Bacteria-Mediated Carbon Flux in Mangrove Waters: A Malaysian Perspective

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Abstract

Sampling was carried out regularly from March 2003 until April 2004, at a fringe mangrove forest at Cape Rachado, Malaysia. Seawater temperature ranged 22–32°C whereas salinity ranged from 23 to 33%. Nitrate ranged from 0.27 to 3.86 μM whereas both nitrite and ammonium ranged 0.04–0.95 μM and 0.73–2.13 μM, respectively. Phosphate ranged 0.16 to 2.42 μM, and silicate ranged 2.44 to 38.67 μM. Chlorophyll a (Chl a) fluctuated within a small range (0.89–5.15 μg L⁻¹), and correlated positively with silicate (R²=0.503, n=9, p<0.05), suggesting silicate limitation. Biovolume measurements were carried out, and carbon conversion factor estimated was 31.6 fg C bacterium⁻¹ and 0.17 fg C protist⁻¹. Bacterial abundance varied from 1.4 to 4.4×10⁶ cells mL⁻¹ whereas protists were from 4.8 to 21.7×10³ cells mL⁻¹. There was a highly significant coupling between bacteria and protists (R²=0.543, n=9, p<0.05), suggesting that bacterial abundance was controlled by protists. Bacterial production (BP) ranged 90 to 169 mg C m⁻³ d⁻¹, and annual BP was calculated at 42.7 g C m⁻³ yr⁻¹ whereas bacterial carbon demand (BCD) was estimated at 474 g C m⁻³ yr⁻¹ (assuming a bacterial growth efficiency of 9%). In this sampling site, annual gross primary production was estimated at 374 g C m⁻³ yr⁻¹, and was not sufficient to support BCD. Other sources of organic matter from mangrove trees could have made up this deficit.

Key Words: Bacterial Production, Inorganic Nutrients, Microbial Abundance, Cape Rachado, Straits of Malacca

INTRODUCTION

Mangroves are ecosystems at the land-sea margin. In the tropics and subtropics, mangroves cover 100,000–230,000 km², and is the major ecosystem fringing the continental margins (Snedaker 1984). Although mangrove forest covers only 2% (or 6500 km²) of Malaysia, mangroves support a large portion of the coastal fisheries. Furthermore, about two-thirds of the important fish and shellfish species in the coastal fisheries are dependent on mangrove habitats (Khoo 1989). This dependence is through an intricate food web where both mangroves and phytoplankton production are sources of organic matter. Bacteria play a central role packaging dissolved organic matter into particulate or utilisable forms (Valiela 1993).

It has been two decades since Azam et al. (1983) introduced the term 'microbial loop' to describe the importance of the microbial food web on the recycling and mineralization of organic matter in aquatic habitats. However research on the microbial food web in Malaysian mangrove waters has been limited to culturable specific bacteria and its participation in nitrogen cycle (Shaiful et al. 1986, Thong et al. 1993). Most of the literature available is on the biology and ecology of penaeid prawn and fish communities in mangroves (e.g. Chong et al. 1990, Chong et al. 2001). Only one study has been reported on the bacterial process rates of mangrove waters (Alongi et al. 2003) in Malaysia.

This study is part of a research initiative to offer new understanding of the microbial food web in
mangrove waters in Malaysia. We also study the significance of bacterial processes in material and energy fluxes in mangrove waters.

MATERIAL AND METHODS

This work was carried out at a small fringing mangrove forest at Cape Rachado. Samanetia alba trees dominated this mangrove forest. Sampling was carried out at Cape Rachado (02°24.8'N, 101°51.5'E, Figure 1) for about one year, March 2003 until April 2004. Cape Rachado is located at the southern end of Port Dickson, a popular seaside town in Malaysia. A global positioning system (Magellan SporTrak MAP, USA) provided the geographic position of the sampling site. Depth at mean tide levels was about 1.5 m. In-situ measurement of salinity was carried out using a salinometer (Atago S/Mill+, Japan) whereas pH, Eh and temperature were measured with a pH meter (Jenway 3071, UK). For dissolved oxygen (DO) determination, sample was collected with 50 mL DO bottles, and fixed immediately with manganese chloride and alkaline iodide solutions. DO concentration was then determined in triplicates using the Winkler method (Grasshoff et al. 1999).

Surface seawater sample was collected about 0.2 m below seawater surface. Sub-sample for the determination of microbial abundance was preserved with filtered (0.2 μm pore size) glutaraldehyde (1% final concentration), whereas the rest of the sample was kept in a cooler box until processing. In the laboratory, seawater sample was filtered through pre-combusted (450°C for 3 h) Whatman GF/F filters, and the filtrate was kept frozen (-20°C) for nutrient analysis. The filter was also kept frozen until chlorophyll a (Chl a) determination. On three occasions, the contribution of phytoplankton from different size-fractions to Chl a were determined. This was carried out by filtering the water samples through selected pre-filters (250 μm net, 20 μm net, and 2 μm polycarbonate filter) before trapping the autotrophs onto the GF/F filters.

Both dissolved inorganic nutrients (nitrate (NO$_3$), nitrite (NO$_2$), ammonium (NH$_4$), phosphate (PO$_4$) and silicate (SiO$_4$)) and Chl a analyses were carried out according to Parsons et al. (1984). Chl a was extracted overnight with 90% ice-cold acetone, and its absorbance at different wavelengths (trichromatic method) were measured with a spectrophotometer (Beckman DU7500i, USA).

For biological parameters, 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 (Bloom et al. 1986).

Microbial biovolume was determined by capturing micrographs with an automatic photomicrographic system (Olympus PM20, Japan). These photographs were then scanned, and these digital images were visually projected onto a white screen for size measurements. A magnification of 3 × 10$^8$ times was achieved. Bacterial biovolume was measured either as a sphere (4/3πA$^3$/6) or an ellipsoid [(4/3AB$^3$/6) where A is the diameter or length, and B is the width of the cell (Kellar et al. 1980). The bacterial biovolume was then converted into carbon biomass using an equation derived from Simon and Azam (1989):

$$f_{gC cell} = 75.9 (\mu m^3 cell^{-1})^{0.59}$$

Bacterial specific growth rate ($\mu$) was measured using a
dilution culture method (Kirchman et al. 1982). Sample was filtered through precombusted GF/F filters, and diluted five-fold with 0.2 μm filtered sample. Incubation was carried out for 24 h in the dark at in-situ temperatures. Subsamples were collected at regular intervals, and the bacterial cell increase over incubation time was measured. μ was calculated using the least-squares method as the slope of the regression analysis of natural logarithmic bacterial cell increase over time. Bacterial production was then estimated by multiplying μ by the bacterial abundance.

Statistical analyses (correlation and regression) were carried out according to Zar (1999). Mean was reported with standard deviation (±SD) when available, and error propagation was carried out wherever applicable.

RESULTS

Figure 2 shows the physico-chemical condition observed at Cape Rachado over the sampling period. Seawater temperature ranged 22–32°C (mean±SD=29±3°C) whereas salinity ranged 23–33 (30±3) (Figure 2A). pH ranged from 7.0 to 8.1 (7.7±0.4) (Figure 2B) whereas dissolved oxygen (DO) varied from 204 to 262 μM (242±32) (Figure 2C).

Nutrient concentrations in this station fluctuated throughout the sampling period (Figure 3). NO₃ ranged from 0.27 to 3.86 μM (1.41±0.99 μM) whereas NO₂ and NH₄ ranged 0.04–0.95 μM (0.38±0.35 μM) and 0.73–2.13 μM (1.29±0.40 μM), respectively (Figure 3A). PO₄ ranged 0.16–2.42 μM (1.21±0.73 μM) (Figure 3B), and SiO₄ varied between 2.44 and 38.67 μM (16.25±11.63 μM) (Figure 3C).

In this study, Chl a fluctuated within a small range (0.89–5.15 μg L⁻¹), and averaged 2.77±1.63 μg L⁻¹ (Figure 4A). Size-fractionation studies were also carried out to determine the major components of primary producers. Results showed the macrophytoplankton fraction (250 μm) was the largest contributor to the Chl a pigment (58%) whereas the microphytoplankton fraction (250 μm, 20 μm) was the smallest (1.6%) (Table 1). Phototrophic nanoplankton contributed

<table>
<thead>
<tr>
<th>Chl a (μg L⁻¹)</th>
<th>01–Sept-03</th>
<th>11–Oct-03</th>
<th>08–Nov-03</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macroplankton &gt;250 μm</td>
<td>2.94±1.07 (54%)</td>
<td>1.17±0.31 (61%)</td>
<td>0.67±0.15 (60%)</td>
</tr>
<tr>
<td>Microplankton, &lt;250 μm, &gt;20 μm</td>
<td>1.52±0.28 (28%)</td>
<td>0.22±0.16 (11%)</td>
<td>0.08±0.08 (79%)</td>
</tr>
<tr>
<td>Nanoplankton &lt;20 μm, &gt;2 μm</td>
<td>1.01±0.06 (19%)</td>
<td>0.53±0.04 (27%)</td>
<td>0.36±0.04 (32%)</td>
</tr>
<tr>
<td>Picoplankton &lt;2 μm</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
</tbody>
</table>
about 26% of total Chl a concentration. The contribution from phototrophic picoplankton was insignificant (less than the detection limit of 0.09 µg L⁻¹). Our study showed bacterial abundance ranged 1.4–4.4×10⁶ cells mL⁻¹ (2.8±1.1×10⁶ cells mL⁻¹) (Figure 4B) whereas protist ranged 4.8–21.7×10³ cells mL⁻¹ (1.1±0.6×10³ cells mL⁻¹) (Figure 4C).

To determine bacterial biomass, the volume of about 160 bacteria was measured. Bacterial cell volume ranged from 0.02 to 2.23 µm³, and the average was 0.28±0.34 µm³ (Figure 5A). The amount of carbon per bacterium was then calculated according to Simon and Azam (1989), and averaged 31.6±19.9 fg C cell⁻¹. For biomass calculation of protists, cell volume of about 50 protists were measured, and ranged 0.98–16.84 µm³ (4.57±3.74 µm³) or in carbon units 0.17±0.08 pg C cell⁻¹ (Figure 5B).

The carbon conversion units were then used to express bacterial production (BP) in carbon units. In this study, bacterial growth rates ranged 0.051–0.091 h⁻¹ (Figure 6A) whereas BP was relatively stable (117±28 mg C m⁻³ d⁻¹), fluctuating between 90 and 115 mg C m⁻³ d⁻¹, but increased to >150 mg C m⁻³ d⁻¹ on two occasions (July and October) (Figure 6B).

**DISCUSSION**

The concentrations for most of physico-chemical variables were relatively constant (Coefficient of Variation, CV < 10%), except DO which fluctuated over a wider range (CV > 20%). This is probably due to the non-conservative nature of DO, affected by both physical and biological processes (e.g., respiration and...
photosynthesis) (Grasshoff et al. 1999). In this study, the redox potential measured was consistently negative (−12 to −72 mV, unpublished data), indicating a reducing environment that is reflective of some mangrove waters (e.g. Ovalle et al. 1990). The relatively constant salinity also indicated a lack of freshwater influx for this station, and on only one occasion, rainfall reduced the salinity to <25.

Of the dissolved inorganic nitrogen (DIN: NH₄, NO₂, NO₃) species, both NH₄ and NO₂ were in low amounts whereas NO₃ was higher. Variation in DIN could be attributed to the NO₃ species alone (R²=0.903, n=13, p<0.001). The nutrient concentrations observed here (including PO₄ and SiO₄) were similar to that previously reported for both Malaysian estuaries and mangrove waters (Table 2).

In this study, Chl a was in the lower range relative to the eutrophic Klang waters (Table 3). The general quality of the water in this sampling site was better than estuarine mangrove waters. Size-fractionation showed that most of the phytoplankton in this study were both the macrophytoplankton and nanophytoplankton fractions. Other studies have shown the importance of nanophytoplankton as a major constituent of the total phytoplankton (Qasim et al. 1972, Ricard 1984).

Phytoplankton requires inorganic nutrients (e.g. NH₄, NO₃, PO₄, and SiO₄) for growth however in this study, Chl a correlated positively only with SiO₄ (Figure 7A: R²=0.503, n=9, p<0.05). This allowed us to suggest SiO₄ limitation for the phytoplankton community, especially diatoms that require relatively more

Table 2. Concentration (μM) of dissolved inorganic nutrients in coastal and mangrove waters in Malaysia

<table>
<thead>
<tr>
<th>Location</th>
<th>NH₄</th>
<th>NO₃</th>
<th>NO₂</th>
<th>DIN</th>
<th>PO₄</th>
<th>SiO₄</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Matang, Perak</td>
<td>1.5–24.7</td>
<td>0–13.8</td>
<td>–</td>
<td>1.9–17.9</td>
<td>2–90</td>
<td></td>
<td>Nixon et al. (1984)</td>
</tr>
<tr>
<td>Kedah</td>
<td>0.0–50</td>
<td>0.1–6.0</td>
<td>–</td>
<td>0.1–6.0</td>
<td>–</td>
<td></td>
<td>Wong (1984)</td>
</tr>
<tr>
<td>Port Dickson</td>
<td>2.80–6.49</td>
<td>0.22–6.27</td>
<td>0.15–1.85</td>
<td>0.31–0.86</td>
<td>–</td>
<td></td>
<td>Law et al. (1988)</td>
</tr>
<tr>
<td>Sg Sementa, Selangor</td>
<td>0.29–3.10</td>
<td>0.11–2.82</td>
<td>0.20–2.25</td>
<td>–</td>
<td>–</td>
<td></td>
<td>Thong et al. (1993)</td>
</tr>
<tr>
<td>Matang, Perak</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>20–33</td>
<td>0.32–0.92</td>
<td>80–190</td>
<td>Tanaka and Choo (2000)</td>
</tr>
<tr>
<td>Cape Rachado</td>
<td>0.73–2.13</td>
<td>0.27–3.86</td>
<td>0.04–0.95</td>
<td>0.16–2.42</td>
<td>2.44–38.67</td>
<td></td>
<td>Present study</td>
</tr>
</tbody>
</table>
Table 3. Chlorophyll a (Chl a) concentration and net primary production around Malaysian mangrove waters. *-Gross primary production (light-dark bottle oxygen method)

<table>
<thead>
<tr>
<th>Location</th>
<th>Chl a (µg L⁻¹)</th>
<th>Production (mg C m⁻³ d⁻¹)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Klang, Selangor</td>
<td>0.53–21.20</td>
<td>10–1068</td>
<td>Lee et al. 1984</td>
</tr>
<tr>
<td>Sg Sangga Besar, Perak</td>
<td>12–32</td>
<td>150–310</td>
<td>Alongi et al. 2003</td>
</tr>
<tr>
<td>Sg Sangga Kecil, Perak</td>
<td>27</td>
<td>350</td>
<td>Alongi et al. 2003</td>
</tr>
<tr>
<td>Cape Rachado</td>
<td>0.89–5.15</td>
<td>1024*</td>
<td>Present study</td>
</tr>
</tbody>
</table>

In this study, carbon conversion factors of 31.6 fg C bacterium⁻¹ and 0.17 pg C protist⁻¹ were used for all calculations. These are comparable to direct measurements of coastal samples (30.2 ± 12.3 fg C bacterium⁻¹) by Fukuda et al. (1998), and constrained value of 0.18 pg C protist⁻¹ suggested by Caron et al. (1995). Our bacterial carbon conversion factor calculated is higher than the commonly adopted carbon conversion factors of 15 fg cell⁻¹ (Caron et al. 1995), and 20 fg cell⁻¹ (Lee and Fuhrman 1987). Some studies used these 'adopted' carbon conversion factors for mangrove waters, and can inadvertently underestimate the importance of bacterial biomass in mangrove ecosystems.

Bacterial abundance was within the range of that reported for the Indus River delta, Pakistan (Bano et al. 1997) whereas protist was on average three orders lower. This concurred with observations by Sanders et al. (1992) that in specific systems, there are usually 1000 bacteria per one protist. In marine environment, protists (especially heterotrophic nanoflagellates) are important grazers or consumers of bacteria (Valiela 1993). Our study showed that protists were the major bacterial grazers, as indicated from the close predator-prey relationship with bacteria (Figure 7B: R² = 0.543, n = 9, p < 0.05). This close coupling showed that bacterial abundance was controlled by grazing pressure from the protists. A similar type of relationship has been reported by Ferrer-Pagès and Gattuso (1998) albeit for coral reef waters.

In order to determine the significance of heterotrophs and autotrophs to the carbon pool, comparison between the bacterial biomass (heterotrophic) and the phytoplankton biomass (autotrophic) was carried out. Our study showed bacterial biomass ranged from 50 to 138 µg C L⁻¹, and is slightly higher than that reported for a mangrove creek in Australia (26–93 µg C L⁻¹) (as cited in Robertson and Blaber 1992). Biomass contribution from protists was less significant, more than one order lower, ranging from 0.8 to 3.7 µg C L⁻¹.

Although phytoplankton biomass was not measured directly in this study, Chl a concentration is often used to represent phytoplankton biomass (Valiela 1995). Phytoplankton biomass was obtained from Chl a concentration assuming a phytoplankton carbon (PC) to Chl a ratio of 100 (Burford et al. 2002). Although PC:Chl a varies over a wide range (22–154) (Valiela 1995), we chose a ratio of 100 on the basis of our

SiO₄ (Valiela 1995). Furthermore, the phytoplankton communities in the coastal waters of Port Dickson are known to be predominantly diatoms (Shamsudin 1987, Lee 2003). This SiO₄ limitation was compounded by the lack of freshwater influx at Cape Rachado. Freshwater or river water is a good source of SiO₄ (Nixon et al. 1984). Although there are several studies that attempt to investigate the connection between nutrient concentrations and Chl a (Thong et al. 1993, Rivera-Monroy et al. 1995), these studies have disregarded the role of SiO₄. We showed that the variability in Chl a may be explained by SiO₄ fluctuations.
similar Chl a concentrations to the site studied by Burford et al. (2002). In this study, the bacteria:PC ratio ranged 0.1–1.2, and the average was 0.5 ± 0.4. This is close to the 0.1–0.5 range reported by Bano et al. (1997), and pointed to the dominance of autotrophy over heterotrophy.

The BP measured in this study was within the BP rates previously reported from tropical estuaries and mangrove waters (Robertson et al. 1993, Bano et al. 1997). Generally, bacteria are dependent on the dissolved organic matter released by phytoplankton, and bacteria-phytoplankton coupling can be observed over different aquatic environments (Cole et al. 1988). In this study, correlation analyses of both bacteria-Chl a and BP-Chl a were not significant (p > 0.05). However the two peaks in BP coincided with Chl a increase that occurred a month earlier, in June and September. The BP increase could be a time lag response to the increase in Chl a earlier.

In this study, annual BP was estimated at 42.7 g C m⁻³ yr⁻¹. In order to determine the carbon flux through the heterotrophic bacterial component, an independent measurement of BGE was carried out, and averaged 9.0±6.0% (ranging from 4.0 to 24.3%) (unpublished data). This was within the range of BGE values previously published (del Giorgio and Cole 2000, Lee et al. 2002). Since the BGE was about 9%, the bacterial carbon demand (BCD) or carbon consumed by the bacteria is estimated at 474 g C m⁻³ yr⁻¹. On one occasion, gross primary production was estimated at 1024 mg C m⁻³ d⁻¹ using the light-dark oxygen method. This is within previously published primary production data (10-1068 mg C m⁻³ d⁻¹) (Ong et al. 1984). We extrapolated on this limited datum, and estimated the annual gross primary production at Cape Rachado at around 374 g C m⁻³ yr⁻¹.

Our study showed that phytoplankton primary production alone cannot support the BCD. This concurred with the finding from other mangrove ecosystems that have been reported as net heterotrophic (e.g. Bano et al. 1997). The presence of net heterotrophic systems has been reported for various habitats (Tranvik 1992, del Giorgio et al. 1997, Lee et al. 2001). In this station, possible sources of organic matter making up the carbon deficit for bacterial consumption could be from mangrove trees via the detrital food web. Examples include leaf litter, leaf leachates and root exudates. Although river input is also considered a major source in most studies, there is no river near this station.

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