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Bacterial Respiration, Growth Efficiency and Protist Grazing Rates in Mangrove Waters in Cape Rachado, Malaysia

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Abstract: This study was carried out at an inter-tidal site at Cape Rachado (02º24′48N, 101º51′31E, Fig. 1) from April 2003 until April 2004. A small mangrove forest populated with *Sonneratia* and *Rhizophora* trees was located nearby. Bacterial respiration ranged 0.818–3.208 µM O_2 h^{-1}, and the amount of carbon respired was 9.80–38.44 µg C L^{-1} h^{-1}. Concurrent bacterial production during the incubation was 1.23–3.28 µg C L^{-1} h^{-1}. Using both these respiration and production values, we calculated the bacterial growth efficiency, and it ranged 4.0–11.1%. The low growth efficiency indicated the lower substrate quality here. However adding nutrients did not significantly increase bacterial growth rates (Student's t-test for matched pairs: $t = 1.883$, $df = 2$, $p > 0.10$). In this study, protists consumed $0.49 - 5.72 \times 10^4$ bacterial cells mL^{-1} h^{-1} or 22 ± 15% of bacterial production. In carbon equivalents, grazing ranged 0.15 – 1.81 µg C L^{-1} h^{-1}. Annual bacterial production at this site is 42.7 g C m^{-3} yr^{-1}, and carbon consumed by the bacteria was estimated at 585 g C m^{-3} yr^{-1}. Of this, only 8 g C m^{-3} yr^{-1} was channeled onto protists. These calculations showed that effectively only 1% of the carbon consumed by bacteria was passed onto protists. The role of bacteria here was essentially that of a remineralizer, and as a sink for carbon.

Key words: Bacterial carbon flux, bacterial carbon transfer, bacterial carbon demand, carbon sink, Straits of Malacca.

Introduction

Mangroves are ecosystems at the land-sea margin. In the tropics and subtropics, mangroves cover 100,000–230,000 km^2, and is the major ecosystem fringing the continental margins (Snedaker, 1984). Mangroves support a large portion of the coastal fisheries, and about two-thirds of the important coastal fish and shellfish species in Malaysia are dependent on mangrove habitats (Khoo, 1989).

The term 'microbial loop' was introduced more than two decades ago (Azam et al., 1983) to describe the importance of the microbial food web on the recycling and mineralization of organic matter in aquatic habitats. As bacteria are the most abundant component responsible for the transformation of organic matter (Cole et al., 1988), bacterial production (BP) becomes a key process in the dissolved organic matter (DOM) flux. However whether the bacteria act as a link (recyclers) or a sink (mineralizers) depends on the bacterial growth efficiency (BGE) (del Giorgio and Cole, 2000). BGE is essentially the ratio of net production over gross production, where gross production has been measured as production plus respiration (Lee et al., 2002) or DOM utilization (Amon and Benner, 1996) or both (Cherrier et al., 1996).

Knowledge of BGE is therefore a prerequisite to understand the function of an aquatic system. BGE is an important parameter to evaluate the fate of organic carbon inputs especially for mangrove waters that receive a considerable amount of mangrove plant litter. However

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there is very little information on BGE values from mangrove waters. Although BP data are widely available, bacterial respiration (BR) and BGE are rare, and most use a common BGE value (i.e. 30%) for their carbon flux calculations (e.g. Bano et al., 1997). Studies of temperate ecosystems have shown that BGE varies in time and space (e.g. Rivkin and Legendre, 2001; Biddanda and Cotner, 2002; Lee et al., 2002), and the independent measurement of BGE is emphasized to avoid uncertainty in conversion factors. Furthermore, studies that include grazing experiments to measure how much of the BP is passed onto higher trophic levels (e.g. Ferrier-Pagés and Gattuso, 1998; Lee et al., 2001) are rare.

This study is part of a research initiative to offer some understanding of the microbial food web in coastal waters in Malaysia. We also wanted to determine the significance of bacterial processes in material and energy fluxes in mangrove waters. An earlier publication focussed on the physical-chemical conditions of the mangrove waters and how this affected microbial abundance and BP (Lee et al., 2005). In this paper, we report for the first time bacterial respiration rates, BGE values, and protist grazing rates from Malaysian mangrove water, and how much of BP is transferred onto higher trophic levels.

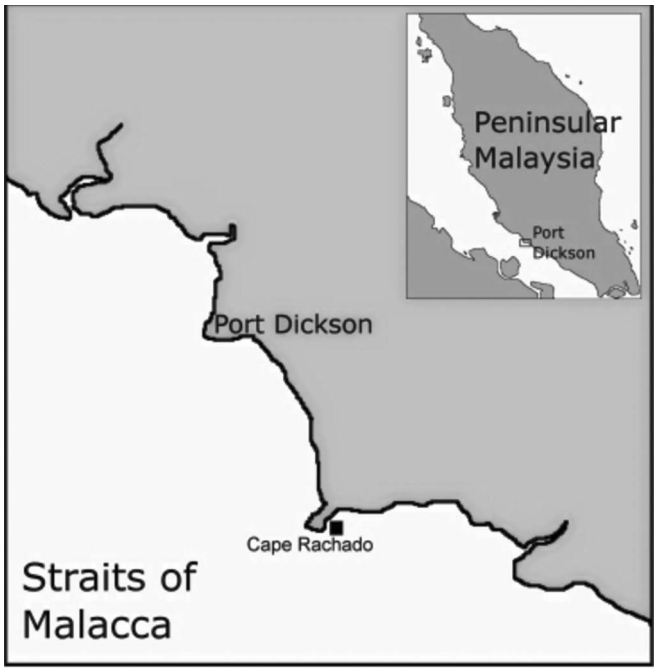

Figure 1: Location of the sampling station (2º24.5′N, 101º51.6′E) at Cape Rachado, Port Dickson. Inset: location of Port Dickson in Peninsular Malaysia (West Malaysia).

Material and Methods

In this study, sampling was carried out at an inter-tidal site (depth at mean tide level was about 1.5 m) at Cape Rachado (02º24'48N, 101º51'31E, Fig. 1) from April 2003 until April 2004. Cape Rachado is located at the southern end of Port Dickson, a popular seaside town in Malaysia. A small mangrove forest populated with *Sonneratia* and *Rhizophora* trees is located nearby. Seawater sample was collected about 0.2 m below seawater surface, and kept in a cooler box until processing. The proximity of this sampling station to our laboratory allowed us to process the water samples in the laboratory within three hours after sampling.

Bacterial Respiration Rate

To determine bacterial respiration (BR), seawater samples were filtered through pre-combusted Whatman GF/F filters to remove particles and bacterial grazers, and then siphoned into acid-washed 50 mL dissolved oxygen (DO) bottles. These bottles were incubated in the dark at in-situ temperatures for <24 h. Change in DO concentration was measured in sets of five DO bottles in a minimum five-time point analysis. BR was calculated by the least-squares method as the rate of decrease in DO concentration with incubation time.

Bacterial Growth Efficiency

In this study, bacterial growth efficiency (BGE) was determined by comparing gross with net bacterial production where the gross bacterial production was measured as net bacterial production (NBP) plus bacterial respiration (BR) (e.g. Lee et al., 2002):

$$BGE = NBP/(NBP+BR)$$

This NBP was determined by measuring the increase in bacterial abundance in the <0.7 μm fraction in another set of bottles ran simultaneously. The μ was calculated using the least-squares method as the slope of the regression analysis of natural logarithmic bacterial cell increase over time. NBP was then estimated by multiplying μ by the bacterial abundance (BA): NBP = μBA. In order to obtain bacterial production rates in carbon units, a carbon conversion factor of 31.6 fg C bacterium^{-1} was used. The method for this carbon conversion determination, and bacterial abundance data was published in Lee et al. (2005).

Protist Grazing Rate

In order to determine the protist grazing rate on bacteria, seawater sample was size-fractionated into both <0.7 µm and <20 µm fractions. The <0.7 µm fraction was essentially a grazer-free environment for the bacteria whereas the <20 µm fraction was a grazer-free environment for protists. These were incubated for <24 h, and the changes in both bacterial and protist abundance in each fraction was determined.

The grazing rates on bacteria were calculated as the difference between the estimated bacterial growth rate in the <0.7 µm fraction ($\mu_{0.7}$) and actual bacterial growth rate in the <20 µm fraction (μ_{20}) which presumably represented the product of both growth and grazing (McManus, 1993). Bacterial activity was assumed the same for all the fractions, and that bacteria grew exponentially within the incubation period. Grazing rate was then estimated using the following equation:

$$\text{Grazing rate } (h^{-1}) = \mu_{0.7} - \mu_{20}$$

Protist grazing rate was later expressed as bacteria eaten per protist per hour (bacteria protist^{-1} h^{-1}).

Microbial Counts, DO Measurements, and Statistical Analysis

When microbial counts were required, bacterial abundance was determined by epifluorescence microscopy on samples filtered onto a black 0.2 µm pore size polycarbonate filter, and then stained with 4'6-diamidino-2-phenylindole (DAPI) (0.1 µg L^{-1} final concentration) for 7 min (Kepner and Pratt, 1994). More than 200 cells were counted for each sample using an epifluorescence microscope (Olympus BX60, Japan). For protists, 10 mL of sample was filtered onto a black 0.8 µm pore size polycarbonate filter, and then stained with the fluorochrome, primulin (Bloem et al., 1986). DO concentration was measured by the Winkler method (Grasshoff et al., 1999).

Statistical analyses (Student's t-test, correlation and regression) were carried out according to Zar (1999). Mean was reported with standard deviation (± S.D.) when available, and error propagation was carried out wherever applicable.

Results

We have earlier shown that in this station, bacterial growth rates ranged 0.051 – 0.091 h^{-1} (Lee et al., 2005). On three occasions, we added nutrients (at final concentrations of 60 µM Glucose, 15 µM NH_4Cl and 2 µM KH_2PO_4) to a similar set of dilution culture carried out simultaneously (Table 1). Adding nutrients, increased the µ by 16 – 48% but was still not significantly different from the control (Student's t-test for matched pairs: $t = 1.883$, $df = 2$, $p > 0.10$).

Table 2 shows that the bacterial respiration ranged 0.818 – 3.208 µM O_2 h^{-1} (2.420 ± 0.816 µM O_2 h^{-1}). Assuming a respiratory quotient of 1.0 (Parsons et al., 1984), the amount of carbon respired was 9.80 – 38.44 µg C L^{-1} h^{-1}. Concurrent NBP measured during the incubation was 1.23 – 3.28 µg C L^{-1} h^{-1}. Using both these BR and NBP values, we calculated the BGE at our sampling site, and it ranged 4.0 – 11.1% (7.3 ± 2.8%).

On three occasions, the < 0.2 µm fraction was collected as a negative control for the respiration experiment i.e. to confirm the decrease in DO concentration was due to biological activity. On all occasions, the decrease was not significant (p > 0.20) (Table 3). We also compared the BR with total or community respiration (CR). CR ranged 3.272 – 9.710 µM O_2 h^{-1}, and BR was about 28% of CR (Table 3).

Table 1: Comparison of µ between dilution cultures without (µ) and with (μ_{GNP}) addition of nutrients.

	μ, h^{-1}	± S.E.	df	p	
µ					
11 February 2004	0.075	0.010	4	0.005	
08 March 2004	0.091	0.010	4	0.003	
21 April 2004	0.084	0.005	6	<0.001	
μ_{GNP}					μ_{GNP}/μ, ± S.D
11 February 2004	0.087	0.008	4	0.001	1.16 ± 0.36
08 March 2004	0.135	0.021	4	0.008	1.48 ± 0.43
21 April 2004	0.113	0.012	6	<0.001	1.34 ± 0.32

df = degrees of freedom, *p* = significance level for the regression analysis, S.D. = standard deviation. Bacterial growth rates without nutrient addition (µ) was obtained from Lee et al. (2005)

Table 2: (a) Bacterial respiration (BR) rates ± standard error (µM O_2 h^{-1} ± S.E.) at Cape Rachado. (b) Concurrent bacterial growth rates (µ ± S.E., h^{-1}) and net bacterial production (NBP).

(a) Bacterial respiration	*µM O_2 h^{-1}*	*±S.E.*	*df*	*p*	*BR ± S.D. (µg C L^{-1} h^{-1})*	
23 May 2003	–0.818	0.343	22	<0.001	9.80 ± 19.71	
29 June 2003	–1.221	0.260	22	0.030	14.63 ± 14.94	
26 July 2003	–2.739	0.181	24	0.001	32.82 ± 10.84	
01 September 2003	–2.834	0.386	22	<0.001	33.96 ± 22.18	
11 October 2003	–3.208	0.275	23	<0.001	38.44 ± 16.14	
08 November 2003	–2.813	0.286	23	<0.001	33.71 ± 16.79	
11 February 2004	–2.716	0.236	24	<0.001	32.55 ± 14.14	
08 March 2004	–2.620	0.232	24	<0.001	31.40 ± 13.90	
21 April 2004	–2.807	0.083	24	<0.001	33.64 ± 4.97	
(b) Bacterial growth and production	*µ, h^{-1}*	*±S.E.*	*df*	*p*	*NBP (µg C L^{-1} h^{-1})*	*BGE(%)*
23 May 2003	0.049	0.007	4	0.006	1.23	11.1
29 June 2003	0.043	0.008	4	0.011	1.74	10.6
26 July 2003	0.052	0.007	4	0.004	1.51	4.4
01 September 2003	0.042	0.008	4	0.015	1.78	5.0
11 October 2003	0.047	0.006	4	0.005	1.62	4.0
08 November 2003	0.047	0.005	4	0.002	1.73	4.9
11 February 2004	0.100	0.027	4	0.035	3.06	8.6
08 March 2004	0.103	0.025	4	0.024	2.76	8.1
21 April 2004	0.080	0.010	4	0.004	3.28	8.9

df = degrees of freedom, *p* = significance level for the regression analysis, S.D. = standard deviation, BGE = bacterial growth efficiency

Table 3: Bacterial respiration (BR) rates ± standard error (µM O_2 h^{-1} ± S.E.), community respiration (CR) ± standard error (µM O_2 h^{-1} ± S.E.), and respiration results from the <0.2 µm fraction (Control) at Cape Rachado.

	µM O_2 h^{-1}	*±S.E.*	*df*	*p*	
Bacterial respiration					*BR ± S.D. (µg C L^{-1} h^{-1})*
20 April 2003	–1.088	0.237	23	<0.001	13.04 ± 13.91
29 June 2003	–1.221	0.260	22	0.030	14.63 ± 14.94
01 September 2003	–2.834	0.386	22	<0.001	33.96 ± 22.18
Community respiration					*BR: CR, ±S.D. (%)*
20 April 2003	–6.398	0.449	29	<0.001	17.0 ± 1.1
29 June 2003	–3.272	0.316	28	<0.001	37.3 ± 1.1
01 September 2003	–9.710	0.510	28	<0.001	29.2 ± 0.7
Control					
23 May 2003	–0.383	0.534	29	>0.40	
26 July 2003	–0.726	0.640	28	>0.20	
11 October 2003	–0.667	0.511	28	>0.20	

df = degrees of freedom, *p* = significance level for the regression analysis, S.D. = standard deviation

Table 4 shows the grazing rates by protists at this sampling site. The consumption rate ranged 0.49 – 5.72 × 10^4 bacterial cells mL^{-1} h^{-1}. In carbon equivalents, grazing ranged 0.15 – 1.81 µg C L^{-1} h^{-1} (0.92 ± 0.55 µg C L^{-1} h^{-1}). We estimated that each protist grazed between 1.01 – 7.90 bacteria h^{-1}.

Discussion

BR in this study was similar to that of other tropical coastal water (5.40 – 25.44 µg C L^{-1} h^{-1}) (Pradeep Ram et al., 2003) but was more than one order higher than temperate water (0.29 – 4.58 µg C L^{-1} h^{-1}) (Lee et al.,

Table 4: Bacterial specific growth rates (μ, h^{-1}) for the grazing experiments (< 0.7 μm and < 20 μm fractions), and grazing rates obtained

	μ, h^{-1}	*Bacteria grazed mL^{-1} h^{-1}*	*Bacteria grazed protist^{-1} h^{-1}*	*Grazing (μg C L^{-1} h^{-1})*
29 June 2003		3.74×10^4	3.21	1.18
<0.7 μm fraction	0.073			
<20 μm fraction	0.039			
01 September 2003		2.70×10^4	2.69	0.85
<0.7 μm fraction	0.069			
<20 μm fraction	0.043			
08 November 2003		2.58×10^4	2.46	0.81
<0.7 μm fraction	0.067			
<20 μm fraction	0.042			
11 February 2004		5.72×10^4	7.90	1.81
<0.7 μm fraction	0.108			
<20 μm fraction	0.062			
08 March 2004		2.20×10^4	4.21	0.70
<0.7 μm fraction	0.080			
<20 μm fraction	0.063			
21 April 2004		0.49×10^4	1.01	0.15
<0.7 μm fraction	0.074			
<20 μm fraction	0.069			

2002). In this respiration experiments, we showed that the DO decrease was due to biological activity as the <0.2 μm fraction incubated did not exhibit any significant DO decrease. We attributed the oxygen utilization in the <0.7 μm fraction to that of BR as only bacteria were observed in this fraction. We also compared the BR with total or community respiration (CR). CR ranged 3.272 – 9.710 μM O_2 h^{-1} whereas BR was about 28% of CR. This was within the lower range reported by Biddanda and Cotner (2002) (20 – 90% BR/CR). Results from this study are consistent with the view that heterotrophic bacteria are responsible for most of the respiration in the water column (del Giorgio et al., 1997; Lee et al., 2002).

In this study, concurrent NBP was measured in an additional set of incubation bottles. Although the preparation was slightly different from the dilution culture experiments (Lee et al., 2005), the μ obtained showed no significant difference between them (Student's t-test for matched pairs: $t = 0.299$, $df = 5$, $p > 0.10$). This indicated the fractionation of the seawater for the respiration experiments did not stimulate nor retard bacterial activity.

BGE from this study was lower than that of tropical estuarine (mean = 18%) or coastal waters (mean = 11%) (Pradeep Ram et al., 2003). If these BGE values were reflective of tropical waters, studies which used BGE values of 30% (e.g. Bano et al., 1997) would have significantly underestimated the carbon flux through bacteria. The variability of BGE has been reported to reflect substrate quality (del Giorgio and Cole, 2000) and water temperature (Rivkin and Legendre, 2001). As the temperature in our site was relatively stable (28.6 ± 2.6ºC, CV = 9%, Lee et al., 2005), the variability of BGE could be better explained by substrate quality. The consistently low BGE values indicated the lower substrate quality here, probably the more refractory organic matter from mangrove plant litter.

However adding excess labile sugars and inorganic nutrients only stimulated bacterial growth rates an average 33%. This suggested that most of the bacterial population in mangrove waters were already adapted to the more refractory organic matter from mangrove plant litter. Probably only a fraction of the bacterial community responded to this nutrient addition.

In this study, the consumption rate of $0.49 – 5.72 \times 10^4$ cells mL^{-1} h^{-1} was similar to $0.6 – 2.4 \times 10^4$ cells mL^{-1} h^{-1} reported for coral reef waters (Ferrier-Pagés and Gattuso, 1998). Protists consumed an average 22 ± 15% of BP. This was lower than 60 – 70% reported by Ferrier-Pagés and Gattuso (1998). As bacterial abundance was relatively stable here (Lee et al., 2005), the fate of the remaining BP could be either lysed by viruses or exported.

Annual BP at this site is 42.7 g C m^{-3} yr^{-1} (Lee et al., 2005). Since the BGE was about 7.3%, the bacterial carbon demand (BCD) or carbon consumed by the

bacteria was estimated at 585 g C m^{-3} yr^{-1}. Of this, only 8 g C m^{-3} yr^{-1} was channeled onto protists. These calculations showed that effectively only 1% of the carbon consumed by bacteria was passed onto protists. We showed that although bacteria consumed a large amount of carbon, relatively little of it was assimilated, and of those assimilated, only 22% was passed onto higher trophic levels. The role of bacteria in this site was essentially that of a remineralizer, and as a sink for carbon.

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