Microbial ecology of Port Klang waters

Choon-Weng Lee, Chui-Wei Bong
Laboratory of Microbial Ecology, Institute of Biological Sciences, University of Malaya, 50603 KUALA LUMPUR, Malaysia

13.1 Introduction
In the early 1970s, the original concept of marine food web was still based on the primary production of the largest sized phytoplankton in the sea. The production of both diatoms and dinoflagellates were consumed by copepods, which in turn were eaten by larger consumers. Bacteria acted as a decomposer in this ‘classic’ marine food chain returning inorganic nutrients (e.g. nitrogen and phosphate) back to the primary producers. However, Pomeroy (1974) suggested that the ‘classic’ food chain was insufficient as the role of microbes (< 60 µm) was generally disregarded. Since then, there have been significant advances in our understanding of microbes and their role in marine food web.

We now know that there is a substantial pool of dissolved organic matter in the sea. The sources of dissolved organic matter include: (i) autochthonous inputs via in-situ primary production, and in-situ regeneration through decomposition processes, (ii) allochthonous inputs via horizontal and vertical transfer, atmospheric deposition, terrigeneous surface-runoff, and river inputs. The main consumer of the dissolved organic matter is bacteria. Bacteria utilize dissolved organic matter, and transfer the carbon to higher trophic levels via grazing or bacterivory thereby completing the microbial loop (Azam et al., 1983) (Figure 1). Other than bacterivory, viral lysis is also an important fate for bacteria. However unlike bacterivory, viral lysis has a net effect of reducing the amount of carbon and energy to the main food web (Fuhrman, 1999).

Most of our present knowledge on aquatic microbial ecology is from studies carried out in temperate regions where there is marked seasonality in temperature. In tropical waters where temperatures are relatively higher and stable, the structure and function of the microbial food web may differ and is worth investigating. Moreover with the rising atmospheric CO₂ concentrations and global warming concerns, we need to understand the major flows of carbon in the upper waters of our aquatic systems i.e. primary production and community respiration especially microbial respiration. To the best of our knowledge, measurements of microbial related processes are still relatively few in Malaysia.

13.2 Location of study
Port Klang is a multipurpose Malaysian gateway port located strategically mid-way on the west coast of Peninsular Malaysia overlooking the Straits of Malacca (Figure 2). 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). In 2004, Port Klang handled a total of 5.2 million twenty-foot equivalent units, and ranked 12th in the World Port Rankings (Barrock, 2005).

Fig. 1. Schematic diagram of the aquatic microbial food web. Modified from Fuhrman (1999).

Fig. 2. 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.
13.3 Marine water quality

Both the growth of the port and the development in the Klang valley have inevitably caused changes to the marine environment. At present, the water quality import in Port Klang waters is poor due to the low dissolved oxygen (DO) concentration (<200 µM) and high total suspended solids (TSS) (>260 mg l⁻¹) (Lee and Bong, 2006). Figure 3 shows some of the marine water quality data from 1990 until 2003 monitored at four stations (North Klang Strait, Pulau Babi Strait, Klang River Estuary and Langat River Estuary) by the Department of Environment, Malaysia. We included our 2004–2005 data in the analyses. Long term data are useful in revealing trends that are not immediately evident. We find significant increases in temperature, salinity, TSS whereas DO concentration decreased significantly over time. pH and *Escherichia coli* counts did not change significantly over time.

Fig. 3. Long term variation of seawater temperature (ºC), salinity (ppt), pH, total suspended solids (TSS, mg l⁻¹), dissolved oxygen (DO, µM) and *Escherichia coli* (ln E.coli, ln MPN 100 ml⁻¹). Data from 1990 until 2003 were obtained from the Department of Environment (DOE), Malaysia for stations Pulau Babi Strait, North Klang Strait, Klang River Estuary and Langat River Estuary (open circles). Closed circles represented Klang River Estuary data from our laboratory. Linear regression line for the DOE data are also shown together with the correlation index, degree of freedom and significance P level of regression tests.
The significant increase in surface seawater temperature could be related to the global warming trend or to the decreasing volume of water flowing into the Klang Strait due to the damming of rivers upstream. The decreasing fresh water inflow could also be the reason for the increasing salinity observed. However, the most disturbing trend was the TSS and DO levels. Although there were gaps of two to five years in this compilation, TSS increased > 130 mg l\(^{-1}\) over the last decade. The increase is usually attributed to land clearing activities for construction projects, mining, agricultural and forest industries, and dredging operations. The increasing TSS could reduce water transparency and indirectly reduce primary production.

The decreasing DO levels are also a cause for concern as all respiring aquatic organisms require oxygen. Low DO concentration or hypoxia (< 62.5 µM) causes stress response in fish and other aquatic organisms. Our analysis revealed that DO decreased about 50 µM over the last decade, and we observed one hypoxic event in the surface water of Klang River Estuary in 2005 (Lee and Bong, 2006). If the trend continues, there will be more occurrences of hypoxia and anoxia in Port Klang waters that would further decrease the marine water quality here.

Dissolved inorganic nutrients measured at Klang River Estuary (Lee and Bong, 2006; 2008) showed that Klang waters are relatively eutrophic with elevated ammonium concentration (Figure 4). Ammonium (± Standard Deviation) (13.5 ± 10.1 µM) was the most dominant nitrogen species, and reflected a reducing environment typical of waters with low DO. Relative to Redfield’s nitrate to phosphate ratio of 16, the nitrate to phosphate ratio observed here was low (3.7 ± 7.0), suggesting a nitrogen limiting condition for primary producers. Silicate was persistently high (11.0 ± 8.5 µM), and is typical of coastal stations with large river systems as freshwater is a source of silicate.

![Box-whisker plot showing the range and median of ammonium (NH4), nitrite (NO2), nitrate (NO3), phosphate (PO4) and silicate (SiO4) concentrations (in µM) measured at Klang River Estuary. Outliers are represented by open circles.](image)
Dissolved organic carbon and dissolved organic nitrogen concentrations were also measured at Klang River Estuary via the high temperature catalytic oxidation method (Lee et al., 2009a) (Figure 5). The concentration of dissolved organic carbon and dissolved organic nitrogen concentrations were $550 \pm 320 \mu M$ and $25 \pm 10 \mu M$, respectively. The ratio of dissolved organic carbon to dissolved organic nitrogen indicates organic matter quality or bioreactivity (Amon and Benner, 1996). In Port Klang waters, the dissolved organic carbon to dissolved organic nitrogen ratio was high (39 ± 24). A possible reason for this is that a substantial amount of organic matter in this region is sourced from leaching e.g. from forest litter, and is usually dominated by refractory humic substances (Steinberg, 2003).

![Box-whisker plot showing the range and median of dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) concentrations (in µM) measured at Klang River Estuary. One outlier for DOC at 2300 µM is not shown.](image)

**Fig. 5.** Box-whisker plot showing the range and median of dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) concentrations (in µM) measured at Klang River Estuary. One outlier for DOC at 2300 µM is not shown.

### 13.4 Microbes

We measured the chlorophyll $a$ concentration as a proxy for phytoplankton biomass whereas bacterial abundance, protists and virus were measured as direct counts via epifluorescence microscopy (Figure 6) (Lee and Bong, 2006; 2008; Bong and Lee, submitted). Average chlorophyll $a$ was $2.9 \pm 0.7 \text{ mg l}^{-1}$ but increased three to ten-fold during phytoplankton bloom. Average bacterial abundance was $4.1 \pm 2.3 \times 10^6 \text{ cells ml}^{-1}$ whereas protists were generally three orders lower ($2.7 \pm 0.9 \times 10^3 \text{ cells ml}^{-1}$). Average virus count was $7.2 \pm 2.2 \times 10^6 \text{ counts ml}^{-1}$, and two times higher than bacterial abundance.

We also calculated the average bacterial size at Klang River Estuary via bacterial biovolume measurements (Figure 7) (Lee and Bong, 2008). The distribution follows a bimodal pattern that suggested a combination of two different bacterial communities could be found probably from different sources e.g. from the river and
from the sea. Relative to other stations that we studied (e.g. Port Dickson or Kuantan), the carbon content per bacterium is highest at Klang River Estuary. The relatively bigger bacterium biovolume and carbon content are probably reflective of the level of eutrophification observed in Klang waters.

Fig. 6. Box-whisker plot showing the range and median of chlorophyll $a$ (Chl $a$, in mg l$^{-1}$), bacteria (cells ml$^{-1}$), protists (cells ml$^{-1}$) and viruses (counts ml$^{-1}$) measured at Klang River Estuary. Outliers are represented by open circles.

Fig. 7. Histogram showing the bacterial carbon content (fg C cell$^{-1}$) distribution measured at Klang River Estuary. The average ± standard deviation is shown.

Figure 8 is an unrooted phylogenetic tree showing the bacterial genera cultured from coastal waters of Peninsular Malaysia including Klang River Estuary (Lee et al., 2009b). Although we did not separate the bacteria according to their sampling stations, the distribution of culturable bacteria is relatively homogenous and is probably reflective of Klang River Estuary (personal communication). Most of the bacteria belonged to the $\gamma$-Proteobacteria class where the most prevalent are from the genera *Pseudoalteromonas*, *Alteromonas* and *Vibrio*. Also important is the low GC, Gram-positive Bacteria where *Bacillus* dominates. Although a culture-dependent approach is a good start to understanding the bacterial community in the Port Klang waters, it still does not reflect total bacterial diversity.
13.5 Primary production and bacterial production rates

Both the primary production rates and bacterial production rates were measured at Klang River Estuary (Figure 9). The bacterial specific growth rate ($\mu$) was measured using a dilution culture method, and then bacterial production was calculated as bacterial abundance $\times \mu$ whereas primary production was measured using the light–dark dissolved oxygen method (Lee and Bong, 2008). Net primary production was $9.7 \pm 3.1 \mu\text{M C h}^{-1}$ whereas bacterial production was $1.6 \pm 1.4 \mu\text{M C h}^{-1}$. These production rates are the highest among the stations studied by our laboratory (e.g. Port Dickson and Kuantan). The bacterial production over net primary production ratio was $0.17 \pm 0.11$, and was similar to various marine and freshwater bodies in the temperate regions (Cole et al., 1988). The ratio of bacterial production over net primary production suggested that primary production was adequate to support bacterial production.

13.6 Bacterial growth efficiency

However the transfer of dissolved organic matter to bacteria is more accurately reflected by bacterial carbon demand or carbon consumption (Jahnke and Craven, 1995) than bacterial production. One way to obtain bacterial carbon demand from bacterial production is through bacterial growth efficiency or growth yield where bacterial growth efficiency is the product of bacterial production over bacterial carbon demand.
In our studies, we measured bacterial growth efficiency as bacterial production / (bacterial production + bacterial respiration) (Lee and Bong, 2007; Lee et al., 2009a).

Average bacterial growth efficiency was 0.18 ± 0.11 whereas bacterial carbon demand was 11.3 ± 6.8 µM C h⁻¹ (Figure 10). The ratio of bacterial carbon demand over net primary production was 1.2 ± 0.7. On average, net primary production alone was inadequate to support bacterial carbon demand. In other words, Port Klang waters are net-heterotrophic in terms of carbon fluxes.

13.7 Bacterial mortality
Among the predators of marine bacteria, protists are important (Sanders et al., 1992). However protistan bacterivory may not always be sufficient to explain bacteria mortality as virus mediated bacterial mortality is another important component (Fuhrman, 1999). Virus production rates were measured via viral decay rates whereas protistan bacterivory was measured using the size fractionation method (Lee and Bong, 2007; Bong and Leé, submitted). Figure 11 shows the viral decay (0.11 ± 0.04 h⁻¹) and viral production rates (7.5 ± 1.6 × 10⁵ virus ml⁻¹ h⁻¹).
From virus production rates, we can estimate the bacterial mortality due to viral lysis via virus burst size. The burst size was estimated from bacterium biovolume (Weinbauer and Peduzzi, 1994), and was 58. Average viral lysis was 0.09 ± 0.02 µM C h⁻¹ whereas average protistan bacterivory was 0.22 ± 0.13 µM C h⁻¹ (Figure 12). In Port Klang waters, protistan bacterivory was more important than viral lysis for bacterial mortality. However bacterial mortality alone accounted only for 20% of bacterial production, and other avenues are possible to explain the fate of bacteria in Port Klang waters.

**Fig. 11.** Box-whisker plots showing the range and median of viral decay rates (VD, h⁻¹) and virus production (VP, virus ml⁻¹ h⁻¹) at Klang River Estuary. Outliers are represented by open circles.

**Fig. 12.** Box-whisker plots showing the range and median of protistan bacterivory (µM C h⁻¹) and viral lysis (µM C h⁻¹) at Klang River Estuary. Outliers are represented by open circles.

### 13.8 Summary
Port Klang waters are eutrophic, and have relatively poor water quality. The deterioration of water quality especially the TSS increase and DO decrease, occurred steadily over time, and was related to the development in the upstream Klang valley.
Primary production was relatively high, but was not enough to support bacterial carbon demand \textit{i.e.} Port Klang waters are net-heterotrophic (Figure 13). The role of allochthonous organic matter is important to support the additional bacterial carbon demand. In terms of bacterial mortality, protistan bacterivory was more important than viral lysis. However, the fate of a substantial portion of bacterial production was still unaccounted for. Other processes \textit{e.g.} precipitation by suspended solids and benthic filter feeders could be important.

**Fig. 13.** Schematic diagram showing the bacterial related carbon fluxes (\(\mu\text{M C h}^{-1}\)) in Port Klang waters. Values represent average process rates and are in \(\mu\text{M C h}^{-1}\). BP = bacterial production, BCD = bacterial carbon demand, NPP = net primary production, DOC = dissolved organic carbon. Diagram is not drawn to scale.

**Acknowledgements**

We are grateful to the National Oceanography Directorate of Malaysia (Grant number: NOD/R&D/P7/0005/03 and 04-01-03-SF0194) and University of Malaya (FP003/2002D, F0377/2002A and FQ009/2007A) for their research grants that supported part of this work. We are also grateful to the Department of Environment, Malaysia for providing the marine water quality data. This paper was written when LCW was a visiting lecturer at the Graduate School of Earth and Environmental Sciences, Hokkaido University, Japan under the Global COE Program (Establishment of Center for Integrated Field Environmental Science), MEXT, Japan.
Microbial Ecology of Port Klang Waters

References