CHAPTER 20

CARBON FLUX THROUGH BACTERIA IN A EUTROPHIC TROPICAL ENVIRONMENT: PORT KLANG WATERS

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1. INTRODUCTION

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 dissolved organic matter (DOM) flux. Heterotrophic and autotrophic processes are the two most fundamental metabolic processes in aquatic ecosystems. In oligotrophic systems, planktonic primary production (PP) is the main source of DOM, and there is tight coupling between BP and PP (Williams, 1998). However in coastal waters, terrigenous DOM is often suggested as an alternative source of organic matter that could be utilized by bacteria, and to explain the "uncoupling" that sometimes occurs between PP and BP (Tranvik, 1992). Understanding these auto- and heterotrophic processes is central to the study of biogeochemical cycles especially the carbon (C) cycle.

Another essential information for C cycle study is the bacterial growth efficiency (BGE). BGEs have great relevance to C dynamics because most aquatic metabolism is microbial (del Giorgio et al., 1997). BGE is an important parameter to evaluate the fate of organic carbon inputs, and whether the bacteria act as a link (recyclers) or a sink (mineralizers) depends on the BGE (del Giorgio and Cole, 2000). BGE is essentially the ratio of net production over bacterial carbon demand (BCD), where BCD has been measured as BP plus bacterial respiration (BR) (Lee et al., 2002) or DOM utilization (Amon and Benner, 1996) or both (Cherrier et al., 1996). Although BP data are available, there are relatively few BR and BGE data, and most studies use a common BGE value (i.e. 30%) for their carbon flux calculations (e.g. Bano et al., 1997; Lee et al., 2001b). However 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

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required to avoid uncertainty in conversion factors. Furthermore, estuarine systems (e.g. Port Klang waters; Figure 1) receive inputs of allochthonous organic matter from terrestrial runoff, and BGE becomes a crucial parameter to evaluate the fate of allochthonous carbon inputs. Other than the work carried out by Pradeep Ram et al. (2003), very little information is available on BGEs from tropical aquatic systems.

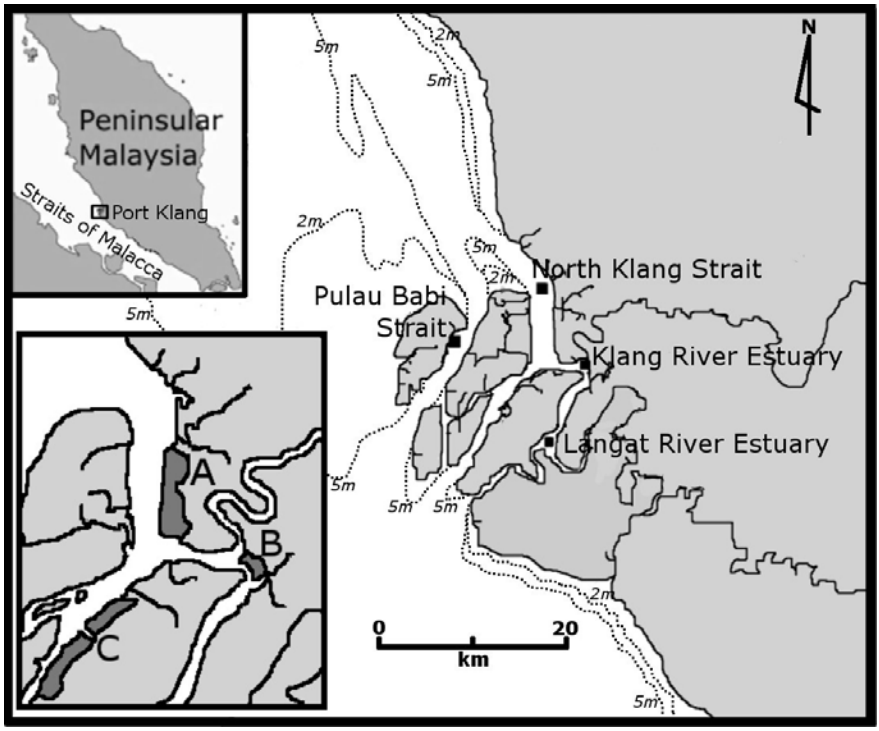

Figure 1. *Map showing the location of Port Klang, and the sampling stations in this study. Isobaths for 2 and 5 m depths are delineated by dotted lines. Lower left inset shows the (A) Northport, (B) Southport and (C) Westport of Port Klang.*

Research on the microbial food web in Malaysian waters is limited to culturable-specific bacteria and its participation in the nitrogen cycle (Shaiful et al., 1986; Thong et al., 1993). Most of the scientific literature focuses on the biology and ecology of penaeid prawn and fish communities in mangroves (e.g. Chong et al., 1990 and 2001). In Malaysia, data on metabolic processes are scarce (Alongi et al., 2003). This study was part of a research initiative to understand the microbial food web in coastal Malaysian waters. It aims to determine the significance of bacterial processes in material and energy fluxes in different aquatic environments. In this paper, microbial process rates including BGE values from a eutrophic tropical aquatic system are reported, as well as the amount of BP grazed, and how much BP

was transferred onto higher trophic levels. A major goal of this research was to evaluate temporal variation in both autotrophic and heterotrophic processes in Port Klang waters, and to determine how these processes affect Port Klang water quality.

2. SITE DESCRIPTION

Port Klang (Figure 1) is a multipurpose Malaysian gateway port located strategically mid-way on the west coast of Peninsular Malaysia overlooking the Straits of Malacca. It offers the first mainline port of call eastbound on the Europe-Asia leg and last port of call westbound on the Asia-Europe leg. Port Klang began more than 100 years ago as a small railway port. Consistent with the rapid growth of the Malaysian economy in the 1970s–1990s, there was a rapid expansion of demand for port facilities at Port Klang. Port Klang now comprises Northport (covering an area of 241 ha), Westport (510 ha) and Southport (48 ha, see Figure 1). Port Klang handled a total of 5.2 million twenty-foot equivalent units (TEUs) in 2004, 12th in the World Port Rankings (Barrock, 2005).

3. METHODS

Sampling was carried out regularly from September 2004 until February 2005 at the Klang River Estuary station (03º00'04 N, 101º23'24E, Figure 1). This station is located at the mouth of the Klang River, near to the Southport. Seawater samples were collected about 0.1 m below seawater surface, and kept in a cooler box until processing within three hours. *In-situ* measurement of salinity was carried out using a salinometer (Atago S/Mill-Σ, Japan) whereas pH and temperature were measured with a pH meter (Jenway 3071, UK). For dissolved oxygen (DO) determination, samples were collected in 50 ml DO bottles, and fixed immediately with manganous chloride and alkaline iodide solutions. DO concentration was then determined by the Winkler titration method (Grasshoff et al., 1999). The theoretical 100% saturation value at the time of sampling was also calculated according to Weiss (1970). One sample for the determination of microbial abundance was obtained each time, and preserved with filtered (0.2 µm pore size) glutaraldehyde (1% final concentration).

3.1. Chemical parameters

In the laboratory, seawater sample was filtered through pre-combusted (500 ºC for 3 h) Whatman GF/F filters, and the filtrate was kept frozen (−20 °C) until nutrient analysis. Filters for chlorophyll *a* (Chl *a*) and total suspended solids (TSS) were also kept frozen until analyses. 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 (using the

trichromatic method) was measured with a spectrophotometer (Beckman DU7500i, US). TSS was measured as the filter weight increase after drying (70 ºC until no more weight loss). The same filter was later combusted in a microwave furnace (CEM MAS7000, US), and the weight loss after combustion was calculated as particulate organic matter (POM).

3.2. Microbiological parameters

Bacterial abundance was determined by epifluorescence microscopy on samples filtered onto a black polycarbonate filter (0.2 μm pore size), 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 300 cells or a minimum of seven fields were counted for each sample using an epifluorescence microscope (Olympus BX60, Japan) with the U–MWU filter cassette (excitor 330–385 nm, dichroic mirror 400 nm, barrier 420 nm). For protist, 10 ml of sample was filtered onto a black polycarbonate filter (0.8 μm pore size), and then stained with the fluorochrome primulin (Bloem et al., 1986). Observation was also carried out using the U–MWU filter cassette. The abundance of phototrophic picoplankton (PPico) was determined by filtering 5 mL of sample onto a black polycarbonate filter (0.2 μm pore size). No fluorochrome was used, and the autofluorescence of the PPico was observed using the U–MWG filter cassette (excitor 510–550, dichroic mirror 570 nm, barrier 590 nm). For the determination of bacterial biovolume, measurements of bacterial diameter and length were obtained using a digital imaging system, analySIS® version 3.2 (Soft Imaging System, Germany). These measurements were then used to calculate the bacterial biovolume. Bacterial biovolume was measured either as a sphere $[(\pi A^3)/6]$ or an ellipsoid $[(\pi AB^2)/6]$ where A is the diameter or length, and B is the width of the cell (Kellar et al., 1980). To obtain a carbon conversion factor, the bacterial biovolume was converted into carbon biomass using an equation derived from Simon and Azam (1989):

$$\text{fg C cell}^{-1} = 75.9(\mu\text{m}^{-3}\text{cell}^{-1})^{0.59} \quad (1)$$

3.3. Bacterial production rate

Bacterial specific growth rate (μ) was measured using a dilution culture method (Lee et al., 2001a). Sample was filtered through pre-combusted Whatman GF/F filters, and diluted five-fold with 0.2 μm filtered sample. Incubation was carried out for 12 h in the dark at *in-situ* temperature. Sub-samples were collected regularly at 4 h intervals, and the bacterial abundance 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 (BP) was then estimated by multiplying μ by the bacterial abundance (BA): BP=μBA. At each sampling, an additional batch of incubation was carried out to

examine whether any nutrient limitation occurred. Before incubation was started, the sample was enriched with nutrients at final concentrations of 60 μM Glucose, 15 μM NH_4Cl and 2 μM KH_2PO_4.

3.4. Bacterial respiration rate

To measure bacterial respiration (BR), seawater sample was filtered through pre-combusted Whatman GF/F filters to remove particles and bacterial grazers, and then siphoned into acid-washed 50 ml DO bottles. These bottles were incubated in the dark at *in-situ* temperatures for 12 h. Change in DO concentration was measured in sets of five DO bottles in a four time-point analysis. BR was calculated by the least-squares method as the rate of DO decrease with incubation time.

3.5. Bacterial growth efficiency

In this study, bacterial growth efficiency (BGE) was determined by comparing gross with net bacterial production where the gross bacterial production or bacterial carbon demand (BCD) was measured as bacterial production (BP_{resp}) plus bacterial respiration (BR) (e.g. Lee et al., 2002):

$$BGE=BP_{resp}/(BP_{resp}+BR) \quad (2)$$

BP_{resp} was determined by measuring the increase in bacterial abundance in another set of bottles incubated simultaneously.

3.6. Protist grazing rate

In order to determine the protist grazing rate on bacteria, the size-fractionation method was used. Seawater sample was size-fractionated into both <0.7 μm and <20 μm fractions. These were then incubated for 12 h, and changes in both bacterial and protist abundance in each fraction was determined. The <0.7 μm fraction was essentially a grazer-free environment for the bacteria, and represented the bacterial growth rates without any grazing pressure ($\mu_{0.7}$) whereas the <20 μm fraction contained both bacteria and protist. The bacterial growth rate in the <20 μm fraction (μ_{20}) presumably represented the product of both growth and grazing (McManus, 1993). Bacterial activity was assumed the same for all the fractions, and grazing rate (h^{-1}) was then estimated using the following equation: $\mu_{0.7}-\mu_{20}$. Protist grazing rate was also expressed as bacteria eaten per protist per h (cell $protist^{-1}$ h^{-1}).

3.7. Gross Primary Production and Community Respiration

To measure gross primary production (GPP) and community respiration (CR), seawater samples were siphoned into acid-washed 50 ml DO bottles. These bottles were then incubated under light (for GPP) and dark (for CR) conditions at *in-situ* temperatures for 12 h. Change in DO concentration was measured in sets of five DO bottles in a four time-point analysis. The rate of DO increase or decrease was analyzed using the least-squares linear regression method. CR was the rate obtained from "dark bottles" whereas GPP was calculated as the rate of DO change in "light

bottles" minus "dark bottles". A photosynthetic quotient of 1.2 and a respiratory quotient of 1.0 was used in the conversion to carbon units (Parsons et al., 1984). For BR, GPP and CR, the multipoint and replicate measurements of DO over a short incubation time is able to yield realistic estimates of respiration (Pomeroy et al., 1994; Biddanda and Cotner, 2002).

4. RESULTS

Surface seawater temperature was relatively invariant, ranging between 29.2 and 30.1 (Coefficient of Variation, *CV*=1%) whereas salinity varied over a wider range (16–29, *CV*=22%) (Figure 2A). Salinity was lowest in late December 2004 and January 2005. pH range was 7.08–7.86 (*CV*=4%), and correlated positively with salinity (R^2=0.678, n=6, p<0.05). DO concentration was generally low (<200 μM, *CV*=42%) (Figure 2B), and decreased to 37 μM in February 2005. Percentage DO saturation can be used to indicate physiological stress (Breitburg, 2002). Although DO saturation value is dependent on both temperature and salinity (Weiss, 1970), temperature plays a more important role. As temperature was stable, DO saturation did not vary much (200–204 μM, Figure 2B). Using both DO saturation and *in-situ* DO concentration, the percentage DO saturation calculated ranged 18–97%. TSS or particulate >0.7 μm size was consistently high, and ranged 260–290 mg l^{-1} (*CV*=4%) (Figure 2C). In comparison to TSS, POM was more variable, fluctuating between 6–13 mg l^{-1} (*CV*=33%). Chl *a* averaged 3.58±1.86 μg l^{-1}, and was between 2.06–3.64 μg l^{-1} throughout most of the sampling period but doubled to 7.16 μg l^{-1} late December 2004.

Figures 3A and 3B show the nutrient concentration measured in this study. Of the three nitrogen species measured, NH_4 (Figure 3A) was consistently highest (>67% of dissolved inorganic nitrogen, DIN). NH_4 (mean ± S.D.=11.76 ± 9.06 μM) fluctuated within 5.46–10.39 μM for most months but increased nearly three fold to 29.78 μM early December 2004. Relative to NH_4, NO_3 (2.78±1.99 μM) and NO_2 (2.39±1.93 μM) made up only 18 and 15% of DIN, respectively. In our study, PO_4 varied nearly ten fold (0.41–5.68 μM), and was also highest early December 2004 (5.68 μM) whereas SiO_4 fluctuated the least (*CV*=39%), and was always >3.51 μM (Figure 3B).

Microbial abundance showed less than one order fluctuation throughout the sampling period (Figure 3C). Bacteria were more than two orders higher than other microbes, and dominated the microbial community. Bacterial abundance ranged 2.5–9.8×10^6 cells mL^{-1} whereas PPico and protists ranged 1.2–6.4×10^4 cells mL^{-1} and 1.3–3.5×10^3 cells mL^{-1}, respectively. Bacterial abundance was relatively stable (2.5–4.0×10^6 cells mL^{-1}) but more than doubled in January 2005 to 9.8×10^6 cells mL^{-1}. For the determination of bacterial biovolume (n=650), the carbon content per bacterium ranged 4.6–97.2 fg C $cell^{-1}$, and the average carbon content per cell (32.8 fg $cell^{-1}$) was used as our constant carbon conversion factor.

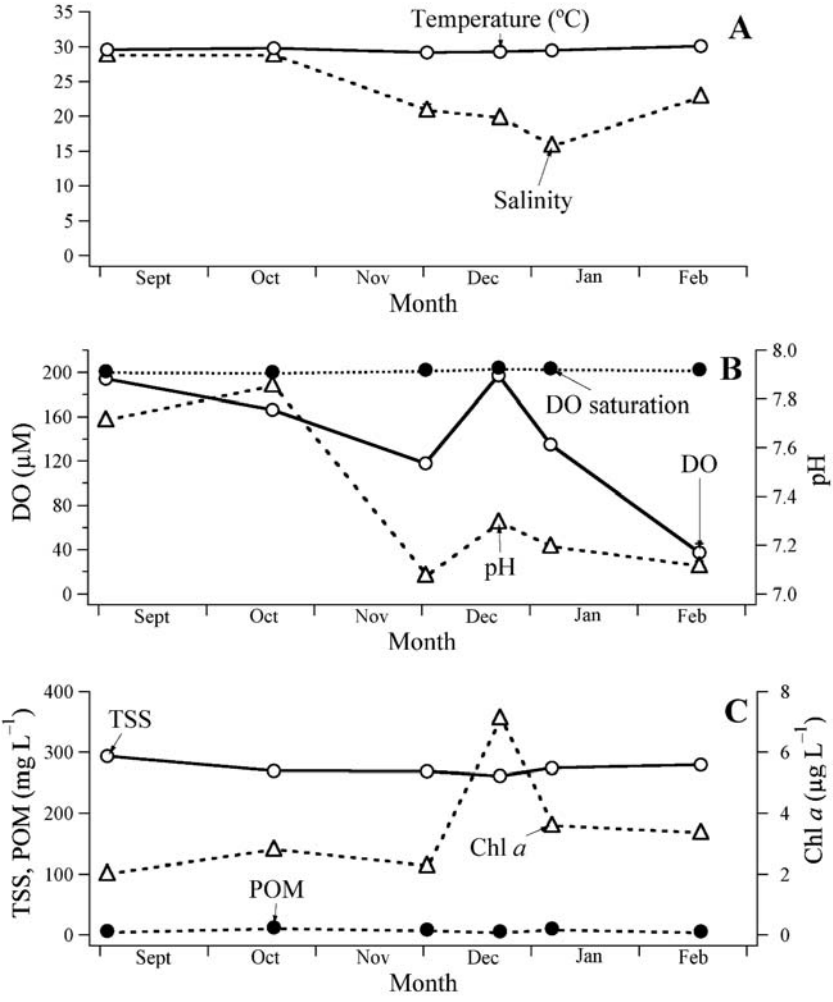

Figure 2. *Temporal variation of (A) temperature and salinity; (B) dissolved oxygen (DO), DO saturation and pH; (C) total suspended solids (TSS), particulate organic matter (POM) and Chlorophyll a (Chl a) at the Klang River Estuary throughout the sampling period. Error bars (±Standard Deviation, S.D.) are shown for DO, except where they are smaller than the symbols.*

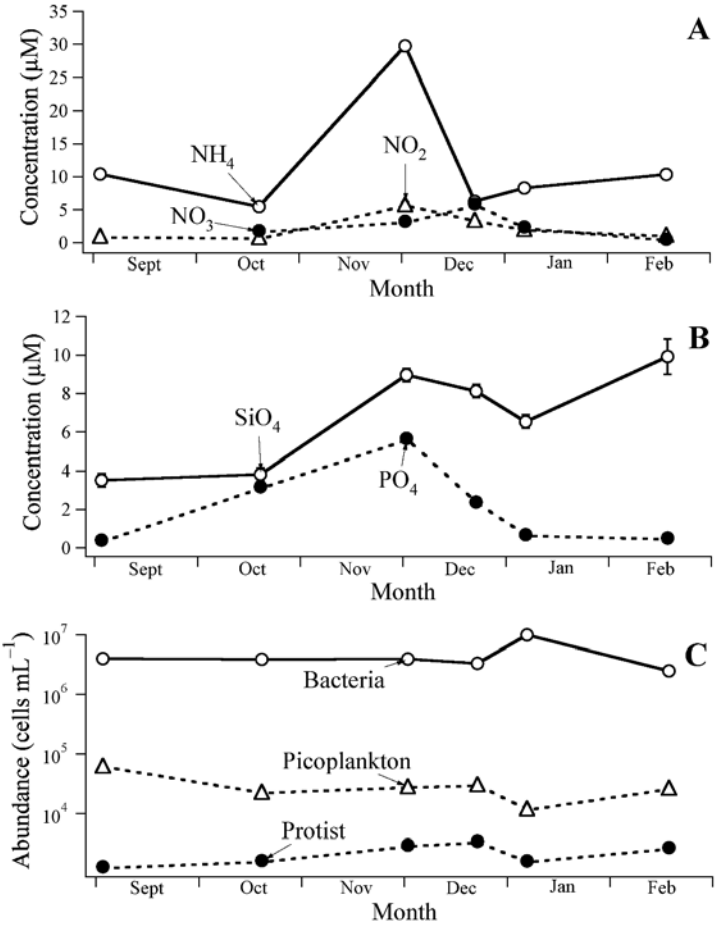

Figure 3. *Temporal variation of (A) NH_4, NO_3 and NO_2; (B) SiO_4 and PO_4; (C) bacterial abundance, protists and phototrophic picoplankton at the Klang River Estuary throughout the sampling period. Error bars (±Standard Deviation, S.D.) are shown for NH_4, NO_3, NO_2, SiO_4 and PO_4, except where they are smaller than the symbols.*

In this study (Figure 4A), GPP fluctuated between 103–265 µg C L^{-1} h^{-1} except late December 2004 when it was highest (407 µg C L^{-1} h^{-1}) and lowest in February 2005 (6 µg C L^{-1} h^{-1}). CR ranged 0.931–7.301 µM O_2 h^{-1} or its carbon equivalent of 11–88 µg C L^{-1} h^{-1}, and was highest late December 2004. CR was lower than GPP on all occasions except February 2005. Bacterial growth rate or µ ranged 0.086–0.221 h^{-1}, and BP ranged 10–71 µg C L^{-1} h^{-1} (Figure 4B). Both µ and BP were highest in January 2005. We also found that adding nutrients did not significantly increase µ (0.098–0.249 h^{-1}) (Student's t-test: t=0.357, df=10, p>0.70). Of this BP,

each protist consumed about 17.8–71.9 bacteria h^{-1}. Protist grazing was about one order lower than BP, ranging 1.5–5.8 µg C L^{-1} h^{-1} (Figure 4C).

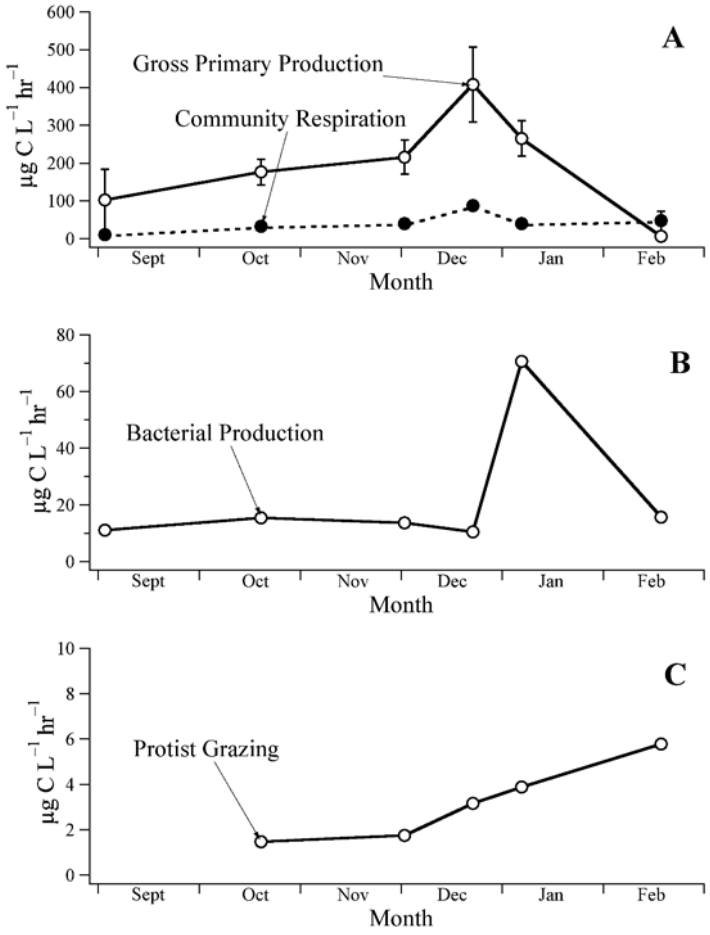

Figure 4. *Temporal variation of (A) Gross Primary Production and Community Respiration; (B) Bacterial Production; (C) Protist Grazing at the Klang River Estuary throughout the sampling period. Error bars (±Standard Deviation, S.D.) are shown for Gross Primary Production and Community Respiration, except where they are smaller than the symbols;*

Table 1 shows both the BR and concurrent BP (BP_{resp}) measured in this study and the BGE estimated for each sampling. In this study, the significant linear change in DO concentration for all the respiration experiments carried out showed there was no "undesirable effects of confinement" (Pomeroy et al., 1994). This could be due to the short incubation time (≤12 h). BR ranged 1.542 to 7.295 µM O_2 h^{-1} or 19.49–87.51 µg C L^{-1} h^{-1} whereas BP_{resp} ranged 3.90–6.08 µg C L^{-1} h^{-1}. The DO decrease

in respiration experiments was due to biological activity because incubation of the <0.2 μm fraction showed no significant DO change (Lee and Bong, *submitted*). Using both BR and BP_{resp} values, BGE calculated ranged 6.4–22.9%. Comparison between the bacterial growth rate in the BR experiments and the dilution culture BP experiments showed no significant difference (Student's t-test: t=0.470, df=7, p>0.30). This indicated that the sample preparation carried out for the respiration experiments did not significantly affect bacterial activity.

Table 1. *(a) Bacterial respiration (BR) rates ± standard error (μM O_2 h^{-1}±S.E.) at Klang River Estuary. (b) Concurrent bacterial growth rates (μ±S.E., h^{-1}) and bacterial production (BP_{resp}). df=degrees of freedom, p=significance level for the regression analysis, S.D.=standard deviation, BGE=bacterial growth efficiency.*

(a) Bacterial respiration (BR)	μM O_2 h^{-1}	±S.E.	df	p	BR±S.D. (μg C L^{-1} h^{-1})	
02 September 2004	–1.542	0.151	19	<0.001	18.49±8.09	
19 October 2004	–2.217	0.108	19	<0.001	26.59±5.82	
01 December 2004	–2.558	0.152	19	<0.001	30.69±8.16	
22 December 2004	–3.781	0.257	19	<0.001	45.35±13.80	
06 January 2005	–1.843	0.132	19	<0.001	22.10±7.11	
16 February 2005	–7.295	0.445	19	<0.001	87.51±23.88	
(b) Bacterial growth	μ, h^{-1}	±S.E.	df	p	BP_{resp}±S.D. (μg C L^{-1} h^{-1})	BGE (%)
02 September 2004	0.132	0.015	3	0.012	5.49±1.24	22.9
19 October 2004	0.167	0.030	3	0.031	3.90±1.40	12.8
01 December 2004	0.124	0.019	3	0.022	6.08±1.85	16.5
22 December 2004	0.134	0.019	3	0.020	5.55±1.60	10.9
06 January 2005	0.154	0.025	3	0.026	3.99±1.31	15.3
16 February 2005	0.187	0.018	3	0.009	5.92±1.11	6.4

5. DISCUSSION

The tropical climate in Peninsular Malaysia is strongly influenced by both North-East (NE) and South-West (SW) monsoons. Towards the end of the year, there is heavier rainfall that coincides with the NE monsoon; otherwise weather conditions are relatively stable with rainfall throughout the year (Uktolseya, 1988). Although our sampling period was only six months, it covered both wet and dry months. We found that salinity was highly variable compared with temperature. Changes in

salinity were strongly influenced by freshwater run-off from large rivers and high rainfall (Uktolseya, 1988). The salinity decreased from early December 2004, and this coincided with the wetter months. The amount of freshwater that flowed from the Klang River diluted the salinity at the Klang River Estuary. This also decreased the pH as freshwater generally has a lower pH than seawater.

The water quality in Port Klang was poor due to the low DO concentration (<200 μM), and high TSS (>260 mg l^{-1}). High TSS levels is a pervasive problem in Malaysia (Dow, 1995). TSS can comprise of both biogenic and non-biogenic particulates. The high TSS here was mainly inorganic (POM <5% of TSS). This could be attributed to land clearing activities for construction projects, mining, agricultural and forest industries, and dredging operations (Dow, 1995). Moreover, the Klang River basin covers the Klang valley that represents the most rapidly developing part of Malaysia.

A good indicator of aquatic health is DO concentration as all respiring organisms require oxygen. Further, low DO concentration (or oxygen deficiency) causes stress response in fish and other aquatic organisms. This stressful level of oxygen-depletion is known as hypoxia. Although there is no universally accepted DO levels to describe hypoxia, the consensus from laboratory or field observations is 125 μM (Rabalais et al., 2002). Another convenient threshold for detecting physiological stress is the <50% DO saturation (Breitburg, 2002). Our study showed two episodes of hypoxia occurring, in early December 2004 and February 2005. In the latter date, DO was close to anoxia (no oxygen). If the Breitburg (2002) threshold is used, only in February 2005 did the oxygen deficiency cause stress (18% of DO saturation). At other periods, the DO levels were 58–97% of DO saturation.

Based on the marine water quality data collected by Department of Environment (DOE) of Malaysia, water quality at and around Port Klang (Pulau Babi Strait, North Klang Strait, Klang River Estuary and Langat River Estuary stations, see Figure 1) deteriorated from 1990 to 2003. Although there were gaps of two to five years in this compilation, TSS increased significantly (F=134.4, df=140, p<0.001) (Figure 5A) whereas DO decreased significantly (F=11.8, df=122, p<0.001) (Figure 5B). From the linear regression equations, we estimated that over the decade (1994–2003), TSS increased 132 mg L^{-1} whereas DO decreased 48 μM. When we included our data in the analysis, TSS and DO continued the existing trend.

Inorganic nutrients were within the range previously reported for estuarine waters in Malaysia (Nixon et al., 1984). NH_4 was the most dominant nitrogen species (mean=67%). This reflects a reducing environment where NH_4 accumulates, and is typical of mangrove waters (Alongi et al., 2003) or waters with low DO. Compared with Redfield's NO_3:PO_4 ratio of 16 (Redfield et al., 1963), the NO_3:PO_4 ratio observed here was extremely low, ranging 0.05–0.38. This suggested a nitrogen limiting condition for phytoplankton which concurred with Law et al. (2001) where they reported that the nutrient limiting factor in the Straits of Malacca is probably nitrogen. SiO_4 was persistently high in this study, and is typical of coastal stations with large river systems (for example the Klang River) as freshwater is a source of SiO_4 (Nixon et al., 1984).

Chl *a* concentration is frequently used as an indicator of phytoplankton biomass (Falkowski et al., 1998) as all primary producers have this photosynthetic pigment.

Chl *a* measured in this study was within the range previously reported for the Klang River (Lee et al., 1984). We observed a small phytoplankton bloom late December 2004 where the Chl *a* concentration doubled. Bacterial abundance measured here was within the range reported for the Indus River delta, Pakistan (Bano et al., 1997), and peaked in January 2005 whereas PPico abundance was within the range for most aquatic systems (Sherr and Sherr, 2000). Although bacteria are closely coupled to primary producers (Cole et al., 1988), we observed a lag in the bacterial increase

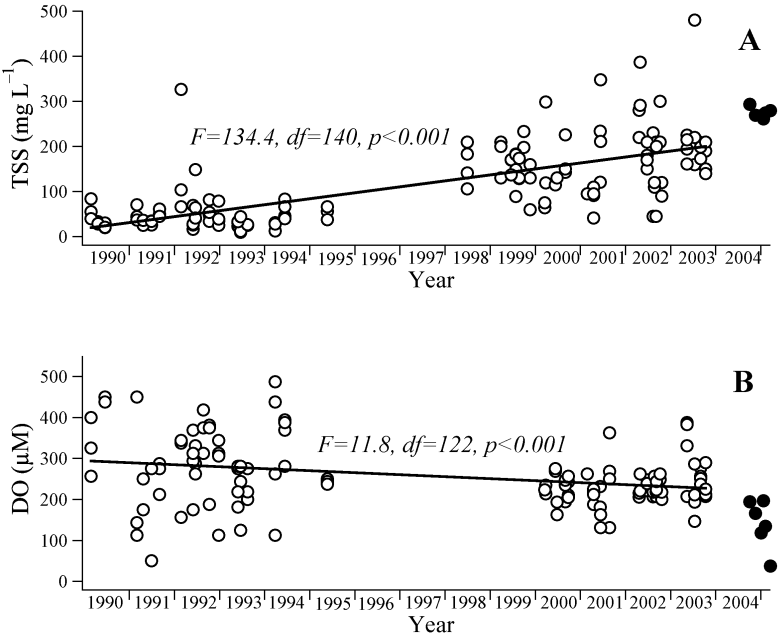

Figure 5. *Temporal variation of (A) Total Suspended Solids; (B) Dissolved Oxygen for stations Pulau Babi Strait, North Klang Strait, Klang River Estuary and Langat River Estuary. Data from 1990 until 2003 are from Department of Environment (DOE), Malaysia (open circles) whereas data from this present study are represented by closed circles. Linear regression line for the DOE data are also shown.*

when compared with phytoplankton bloom late December 2004. This could be due to the fact that bacterial biomass increases slower than phytoplankton biomass (Sanders et al., 1992; Simon et al., 1992).

Protist abundance was three orders lower than bacteria, and this conformed to the observation by Sanders et al. (1992) that in specific systems, there are usually 1000 bacteria per one protist. In this study, the carbon conversion factor of 32.8 fg C cell^{-1} was used, and this is comparable to other direct measurements of coastal samples (Fukuda et al., 1998). However it is about twice the factor used for open ocean systems (Caron et al., 1995). The wide range of conversion factor shows the necessity of measuring the carbon content per bacterium for different ecosystems.

Our GPP and CR results showed the eutrophic nature of Port Klang waters where both were one order higher than that reported for a mangrove estuarine system at Matang, Malaysia (Alongi et al., 2003). The phytoplankton bloom observed late December 2004 coincided with a two times increase in GPP. There was also a 70% increase in DO that could be attributed to primary production as photosynthesis releases oxygen (Grasshoff et al., 1999). However there was hypoxia in February 2005 due to reduced photosynthesis as GPP was lowest, and was 40 times less. This drastic reduction in GPP could be due to nutrient limitation. We found that GPP correlated significantly with NO_3 (R^2=0.867, n=5, p<0.05). The low NO_3 concentration in February 2005 could have limited GPP, and indirectly triggered hypoxia. This was further evidence of nitrogen limitation for primary producers. GPP is the basis of aquatic food web from where all autochthonous organic matter originates (Valiela, 1995). Excluding the data in February 2005, we found a highly significant correlation between GPP and CR (R^2=0.956, n=5, p<0.01). However in February 2005, there was uncoupling between primary production and heterotrophy as CR was probably supported by allochthonous organic matter from the Klang River.

Bacterial growth rates were within the range reported for an estuarine mangrove ecosystem at Matang, Malaysia (Alongi et al., 2003) but BP was up to six times higher. This was because the bacterial abundance here was generally higher, and the bacterial carbon conversion factor adopted by Alongi et al. (2003) was lower (25 fg C cell^{-1}). Although GPP was limited by nutrients, adding nutrients to our BP experiments did not stimulate bacterial growth rates indicating that there was no nutrient limitation for bacteria. This showed the catabolic flexibility of the bacterial community in obtaining its essential nutrients. Bacterial growth rates were not significantly stimulated even with the addition of glucose.

In marine environment, protists are important grazers or consumers of bacteria (Valiela, 1995). In this study, the consumption rate ranged 5.5–26.9×10^4 cells mL^{-1} h^{-1}, higher than that reported for coral reef waters (Ferrier-Pagés and Gattuso, 1998). This consumption rate was only 19±14% of BP, lower than the 60–70% reported by Ferrier-Pagés and Gattuso (1998). Ingestion rates ranged 18–72 cells protist^{-1} h^{-1}, higher than 9–36 cells protist^{-1} h^{-1} reported in North Atlantic waters (Weisse and Scheffel-Möser, 1991). By observing the temporal change in GPP, BP and grazing, we observed that there was a lag response to earlier events. Maximum GPP was in late December 2004, followed by maximum BP in January 2005 and then by maximum grazing in February 2005.

BR was higher than the range 5–25.44 μg C L^{-1} h^{-1} reported for tropical coastal waters near Goa, India (Pradeep Ram et al., 2003). BR:CR was about 0.29±0.16, and this was on the lower end of the range reported by Biddanda and Cotner (2002). Earlier studies have shown that BR:CR tends to decline as nutrient concentration increases (Schwaerter et al., 1988; del Giorgio et al., 1997). We determined the BGE at each sampling as BGE can have a wide range (del Giorgio and Cole, 2000). BGE was within the range reported for tropical estuarine and coastal waters (Pradeep Ram et al., 2003), and was generally >10%, but decreased to 6% in February 2005. The variability of BGE reflects both substrate quality (del Giorgio and Cole, 2000) and water temperature (Rivkin and Legendre 2001). As temperature was relatively

stable, the sudden reduction of BGE in February 2005 was probably caused by the consumption of a more refractory organic matter. This further pointed to heterotrophy uncoupling from primary production.

With these BGE values, we calculated the carbon consumed by bacteria or the bacterial carbon demand (BCD). Table 2 shows the BCD, and the amount of carbon flux through the bacterial component. BCD ranged 1.16–11.08 g C m^{-3} d^{-1}. To determine whether there was net heterotrophy (BCD>GPP) and net autotrophy (BCD<GPP) at each sampling, the ratio BCD:GPP was estimated. It ranged over more than two orders. The lowest ratio (0.47) occurred when there was a phytoplankton bloom in late December 2004, and primary production alone could have supported BCD. The highest ratio (77.38) was in February 2005 when primary production was limited by NO_3, and could not support BCD. Uncoupling between bacteria and phytoplankton occurred. BCD:GPP ratio was also high (3.48) in January 2005, a post-bloom period. There was elevated BCD (highest at 11.08 g C m^{-3} d^{-1}) that was probably supported by excess primary production that occurred during the phytoplankton bloom earlier (Biddanda and Cotner, 2002). On other occasions, the BCD and GPP seemed in balance, and BCD:GPP ratio averaged 1.03. Of the C consumed by bacteria, only 2% was consumed by protists. Although microbial loop (organic matter–bacteria–protists) is essential to recycling of organic matter (Azam et al., 1983), we observed that in this eutrophic and tropical coastal system, it was not an efficient pathway as a substantial amount of carbon was lost.

Table 2. *Amount of carbon flux through the bacterial component. GPP=Gross Primary Production ((g C m^{-3} d^{-1}), BP=Bacterial Production (g C m^{-3} d^{-1}), BGE=Bacterial Growth Efficiency (%), BCD=Bacterial Carbon Demand (g C m^{-3} d^{-1}), n.d.=not detectable.*

	GPP	BP	BGE	BCD	Grazed	Amount of bacteria consumed (%)	Amount of BCD transferred to higher trophic level (%)
02 Sep. 2004	1.23	0.27	22.9	1.16	n.d.	n.d.	n.d.
19 Oct. 2004	2.12	0.37	12.8	2.90	0.04	9.5	1.2
01 Dec. 2004	2.59	0.33	16.5	1.99	0.04	12.8	2.1
22 Dec. 2004	4.89	0.25	10.9	2.30	0.08	30.3	3.3
06 Jan. 2005	3.18	1.70	15.3	11.08	0.09	5.5	0.8
16 Feb. 2005	0.08	0.38	6.3	5.92	0.14	37.0	2.3

6. CONCLUSION

Port Klang waters is eutrophic and long term data showed that its water quality is deteriorating. We observed the occurrence of hypoxia due to very low GPP that was limited by NO_3. Although primary production is the basis of aquatic food web, and supported both CR and BCD, episodes of uncoupling were observed. Uncoupling occurred especially when GPP was very low, and generally BCD and GPP were balanced. Our results showed that only 2% of C consumed by bacteria were passed

onto protists. This suggested that the microbial loop was not an efficient pathway to recycle organic matter as a substantial amount of carbon was lost.

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