EFFECT OF VARYING pCO_2 ON BACTERIAL ACTIVITIES IN TROPICAL COASTAL AND ESTUARINE WATERS

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2019

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DISSERTATION SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

INSTITUTE OF BIOLOGICAL SCIENCES
FACULTY OF SCIENCE
UNIVERSITY OF MALAYA
KUALA LUMPUR

2019

UNIVERSITY OF MALAYA

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Name of Degree: **MASTERS OF SCIENCE**

Title of Project Paper/Research Report/Dissertation/Thesis ("this Work"):

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Field of Study:

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EFFECT OF VARYING pCO_2 ON BACTERIAL ACTIVITIES IN TROPICAL COASTAL AND ESTUARINE WATERS

ABSTRACT

The rise of pCO_2 levels in oceanic surface waters may have a potential effect on marine bacteria although experimental results related to the effects of pCO_2 on marine microbes are rather inconsistent and at times conflicting. In the present study, we investigated (i) the temporal variation of pCO_2 and (ii) the effect of elevated pCO_2 in seawater on bacterial production (BP), bacterial respiration (BR) and bacterial growth efficiency (BGE) at two different locations which are Port Klang and Port Dickson. Estimated pCO_2 in the estuary and coastal water ranged from 345 to 5365 µatm and 421 to 1283 µatm respectively. A total of six sets of experiment were conducted for each location with different concentrations of pCO_2 ranging from 305 to 10255 µatm. Estimates of bacterial production and bacterial respiration obtained ranged between 0.06 to 1.21 µM C h^{-1} and 0.18 to 10.05 µM O_2 h^{-1}, respectively. Bacterial growth efficiency was calculated as bacterial production/ (bacterial production + respiration) and ranged from 0.09 to 0.70. pCO_2 and bacterial growth efficiency were significantly correlated ($r^2 = 0.25$, $P < 0.001$, $n = 60$). The result from this experiment suggested that an increase in atmospheric CO_2 might affect BGE, and may have implications towards understanding the ocean carbon flux.

Keywords: Ocean acidification, pCO_2, bacterial growth efficiency, tropical

KESAN PERUBAHAN pCO_2 KE ATAS AKTIVITI BAKTERIA DI PANTAI DAN MUARA SUNGAI IKLIM TROPIKA

ABSTRAK

Peningkatan tahap pCO_2 di kawasan permukaan lautan mungkin mempunyai potensi untuk memberi kesan kepada bakteria marin, walaupun keputusan eksperimen yang berkaitan dengan kesan pCO_2 pada mikrob marin agak tidak konsisten dan kadang-kadang bertentangan atara satu sama lain. Dalam kajian ini, kami menyiasat (i) variasi jangka masa panjang pCO_2 dan (ii) kesan peningkatan pCO_2 dalam air laut mengenai penhasilan bakteria (BP), respirasi bakteria (BR) dan kecekapan pertumbuhan bakteria (BGE) di dua lokasi berbeza iaitu Port Klang dan Port Dickson. Anggarkan pCO_2 di muara air dan air pantai berjulat antara 345 hingga 5365 µatm dan 421 hingga 1283 µtam masing-masing. Sebanyak enam set eksperimen telah dijalankan bagi setiap lokasi dengan jumlah pCO_2 yang berbeza antara 305 hingga 10255 µatm. Anggaran penghasilan bakteria dan respirasi bakteria yang diperoleh antara 0.06 hingga 1.21 µM C h^{-1} dan 0.18 hingga 10.05 µM O_2h^{-1}, masing-masing. Kecekapan pertumbuhan bakteria dikira sebagai penghasilan bakteria / (penghasilan bakteria + respirasi bakateria), dan berjulat antara 0.09 hingga 0.70. pCO_2 dan kecekapan pertumbuhan bakteria berkorelasi secara positif (r^2 = 0.25, P < 0.001, n = 60). Hasil daripada eksperimen ini mendapati peningkatan CO^2 atmosfera mungkin akan memberi kesan kepada BGE, dan mungkin mempunyai implikasi ke arah pemahaman fluks karbon laut.

Keywords: Pengasidan laut, pCO_2, BGE, tropika

ACKNOWLEDGEMENTS

Glory and praise be to Allah (SWT), The Omnipotent, Omniscient and Omnipresent, for providing me with the strength and perseverance to complete this thesis despite several obstacles encountered throughout this research.

I would like to express my sincere and wholehearted gratitude to my supervisors, Prof Madya Dr Lee Choon Weng for his witty remarks inspired my Master Yoda from Star Wars when I said I could not do it anymore and Dr Bong Chui Wei for my first life lesson with her when I broke an expensive glass beaker. Their paramount advice, continuous support, expertise, and patience that have taught me not just about lab works and science but also life lessons that I will continue to hold on to for the rest of my life.

Thanks, are extended to my fellow lab mates who accompanied me to the sampling sites to sleepovers in the lab for late night experiments. I would like to convey my gratitude to a significant number of people for pushing me to complete this research; Shoba, Ben, Nasha, Van, Kak Syud and others that I could not mention here. Last, but not least, to Ibu and Dadi, this is for you.

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LIST OF SYMBOLS AND ABBREVIATIONS

m	:	Meter
mm	:	Millimeter
µm	:	Micrometer
nm	:	Nanometer
°N	:	Degree North
°S	:	Degree South
°E	:	Degree East
°C	:	Degree Celsius
%	:	Percentage
>	:	More than
<	:	Less than
E	:	Energy
µM	:	Micromolar
L	:	Liter
ml	:	Milliliter
µl	:	Microliter
p	:	Partial
rpm	:	Rotation per minutes
N	:	Normality
C	:	Carbon
µmol	:	Micromole
ppt	:	Parts per thousand
µEq	:	micro Equivalents

µatm	:	micro atmosphere
g	:	Gram
mg	:	Milligram
µg	:	Microgram
pg	:	Picogram
fg	:	Femtogram
y	:	Year
d	:	Day
h	:	Hour
s	:	Second
CV	:	Coefficient of Variant
S.D.	:	Standard Deviation

LIST OF APPENDICES

CHAPTER 1: INTRODUCTION

"Global warming is not a prediction. It is happening"—James Hansen

1.1 Climate change: is it real?

In these recent years, climate change or recently known as climate crisis has been one of the most highly debated subjects amongst scientists and politicians around the world. Climate change, also known as global warming is the periodic variation of Earth's climate due to changes in the atmosphere and interactions between the atmosphere and other biological, chemical, geological, and geographic factors within the Earth system (Jackson, 2018).

Although natural forces play essential roles in changes in the climate; most of the scientists agree that human activities such as industrialisation, urbanisation, burning coal and gas for energy, burning of forest for agriculture and other human-induced perturbations are the primary drivers accelerating changes in the climate around the world (Anderegg *et al*., 2010; Cook *et al*., 2016).

These activities have resulted in the increase of greenhouse gases (**Figure 1.1**) mainly methane and carbon dioxide (CO_2), contributing to global climate change (Pachauri ***et al.***, 2008; IPCC 2014). Greenhouse gases emissions are primarily determined by economic activity, population size, technology and climate policy (IPCC, 2014). Developed countries such as the United States of America and China are the main contributors to the greenhouse gases emission. In 2018, the atmospheric concentrations of CO_2 reported has surpassed 400 ppm and by far has exceeded the natural range of CO_2, more than they were over 800 000 years ago (IPCC, 2014). This rapid phenomenon—at least ten times faster—is expected to continue and rise rapidly (Rhein ***et al.,*** 2014; IPCC, 2014).

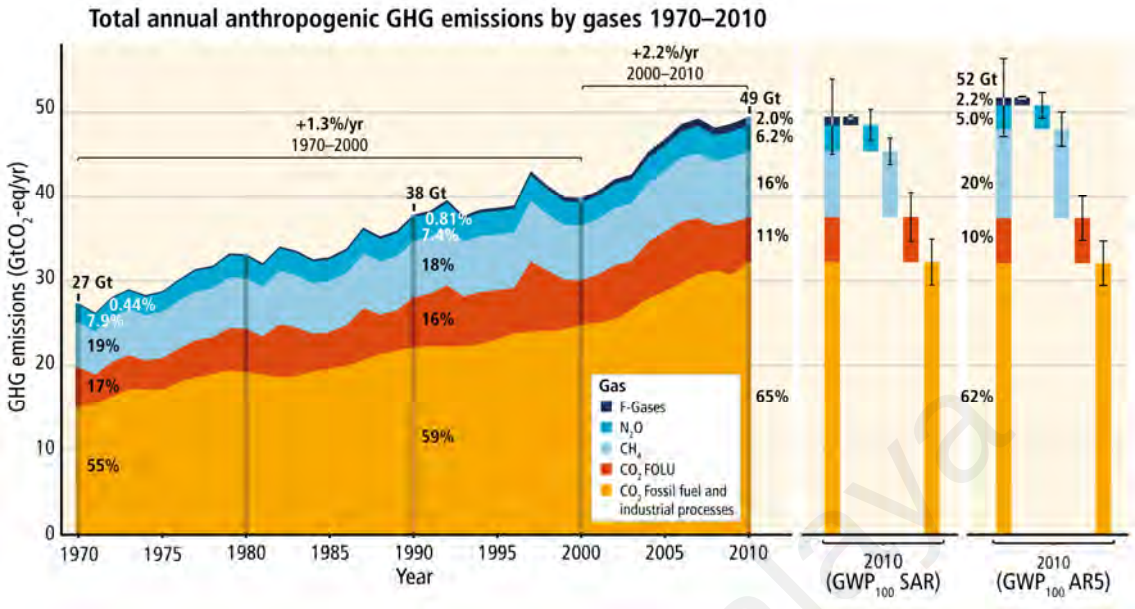


Figure 1.1: Total annual anthropogenic greenhouse gasses (GHG) emissions by gasses from 1970 to 2010. It is adapted from IPCC (2014) report.

The greenhouse gases trap heat from going out of the lithosphere, thus, increasing the temperature of the earth. **Figure 1.2** shows the annual temperature anomaly of the global average near-surface air temperature over land and the surface sea temperature (SST) from 1981 to 2017 based on data from the Japan Meteorological Agency (JMA). The global average surface temperatures have ascended at a rate of about 0.73°C per century. In 2016, the warmest temperature was recorded in this century with an increase of 0.45°C above the average. If the emission of the greenhouse gas continues, the Intergovernmental Panel on Climate Change (IPCC) predicted that the earth temperature will tremendously increase by 4 °C warmer than the beginning of the industrial revolution and lead to other lines of climate change evidence including warming of the ocean (Levitus *et al.,* 2009), decreasing of the Arctic ice coverage (Kwok and Rothrock, 2009; Polyak *et al.,* 2009), and rising of the sea level (IPCC, 2014).

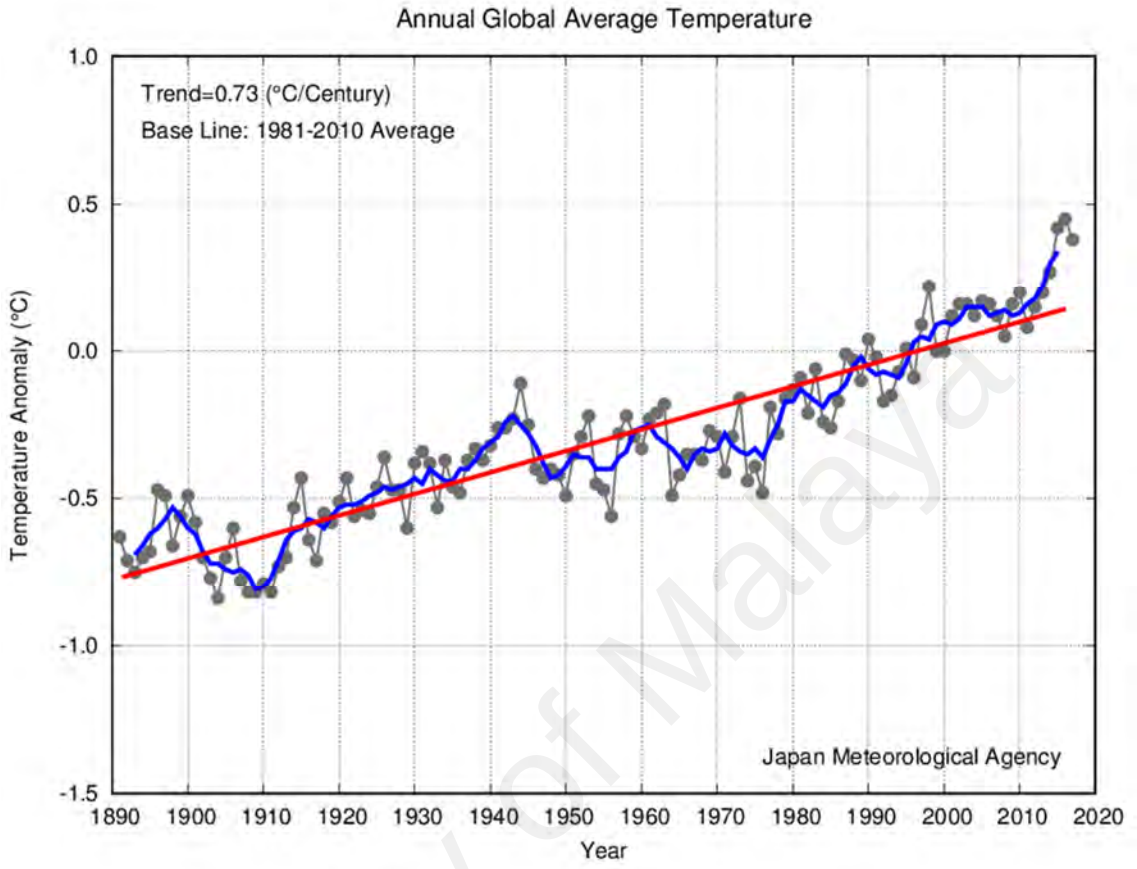


Figure 1.2: Annual global average temperature from 1891 to 2017 from the Japan Meteorological Agency. The thin black line - surface temperature anomaly of each year; blue line - 5 year running mean; red line - the long-term linear trend. Retrieved on 24 July, 2018 from: http://ds.data.jma.go.jp/tcc/tcc/products/gwp/temp/ann_wld.html

Current climate variability has shown an increased risk of extreme events (Kunkel *et al.*,2012; 2013) such as cyclones, drought, flood, heat waves, wildfires which poses threats to human and the ecosystem. The Climate Change 2014 Synthesis Report by IPCC has reported the alarming impacts of climate change in the near future. By the year 2100, the seawater level is predicted to rise up to 1 meter. Besides that, extreme weather with events such as intense hurricanes may become more prevalent. With the combination of higher temperature and less rain (expected to drop by 30%), the mid-altitude region is predicted to face extreme drought. These changes will lead to a problem in global food security where worldwide crop yield is expected to decrease by over 30% by the year 2050. In the past, all mass extinctions happened due to extreme changes in the climate (Twitchett, 2006). At this rate, many organisms may be unable to adapt quickly to the rapid changes in temperature; therefore, poses a threat to biodiversity. Up to almost 40% of the world, ecosystem is expected to be affected within this century. In the near future, we are also expected to observe an increase in respiratory-related health issues (IPCC, 2014).

1.2 Ocean acidification: changes in the ocean carbonate system

Most of the CO_2 released remains in the atmosphere; however, part of it dissolves in the oceans. The ocean is known to be one of the largest CO_2 reservoirs, and takes up almost 30% of anthropogenic CO_2 released into the air (Sabine *et al*., 2004; Feely *et al*., 2009), resulting in an unprecedented shift in seawater carbonate chemistry and a decline in oceanic pH known as ocean acidification.

For instance, when anthropogenic CO_2 increases in the atmosphere, this creates air flux between the atmosphere and the ocean. CO_2 dissolves in the surface ocean, and the aqueous carbon dioxide (CO_2 (aq)) will react with water to form carbonic acid (H_2CO_3)

$$[CO_2] + [H_2O] \rightleftharpoons [H_2CO_3] \quad (1.1)$$

Carbonic acid immediately dissociates releasing hydrogen ions (H^+) and bicarbonate ions (HCO_3^-):

$$[H_2CO_3] \rightleftharpoons [H^+] + [HCO_3^-] \quad (1.2)$$

The additional hydrogen ions react with carbonate ions (CO_3^{2}) to form bicarbonate ions:

$$[H^+] + [CO_3^{2-}] \rightleftharpoons [HCO_3^-] \quad (1.3)$$

Hence, the result of dissolving CO_2 in seawater is an increase in the concentrations of aqueous carbon dioxide, carbonic acid, bicarbonate ions, hydrogen ions and a decrease in carbonate ions. The increase in H^+ results in a decrease in pH. This, in turn, has influenced changes in the oceanic carbonate system. For a long-term climate change and the ocean carbon uptake indicator (Tjiputra *et al.,* 2014) the term surface ocean partial pressure of carbon dioxide (pCO_2) is used for the overall representation of the seawater carbonate

chemistry taking into account other parameters such as pH, temperature, salinity and alkalinity.

The planetary boundary approach (**Figure 1.3**) proposed by Steffan *et al.* in 2015 suggested the current state of ocean acidification from human perturbation activities is still within the safe boundary as long as the climate change boundary of 350 ppm CO_2 remains unchanged and not exceeded. However, in recent years, CO_2 concentrations have reached ~400 ppm (**Figure 1.4**). If this worrying trend continues, the safe boundary may be transgressed, and ocean acidification could pose a threat to the marine ecosystem. It is worth to note that the trend of increase in atmospheric CO_2 by far has shown to never exceed 500 ppm (pH > 8.0) in the past twenty-four million years. Currently, the pH of the ocean has decreased by about 0.1 unit (Blackford & Gilbert 2007; Caldeira & Wickett 2003), corresponding to a 26% increase in acidity (IPCC, 2014). If the trends of carbon emissions continue to rise, the pH of the ocean may drop by ca. 0.35 units (1000 µatm) by the year 2100, resulting in 150% more acidic than they were at the beginning of the Anthropocene (IPCC, 2007). This trend could be further extrapolated to even drop by ca. 0.7 units (1900 µatm) over the next 2000 years (Caldeira & Wickett, 2003). According to Doney *et al*. (2009), the changes to ocean carbonate chemistry at this point are irreversible.

Ocean acidification likely will affect the biogeochemical dynamics of calcium carbonate, organic carbon, nitrogen, and phosphorus in the ocean as well as the seawater chemical speciation of trace metals, trace elements, and dissolved organic matter (IPCC,2014). Acidification impacts process so fundamental to the overall structure and function of marine ecosystems that any significant changes could have far-reaching consequences for the oceans of the future and the millions of people that depend on its food and other resources for their livelihoods.

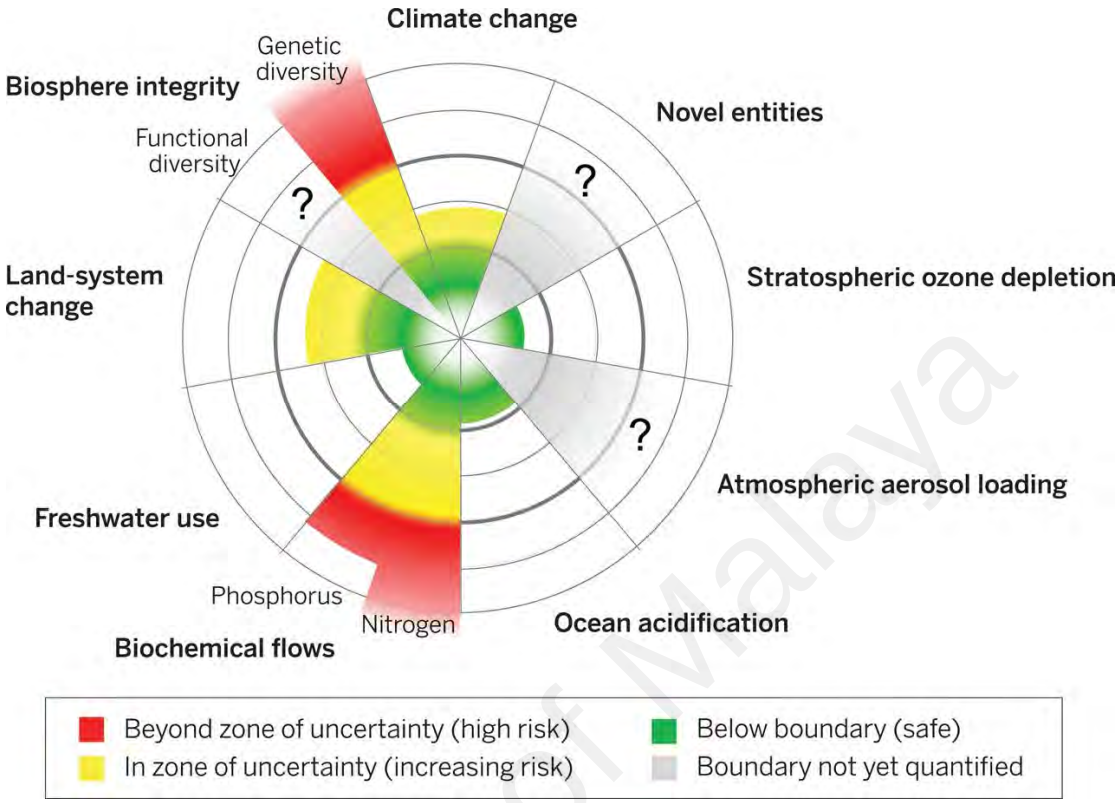


Figure 1.3: The current status of the control variables for seven of the nine planetary boundaries. The colours represent; green - the safe operating space (below the boundary), yellow - the zone of uncertainty (increasing risk), and red - the high-risk zone. The planetary boundary itself lies at the inner heavy circle. Adapted from Steffan ***et al.*** *(*2015)

In this thesis, we compile literature from water bodies in tropical and subtropical regions to get a general idea of the spatial variation of pCO_2 in these regions (**Table 1.1**). We notice that most of the research regarding carbon dioxide emissions are concentrated in the temperate region, and a small number of studies on this topic are from the tropical area. From the data sets collected, most of the estuaries have a wide range of pCO_2 compared to the open oceans or a closed system. In Malaysia, apparent acidification of oceans, coastal and estuaries has not been observed clearly—only a few acidification studies were conducted on the aquatic environment. For instance, reports by Acid Deposition Monitoring Network in East Asia (EANET) covered only two areas of study, mainly Semenyih Dam, Selangor and Sungai Tembeling, Pahang. High alkalinity readings and pH measurements were observed, indicating that the Semenyih Dam and Sungai Tembeling (Kantasamy *et al.,* 2007) are not currently experiencing acidification due to relatively high buffering capacity. However, pCO_2 were not determined in these two study areas. Besides that, extensive research was conducted pertaining to two estuaries which are Sungai Lupar and Saribas in the east of Malaysia, and consider as the first record of pCO_2 concentrations in Malaysia (Müller *et al.*, 2015). There are no data available for open ocean and coastal water in Malaysia up to this date. For instance, the nearest National Oceanic and Atmospheric Administration Earth System Research Laboratory (NOAA ESRL) Carbon Cycle Cooperative Global Air Sampling of the terrestrial environment nearest to Malaysia is located at Bukit Kototabang, Indonesia (**Figure 1.4**). From the data set, the current CO_2 in Southeast Asia is documented to be approximately around ~400 ppm.

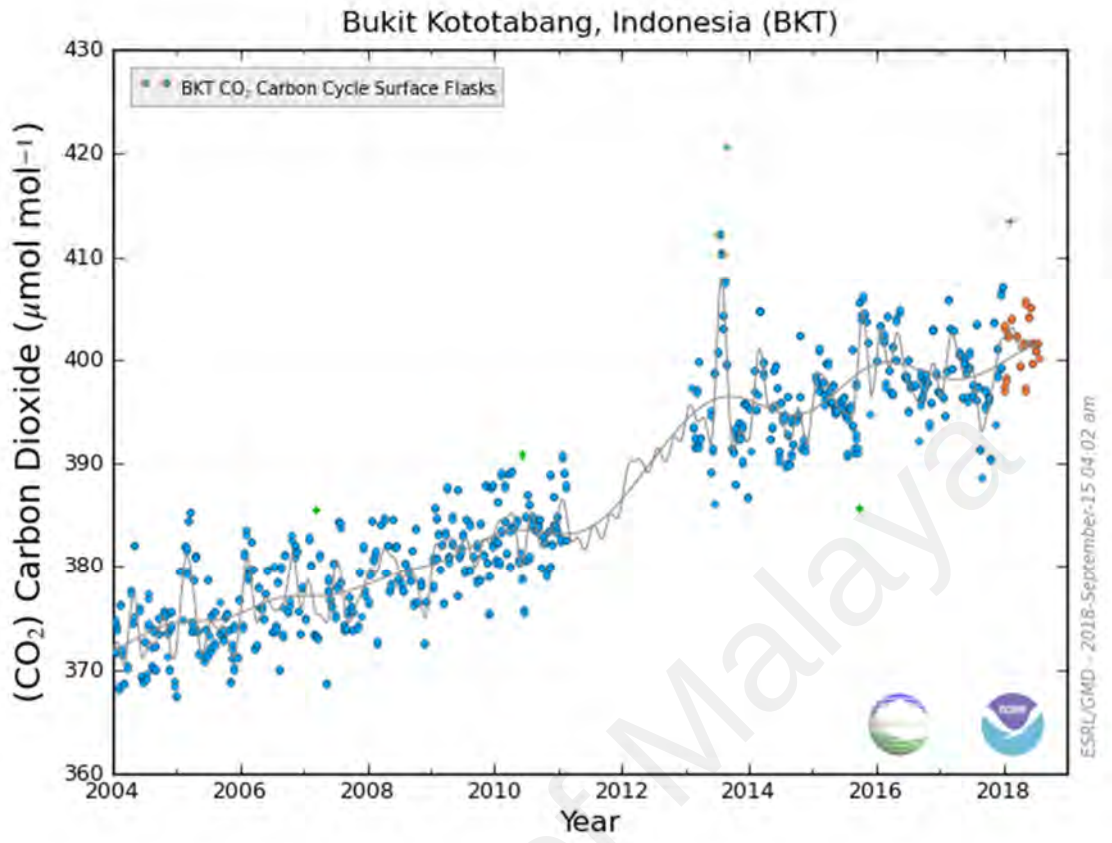


Figure 1.4: Carbon dioxide measurement collected from NOAA ESRL Carbon Cycle Cooperative Global Air Sampling Bukit Kototabang, Indonesia from 2004 to 2018. Retrieved on 23 July, 2018 from: https://www.esrl.noaa.gov/gmd/dv/iadv/graph.php?code=BKT&program=ccgg&type=ts

Table 1.1: Comparison of *partial* carbon dioxide for different tropical and subtropical sites.

Reference	Study site	Climate	Location	Latitude	pCO_2 range (µatm)
Müller *et al.* (2015)	Estuaries in Sarawak	Tropical	Malaysia	0°57'N, 110°37' E	640−5065
Cotovicz Jr. *et al.* (2015)	Guanabara Bay	Subtropical	Brazil	-	22−3715
Noriega & Araujo (2014)	Brazilian estuaries	Subtropical	Brazil	-	162−8638
Muduli *et al.* (2012)	Chilika	Tropical	India	19º28′−19º54N: 85º06′−85º35′ E	104−22487
Sarma *et al.* (2012)	Indian estuaries	Tropical	India	-	263−26521
Sarma *et al.* (2011)	Godavari estuary	Tropical	India	-	100−33391
Yuan *et al.* (2010)	Pearl River	Subtropical	China	-	263−26521
Roland *et al.* (2010b)	Cerrado hydroelectric reservoirs	Subtropical	Brazil	20º39′S, 46º18′W 20º16′S, 47º03′W 14º52′S, 55º46′W 22º35′S, 44º35′W	291−3079
Shim *et al.* (2007)	East China Sea	Subtropical	Korea	31º30′−34º00′N: 124º00′−127º30′ E	236−517

1.3 Factors controlling pCO_2 in the seawater

The ocean plays a vital role in regulating the Earth's atmospheric CO_2 (Falkowski *et al*., 2000) by sequestering one-third of the world CO_2 (Khatiwala *et al*., 2009). The carbon cycle is a sequence of events (i.e. surface mixing) and different concentrations where atmospheric CO_2 exchanges rapidly between terrestrial systems and the oceans in order to reach an equilibrium state. However, the buffering capacity of the ocean is not fixed as the availability of carbonate ions (CO_3^{2-}) will eventually limit how much bicarbonate (HCO^{3-}) can be formed thereby decreasing the capacity of the surface oceans to absorb CO_2 (Egleston *et al*., 2010). The vertical gradient of carbon can be explained by both physicochemical and biological processes.

The biological pump helps to reduce the partial pressure of CO_2 concentration by 150–200 ppm at the surface area (Falkowski *et al*., 2000) via photosynthesis:

$$6CO_2 + 6H_2O + \text{light energy} \longrightarrow C_6H_{12}O_6 + 6O_2 \quad (1.4)$$

At the sunlit layer, phytoplankton takes up nutrients and inorganic carbon to synthesis organic compounds using light energy and known as primary producer. Primary producer represents the base of the marine food web where non-photosynthetic organisms get their food. Through photosynthesis, CO_2 from the sea surface is transferred to the living organism. After cell death, the organic material exits the surface layer and sinks into the interior of the ocean. Before going into the deep ocean, some of the phytoplankton and zooplankton consume some of the phytoplankton, releasing some dead cells, detrital matter and faecal pellets as particulate organic carbon (POC). Majority of the phytoplankton is indirectly consumed through heterotrophic bacteria and find its way back to the surface as dissolved inorganic carbon (DIC) through respiration. Only 0.1 to 1% remaining organic carbon reaches the mesopelagic zone (4,000 – 6,000 m) and an

even smaller fraction to deep ocean sediments where it can be buried for millennia and turn into fossil fuel (IPCC, 2007).

Vertical gradient of carbon distribution can be explained through the other series of processes combining physicochemical activities. The temperature of the seawater plays a vital role in the carbon fluxes. The cooling of the surface water increases the ability of seawater to take up CO_2 (increased in gas solubility). Most CO_2 is found in the upper ocean, with approximately 30% located at depths shallower than 200 m and almost 50% at depths above 400 m (Sabine *et al.*, 2004). This mechanism of physical mixing known as the solubility pump is balanced when the waters from the ocean interior are brought back to the warm surface waters, decades to several hundred years later (Falkowski *et al.*, 2000).

1.4 Impact of ocean acidification on marine organisms

In the past years, numerous international research initiatives have been launched to investigate the effects of ocean acidification on marine organisms. Growth and reproduction of marine organisms such as benthos, corals, fishes and planktonic copepod were reported to be affected by the elevated pCO_2 concentrations with both positive and negative consequences (Kline *et al.,* 2009; Kikkawa *et al.,* 2004; Kurihara *et al.,* 2004; Sedlacek *et al.,* 2009).

The increasing amount of carbonic acid has increased the acidity of the seawater and at the same time, reduced the availability of the carbonate ions. This phenomenon poses a threat to calciferous organisms such as coral, calcareous algae, echinoderms (Barry *et al.,* 2002; Miles *et al.,* 2007), mollusc (Feely *et al.*, 2004; Gazeau *et al.*, 2007) and other shell-forming organisms (Watanabe *et al.,* 2006; Metzger *et al.,* 2007) which use calcium carbonate ($CaCO_3$) as the fundamental material to build new shells and skeletons. The

increase in acidity has led to the struggle in maintaining the existing structures. As this group plays vital roles in the ecosystem the changes in the community may affect the function of this group as shelter, food provider to the higher trophic levels and may change the biogeochemical cycle in the ocean (Joint ***et al.,*** 2011).

Reduction in pH has also has revealed detrimental consequences to higher trophic organism due to acidosis or hypercapnia where pH of the body fluid decreases rapidly due to the presence of excess carbon dioxide in the blood. The internal pH of the organism decrease as the CO_2 in the water diffuses across the biological membrane to form hydrogen and carbonate ions. Most marine organisms usually regulate their internal pH through passive buffering of the intro and extracellular compartments, consumption of proton, metabolic production and active proton transport (Michaelidis ***et al***., 2005). However, taxa with low buffering capacity are most likely susceptible to extinction under the elevated CO_2 environment (Kiessling & Simpson, 2011). For instance, most fish have shown a 100% mortality rate under the exposure of elevated CO_2 (Hayashi ***et al***., 2004). When an organism is exposed to an acidic environment, more energy is needed to regulate internal pH, which then leads to less energy for growth and reproduction (Williamson ***et al***., 2013). Interestingly, some organisms have managed to thrive in low pH environment such as the sea star, ***Pisaster ochraceus*** (Gooding, 2009).

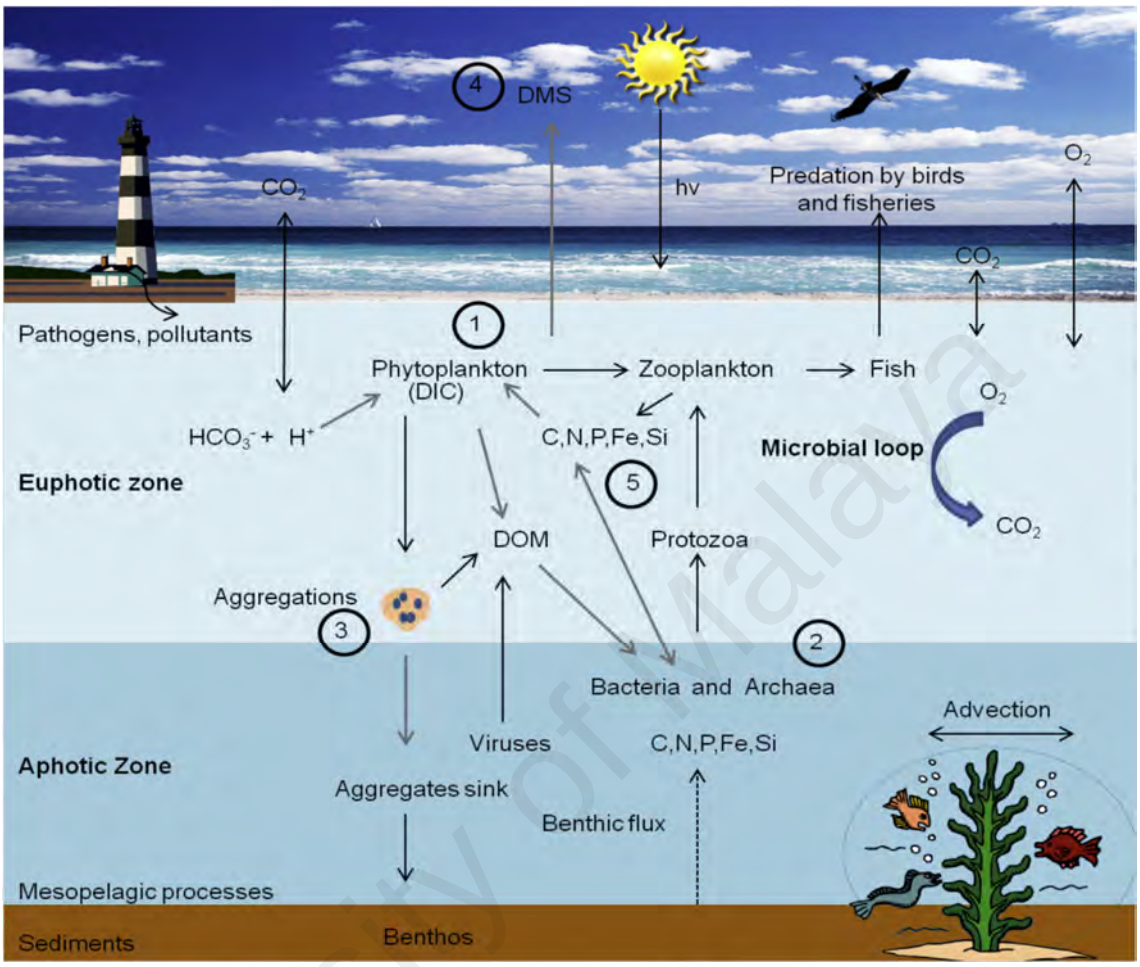


Figure 1.5: Microbial loop. Grey arrows represent processes that may be effected with an increase of CO_2. Adapted from Das & Mangwani (2015).

1.5 Impact of ocean acidification on marine microorganisms

Studies on the effect on marine microorganisms on the other hands have shown robust and at time conflicting results. Primary productivity showed reduced productivity under elevated CO_2 (Gao *et al.,* 2012). In contrast, the growth rate of specific diatom-dominated phytoplankton assemblages is unaffected (Tortell *et al.,* 2000). So far, ocean acidification is known to affect the calcification of phytoplankton and zooplankton (Reibesell *et al.,* 2000). Physiologically, the marine ecosystem does not work in a *silo*. All marine microorganisms in the microbial loop are interconnected. If phytoplankton is affected (whether positively or negatively) under elevated pCO_2, the response of heterotrophic bacteria will also change (Allgaier *et al.*, 2008).

1.6 Impact of ocean acidification on marine bacteria

In this sub-chapter, we focus on the effect of ocean acidification on heterotrophic bacteria and bacterial dynamics. In the marine food web (**Figure 1.5**), we recognise the role of bacteria as the main respirers besides decomposing a large pool of dissolved organic matter and remineralising inorganic nutrient (Azam *et al.,* 1983; Cole *et al.,* 1988; Pomeroy *et al.,* 2007). Phytoplankton exudates and organic materials and detritus released from zooplankton or protozoan grazing are amongst the critical carbon source for marine bacteria. The microbial loop is a significant pathway of organic carbon in the ocean, channelling about 50% of primary production (Azam, 1998).

The recent projected increase in oceanic pCO_2 has shown to have profound consequences on marine phytoplankton community (Liu *et.,* 2010). Since marine heterotrophic bacteria are closely related to phytoplankton and make up for the majority of biomass in the seawater, they utilise the majority of organic carbon in the surface oceans (Robinson & Williams, 2005; Joint *et al.,* 2011); they play vital roles in taking up

carbon into anabolic and catabolic processes. Therefore, measuring bacterial production and bacterial respiration is crucial to estimating carbon metabolism to understand the impact of bacterial changes on marine biogeochemical cycles.

Nonetheless, previous studies have shown that the rise of CO_2 levels in oceanic surface waters have the potential effect on marine bacteria although experimental results related to the effects of pCO_2 on marine bacteria are somewhat inconsistent and at times conflicting (Liu *et al.,* 2010). Interestingly, Joint *et al.* (2011) argue that marine microorganisms have already been exposed to changes in environmental pH in certain habitats such as estuaries and freshwater lakes. Other than the effect on the calcifying microbes, no prevalent evidence were observed. Hence, he concluded that without these evidence, the oceanic biogeochemical cycle would remain unchanged. In **Table 1.2**, we compile studies and findings related to the effect of elevated pCO_2 on bacterial dynamics to understand the current findings on the topic of ocean acidification.

Table 1.2: Compilation of various pCO_2 enrichment studies related to bacterial dynamics. BA: Bacterial abundance; BP: Bacterial production; BPP: Bacterial protein production; BR: Bacterial respiration; BCD: Bacterial carbon demand; BCM: Bacterial cell multiplication; BGE: Bacterial growth efficiency; DVC: Direct viable count; HPR: Heterotrophic prokaryotic production rate; TCC: Total cell count; TEP; µ: Growth rates.

Reference	Region	Coordinates	Process	State of Knowledge
Coffin *et al.* (2004)	United States of America	-	BP	Reduced bacterial production was observed primarily in longer incubation times and warmer temperature when exposed to lower pH
Grossart *et al.* (2006)	Norway	60.3°N, 6.2°E	BA, BPP	BA did not correlate with the increase in pCO_2 except for attached bacteria. There was an increase in BPP and µ observed at elevated pCO_2, especially BPP of the attached bacteria. Similarly, α and β glucosidase were as the highest at elevated pCO_2. The indirect effect on bacterial activities was mainly linked to phytoplankton. BCM of attached bacteria was observed to increase in the experiment. Also, a shift in particle quality and dynamics were induced by pCO_2
Allgaier *et al.* (2008)	Norway	62.2°N, 5.1°E	BA, BPP	There was no effect observed between BPP and BA with the increase in pCO_2. The indirect effect of changes in perturbation experiment was mainly related to phytoplankton carbon consumption, DOC exudation as well as TEP formation and

Table 1.2, continued.

Reference	Region	Coordinates	Process	State of Knowledge
				Subsequent sedimentation. Besides that, the study also observed changes in the community structure of free-living, and not attached bacteria
Yamada *et al.* (2009)	Japan	32°00'N, 138°00'E	TCC, HPR, DVC	Acidification could suppress HPR. However, TCC was only slightly affected, thus implying that seawater acidification could potentially alter heterotrophic activities and community structure of the bathypelagic prokaryotes
Krause *et al.* (2012)	Germany	54°11.3'N, 7°54.0'E	BA, Bacterial community composition	BA was not susceptible to the changes in pH. Conversely, bacterial composition showed a potential shift at lower pH
Motegi *et al.* (2012)	Norway	78°56.2'N, 11°53.6'E	BP	BP of attached bacteria is lower at elevated pCO_2
Motegi *et al.* (2013)	Norway	78°56.2'N, 11°53.6'E	BP, BR, BCD and BGE	The study observed an increase in BA and a decrease in BP at a specific time. However, no changes on BR, BCD and BGE were observed. It is worth to note that bacterial activities were influenced by the interactive effect of multiple factors such as phytoplankton, nutrient, temperature and others
Roy *et al.* (2013)	Norway	78°56.2'N, 11°53.6'E	Bacterial community composition	The direct impact of pCO_2 was found to be insignificant, except for 15 rare taxa that might have an impact on ocean biogeochemical process

Table 1.2, continued.

Reference	Region	Coordinates	Process	State of Knowledge
Pointek *et al.* (2013)	Norway	78°56.2'N, 11°53.6'E	BA, BPP	Natural extracellular enzyme assemblage increased in response to acidification. No changes were observed in BA and BPP
Maas *et al.* (2013)	Antarctica	-	BA, BP, Bacterial community composition	BA increased in the acidified incubation, while there was no consistent response in BP. The loss in diversity was observed at lower pH
Siu *et al.* (2014)	United States of America	48°30.32'N, 23°15.5'E	BA, BP, BR	This study highlights the ability of the bacterioplankton communities to respond to ocean acidification both structurally and metabolically. BP rates decreased while BR increased under lower pH condition
Endres *et al.* (2014)	Norway	60.31°N, 51.16°E	BA, Bacterial growth	Highest BA was recorded at the highest pCO_2. Similarly; bacterial growth was stimulated in the low pH mesocosms
Sala *et al.* (2015)	Spain	41°40'N, 21°48'E	BA	No apparent differences were observed in bacterial abundance and diversity in regards to ocean acidification in summer and winter
Hornick *et al.* (2017)	Finland	59°51.5'N, 23°15.5'E	BPP	There was no direct effect of CO_2 on BPP, but effect was evident indirectly, through either alteration of physicochemical parameters or the composition of the microbial community

Table 1.2, continued.

Reference	Region	Coordinates	Process	State of Knowledge
James *et al.* 2017	United States of America and French	32°10'N, 64°30'W 32°24'N, 119°50'W 17°36'S,149°43'W	BCD, BGE	BGE was lower in the elevated pCO_2 due to high bacterial respiration. The direct effect of elevated pCO_2 on bacterial carbon content was negligible. The removal of organic carbon by bacterioplankton communities was enhanced by the increase in pCO_2.

The vast majority of the studies on the effect of bacterial dynamics are mostly concentrated at the temperate regions, leaving other countries and even regions, left unexplored, despite of its biological vulnerability of the latter to the future global changes (Dupont & Pörtner, 2013). Little is known about the effect of ocean acidification on bacterial in this region, although, Southeast Asia is considered as one of the hotspot regions for CO_2 emission to the atmosphere (Regnier *et al*., 2013).

1.7 Relationship of Bacterial Respiration (BR), Bacterial Production (BP) and Bacterial Growth Efficiency (BGE)

In order to understand how ocean acidification may affect the roles of BR, BP and BGE in the biogeochemical cycle, we must first understand the relationship of bacterial dynamics under normal conditions.

Previous studies have shown BR in the tropical seawaters is usually a magnitude higher than BP (Lee *et al*., 2009). Therefore, suggesting that most of the bacterial energy is concentrated in respiration rather than to biosynthesis (Ram *et al.,* 2003). Temperature mainly regulates BR.

BGE is the quantity of biomass synthesised per unit of substance assimilated where conversion of various compounds, elements and minerals into a cell using the energy source. (del Giorgio & Cole, 1998) Understanding the key functions of BGE are crucial to understanding the roles of bacterial in regulating the biogeochemical cycle. BGE in the tropical seawaters is generally lower than the temperate seawaters (Amado ***et al.,*** 2013), usually, attributed to the high bacterial respiration in this region. BGE is also known as a function of Bacterial Carbon Demand (BCD). BCD is the sum of BR and BP. The relationship between BR and BP usually covary, however in some instances they could function independently. Uncoupling of these two controls helps to modulate the metabolism of BGE in the ever-changing environment.

1.8 Aims of the study

In this study, the effects of ocean acidification on marine bacteria are investigated in small-scale microcosms. The present study focusses on two different environments– oligotrophic (Port Dickson) and eutrophic (Port Klang) waters. Most of the studies on pCO_2 focused on estuaries where the coastal ocean is underrepresented. This study addresses two central questions. First, what is the temporal variability of pCO_2 in the coastal and estuarine waters? Second, what is the potential effect of elevated pCO_2 on BR, BP and BGE? In order to answer these questions, environmental data were collected at the estuarine and coastal water, and a series of experiments were conducted by exposing bacteria to elevated pCO_2 in short incubations in the laboratory. Our study helps to fill a gap in current knowledge on pCO_2 as data from the tropical waters, specifically in the South East Asia region, are relatively limited. This study helps to set baseline data for the present condition in tropical waters, and therefore help provide insights on the impact of ocean condition of the tropical in the future.

The specific research objectives are as follows:

i. To determine spatial variation of environmental conditions in Port Klang and Port Dickson
ii. To determine the temporal variation of pCO_2 in Port Dickson and Port Klang
iii. To investigate the effect of elevated pCO_2 in the seawater on bacteria; production (BP), bacterial respiration (BR) and bacterial growth efficiency (BGE) at Port Dickson and Port Klang

CHAPTER 2: MATERIALS AND METHODS

2.1 Sampling

Our study focused on two water bodies in Peninsular Malaysia which are Port Dickson (02°29.5'N, 101°50.3'E) and Port Klang (03°00.1'N, 101°23.4'E), located on the west coast of Peninsular Malaysia (**Figure 2.1**). Port Klang is one of the busiest ports in Malaysia and is an estuarine system with the Klang river flowing into it. The Klang river flows through the Klang Valley, which is the most developed region in Malaysia and has the highest population density. Therefore, it is known that the Klang river experiences enhanced loading of nutrient, organic and inorganic pollutants. Port Dickson, on the other hand, is a sandy beach and a tourist attraction for sea-related recreational activities. Generally, the coastal waters of Port Dickson are shallow about (20 m) (Law ***et al.***, 2002).

Surface seawater samples were collected from Port Dickson and Port Klang using acid-cleaned jerry can. A total of thirteen and sixteen samplings were conducted from July 2011 to March 2013 except for a long gap between July to October and Jun to September 2012 for Port Dickson and Port Klang, respectively. ***In situ*** temperature, salinity and dissolved oxygen (DO) concentrations were measured using Scientific Thermo Orion 5-Star pH/RDO/Conductivity Portable Multiparameter Meter. Subsamples for ***in situ*** total alkalinities (A_T) were collected in a 200 ml bottle and poisoned $HgCl_2$ with whereas, for bacterial abundance (BA), subsamples were preserved with glutaraldehyde (1% final concentration). Subsamples were collected for ***in situ*** A_T and BA. All subsamples for ***in situ*** measurements were collected in duplicates.

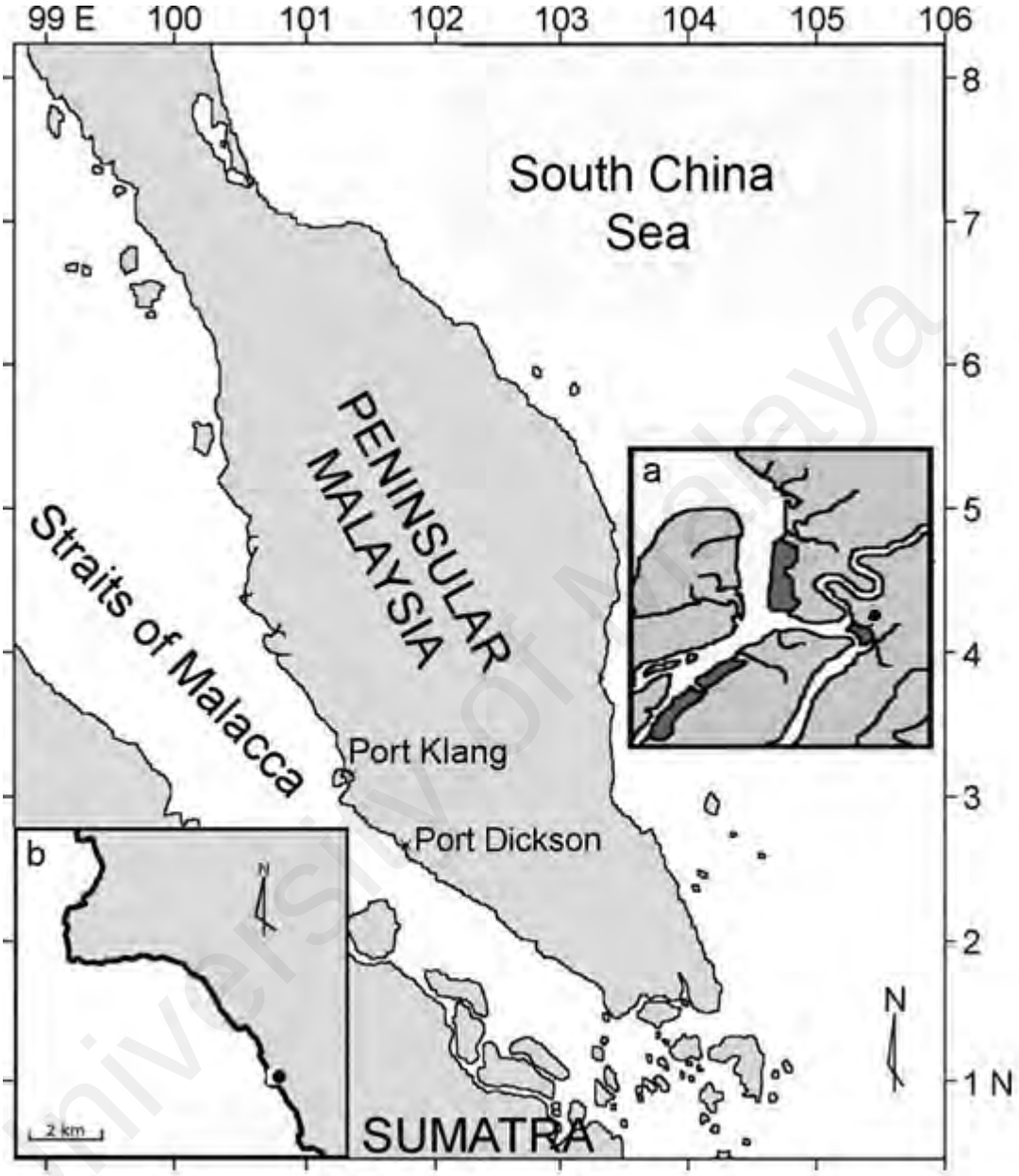


Figure 2.1: Location of sampling sites. (a) Port Klang (03˚00.1`N, 101˚23.4E). (b) Port Dickson (02˚29.5`N, 101˚50.3`E). Adapted from Lee & Bong (2006)

2.2 Chlorophyll (Chl) *a* measurement

Water samples for chl *a* were filtered through pre-combusted (450°C for 5 hours) Whatman GF/F and filters were frozen at -25°C before analysis. Chl *a* was extracted using acetone and measured using a spectrophotometer (Hitachi U–1900, Japan). Chl *a* was then calculated according to the tri-chromatic equation with absorbance at wavelengths of 630, 647, 664 and 750 nm (Parsons *et al*. 1984): $C = 11.85 (Abs_{664} - Abs_{750}) - 1.54 (Abs_{647} - Abs_{750}) - 0.08 (Abs_{630} - Abs_{750})$, where, Chl *a* ($\mu g\ L^{-1}$) = (C × volume of acetone) / (volume of sample filtered × 10).

2.3 Dissolved inorganic and organic nutrients

Water samples for nutrient analysis were also filtered through pre-combusted Whatman GF/F filters and frozen at -20°C until further analysis. Nitrate (NO_3), nitrite (NO_2), ammonium (NH_4) phosphate (PO_4), and silicate (SiO_4), were determined as in Parson *et al.* (1984). All nutrient measurements were carried out in triplicates.

NO_3 was first reduced using granulated copper-cadmium before being measured as NO_2. As NO_3 is reduced to NO_2, the sum of NO_3 and NO_2 was measured. The increase in NO_2 after the column reduction was assumed to be NO_3 concentration. For NO_2 measurement, sulfanilamide acid solution was used to react with the NO_2 in the seawater sample to form diazo compound was then reacted with N-(1-naphthyl)-ethylenediamine. The coloured azo dye formed was measured with a spectrophotometer at 543 nm.

PO_4 was determined by treating the water sample with mixed-reagent containing ascorbic acid, trivalent antimony and molybdic acid test. The dark blue phosphomolybdenum complex was formed and the absorbance was measured at 880 nm.

Water sample for SiO_4 measurement was treated with sulphuric acid and molybdate to form silicomolybdate complex. The sample was then reduced using ascorbic acid to form a blue coloured solution. The colour intensity was then measured at 810 nm. This test was carried out using plastic bottles.

NH_4 was determined based upon the oxidation reaction with hypochlorite to form indophenol blue dye. Sodium nitroprusside was then added to strengthen the dye formation, and the absorbance was measured at 640 nm. Standard was measured in approximately at the same time frame.

Water samples for dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) were filtered through pre-combusted (450°C for 5 hours) and then, preserved using concentrated hydrochloric acid. DON and DOC were analysed using high-temperature catalytic oxidation method via our collaborator in Japan. DON and DOC data are available only for four sets of CO_2 enrichment experiment at each location respectively.

2.4 Total alkalinity (A_T) and pCO_2

Samples for A_T were analysed according to the Gran titration analysis. pH meter (Orion 4 star pH.ISE Benchtop) was calibrated against three National Bureau of Standards (NBS) standards (Dickson & Goyet, 1994). Preserved water samples were titrated with the sulfuric acid solution to an endpoint of pH 3.5. Data points were plotted on Microsoft Excel (MS Office 2012, USA) to determine F1 Gran function: $(V_0 + V) \times 10^{-pH}$, where, V_0 is the volume of the sample and V is the volume of titrant. An X-axis and Y-axis graph were tabulated to determine the X intercept (titrant volume necessary to neutralise the

sample), where the value of R is ≥ 0.999. Total alkalinity (μEq^{-1}) was equal to (Volume of titrant × Normality of titrant) / (sample of water in litres).

pH, $A_{T,}$ and physical parameters were used to calculate the seawater pCO_2 using Excel Macro CO2SYS software version 2.1 (Lewis & Wallace, 1998). pCO_2 was calculated using Peng *et al.* (1987) and refitted using Mehrbach *et al*. (1973) for dissociation constants (K_1 and K_2). The approximate precision of K_1 and K_2 are 1.2% and 2% respectively. The pH_{NBS} scale was used for calculations in CO2SYS using pH_{NBS} electrode data under *in situ* temperature of 25°C.

2.5 Bacterial abundance (BA)

Bacterial abundance was determined by DAPI (4',6-diamino-2-phenylindole) direct counting method (Kepner *et al.,* 1994). Bacterial abundance was enumerated, and digital images were captured using an epifluorescence microscope (Olympus BX60; Japan) with a U-MWU filter cassette (excitation, 330 to 385nm: dichroic mirror, 400nm; barrier 420nm). Each field was also viewed under a U-MWG filter cassette (excitor, 510 to 550nm; dichroic mirror, 570nm; barrier, 590nm) to exclude photoautotroph. Stained bacterial cells on the digital images were acquired and counted with ImageJ 1.46r (Wayne Rasband National Institutes of Health, USA)

2.6 Bacterial production (BP), respiration (BR) and Growth Efficiency (BGE)

Water samples were filtered through pre-combusted (450°C for 5 hours) Whatman GF/C. Seawater was syphoned into approximately 60 ml dissolved oxygen (DO) bottles and incubated in the dark at 25°C. Filled DO bottles were then subsampled at 30 minutes after incubation (as initial 0-hour) and 12-hour for BP and BR.

Bacterial growth rate (μ) was calculated using the least-squares method as the slope of the linear regression analysis of natural logarithmic bacterial abundance over incubation time (12 hours). Bacterial production was then calculated as the product μ × initial bacterial abundance. In order to obtain bacterial production in carbon equivalents, the bacterial carbon content of 17.3 fg C cell $^{-1}$ (Port Dickson) and 32.8 fg C cell^{-1} (Port Klang) was used as constant conversion factor (Lee & Bong, 2008).

Bacterial respiration was estimated by measuring the change in DO concentration by the least-squares linear regression method after 12-hour dark incubation. DO concentration was determined using the Winkler method (Grasshoff *et al*., 1999). Change in DO concentration was replicated in sets of five. Subsequently, BGE was estimated with the following equation: BGE = BP/(BP+BR)

2.7 Experimental set up

Figure 2.2 shows the experimental set up of the study. Six sets of experiments were conducted with samples from Port Dickson and Port Klang in a laboratory setting. Enrichment studies were conducted in March, April, May, June, November 2012 and January 2013 for Port Dickson and January, March (twice), May, December 2012 and January 2013 for Port Klang. Water samples were filtered through pre-combusted (450°C for 5 hours) Whatman GF/C. Filtered seawater (2 L) was transferred into four separate 2 L bottles and bubbled with air containing CO_2 at the flow rate of approximately (10 ml/min) for 2, 5, 7 and 10 minutes, respectively. Seawater samples not treated with additional CO_2 were used as controls.

CO_2 treated and untreated seawaters were syphoned into 60 ml DO bottles and reagent bottles for pH, temperature, bacterial abundance, DO and total alkalinity later in the

experiment. A set of fourteen DO bottles and one 300 ml DO bottle were incubated in the dark at temperature 25°C for each treatment. Filled DO bottles and reagent bottle were then subsampled at 30 minutes after incubation (as initial 0-hour) and 12-hour. The following variables (pH, temperature, bacterial abundance, DO and total alkalinity) were measured. Change in bacterial abundance and DO were also used to calculate for BP and BR.

2.8 Statistical analysis

Statistical analysis such as Student's t test; correlation linear regression and multiple correlation were carried out according to Zar (1999). Student's t-test was mainly used to compare spatial variation between Port Klang and Port Dickson. Relationship between various parameters measured were determine using multiple correlation test. All statistical tests were conducted using Microsoft Excel.

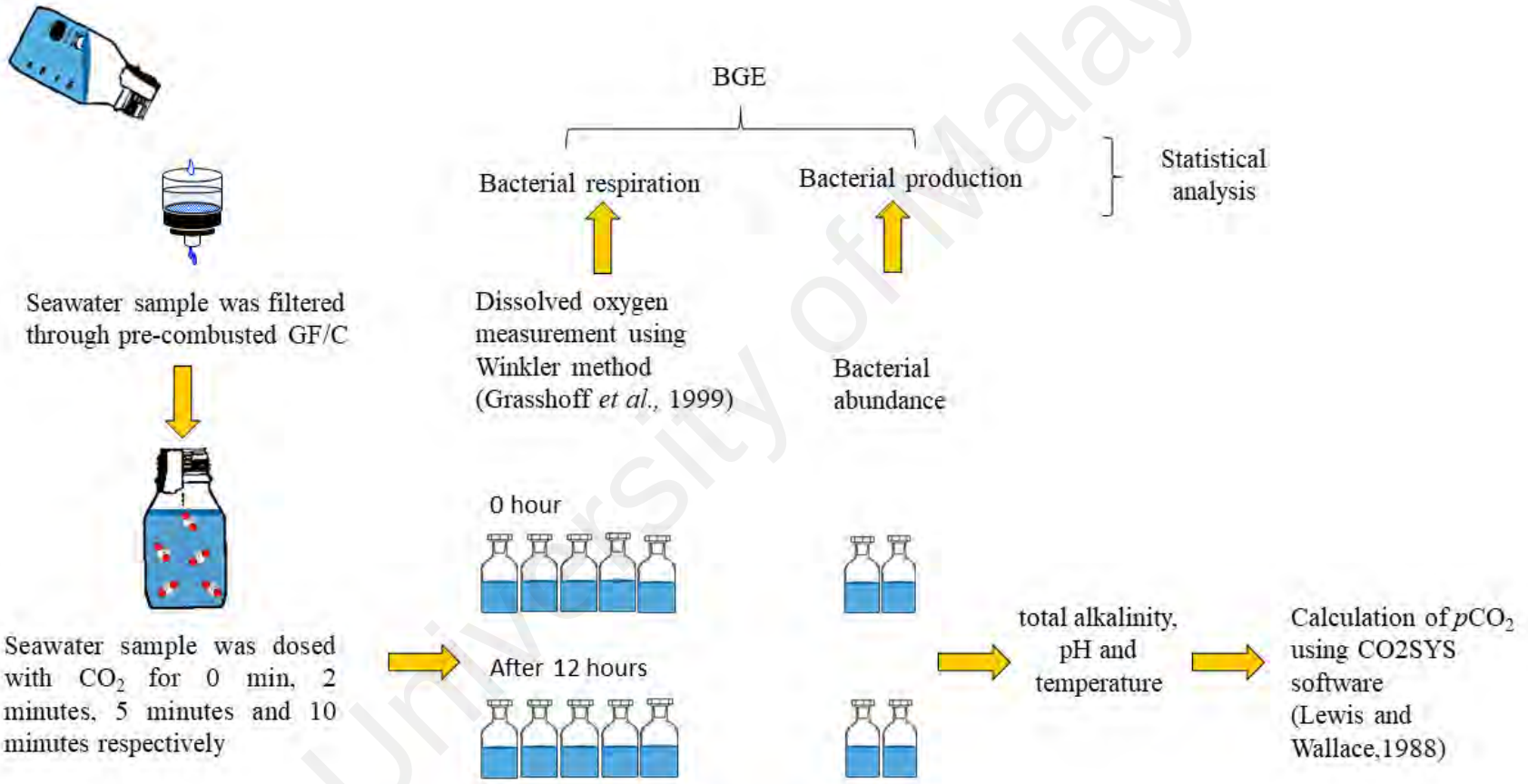


Figure 2.2: Flowchart of CO_2 enrichment experiment

CHAPTER 3: RESULTS

3.1 Environmental conditions

Table 3.1 shows the physicochemical parameters measured at both stations. Seawater temperatures in the study ranged from 27.7 to 33.5 °C. Salinity at Port Klang varied over a broader range (8.9 − 32.6 ppt) whereas salinity at Port Dickson showed a narrower range from 24.4 to 33.8 ppt. **Figure 3.1** shows the temporal variation of temperature and salinity. DO concentrations were higher at Port Dickson relative to Port Klang (Student's t-test: $t = -5.27$, $df = 21$ and $P < 0.001$).

In contrast, all dissolved inorganic nutrient concentrations were significantly lower at Port Dickson compared to Port Klang. NH_4 was the major contributor of Dissolved Inorganic Nitrogen (DIN), accounting for 70% in Port Dickson and 69% in Port Klang. **Figure 3.2** shows the temporal variation of NO_3, NO_2, and NH_4 in the estuary and coastal water. NO_3 generally range from 0.03 to 6.37 µM except April 2012, where the highest concentration was recorded (16.60 µM). Average NO_2 concentrations observed in Port Klang and Port Dickson were 3.27 ± 2.84 µM and 0.15 ± 0.13 µM respectively. Highest SiO_4 (114.40 µM) and PO_4 (6.60 µM) concentrations were recorded in Port Klang, in July and December respectively (**Figure 3.3**). Contrary to the dissolved inorganic nutrients, dissolved organic nutrients' concentrations were not significantly different between the two sites. Concentrations of DOC were the highest followed by DON, ranged from 714 to 1510 µM and 9 to 537 µM respectively.

Table 3.1: Environmental parameters at Port Klang and Port Dickson. Values shown are average (± standard deviations). *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$.

Variable	**Port Dickson**	**Port Klang**
Location	02°29.5'N, 101°50.3'E	03°00.1'N, 101°23.4'E
Temperature (°C)	29.2 ± 1.4	29.4 ± 0.7
Salinity (ppt)**	28.7 ± 2.9	24.4 ± 6.9
pH***	8.0 ± 0.1	7.6 ± 0.3
DO (µM)	212 ± 16	155 ± 40
Total alkalinity (µeq L^{-1})*	1912 ± 188	1787 ± 316
pCO_2 (µatm)**	788 ± 256	2168 ± 1513
Chl *a* (µg L^{-1})	2.59 ± 0.94	3.21 ± 2.59
Bacterial abundance (cell L^{-1})***	$1.20\times10^6 \pm 0.38\times10^6$	$2.80\times10^6 \pm 1.16\times10^6$
NH_4 (µM)**	2.70 ± 3.29	17.69 ± 18.42
NO_3 (µM)**	1.04 ± 0.69	4.58 ± 4.06
NO_2 (Mm***	0.15 ± 0.13	3.27 ± 2.84
PO_4(µM)*	0.26 ± 0.16	1.11 ± 1.50
SiO_4(µM)***	10.66 ± 5.14	34.73 ± 27.62
DOC (µM)	1331 ± 777	1379 ± 1567
DON (µM)	127 ± 201	202 ± 190

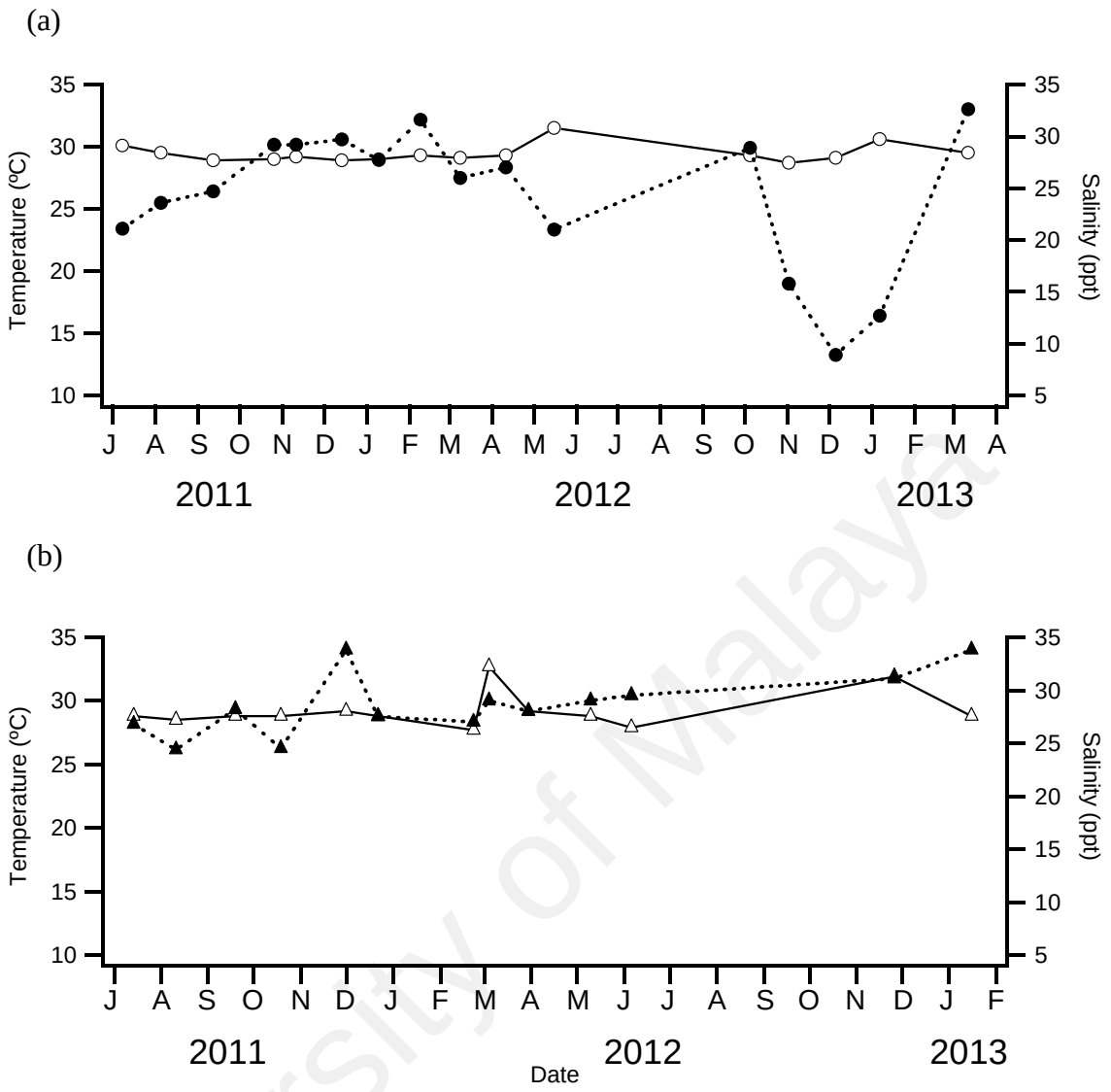


Figure 3.1: Temporal variation of temperature and salinity in (a) Port Klang and (b) Port Dickson. Closed circles/rectangles refer to salinity: Open circles/rectangles refer to temperature.

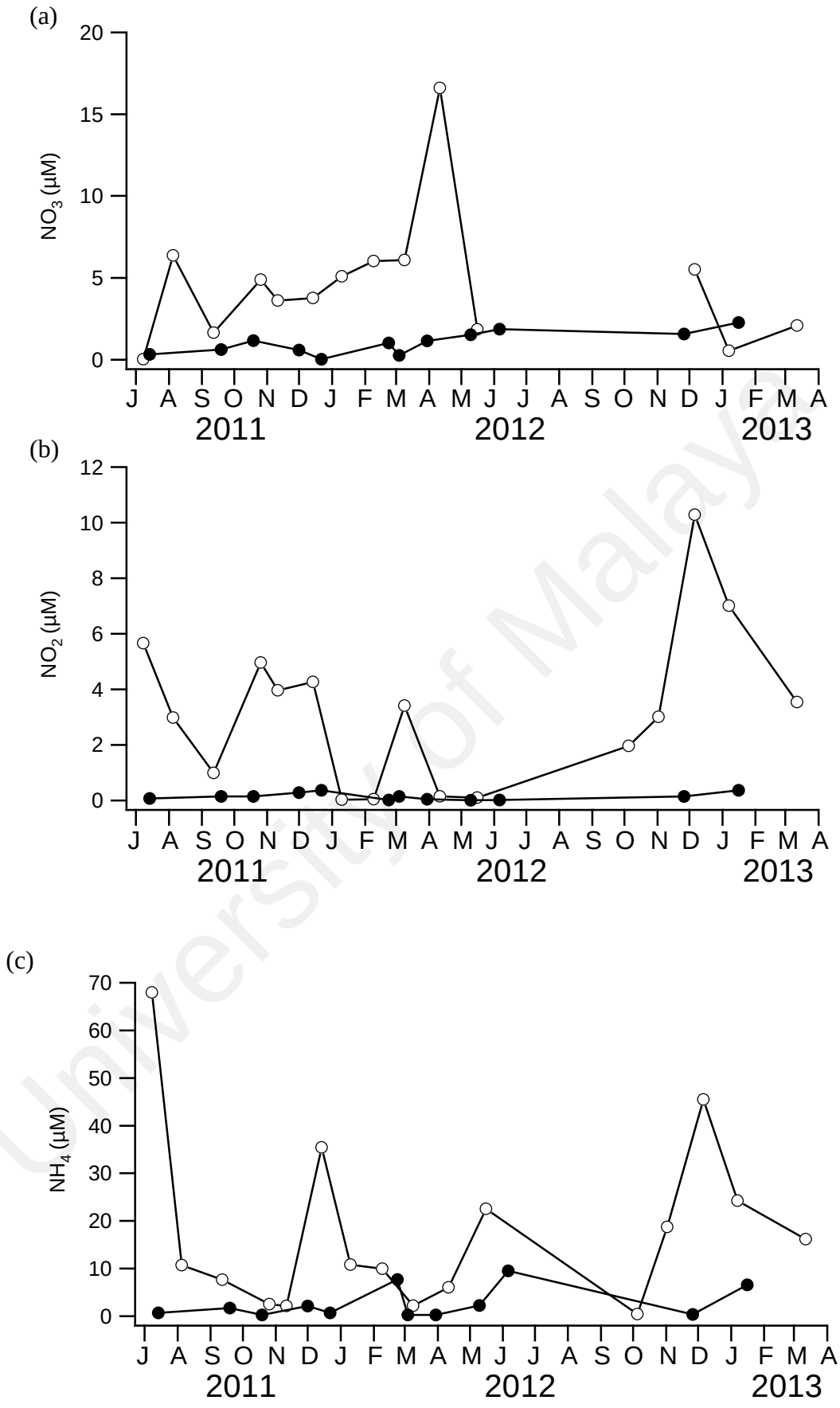
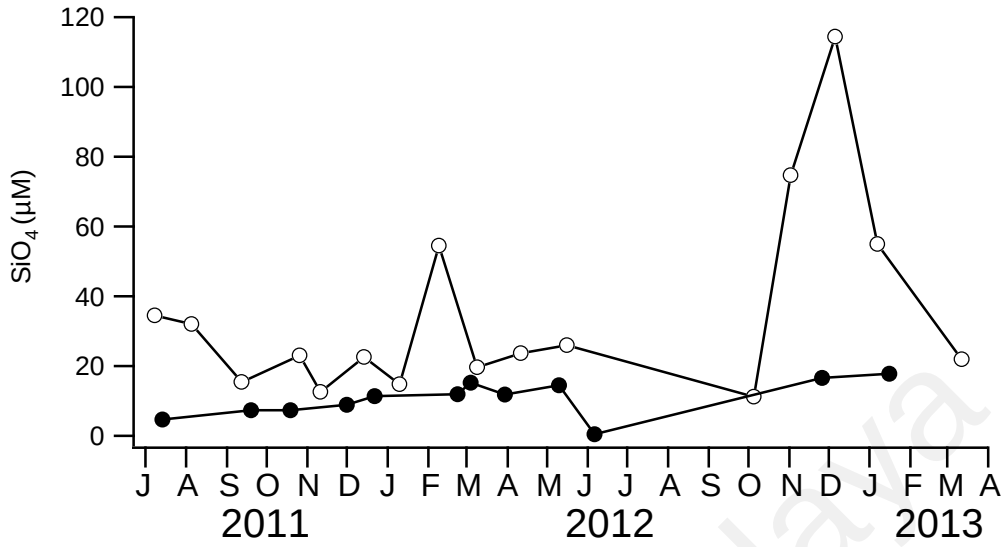


Figure 3.2: Temporal variation of (a) nitrate, (b) nitrite and (c) ammonia. Closed circles refer to Port Dickson: Open circles refer to Port Klang.

(a)



(b)

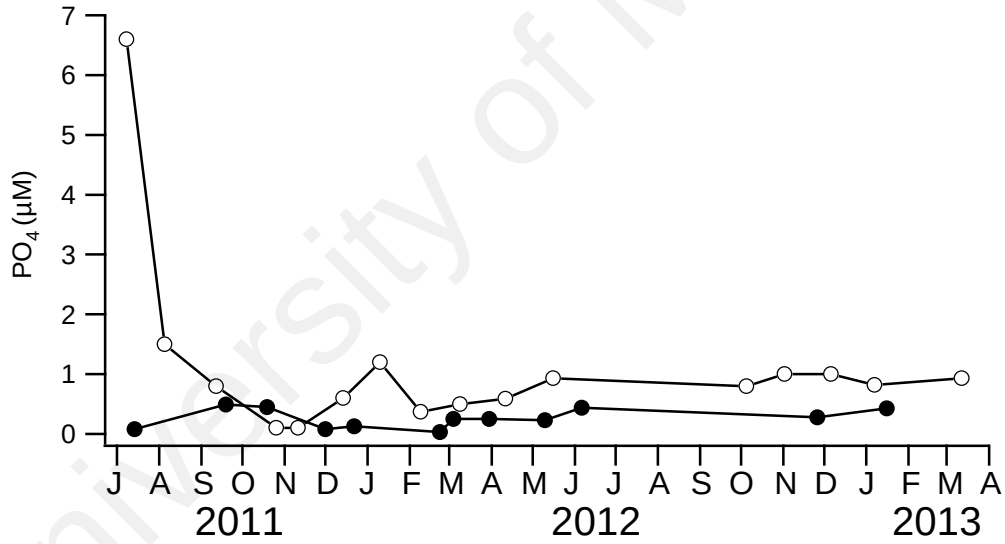


Figure 3.3: Temporal variation of (a) silicate, (b) phosphate. Closed circles refer to Port Dickson: Open circles refer to Port Klang.

3.2 Temporal variation

Figure 3.4 shows the temporal variation of pH, A_T and pCO_2. A_T measured at Port Klang ranged from 1061 to 2171 µEq L^{-1} and fluctuated widely (*CV* = 17%). Relatively higher and more stable A_T was found at Port Dickson, ranging from 1641 to 2228 µEq L^{-1} (*CV* = 9 %). These properties were reflected in the pH scale. A broader range of pH (7.03 to 8.25) was measured at Port Klang. Contrastingly, we observed a relatively stable and higher pH (7.78 to 8.13) in Port Dickson (Student's t-test: t= −3.29 df= 23 and P < 0.01). pCO_2 at Port Klang generally ranged from 345 to 1599 µatm. pCO_2 was outside this range when three apparent peaks in Port Klang were observed in April 2011, November 2011 to January 2012 and November 2012 to January 2013 (**Figure 3.4c**). A relatively stable pCO_2 was observed at Port Dickson (*CV* = 33%) compared to Port Klang (*CV* = 70%). We carried out multiple univariate correlation analysis to determine whether the temporal variation of pCO_2 was coupled to any of the environmental variables available (**Table 3.2)**.

Table 3.2: Correlation matrix of pCO_2 with environmental parameters.

	Temp. (°C)	Sal. (ppt)	pH	DO (µM)	Chl *a* (µg L^{-1})	BA (cell L^{-1})	BP	BR	BGE
***p*CO₂**	-0.0133	-0.7848***	-0.9703***	-0.7522***	-0.0152	0.5135**	0.2042	-0.0858	0.3230

Values for the coefficient of multiple correlations (R) are shown. A negative sign denotes an inverse relationship. *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$.

Table 3.2, continued.

	NO_3 (µM)	NO_2 (µM)	SiO_4 (µM)	PO_4 (µM)	NH_4 (µM)	A_T (µmeq)	*p*CO₂
***p*CO₂**	0.2451	0.7436***	0.7827***	0.0596	0.3806*	-0.6600***	1

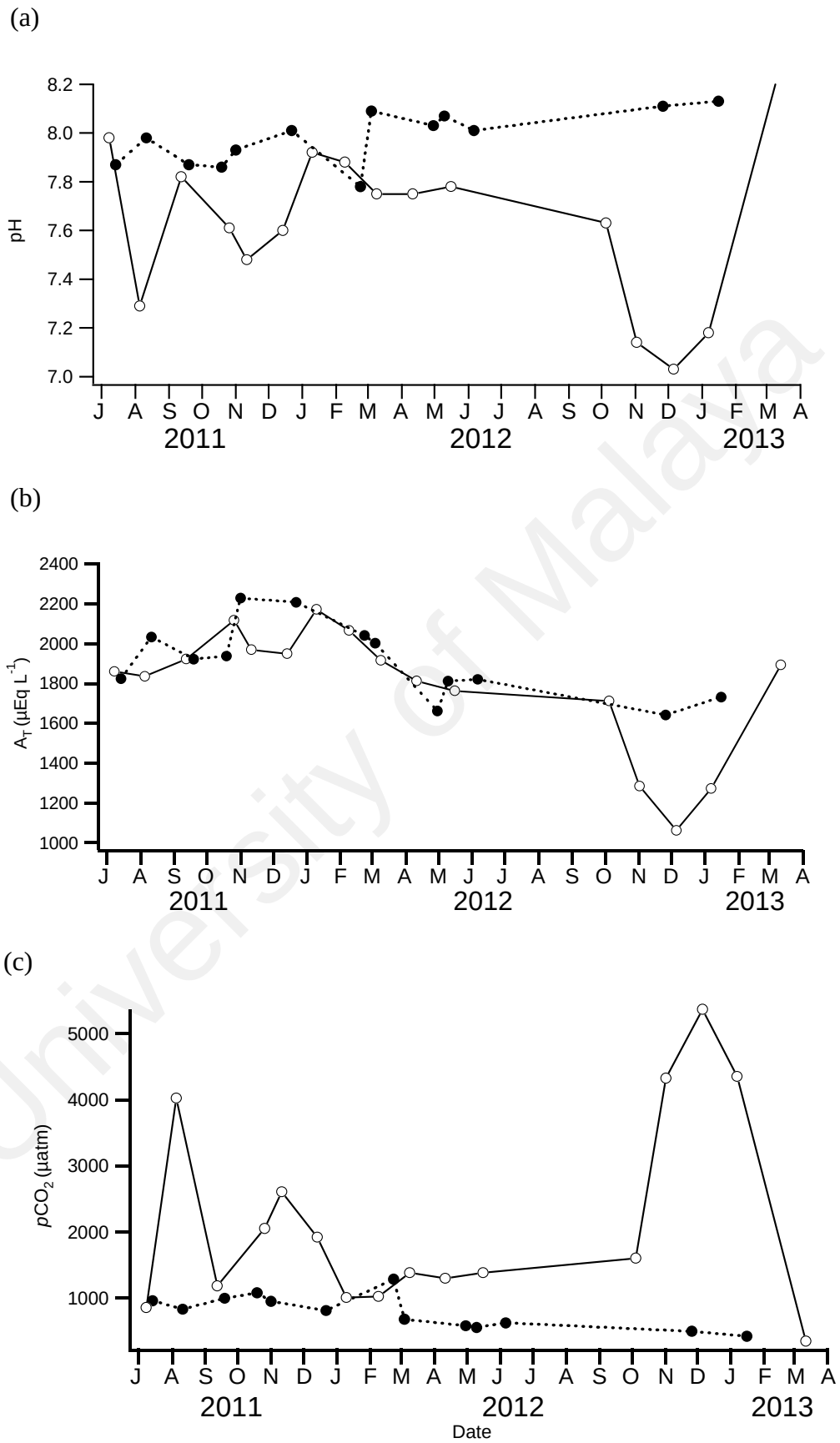


Figure 3.4: Temporal variation of (a) pH, (b) total alkalinity and (c) *partial* carbon dioxide. Closed circles refer to Port Dickson: Open circles refer to Port Klang.

The average Chl *a* concentration at Port Klang and Port Dickson, were 3.21 ± 2.59 µg L^{-1} and 2.61 ± 0.97 µg $L^{-1,}$ respectively (**Figure 3.5b**). Higher fluctuation of Chl *a* concentration was observed at Port Klang (*CV* = 81%) compared to Port Dickson (*CV* = 39%). A two-fold in Chl *a* concentrations relative to the mean was observed in May 2012, January and March 2013 at Port Klang. Higher bacterial abundance was observed at Port Klang with an average of $2.8 \times 10^6 \pm 1.2 \times 10^6$ cell L^{-1} compared to Port Dickson with an average of bacterial abundance, $2.8 \times 10^6 \pm 1.2 \times 10^6$ cell L^{-1}. The highest bacterial abundance (**Figure 3.5a**) recorded in the study was in January 2013 at Port Klang (5.06×10^6 cell L^{-1}). TSS in the seawaters in Port Dickson and Port Klang ranged from 27.4 to 164.7 mg L^{-1}.

Figure 3.6 shows the temporal variation of bacterial dynamics in coastal water and estuaries. $BP_{temporal}$ ranged between 0.12 to 0.87 µM C h^{-1} and 0.09 to 0.21 µM C h^{-1} at Port Klang and Port Dickson, respectively. $BP_{temporal}$ were higher in estuarine compared to coastal water (Student's t-test: t= 2.94 df= 6 and $P < 0.05$). $BR_{temporal}$ (**Figure 3.6a**) for the experimentation sets at Port Klang, varied from 0. 51 to 2.26 µM O_2 h^{-1} (*CV* = 59 %) while $BR_{temporal}$ at Port Dickson (**Figure 3.6a**) ranged from 0.38 to 0.85 µM O_2 h^{-1}. BGE (**Figure 3.6c**) was estimated based on BP to Bacterial Carbon Demand (BP + BR) ratio. Average $BGE_{temporal}$ at Port Dickson and Port Klang were 0.19 ± 0.06 and 0.28 ± 0.13 respectively.

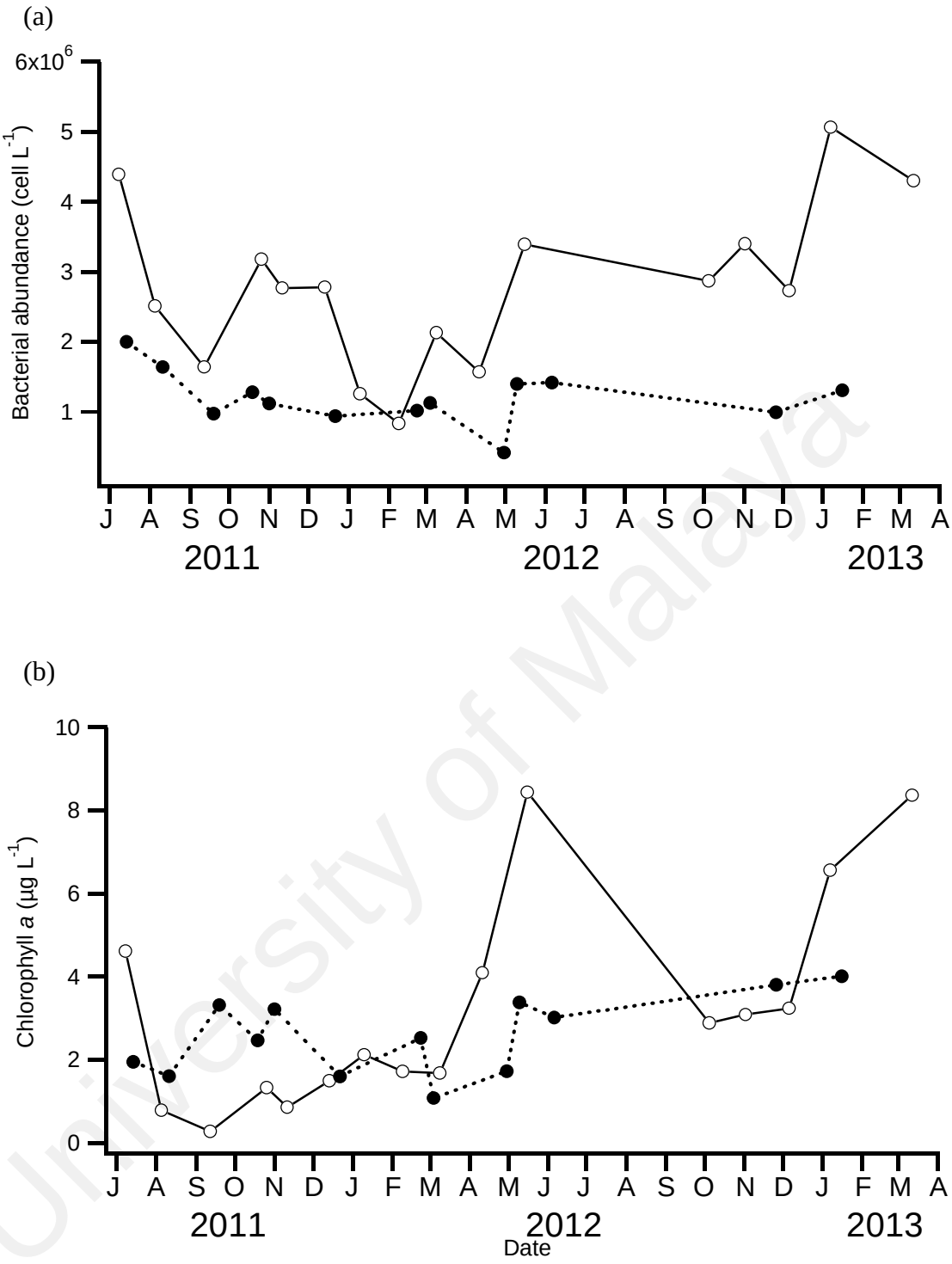


Figure 3.5: Temporal variation of (a) bacterial abundance and (b) chlorophyll a. Closed circles refer to Port Dickson: Open circles refer to Port Klang.

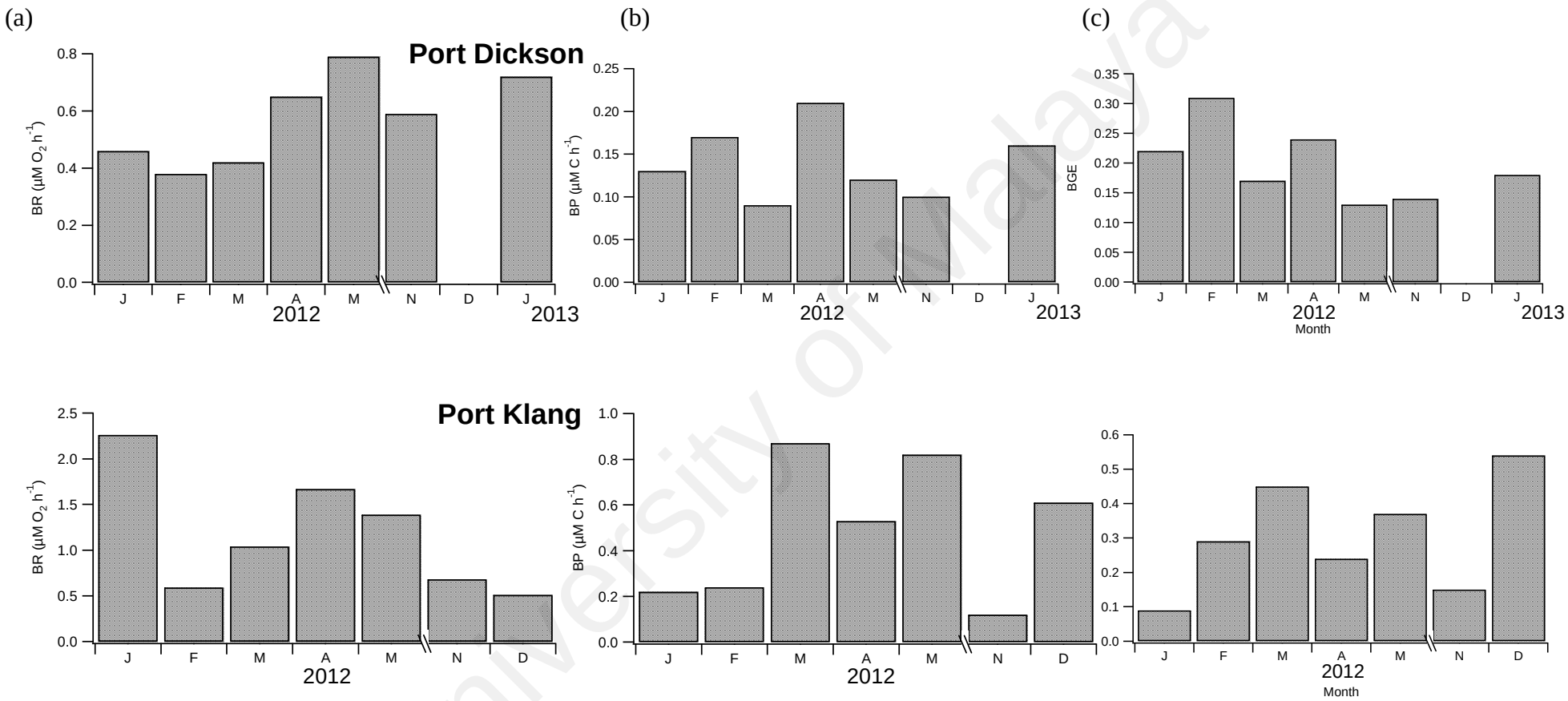
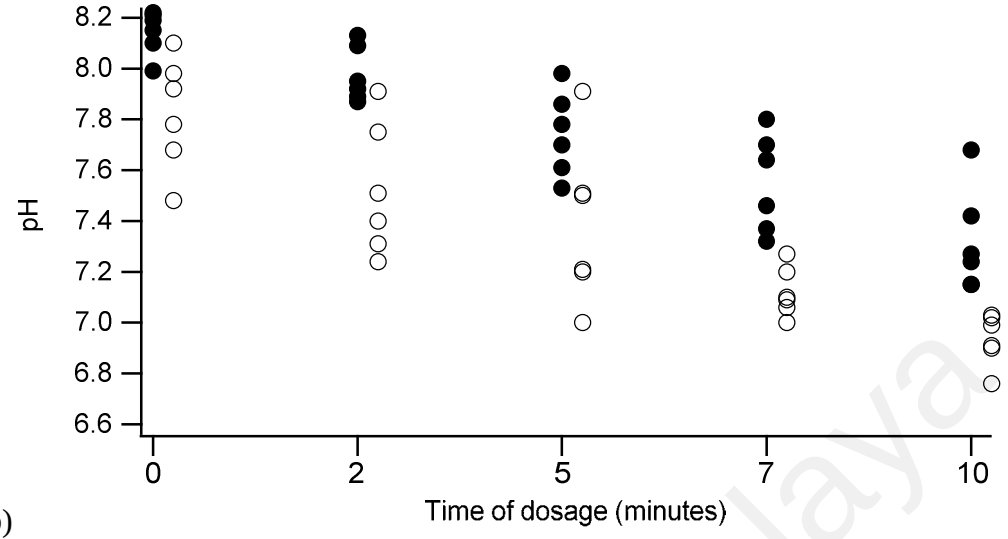


Figure 3.6: Temporal variation of bacterial respiration (a), bacterial production (b) and bacterial growth efficiency (c) at Port Dickson and Port Klang

3.3 CO_2 enrichment experiment

In the experiment, gradients in pH (**Figure 3.7a**) and pCO_2 (**Figure 3.7b**) were controlled among the treatments by bubbling the CO_2 in the seawater within specific dosage time. Initial pCO_2 (control) without CO_2 treatment were used to manipulate the CO_2 system ranged from 462 to 1926 µatm and 305 to 938 µatm for Port Klang and Port Dickson, respectively. During 2, 5, 7, and 10 minutes dosage time, pCO_2 for Port Klang ranged from 1129 to 10255 µatm, and pCO_2 for Port Dickson ranged from 610 to 7890 µatm. Similarly, seawater pH during 2, 5, 7, and 10 minutes dosage time were 7.48−8.10, 7.24−7.91, 7.00−7.91, 7.27−6.82 and 7.03−6.76, respectively for Port Klang and 8.22−7.99, 8.13−7.87, 7.98−7.53, 7.80−7.32, and 7.68−7.15, respectively for Port Dickson. There was no apparent trend observed on BP, BR and BGE on the elevated pCO_2 experiment. In order to understand the relationship between pCO_2 and bacterial activities, correlation graphs of the relationship between pCO_2 enrich waters and BR, BP and BGE are plotted and shown in **Figure 3.8.**

(a)



(b)

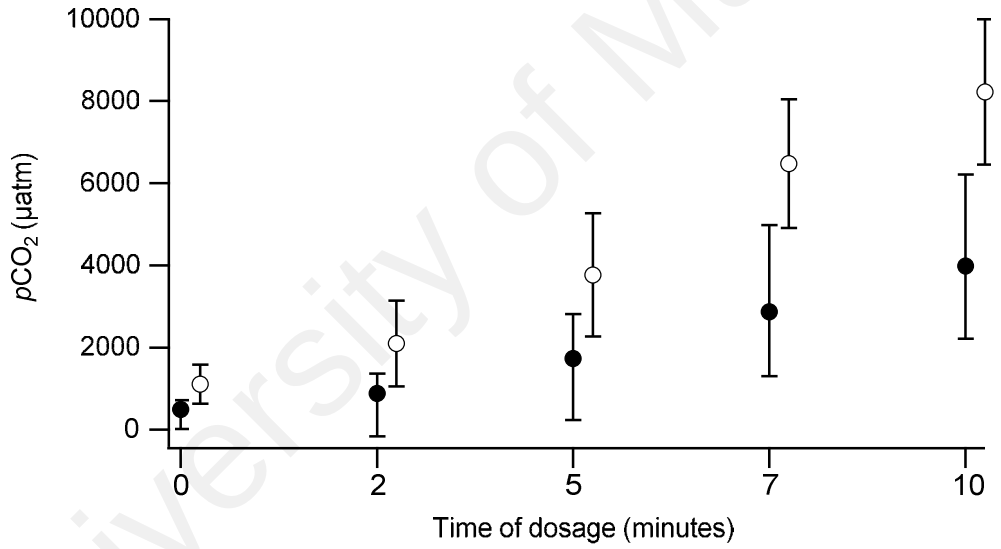


Figure 3.7: The time of carbon dioxide dosage and its association with (a) pH and (b) *partial* carbon dioxide. Closed circles refer to Port Dickson: Open circles refer to Port Klang. Error bars represent the S.D. of the mean

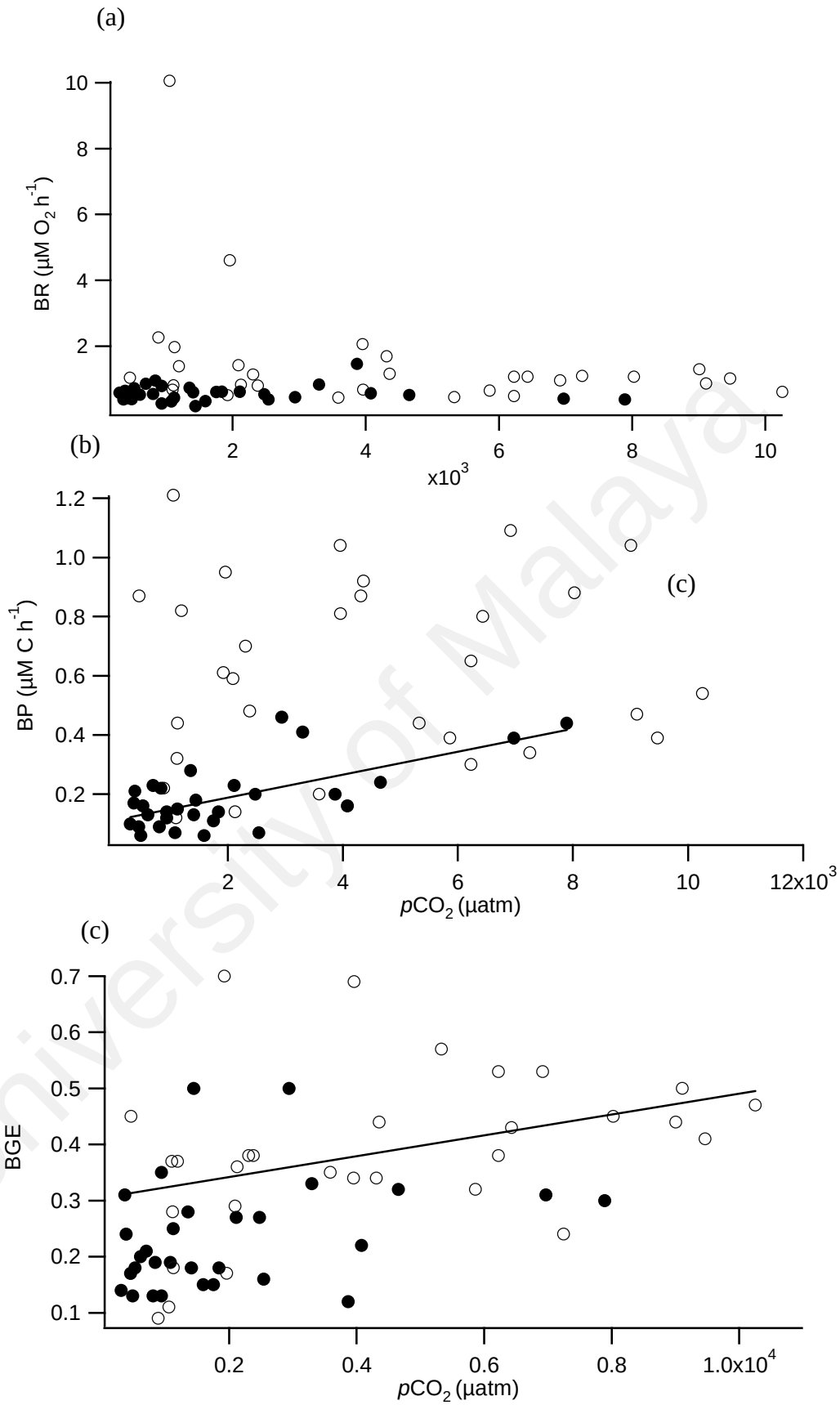


Figure 3.8: Relationship between *partial* CO_2 and bacterial respiration (a), bacterial production (b), bacterial growth efficiency (c). Closed circles refer to Port Dickson: Open circles refer to Port Klang.

CHAPTER 4: DISCUSSION

4.1 Environmental conditions

Surface water temperatures observed in this study is consistent with previous works (Lee & Bong, 2008; Lee *et al.,* 2009). In this study, salinity observed in Port Klang fluctuated over a broader range compared to Port Dickson due to the series of fresh river water input streaming into the Port Klang estuary (Lee *et al.,* 2009). Dissolved inorganic nutrient concentrations were within the range for coastal water and estuarine in Malaysia (Lee *et al.,* 2009). High accumulation of nutrients—particularly NH_4—and productivity coupled with the low concentration of dissolved oxygen at Port Klang are reflective of a eutrophic environment. Rapid development and industrialisation taking place upstream contributed to the reducing environment (eutrophication) at Port Klang (Lee & Bong, 2006). In contrast, Port Dickson exhibited characteristics of an oligotrophic environment. The spikes in Chl *a* concentrations observed at Port Klang is a regular phenomenon attributed to high rainfall, though, the Chl *a* concentrations observed in this present study were relatively lower compared to previous reports (Lim *et al.,* 2017)

4.2 Relationships between pCO_2 and environmental variables

In this study, pCO_2 in Port Klang ranged from 345 to 5365 µatm and were within the range observed for estuaries in the subtropical and tropical regions (**Table 1.1**). However, pCO_2 in Port Dickson was relatively more stable, ranging from 421 to 1282 µatm. Estuaries are known to show significant supersaturation of CO_2 relative to the atmosphere. In an observation collected over 40 years at North Atlantic, North and South Pacific and Southern Oceans by Takahashi *et al.* (2009), it was reported that surface water

pCO_2 values are increasing everywhere at about the same rate as atmospheric CO_2. As most previous studies were focused on estuaries, no data from tropical coastal water was available for us to compare. Nonetheless, the range observed here for pCO_2 were within the range for marine-dominated estuaries where salinity is above 25 (Cotovicz Jr. *et al.*, 2015).

We observed two peaks in pCO_2 measurements at Port Klang from November to January in both 2011 and 2012 (**Figure 3.4c**). Samplings during this period were conducted during the intermonsoon and wet seasons, suggesting differences in pCO_2 between dry and wet seasons' pCO_2 concentrations. This was expected as freshwater input would dominate the system due to high riverine runoff and intense rainfall. Our observations were concurrent with studies in the tropical waters during the wet season (Sarma *et al.,* 2011; Cotovicz Jr. *et al.,* 2015). However, this is in contrast to a study by Müller *et al.* (2016) in Sarawak estuaries (in East Malaysia), who reported no correlation between rainfall periods and pCO_2 based on a single cruise sampling. Therefore, a comprehensive study during the peak of the wet season would help us to confirm this hypothesis where intense rainfall often resulted in higher pCO_2 concentration.

From **Table 3.2,** average pCO_2 was higher in Port Klang and varied over a broader range. This observation was proven in the inverse coupling between pCO_2 with salinity (**Figure 4.1a**) in estuaries (Frankignoulle *et al.,* 1998), caused by the episodic input of freshwaters into the estuaries (Müller *et al.,* 2016). However, this relationship was not observed in the coastal water of Port Dickson ($r^2 = -0.23$, $P > 0.05$, $n = 13$) suggesting a high buffering capacity in the coastal water carbonate system. This was strongly evident in a positive relationship between pCO_2 and total alkalinity. Presently, we observed an absence of the relationship between pCO_2 and temperature **(Figure 4.1b)** observed in the temperate countries. This may be due to the relatively more stable and less variable

temperature measured (*CV* = 4%) in this study. Previous study at East China Sea; a temperate region, has also shown that this lack of relationship may be attributed to seasonal freshwater input and shallow waters (Shim *et al.,* 2007).

Temporally, pCO_2 was inversely correlated with DO ($r^2 = 0.57$, $P < 0.001$, $n = 28$), however, spatially, pCO_2 only correlated with dissolved oxygen in Port Klang ($r^2 = 0.45$, $P < 0.01$, $n = 16$) and not in Port Dickson ($r^2 = 0.03$, $P > 0.05$, $n = 12$). A possible reason for this is that natural and anthropogenic enrichment of nutrients in estuaries may enhance the production and subsequent remineralisation of organic matter leading to hypoxia and low pH water (Feely *et al.,* 2010). Although pCO_2 was relatively low in the coastal water, no apparent correlation with the DO suggests that there is more oxygen available in Port Dickson. However, low *CV* of pCO_2 in this case might also affect the lack of correlation between these two variables in coastal water. In this study, we found that pCO_2 was positively correlated with the dissolved nutrients NO_2, NH_4 and SiO_4 but not NO_3 and PO_4. Correlation of pCO_2 with ammonia suggested a manifestation of nitrification that may have an impact on increasing pCO_2 (Sarma *et al.,* 2011). Other studies showed that anthropogenic organic matter could enhance pCO_2 through an increase in respiration rate (Yuan *et al.,* 2010).

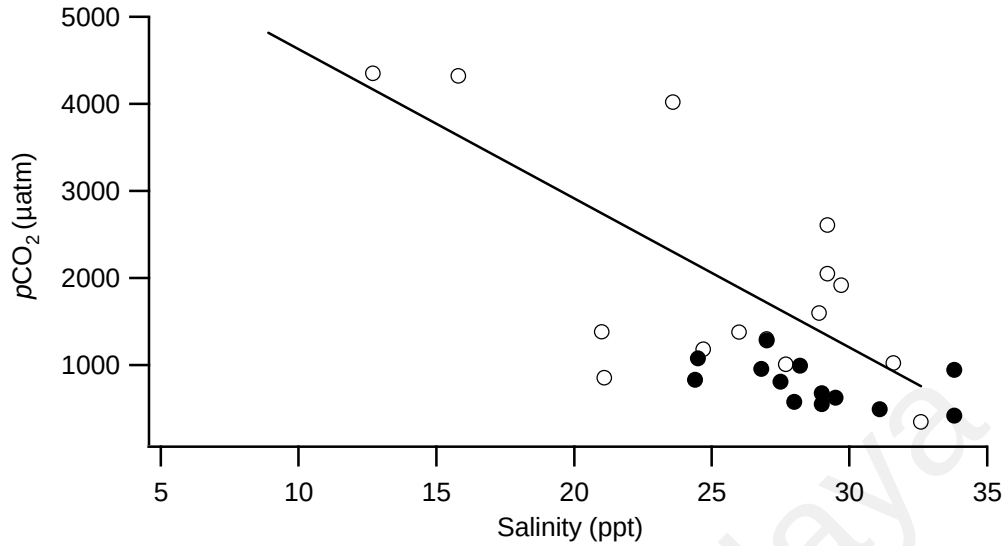
4.3 Bacterial dynamics in estuarine and coastal water

In general, $BP_{temporal}$ in this study ranged from 0.09 to 0.87 µM C h^{-1}. These values are comparable to *in situ* production rates observed in tropical waters (Farjalla *et al.,* 2009; Roland *et al.*, 2010a). Average bacterial production at Port Klang (0.49 µM C h^{-1}) was higher than Port Dickson (0.14 µM C h^{-1}), due to the eutrophication in Port Klang (Lee & Bong, 2008; Lee *et al.,* 2009). We found an absence in the coupling between $BP_{temporal}$ and $BR_{temporal}$ ($r^2 = 0.13$, $P > 0.05$, $n = 14$) suggesting a disconnect between catabolism

and anabolism activity (Ram *et al.*, 2003). In this study, $BGE_{temporal}$ was correlated with $BP_{temporal}$ ($r^2 = 0.61$, $P < 0.001$, $n = 14$), but not $BR_{temporal}$ ($r^2 = 0.03$, $P > 0.05$, $n = 14$) resulting in the variation in BGE (Roland & Cole, 1999). Therefore, BGE could be expressed as a ratio of BP, thus indicating that a relatively higher fraction of energy is allocated for the bacteria in anabolism.

We observed a correlation between temporal pCO_2 with bacterial abundance ($r^2 = 0.26$, $P < 0.01$, $n = 29$) but not with $BP_{temporal}$ and $BR_{temporal}$. Although respiration could be one of the controls on the pH (Müller *et al.*, 2016), we found no relationship between pH and bacterial respiration in Port Dickson ($r^2 = 0.08$, $P > 0.05$, $n = 7$) and Port Klang ($r^2 = 0.37$, $P > 0.05$, $n = 7$). We did not consider benthic respiration as there were no benthic measurements taken during the study. However, through the relationship above, we could infer that pH and not respiration drove the pCO_2 patterns in Port Klang and Port Dickson.

(a)



(b)

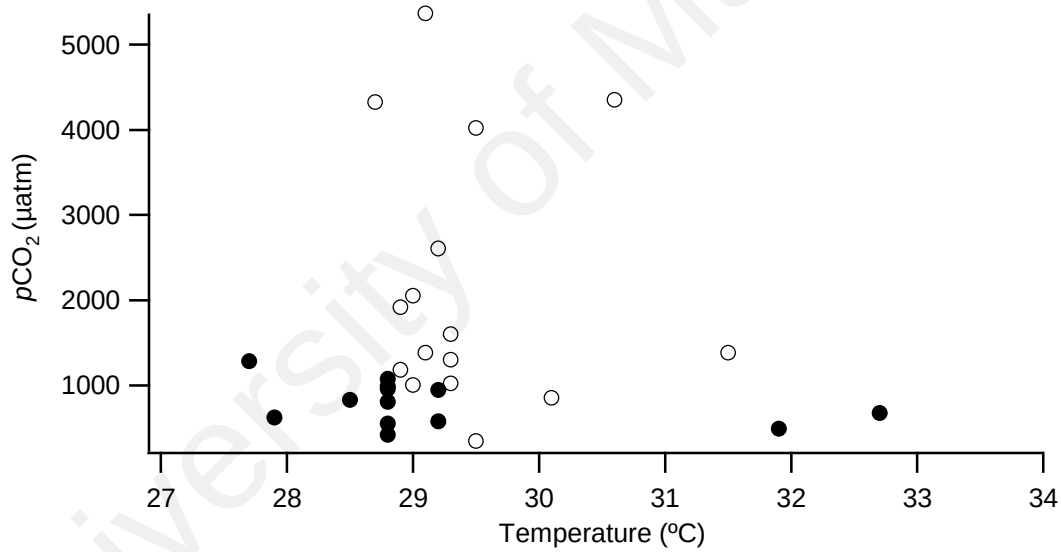


Figure 4.1: Relationship of *partial* carbon dioxide with surface seawater (a) temperature and (b) salinity. Closed circles refer to Port Dickson: Open circles refer to Port Klang.

4.4 CO_2 enrichment experiment

We used our understanding of the data collected from the temporal variation study as the basis for setting future possible pCO_2 concentration. In the experimental setup, gradients in pH were controlled among the treatments by bubbling CO_2 in the seawater within specific dosage time. This method would be a better representation of the natural condition and its effect on the carbonate system compared to the use of acid to acidify the water samples (Gattuso *et al.,* 2010). Although constant bubbling rates were used during the experiment, the uptake of CO_2 into the estuarine and coastal water samples varied accordingly depending on the initial pCO_2 content and the source of the water samples. This may be attributed to the disequilibrium flow rates of the air bubbling CO_2 concentration with the water samples (Yoshimura *et al.*, 2009). The average unit of pH reductions were 0.22 (2 mins), 0.44 (5 mins), 0.74 (5 mins), 0.89 (10 mins) unit of reduction in pH unit in Port Klang and 0.17 (2 mins), 0.40 (5 mins), 0.59 (7 mins), 0.82 (10 mins) in Port Dickson. Henceforward, we refer to the 0, 2, 5, 7 and 10 minutes dosage time based on the calculated pCO_2 values. The pCO_2 chosen in this study were relatively higher in comparison to other studies (James *et al.,* 2017; Endres *et al.,* 2014; Silyakova *et al.,* 2012). Expected change in pCO_2 will result in pH drop by ca. 0.35 units (1000 µatm) by the year of 2100 and may even drop by ca. 0.7 units (1900 µatm) over the next 2000 years (Caldeira & Wickett, 2003; Raven *et al.,* 2005). According to Melzner *et al.* (2013) even at current conditions—when all oxygen was consumed at salinities between 35 to 20 ppt—maximum pCO_2 values of 1700−3200 µatm up to 4,500 µatm can be achieved. However, in the present study, the bacterial community was exposed to a pCO_2 gradient ranging from 305 to 7890 µatm for Port Dickson and 462 to 10255 µatm for Port Klang to represent real changes in pCO_2 in the future at both locations after taking account the temporal range observed and the variability of habitats in this case coastal water and estuarine.

4.5 The relationship between pCO_2 and bacterial activities

In our initial analysis, we pooled the data from both locations to observe general patterns of response of bacterial activities to pCO_2 enrichments (**Figure 3.8**). BP (r^2 = 0.17, P < 0.001, n = 60) and BGE (r^2 = 0.25, P < 0.001, n = 60) showed significant correlations with pCO_2 whilst there was no correlation observed for BR and pCO_2. Our study reveals evidence that pCO_2 induced changes in bacterial production and bacterial growth efficiency, thus could profoundly affect organic matter cycling and sinking flux which may have significant implications for the ocean's response to global climate change. The effect of elevated pCO_2 on bacterial dynamics has been investigated in the previous study. However, they were ambiguous and at time, conflicting. Results from our study agree with studies by Maas *et al.* (2013) on bacterial production, Endres *et al.* (2014), Arnosti *et al.* (2011) on bacterial abundance but is in contrast with other studies (Yamada *et al.*, 2010; Siu *et al.*, 2014).

We next analysed the data from each location separately, searching for common responses to pCO_2 enrichment in each location. BP in Port Dickson ($r^2 = 0.44$, $P < 0.001$, $n = 30$) showed a positive correlation with pCO_2 enrichment while there was no correlation observed in Port Klang ($r^2 = 0.00$, $P > 0.05$, $n = 30$). Uncoupling of BP and pCO_2 in Port Klang may suggest a coping mechanism in the microbial processes with the ever-changing pCO_2 in estuarine (*CV*= 70%). Conversely, we observed coupling between BP and pCO_2 in the coastal water ($r^2 = 0.3$, $P < 0.001$, $n = 30$) suggesting that pCO_2 would positively affect the association between the uptake of energy to regulate respiration and biosynthesis. There was no trend of BR in elevated pCO_2 at both locations. Surprisingly, BGE was positively correlated with elevated pCO_2 in Port Klang ($r^2 = 0.14$, $P < 0.05$, $n = 30$) and not in Port Dickson ($r^2 = 0.09$, $P > 0.05$, $n = 30$). In Port Klang, BGE was correlated with both BP and BR in Port Dickson (BR: $r^2 = 0.14$, $P < 0.001$, $n = 30$; BP: $r^2 = 0.25$, $P < 0.05$, $n = 30$). Based on the r^2 values, we could see that both BP and BR play distinct roles in BGE, and thus would have a different response in coping the environmental changes. Joint *et al.* (2011) recently argued that microbe-dependent processes would not substantially change in a more acidic ocean, as marine microbes already experience large regional, temporal and depth-dependent pH variability. However, this null-hypothesis was challenged by Liu *et al.* (2010). He uses meta-analysis as a tool to derive a conclusion that the microbial processes will be affected either positively or negatively. Given the conflicting arguments in these two settings, the question remains in the different coping mechanisms in these locations, i.e. whether the response of anabolic processes towards elevated pCO_2 was acclimatisation (temporary change) towards the environmental changes or, it was an adaptation (permanent change) in the microbial processes. The answer to this question is very important to determine whether changes in microbial process can compensate for the adverse effect of ocean acidification and maintain the ecosystem services and functions (Sunday *et al.,* 2014).

Although most ocean acidification studies are conducted in a mesocosm setting to replicate a semi-natural environment, it is often tedious and not highly replicated. A small microcosm like ours is important to help provide useful insights on the impact of ocean acidification (Das & Mangwani, 2015) because it is highly replicated (Krause ***et al.,*** 2012).

CHAPTER 5: CONCLUSION

In this study, we have discussed the estimated pCO_2 and its controlling factors in two different environments. The estimates collected in this study is useful as a baseline for future predictions/studies on the response of this region to the increase of anthropogenic carbon dioxide. Our findings help to elucidate the potential bacterial dynamic response to elevated pCO_2 that might potentially affect the biogeochemical cycle of the seawater. Increase in BP and BGE could potentially enhance the role of bacteria as the recycler and mineraliser in the seawater. Future study, combining multiple parameters that could possibly accompany ocean acidification such as an increase in temperature, de-oxygenation eutrophication and alteration of the availability of nutrient and light (stratification) is crucial to understand the effect of ocean acidification in this region holistically. Future research should also focus on the synergistic response of communities that are highly dependent on one another, i.e. phytoplankton and bacteria.

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