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

Jessica L. McKenzie*, Gerry P. Quinn, Ty G. Matthews, Jan Barton, Alecia Bellgrove

School of Life and Environmental Sciences, Deakin University, P.O. Box 423, Warrnambool, Victoria 3280, Australia

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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 southwest Victoria, Australia. For each estuary, we measured sediment organic matter, microphytobenthic chlorophyll *a* and microbial utilization 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 in 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 utilization 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 utilized different carbon sources between locations and the only significant interaction between location and time was post flood with change in carbon sources utilized 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.

1. Introduction

Estuaries are dynamic environments and form an integral part of the coastal zone. Lying at the interface between marine, freshwater and terrestrial systems, estuaries are well known for being highly productive environments, which provide essential nursery habitats for many fish species and feeding grounds for numerous bird species (Robertson and Duke, 1987; Laegdsgaard and Johnson, 1995; Grimes and Kingsford, 1996; Nagelkerken et al., 2001; Dagg and Breed, 2003; McLusky and Elliott, 2004; Smith and Hindell, 2005; Becker and Laurenson, 2008). Estuary outflow discharges into offshore waters in the form of a plume. The importance of these plumes has been studied for large rivers (e.g. Amazon, Mississippi) and have shown to increase primary productivity by delivering 'fresh' nutrient loads and depositing organic material from the catchment to coastal waters (Calef and Grice, 1967; Trefry et al., 1994; Grimes and Kingsford, 1996; Smith and Demaster, 1996; Lohrenz et al., 1999; Perissinotto et al., 2000; Rabalais et al., 2000; Dagg and Breed, 2003; Liu and Dagg, 2003; Dagg et al., 2004; Green et al., 2006; Schlacher et al., 2009).

In temperate Australia and South Africa, seasonal or intermittently open estuaries are common; such systems can experience separation from the ocean by the presence of a sandbar at the mouth, particularly during periods of low rainfall and high seas (Eyre and France, 1997; Perissinotto et al., 2000; Cowley et al., 2001;

* Corresponding author.
E-mail address: jlmck@deakin.edu.au (J.L. McKenzie).

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Barton and Sherwood, 2004; Harrison, 2004; Gladstone et al., 2006; Haines et al., 2006; Hastie and Smith, 2006; Matthews, 2006; Pope, 2006; Allan and Froneman, 2008; Anandraj et al., 2008). In these types of estuaries prolonged mouth closure is common, especially during droughts, and therefore the estuary might be artificially opened to protect assets of adjacent landholders from flooding. Artificial opening of the mouth usually involves digging a trench through the sandbar at the mouth and re-linking the estuary to the ocean (Barton and Sherwood, 2004).

In Australia, although small estuaries discharging onto open coastlines are very common, few studies have investigated plume traits and their influence on adjacent coasts for these smaller systems (Kingsford and 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 and Finucane, 1991; Kingsford and Suthers, 1994; Grimes and Kingsford, 1996; Eyre and France, 1997; Lohrenz et al., 1999; Cowley et al., 2001; Froneman, 2002; Gladstone et al., 2006; Hermand et al., 2008; Chumen et al., 2009). 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; Connolly et al., 2009; Schlacher and 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 (unpublished data). Sampling of fish was impractical because of the exposed nature of the shores and there were no data on nearby commercial or recreational fish catches. Instead, this study specifically investigated the influence of estuarine outflow 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 so this study focused on the influence of estuary outflow 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., 2000,2002; Nozais et al., 2001), and therefore microphytobenthos was the focus as opposed to phytoplankton. We compared these variables for two intermittent estuaries in southeastern 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 estuary outflow on sediment organic matter, microphytobenthos and microbiota. We hypothesized 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. We also hypothesized that in response to continued discharge and increased flow from a natural flood, sediment organic matter, microphytobenthos and microbiota would continue to increase in the estuary swash. Results from this study provide a unique assessment of the impact of the outflow of small intermittently open estuaries on sediment productivity, providing valuable tools for estimating future effects of climate change and drought.

2. Material and methods

2.1. Study sites

Intermittent estuaries in southeastern Australia such as Aire, Anglesea, Barham, Curdies, Gellibrand, Hopkins, Merri and Painkalac have shown to be enriched in nutrients (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 the values recommended in the ANZECC and ARMCANZ (2000) guidelines of 0.08 mg/L and 0.035 mg/L respectively (ANZECC and 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 located in southwest Victoria, Australia (Fig. 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 ~1200 km^2, 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 (Barton and Sherwood, 2004; Sherwood et al., 2008). Generally, the mouth closes over in the drier months, December to June (Barton and 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 and Sherwood, 2004). The Anglesea estuary (144°11′28"E, 38°24′59"S) catchment covers an area of ~125 km^2 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 (Pope, 2006; Sherwood et al., 2008). The estuary is open much of the year, but during the past four 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 SE Australia that began in 1997. Since that time river discharge has been well below the long term average (Matthews, 2006; Lester and Fairweather, 2009). 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 ML 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 m^3/s (Pope, 2006).

Sampling to test for the effect of estuarine outflow on sediment organic matter, microphytobenthos and microbiota occurred during two events at each estuary: (1) *artificial mouth opening* where the adjacent coast received discharge from the previously closed estuary; (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 (*1 week post and 9 weeks post,* respectively). The Curdies was artificially opened on 14th July 2007

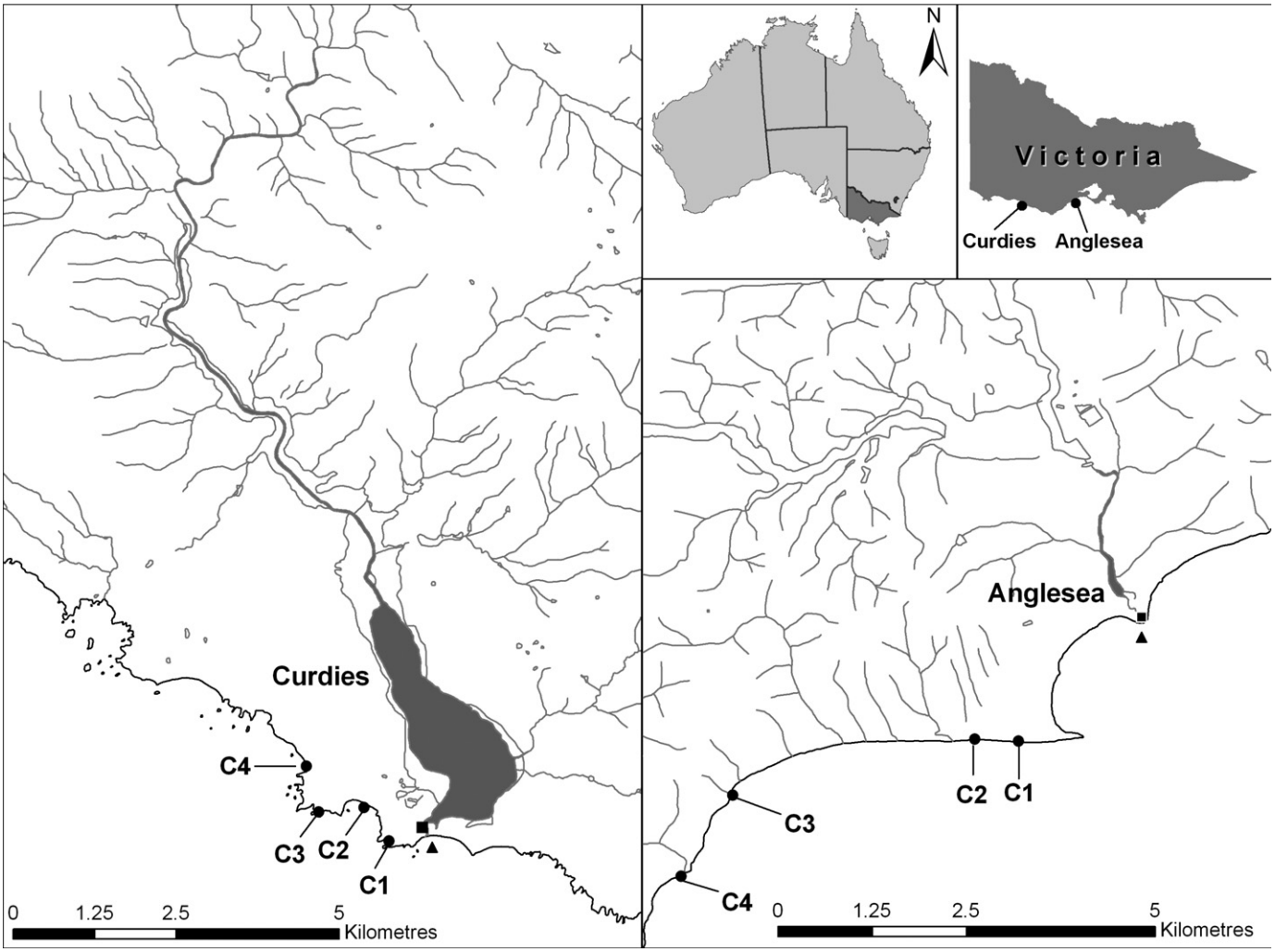

Fig. 1. Location of study sites along the southwestern Victoria coastline. Black circles denote control beaches 1–4, squares is the estuary mouths and triangles are estuary swash locations that were sampled. Insert: Location of Curdies and Anglesea estuaries within Victoria and southeastern Australia.

(hence, sampled during June, July and August 2007) and the Anglesea on 13th Aug 2007 (sampled July, Aug, Sep 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 Nov 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*) (Fig. 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 × 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 Fig. 1 *C2–C4*). Sampling of all locations for each estuary was conducted at low tide, within 4 h of each other.

Standard techniques were used for organic matter and microphytobenthos analysis (Lorenzen, 1967; Winter et al., 1996; Arar, 1997; Light and 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 utilization (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 and Mills, 1991; Preston-Mafham et al., 2002; Winding and Hendriksen, 2007; Chen et al., 2008; Weber et al., 2008; Kunito and Nagaoka, 2009); few studies have used this technique in marine environments (Garland and Mills, 1991; Tam et al., 2003; Sala et al., 2005,2006; Barton, 2006). While literature has addressed limitations in using BIOLOG plates (Konopka et al., 1998; Preston-Mafham et al., 2002; Christian and Lind, 2006; Weber et al., 2008), we 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 and del Giorgio, 2002; Henriques et al., 2004; Hawkins and Purdy, 2007; Sorensen et al., 2007; Zhang et al., 2007; Chiu et al., 2008).

2.2. Sampling- benthic organic matter

There was no visual difference in sediment particle size between locations, except that sediment from estuary mouths was slightly

finer compared to the estuary and control swash locations (pers. observation). Five replicate, 3-cm long sediment cores were randomly collected with a modified 60-mL syringe (30-mm diameter, 7.07-cm^2 area), transported on ice and frozen in the laboratory. Organic matter was determined following a modified method from Light and Beardall (1998) where samples were initially dried at 60 °C for 24 h. After stabilizing 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.

2.3. 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). In this study an optimal sample size determined by Rodriguez (1993) was used, as it has been shown to be least affected by water content (Snow et al., 2000). Ten replicate benthic microalgal sediment cores, 1-cm long (30-mm diameter, 7.07-cm^2 area), were randomly collected at each location. Samples were collected from all locations within 4 h under similar light conditions and were stored in the dark on ice until they could be frozen. Pigment extraction occurred within one month of sample collection. Chlorophyll *a* was extracted using acetone, following a modified method of Light and Beardall (1998). Technical details followed USEPA Method 446.0 (Arar, 1997) for determining chlorophyll *a* by visible spectrophotometery. 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 and Simpson, 1996).

Each sample was put into 35-mL of 100% acetone, and mechanically mixed before being refrigerated at 4 °C for 24 h. 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 Shimaduzu 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)).

2.4. Benthic microbiota

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 × 15-cm deep), were collected from unvegetated sediment. A sub-sample (22-mm diameter × 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.

2.5. 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 and Keough, 2002); subsequently, organic matter and chlorophyll *a* data were log $(x + 1)$ transformed to meet these assumptions. The two estuaries were analyzed 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 and 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 hypotheses were tested 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 utilization (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. Results

3.1. 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 × location interaction; Table 1, Fig. 2a). There was no change in organic matter at the estuary swash one week after the opening; however there was a significant difference between estuary swash and control swash before compared to nine weeks, with both locations increasing (Table 1, Fig. 2a). 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 (Fig. 2a). At the Anglesea estuary, only the effect of

Table 1
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 × estuary mouth versus estuary swash one week post mouth event. (2) Estuary mouth versus estuary swash before mouth event × estuary mouth versus estuary swash nine weeks post mouth event. (3) Estuary swash versus control swash before mouth event × estuary swash versus control swash one week post mouth event. (4) Estuary swash versus control swash before mouth event × estuary swash versus control swash nine weeks post mouth event.

Source	Curdies artificial opening ($\log(x+1)$ transformed)				Anglesea artificial opening ($\log(x+1)$ transformed)				Curdies natural flood opening ($\log(x+1)$ transformed) (Control = Average of 4 controls)				Anglesea natural flood opening ($\log(x+1)$ transformed) (Control = Average of 4 controls)			
	df	MS	*F*	*P*	*df*	MS	*F*	*P*	*df*	MS	*F*	*P*	*df*	MS	*F*	*P*
Time	2	0.002	12.614	<0.001	2	0.001	6.222	0.005	2	0.004	0.305	0.738	2	0.001	89.991	<0.000
Location	2	0.004	31.108	<0.001	2	0.000	1.609	0.214	5	0.011	0.833	0.531	5	0.000	25.864	<0.000
Time × Location	4	0.001	6.355	0.001	4	0.000	1.339	0.274	10	0.014	1.052	0.410	10	0.000	7.514	<0.000
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		

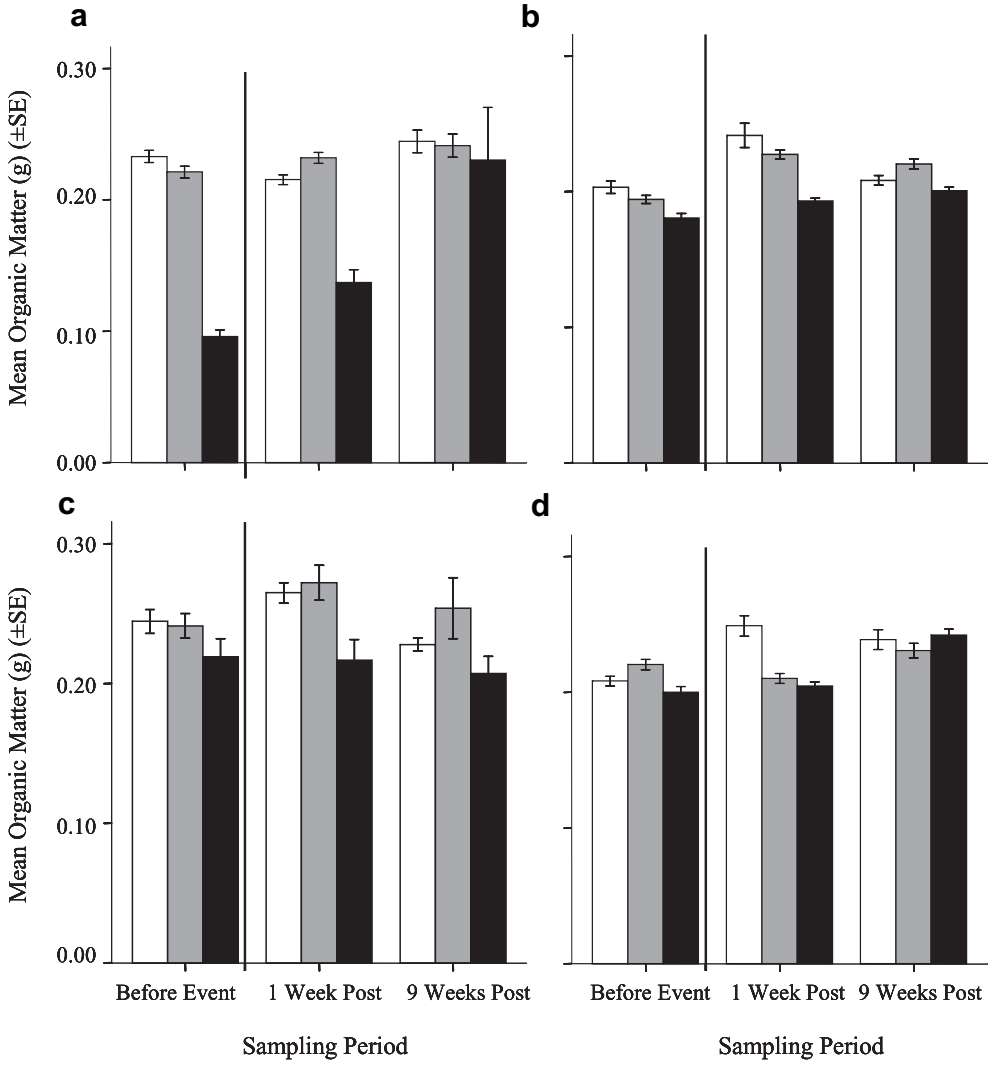

Fig. 2. Mean ± SE organic matter (grams) of benthic samples collected at a) Curdies and b) Anglesea estuaries during the artificial mouth opening and c) Curdies and d) Anglesea estuaries during the natural flood event. Estuary mouth is *white*, Estuary swash is *grey* and control swash or average of the four control swash locations for the natural flood event is *black*. Line defines mouth event.

time on organic matter was significant for the artificial opening (Table 1, Fig. 2b).

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 1, Fig. 2c). 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 1). 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 1, Fig. 2d).

3.2. Microphytobenthos

The Curdies estuary showed temporal variability in microphytobenthic chlorophyll *a* at different locations following the artificial opening of the estuary mouth (time × location interaction; Table 2, Fig. 3a), 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 (Fig. 3a). It is clear from Fig. 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 2) following the artificial mouth opening, but no interaction with time. 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 2), 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 (Fig. 3b).

Curdies estuary showed temporal variability in microphytobenthic chlorophyll *a* concentrations at the different locations following the flood event (time × location interaction; Table 2, Fig. 3c). 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 2, Fig. 3c). The control swash showed little change in chlorophyll *a* from before to one or nine weeks post flood (Table 2, Fig. 3c). 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 2, Fig. 3d). 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 (Fig. 3d).

3.3. Benthic microbial bacteria

There was temporal variability in carbon utilization patterns at the different locations following the artificial opening of the Curdies estuary mouth (time × location interaction; Table 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 utilization 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 utilization patterns between locations after the artificial mouth opening, but no interaction through time (Table 3). The multivariate differences in carbon utilization 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 utilization (time × location interaction; Table 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 utilization 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

Table 2
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 × estuary mouth versus estuary swash one week post mouth event. (2) estuary mouth versus estuary swash before mouth event × estuary mouth versus estuary swash nine weeks post mouth event. (3) estuary swash versus control swash before mouth event × estuary swash versus control swash one week post mouth event. (4) estuary swash versus control swash before mouth event × estuary swash versus control swash nine weeks post mouth event.

Source	Curdies artificial opening ($\log(x+1)$ transformed)				Anglesea artificial opening ($\log(x+1)$ transformed)				Curdies natural flood opening ($\log(x+1)$ transformed) (Control = Average of 4 controls)				Anglesea natural flood opening ($\log(x+1)$ transformed) (Control = Average of 4 controls)			
	df	MS	*F*	*P*	*df*	MS	*F*	*P*	*df*	MS	*F*	*P*	*df*	MS	*F*	*P*
Time	2	8.944	121.284	<0.001	2	0.345	4.615	0.013	2	3.565	22.714	<0.001	2	0.612	7.917	0.001
Location	2	0.524	7.099	0.001	2	7.235	96.771	<0.001	5	2.371	15.106	<0.001	5	5.178	66.935	<0.001
Time × Location	4	0.811	10.994	<0.001	4	0.091	1.220	0.309	10	0.885	5.640	<0.001	10	0.277	3.583	<0.001
Contrasts																
(1)	1	0.977	13.254	<0.001	1	0.134	1.787	0.185	1	0.898	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		

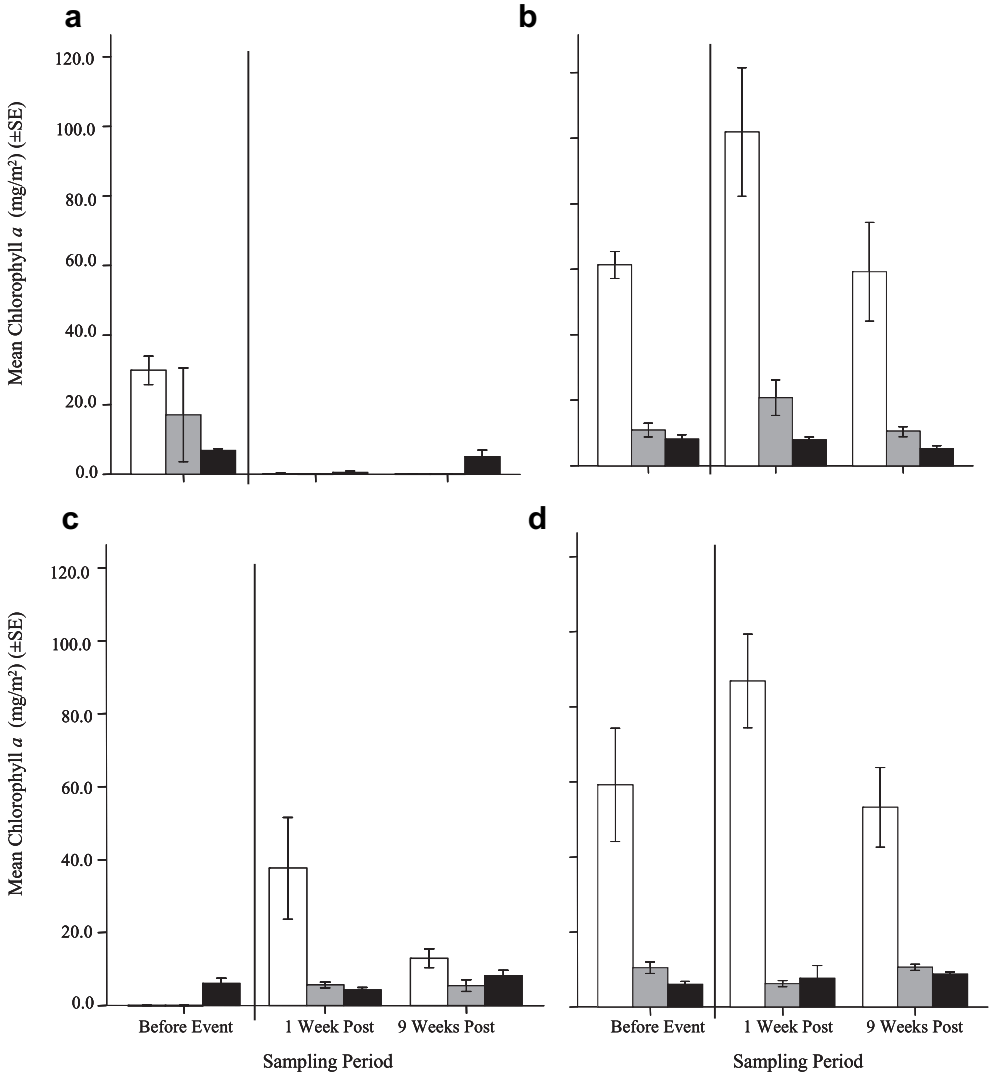

Fig. 3. Mean ± SE chlorophyll *a* of benthic samples collected at a) Curdies and b) Anglesea estuaries during the artificial mouth opening and c) Curdies and d) Anglesea estuaries during the natural flood event. Estuary mouth is *white*, Estuary swash is *grey* and control swash or average of the four control swash locations for the natural flood event is *black*. Line defines mouth event.

(14.33–32.31, 20.76–34.27). Carbon utilization 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 utilization compared to Times 1 and 3 (T1 vs T3 = 32.94).

Similarly to Curdies, the Anglesea estuary showed temporal variability in carbon utilization at different locations after the natural flood event (Table 1). 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

Table 3
Two way PERMANOVA comparing carbon utilization during (a) artificial opening (b) natural flood event.

Source	(a) Artificial opening							(b) Natural flood event						
	Curdies estuary				Anglesea estuary			Curdies estuary				Anglesea estuary		
	df	MS	F	P(perm)	MS	F	P(perm)	df	MS	F	P(perm)	MS	F	P(perm)
Time	2	757.54	6.31	0.0001	1269.67	1.03	0.3925	2	3030.69	4.77	0.0001	3622.9	4.99	0.0001
Location	2	5208.78	4.66	0.0003	3556.83	10.99	0.0001	5	2636.46	4.15	0.0001	3559.27	4.91	0.0001
Time × Location	4	3912.21	3.49	0.0001	1638.3395	1.33	0.1459	10	1137.44	1.79	0.0001	2846.65	3.93	0.0001
Residual	36	1117.96			1232.51			72	635.44			725.26		
Total	44							89						

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.

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 and 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; Schlacher and Connolly, 2009; Schlacher et al., 2009). We studied two small intermittent estuaries, both before and after an artificial mouth opening together with a separate natural flood. Freshwater outflow from artificial mouth openings and an increase in flow from floods did correspond with a change in sediment organic matter, concentrations of sediment chlorophyll *a* and microbial diversity of adjacent coasts. However the responses of organic matter, chlorophyll *a* and bacteria at both estuaries were not consistent for the artificial mouth openings and natural flood events. This highlights the difficulties of detecting whether the output of relatively small intermittent estuaries makes a significant contribution to the organic matter, microphytobenthos and microbiota of adjacent coastal sediment.

Results for sediment organic matter at both estuaries showed different patterns to that 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, similar to that discussed by (Froneman (2002), Perissinotto et al. (2002), Lane et al. (2007), Anandraj et al. (2008)) may explain these anomalies in sediment organic matter. 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.

Microphytobenthic chlorophyll *a* concentrations at the estuary swash and mouth during the artificial mouth opening at Curdies estuary, didn't show what was expected, with the highest concentration occurring during the closed phase, and concentrations decreased at one and nine weeks after the opening. Similar results were shown by Nozais et al. (2001) and Froneman (2002) 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 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 and Adams, 2007; Anandraj 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, 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 fairly 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 inflow were associated with an increase in chlorophyll *a* (Adams et al., 1999; Froneman, 2002; Perissinotto et al., 2002; Allan and 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 and Bate, 1999; Adams et al., 1999; Lohrenz et al., 1999; Froneman, 2002; Snow and Adams, 2007; Allan and Froneman, 2008; Schlacher et al., 2009). 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 estuary outflow can be on sediment microphytobenthos between estuaries.

As well as nutrients, other factors such as sediment characteristics and light have been shown to regulate microphytobenthic biomass (Cahoon, 1999; Nozais et al., 2001; Snow and Adams, 2007; Brito et al., 2009; Du et al., 2009). This study clearly shows that the Anglesea estuary has higher concentrations of chlorophyll inside the estuary mouth and swash during both mouth events compared to Curdies. The type of sediment at Anglesea is fine sand (2.5 phi units; Sherwood et al., 2008), compared to Curdies which is medium sand (1.5 phi units; Sherwood et al., 2008). Microalgal biomass has been shown to be higher in fine muddy sediments, and much lower in sandy sediments (Adams et al., 1999; Snow and Adams, 2007; Brito et al., 2009; Du 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 should be addressed 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 utilization of different carbon sources being 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 estuary outflow 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 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 and Cole, 1994; Froneman, 2006); an increase in freshwater flow 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 and 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 and Graf, 1992), increasing the bacteria available to utilize more carbon sources post event, as evident at Curdies.

In contrast, the artificial mouth opening at Anglesea did not result in predicted effects on sediments, with results showing a difference in carbon source utilization 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 smaller compared to the Curdies, suggesting that groundwater seepage may be occurring into the coastal areas, contributing to these similarities in carbon source utilization 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 utilization 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 we 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 also differs with Anglesea (125 km^2) being much smaller than the Curdies (1200 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 and Duke, 1987; Laegdsgaard and Johnson, 1995; Nagelkerken et al., 2001; McLusky and Elliott, 2004) together 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 and Kingsford, 1996; Dagg and Breed, 2003). Tracing the spatial and temporal extent of the influence of these nutrient-carrying plumes (Kingsford and 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 most estuaries worldwide.

The influence of plumes for contributing to productivity in coastal waters has been reported for large rivers such as the Amazon and Mississippi (Calef and Grice, 1967; Trefry et al., 1994; Smith and Demaster, 1996; Lohrenz et al., 1999; Rabalais et al., 2000; Liu and 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 southwest 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 (6300,000,000 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 our study was conducted during periods of low flow and extensive drought, we found it 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, a feature not as clear as previously thought.

Assessing the influence of estuary outflow on coastal sediment is complex because of the interactions and processes involved in this environment. Therefore, studies such as this 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 near shore coastal ecosystems. Ideally, similar future studies need to be repeated during periods of higher rainfall as well as potentially measuring variables such as nutrients, light and phytoplankton, 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 and Durack, 2005). The consequences of reduced estuary outflows (e.g. reduced fisheries production) for adjacent coastal waters of southeastern Australia remain largely unknown.

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