. + = present, - = absent.

<table>
<thead>
<tr>
<th>Species</th>
<th>Estuary Shores</th>
<th>Control Shores</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Curdies R1 R2</td>
<td>Bay of Islands R1 R2</td>
</tr>
<tr>
<td>Rhodophyta</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caprodia impexa</td>
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<td>-</td>
</tr>
<tr>
<td>Ceramium sp.</td>
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<td>-</td>
</tr>
<tr>
<td>Corallina officinalis</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Encrusting Coralline</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Laurencia sp.</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Polysiphonia sp.</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Unidentified red crust</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Unidentified dark red crust</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Unidentified filamentous red</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Chlorophyta</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caulerpa cactoides</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Codium pomoides</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Diatom film.</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ulva spp.</td>
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<td>+</td>
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### Table 4.3 Continued.

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<td><strong>Phaeophyceae</strong></td>
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<tr>
<td>Caulocystis sp.</td>
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<td>-</td>
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<td>Cladostephus spongiosus</td>
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<tr>
<td>Colpomenia simosa</td>
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<td>+</td>
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<tr>
<td>Cystophora sp.</td>
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<td>Cystophora torulosa</td>
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<td>Dicytota sp.</td>
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<td>Ectocarpus fasciculatus</td>
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<td>+</td>
</tr>
<tr>
<td>Halopteris gracilesens</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Hormostra banksii</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Notheira sp.</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Petalonia fascia</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Rafisia sp.</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Sargassum sp.</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Splachnidium rugosum</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sycosiphon lomentaria</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Xiphophora sp.</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Cyanobacteria</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rivularia firma</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Total Number of species</strong></td>
<td>9</td>
<td>6</td>
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</table>
Figure 4.3  MDS ordination plot based on Bray-Curtis dissimilarity of established algae on rocky shores at four estuary rocky shores and four control rocky shores (Survey data). Stress = 0.19. Different shapes represents estuaries; Δ = Curdies, □ = Barham; ○ = Skenes; ♦ = Kennett. Symbols for estuary rocky shores are filled and control rocky shores are open.

4.3.2. **Effect of estuarine discharge on algal recruit assemblages**

The Curdies estuary was closed at the beginning of the recruitment experiment, however it was artificially opened during the first recruitment experiment (July 2007) and remained open for the rest of the sampling period (July-October 2007). Barham, Skenes and Kennett estuaries were open for the entire sampling period (June-October 2007).

4.3.2.1. **Total cover of algal recruits**

During the first recruitment experiment (June-August 2007), the differences in total algal cover between rocky shore types were not consistent amongst estuaries (Table 4.4a). There were significant differences in algal cover between shore types at Barham, Skenes and Kennett, with Barham and Skenes
estuary rocky shores having significantly higher algal recruit cover compared to their respective control sites (Table 4.5a; Figure 4.4a). Kennett showed the opposite pattern, with the estuary rocky shore having lower recruit cover compared to the control shore (Table 4.5a; Figure 4.4a). In contrast, there were no significant differences between rocky shore types at Curdies (Table 4.5a). Similar patterns were shown at most estuaries during the second recruitment experiment (August-October 2007) (Table 4.6a). Barham and Skenes continued to have significantly higher algal recruit cover at estuary rocky shores compared to control shores (Table 4.7a; Figure 4.4a). While at Curdies and Kennett estuaries there were no significant differences in algal recruit cover between rocky shore types (Table 4.7a).

There were no significant differences in the taxon richness of algal recruits between rocky shore types during the first recruitment experiment (June-August 2007) and similar richness was found for each estuary (Table 4.4b; Figure 4.4b). However, this changed over the following months during the second recruitment experiment (August-October 2007), but not consistently across estuaries (Table 4.6b; Figure 4.4b). Curdies and Skenes estuary rocky shores had significantly higher richness of recruits compared to the control shores (Table 4.7b; Figure 4.4b). In contrast, there were no differences in taxon richness between the two rocky shore types for Kennett and Barham estuaries (Table 4.7b; Figure 4.4b).

The percentage cover of Ulva spp. recruits between shore types was not consistent across estuaries during the first recruitment experiment (Table 4.4c). Rocky shores adjacent to the estuary mouths of Barham and Skenes showed a significant difference in Ulva spp. cover compared to the control rocky shores, but differed in direction (Table 4.5b; Figure 4.4c). The Skenes estuary rocky shore had significantly lower Ulva spp. recruits compared to the control rocky shore, while the Barham estuary rocky shore had significantly higher Ulva spp. recruits, showing double the amount of recruitment than the control rocky shore (Figure 4.4c). In contrast, Curdies and Kennett estuary rocky shores
showed no difference in *Ulva* recruitment to the control sites (Table 4.5b). Recruitment of *Ulva* spp. was lower in the following months (August-Oct), with no significant difference in percentage cover between rocky shores types or across estuaries during the second recruitment experiment (Table 4.6c). The opportunistic brown alga, *Ectocarpus* sp. recruited onto the panels in high abundances at the Curdies estuary rocky shore (compared to control) during both recruitment experiments however, it was not found at any of the other sites (results not presented).

Table 4.4 Recruitment experiment 1: Two-way ANOVAs comparing (a) Total Algal Cover (%); (b) Taxon Richness; (c) *Ulva* spp. Cover (%) of algal recruits between estuaries and rocky shore types.

<table>
<thead>
<tr>
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<th>df</th>
<th>MS</th>
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<th>P</th>
</tr>
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<tbody>
<tr>
<td><em>(a) Total Algal Cover (%)</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estuary</td>
<td>3</td>
<td>1305.73</td>
<td>2.454</td>
<td>0.071</td>
</tr>
<tr>
<td>Rocky shore type</td>
<td>1</td>
<td>238.51</td>
<td>0.448</td>
<td>0.505</td>
</tr>
<tr>
<td>Estuary*Rocky shore type</td>
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<td>6078.71</td>
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<tr>
<td>Residual</td>
<td>67</td>
<td>532.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>(b) Taxon Richness</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estuary</td>
<td>3</td>
<td>2.34</td>
<td>3.74</td>
<td>0.015</td>
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<tr>
<td>Rocky shore type</td>
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<td>1.12</td>
<td>1.78</td>
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<td>1.26</td>
<td>2.02</td>
<td>0.120</td>
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<tr>
<td>Residual</td>
<td>67</td>
<td>0.63</td>
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<tr>
<td><em>(c) Ulva spp. Cover (%)</em></td>
<td></td>
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<td>Estuary</td>
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<td>3416.23</td>
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<td>Estuary*Rocky shore type</td>
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<tr>
<td>Residual</td>
<td>67</td>
<td>433.57</td>
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Table 4.5  Recruitment experiment 1: One-way ANOVAs comparing (a) Total Algal Cover (%); (b) Ulva spp. cover (%) of algal recruits between rocky shore types for each estuary. Simple main effects used for this analysis from Table 4.4, where there was a significant estuary by rocky shore type interaction.

<table>
<thead>
<tr>
<th>Estuary</th>
<th>Source</th>
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<th>F</th>
<th>P</th>
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</thead>
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<tr>
<td></td>
<td>(a) Total Algal Cover (%)</td>
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<td></td>
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<td></td>
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<tr>
<td>Curdies Estuary</td>
<td>Between rocky shore types</td>
<td>1</td>
<td>1140.41</td>
<td>2.14</td>
<td>0.148</td>
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<td></td>
<td>Residual</td>
<td>67</td>
<td>532.11</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>(b) Ulva spp. cover (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Between rocky shore types</td>
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<td>Residual</td>
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<td>433.57</td>
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<td></td>
</tr>
<tr>
<td>Barham Estuary</td>
<td>(a) Total Algal Cover (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Between rocky shore types</td>
<td>1</td>
<td>2838.67</td>
<td>5.33</td>
<td>0.024</td>
</tr>
<tr>
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<td>Residual</td>
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<td>532.11</td>
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<td>(b) Ulva spp. cover (%)</td>
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<tr>
<td></td>
<td>Between rocky shore types</td>
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<td>6364.96</td>
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<tr>
<td>Skenes Estuary</td>
<td>(a) Total Algal Cover (%)</td>
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<tr>
<td></td>
<td>Between rocky shore types</td>
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<td>3515.54</td>
<td>6.61</td>
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<td>Residual</td>
<td>67</td>
<td>532.11</td>
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<tr>
<td></td>
<td>(b) Ulva spp. cover (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Between rocky shore types</td>
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<td>6435.31</td>
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<tr>
<td>Kennett Estuary</td>
<td>(a) Total Algal Cover (%)</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Between rocky shore types</td>
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<td>108385.50</td>
<td>20.37</td>
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<td>(b) Ulva spp. cover (%)</td>
<td></td>
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<td></td>
<td>Between rocky shore types</td>
<td>1</td>
<td>3.81</td>
<td>0.009</td>
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<td>433.57</td>
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</table>
Figure 4.4 Means ±SE of algal species recruiting onto panels during recruitment experiments one and two: (a) Total algal cover (%) (b) Taxon richness; (c) Total Ulva spp. cover (%). Grey bars represent rocky shores at estuary mouths, white bars represent rocky shores at control beaches.
Table 4.6 Recruitment experiment 2: Two-way ANOVAs comparing (a) Total Algal Cover (%); (b) Taxon Richness; (c) *Ulva* spp. Cover (%) of algal recruits between estuaries and rocky shore types.

<table>
<thead>
<tr>
<th></th>
<th>df</th>
<th>MS</th>
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<tr>
<td>(a) Total Algal Cover (%)</td>
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<td></td>
</tr>
<tr>
<td>Estuary</td>
<td>3</td>
<td>3453.25</td>
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<td>7.46</td>
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<tr>
<td>Residual</td>
<td>69</td>
<td>894.80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(b) Taxon Richness</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Estuary</td>
<td>3</td>
<td>2.154</td>
<td>3.83</td>
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<tr>
<td>Residual</td>
<td>69</td>
<td>0.562</td>
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<tr>
<td>(c) Ulva spp. Cover (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
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<td>46.762</td>
<td>1.66</td>
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<td>4.38</td>
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<tr>
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<td>71.985</td>
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<tr>
<td>Residual</td>
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<td>28.165</td>
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Table 4.7  Recruitment experiment 2: One-way ANOVAs comparing (a) Total Algal Cover (%); (b) Taxon Richness of algal recruits between rocky shore types for each estuary. Simple main effects used for this analysis from Table 4.6, where there was a significant estuary by rocky shore type interaction.

<table>
<thead>
<tr>
<th>Estuary</th>
<th>df</th>
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<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Curdles Estuary</strong></td>
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<td></td>
<td></td>
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<tr>
<td><em>(a) Total Algal Cover (%)</em></td>
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</tr>
<tr>
<td>Between rocky shore types</td>
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<td>0.384</td>
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<tr>
<td>Residual</td>
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<td>894.80</td>
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<td></td>
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<tr>
<td><em>(b) Taxon Richness</em></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Between rocky shore types</td>
<td>1</td>
<td>6.050</td>
<td>10.76</td>
<td><strong>0.002</strong></td>
</tr>
<tr>
<td>Residual</td>
<td>69</td>
<td>0.562</td>
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</tr>
<tr>
<td><strong>Barham Estuary</strong></td>
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<tr>
<td><em>(a) Total Algal Cover (%)</em></td>
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<td></td>
</tr>
<tr>
<td>Between rocky shore types</td>
<td>1</td>
<td>7323.58</td>
<td>8.18</td>
<td><strong>0.006</strong></td>
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<tr>
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<td></td>
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<tr>
<td><em>(b) Taxon Richness</em></td>
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<tr>
<td>Between rocky shore types</td>
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<td>1.800</td>
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<td>Residual</td>
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<td><strong>Skennes Estuary</strong></td>
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<td><em>(a) Total Algal Cover (%)</em></td>
<td></td>
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</tr>
<tr>
<td>Between rocky shore types</td>
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<td>18.32</td>
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<tr>
<td>Residual</td>
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<td></td>
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<tr>
<td><em>(b) Taxon Richness</em></td>
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</tr>
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<td>Between rocky shore types</td>
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<td><strong>Kennett Estuary</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>(a) Total Algal Cover (%)</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between rocky shore types</td>
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<td>1390.07</td>
<td>1.55</td>
<td>0.217</td>
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<tr>
<td>Residual</td>
<td>69</td>
<td>894.80</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>(b) Taxon Richness</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between rocky shore types</td>
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<td>2.025</td>
<td>3.60</td>
<td>0.062</td>
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<tr>
<td>Residual</td>
<td>69</td>
<td>0.562</td>
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</table>
Nine different species of macroalgae recruited onto the artificial panels attached to estuary and control rocky shores, with eight species on estuary shores and eight recruiting on control shores (Table 4.3). Six species recruited during the first recruitment experiment and an additional three species (Diatom film, *Dictyotyota* sp. Unidentified filamentous red sp.) recruited during the second recruitment experiment (Table 4.3). Species composition of algal recruits differed between recruitment experiments one and two. During the first experiment (June-August 2007) the differences in recruit assemblages amongst rocky shore types was not consistent across estuaries (PERMANOVA $F_{3,66} = 10.94$, $P = 0.001$). All estuaries showed significant differences in species composition between rocky shore types (PERMANOVA Curdies: $F_{1,16} = 13.53$, $P = 0.001$; Barham: $F_{1,16} = 9.82$, $P = 0.001$; Skenes: $F_{1,17} = 18.70$, $P = 0.001$; Kennett: $F_{1,16} = 4.27$, $P = 0.01$), and this can be seen in the MDS plot, with different rocky shore types showing grouping for each estuary (Figure 4.5a). SIMPER identified four species driving differences between rocky shore types, but there were inconsistencies across estuaries. *Scytosiphon lomentaria* contributed 34 – 63 % of the dissimilarity in recruited algae between rocky shore types, but it was more abundant at control shores of Curdies, Barham and Kennett, and the estuary shore of Skenes. Similarly *Ralfsia* contributed 20 % of dissimilarity but differed in relative abundance between estuaries, higher at estuary shore of Barham, but control shore of Kennett. Ephemeral species such as *Ulva* spp. and *Ectocarpus* sp. accounted for 12 % (Curdies), 19 % (Kennett), 37 % (Skenes, Barham) (*Ulva* spp.); and 30 % (Curdies - *Ectocarpus* sp.) of the dissimilarity in recruited algae between rocky shore types. However, there were again inconsistencies with greater abundances of *Ulva* spp. and *Ectocarpus* sp. at estuary shores of Kennet, Barham and Curdies (*Ectocarpus* sp.), while greater abundances of *Ulva* spp. occurred at control shores of Skenes and Curdies.
For the second recruitment experiment (August-October 2007) again the
difference in species composition of algal recruits occurring between rocky
shore types were not consistent amongst estuaries (PERMANOVA $F_{3,37} =
13.48, P = 0.001$). The MDS plot clearly shows grouping between rocky shore
types for each estuary (Figure 4.5b), and this was supported by a one-way
PERMANOVA, where results showed a significant difference between rocky
shore types for each estuary (PERMANOVA: Curdies $F_{1,18} = 27.78, P = 0.001$;
Barham $F_{1,12} = 7.92, P = 0.002$; Skenes $F_{1,14} = 8.06, P = 0.001$; Kennett $F_{1,14} =
8.02, P = 0.003$). Algal species driving the differences between rocky shore
types during the second experiment were different to those seen for the first
recruitment experiment, and again patterns differed amongst estuaries.
Diatoms accounted for 25 – 58 % of the dissimilarity of recruited algae
between rocky shore types, and were more abundant at estuary shores of
Barham, Skenes and Kennett, but higher at the control shore of Curdies.
Similarly, $S. lomentaria$ contributed 21 and 48 % of the dissimilarity but
differed in relative abundance between estuaries; being higher at the estuary
shore of Curdies but higher at the control shore of Kennett. An unidentified
filamentous red alga was consistently more abundant at estuary shores than
control shores, contributing 50 % and 34 % of the variation of Barham and
Skenes respectively. $Colpomenia sinuosa$ was more abundant at the estuary
shore of Curdies but greater at the control shore of Kennett, however it only
contributed 13 % and 16 % (respectively) of the dissimilarity between recruited
algae.
Figure 4.5  MDS ordination plot based on Bray-Curtis dissimilarity of algae recruiting (species composition) onto rocky shores at four estuary rocky shores and four control rocky shores during recruitment experiments (a) one: stress = 0.09 and (b) two: stress = 0.09. Different shapes represent estuaries; $\Delta =$ Curdies, $\Box =$ Barham; $\bigcirc =$ Skenes; $\Diamond =$ Kennett. Symbols for estuary rocky shores are filled and control rocky shore are open.
4.3.2.2. Chlorophyll a content of algal recruits

The differences in concentrations of chlorophyll a on recruitment panels between rocky shore type were not consistent amongst estuaries for the first recruitment experiment (June-August 2007) (Table 4.8a). Three of the four estuary rocky shores were significantly different in chlorophyll a concentrations compared to the control shores (Table 4.9), with the Curdies and Skenes estuary rocky shores having significantly higher concentrations than the control shores. Kennett estuary rocky shore had significantly lower concentrations compared to the control and Barham estuary rocky shore showed similar concentrations to the control rocky shore (Table 4.9; Figure 4.6). The interaction in chlorophyll a concentration between estuary and rocky shore type was also significant during the second recruitment experiment (August-October 2007); with all estuary rocky shores showing significant higher concentrations than the control rocky shores, except for Kennett (Table 4.10a; 4.11; Figure 4.6).

4.3.2.3. Biomass of algal recruits

During the first recruitment experiment (June-August 2007), there were only differences in algal biomass between rocky shore types at Kennett estuary, with the estuary rocky shore having significantly lower algal biomass compared to the control rocky shore (Table 4.8b, 4.9; Figure 4.6). Different patterns were shown for the second recruitment experiment (August-October 2007), with there being differences in algal biomass between rocky shore types across all estuaries (Table 4.10b & 4.11). Curdies, Barham and Skenes estuary rocky shores had significantly higher algal biomass compared to the control rocky shores; however Kennett estuary rocky shore was significantly lower in algal biomass compared to the control rocky shore (Table 4.11; Figure 4.6).
Table 4.8 Recruitment experiment 1: Two-way ANOVAs comparing (a) Log chlorophyll \( a \) and (b) Log algal biomass of algal recruits between estuaries and rocky shore types.

<table>
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<tr>
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<td>0.411</td>
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<td>(b) Algal Biomass</td>
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Table 4.9 Recruitment experiment 1: One-way ANOVAs comparing Log chlorophyll $a$ and Log algal biomass of algal recruits between rocky shore types for each estuary. Simple main effects used for this analysis from Table 4.8, where there was a significant estuary by rocky shore type interaction.

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Figure 4.6 Mean ±SE Log Chlorophyll $a$ (mg/L) and Log algal biomass (g) of algal recruits from estuary and control rocky shores during recruitment experiments one and two. Vertical grey bars represent estuary rocky shores, white represent control rocky shores. Horizontal bars denote mouth status during the experiments, solid black bar mouth is closed, white is mouth open.
Table 4.10 Recruitment experiment 2: Two-way ANOVAs comparing (a) Log chlorophyll $a$ and (b) Log algal biomass of algal recruits between estuaries and rocky shore types.

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Table 4.11  Recruitment experiment 2: One-way ANOVAs comparing Log chlorophyll $a$ and Log algal biomass of algal recruits between rocky shore types for each estuary. Simple main effects used for this analysis from Table 4.10, where there was a significant estuary by rocky shore type interaction.

<table>
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<td>Between Rocky Shore Type</td>
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<td>Residual</td>
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<td>0.081</td>
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<td><strong>Barham Estuary</strong></td>
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<tr>
<td>Algal Biomass</td>
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<td>9.083</td>
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<td>0.081</td>
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<td><strong>Kennett Estuary</strong></td>
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<td>Chlorophyll $a$</td>
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</table>
4.3.3. Assimilation of terrestrial/estuarine food sources by intertidal mussels

There was a significant difference in carbon isotopes ($\delta^{13}$C) of mussels between rocky shore types for all estuaries except Kennett (Table 4.12 & 4.13). Mussels close to the estuary mouths of Barham, St. George and Anglesea had isotope ratios that were significantly depleted in $\delta^{13}$C compared to the mussels at the control sites (Table 4.13; Figure 4.7). Nitrogen isotopes ($\delta^{15}$N) for mussels from estuary rocky shores were more enriched relative to controls, particularly for the Barham and Anglesea estuaries, although the differences were statistically significant for the Anglesea only (Table 4.12; 4.13; Figure 4.7b). The Kennett rocky shore was the only location that showed no significant differences in carbon and nitrogen isotope ratios compared to the control site (Table 4.13; Figure 4.7).

Table 4.12 Two-way ANOVAs comparing $\delta^{13}$C and $\delta^{15}$N of mussels between estuaries and rocky shore types.

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Table 4.13 One-way ANOVA’s comparing $\delta^{13}$C and $\delta^{15}$N of mussels between rocky shores types for each estuary. Simple main effects used for this analysis from Table 4.12, where there was a significant estuary by rocky shore type interaction.

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</tbody>
</table>
Figure 4.7 Mean ±SE (a) Carbon isotope signature ($\delta^{13}$C) (b) Nitrogen isotope signature ($\delta^{15}$N) of mussels (*Austromytilus rostratus*) from intertidal rocky shores adjacent to estuary mouths (△) and open beaches acting as control sites (○) paired with each estuary site. ($n = 5$)
4.4. Discussion

Estuary plumes from large and small systems have been shown to be important for the delivery of nutrients and organic material from the catchment to coastal areas (Smith 1996, Lohrenz et al. 1999, Rabalais et al. 2000, Dagg et al. 2004, Gaston et al. 2006, Haines et al. 2006, Ostrander et al. 2008, Schlacher & Connolly 2009, Schlacher et al. 2009). Surprisingly, the extent to which estuarine discharge can be detected within adjacent coastal areas remains unclear for many of the world’s estuaries (Dagg et al. 2004, Gaston et al. 2006, Schlacher et al. 2008, Schlacher et al. 2009). The present study demonstrates that material exported from intermittent estuaries can influence algal composition and recruitment, and is assimilated by invertebrates on adjacent rocky shores. This was illustrated by differences in algal growth, taxon richness, dominance of ephemeral species and differences in isotope signatures of intertidal mussels, relative to control shores. There was spatial variability in the variables measured between estuaries, which may be a reflection of differences in propagule supply and recruitment, estuary mouth status, size of the catchment, along with nutrient supply, salinity levels and land practices. These potential contributing factors are discussed below.

4.4.1. Effects of estuarine discharge on algal assemblages and primary productivity

The spatial variability in algal cover of established assemblages observed between estuaries may be related to differences in beach exposure and estuary characteristics, such as volume of flow and nutrient concentrations. However, it is most likely to be related to a number of pre- and post-recruitment processes that can influence the structure and dynamics of macroalgal assemblages. In addition to the spatial differences in algal recruitment, numerous other studies have shown that predation/herbivory (Petratis 1990, McCook & Chapman 1993); competition (Dayton 1971, Paine 1984, Reed 1990) and disturbance (Dayton 1971, Farrell 1989) are important post-
recruitment mortality pressures in marine systems. Measuring the contribution of these processes was beyond the scope of the present study.

Rocky shores exposed to Barham and Skenes estuarine discharge exhibited higher recruitment cover compared to their respective control shores during the months of June – August 2007. However, Curdies estuary shores showed no difference while the Kennett estuary showed lower algal cover of recruits compared to the control shore. Bellgrove et al. (2004) found similar peaks in algal recruitment during the winter months, identifying that the supply of propagules and recruitment of most intertidal macroalgae in south-eastern Victoria was greatest between April and August, with opportunistic species such as Ulva spp. continuing into October. The Curdies estuary rocky shore had higher algal cover of established assemblages, however there were no differences in recruitment of algae between rocky shore types. Conversely, total algal cover of established assemblages at Barham, Skenes and Kennett showed no differences between rocky shore types, yet there was greater cover of recruits at Barham and Skenes estuary rocky shores, while Kennett had lower recruitment of algal cover compared to its control shore. These patterns are consistent with Bellgrove et al. (2004) demonstrating that algal recruitment is not directly linked with established assemblages.

In contrast to our predictions and other studies of nutrient-influenced shores (Fairweather 1990, Lopez Gappa et al. 1990, Bellgrove et al. 1997, Valiela et al. 1997, Wear & Tanner 2007) , taxon richness was not lower at shores exposed to estuary outflow. Established algae and recruitment at all estuary shores showed the same or greater taxon richness compared to the control shores. Estuary rocky shores exposed to the plumes of Curdies and Skenes were the two estuaries that showed significantly greater taxon richness in algal recruits compared with the control shores, but this was not consistent across the two experimental periods. The Curdies estuary was the only estuary to be closed at the start of the recruitment experiment. The estuary mouth was artificially opened during the first recruitment experiment (14th July 2007),
releasing volumes of water $>1400$ ML/day (Chapter 2) into the coastal zone and remained open for the remaining sampling periods. It was not until a month after the artificial mouth opening (second recruitment experiment) that there was a difference in taxon richness detected between the estuary and control shores; however this difference was caused by a decrease in species richness at the control shore. Considering that the Curdies is the largest estuary examined in the present study, it was expected that any estuarine influence would be most pronounced at this estuary. In contrast, results suggest that the volume of discharge following an artificial mouth opening may need to be larger than the opening event in July 2007, to be able to detect an influence of estuarine discharge on macroalgal taxon richness in these smaller systems. Given that sampling occurred during a period of drought (Matthews & Fairweather 2003, Timbal 2009) these results are not surprising.

Estuarine discharge provides a source of high nutrients (from terrestrial inputs) into the marine receiving waters (Grimes & Kingsford 1996, Lohrenz et al. 1999, Perissinotto et al. 2000, Dagg & Breed 2003, Dagg et al. 2004, Schlacher et al. 2009), potentially causing floristic changes similar to those associated with other nutrient inputs into coastal environments, such as from treated sewage outfalls. Greater abundances of Ulva spp. were observed in established assemblages at rocky shores close to estuaries compared to remote shores, consistent with findings for opportunistic ephemeral green algae surrounding sewage outfalls (Borowitzka 1972, May 1985, Brown et al. 1990, Fairweather 1990, Chapman 1995, Bellgrove et al. 1997, Bellgrove et al. 2010, Firstater et al. 2010). There was however, considerable temporal and spatial variability in both the recruitment and abundance on the shore of ephemeral species, with shores exposed to Barham, Skenes and Kennett estuaries having greater algal cover of Ulva spp. in established assemblages compared to their respective control shores. While for recruitment, Barham estuary rocky shore was the only estuary to have higher cover of Ulva spp. recruits compared to the control shore. This variability in Ulva spp. abundance and recruitment is possibly due to variability in propagule (Bellgrove et al. 2004) and nutrient
supply (from the estuaries) during the sampling period of August-October 2007 (McGlathery et al. 1996, Fry et al. 2003).

Specifically, nitrogen is a critical nutrient for marine algal growth (May 1985, Valiela et al. 1997). Estuaries receive nitrogen from riverine/terrestrial organic material, fertilizers and human and animal wastes (McClelland et al. 1997). The concentrations of nitrogenous inputs are variable (McGlathery et al. 1996, Fry et al. 2003) and depend upon the land practices surrounding the estuaries and upstream (Schlacher et al. 2008). For example, Barham may receive higher nitrogenous inputs because the catchment is dominated by agriculture, potentially causing high recruitment of Ulva spp., while Skenes is surrounded by forest and may not receive such high nitrogenous inputs (perhaps driving lower recruitment). Examining nitrogen concentrations of the water column (McGlathery et al. 1996, Cohen & Fong 2006, Hill et al. 2006, Hill et al. 2008) or stable isotope ratios of $\delta^{15}$N of algal tissue (Peterson & Fry 1987, McClelland et al. 1997, Hill et al. 2006, Thornber et al. 2008, Firstater et al. 2010) such as the ephemeral species Ulva due to its dominance on estuary rocky shores, may improve assessment of the effect of nutrient sources onto coastal zones.

Species composition of established assemblages varied with respect to abundances between the estuary and control rocky shores. As predicted, ephemeral taxa, such as Ulva spp. and Ectocarpus sp., were in high abundance for most estuary shores, with perennial species such as Corallina officinalis, Hormosira banksii and Scytosiphon lomentaria showing spatial variability between estuaries. The differences in species composition between rocky shores are consistent with those described in previous studies (Borowitzka 1972, Brown et al. 1990, Fairweather 1990, Bellgrove et al. 1997, 2004) of algal assemblages affected by sewage outfalls, where brown algae are reduced and opportunistic ephemeral greens are a dominant taxon (Borowitzka 1972, May 1985, Brown et al. 1990, Fairweather 1990, Lopez Gappa et al. 1990, Bellgrove et al. 1997).
The low abundance of *H. banksii* in established assemblages of some estuary shores, relative to controls, may be in response to very high nutrients and reduced salinities (Dobbin & Clayton 1995). During the present study the lowest salinities recorded in the estuary swash (adjacent to the estuary mouths) were 6 psu and 15 psu (Barham and Curdies respectively) and 27 - 33 psu (Kennett, Skenes) during January 2007. Dobbin and Clayton (1995) found that a high concentration of nutrients from sewage effluent and low salinity (in the range measured here) can inhibit zygote germination and embryo growth in *H. banksii*. Additionally, there was almost complete loss of *H. banksii* reported in Doubtful Sound, New Zealand following prolonged freshwater discharge from riverine inputs (Boyle et al. 2001).

The differences in the composition between algal recruits and established algae, suggests that the composition of established macroalgal assemblages does not necessarily correlate with the supply of propagules and recruitment (consistent with (Bellgrove et al. 2004)). There was spatial variability between estuaries in chlorophyll *a* and algal biomass of recruited algae during the first recruitment experiment, with some estuary rocky shores having greater concentrations compared to control shores. However, by the middle of winter (second recruitment experiment), all estuary rocky shores except Kennett had higher concentrations of chlorophyll *a* and algal biomass of recruited algae compared to their respective control shores. These results are consistent with what was shown for the total algal cover of recruits, potentially suggesting that there were greater nutrients being discharged out of the estuaries during the second recruitment experiment (August – October 2007); limited discharge data are available for these estuaries, however Curdies recorded large discharge events in August 2007 (1425 ML/day) and October 2007 (1396 ML/day). Similar results have been shown for microphytobenthic chlorophyll *a* concentrations in estuaries of South Africa, where flood events can introduce high concentrations of nutrients, sediment, dissolved and suspended organic matter into adjacent coasts, stimulating microphytobenthic and algal growth (May 1985, Valiela et al. 1997, Adams et al. 1999, Lohrenz et al. 1999,
Froneman 2002, Perissinotto et al. 2002). The high recruitment occurring during the high flow period (August-October 2007), would result in greater surface area of algal cover and therefore chlorophyll a pigment would be more abundant contributing to these high concentrations that were recorded during the flood period.

4.4.2. Assimilation of terrestrial/estuarine food sources by intertidal mussels

Further indirect evidence that estuarine discharge is stimulating production of adjacent coastal ecosystems was illustrated by the carbon and nitrogen isotopes in intertidal mussel populations. Mussels exposed to estuarine plumes of the Barham, St. George and Anglesea estuaries were significantly lower in carbon isotope ratios, indicating the presence of a terrestrial/estuarine signal, compared to mussels at marine control shores, which reflected marine food sources. These results suggest that mussels receiving estuarine plumes are assimilating terrestrial/estuarine sources, which are being discharged out of the estuary. Similar results have been shown for beach clams exposed to estuary plumes in southern Queensland (Schlacher & Connolly 2009). There are very few isotope signatures for coastal/estuarine bivalves that exist in southern Australia. However, one local study looking at the diet of the infaunal bivalve Solestellina alba, inside the Hopkins estuary (Lautenschlager unpublished data 2009); found the estuarine bivalve S. alba to be significantly more depleted in carbon (mean -30.22) compared to values recorded for A. rostratus in this present study (mean -21.8), suggesting that potentially there is a dilution of terrestrial/estuarine nutrients once released into the coastal zone. In contrast to mussels from Barham, St. George and Anglesea estuary rocky shores, mussels at the Kennett shore showed similar carbon isotope ratios to the marine control site. Kennett is part of a relatively small catchment with the lowest river discharge, potentially resulting in limited availability of terrestrial/estuarine nutrients for mussels to consume. Similar variability in mussel diet was shown by Hill et al. (2008), who monitored the diet of mussels living on a rocky intertidal zone in close proximity to three permanently open estuaries in South
Africa over a 14 month period, to identify whether mussels were feeding on nearshore, offshore or estuarine suspended particulate matter. Hill et al. (2008) identified carbon ratios of mussel tissue to be more enriched or similar to the values of nearshore suspended particulate matter in 11 out of the 14 sampling months. While for the other three months, carbon ratios of mussels fell between values of nearshore and offshore suspended particulate matter (Hill et al. 2008). Consequently, the variability of mussel diet shown in the present study, suggests that mussels may not have a feeding preference and may feed on whatever is available (Hill et al. 2006, Hill et al. 2008), whether that is an offshore marine source or passively feed on a diet of terrestrial/estuarine organic material released from the nearby estuary. It suggests a small contribution of terrestrial matter to the diet of marine consumers in the immediate vicinity of estuarine plumes.

Enriched $\delta^{15}N$ signatures were detected in mussels associated with estuary mouths (relative to controls), consistent with an assimilation of organic material from anthropogenic terrestrial and estuarine sources. This study did detect variability in the scale of the difference, with Anglesea and Barham estuaries having the greatest influence. These nitrogen results are in close agreement with previous research, which have shown nitrogen isotope ratios to reflect the catchments’ land practices (Peterson & Fry 1987, McClelland et al. 1997). Anglesea is the most urbanised estuary in this study, while the Barham catchment is surrounded by agriculture and native vegetation. Both upper catchments are likely to receive high nitrogen inputs through freshwater runoff of human/animal wastes and fertilizers being transported downstream to the estuary and coastal zone, available for the uptake of organisms such as mussels (McClelland et al. 1997). In contrast, $\delta^{15}N$ signatures in mussels from Kennett and St. George estuary shores were less enriched. The catchments of these two estuaries are small and surrounded by forest (Otways temperate rain forest). The townships located on the estuaries have very few permanent residences, therefore potentially these estuaries do not receive high nitrogen loads (Mondon et al. 2003, Sherwood et al. 2008). Higher nitrogen values (highest
9.7) were shown for *S. alba* inside the Hopkins estuary (Lautenschlager unpublished data 2009) compared to *A. rostratus* (highest mean 8.3). The Hopkins estuary is one of the largest estuaries in Victoria and is more urbanised than the Anglesea (Sherwood *et al.* 2008). Consequently, it is not surprising that bivalves living inside the Hopkins estuary mouth are more enriched in nitrogen, once again reflecting the catchments land practices. This study identified that even during periods of drought (Matthews & Fairweather 2003, Timbal 2009) estuaries are an important link between catchments and nearshore marine assemblages, by supplying food sources for bivalve populations.

The size of the catchment, freshwater flow, land practices and time of year are all important factors contributing to the influence of estuary discharge on coastal productivity (McClelland *et al.* 1997, Howarth *et al.* 2000, Schlacher *et al.* 2008). It is not surprising that this study detected variability in the extent of estuarine influence amongst estuaries, given the considerable difference in estuaries, catchment size, catchment use and variability in the surrounding coastal areas. However, it is of significant interest that the results of this study for small, intermittent estuaries with small pulse events are consistent with results of both large and small permanently opened estuaries, in being able to detect an influence of plumes on nearby coastal areas. With increasing variability in rainfall and prolonged drought periods being experienced throughout Australia during the sampling period (Matthews & Fairweather 2003, Timbal 2009), this study highlights that it is not only important to manage our water resource to ensure the health of our rivers and estuaries (Sherwood 1988, Sherwood *et al.* 2008), but also to maintain the productivity of our incredibly diverse (Phillips 2001) nearshore marine assemblages.
CHAPTER 5

5. General Discussion

This study has explored a subset of ecological responses by coastal ecosystems to estuarine discharge from intermittently open estuaries. The research was conducted during a period of extended drought, with flows for all rivers in south-west Victoria being well below the long-term average annual flow (DSE 2010). Despite the period of reduced flow, this research did detect a coastal ecosystem response to estuarine discharge, albeit very variable for different measures and across different estuaries. Chapter 2 showed that artificial opening of the estuary mouths leads to changes in water temperatures and salinity of the estuary swash with marine waters becoming slightly colder, shifting to almost completely fresh conditions, and dissolved oxygen levels increasing, presumably through turbulent mixing of estuarine and marine waters. The initial drop in salinity at the estuary swash was not sustained, with salinity levels gradually returning to marine levels (35 psu) by 3 – 4 months after the opening. These findings suggest that physical forces of the ocean such as currents, winds, tidal mixing and waves are likely to be important controlling factors that determine where and how long the estuarine plume would remain concentrated at the mouth of the estuary, before river flow and the estuary plume dissipated (Lohrenz et al. 1990, Dagg & Whitledge 1991, Grimes & Kingsford 1996, Lohrenz et al. 1999, Devlin et al. 2001). This in turn would influence the degree to which coastal environments are affected by estuarine discharge.

More significantly, the discharge of these small intermittent estuaries is detectable during periods of significantly reduced flow. For example, estuarine discharge appeared to influence microphytobenthic chlorophyll a and organic matter content of the sediment (Chapter 2), microbial diversity (Chapter 3) and rocky shore algal assemblages (Chapter 4). Stable isotope analyses of an intertidal mussel also provided evidence for assimilation of material derived
from estuarine discharge (Chapter 4). However the spatial extent of the influence of intermittent estuarine discharge on nearshore marine environments remains unknown, and should be addressed in future studies.

5.1. Coastal water response detected during a period of reduced flow

Large temporal changes in physico-chemical conditions observed inside the estuary are typical of estuarine environments, not just in Australia (Matthews 2000, Arundel 2003, Becker 2007) but also in temporarily open/closed estuaries in South Africa, depending upon the amount of flow and exchange with the marine environment (Mackay & Cyrus 2001, Whitfield & Bate 2007). In South Africa, two estuaries (Siyaya and Nhlabane) also experienced a period of drought during 1992-1994, where minimal freshwater runoff lead to the closure of the estuary mouths, recording the longest period of isolation from the marine environment that these two systems had experienced on record (Mackay & Cyrus 2001). Variability in salinity was more extreme than the findings of the present study, with salinity of the two South Africa estuaries shifting from estuarine to freshwater from the mouth of the estuary to the head (Mackay & Cyrus 2001). Salinity levels of both estuaries decreased substantially due to dilution from river flow, and never exceeded 6 psu throughout the entire estuary during the two year drought (Mackay & Cyrus 2001). Intermittent estuaries not only experience periods of low salinity, but hypersaline (Whitfield & Bate 2007, Sherwood et al. 2008, Froneman & Henninger 2009) and hypoxic conditions (dissolved oxygen levels < 2-3 mg/L) (Whitfield & Bate 2007) can also occur inside the estuary during periods of mouth closure. Although flow was low for estuaries in the present study, there was sufficient inflow, even during periods of mouth closure, to prevent the estuaries from experiencing conditions of hypersalinity or hypoxia within shallow waters (< 2m) during the study period.

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As predicted, Chapter 2 identified increased nutrient concentrations in the coastal zone following the artificial mouth opening of the Curdies estuary. This contribution of nutrients from the Curdies to the coastal zone is similar to what has been observed for larger, permanently open systems in both northern Australia (Gaston et al. 2006, Schlacher et al. 2008, Schlacher et al. 2009) and larger river systems in the northern hemisphere (Lohrenz et al. 1990, Smith & Demaster 1996, Lohrenz et al. 1999, Dagg & Breed 2003, Dagg et al. 2004). This implies that the effects of nutrient-rich plumes of large river systems might scale down to small intermittently open estuaries. Numerous studies have shown that the delivery of nutrients and organic material from permanently open estuaries to the coastal zone is an important factor for phytoplankton and microphytobenthic growth (Lohrenz et al. 1990, Mallin et al. 1993, Mallin & Paerl 1994, Lohrenz et al. 1997, Adams et al. 1999, Froneman 2002, Dagg & Breed 2003, Dagg et al. 2004, Snow & Adams 2007, Schlacher et al. 2008, Schlacher et al. 2009). To my knowledge, the present study is the first to demonstrate that intermittently open estuaries provide an important source of nutrients and organic material for coastal productivity in southern Australia. The distance that estuary plumes can extend and transport materials to offshore waters in southern Australia is unknown for these small intermittent systems, however for large systems such as the Mississippi River, nutrients associated with the river plumes has been observed 10 - 100 km from the mouth several days after discharge (Lohrenz et al. 1990, Dagg & Whitledge 1991, Lohrenz et al. 1997). These great distances of influence are unlikely for small intermittent systems, however it does highlight how far the influence of estuaries can extend and that these smaller systems could potentially influence surrounding coasts.
5.2. Influence of estuarine discharge on primary productivity of coastal sediments

5.2.1. Coastal sediment response to artificial mouth openings

The research undertaken in Chapters 2 and 3 identified inconsistent patterns in the response of several variables associated with coastal sediments following artificial mouth openings. There was considerable variability between estuaries for microphytobenthos at estuary swash locations, with chlorophyll \( a \) concentrations decreasing following the artificial mouth opening of Curdies and Barham River estuaries, while only the Anglesea estuary swash exhibited increased microphytobenthic chlorophyll \( a \) concentrations one week after the opening of the estuary mouth. Initial predictions were that increases in organic matter content at the estuary swash would occur following the artificial mouth opening. However, in contrast to this, both Chapters 2 and 3 detected no change in organic matter content at the estuary swash.

Microphytobenthic chlorophyll \( a \) concentrations reported in this study are in agreement with results shown by Froneman (2002) and Nozais et al. (2001) in temporarily open/closed estuaries in South Africa, where microphytobenthic biomass decreased by 60-98 % when the estuary mouth was opened. The results of Chapters 2 and 3 showed that microphytobenthic chlorophyll \( a \) concentrations declined and organic matter did not change at the estuary swash following the artificial mouth openings of Curdies and Barham. This is likely to be associated with strong mixing between freshwater and tidal flow, which causes scouring of the sediment, increasing turbidity and resuspending benthic microalgae (Snow & Adams 2007, Froneman 2002; Perissinotto, Nozais et al. 2002; Anandraj, Perissinotto et al. 2008). Such mixing may cause a reduction in light penetration, decreasing light availability to the sediments and leading to limited microphytobenthic growth (Nozais et al. 2001, Perissinotto et al. 2002). In contrast, at Anglesea, the scouring of sediment upon opening of the mouth may have dislodged microphytobenthos from inside the estuary mouth,
resuspending and then depositing them into the estuary swash, resulting in this initial increase in concentration (Adams et al. 1999, Perissinotto et al. 2002). Additionally, the Anglesea estuary flows out into a protected beach compared to the Curdies and Barham, and these calmer waters at Anglesea may allow the accumulation and growth of microphytobenthos at the estuary swash to occur one week post opening.

It was envisaged that stable isotope analysis would identify a change in terrestrial/estuarine sources of organic material throughout the catchment, following the artificial opening of the Curdies estuary mouth (August 2008), resulting in terrestrial and estuarine sources being deposited downstream and into adjacent coastal zones (Chapter 2). However, the amount of organic material in the sediment samples for most of the sampling locations throughout the Curdies estuary was so low, that it prevented stable isotope signatures to be detected for all locations except the freshwater and upper estuary locations. Therefore to obtain a thorough understanding of whether terrestrial/estuarine inputs of organic material are being deposited into the estuary and adjacent coastal sediments following rainfall or mouth opening events, future studies need to collect greater amounts of sediment, use sediment traps (Wakeham et al. 1984, Budge & Parrish 1998) or alternatively use a different indicator for stable isotope analyses such as flora or fauna that are abundant throughout the entire catchment or at the coastal zone (see Chapter 4).

5.2.2. Coastal sediment response to an estuary mouth closure

Chapter 2 also showed that concentrations of microphytobenthic chlorophyll a and organic matter content of the estuary swash at both the Curdies and Barham estuaries remained the same following the closure of the estuary mouths. When an estuary mouth closes over, there is generally a reduction in tidal exchange between the estuarine and marine environment, preventing release of organic material and nutrients into the estuary swash, and therefore no ‘fresh’ estuarine organic material or nutrients are available to stimulate

5.2.3. Coastal sediment response to a flood event

The natural flood event produced temporal variability in microphytobenthic chlorophyll $a$ and organic matter concentrations at the estuary swash of Curdies and Anglesea River estuaries (Chapter 3). Microphytobenthic chlorophyll $a$ concentration increased in the Curdies estuary swash one week post flood and remained at these elevated concentrations nine weeks post flood, while there was no change across time in the Anglesea estuary swash. Similarly, variable results were shown for sediment organic matter, with no change to Curdies estuary swash, but an increase at nine weeks post flood only in the Anglesea estuary swash. The increase in microphytobenthic chlorophyll $a$ at the Curdies estuary swash is consistent with results of earlier studies in South Africa, where increased freshwater flow was associated with an increase in microphytobenthic chlorophyll $a$ (Froneman 2002, Snow & Adams 2007). Previous studies have shown that flood events can introduce high concentrations of nutrients and organic matter into an estuary from the upper catchment (Mallin & Paerl 1994, Adams et al. 1999, Froneman 2000, Grange et al. 2000, Froneman 2002). When river flows recede, these inputs of organic materials and nutrients settle out and are deposited on the bottom of the estuary, where mineralization of nutrients occurs stimulating microphytobenthic growth (Lohrenz et al. 1990, Mallin et al. 1993, Pace & Cole 1994, Adams & Bate 1999, Adams et al. 1999, Lohrenz et al. 1999, Froneman 2002, Perissinotto et al. 2002, Dagg & Breed 2003). The differing results between Curdies and Anglesea estuary swash locations in microphytobenthic chlorophyll $a$ and organic matter in response to flooding (Chapter 3), highlight how spatially variable the influence of estuarine discharge can be on coastal sediments.
5.2.4.  

**Benthic microbial bacteria response to estuarine outflow**

Chapter 3 showed that changes in microbial diversity in the estuary swash could be detected following estuarine discharge although results were again spatially variable. Results from the Curdies estuary suggest that estuarine discharge may be contributing to a change in microbial diversity at the estuary swash. As predicted, the largest change in microbial utilisation of different carbon sources occurred at one and nine weeks after the artificial mouth opening and flood events at the Curdies estuary, with similar bacterial communities occurring at the estuary mouth and swash after the mouth events, but not before. These findings may be caused by different bacterial communities being deposited outside the estuary after the mouth events, along with ‘new’ material being delivered by freshwater inputs (Mallin & Paerl 1994, Lohrenz et al. 1999, Froneman 2002, Schlacher et al. 2009). This ‘new’ supply of nutrients and organic material (Adams et al. 1999, Lohrenz et al. 1999, Froneman 2002, Schlacher et al. 2009), is likely to stimulate bacterial growth and production (Wainright 1987, Ritzrau & Graf 1992), as well as increasing the bacterial communities available to utilise more carbon sources post event, as evident at Curdies estuary.

In contrast to what was predicted and to the observations made at the Curdies, the Anglesea estuary only showed differences in carbon source utilisation between locations and not between before compared to after the artificial mouth opening event. In the estuary swash, bacteria used similar carbon sources to those used at the mouth, suggesting similar bacterial communities living at the estuary mouth and swash (Hopkinson 1987, Wainright 1987, Seymour et al. 2007, Celussi 2008). Results during the flood event at Anglesea identified the greatest difference in carbon sources at the estuary swash to occur between before and at one week post flood, but this difference had disappeared by nine weeks. These findings suggest that any new bacteria may have dispersed or been washed out to sea by nine weeks post flood, causing carbon utilisation to show similar microbial diversity as before the flood.
5.3. **Effect of estuarine discharge on adjacent rocky shore communities**

5.3.1. **Macroalgal assemblage structure and recruitment response to estuary outflow**

Chapter 4 demonstrated that estuarine discharge from intermittent estuaries can influence macroalgal assemblage structure and recruitment of algal species living on rocky shores adjacent to estuary mouths, in ways similar to those driven by elevated nutrients from sewage outfalls. Differences in total algal cover of established assemblages between rocky shore types were not spatially consistent. The rocky shore exposed to discharge from the Curdies estuary had higher algal cover compared to its control shore, while there were no differences between rocky shore types for Barham, Skenes and Kennett. This spatial variability observed for algal cover of established assemblages may be related to differences in beach exposure and estuary characteristics, such as volume of flow and nutrient concentrations that the estuary shores are exposed to, but is most likely to be related to a number of post-recruitment mortality pressures that have been shown to influence the structure and dynamics of macroalgal assemblages. Numerous studies have shown that predation/herbivory (Petrailitis 1990, McCook & Chapman 1993); competition (Dayton 1971, Paine 1984, Reed 1990) and disturbance (Dayton 1971, Farrell 1989) are important post-recruitment mortality pressures in marine systems. Therefore future studies need to investigate some of these pressures as well as algal cover of established and recruited macroalgae to obtain a clear understanding of macroalgal responses to estuarine discharge.

Similar spatial variability was shown between estuaries for algal cover of recruits where rocky shores exposed to Barham and Skenes estuarine discharge exhibited higher recruitment of macroalgae cover compared to their respective control shores during the months of June – August 2007, while Curdies estuary shores showed no difference in algal cover of recruits compared to their control
shores and Kennett estuary showed lower recruitment at shores exposed to estuary outflow. Bellgrove et al. (2004) found similar peaks in algal recruitment during the winter months, identifying that the supply of propagules and recruitment of most intertidal macroalgae in south-eastern Victoria was greatest between April and August, with opportunistic species such as Ulva spp. continuing into October. Furthermore, Curdies estuary rocky shore had higher algae cover of established assemblages compared to its control rocky shore, however there was no difference in algal cover of recruits between rocky shore types. Conversely, Barham, Skenes and Kennett showed no differences in total algal cover of established assemblages between rocky shores types, however greater cover of recruits were shown at rocky shores exposed to Barham and Skenes estuaries, while Kennett had lower recruitment of algal cover compared to the control shore. These results demonstrate patterns consistent with Bellgrove et al. (2004) where patterns of algal recruitment were not directly linked with established assemblages. Additionally, in contrast to what was expected, and findings of others studies on nutrient-influenced shores (Fairweather 1990, Lopez Gappa et al. 1990, Bellgrove et al. 1997, Valiela et al. 1997, Wear & Tanner 2007), species richness of established and recruited algae was not lower at shores exposed to estuary outflow. In fact, macroalgal species richness on shore adjacent to estuaries was the same or greater for established and recruited algae compared to control shores.

Like sewage outfalls, estuarine discharge provides a source of high nutrients into the marine receiving waters (Grimes & Kingsford 1996, Lohrenz et al. 1999, Dagg & Breed 2003, Dagg et al. 2004, Schlacher et al. 2009). Although the rates of delivery are likely to be different between sewage effluent outfalls and intermittently open estuaries, nutrient concentrations discharging out of the estuary are elevated enough to potentially have an impact on macroalgal assemblages. For example, the sewage outfall at Boags Rocks (South Eastern Purification Plant Victoria, Australia: 144°53’E, 38°30’S) (Bellgrove et al. 1997), discharges 437 ML/day with an organic nitrogen concentration of 3.1 mg/L (Bellgrove et al. 1997), compared to Curdies River estuary where the
largest flood event occurring during the sampling period was 1425 ML/day (Sherwood et al. 2008), with a total nitrogen concentration of 3.0 mg/L inside the estuary mouth, diluted to 2.0 mg/L when discharged into the coastal zone (Chapter 2). The differences may lay in the responses of macroalgae to press (sustained sewage effluent disposal) and pulse (periodic flooding and variable discharge) disturbances.

However, there was evidence that estuarine discharge is causing some floristic changes (Chapter 4), similar to those associated with other nutrient inputs into coastal environments, such as from treated sewage outfalls (Borowitzka 1972, Brown et al. 1990, Bellgrove et al. 1997). The research demonstrated greater abundances of opportunistic species (such as Ulva spp. and Ectocarpus sp.) in established assemblages at rocky shores close to estuaries compared to remote shores. However, there was temporal and spatial variability in both the recruitment and abundance on the shore of ephemeral species, with shores exposed to Barham, Skene and Kennett estuaries having greater algal cover of Ulva spp. in established assemblages compared to their respective control shores. While for recruitment, Barham estuary rocky shore was the only estuary shore to have higher cover of recruits compared to the control shore. The variability in abundance and recruitment of Ulva spp. may be due to variability in propagule availability and nutrient supply during the sampling period of August-October 2007 (McGlathery et al. 1996, Fry et al. 2003, Bellgrove et al. 2004).

Nitrogen has been shown to be a critical factor in marine algal growth (May 1985, Valiela et al. 1997). The land practices surrounding the Curdies, Skene and Barham river estuaries are dominated by agriculture (98 %, 91 % and 36 % respectively) (Chapter 2), and from this, these estuaries would receive additional nitrogen inputs from runoff of fertilizers and animal wastes, compared to the Kennett estuary which is dominated by forest, only receiving nitrogen inputs from riverine/terrestrial organic material through land runoff (McClelland et al. 1997, Fry et al. 2003, Schlacher et al. 2008). With these
high inputs of nitrogen into the estuaries, it would be expected that estuarine discharge of Curdies, Skene and Barham would have strong effects on ephemeral species such as *Ulva* spp. However this was only reflected in the abundance of *Ulva* spp. established on the shore (Chapter 4), with recruitment of *Ulva* spp. also being elevated relative to controls at Barham estuary only.

The concentration of chlorophyll *a* and algal biomass of recruited algae reflected similar patterns to what was seen for total algal cover of recruits, with greater concentrations of chlorophyll *a* and algal biomass occurring at rocky shores exposed to estuaries, peaking during the second recruitment experiment. This was not unexpected since greater amounts of algae recruiting onto the artificial panels would result in concentrations of chlorophyll *a* and organic material also being higher. Furthermore, these findings suggest that there were potentially greater concentrations of nutrients being discharged out of the estuaries during the second recruitment experiment (August – October 2007; limited discharge data are available for these estuaries, however Curdies recorded large discharge events in August 2007 (1425 ML/day) and October 2007 (1396 ML/day)). The large discharge events that occurred in August 2007 and October 2007 may have resulted in high concentrations of nutrients, sediment, dissolved and suspended organic matter being discharged into adjacent coasts, stimulating microphytobenthic and algal growth (May 1985, Valiela *et al.* 1997, Adams *et al.* 1999, Lohrenz *et al.* 1999). Alternatively, this temporal variability of chlorophyll *a* and algal biomass may be a result of seasonality of algal recruitment and growth (Bellgrove *et al.* 2004). Further temporal sampling to identify seasonality in algal recruitment and growth was beyond the scope of this research.
5.3.2. Assimilation of estuarine-derived organic material by intertidal mussels

Further evidence that estuarine discharge contributes to the productivity of adjacent coastal ecosystems, even during a period of extended drought, came from the examination of carbon and nitrogen isotopes in a common filter-feeding, intertidal mussel. The intertidal mussel *Austromytilus (Brachidontes) rostratus* living in coastal waters exposed to estuary plumes assimilated organic material discharged from the estuary. Carbon isotopes revealed distinct differences between mussels living at rocky shores exposed to estuarine discharge and from shores remote from estuary mouth. Mussels exposed to estuarine plumes of Barham, St. George and Anglesea exhibited lower carbon isotope ratios compared to the control shores, indicating the presence of a terrestrial/estuarine signal. Similar results have been shown for beach clams exposed to a permanently open estuary in southern Queensland, where beach clams were assimilating distinct organic material being discharged out of the estuary (Schlacher & Connolly 2009). However, the intermittent estuaries in the present study did show spatial variability between estuaries, with mussels from Kennett estuary rocky shore having similar carbon ratios to the control shore. Similar variability in mussel diet was shown by Hill et al. (2008), who monitored the diet of mussels living on a rocky intertidal zone exposed to estuarine discharge from permanently open estuaries in South Africa. The South African study found mussels to feed on both nearshore and offshore sources of particulate organic matter at different stages throughout the sampling period, suggesting that mussels in the present study may not have a feeding preference and may feed on whatever is available (Hill et al. 2006, 2008).

Enriched nitrogen ratios of mussels associated with estuary mouths (relative to controls), consistent with an assimilation of organic material from anthropogenic terrestrial and estuarine sources, were also detected. However, there was variability in the scale of the difference between estuaries, with
mussels from Anglesea (the most urbanised estuary in this study) and Barham (surrounded by agriculture and native vegetation) estuary shores having the greatest difference in nitrogen ratios compared to mussels from control shores. These results suggest that Anglesea and Barham estuaries have higher inputs of terrestrial and estuarine nitrogenous sources compared to Kennett and St. George (dominated by dense forest), which in turn reflects the land practices of their surrounding catchment: agriculture (e.g. high nitrogen inputs from fertiliser) and a highly urbanised catchments (Peterson & Fry 1987, McClelland et al. 1997, Dagg & Breed 2003). These findings reported in Chapter 4 not only demonstrate that nutrients released from estuaries are being assimilated by mussels on adjacent rocky shores, but also demonstrate that the types of land practices that occur within the catchment would potentially dictate the availability of nutrients for primary production.

Consumers such as bivalves can provide a better time-integrated indicator of food sources in the water column rather than phytoplankton or suspended particulate organic matter (SPOM) (Cabana & Rasmussen 1996, O'Reilly et al. 2002). This is due to isotopic ratios of primary producers being subject to more variability than higher trophic levels, due to the variability of coastal hydrography which may drive changes in nutrient sources. Furthermore, SPOM measurements are only a snapshot in time and are not necessarily an indication of time-averaged measurement, so it doesn’t seem reasonable to relate SPOM measurements to time-integrated mussel tissue (Cabana & Rasmussen 1996, Hill et al. 2008). Therefore mussels provide a better insight into whether estuarine discharge is a valuable food source for invertebrates rather than solely measuring SPOM.
5.4. Estuaries and coastal outwelling

The present study relates to aspects of the ‘outwelling hypothesis’, which is defined as marsh-estuarine ecosystems producing more material than can be utilised or stored within the system, and therefore excess material is exported to the nearshore marine environment where it supports coastal ocean productivity (Odum 1968, Dame et al. 1986). This hypothesis was developed after early observations that material from salt marshes inside estuaries may be transported to the ocean (Teal 1962). Historically, there have been two ways by which studies have tested the outwelling hypothesis (Dame et al. 1986). One technique is indirect and involves the measurement and development of the production and consumption budgets for either salt marsh-estuarine systems (Pomeroy & Wiegert 1981) or the nearshore marine environment. Any differences found in the budgets were attributed to either export or import of organic material (Dame et al. 1986, Dame & Allen 1996). The second technique involves directly measuring the exchange of water and organic material between the estuarine and marine environment (Taylor & Allanson 1995, Cunha et al. 2003, Dagg & Breed 2003, Gaston et al. 2006, Lopes et al. 2008, Schlacher et al. 2008). In the present study, I have used this direct approach, and tested the effect of estuarine discharge on coastal productivity, by exploring a subset of ecological responses by coastal ecosystems to estuarine discharge from intermittently open estuaries as well as tracing elements that have been delivered into the coastal zone and assimilated by organisms.

In the past there has been controversy around Odum’s (1968) outwelling hypothesis in relation to the ability of salt marshes to export organic matter to adjacent waters (Haines 1976, 1977, Dame et al. 1986, Taylor & Allanson 1995). The size and elevation of salt marsh plants has been shown to influence whether the plant acts as an importer (tall/high elevation) or exporter (low elevation) of organic material with adjoining waters (Dankers et al. 1984, Jordan & Correll 1991, Baird & Winter 1992, Taylor & Allanson 1995).
Numerous studies have investigated outwelling effects further and concentrated not only on testing the interactions between salt marsh-estuarine habitats and coastal waters (Dankers et al. 1984, Jordan & Correll 1991, Baird & Winter 1992, Taylor & Allanson 1995), but more recent studies, such as the present study and others of permanently open systems (Dame & Allen 1996, Lohrenz et al. 1999, Dagg & Breed 2003, Gaston et al. 2006, Schlacher et al. 2008, Schlacher & Connolly 2009, Schlacher et al. 2009), have also assessed the link between estuarine outwelling and the marine environments. These studies have shown outwelling of estuaries to be important in delivering nutrient-rich waters to nearshore marine environments and generating hot spots of biogeochemical and biological activity, consequently these outwelling regions are amongst the most productive regions of the world’s oceans (Grimes & Kingsford 1996, Dagg & Breed 2003, McKee et al. 2004). Estuarine outwelling areas provide favourable conditions for primary productivity of both the water column and in the underlying benthic sediment (Dagg & Breed 2003, Dagg et al. 2004, Gaston et al. 2006, Schlacher et al. 2008, Connolly et al. 2009, Schlacher & Connolly 2009, Schlacher et al. 2009), and therefore enhancing productivity of higher trophic levels (Dagg & Govoni 1996, Dagg & Breed 2003, Darnaude et al. 2004, Darnaude 2005, Schlacher et al. 2008, Schlacher et al. 2009). Outwelling of estuaries also provides a mode of transport for fish and invertebrate larvae, which rely on estuarine discharge for dispersal (Grimes & Kingsford 1996), however the assessment of outwelling effects on fish and invertebrate dispersal for intermittent systems was beyond the scope of the present study.
5.5. Further Research

While the findings of this research have contributed significantly to the knowledge of small intermittently open estuaries and the role they play in delivering nutrients available for coastal productivity, further work is required to better our understanding of the importance of estuarine discharge on coastal productivity.

- Sampling occurred during a period of prolonged drought and reduced flows. It would be useful to repeat this study during a period of high rainfall, as this would allow for an understanding of whether the patterns experienced during low flow are stronger and potentially more consistent across estuaries during periods of high flow. However, this may be difficult in the future, due to low rainfall periods being more common under climate change predictions. Additionally, a longer sampling period (for example 2 - 3 years) may help to address specific implications that mouth openings, closures and floods may have on the productivity of adjacent coasts.

- Measurements of nutrient concentrations of the water column were recorded from a single estuary on three occasions only, which targeted a single mouth opening event. Inclusion of more estuaries was limited by the time and cost to process additional nutrient samples. Examining nutrients in the water column of adjacent coasts over broader temporal scales would facilitate a better understanding of the nutrient levels of coastal systems and how long these nutrient rich plumes remain in the coastal zone.
• The degree of influence that individual estuaries have on adjacent coasts is very variable and appears to relate to differences in size of the catchment, land practices and beach dynamics. Therefore further studies could specifically monitor a broader spectrum of estuaries with varying catchment uses in southern Australia, to obtain a thorough understanding of characteristics of estuary plumes and their potential impacts on nearshore marine environments.

• This study focused on the nutrient effects on coastal productivity, however light and mixing are also important factors influencing productivity. It would be beneficial for future work to measure light penetration and the effect of turbulence (particularly associated with flooding events) on productivity of estuarine/ocean interfaces, to increase our knowledge on the physical factors that would be affecting primary productivity.

• Estuary plumes from large river systems have been shown to be transported and detected 10 – 100 km away from the point of discharge (Dagg & Breed 2003, Dagg et al. 2004), however the distance estuary plumes from these small intermittent estuaries can travel, still remains unknown. Future studies could conduct gradient sampling to investigate how far estuary plumes of these smaller systems travel and whether they can be detected within pelagic and benthic environments.
5.6. Conclusion

This thesis has detected the influence of estuarine discharge from intermittently open estuaries on a number of biological variables, indicative of productivity, in the coastal environment, during a period of extended drought and low river flow. These small estuaries deliver nutrients and organic matter, which are important components of the estuary plume that are in turn stimulating coastal productivity. Patterns were not consistent between estuarine locations, which is probably attributable to considerable differences in estuary catchment size, land use and quantity of freshwater inflows, which have been shown to influence the degree of coastal productivity (Howarth et al. 2000, Schlacher et al. 2008). Assessing the influence of estuarine discharge on coastal productivity is complex because of the interactions between the estuary and its different inputs from land runoff, upper riverine reaches, upper estuary and exchange with the adjacent coastal environment. However, some of the patterns observed for these small intermittent estuaries are consistent with studies involving much larger permanently open estuaries (Lohrenz et al. 1999, Dagg et al. 2004, Gaston et al. 2006, Wetz et al. 2006, Schlacher et al. 2008, Schlacher & Connolly 2009, Schlacher et al. 2009). With increasing variability in rainfall and prolonged drought periods being experienced throughout Australia (Matthews & Fairweather 2003, Timbal 2009), this study highlights that it is not only important to manage our water resource to ensure the health of our rivers and estuaries (Sherwood 1988, Sherwood et al. 2008), but to also maintain the productivity of our incredibly diverse (Phillips 2001) nearshore coastal environments.
APPENDIX 1 Manuscript

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Influence of intermittent estuary outflow on coastal sediments of adjacent sandy beaches

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Abstract

Outflows from estuaries potentially contribute to the productivity of adjacent coastal waters, although most previous work has been on estuaries with considerable river discharge. We investigated the influence of estuary outflow on aspects of coastal sediments adjacent to two seasonally intermittent estuaries, the Curdies and Anglesea Rivers, in south-west Victoria, Australia. For each estuary, we measured sediment organic matter, microphytobenthic chlorophyll a and microbial utilisation of carbon sources at three locations associated with each estuary: (1) inside estuary mouth; (2) estuary swash; and (3) control swash (an open beach distant from any estuarine influences). Sampling occurred one week before and at one and nine weeks after both an artificial mouth opening and a separate natural flood at both estuaries. Significant temporal changes were detected for all three variables at the estuary mouth and estuary swash, but the direction of change was inconsistent across the two estuaries and between the artificial mouth opening and natural flood. Organic matter at both estuaries showed no difference after the artificial mouth openings. Only Anglesea showed an increase in organic matter in the estuary mouth and estuary swash after the floods. Microphytobenthic chlorophyll a concentrations were highest when the estuary mouths were closed. Concentrations decreased at all locations at Curdies after the mouth was artificially opened. The estuary mouth at Anglesea sustained high chlorophyll concentrations and the estuary swash increased one week post artificial opening. The flood event resulted in an increase in chlorophyll a at the estuary mouth and swash at both estuaries, one week post flood. At Curdies, the microbial utilisation of different carbon sources changed after both mouth events; estuary mouth and estuary swash showed similar patterns at one and nine weeks post opening. At Anglesea, the bacteria utilised different carbon sources between locations the only significant interaction between location and time was post flood with change in carbon sources utilised by bacteria in the estuary mouth and estuary swash for one and nine weeks post flood. The southern coastline of Australia is characterized by estuaries with small catchments. This study highlights the spatial and temporal variability in the effects of the output of relatively small, intermittent estuaries on coastal sediment of adjacent beaches, particularly during prolonged periods of drought.
APPENDIX 2 Conference Presentations


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