

# Carbon and nitrogen cycling on intertidal mudflats of a temperate Australian estuary.

## I. Benthic metabolism

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**ABSTRACT:** The light and dark inundated fluxes of O<sub>2</sub> and total CO<sub>2</sub> (TCO<sub>2</sub>), as well as the concentrations of chlorophyll *a* and phaeopigments, were measured (*ex situ*) on the upper and lower portions of 2 intertidal mudflats—1 in the upper Huon Estuary (salinity 4 to 32) and 1 in a marine side-arm of the estuary (salinity 17 to 34)—over 4 seasons. Dark-exposed fluxes of O<sub>2</sub> and CO<sub>2</sub> were also measured on the upper and lower mudflats of both sites over 2 seasons. Exposed fluxes of O<sub>2</sub> were generally not significantly different to the fluxes measured during inundation. Exposed fluxes of CO<sub>2</sub> were generally 3 to 5 times lower than inundated fluxes of TCO<sub>2</sub>. At the more sheltered site in the upper estuary, significantly greater rates of primary production were measured on the upper mudflat. In contrast, the more marine site had lower rates of primary production, and no significant difference in rates of primary production were observed across the inundation gradient. It is proposed that a greater exposure to wave energy (as indicated by sediment grain size) at the marine site was the cause of the lower rates of primary production. Rates of TCO<sub>2</sub> consumption in the light were generally greater than those of O<sub>2</sub> production. It is suggested that O<sub>2</sub> effluxes are greatly reduced in the light as a consequence of the re-oxidation of sulphides within the sediments.

**KEY WORDS:** Microphytobenthos · TCO<sub>2</sub> · O<sub>2</sub> · Alkalinity · Primary production · Respiration · Mudflat · Benthic metabolism · Gas exchange

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### INTRODUCTION

Intertidal sediments act as important zones for organic matter deposition within estuaries (Jickells & Rae 1997). As such, high rates of organic matter remineralisation may be observed within these zones (Middelburg et al. 1996). An availability of light and nutrients means that intertidal sediments may also be an important habitat for microphytobenthos (MPB) (Heip et al. 1995). MPB have been shown to contribute significantly to total primary production in a number of estuaries (Underwood & Kromkamp 1999). Intertidal sediments are, therefore, likely to play an important

role in both the production and remineralisation of organic matter within estuarine ecosystems.

In order to evaluate the importance of primary production and respiration on mudflats, and the balance between these 2 processes, it is necessary to consider their rates across spatial and temporal scales likely to exert important influences on them. Environmental factors controlling primary production by MPB include light, sediment type, exposure and temperature (MacIntyre et al. 1996, Underwood & Kromkamp 1999). Seasonality is well recognised as exerting an important influence on the biomass and rates of primary production by MPB. Peaks in primary production have gener-

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ally been observed during spring and sometimes in autumn (MacIntyre et al. 1996). Of key importance on mudflats is the inundation gradient, which gives rise to large changes in some of these important environmental variables, in particular irradiance. This gradient has been shown to significantly affect MPB biomass and production in a number of turbid macrotidal systems, with greater biomass and productivity generally being observed in the upper intertidal zone (Underwood & Kromkamp 1999). Exposure to hydrodynamic energy, as indicated by sediment grain size, has also been shown to be a significant factor affecting microalgal biomass and primary production on intertidal flats; lower biomass and rates of primary production have generally been observed at more exposed sites (MacIntyre et al. 1996). Because organic matter derived from MPB is likely to drive secondary production by both bacteria (Cammen 1991, Middelburg et al. 2000) and macrofauna (Herman et al. 2000) in intertidal sediments, sediment respiration rates are likely to reflect the input of organic matter derived from MPB.

The measurement of benthic metabolism may be carried out either using total oxygen uptake or the production of  $\text{TCO}_2$  (Heip et al. 1995). To date, the vast majority of studies on mudflats have only used oxygen fluxes as a measure of benthic metabolism (e.g. Underwood & Kromkamp 1999) and very few have used both  $\text{O}_2$  and  $\text{TCO}_2$  fluxes.  $\text{O}_2$  and  $\text{TCO}_2$  fluxes can provide very different measures of benthic metabolism due to dissolution and precipitation of carbonates and the net oxidation and reduction of reduced compounds (Therkildsen & Lomstein 1993, Ferguson et al. 2003). Alkalinity fluxes can be used to gain insights into the discrepancies between  $\text{O}_2$  and  $\text{TCO}_2$  fluxes (Eyre & Ferguson 2002), but these are rarely measured and have never been measured on intertidal sediments before the present study. Furthermore, the exchange of  $\text{CO}_2$  and  $\text{O}_2$  between the sediment and the atmosphere will continue during periods of mudflat exposure, but data on the relative rates of  $\text{O}_2$  and  $\text{TCO}_2$  exchange during sediment exposure and inundation are scarce (Alongi et al. 1999, Gribsholt & Kristensen 2003).

The aim of this paper was to compare the rates of respiration and primary production at sample sites on the upper and lower regions of 2 mudflats, one located at the marine end-member of the estuary, the other in the upper, river-dominated part of the estuary over 4 seasons. Primary production and respiration during inundation were measured using  $\text{O}_2$  and  $\text{TCO}_2$  fluxes calculated from pH and alkalinity. Furthermore, dark rates of gaseous  $\text{O}_2$  and  $\text{CO}_2$  exchange were also measured during exposure, allowing a comparison of the exchange rates between inundation and exposure.

## MATERIALS AND METHODS

**Study site.** The Huon Estuary is a drowned river valley, and begins just above Ranelagh, south of Hobart, stretching for 39 km southeast to Huon Island (Fig. 1). The entire estuary has an estimated mean flushing time of 7 d, with a riverine input averaging  $\sim 90 \text{ m}^3 \text{ s}^{-1}$ . It is microtidal (mean tidal amplitude  $\sim 1 \text{ m}$ ), with a salt-wedge system in the upper reaches and a tendency to become partially mixed near the mouth. The system could be classed as mesotrophic (J. Parslow pers. comm.), and has a low nutrient input from the catchment, which consists predominantly of intact forest in the upper catchment, and small-scale low-intensity agriculture in the lower catchment. Nutrient budgets show that the estuary imports nutrients from coastal waters and that loads of inorganic nutrients from the catchment and aquaculture are small in comparison (Butler et al. 2000). The concentrations of dissolved inorganic nitrogen (DIN) in the surface layer of the estuary are usually very low ( $< 1 \mu\text{M}$ ) between October and April as a consequence of biological uptake. Between April and October DIN concentrations increase and are dominated by  $\text{NO}_3^-$  derived from coastal waters which have a  $\text{NO}_3^-$  concentration of  $\sim 3$  to  $5 \mu\text{M}$ . Concentrations of DIN in the Huon River are negligible ( $< 1 \mu\text{M}$ ). The waters of the Huon River are highly coloured by elevated concentrations of coloured dissolved organic matter (CDOM) derived from a range of subalpine and 'buttongrass' (*Gymnoschoenus sphaerocephalus*) moorlands as well as ti-tree stands (*Leptospermum* spp.) in the upper catchment. Dis-

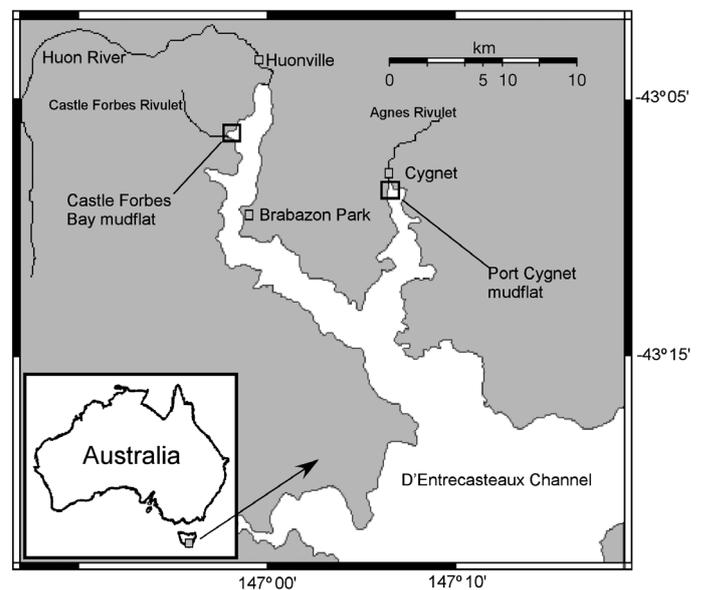


Fig. 1. Huon Estuary, southeast Tasmania, showing locations of study sites at Castle Forbes Bay and Port Cygnet

solved organic nitrogen (DON) is the dominant form of dissolved nitrogen in the Huon Estuary with DON concentrations generally between 9 to 16  $\mu\text{M}$  being observed in the Huon River (Butler et al. 2000).

Intertidal areas do not represent a large portion of the estuary as a whole; however, a number of large mudflats exist in depositional zones such as upper Port Cygnet and around the fluvial delta region of the Huon River (Fig. 1). The mudflats chosen for this study were located at Port Cygnet (Site PC) and Castle Forbes Bay (Site CF). Port Cygnet is a somewhat isolated, marine dominated side-arm of the estuary, with small and sporadic discharge from 2 rivulets. It has a southerly aspect and is, therefore, more exposed to the prevailing SW winds than Site CF, which has an easterly aspect. Site CF was located in the upper estuary which is heavily influenced by discharge from the Huon River, resulting in the waters at this site being much more coloured and having a lower salinity than at Site PC. These 2 mudflats were selected as they represented 2 endmembers of salinity and exposure to wave energy, which were both thought to be potentially important factors controlling the activity of MPB, and carbon and nitrogen cycling. It was also considered likely that there would be gradients across the intertidal zone in terms of the type of organic matter deposited, light availability and also in terms of the rates and importance of the various biogeochemical cycling processes. A hierarchical sampling design was therefore employed so that the cycling of carbon and nitrogen could be investigated across 2 spatial scales likely to influence the processes of interest, namely, site within the estuary and position on the mudflat.

There is anecdotal evidence to suggest that the mudflat at Site PC has only accreted over the past 100 yr due to land-clearing in the lower catchment. In spring and summer this mudflat had an approximately 50% cover of the macroalga *Gracillaria* sp. During autumn and winter this was greatly reduced to ~10%. Port Cygnet is considered to be part of the marine end of the estuary (Butler et al. 2000). The mudflat at Castle Forbes Bay was unvegetated at all times of the year. This site was located at the transition between the fluvial and the mixing zones of the estuary (Butler et al. 2000). The elevation of each of the sampling locations above lowest astronomical tide (LAT) was estimated by measuring the water depth at high tide. The exposure time for the upper and lower zones of each mudflat were calculated using the elevation of each position and hourly tidal data (National Tidal Facility, Hobart Tide Gauge, unpubl. tidal data). These data are presented in Table 1.

**Sampling and incubation.** Samples were collected from Castle Forbes Bay (Site CF) in November 2000,

March, June, September and December 2001. At Port Cygnet (Site PC) samples were collected during April, June, September and December 2001. To facilitate comparison with other measurements, March and April are referred to as autumn. June, September, November and December are referred to as winter, early spring, late spring, and summer, respectively. We took 4 intact sediment cores ( $25 \times 14.5$  cm inner diameter) randomly by hand at low tide from the upper and lower mudflat at each site, carefully avoiding vegetation. Samples at each site were collected at lower tide, when the water on the lower mudflat was  $<0.5$  m deep. The cores were then placed in a padded box and returned to the laboratory within 1 h. Samples for determination of water-column nutrients were collected from the site and filtered immediately into polypropylene vials. Upon return to the laboratory, the cores were placed in a water bath of site water (160 l) at *in situ* temperature. Gaseous exchange rates (non-illuminated) of  $\text{CO}_2$  and  $\text{O}_2$  were then measured on the sediments from which all overlying water had been removed. Gas-tight inserts were placed in the cores leaving a gas headspace of ~1 cm above the sediment. We then took 4 to 5 gas samples of 50  $\mu\text{l}$  each over a time series of ~4 to 6 h using a gas-tight syringe and analysed them immediately. Following the gas-exchange measurements, the cores were carefully in-undated avoiding sediment re-suspension, and transparent lids with a paddle stirrer ( $4.5 \times 1.5$  cm) rotating at 60 rpm were then propped over the cores such that a free exchange of water between the cores and the water bath could take place. The paddle stirrer in each core was driven by an electric motor mounted on the edge of the lid. This stirring set-up was chosen as it was thought to realistically mimic the diffusive boundary layer *in situ* and has been used extensively on cohesive sediments in a range of estuaries (Dalsgaard et al. 2000).

Rates of dissolved  $\text{O}_2$ ,  $\text{TCO}_2$  and nutrient exchange (nutrient data presented in Cook et al. 2004a, this volume) in the light were measured the day after col-

Table 1. Summary of elevations of each sample site (metres above lowest astronomical tide, LAT), percent of time mudflat was exposed, and distance of each sample site from terrestrial edge of mudflat (defined as beginning of rooted macrophytic marsh vegetation)

Site		Elevation	% time exposed	Distance from mudflat edge
Castle Forbes	upper	0.5	28	12
	lower	0.1	0.28	60
Port Cygnet	upper	0.7	43	5
	lower	0.2	0.76	54

lection coincidental with the period of *in situ* low tide. Cores were illuminated at  $500 \mu\text{E m}^{-2} \text{s}^{-1}$  at the sediment surface using a 50 W halogen lamp placed above each core. Measurements showed that this was reduced to  $\sim 250 \mu\text{E m}^{-2} \text{s}^{-1}$  over an area equivalent to  $\sim 10\%$  of the core immediately below the electric motor. Before commencing the flux measurements, the cores were pre-incubated under illumination for  $\sim 1$  h. The water column in the cores was then flushed with fresh site water, caps were placed on the cores, and flux measurements commenced. Rates of  $\text{O}_2$  and  $\text{TCO}_2$  and nutrient exchange were similarly made in the dark on the following day (at least 12 h after the light-flux measurements) using the same cores. We took 4 samples of the water (for nutrient and alkalinity determinations) in a time series from the water overlying the sediment core by withdrawing 65 ml of sample into a plastic syringe through a Luer Lock valve fitted to the lid. The water withdrawn was simultaneously replaced with water from a gravity-fed reservoir. Water samples taken for nutrients and alkalinity were filtered through a pre-combusted Whatman GF/F filter into 10 ml screw-cap polypropylene containers; alkalinity samples were stored in the dark at  $4^\circ\text{C}$  and analysed within 2 wk. Dissolved oxygen (DO) and pH were measured in water overlying the sediment during the incubations using electrodes. An incubation time was used which allowed the DO in the dark incubation to drop by  $\sim 20\%$  from its saturated concentration, this ranged from 3 to 6 h. In the light, DO readings were taken before bubble formation. Observed DO and  $\text{TCO}_2$  fluxes were always linear and generally had an  $r^2$  of  $>0.98$  (data not shown). The flux across the sediment–water interface was calculated using linear regression of the data, typically using 4 data points. The flux was only taken as being significant if the standard error of the slope was less than the magnitude of the flux. After the completion of core incubations, the top 0.5 cm of sediment was collected from half of the core and frozen in liquid nitrogen for pigment analysis. The other half of the core had 1 to 2 sub-cores taken for the measurement of denitrification (data presented in Cook et al. 2004a).

Gross rates of production in the light were calculated for  $\text{O}_2$  and  $\text{TCO}_2$  as follows: gross benthic  $\text{O}_2$  production (sediment efflux) = light net  $\text{O}_2$  flux (positive) – dark  $\text{O}_2$  flux (negative); gross benthic carbon production (sediment influx) = light net  $\text{TCO}_2$  flux (negative) – dark  $\text{TCO}_2$  flux (positive).

A conservative estimate of the photoperiod for each site and season sampled was made as follows. The day for each sampling times at  $43^\circ\text{S}$  was calculated using the equations given in Kirk (1994). It was observed that when there was an onshore breeze at each of the sites, the water became highly turbid, thus potentially limit-

ing photosynthesis. For calculation purposes, it was assumed that when the wind blew onshore at each of the sites (easterly for Site CF and southwesterly for Site PC), no photosynthesis occurred, even during periods of exposure. We consider this reasonable as the upper mudflat at both sites was inundated for more than 50% of the time, and also disturbance caused by an onshore wind most probably meant that photosynthesis would not resume immediately upon re-exposure because MPB would require time to migrate back to the sediment surface. The period of time the wind blew onshore at each site during daylight hours for each season (as defined above) was calculated from half-hourly meteorological data on wind speed and direction recorded at Hobart (approx. 40 km north of the study sites). The effective photoperiod was then calculated as the average day length less daily onshore wind hours, and less a further 2 h (the 2 h were subtracted as it was assumed that significant rates of photosynthesis were not likely to be occurring at dawn and dusk). It was assumed that MPB production was saturated at the irradiance of  $500 \mu\text{E m}^{-2} \text{s}^{-1}$  used in the laboratory, and that MPB production was also always saturated in the field during the effective photoperiod.

**Analytical methods.**  $\text{O}_2$  and  $\text{CO}_2$  in the gas phase were quantified using a Varian Aerograph GC equipped with an Alltech CTR I column and a thermal conductivity detector. Dissolved  $\text{O}_2$  was measured in the cores using a calibrated dissolved oxygen probe (WTW brand) (precision  $\pm 5\%$ ).  $\text{TCO}_2$  was calculated from pH (precision  $\pm 0.002$ ) and alkalinity (precision  $\pm 1\%$ ) according to Almgren et al. (1983); dissociation constants of carbonic acid were calculated according to Roy et al. (1993). pH was measured in the core using an Orion (91-55) pH probe connected to a Radiometer PHM 85 pH meter. Alkalinity was measured using Gran titration performed with an Orion 960 Autochemistry system. Chlorophyll *a* and phaeopigments were extracted twice in 90:10 acetone:water and analysed by HPLC according to Wright et al. (1991) (for more details see (Cook et al. 2004b, this volume), except for the November 2000 samples which were determined spectrophotometrically according to Jeffrey & Humphrey (1975). Sediment grain size was determined by wet-sieving using Endecott test-sieves through 500, 250, 150 and 63  $\mu\text{m}$  meshes. Photosynthetically available radiation (PAR) at the sediment surface in the laboratory was measured using a Biospherical Instruments QSL-100 light meter.

$\text{NH}_4^+$  was analysed using *o*-phthalaldehyde (OPA) derivatisation and fluorescence detection (R. Watson et al. unpubl. data); the precision of the method was generally between 5 and 8%, with a detection limit of 0.07 to 0.2  $\mu\text{M}$ .  $\text{NO}_2^-$  and  $\text{NO}_3^-$  were determined on a Technicon Autoanalyzer using sulphanilamide derivatisa-

tion of  $\text{NO}_2^-$ ;  $\text{NO}_3^-$  was determined as  $\text{NO}_2^-$  after cadmium reduction to  $\text{NO}_2^-$  (modified from Grasshoff 1976). The limit of detection of the analysis was  $0.05 \mu\text{M}$  for  $\text{NO}_2^-$  and  $0.1 \mu\text{M}$  for  $\text{NO}_3^-$ ; the precision for both analyses was typically less than 3%. Total dissolved nitrogen was determined as  $\text{NO}_3^-$  after a persulphate digestion modified from Valderrama (1981); the precision was generally <5% and the limit of detection was  $5 \mu\text{M}$ . Dissolved organic nitrogen (DON) was calculated as the difference between total nitrogen and DIN ( $\text{NO}_2^- + \text{NO}_3^- + \text{NH}_4^+$ ); the precision for this analysis was 10%.

**Statistical analysis.** Statistical analysis was carried out using Statistica Version 6.0 (StatSoft). A 1-way and a 2-way analysis of variance (ANOVA) were carried out on log-transformed data with time and position as factors. Correlation and multiple regression analysis were used to explore relationships between variables. The level of significance for rejection of the null hypothesis was set at  $p < 0.05$ .

## RESULTS

### Tidal water and site characteristics

The salinity at Site PC was greater and less variable than that at Site CF, reflecting the lower freshwater discharge into Port Cygnet (Fig. 2). During September, the salinity at Site PC dropped markedly, reflecting a heavy rainfall event the week before sampling. The salinity at Site CF was highly variable, reflecting its position in the mixing zone of the estuary, where 'out-crops' of marine waters are observed (Butler et al. 2000). Water temperature varied with season, consistently remaining at temperatures around  $18^\circ\text{C}$  between late spring and autumn and between  $5$  and  $10^\circ\text{C}$  during winter and early spring at both sites.  $\text{NO}_2^- + \text{NO}_3^-$  ( $\text{NO}_x$ ) concentrations were low, reflecting the low nutrient runoff from the catchment, peaking during winter when it was derived from the marine end-member of the estuary (Butler et al. 2000).  $\text{NO}_3^-$  was always the dominant  $\text{NO}_x$  species, but  $\text{NO}_2^-$  concentrations at Site PC were up to 50% of those of  $\text{NO}_3^-$  previously observed at Port Cygnet (Butler et al. 2000).  $\text{NH}_4^+$  concentrations were generally low and variable, reflecting the low inputs to the estuary and its high bio-availability to phytoplankton. Dissolved organic nitrogen (DON) was the dominant form of dissolved nitrogen. DON concentrations ranged between  $8$  and  $19 \mu\text{M}$  at Site CF and between  $7$  and  $12 \mu\text{M}$  at Site PC. Average sediment porosity (0 to 6 cm) was lowest on the upper mudflats, being  $0.64$  at Site PC upper and  $0.73$  at Site CF upper. On the lower mudflats, the porosity was  $0.8$  and  $0.9$  at Sites PC and CF, respectively (data not shown).

### Sediment grain size

Mean sediment grain size was finer at Site CF (ranging from  $41$  to  $61 \mu\text{m}$ ) than at Site PC, where the mean sediment grain size ranged from  $150$  to  $230 \mu\text{m}$  (Table 2). The consistently larger sediment grain size at Site PC suggests that this is a higher hydrodynamic energy environment than Site PC. Sediment organic content for these sediments is presented in Cook et al. (2004b).

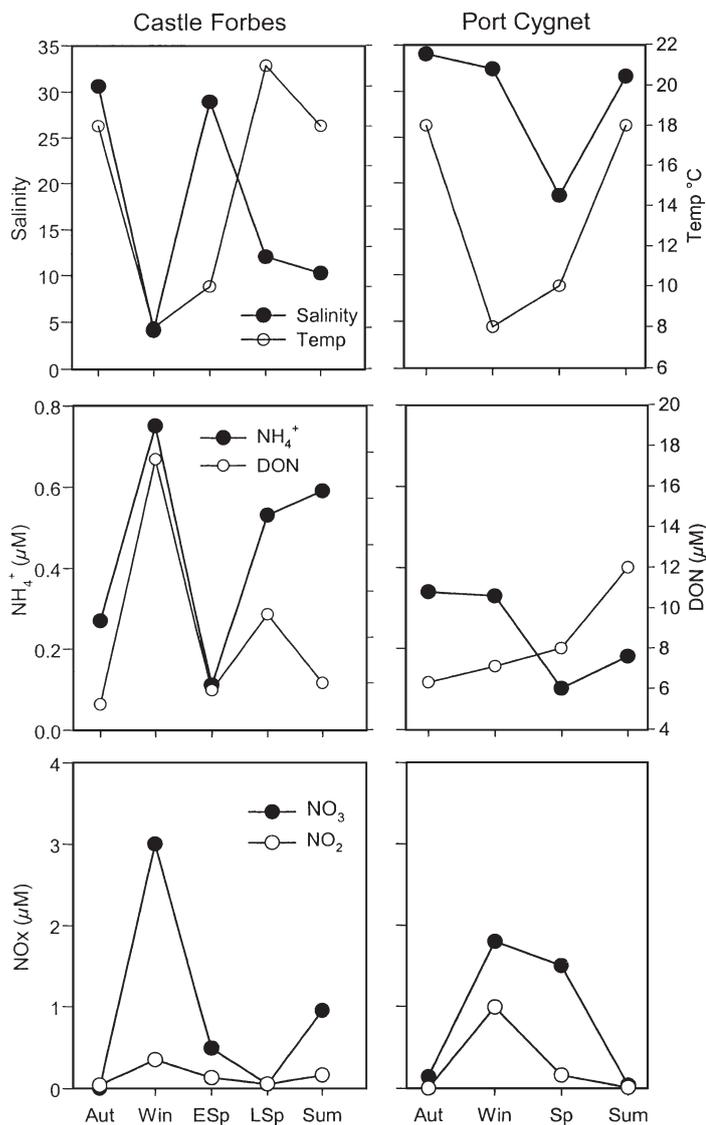


Fig. 2. Temperature and salinity,  $\text{NH}_4^+$  and dissolved organic nitrogen (DON) concentrations,  $\text{NO}_3^-$  and  $\text{NO}_2^-$  ( $\text{NO}_x$ ) concentrations in water column on days of sampling for sediment flux measurements at Castle Forbes Bay and Port Cygnet in autumn (Aut), winter (Win), spring (Sp) and summer (Sum) in 2001. ES, LSp: early and late spring, respectively

Table 2. Mean (SD) grain sizes ( $\mu\text{m}$ ) of sediments in autumn (Aut) and spring (Spr) on upper and lower mudflats at Castle Forbes Bay (CF) and Port Cygnet (PC)

CF upper		CF lower		PC upper		PC lower	
Aut	Spr	Aut	Spr	Aut	Spr	Aut	Spr
41	43	54	61	167	230	153	150
(58)	(59)	(60)	(87)	(220)	(240)	(200)	(189)

### Chlorophyll *a* and degradation products

At Site CF, chlorophyll *a* (chl *a*) concentrations were significantly greater on the upper mudflat than on the lower mudflat, and time affected the upper and lower mudflats differently (Fig. 3, Table 3). A Fisher LSD post-hoc analysis showed that chl *a* on the lower mudflat did not vary significantly throughout the year; however, on the upper mudflat chl *a* was highest in

spring and lowest in autumn. Summer and winter chl *a* concentrations were not significantly different. At Site PC, chl *a* concentrations were significantly greater on the upper mudflat, and time did not significantly affect chl *a* concentrations throughout the year on the upper or lower mudflats (Table 3).

### Sediment primary production

The sediments at Site CF were net-productive (as measured by  $\text{O}_2$  and  $\text{TCO}_2$  fluxes) in the light throughout the year on both the upper and lower mudflats (Fig. 4). Gross rates of productivity ranged from  $650 \mu\text{mol m}^{-2} \text{h}^{-1} \text{TCO}_2$  on the lower mudflat in winter to  $15000 \mu\text{mol m}^{-2} \text{h}^{-1} \text{TCO}_2$  on the upper mudflat during early spring. In contrast, the sediments at Site PC were generally less productive, with rates of gross primary production ranging from  $1100 \mu\text{mol m}^{-2} \text{h}^{-1} \text{TCO}_2$  on the lower mudflat in summer to  $7700 \mu\text{mol m}^{-2} \text{h}^{-1} \text{TCO}_2$  on the lower mudflat in autumn. Gross production (as measured by  $\text{O}_2$  and  $\text{TCO}_2$  fluxes) was significantly greater on the upper mudflat at Site CF, but at Site PC there was no significant difference between rates of gross production on the upper and lower mudflats (Table 3). The significant interaction between position and time at Site CF (Table 3) was caused by a much greater increase in rates of primary production on the upper mudflat compared to the lower mudflat in spring (Fig. 4). At site CF, Community production quotients (CPQ =  $\text{O}_2/\text{CO}_2$  flux ratio) for gross primary production averaged 0.88 and ranged between 0.57 and 1.7; this is similar to Site PC, where the CPQ for gross production averaged 0.87 and ranged between 0.39 and 1.31. Sediment primary production rates were not correlated with temperature at either site.

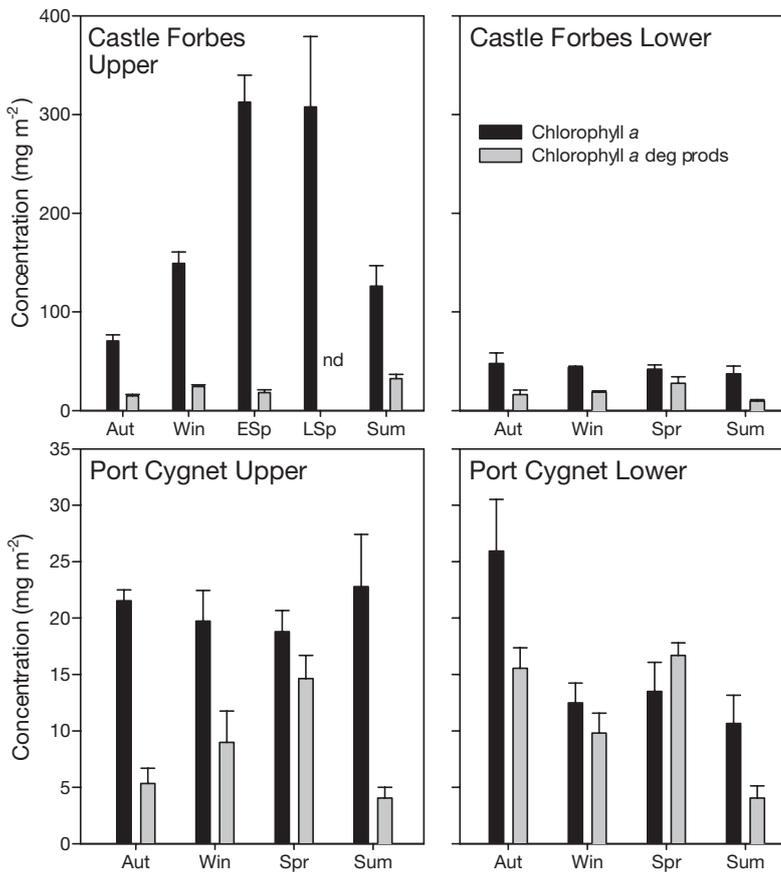


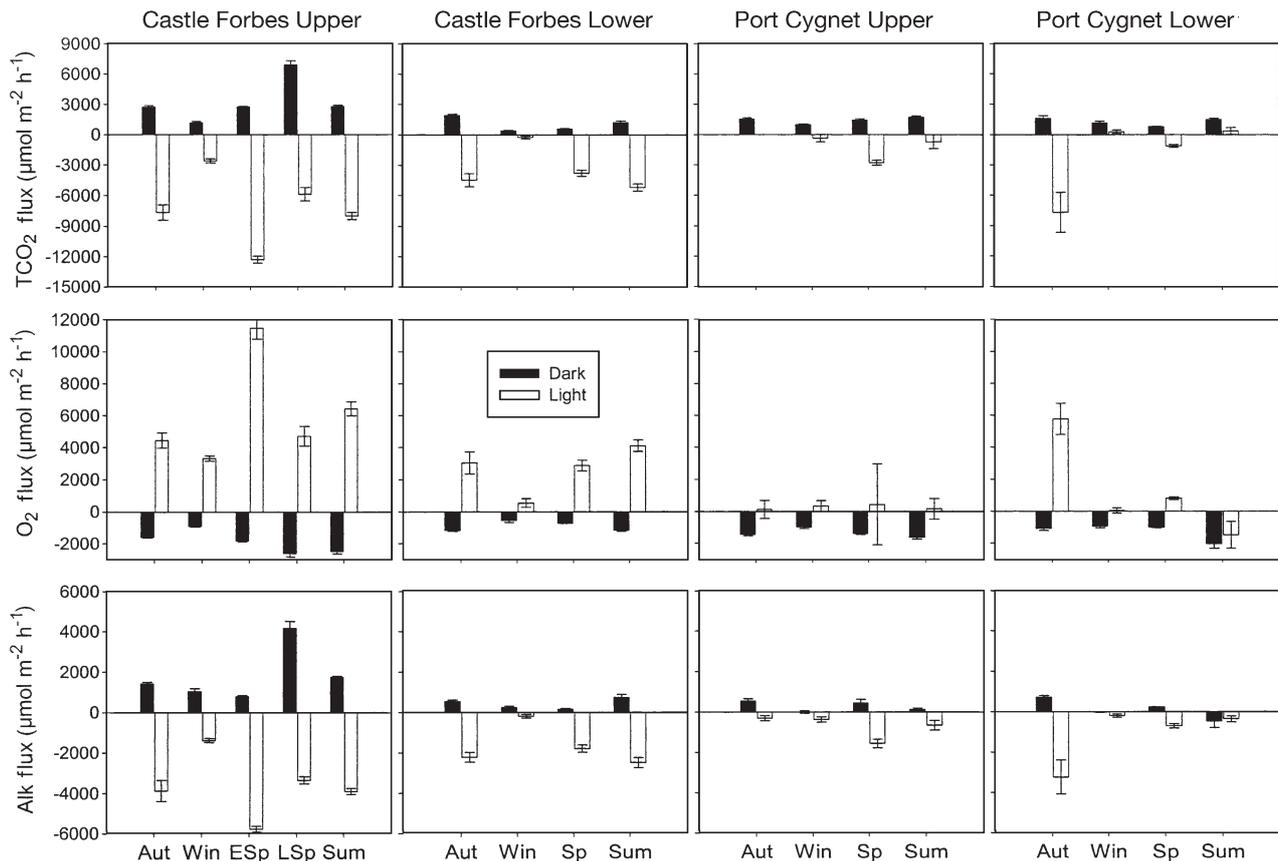
Fig. 3. Chlorophyll *a* and chlorophyll *a* degradation products in autumn (Aut), winter (Win), spring (Spr) and summer (Sum) on upper and lower mudflats at Port Cygnet and Castle Forbes Bay. ESpr, LSpr: early and late spring, respectively. Error bars = SE of replicate samples ( $n = 3$  to 4). nd: not determined. Note different vertical scale for the 2 sites

### Sediment respiration

Sediment respiration rates at Site CF ranged between  $390 \mu\text{mol m}^{-2} \text{h}^{-1} \text{TCO}_2$  on the lower mudflat during winter and  $6900 \mu\text{mol m}^{-2} \text{h}^{-1} \text{TCO}_2$  on the upper mudflat during late spring (Fig. 4). The sediment respiration rates at Site PC had a lower range and varied between  $790 \mu\text{mol m}^{-2} \text{h}^{-1} \text{TCO}_2$  on the lower mudflat during winter and  $1700 \mu\text{mol m}^{-2} \text{h}^{-1} \text{TCO}_2$  on the upper mudflat during summer. At Site CF, community respiration quotients (CRQ =  $\text{TCO}_2/\text{O}_2$  flux ratio) averaged 1.4 and ranged from 0.71 to 2.7. In comparison, the CRQ at

Table 3. Results of ANOVA tests comparing sediment chlorophyll *a* content, gross primary production and sediment respiration rates on the upper and lower mudflats at Castle Forbes Bay (CF) and Port Cygnet (PC)

Comparison	Statistical test	Result
<b>Chlorophyll <i>a</i></b>		
CF upper and lower mudflats	2-way ANOVA with position and time as factors	Significantly higher on upper mudflat ( $p < 0.01$ ) Significant interaction with position $\times$ time ( $p < 0.01$ ) Fisher post-hoc analysis showed chl <i>a</i> on lower mudflat did not vary significantly over year
PC upper and lower mudflats	2-way ANOVA with position and time as factors	Significantly higher on upper mudflat ( $p < 0.01$ ) No significant interaction with position $\times$ time
<b>Gross primary production (CO<sub>2</sub> fluxes)</b>		
CF upper and lower mudflats	2-way ANOVA with position and time as factors	Significantly higher on the upper mudflat ( $p < 0.001$ ) Significant interaction with position $\times$ time ( $p < 0.001$ )
PC upper and lower mudflats	2-way ANOVA with position and time as factors	Not significantly different Significant interaction between position $\times$ time ( $p < 0.01$ )
Comparison of all 4 sample sites	1-way ANOVA with site as factor, Fisher post-hoc analysis	CF upper had significantly highest production rates ( $p < 0.001$ )
<b>Sediment respiration (CO<sub>2</sub> fluxes)</b>		
CF upper and lower mudflats	2-way ANOVA with position and time as factors	Significantly higher on the upper mudflat ( $p < 0.001$ ) Significant interaction with position $\times$ time ( $p < 0.001$ )
PC upper and lower mudflats	2-way ANOVA with position and time as factors	Not significantly different Significant interaction between position $\times$ time ( $p < 0.01$ )
Comparison of all 4 sample sites	1-way ANOVA with site as factor, Fisher post-hoc analysis	Respiration was significantly highest on Site CF upper mudflat and significantly lowest on Site CF lower mudflat ( $p < 0.001$ )

Fig. 4. Total CO<sub>2</sub> (TCO<sub>2</sub>), O<sub>2</sub> and alkalinity fluxes measured under light and dark conditions in autumn (Aut), winter (Win), spring (Sp) and summer (Sum) on upper and lower mudflats at Port Cygnet and Castle Forbes Bay. ESp, LSp: early and late spring, respectively. Error bars = SE of replicate samples (n = 3 to 4)

Site PC averaged 1.0 and ranged between 0.72 and 1.48. The highest respiration quotient was measured at CF in the upper mudflat during late spring, when respiration rates were highest. At Site CF, respiration was significantly greater on the upper mudflat than on the lower mudflat, but was not significantly different between the upper and lower mudflat at Site PC (Table 3).

At Site CF, respiration rates (TCO<sub>2</sub> effluxes) were correlated with chl *a* ( $r = 0.75$ ,  $p < 0.05$ ) and gross production (TCO<sub>2</sub>) ( $r = -0.74$ ,  $p < 0.05$ ). Standard multiple regression analysis showed that chl *a*, temperature (*T*) and net production (NP, TCO<sub>2</sub> uptake) controlled 84% (adjusted  $r^2$ ) of the variability for respiration; the resulting regression equation was: TCO<sub>2</sub> flux =  $-2458 + 13.9 \text{ chl } a + 264 T + 0.15 \text{ NP}$  ( $F_{2,30} = 57$ ,  $p < 0.001$ ). Chl *a*, temperature and net production all made a significant unique contribution to the model as indicated by the significance of the semi-partial correlations (Student's *t*-test,  $p < 0.01$ ).

At Site PC, respiration was significantly correlated to temperature ( $r = 0.78$ ,  $p < 0.05$ , TCO<sub>2</sub> flux). Multiple regression analysis indicated that only temperature, and not chl *a*, contributed significantly to the variability in dark TCO<sub>2</sub> fluxes.

#### Sediment–air exchange of O<sub>2</sub> and CO<sub>2</sub>

The exchange rates of CO<sub>2</sub> measured during exposure were consistently and significantly much lower than the rates of TCO<sub>2</sub> exchange measured during inundation, with the ratio of water:gas exchange falling in the range of 1.6 to 5.2 (Table 4). In contrast, the exchange of O<sub>2</sub> across the gas–sediment interface was generally not significantly different to that measured during exposure. The average ratios of the water:gas O<sub>2</sub> exchange were generally in the range of 1 to 2, except for those measured during winter at Site CF, when the water:gas O<sub>2</sub> exchange was ~0.5.

#### Alkalinity fluxes

Alkalinity fluxes were generally positive in the dark and negative in the light, with the exception of the lower mudflat at Site PC, which had a significant negative alkalinity flux during the dark in summer (Fig. 4). Alkalinity fluxes were generally similar to TCO<sub>2</sub> fluxes, with the highest uptake occurring during early spring on the upper mudflat at Site CF in the light ( $-5800 \mu\text{Eq m}^{-2} \text{ h}^{-1}$ ) and the highest efflux occurring during late spring in the dark ( $4200 \mu\text{Eq m}^{-2} \text{ h}^{-1}$ ) at the same site.

## DISCUSSION

### O<sub>2</sub> and TCO<sub>2</sub> exchange during inundation and exposure

A comparison of the gas-exchange rates between the sediment–air and sediment–water interfaces has rarely been undertaken on intertidal mudflats, with the notable exceptions of Alongi et al. (1999) and Gribsholt & Kristensen (2003). In agreement with our study, Gribsholt & Kristensen (2003) found that exposed CO<sub>2</sub> fluxes measured on unvegetated mudflats were 2.5 times higher during inundation than exposure. As discussed by Gribsholt & Kristensen (2003), the observed differences between the exposed and inundated gas fluxes could arise from biological factors such as changes in bio-irrigation, or from physical effects such as changes in the thickness of the diffusive boundary layer during exposure. These factors, however, cannot explain the greatly reduced CO<sub>2</sub> flux relative to the O<sub>2</sub> flux that we observed during exposure.

Conceptualising the mechanism for TCO<sub>2</sub> fluxes in sediments is rather more complicated than for O<sub>2</sub> because of the carbonate equilibria, which means that TCO<sub>2</sub> in the sediment may actually diffuse as several species, depending on the sedimentary processes taking place. When the source of TCO<sub>2</sub> within the sediment is accompanied by the generation of alkalinity, as observed in this study, then a portion of the transport of TCO<sub>2</sub> across the sediment–water interface will be driven by HCO<sub>3</sub><sup>-</sup> and CO<sub>3</sub><sup>2-</sup> as well as by aqueous CO<sub>2</sub>. During exposure there can no longer be a transport of these ion species out of the sediment, and thus only the CO<sub>2</sub>-driven portion of the flux is lost from the sediment to the atmosphere. From Fig. 5, it is evident that when the alkalinity fluxes are subtracted from the inundated TCO<sub>2</sub> fluxes, there is closer agreement with the exposed CO<sub>2</sub> fluxes measured in each core. This high-

Table 4. Ratio of fluxes measured during inundation (ind) and exposure (exp) on upper and lower mudflats at Castle Forbes Bay (CF) and Port Cygnet (PC) in autumn (Aut) and winter (Win). Ratios shown are average of 3 to 4 cores. \*Significant difference between inundated and exposed fluxes determined using a 2-tailed *t*-test,  $p < 0.05$

Site, season	O <sub>2</sub> ind/O <sub>2</sub> exp	CO <sub>2</sub> ind/CO <sub>2</sub> exp
CF upper, Aut	1.20	3*
Win	0.53	2.6*
lower, Aut	1.6	5.2*
Win	0.54*	1.6
PC upper, Aut	2	3.1*
Win	1.20	2.2*
lower, Aut	1.5	2.3*
Win	1.6*	4

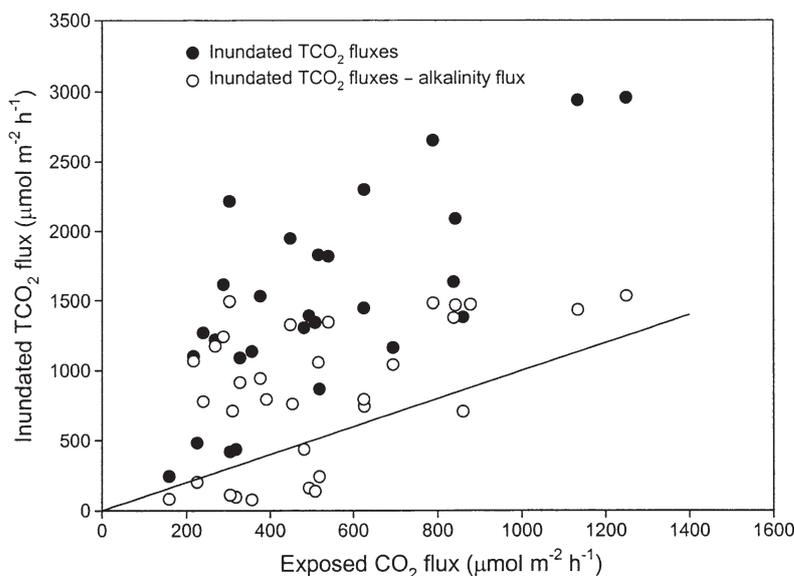


Fig. 5. Inundated fluxes of  $\text{TCO}_2$  versus exposed fluxes of  $\text{CO}_2$  (●) and inundated fluxes of  $\text{TCO}_2$  minus alkalinity versus exposed fluxes of  $\text{CO}_2$  (○) measured in each core in autumn and winter on upper and lower mudflats of Castle Forbes Bay and Port Cygnet. Line: inundated  $\text{TCO}_2$  flux = exposed  $\text{CO}_2$  flux

lights the importance of considering alkalinity fluxes when comparing the exchange of  $\text{TCO}_2$  between inundated and emersed sediments.

### Spatial patterns in primary production

At Site CF, there was a clear spatial difference in primary production in the upper and lower mudflat. Greater MPB biomass and benthic productivity were measured in the upper zone of the mudflat than in the lower zone. Likely factors giving rise to changes in primary production across the inundation gradient of mudflats include light and grazing pressure. In several previous studies, light has been identified as the key factor giving rise to observed differences in primary production across the inundation gradients of mudflats (Underwood & Kromkamp 1999). The water at Site CF was heavily influenced by the incoming freshwater discharge from the Huon River, which is darkly coloured by humic material (Butler et al. 2000). Using data from Butler et al. (2000) at Brabazon Park, approximately 7 km downstream from Site CF (Fig. 1), we estimated an annual average light-attenuation coefficient of 2 to 4  $\text{m}^{-1}$  (at 440 nm) for this portion of the estuary. Given this light-attenuation coefficient, the lower mudflat at Site CF would receive significantly less light during periods of inundation.

We made no measurements of water-column or sediment-based grazers, and cannot therefore rule out

the possibility that different grazing rates gave rise to the observed gradient in MPB biomass and productivity. We did not, however, detect phaeophorbides in significant quantities in our pigment analyses, suggesting that there was not a heavy grazing pressure at these sites (Bianchi et al. 1988). We therefore conclude that light limitation of MPB at on the lower mudflat is most probably the cause of the lower MPB biomass and productivity observed there.

In contrast to Site CF, the highest productivity recorded at Site PC was observed on the lower mudflat (Fig. 4), and there was no consistent pattern between the upper and the lower mudflat. Davis & MacIntyre (1983) interpreted the lack of any observable gradient in production in the intertidal zone as indicating that light was not a limiting factor to primary production. The waters of Site PC generally have a salinity  $>30$  (Butler et al. 2000), and thus CDOM concentrations are negligible, and light attenuation is less than at Site CF, resulting in significantly higher light penetration at Port Cygnet than in the upper estuary (Parker 2002). Despite the apparent absence of light limitation, rates of primary production and chl *a* at Site PC were significantly less than at Site CF. As such, a factor other than light appears to be the dominant control on biomass and primary production.

Factors that might influence primary production between the 2 sites include salinity, nutrient availability, grazing pressure and wave energy. The salinity differences between Sites PC and CF (Fig. 2) are unlikely to have a negative affect on rates of primary production, as previous studies either showed that salinity did not significantly affect MPB production on intertidal flats (Admiraal 1977, van Es 1982), or that it had a positive affect up to a salinity of 30 (Rasmussen et al. 1983). Nutrient availability is also unlikely to be the factor giving rise to the differences in primary production, since  $\text{NH}_4^+$  concentrations in the porewater at Site PC were generally greater than those at Site CF (Cook et al. 2004a). Sediment fauna were not quantified at these field sites and therefore no statement on the role of grazing can be made. As for Site CF, phaeophorbides, which have been used as an indicator of grazing (Bianchi et al. 1988), were never detected in significant quantities at Site PC (data not shown), which may indicate a lack of grazing pressure.

Sediment resuspension may be an important factor controlling biomass and primary production of MPB (MacIntyre et al. 1996), and it has previously been observed that primary production was lower on a more exposed mudflat in the Bay of Fundy (Hargrave et al. 1983). The mudflat at Site PC has a southerly aspect, and was thus much more exposed to the southwesterly

winds that prevail in this region (Butler et al. 2000) than Site CF, which has an easterly aspect. Using sediment grain size as a proxy for hydrodynamic energy, it has been found that biomass is generally lower in high-energy environments where grain size is larger (de Jong & de Jonge 1995, Lucas & Holligan 1999). The sediments at Site PC were coarser than those at Site CF (Table 2), further suggesting that the mudflat at PC is a higher wave-energy environment. It therefore seems most likely that the lower rates of primary production and biomass at Site PC were caused by greater exposure to wave energy.

### Spatial patterns of respiration

Only at Site CF was there a clear spatial difference in respiration rates between the upper and the lower mudflats. The supply of organic matter and temperature are both likely to be important regulators of respiration rates within sediments, and this is supported by the regression analysis. Given that temperature was very similar for PC and CF during each season (Fig. 2) and that the upper and lower mudflat sediments were always incubated at the same temperature, it is most probable that differences in organic matter supply led to the observed differences in the rates of respiration. At these study sites, bacterial respiration was driven by organic matter derived from MPB, as indicated by the compound-specific stable isotope analysis of bacterial fatty acids (Cook et al. 2004b). It is thus most likely that the significantly higher sediment respiration rates in the upper mudflat at Site CF arose as a direct consequence of the higher biomass and productivity rates measured at this site.

At this point, air exposure of the sediments should be considered as a possible cause of the observed differences in the rates of respiration measured between the upper and lower mudflats at Site CF. It was observed that the CO<sub>2</sub> gas fluxes were ~3 times lower during exposure than during inundation. Assuming that the respiration rates measured during exposure do not represent the true TCO<sub>2</sub> production rates for the reasons discussed above, one would expect the rates of TCO<sub>2</sub> release to be higher during inundation to compensate for the low release during exposure. Given that the upper mudflat at Site CF was exposed for a ~28% longer period than the lower mudflat, the TCO<sub>2</sub> release may have been 20% higher during inundation on the upper mudflat to compensate for the build-up during exposure (this calculation assumes that the release of the built-up TCO<sub>2</sub> occurs gradually over the entire period of exposure and not as a pulsed release upon inundation). Given that the respiration rates at Site CF were 30 to 80% higher on the upper than on

the lower mudflat, the expected 20% increase in CO<sub>2</sub> release during exposure cannot explain the higher respiration rates observed on the upper mudflat. Furthermore, our cores were inundated for 2 d before respiration rates were measured and therefore it seems unlikely that TCO<sub>2</sub> release would still be 20% higher after exposure in the field. The fact that no significant differences in TCO<sub>2</sub> fluxes were observed between the upper and lower mudflats at Site PC further suggests the higher TCO<sub>2</sub> fluxes on the upper mudflat at Site CF were not an artefact of exposure.

### Trophic status

The trophic status (heterotrophy versus autotrophy) of a system can provide a useful tool for assessing ecosystem stability (de Wit et al. 2001), and the likely effect of a benthic system on water quality (Rizzo et al. 1996) and nutrient cycling (Eyre & Ferguson 2002, Risgaard-Petersen 2003). A number of indices have been used to classify the status of benthic production versus respiration. The benthic trophic state index (BTSI) (Rizzo et al. 1996) and the trophic oxygen state indicator (TOSI) (Viaroli et al. 1996) both give an indication of the balance between production and respiration. Here, we have applied these indices to the TCO<sub>2</sub> data. Applying these indices (Fig. 6a), it can be seen that Site CF sediments were generally highly autotrophic (BTSI 3 region, see Fig. 6 legend for BTSI definitions), while the Site PC sediments ranged from net-heterotrophic to highly autotrophic (BTSI 1 to 3). TOSI and BTSI do not take into account the trophic status over a diurnal cycle; therefore, a sediment that appears to be autotrophic on the basis of these indices may in fact be net-heterotrophic over a diurnal cycle when the day length is short.

More traditional P:R ratios, as applied by Eyre & Ferguson (2002), give an indication of the trophic status of the sediments over a diurnal cycle. Such ratios may thus be more ecologically relevant, especially in more temperate latitudes where day length can vary greatly between winter and summer. Daily respiration was simply calculated as hourly dark TCO<sub>2</sub> flux multiplied by 24 h. Daily production was calculated as hourly gross production multiplied by the estimated photoperiod, estimated as described in 'Materials and methods'. Using P:R ratios, the sediments are classified as either net-heterotrophic (where P:R < 1) or net-autotrophic (where P:R > 1, Fig. 6b). For this data set, all sediments with a classification of highly autotrophic (BTSI 3) were also classified as net-autotrophic using P:R ratios. Sediments classified as net-heterotrophic and net-autotrophic (BTSI 1, 2), were net-heterotrophic using P:R ratios. As such, it appears that the 2 ap-

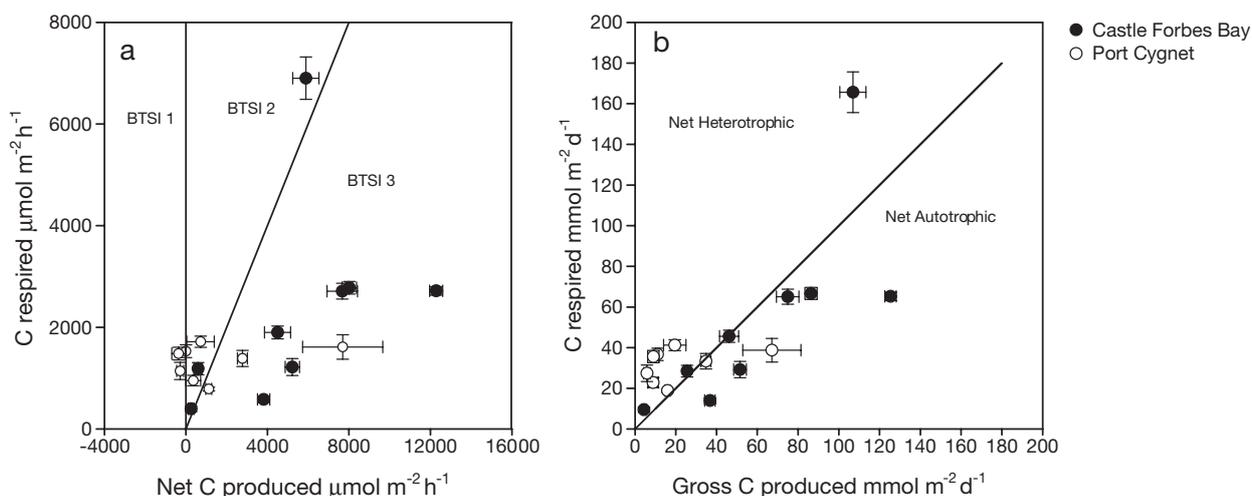


Fig. 6. (a) Dark  $\text{TCO}_2$  flux versus light  $\text{TCO}_2$  flux (after Viaroli et al. 1996 for oxygen fluxes) over study period at Castle Forbes Bay and Port Cygnet. BTSI: benthic trophic state index of sediments applied by Rizzo et al. (1996); BTSI 1: net-heterotrophic; BTSI 2: net-autotrophic; BTSI 3: highly autotrophic. Error bars: SE of replicate measurements ( $n = 3$  to 4). (b) Balance of respiration and primary production at the 2 study sites estimated on daily basis using  $\text{TCO}_2$  fluxes measured in laboratory and effective photoperiod calculated as in 'Materials and methods'

proaches result in similar trophic classifications in this instance.

In the current study, it was found that the most autotrophic sediments were generally those at Site CF, which were the most fine and organic-rich sediment measured (Cook et al. 2004b) sediments. This is in contrast to the findings of Rizzo et al. (1996), who investigated a range of factors affecting BTSI and came to the conclusion that the most autotrophic sediments were coarser sandy sediments. Rizzo et al. (1996) suggested that this was primarily due to increased respiration rates in the more organic-rich, finer sediments. Sundbäck et al. (2000) found no clear differences in the trophic status of a silty and a sandy site. Therefore it seems that no clear generalisations can be made about the effect of hydrodynamic energy and grain size on the trophic status of the sediment.

### Light $\text{O}_2$ and $\text{TCO}_2$ stoichiometry

The use of both  $\text{O}_2$  and  $\text{TCO}_2$  fluxes in this study has allowed 2 estimates of production using the net flux or 'bell jar' technique. The average production quotients (PQs) for Sites PC and CF were very similar to the value of 0.87 reported by Kristensen (1993) using the same methodology, but were well below the ratio of 1.1 to 1.4 (Fig. 7) expected for the balanced growth of phytoplankton (Laws 1991). During times of unbalanced growth, MPB may excrete most of their cellular production as extracellular carbohydrates (Goto et al. 1999). Given that the PQ expected for carbohydrates is 1 (Laws 1991), a lower PQ value of 1 might be expected. The average PQ value, however, was  $<1$  at

both sites; this most probably further highlights the limitation of measuring gross sediment production using direct fluxes. Measuring gross production in this way assumes that respiration rates in the dark will be

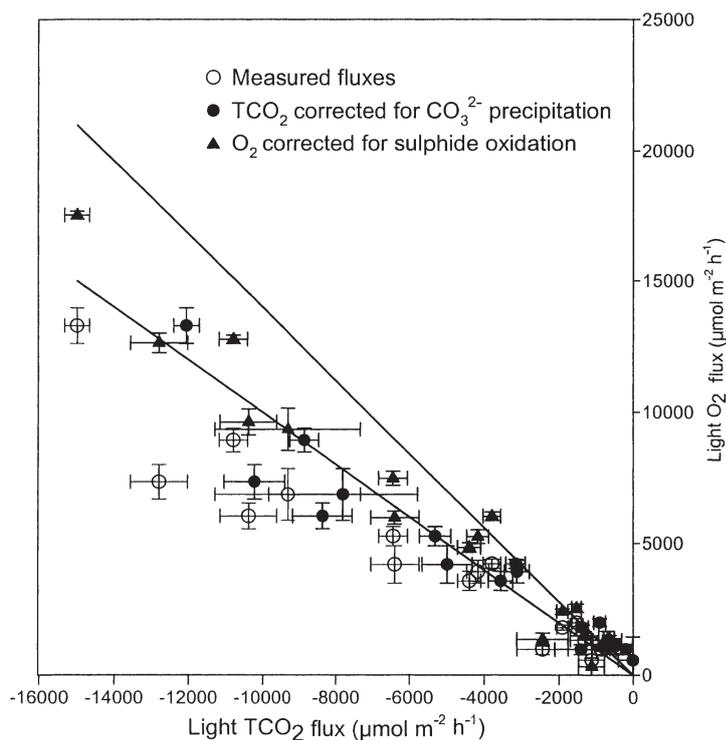


Fig. 7. Gross light  $\text{O}_2$  flux versus gross light  $\text{TCO}_2$  flux (O), gross light  $\text{O}_2$  flux versus gross light  $\text{TCO}_2$  flux corrected for  $\text{CO}_3^{2-}$  precipitation (●) and gross light  $\text{O}_2$  flux corrected for  $\text{FeS}$  oxidation versus gross light  $\text{TCO}_2$  flux (▲). Data are from the 2 study sites over course of a year. Error bars = SE of replicate measurements ( $n = 3$  to 4)

the same as those in the light. This has been shown not to be the case in intertidal sediments (Epping & Jørgensen 1996), and is most probably a consequence of increased respiration by both algae and bacteria as well as increased chemical oxygen demand (Epping & Jørgensen 1996, Epping et al. 1999, Fenchel & Glud 2000). Increased respiration by algae and bacteria in the light will not significantly alter the ratio of  $O_2$  released to  $TCO_2$  consumed, or appreciably alter alkalinity fluxes. Therefore, it is not possible to make an estimate of the magnitude of the increased rates of respiration using this data set. At times of deeper oxygen penetration caused by MPB photosynthesis, the reoxidation of  $Fe^{2+}$ ,  $FeS$  and  $HS^-$  will consume alkalinity and  $O_2$  without producing  $TCO_2$ . Removal of  $TCO_2$  and increases in pH caused by photosynthesis may promote precipitation of  $CaCO_3$  (Bernier 1971), consuming  $TCO_2$  and alkalinity without affecting  $O_2$  fluxes. Thus, carbonate precipitation and the reoxidation of reduced solutes are both possible explanations for the observed negative alkalinity fluxes and low PQ.

To estimate the probable magnitude of  $CaCO_3$  precipitation and sulphide oxidation, gross fluxes of  $O_2$  and  $TCO_2$  were corrected, using alkalinity data, as follows: alkalinity fluxes in the dark and light were first corrected by adding the sediment  $NO_3^-$  flux and subtracting the  $NH_4^+$  flux (Berelson et al. 1996). Primary production by 'Redfield-type' algae assimilating  $NH_4^+$  will reduce alkalinity by 14 equiv. for every 106 mol of  $TCO_2$  consumed (Gattuso et al. 1999). This alkalinity flux, attributable to photosynthesis (calculated as gross  $TCO_2$  flux  $\times$  14/106), was also subtracted from the alkalinity flux measured in the light. It was assumed that processes giving rise to positive alkalinity fluxes in the light continued during the dark. Gross alkalinity fluxes in the light were estimated by subtracting the dark alkalinity fluxes from the light fluxes.

The amount of  $O_2$  consumed in relation to alkalinity consumed will depend upon the stoichiometry of the oxidation reaction. Here we have considered 2 scenarios, the oxidation of  $FeS_2$  as suggested by Fenchel & Glud (2000), and the precipitation  $CaCO_3$ . For the sulphide oxidation scenario, the  $O_2$  fluxes were corrected assuming that 1.09 alkalinity equivalents were consumed  $mol^{-1}O_2$  according to the following equation (Stumm & Morgan 1996):  $FeS_2(s) + 1\frac{1}{4}O_2 + 3\frac{1}{2}H_2O \rightarrow Fe(OH)_3(s) + 4H^+ + 2SO_4^{2-}$ . For the carbonate precipitation scenario,  $TCO_2$  fluxes were corrected for  $CaCO_3$  precipitation assuming that 2 mol alkalinity were consumed  $mol^{-1}CaCO_3$  precipitated (Berelson et al. 1996).

As evident from Fig. 7, the data corrected for either  $CaCO_3$  precipitation or sulphide oxidation fall closer to the expected  $O_2:TCO_2$  of 1.0 to 1.4:1 than the uncorrected data. Both the corrected data sets had an average PQ value of 1.2. Precipitation of carbonates and/or

sulphide oxidation in the light are therefore plausible explanations for the observed fluxes of alkalinity and the deviation of the production quotient from the expected range (1 to 1.4).

## CONCLUSIONS

Rates of primary production were significantly greater on the upper mudflat at Site CF. This difference was most probably caused by the presence of high concentrations of CDOM in the water, which severely limited light penetration across the inundation gradient. At Site PC, no significant difference was observed between the upper and the lower mudflats. It is proposed that primary production at Site PC was limited by an exposure to greater wave energy compared to Site CF, as indicated by its southerly aspect and sediment grain size. Sediment respiration rates were highest on the upper mudflat at Site CF, reflecting the higher input of organic matter derived from MPB at this site.

The gaseous exchange of  $CO_2$  across the sediment–air interface was 3 to 5 times lower than the  $TCO_2$  exchange across the sediment–water interface. This was because a portion of the  $TCO_2$  flux across the sediment–water interface was transported in the form of  $HCO_3^-$  and  $CO_3^{2-}$ , and this ceased upon sediment exposure.

The use of  $O_2$  and  $TCO_2$  fluxes provided different measures of benthic primary production on the mudflats. High alkalinity fluxes into the sediment during sediment illumination suggested that either the reoxidation of reduced solutes in the sediments was taking place, or that precipitation of carbonates was occurring, decoupling the fluxes of  $O_2$  and  $TCO_2$ .

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