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Microbial Abundance and Nutrient Concentration in Riverine and Coastal Waters of North-East Langkawi

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ABSTRACT We measured both biotic and abiotic variables at 19 stations along the three river systems and also off the Langgun Island in the North-East (NE) Langkawi region. Temperature ranged between 29.8–31.3°C whereas salinity ranged between 22–32. pH was between 7.4 and 8.2 whereas redox potential ranged between –82 to –32. Dissolved oxygen (DO) concentration ranged between 120–280 µM. Both pH and DO concentration were lower upstream of the rivers. For inorganic nutrient concentrations; ammonium (NH_4) ranged between 0.56–4.30 µM, nitrate (NO_3) between 0.31–1.46 µM, nitrite (NO_2) between 0.04–0.38 µM and silicate (SiO_4) between 1.36–15.23 µM. Of the nitrogen species, NH_4 was dominant, making up to 79% of total dissolved inorganic nitrogen (mean=57%). Total suspended solids (TSS) in the water samples were high, ranging between 270 to 330 mg L^{-1} whereas particulate organic matter (POM) constituted a very small component of TSS (<5%, 3.6–14.4 mg L^{-1}). For biotic variables, chlorophyll *a* (Chl *a*) concentration that represented the photoautotrophs present in the water, with the range of 1.49–8.24 µg L^{-1}. Bacteria ranged between 1.5–5.3×10^6 cells mL^{-1} whereas phototrophic picoplankton and protists ranged between 1.7–4.3×10^5 cells mL^{-1} and 0.8–1.6×10^4 cells mL^{-1}, respectively. Marine bacteria cultured ranged from 4×10^2 to 1.2×10^5 cfu mL^{-1}. Although our limited sampling was inadequate to detect controlling factors for bacterial abundance, our study showed that bacteria could have caused the lower DO concentration in the rivers in NE Langkawi.

ABSTRAK Kami mengukur kepekatan parameter biotik dan abiotik 19 stesyen sepanjang tiga sistem sungai dan Pulau Langgun di sekitar timur-laut (NE) Langkawi. Suhu air adalah antara 29.8 – 31.3°C manakala saliniti antara 22 – 32. pH adalah antara 7.4 dan 8.2 manakala keupayaan redoks adalah antara –82 hingga –32. Kepekatan oksigen terlarut (DO) pula antara 120–280 µM. Kedua-dua pH dan kepekatan DO adalah lebih rendah di bahagian hulu sungai. Bagi kepekatan nutrien inorganik; ammonia (NH_4) adalah antara 0.56–4.30 µM, nitrat (NO_3) antara 0.31–1.46 µM, nitrit (NO_2) antara 0.04–0.38 µM manakala silikat (SiO_4) antara 1.36–15.23 µM. Daripada spesis nitrogen, NH_4 adalah paling dominan, membentuk sehingga 79% jumlah nitrogen inorganik terlarut (purata=57%). Keputusan kami menunjukkan jumlah pepejal tak mendap (TSS) adalah tinggi, antara 270–330 mg L^{-1} manakala bahan organik partikulat (POM) merupakan komponen kecil TSS (<5%, 3.6–14.4 mg L^{-1}). Bagi pembolehubah biotik, kepekatan klorofil *a* (Chl *a*) adalah antara 1.49–8.24 µg L^{-1}. Bakteria adalah antara 1.5–5.3×10^6 sel mL^{-1} manakala pikoplankton fototrofik dan protis masing-masing adalah diantara 1.7–4.3×10^5 sel mL^{-1} dan 0.8–1.6×10^4 sel mL^{-1}. Bakteria marin yang dikultur antara 4×10^2 hingga 1.2×10^5 cfu mL^{-1}. Walaupun bilangan penyampelan kami terhad, dan tidak mencukupi untuk mengesan faktor pengawal kelimpahan bakteria, kajian kami menunjukkan bahawa bakteria mungkin telah menyebabkan kepekatan DO yang rendah dalam air sungai di NE Langkawi.

(bacteria, picoplankton, inorganic nutrients, chlorophyll *a*)

INTRODUCTION

It has been two decades since the term 'microbial loop' was introduced to describe the importance of the microbial food web on the recycling and mineralization of organic matter in aquatic habitats [1]. Evidences suggest that the microbial food web is a key component in both coastal and estuarine ecosystems [2]. However, research on the microbial food web in Malaysian waters has been limited to culturable-specific bacteria and its participation in nitrogen cycle [3, 4]. This study

is part of a research initiative to study both the environmental conditions and biodiversity in the North-East (NE) Langkawi area. This endeavor was to provide scientific data for a proposal to conserve this area, and establish it as a National Park.

Langkawi is a group of islands located North-West of peninsular or West Malaysia. The island of Langkawi is the largest island, and is a popular resort town for both locals and foreigners. The NE Langkawi is a popular spot for ecotourism e.g. mangrove forest exploration, boat rides, eagle feeding etc. There are three main river systems here; Sg Kisap, Sg Kilim and Sg Ayer Hangat. These rivers have many tributaries, and mangrove forests lined both sides of these rivers.

In this study, we presented new data on the microbial abundance of tropical mangrove waters for the NE Langkawi region. We measured both biotic and abiotic variables in 19 stations, and observed significant differences between some stations.

MATERIALS AND METHODS

A total of 19 sampling stations were chosen, and sampled throughout a four-day period (5 April until 8 April, 2004). The location and time of sampling are shown in Table 1. Stations (Stations) 1–5 were selected along Sg Kisap whereas Stations 6–11 and Stations 12–15 were from Sg Kilim and Sg Ayer Hangat, respectively (Figure 1). Four stations (Stations 16–19) were coastal water sites located off the Langgun and Tg Dendang islands.

Table 1. Location of sampling stations and their sampling dates.

Station number	Location		Date	Time
1	6'23'35N	99'54'12E	5 April 2004	11:56:51
2	6'23'16N	99'53'46E	5 April 2004	12:28:15
3	6'23'06N	99'53'10E	5 April 2004	12:33:34
4	6'22'42N	99'52'24E	5 April 2004	13:00:08
5	6'23'10N	99'52'12E	5 April 2004	14:07:52
6	6'23'20N	99'51'58E	5 April 2004	14:35:30
7	6'24'22N	99'51'50E	6 April 2004	9:29:35
8	6'24'50N	99'51'55E	6 April 2004	9:47:14
9	6'25'30N	99'52'05E	6 April 2004	10:07:35
10	6'25'27N	99'51'33E	6 April 2004	13:27:42
11	6'25'00N	99'52'34E	6 April 2004	13:47:43
12	6'27'41N	99'49'42E	7 April 2004	9:45:36
13	6'26'42N	99'49'53E	7 April 2004	10:13:22
14	6'26'35N	99'49'03E	7 April 2004	10:35:30
15	6'25'45N	99'49'56E	7 April 2004	11:02:57
16	6'25'31N	99'53'10E	8 April 2004	9:09
17	6'25'00N	99'53'58E	8 April 2004	9:30
18	6'24'57N	99'55'00E	8 April 2004	10:15
19	6'24'57N	99'55'00E	8 April 2004	10:37

In-situ parameters measured in this study included salinity (Atago S/Mill-E, Japan), temperature, pH and redox potential (Eh) (Jenway 3071, UK). DO concentration was also measured with a DO electrode (Jenway 970, UK). Water sample was taken at about one meter depth with a Van Dorn water sampler. Subsample for the determination of bacteria, phototrophic picoplankton and protists abundance was preserved with filtered (Millipore filter, 0.2 µm pore size) glutaraldehyde (final concentration of 1%). The rest of the sample was kept in a cooler box prior to processing.

Seawater sample was filtered through pre-combusted (450°C for 3 hrs) Whatman GF/F filter. The filtrate was preserved with suitable preservatives (either $HgCl_2$ or HCl), and stored at 4°C prior to analysis. Dissolved inorganic nutrients e.g. ammonium (NH_4), nitrite (NO_2), nitrate (NO_3) and silicate (SiO_4) were measured [5]. The method used for sample preservation was tested with a control sample from Port Dickson, and results showed that the preservation method used had no significant effect on nutrient concentration when compared with unpreserved sample (data not shown).

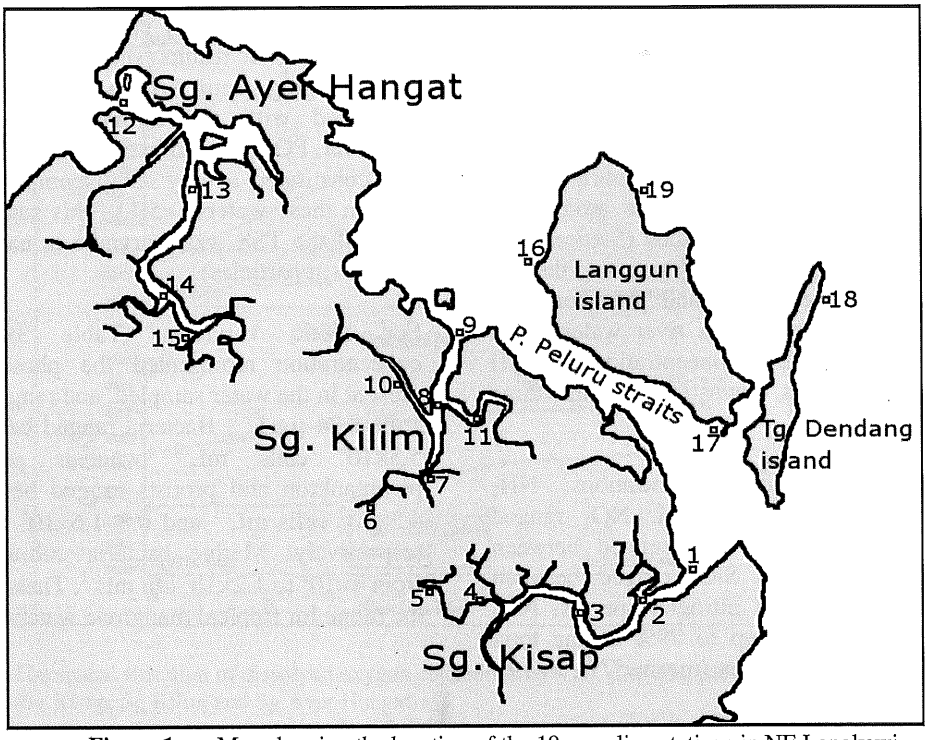

Figure 1. Map showing the location of the 19 sampling stations in NE Langkawi

The GF/F filter was stored at –20°C until extraction for chlorophyll *a* (Chl *a*). Chl *a* was extracted with 90% acetone, and measured with a spectrophotometer (Beckmann DU7500i, US) [5]. Filters for total suspended solids (TSS) and particulate organic matter (POM) were also kept. For TSS, filters were dried (70°C for 1 d), and any weight increase detected was the amount of TSS in the seawater. The same filter was then combusted to remove any organic matter. The weight difference before and after combustion gave us the POM of the water sample.

For the determination of bacterial abundance, sample was stained with 4'6-diamidino-2-phenylindole (DAPI) (0.1 µg L^{-1} final concentration) for 7 min [6]. Prepared filter was examined under an epifluorescence microscope (Olympus BX60, Japan), and >300 cells were counted for each slide. Unstained sample was also prepared, and examined for autofluorescence from the phototrophic picoplankton (both prokaryotic and eukaryotic types). A minimum of 15 fields were counted. 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 [7]. Marine bacteria was also cultured in this study using the ZoBell 2216E medium [8], and colony forming units (CFUs) were calculated according to standard methods [9].

RESULTS AND DISCUSSION

Table 2 shows the values of abiotic factors measured. Temperature ranged between 29.8–31.3°C whereas salinity ranged 22–32. The high salinity was typical of river systems under tidal influence. Saltwater intrusion was predominant, and seawater moved up in all the three river

systems. pH was between 7.4 and 8.2 whereas redox potential that correlated negatively with pH (R=–0.861, n=19, p<0.001) ranged between –82 to –32. DO concentration ranged between 120–280 µM. These variables fluctuated throughout each river system except for pH and DO which were higher downstream (Figure 2). The DO concentration appears to regulate pH as reflected in the correlation between DO and pH (R=0.523, n=19, p<0.05). A similar pattern has been observed in Sg Sangga, Perak, and is attributed to the rapid oxygen consumption which resulted in lower DO concentration and pH [10]. However, the dilution caused by seawater could also bring about the increase in both DO concentration and pH downstream. Sampling stations around the Langgun and Tg Dendang islands (Stations 16–19) were the furthest from the rivers, and the DO concentrations were higher, and pH about 8.0. The mixing of seawater and river water could have increased the DO concentration and pH values in the stations downstream of the three river systems.

For inorganic nutrient concentrations; NH_4 ranged between 0.56–4.30 µM, NO_3 ranged between 0.31–1.46 µM, NO_2 ranged between 0.04–0.38 µM whereas SiO_4 ranged between 1.36–15.23 µM. Of the nitrogen species, NH_4 was dominant, making up to 79% of the total dissolved inorganic nitrogen (mean=57%, n=19). This is typical as tropical mangrove waters are usually dominated by NH_4 [11]. In this study, NH_4, NO_2 and NO_3 were within the range previously reported for tropical mangrove waters [11, 12] whereas SiO_4 was similar to tropical mangrove waters with high salinity [12].

Other abiotic factors measured included TSS and POM. TSS affects the water turbidity, and can result in lower primary productivity due to poor irradiance. Our results showed that TSS was high, ranging from 270 to 330 mg L^{-1}. This concurred with the turbid waters observed. However, POM ranged between 3.6–14.4 mg L^{-1}, and constituted a very small component of the TSS in these waters (<5%). This suggested that most of the TSS was inorganic in nature (either clay or silt particles).

For biotic variables (Table 3), Chl *a* concentration represented the photoautotrophs present in the water sampled, and ranged between 1.49–8.24 µg L^{-1}. Bacteria ranged between 1.5–5.3×10^6 cells mL^{-1} whereas phototrophic picoplankton and protists ranged between 1.7–4.3×10^5 cells mL^{-1} and 0.8–1.6×10^4 cells mL^{-1}, respectively. Marine bacteria cultured ranged from 4×10^2 to 1.2×10^5 cfu mL^{-1}. These are within the range for tropical mangrove waters [10, 13].

Table 2. The values of abiotic factors for waters around NE Langkawi.

Station	Temperature (°C)	Salinity	pH	Eh (mV)	DO (µM)	NH_4 (µM)	NO_3 (µM)	NO_2 (µM)	SiO_4 (µM)	TSS (mg L^{-1})	POM (mg L^{-1})
1	31.3	25	8.2	-75	190	4.30	0.91	0.22	6.36	300	8.4
2	30.9	28	8.2	-65	180	1.96	1.11	0.04	5.23	330	12.0
3	31.1	28	8.2	-71	180	0.84	0.31	0.13	2.04	–	–
4	30.8	30	8.0	-68	160	0.73	0.71	0.20	5.23	320	10.2
5	30.8	30	7.6	-40	120	0.56	1.35	0.16	15.23	–	–
6	30.7	25	7.4	-32	120	1.17	0.88	0.18	9.54	310	11.6
7	29.8	30	7.4	-34	190	0.78	1.46	0.18	7.96	280	5.4
8	30.3	30	7.8	-50	230	–	–	–	–	–	–
9	30.6	30	8.1	-78	240	1.01	0.60	0.18	8.64	280	5.4
10	30.7	25	8.7	-78	250	1.01	0.84	0.13	3.86	340	13.2
11	30.8	27	8.1	-81	260	0.95	0.62	0.33	3.64	330	12.2
12	30.7	30	7.9	-66	220	0.84	0.71	0.22	6.59	290	4.2
13	30.7	22	7.8	-62	220	0.84	0.69	0.11	4.54	320	12.8
14	30.6	29	7.6	-53	210	1.96	0.69	0.11	5.91	290	7.0
15	30.6	26	7.6	-52	190	2.29	0.80	0.26	9.09	280	6.2
16	30.7	30	8.0	-67	260	2.29	0.58	0.29	11.59	330	14.4
17	30.9	32	8.1	-78	260	2.46	0.62	0.33	1.36	280	4.4
18	31.3	30	8.1	-82	280	1.06	0.58	0.38	7.50	280	3.6
19	31.2	29	8.0	-78	280	2.24	0.53	0.22	5.00	270	5.6

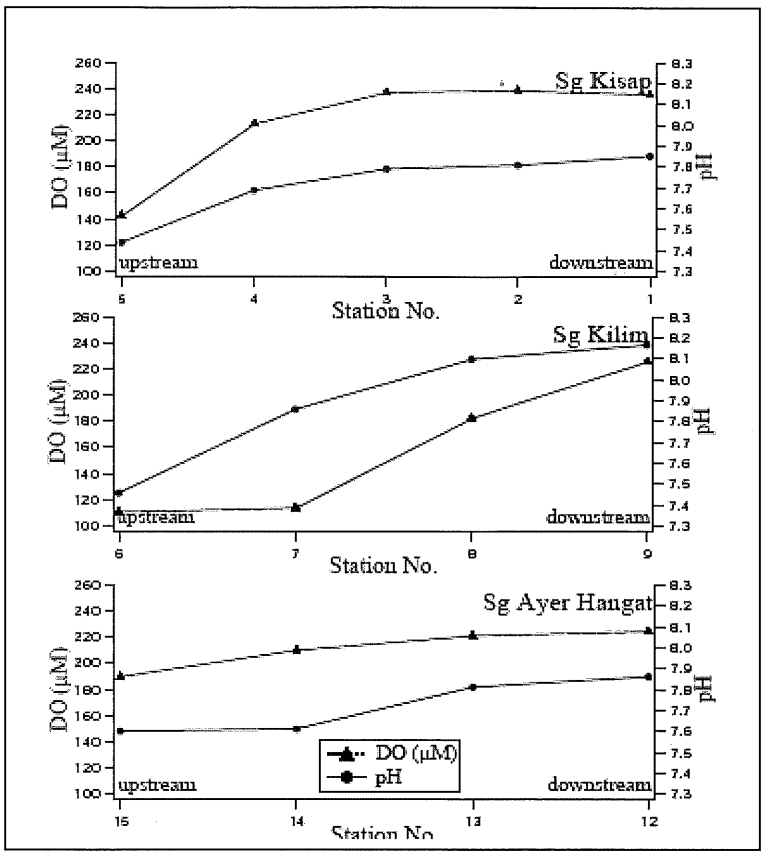

Figure 2. The spatial variation of dissolved oxygen concentration (DO, μM) and pH along the three river systems (Sg Kisap, Sg Kilim and Sg Ayer Hangat)

Table 3. The values of biotic factors for waters around NE Langkawi

Stations	Chl *a* (μg L^{-1})	Bacteria (cells mL^{-1})	Culturable marine bacteria (CFU mL^{-1})	Phototrophic picoplankton (cells mL^{-1})	Protist (cells mL^{-1})
1	4.22	2.1×10^{6}	1.2×10^{5} est	4.1×10^{5}	0.9×10^{4}
2	8.24	2.3×10^{6}	2.0×10^{2} est	3.5×10^{5}	1.0×10^{4}
3	5.36	2.5×10^{6}	5.0×10^{3} est	3.0×10^{5}	1.2×10^{4}
4	0.40	2.6×10^{6}	4.6×10^{3}	3.1×10^{5}	1.1×10^{4}
5	5.72	1.5×10^{6}	9.2×10^{4}	3.2×10^{5}	1.2×10^{4}
6	5.71	2.3×10^{6}	1.2×10^{4}	3.3×10^{5}	0.8×10^{4}
7	5.04	2.6×10^{6}	2.4×10^{5} est	3.4×10^{5}	1.6×10^{4}
8	3.20	5.3×10^{6}	–	3.6×10^{5}	0.9×10^{4}
9	5.91	5.3×10^{6}	8.0×10^{2} est	3.7×10^{5}	1.2×10^{4}
10	6.26	4.1×10^{6}	4.0×10^{2} est	3.6×10^{5}	1.0×10^{4}
11	6.04	4.6×10^{6}	–	4.3×10^{5}	1.2×10^{4}
12	4.26	3.3×10^{6}	6.6×10^{3}	2.9×10^{5}	0.8×10^{4}
13	3.47	3.7×10^{6}	1.2×10^{3}	2.7×10^{5}	1.0×10^{4}
14	4.58	4.5×10^{6}	1.4×10^{3}	2.5×10^{5}	1.2×10^{4}
15	4.90	4.2×10^{6}	3.5×10^{3}	2.1×10^{5}	1.0×10^{4}
16	1.49	2.5×10^{6}	8.1×10^{2}	1.9×10^{5}	1.1×10^{4}
17	3.86	1.5×10^{6}	7.2×10^{2}	1.7×10^{5}	1.1×10^{4}
18	6.43	1.6×10^{6}	4.7×10^{2}	1.9×10^{5}	1.0×10^{4}
19	6.32	1.5×10^{6}	1.5×10^{3}	1.8×10^{5}	1.2×10^{4}

CFU – colony forming unit
est – estimated, according to [9]

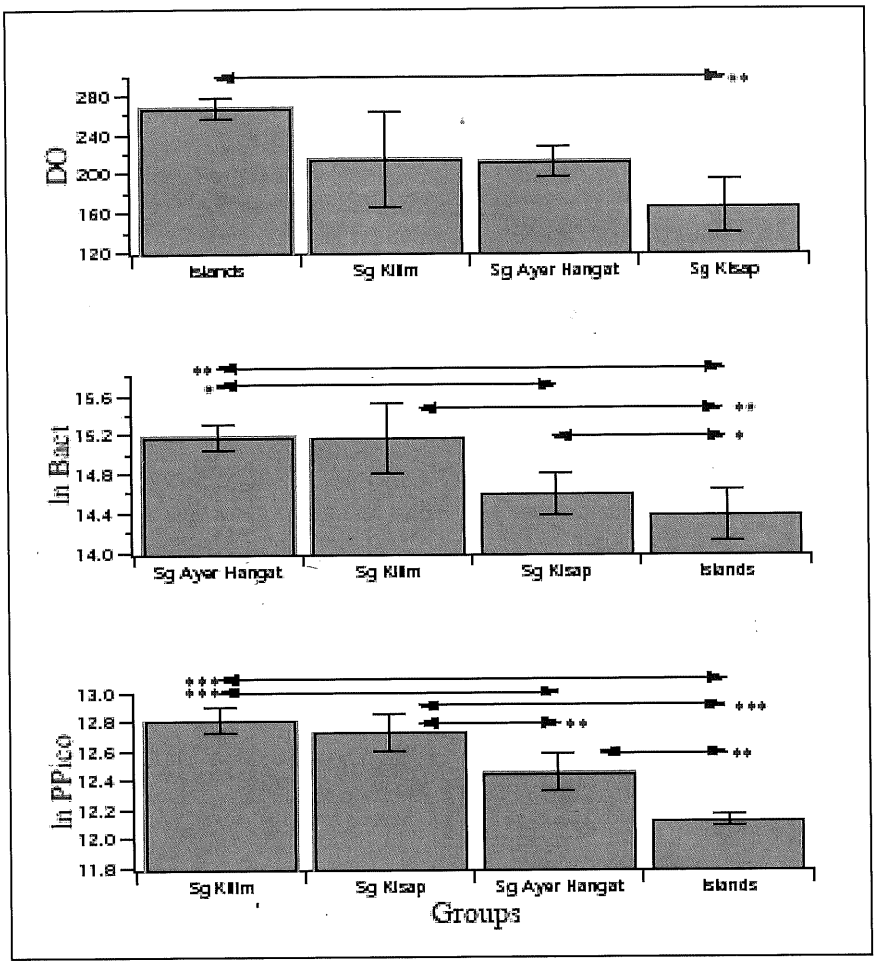

Figure 3. Column chart showing the differences in dissolved oxygen concentration (DO, μM), ln bacterial abundance (ln Bact, ln cells mL^{-1}) and ln phototrophic picoplankton (ln PPico, ln cells mL^{-1}). Error bar shows the standard deviation of the mean values from each group of sampling stations. Significant differences between groups are shown by horizontal lines with arrows. Level of significance is shown by asterisks; *–p<0.05, **–p<0.01, ***–p<0.001

Although microbial abundance is affected by both top-down and bottom-up controls [2], we were not able to detect any evidence of these controls. This could be due to our simple sampling design that did not cover over temporal scales. We then proceeded to analyze whether there was any difference in both abiotic and biotic variables between these sampling stations.

For our data analysis, we separated all the sampling stations into four groups; Sg Kisap contained Stations 1–5, Sg Kilim contained Stations 6–11, Sg Ayer Hangat contained Stations 12–15 whereas the islands contained Stations 16–19. Each group represented one river system whereas the islands represented the coastal water stations off Langgun and Tg Dendang islands. We carried out analysis of variance (ANOVA) to determine whether there is any difference in both abiotic and biotic factors between the four groups. Of all the abiotic and biotic factors, only DO concentration (F=7.0, df=18, p<0.01), bacterial abundance (F=10.5, df=18, p<0.001) and phototrophic picoplankton abundance (F=40.3, df=18, p<0.001) showed highly significant differences between the four groups. We then carried out the Tukey test [14] to compare between these four groups (Figure 3).

Results showed that DO concentration at the islands was significantly higher than at Sg Kisap whereas both bacterial and phototrophic picoplankton abundance were lowest at the islands. Bacteria are major consumers of oxygen,

and the higher abundance in the rivers could explain their lower DO concentration. Although not measured, organic matter concentration is known to affect bacterial abundance [15]. The higher bacterial abundance in the three river systems could indicate a higher level of organic content. The source of this organic matter could be due to anthropogenic activities around these sites.

Our study is the first report on the microbial abundance of NE Langkawi waters. Both nutrient concentration and microbial abundance were within the range of tropical mangrove waters previously reported. Due to the limited sampling, we were not able to determine the controlling factors that affected microbial abundance. However, we observed significant differences between the islands and the river systems, where the higher bacterial abundance in these three river systems could have reduced the DO concentration.

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REFERENCES

1. Azam, F., Fenchel, T., Field, J.E., Gray, J. S., Meyer-Reil, L.A. and Thingstad, F. (1983). The ecological role of water column microbes in the sea. *Mar. Ecol. Prog. Ser.* **157**: 1–12.
2. Sherr, E. and Sherr, B. (2000). Marine microbes: An overview. In: *Microbial Ecology of the Oceans* (ed. Kirchman, D.L.) Wiley-Liss, New York, pp. 13–46.
3. Shaiful, A.A.A., Abdul Manan, D.M., Ramli, M.R. and Veerasamy, R. (1986). Ammonification and nitrification in wet mangrove soils. *Mal. J. Sci.* **8**: 47–56.
4. Thong, K.L., Sasekumar, A. and Marshall, N. (1993). Nitrogen concentrations in a mangrove creek with a large tidal range, Peninsular Malaysia. *Hydrobiologia* **254**: 125–132.
5. Parsons, T.R., Maita, Y. and Lalli, C.M. (1984). *A Manual of Chemical and Biological Methods for Seawater Analysis.* Pergamon Press, Oxford.
6. Kepner, R.L. Jr and Pratt, J.R. (1994). Use of fluorochromes for direct enumeration of total bacteria in environmental samples: past and present. *Microbiol. Rev.* **58**: 603–615.
7. Bloem, J., Bär-Gilissen, M.J.B. and Cappenberg, T.E. (1986). Fixation, counting, and manipulation of heterotrophic nanoflagellates. *Appl. Environ. Microbiol.* **52**: 1266–1272.
8. Morita, R.Y. and ZoBell, C.E. (1955). Occurrence of bacteria in pelagic sediments collected during the Mid-Pacific expedition. *Deep-Sea Res.* **3**: 66–73.
9. Swanson, K.M.J., Busta, F.F., Peterson, E. H. and Johnson, M.G. (1992). Colony count methods. In: *Compendium of methods for the microbiological examination of foods, 3rd ed.* (eds. Vanderzant, C. and Splittstoesser, D.F.) APHA, Washington, pp. 75–95.
10. Alongi, D.M., Chong, V.C., Dixon, P., Sasekumar, A. and Tirendi, F. (2003). The influence of fish cage aquaculture on pelagic carbon flow and water chemistry in tidally dominated mangrove estuaries of peninsular Malaysia. *Mar. Environ. Res.* **55**: 313–333.
11. Alongi, D.M., Boto, K.G. and Robertson, A. I. (1992). Nitrogen and phosphorus cycles. In: *Tropical mangrove ecosystems* (eds. Robertson, A.I. and Alongi, D.M.) American Geophysical Union, Washington, D. C., pp. 251–292.
12. Tanaka, K. and Choo, P.S. (2000). Influences of nutrient outwelling from the mangrove swamp on the distribution of phytoplankton in the Matang mangrove estuary, Malaysia. *J. Oceanogr.* **56**: 69–78.
13. Lee, C.W. (2003). The effects of thermal effluent on marine diatoms and bacteria. *Mal. J. Sci.* **22**: 23–27.
14. Zar, J.H. (1999). *Biostatistical analysis, 4th ed.* Prentice Hall, Upper Saddle River, N.J.
15. Cole, J.J., Findlay, S. and Pace, M.L. (1986). Bacterial production in fresh and saltwater ecosystems: a cross-system overview. *Mar. Ecol. Prog. Ser.* **43**: 1–10.