PRODUCTION AND LOSS RATES OF PICOCYANOBACTERIA IN TROPICAL COASTAL WATERS

HENG PEI LI

FACULTY OF SCIENCE

UNIVERSITY OF MALAYA

KUALA LUMPUR

2014

PRODUCTION AND LOSS RATES OF PICOCYANOBACTERIA IN TROPICAL COASTAL WATERS

HENG PEI LI

DISSERTATION SUBMITTED IN FULFILLMENT OF THE REQUIREMENTS OF THE DEGREE OF MASTER OF SCIENCE

INSTITUTE OF BIOLOGICAL SCIENCES

FACULTY OF SCIENCE

UNIVERSITY OF MALAYA

KUALA LUMPUR

2014

UNIVERSITI MALAYA

ORIGINAL LITERARY WORK DECLARATION

Name of Candidate: **HENG PEI LI** (I.C/Passport No: **860725-43-5694**)

Registration/Matric No: **SGR 100055**

Name of Degree: **MASTER OF SCIENCE (Except Mathematics & Science Philosophy)**

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

PRODUCTION AND LOSS RATES OF PICOCYANOBACTERIA IN TROPICAL COASTAL WATERS.

Field of Study: **MICROBIAL ECOLOGY**

I do solemnly and sincerely declare that:

(1) I am the sole author/write of this Work;

(2) This Work is original;

(3) Any use of any work in which copyright exists was done by way of fair dealing and for permitted purposes and any excerpt or extract from, or reference to or reproduction of any copyright work has been disclosed expressly and sufficiently and the title of the Work and its authorship have been acknowledged in this Work;

(4) I do not have any actual knowledge nor do I ought reasonably to know that the making of this work constitutes an infringement of any copyright work;

(5) I hereby assign all and every rights in the copyright of this Work to the University of Malaya ("UM"), who henceforth shall be owner of the copyright in this Work and that any reproduction or use in any form or by any means whatsoever is prohibited without the written consent of UM having been first had and obtained;

(6) I am fully aware that if in the course of making this Work I have infringed any copyright whether intentionally or otherwise, I may be subject to legal action or any other action as may be determined by UM.

Candidate's Signature Date

Subscribed and solemnly declared before,

Witness's Signature Date

Name:
Designation:

ABSTRACT

We studied temporal variation of picocyanobacteria and their production (μ) and loss (g) rates for over two years of period at Port Klang (PK) estuarine waters [03º00.1'N, 101º23.4'E] and Port Dickson (PD) coastal waters [02º29.5'N, 101º50.3'E] along the Straits of Malacca. PK waters showed higher level of eutrophication, lower euphotic depth and higher TSS level as compared to PD. Heterotrophic bacterial abundance at PK ($2.78 \pm 1.58 \times 10^6$ cell ml^{-1}) was generally higher than PD ($1.39 \pm 0.49 \times 10^6$ cell ml^{-1}) (Student's t-test: $t = -5.30$, $df = 47$, $p < 0.001$). In contrast, picocyanobacterial abundance at PD ($1.33 \pm 0.47 \times 10^5$ cell ml^{-1}) was always higher than at PK ($0.28 \pm 0.17 \times 10^5$ cell ml^{-1}) (Student's t-test: $t = 10.44$, $df = 30$, $p < 0.001$). μ and g of picocyanobacteria were tightly coupled with each other ($R^2 = 0.47$, $df = 459$, $p < 0.001$) and similar at both sites ($p > 0.05$). μ ranged from – 0.03 to 1.57 d^{-1} while g ranged from 0.12 to 1.80 d^{-1} at PK whereas at PD, μ and g averaged at 0.99 ± 0.28 d^{-1} and 0.83 ± 0.42 d^{-1}, respectively. Temperature limitation was weak at both sampling sites ($p > 0.05$) but there was tight coupling between Secchi depth and abundance of picocyanobacteria at both sites ($R^2 = 0.43$, $df = 45$, $p < 0.01$). Picocyanobacterial abundance also decreased with increasing siltation ($R^2 = -0.70$, $df = 45$, $p < 0.01$) which suggested light availability as a factor for picocyanobacteria distribution. Via a two-factorial experiment, we showed that light had a significant effect on production but only at PD ($F = 5.94$, $p < 0.05$) whereas nutrient enrichment was not an important factor. The contribution of picocyanobacteria to total primary production and net production was also higher at PD which suggested that at PD, environmental conditions were more favourable towards picocyanobacteria as compared to PK.

ABSTRAK

Kami mengkaji variasi picocyanobacteria dan kadar produksi (μ) serta kehilangan (g) mereka selama dua tahun di perairan muara Port Klang (PK) [03º00.1'N, 101º23.4'E] dan perairan pantai Port Dickson (PD) [02º29.5'N, 101º50.3'E] yang terletak di sepanjang Selat Melaka. Perairan PK menunjukkan tahap eutrofikasi yang lebih tinggi, kedalaman eutrofik yang lebih rendah and jumlah pepejal terampai yang lebih tinggi berbanding dengan PD. Bilangan bakteria heterotrofik di PK ($2.78 \pm 1.58 \times 10^6$ sel ml^{-1}) adalah lebih tinggi dari PD ($1.39 \pm 0.49 \times 10^6$ sel ml^{-1}) (Student's *t*-test: $t = -5.30$, $df = 47$, $p < 0.001$). Sebaliknya, bilangan picocyanobacteria di PD ($1.33 \pm 0.47 \times 10^5$ sel ml^{-1}) adalah lebih tinggi berbanding dengan PK ($0.28 \pm 0.17 \times 10^5$ sel ml^{-1}) (Student's *t*-test: $t = 10.44$, $df = 30$, $p < 0.001$). μ dan g untuk picocyanobacteria bergandingan dengan ketat ($R^2 = 0.47$, $df = 459$, $p < 0.001$) dan serupa di kedua-dua tapak persampelan ($p > 0.05$). Di PK, μ adalah di antara – 0.03 dan 1.57 d^{-1} sementara g adalah di antara 0.12 dan 1.80 d^{-1} sementara di PD, purata μ dan g adalah 0.99 ± 0.28 d^{-1} dan 0.83 ± 0.42 d^{-1}. Di kedua-dua tapak persampelan, pembatasan suhu didapati lemah ($p > 0.05$) tetapi gandingan ketat antara kedalaman Secchi dan bilangan picocyanobacteria amatlah jelas ($R^2 = 0.43$, $df = 45$, $p < 0.01$). Penurunan bilangan picocyanobacteria mengikut peningkatan pemendapan ($R^2 = -0.70$, $df = 45$, $p < 0.01$) juga mencadangkan bahawa keadaan cahaya merupakan factor yang mempengaruhi taburan picocyanobacteria. Melalui eksperimen dua-faktorial, kami mendapati bahawa cahaya hanya memberi kesan pada kadar produksi di PD ($F = 5.94$, $p < 0.05$) manakala pengayaan nutrient tidak memainkan perana yang penting. Sumbangan picocyanobacteria terhadap jumlah produksi primer dan produksi bersih yang lebih

tinggi di PD juga menunjukkan bahawa di PD, keadaan alam sekitar adalah lebih sesuai untuk picocyanobacteria berbanding dengan keadaan di PK.

ACKNOWLEDGEMENT

This project would not have been possible without the support from many people. Thus, I would like to express my gratitude to all the people who help me directly or indirectly throughout the whole process. To my supervisor, Associate Professor Dr Lee Choon Weng, for his guidance and advice that make the completion of this project possible. To Dr Bong Chui Wei and my fellow labmates, for the help and supports that keep me going despite all the difficulties I have to endure. To my family and friends, who provided me with their unconditional love and support throughout this process. Finally, to University of Malaya, for the support in funding i.e. research grant PV016-2011A, UM.C/625/1/HIR/050, and MOSTI (04-01-03-SF0671) that allow me to carry out my project smoothly.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDIX

LIST OF SYMBOLS AND ABBREVIATIONS

m	Meter
mm	Milimeter
µm	Micrometer
nm	Nanometer
°N	Degree North
°S	Degree South
°E	Degree East
°C	Degree Celcius
%	Percentage
>	More than
<	Less than
E	Energy
µM	Micromolar
L	Litre
ml	Millilitre
µl	Microliter
rpm	Rotation per minutes
N	Normality
C	Carbon
µmol	Micromole
ppt	Parts per thousand
g	Gram
mg	Milligram

µg	Microgram
pg	Picogram
fg	Femtogram
y	Year
d	Day
h	Hour
s	Second
CV	Coefficient of Variation
S.D.	Standard Deviation
S.E.	Standard Error

INTRODUCTION

Picocyanobacteria are defined as cyanobacteria with sizes between 0.2 to 2.0 µm (Johnson and Sieburth, 1982). Only two genera in picocyanobacteria, *Synechococcus* (**Figure 1.1**) and *Prochlorococcus* (**Figure 1.2**) have been recorded *(*Scanlan *et al*., 2009). *Synechococcus* (0.6 to 2.1 µm in diameter) has a larger average size than *Prochlorococcus* (0.5 to 0.7 µm in diameter). *Prochlorococcus* is the smallest known photosynthetic organism and is believed to be the most abundant photosynthetic organism in the ocean (Morel *et al*., 1993).

Marine picocyanobacteria have small genomes when compared to most pelagic marine bacteria (Scanlan *et al.,* 2009). They are able to lower their cell volume which leads to higher surface area to volume ratio and thus, allowing them to thrive better in resource-limited environment (Raven *et al.,* 1998). Their diversity is largely determined by the physiochemical properties of dominant water masses and resultant trophic conditions (Choi *et al.*, 2011).

Based on 16S rRNA gene sequences (**Figure 1.3**), *Prochlorococcus* is divided based on their light-adaptation to high-light (HL) and low-light (LL) ecotypes. HL-adapted ecotypes are mostly found in surface waters whereas LL-adapted ecotypes are distributed from surface to deep waters in water column. As for *Synechococcus*, they are more genetically diverse and can be divided into three subclusters, with subcluster 5.1 being subdivided into at least 10 genetically distinct clades. Clade I and IV are more commonly found in coastal or temperate mesotrophic open ocean waters above 30 °N and below 30 °S whereas clade III thrives in ultraoligotrophic open ocean waters. Clade II is dominant in the upper euphotic zone of tropical and subtropical oceanic waters (Toledo and Palenik, 2003; Zwirglmaier *et al.*, 2008).

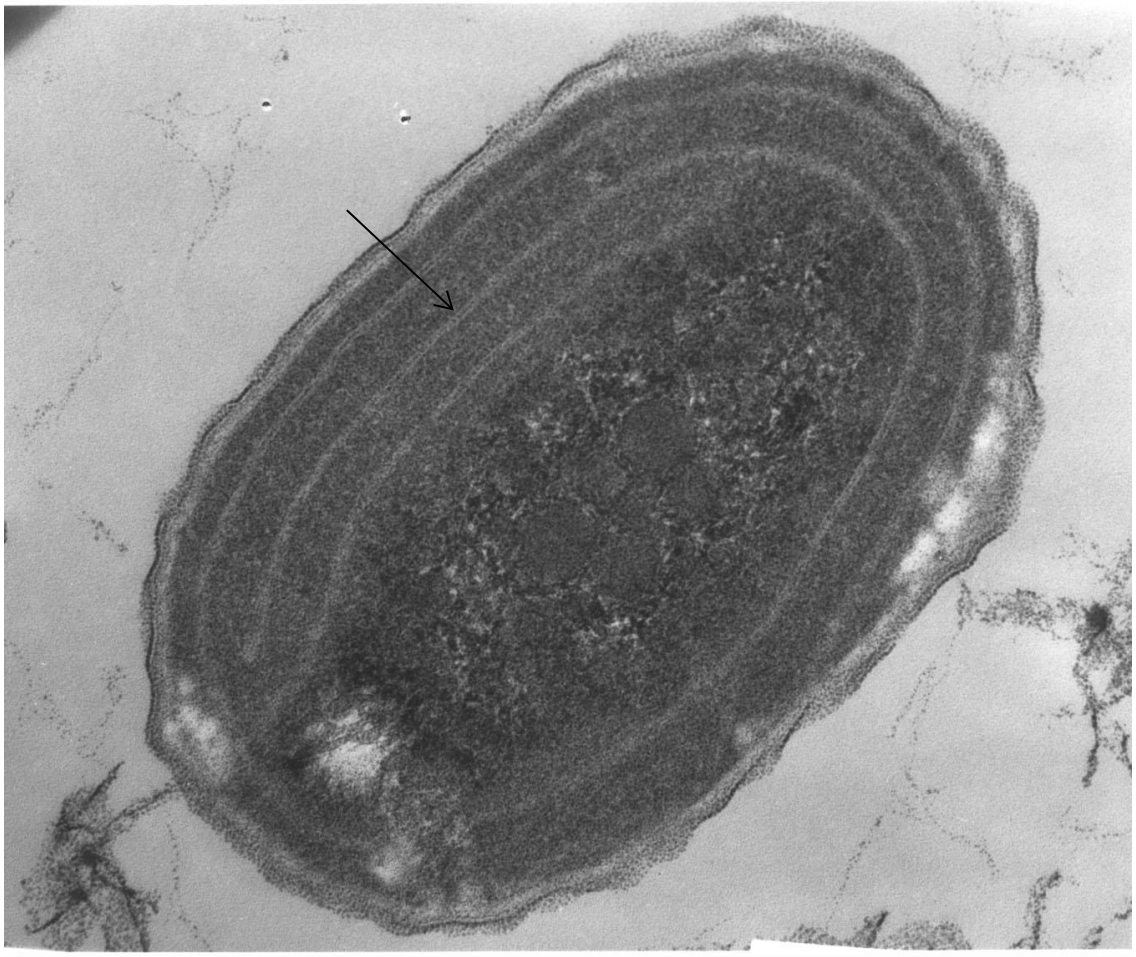

Figure 1.1: Electron micrographs of *Synechococcus* sp. WH7803. The concentric lines at the periphery of the cells are thylakoidal membranes (arrow). They are the centres of photosynthesis, and their number varies inversely with the intensity of light provided during growth (Kana and Glibert, 1987).

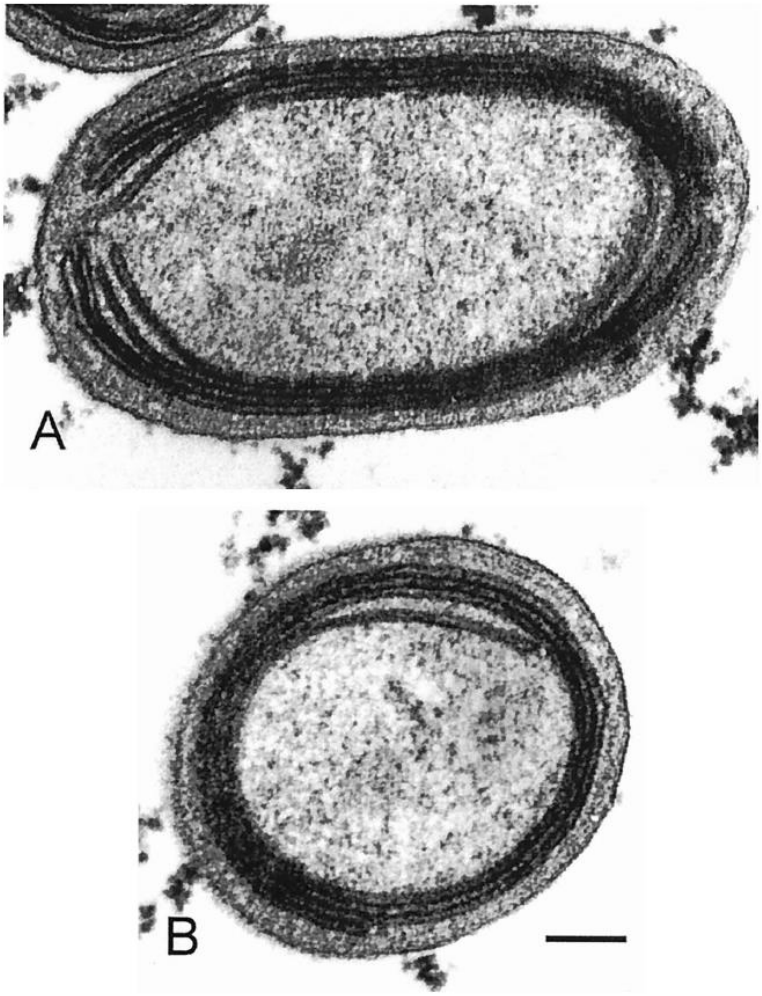

Figure 1.2: Electron micrographs of (A) longitudinal and (B) cross sections of *Prochlorococcus* strain MIT9313 showing tightly appressed thylakoids at the periphery of the cell (Partensky *et al.*, 1999).

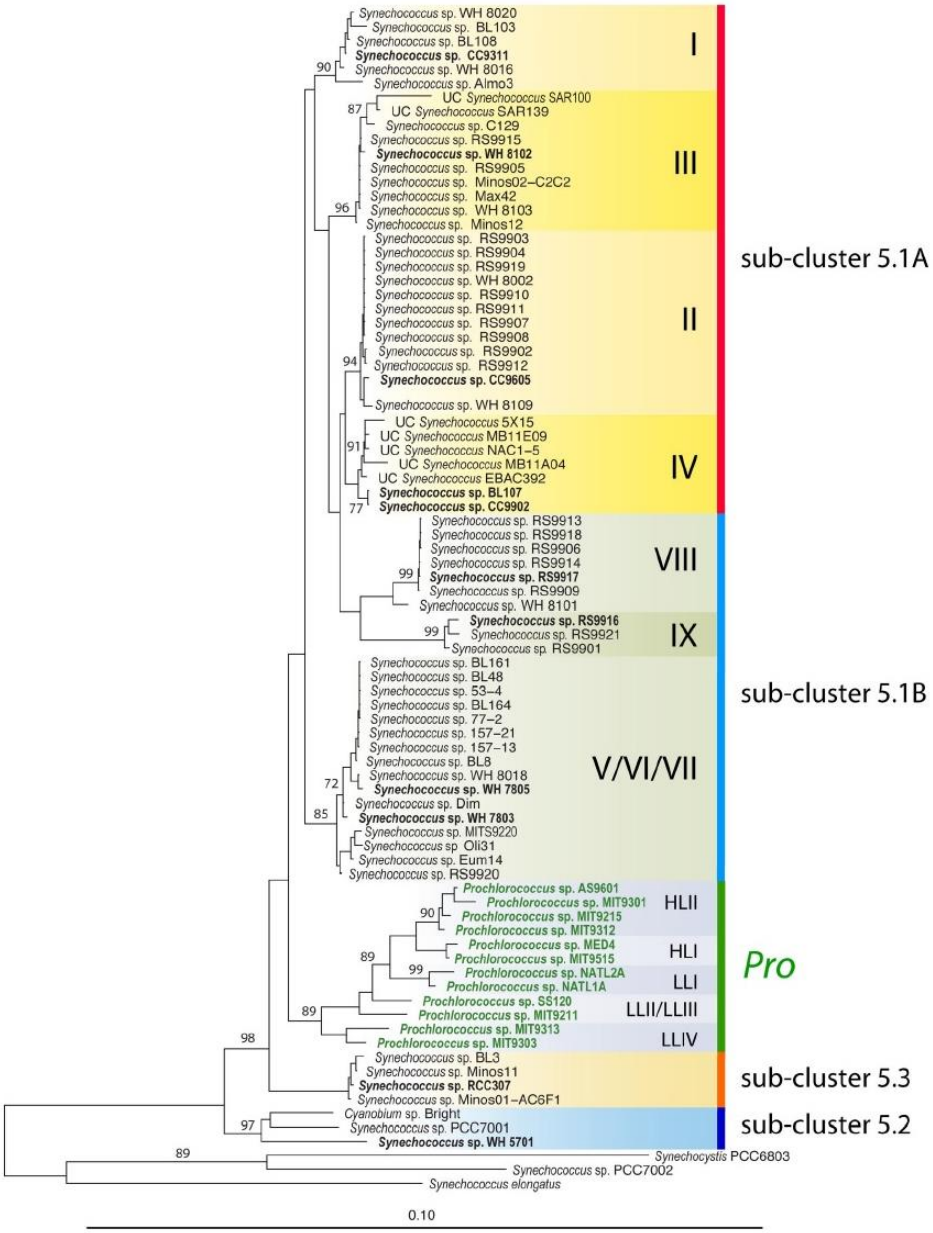

Figure 1.3: Phylogenetic relationships among marine picocyanobacteria based on 16S rRNA gene sequences. Bootstrap values of > 70 % are shown. (Scanlan *et al*., 2009).

1.1 *Contribution and distributions of picocyanobacteria*

The world's oceans are estimated to contribute around half of the net global primary productivity. Of this, approximately 25 % are in oligotrophic regions, which are predominated by picocyanobacteria (West and Scanlan, 1999; Winder, 2009). Recent estimates suggest that marine cyanobacteria could contribute up to 25 % of ocean net primary productivity (Flombaum *et al.*, 2013) and more than 50 % of biomass (**Table 1.1**). In warm and oligotrophic waters, picocyanobacteria are the most important for cycling of carbon and elements in the planktonic food web (Agawin *et al*., 2000a).

Response of phytoplankton in terms of abundance and biomass towards climate change has been studied extensively but reports that focus on the smaller-size primary producers such as picophytoplankton and picocyanobacteria are fairly limited (Morán *et al.*, 2010). The abundance of marine picocyanobacteria is expected to increase while their community structure change as ocean temperature increases due to climate change, though the magnitude differs regionally (Flombaum *et al.*, 2013). The significance of changes caused by climate change is expected to be higher as the magnitude of changes in atmospheric CO_2 concentration and resulting ocean temperature is still unclear.

Distribution of *Prochlorococcus* and *Synechococcus* differs due to the variation in their ability to survive under certain circumstances. *Synechococcus* has a wider regional distribution as compared to *Prochlorococcus* (Partensky *et al.*, 1999). In a study carried out by Flombaum *et al.* (2013), *Synechococcus* is absent in subzero waters but showed peak abundance at mid latitudes. Their highest abundance is found at 10 °C and decreased as temperature increases until 20 °C, after which their abundance show relatively small increase. They are also found to be most abundant at intermediate nutrient and chlorophyll concentrations (Chen *et al*., 2011; Guo *et al.*,

2013). As for *Prochlorococcus*, they are most abundant in warm oligotrophic waters and their importance deteriorates beyond 40 °N and 40 °S region (Flombaum *et al.*, 2013).

Table 1.1: Compiled data from published literature. PP – Total primary production, B – Biomass, C – Chlorophyll.

Reference	**Location**	**Latitude**	**Climate**	**Contribution (%)**	**Abundance (x 10^3cell ml^{-1})**	***μ*** **(d^{-1})**	***g*** **(d^{-1})**
Liu *et al*., 1995	Central Pacific Ocean	22°45'N	Tropical	< 5 (PP)	10.00 – 100.00	0.54 – 0.70	0.20 – 0.39
Reckermann & Veldhuis, 1997	Western Arabic Sea	4° – 16°N	Tropical	NA	43.69 – 142.23	0.40 – 1.12	0.04 – 1.19
Charpy & Blanchot, 1998	South Pacific Ocean	14°30' – 18°03'S	Tropical	1.4 – 73.4 (C) 1.4 – 96 (B) 44.1 (PP)	0.10 – 369.70	NA	NA
Gin *et al*., 2003	Singapore & Johor Strait	1°10' – 1°30'N	Tropical	18.1 – 46.6 (C)	12.40 – 115.20	NA	NA
André *et al.*, 1999	Equatorial Pacific Ocean	0°	Tropical	NA	6.00 – 12.00	0.2 – 0.9	0.2 – 0.9

Table 1.1, continued.

Reference	Location	Latitude	Climate	Contribution (%)	Abundance (x 10^3cell ml^{-1})	μ (d^{-1})	g (d^{-1})
Brown *et al.*, 1999	Arabian Sea	10° – 19°N	Tropical	12.3 – 45.4 (B) 5.9 – 102 (PP)	45.00 – 123.00	0.46 – 1.12	0.33 - 0.72
Agawin *et al.*, 2003	**South China Sea**	**11.10° – 16.55 °N**	**Tropical**	**0.01 – 16.0 (C)**	**0.13 – 4.26**	**0.20 – 1.28**	**NA**
Lee *et al.*, 2006	**Cape Rachado, Malaysia**	**2º24.8'N**	**Tropical**	**NA**	**180 - 1460**	**NA**	**NA**
Liu *et al.*, 2007	Northern South China Sea	18°N	Tropical	60 – 80 (C)	10.00 – 1000.00	NA	NA
Chen *et al.*, 2009	Western South China Sea	11 - 15.75°N	Tropical	NA	43.00 ± 46.00	0.14 - 1.83	0 - 1.04
Nakamura *et al.*, 1993	Seto Inland Sea	34°40'N	Subtropical	NA	7.00 – 57.00	0.585	NA
Affronti & Marshall, 1994	Chesapeake Bay	36°58'N	Subtropical	NA	7.36 – 928.00	0.62	NA

Table 1.1, continued.

Reference	Location	Latitude	Climate	Contribution (%)	Abundance (x 10^3cell ml^{-1})	μ (d^{-1})	g (d^{-1})
Hamasaki *et al*., 1999	Sagami Bay	35°09'N	Subtropical	0.97 – 18 (B) 16 – 45 (PP)	4.20 - 78.00	0.84 - 1.9	NA
Ning *et al.*, 2000	San Francisco Bay	37.64° - 38°N	Subtropical	0.4 - 37.5 (PP)	114.00	NA	NA
Worden & Binder, 2003	Sargasso Sea	26°00' - 38°25'N	Subtropical	NA	7.00 - 42.00	0.42 - 0.69	0.09 - 0.49
Worden *et al*., 2004	Pacific Ocean	32°53'N	Subtropical	NA	33.00 - 100.00	0.52 - 0.86	0.15 - 0.39
Vidal *et al.*, 2007	Atlantic Ocean	34°20' - 34°54'S	Subtropical	4.2 - 96.6 (C)	20.00 – 150.00	NA	NA
Hirose *et al.*, 2008	Uwa sea, Japan	33°2'N	Subtropical	NA	1.20 – 460.00	0.25 - 1.39	0.62 - 1.54
Berninger *et al*., 2005	Gulf of Aqaba	27°30' - 29°30'N	Subtropical	NA	4.50 - 43.50	-2.74 - 0.56	-2.78 - 0.19
Chang *et al.*, 2003	East China Sea	25 - 32°N	Subtropical	5 – 63 (PP)	10.00 – 60.00	0.42	0.21

Table 1.1, continued.

Reference	Location	Latitude	Climate	Contribution (%)	Abundance (x 10^3cell ml^{-1})	μ (d^{-1})	g (d^{-1})
Zhao *et al*., 2013	Yellow Sea, China	33.5 - 37.5 °N	Subtropical	0.13 - 2.19 (B)	1.90 - 10.17	NA	NA
Guo *et al*., 2013	East China Sea	25 - 32°N	Subtropical	2 – 88 (B)	0.74 - 97.63	0.39 - 1.08	0.29 - 1.11
Agawin & Agusti, 1997	Northwest Mediterranean Sea	40°21' -41°37' N	Temperate	NA	1.70 - 12.94	0.23 - 1.76	1.65 ± 0.08
Agawin *et al*., 1998	Mediterranean Bay	41°40'N	Temperate	> 20 (B) > 30 (PP)	0.50 – 70.00	0.2 - 1.5	NA
Jacquet *et al*., 1998	Northwestern Mediterranean Sea	43°41'N	Temperate	NA	43.00	0.69 - 1.25	0.5 - 1.0
Kuipers *et al*., 2003	Faroe-Shetland Channel	60 - 62°N	Temperate	NA	5.00 – 25.00	NA	0.075 - 0.275
Martin *et al*., 2005	Celtic Sea	50°45'N	Temperate	NA	25.00 – 150.00	NA	NA

1.2 *Factors affecting picocyanobacterial distributions*

Both bottom-up and top-down factors are involved in picocyanobacterial distribution but the significance of these factors changes in different regions (Guo *et al.*, 2013). The environmental clines in water temperature, nutrient levels, light availability and grazing (Chang *et al.*, 2003; Hirose *et al.*, 2008; Mackey *et a*l., 2009) is found to alter the distribution and abundance of picocyanobacteria. Physical factors such as temperature and salinity are responsible for composition of picocyanobacteria community whereas other factors such as nutrients, light and grazing control their growth (Uysal, 2001; Vidal *et al.*, 2007).

1.2.1 *Temperature*

Changes in picocyanobacteria community structure seems to be regulated mainly by latitudinal difference which also influences the average temperature (Zhang *et al.*, 2008; Flombaum *et al*., 2013). Also, Morán *et al*. (2010) attributed 73 % of variation in picophytoplankton contribution to total phytoplankton biomass solely to temperature. In temperate studies, *Synechococcus* is more abundant during summer than winter. This is probably due to the observed near maximal growth rates during summer but lower growth rates during winter (Agawin *et al.*, 1998).

In tropical regions where temperature is not limiting, biological activity may not be significantly affected by the small variation in temperature as compared to temperate or subtropical regions (Agawin *et al.*, 1998). However, other studies have shown that microbial activity such as bacterial respiration and viral decay in tropical areas are affected by temperature (Ayukai, 1992; Lee *et al.*, 2009; Lee and Bong, 2012). Lee *et al.* (2013) also showed the frequency of dividing cells (FDC) of picocyanobacteria

were correlated with temperature. Although the effects of temperature are clear in temperate and colder waters, its influence in tropical and warm waters are still unresolved.

1.2.2 *Light*

Light is found to display a rather complex constrain upon picocyanobacteria. In surface waters, division may be controlled negatively by ultra-violet (UV) irradiance (Agawin *et al.*, 2002). *Prochlorococcus* abundance reduces by 30 % at high photosynthetically active radiation (PAR) intensities (>10 E m^{-2} d^{-1}) in tropical surface waters, where photoinhibition or UV radiation damage occurs and lower growth rates and overall abundances (Flombaum *et al.*, 2013). However, a low level of PAR (< 0.06 E m^{-2} d^{-1}) would inhibit their growth as well as this level of light would not be sufficient to support autrophic activity.

Even though high light intensity (>10 E m^{-2} d^{-1}) was shown to have inhibitory effect upon growth in tropical surface waters (Flombaum *et al.*, 2013), light availability could be greatly reduced by siltation introduced by terrestrial run-off in coastal waters (Lee *et al*., 2006). Agawin *et al.* (2003) showed that production and relative biomass of *Synechococcus* decreases along with increasing suspended solids concentrations which reflect the deterioration of water transparency (Schubert *et al.*, 2001). Light attenuation coefficient which increases along siltation gradients as suggested that *Synechococcus* can be light limited even with their ability to survive in low light (Raven, 1998).

1.2.3 *Nutrient*

Nitrogen has been identified as the primary limiting nutrient for phytoplankton on a region-wide and year-round basis. Ammonium is the preferred form of nitrogenous nutrients for *Synechococcus* due to their small size whereas the majority of nitrate uptake is accounted for by large cells (Scanlan *et al.*, 2009). Even though cell abundance could not be directly related to nutrients availability, *Synechococcus* has been reported to respond rapidly to increasing nutrients when other factors are not limiting (Agawin *et al*., 2000b; Uysal, 2001). For example, nutrient limitation was found to be important in *Synechococcus* variation when temperature limitation is absent (Li, 1998). Chang *et al.* (2003) also suggested that at higher temperature (> 16 ℃), nutrients show greater influence.

In contrast, relationship between dissolved inorganic nitrogen (DIN) and picocyanobacetria can be absent in tropical coastal waters where DIN is high (Lee *et al.*, 2013), their growth rates are found to be uncoupled from nutrients (> 8 µM) (Agawin *et al.*, 2000b). In addition, the dominance of picocyanobacteria decreases as Chl *a* and nutrient concentration increases (Agawin *et al.*, 2000a).

1.2.4 *Loss processes*

Loss processes such as grazing and viral lysis play a crucial role in tropical coastal waters (Agawin *et al.*, 2003). Activities of protozoan grazers are found to be one of the major controls of picocyanobacteria distribution as they have great influence on loss processes (Agawin and Agusti, 1997; Guo *et al.*, 2013). However, grazing rates are controlled by temperature, and a larger fraction of *Synechococcus* is consumed in warmer waters (Chang *et al*., 2003).

In addition, an increase in nutrients may also improve the nutritional quality of picocyanobacteria as grazing mortality of picocyanobacteria increased with nutrient addition (Worden and Binder, 2003). On the other hand, viral lysis which is responsible for about 30 % of cyanobacteria mortality (Proctor and Fuhrman, 1990) is not triggered by increased nutrients. Viral lysis is dependent upon the ambient population of host and cyanophage, along with the temperature and level of productivity (McDaniel and Paul, 2005).

1.3 *Picocyanobacteria in tropical waters.*

Picocyanobacteria can contribute more than 50 % of the biomass and primary production in warm oligotrophic tropical and subtropical open oceans (**Table 1.1**) but when total Chl *a* concentrations exceed 1 μg L^{-1}, their importance reduce significantly (Veldhuis *et al*., 2005). As observed in tropical open oceans, *Prochlorococus* is the most abundant primary producer while *Synechococcus* is constantly less than 10 % of the phototrophic biomass (Blanchot *et al.*, 2001). But as tropic condition shifts near shores, *Synechococcus* replaces *Prochlorococcus* as the dominant contributor towards primary production and ultraphytoplankton biomass (Chen *et al*., 2009).

In coastal waters of South China Sea, *Synechococcus* is in the lower range in terms of abundance and biomass and is suggested to be a minor contributor to primary producers (**Table 1.1**; Agawin *et al*., 2003; Lee *et al.*, 2006). Although this could be attributed to the reduction in size of picocyanobacteria as temperature increases (Morán *et al.*, 2010), the higher contribution to primary production by picocyanobacteria despite the lower biomass contribution suggests that picocyanobacetria are contributing more in coastal waters towards carbon cyling (**Table 1.1**: Hamasaki *et al*., 1999). The importance of picocyanobacteria in tropical coastal waters could be greater than expected.

Picocyanobacterial distribution is affected by short-term episodic and human-derived disturbances which are common in coastal waters. However, concurrent measurements of picocyanobacterial production and loss rates are rare, and virtually absent especially in Sunda Shelf waters (Agawin *et al*., 2003).

By using Landry and Hasset dilution method, we measured picocyanobacterial production and loss rates concurrently. In this approach, prey consumption is assumed to be directly proportional to the abundance of prey present. As dilution will reduce the

encounter rates between grazers and prey, grazing pressure reduced as dilution increased, and thus encouraged the growth of picocyanobacteria. Production and grazing/loss rates are then derived.

With the availability of both production and loss rates, we could establish if increased loss rates caused a reduction in picocyanobacteria in productive waters. The availability of these rates could also help us constrain the magnitude of carbon fluxes mediated by picocyanobacteria as picocyanobacterial grazing loss rates can vary over a wide range (up to 1.65 d^{-1}), and differ among study sites. Therefore our study fills an important data gap in our quest to understand the ecology of picocyanobacteria.

OBJECTIVES

Even though picocyanobacteria serves as important primary producer worldwide, information on their distribution, production and loss rates in tropical waters, is relatively limited. In Malaysia, only two studies are available i.e. on their diel variation in mangrove waters (Lee *et al*., 2006) and their spatial distribution in tropical estuary (Lee *et al.*, 2013). As their importance was found to vary across different regions, it is important for us to investigate their distribution. Therefore, the objectives of this study are

i. To investigate the temporal variation of picocyanobacterial abundance in tropical coastal waters.
ii. To determine the balance between picocyanobacterial production and loss rates in tropical coastal waters.

MATERIALS AND METHOD

2.1 *Sampling sites*

Surface seawater samples (about 0.1 m depth) were collected monthly at nearshore stations (**Figure 2.1**) i.e. Port Klang (PK) estuarine waters [03º00.1'N, 101º23.4'E] and Port Dickson (PD) coastal waters [02º29.5'N, 101º50.3'E] along the Straits of Malacca. Port Klang is an estuarine located at the mouth of Klang river.In previous study, it was found to have high eutrophication caused by rapid development and industrialization taking place upstream (Lee *et al*., 2009). As for Port Dickson, it is a beach and holiday destinations for tourists where it is less polluted as compared to Port Klang. Sampling was carried out for about two years from March 2010 until March 2012 i.e. March 2010 until February 2011 for first year and March 2011 until March 2012 for second year (PK: n = 26; PD: n = 25).

2.2 *Sample collection*

Physical parameters such as salinity, temperature and water transparency were measured in-situ. Seawater temperature and salinity and were measured in-situ using a digital thermometer (Comark, USA) and a conductivity meter (YSI-30, USA), respectively whereas water transparency was measured as Secchi disc depth. pH was measured using a pH meter (Thermo Scientific, Orion 4 star, USA) upon arrival in the laboratory. Samples were also collected for dissolved oxygen (DO) concentration measurements via the Winkler method (Grasshoff *et al*., 1999).

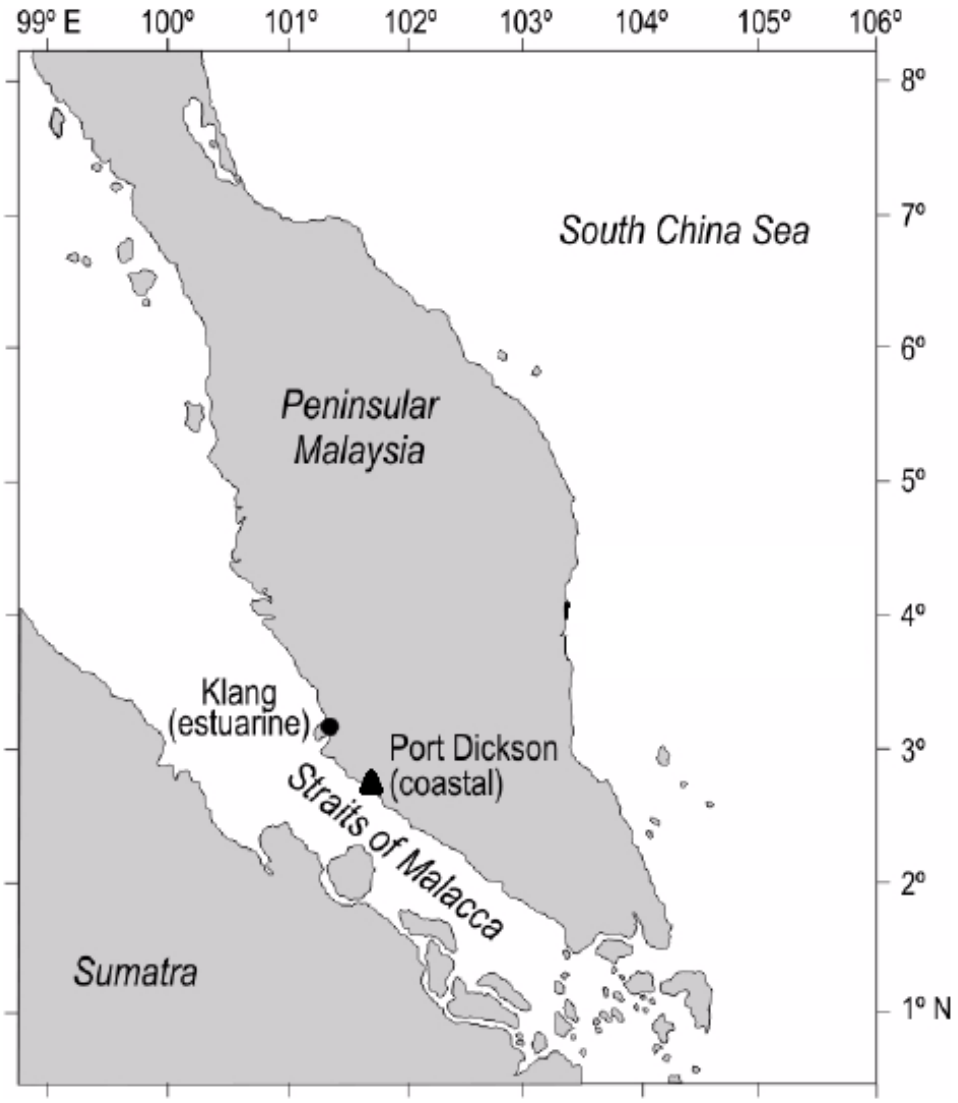

Figure 2.1: Location of sampling stations at Port Klang (filled circle) and Port Dickson (filled triangle) (adapted from Lee *et al.*, 2009).

2.3 *Total suspended solids*

In the laboratory, a known volume of samples (V) were filtered through preweighed (W1) Whatman GF/F filters (precombusted at 500 °C for 3 hours) and the filters were then dried at 50 °C until a constant reading (W2) was obtained. Total suspended solids (TSS) were measured as a net increase in weight.

$$\text{TSS (mg L}^{-1}) = \frac{W2 - W1}{V}$$

2.4 *Dissolved oxygen (DO) concentration* (Grasshoff *et al*., 1999)

Samples were collected in triplicate and fixed immediately with manganese (II) chloride and alkaline potassium iodide solution. Samples were then mixed well to allow formation of hydroxide precipitate and brought back to laboratory. In the laboratory, the hydroxide precipitate was dissolved by acidification to a pH between 1 and 2.5. Titration with thiosulphate solution was carried out and the endpoint of titration was indicated by using starch indicator. Dissolved oxygen concentration was calculated from the volume of titrant used.

2.5 *Chlorophyll a (Chl a) concentration*

A known volume of samples were filtered through precombusted (500 °C, 3 h) GF/F filter (47mm diameter, Whatman) and filters were kept frozen at – 20 °C until analysis. Chl *a* was extracted from the filters using 90% ice-cold acetone at – 20 °C overnight and the samples were centrifuged at 3000 rpm for 5 minutes. Absorbance of extracted Chl *a* was read using spectrophotometer (Hitachi U – 1900, Japan) at 750, 665, 664, 647, 630, 510 and 480 nm. A drop of 0.1 N hydrochloric acid (HCl) was added for phaeopigment correction and the absorbance was read again at 750 and 665

nm. Chl *a* concentration was calculated according to Parsons *et al.* (1984). The concentrations were measured in triplicates for each sampling.

2.6 *Dissolved inorganic nutrient*

Dissolved inorganic nutrient concentrations [phosphate (PO_4), silicate (SiO_4), nitrate (NO_3), nitrite (NO_2), ammonium (NH_4)] were measured according to Parsons *et al*. (1984). Absorbance was measured using spectrophotometer (Hitachi, U-1900, Japan), and all measurements were in triplicates.

For PO_4 test, compound formed by PO_4 under acidic condition was reduced by ascorbic acid with addition of reagent containing potassium antimonyl tartrate. Phosphomolybdenum blue was formed and the absorbance was measured at 880 nm.

For SiO_4 test, formation of silicomolybdate complex from SiO_4 was allowed. The complex was then reduced to produce a blue solution. The colour intensity was then recorded at 810 nm wavelength.

As for NH_4 test, NH_4 reacted with alkaline phenol and hypochlorite to form indophenol blue dye. Sodium nitroprusside was then added to strengthen the dye formation and the absorbance was measured at 640 nm.

NO_2 was allowed to react with sulfanilamide to form diazo compound. α-naphthyl-ethylenediamine hydrochloride was then added to react with diazo compound to form diazo dye which its intensity was measured at 543 nm.

To determine NO_3 concentrations, sample was run through a copper-cadmium column for reduction of NO_3 to NO_2. Increase in NO_2 concentration was measured as NO_3 concentration.

2.7 *Bacterial abundance*

Bacterial abundance was measured via direct enumeration under epifluorescence microscope. Samples were collected with sterile polypropylene tube and were preserved *in-situ* using glutaraldehyde (1% final concentration). The samples were then kept on ice until processing within four hours. Upon arrival in laboratory, samples were filtered onto a black polycarbonate filter (0.22 µm, Millipore) and then stained with 4'6 – diamidino – 2 – phenylindole (DAPI, 1 µg ml^{-1} final concentration) for 7 minutes (Kepner and Pratt, 1994). A minimum of 30 random fields or 300 cells were observed under epifluorescence microscope (Olympus BX60, Japan) with a U-MWU filter cassette (exciter 330 – 385 nm, dichroic mirror 400 nm, barrier 420 nm). Each field was also viewed under the U-MWG filter cassette (exciter 510 – 550 nm, dichroic mirror 570 nm, barrier 590 nm) for autofluorescence of chlorophyll pigment to eliminate phototrophs from our count.

2.8 *Picocyanobacterial abundance* (Agawin *et al.*, 2003)

Samples were collected using sterile polypropylene tubes and preserved using glutaraldyhyde (1% final concentration) *in-situ*. Samples were kept on ice until processing (within 4 hours). Upon arrival in laboratory, samples were filtered through 0.22 µm black polycarbonate filters (25 mm diameter, Millipore) and the filters were mounted with immersion oil. Then, direct enumeration of autofluorescing cells were carried out under epifluorescence microscope (Olympus BX60, Japan) with U-MWG filter cassette (exciter 420 – 490 nm, dichroic mirror 510 nm, barrier 520 nm) (Agawin *et al.*, 2003). A minimum of 50 fields or 500 cells were observed.

2.9 *Production (μ) and loss rates (g) of picocyanobacteria*

Determination of production and loss rate of picocyanobacteria was based on the dilution method of Landry and Hassett (1982). Water samples were collected with cleaned bottles. In the laboratory, samples were filtered through 0.2 μm filter to obtain particle-free water (< 0.2 μm). The particle-free seawater was then used to dilute the natural seawater samples to 0.2, 0.4, 0.6, 0.8 and 1.0 (undiluted) fractions. Each dilution were duplicated and incubation was then carried out at *in-situ* temperature for 16 h. We found this experimental setup to be no different from a 16 h light: 8 h dark incubation (Student's *t*-test: $t = 1.78$, $df = 8$, $p = 0.11$) and 12 h light: 12 h dark incubation regime (Student's *t*-test: $t = 1.95$, $df = 9$, $p = 0.08$). Production rate of picocyanobacteria for each dilution (μ_i) was then calculated according to formula,

$$\mu_i = \ln N_t - \ln N_0$$

where N_t is the picocyanobacteria cell abundance after incubation and N_0 is the initial picocyanobacteria cell abundance. Production (μ) and loss rate (g) were then derived from linear regression analysis of production rate against dilution factor (**Figure 2.2**), where Y-axis intercept is μ and gradient of negative slope is g. For the carbon conversion of both μ and g, biovolume of picocyanobacteria was measured and converted using a conversion factor of 0.123 pg C μm^{-3} (Agawin *et al.*, 2003).

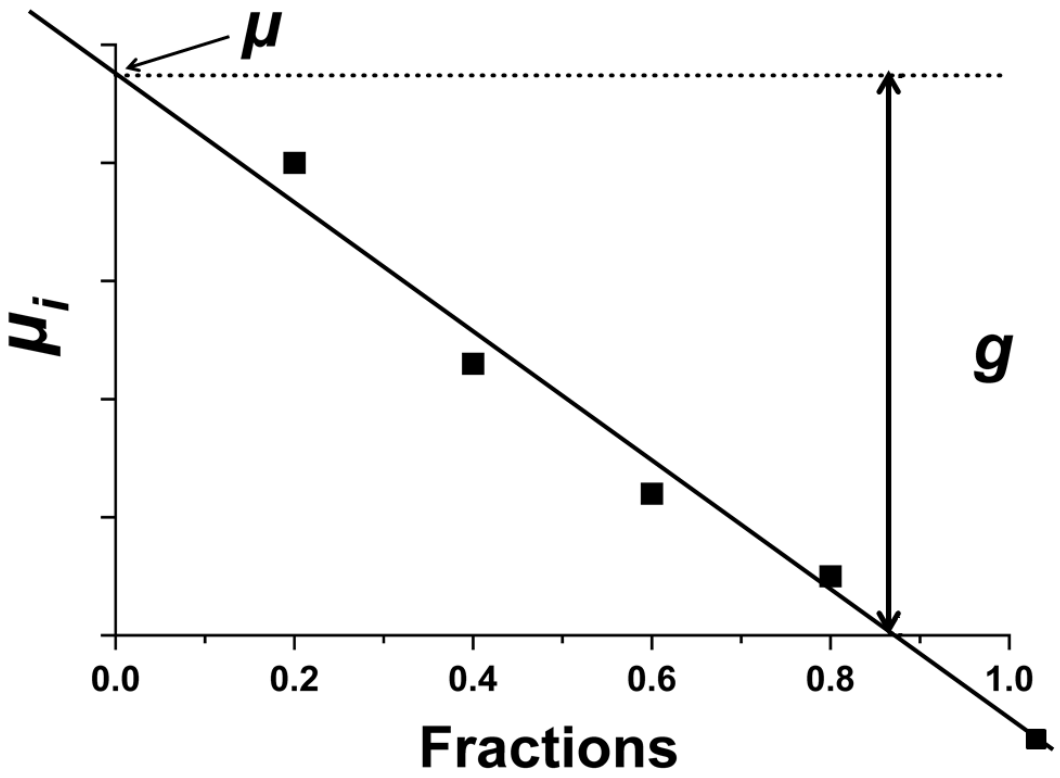

Figure 2.2: Example of linear regression analysis for Landry and Hassett (1982)

2.10 *Two-factorial experiment*

The effects of light and inorganic nutrients on μ and g rates were investigated using a full factorial two level experimental design. For light as a factor, we used 100 µmol m^{-2} s^{-1} and 340 µmol m^{-2} s^{-1} as the two levels whereas for inorganic nutrients, we used one control and one enriched with both 5 µM NH_4Cl and 1 µM NaH_2PO_4.This experimental setup was carried out six times at each station.

2.11 *Statistical analysis*

Unless otherwise mentioned, all values were reported as mean ± standard deviation (S.D.). In order to compare the different parameters between Port Klang and Port Dickson, Student's *t*-test was used. Correlation was used to show relationships between the different parameters measured. All statistical tests including the full factorial two level experiments were carried out with the software PAST (Hammer *et al*., 2001) unless otherwise stated.

RESULTS

3.1 *Physico-chemical analysis*

Seawater temperature showed similar fluctuations at both site (**Figure 3.1**), ranging from 27 °C to 32 °C. Port Klang (7.60 ± 0.28) showed significantly lower pH as compared to PD (7.88 ± 0.19) (**Figure 3.1**: Student's *t*-test: $t = 4.18$, $df = 44$, $p < 0.001$). Salinity (**Figure 3.1**) was higher and fluctuated over a wider range in PK ($CV > 25$ %) (from 12 to 31.6 ppt) whereas salinity at PD varied within a narrower range ($CV < 10$ %) (between 20.8 and 33.8 ppt). (Student's *t*-test: $t = 2.11$, $df = 35$, $p < 0.05$).

Total suspended solids (**Figure 3.2**) (Student's *t*–test: $t = 3.20$, $df = 42$, $p < 0.01$) varied from 36.4 to 85.8 mg L^{-1} at PD whereas at PK, TSS was higher, ranging from 33.6 to 93.6 mg L^{-1}. Water was also clearer at PD where Secchi disc depth ranged from 0.29 m to 1.78 m (**Figure 3.2**) (Student's *t*-test: $t = -5.15$, $df = 31$, $p < 0.001$). **T**emporal variations of DO at both sites are shown in **Figure 3.3.** Dissolved oxygen at PK (147.30 ± 30.33 µM) was lower than PD (203.51 ± 11.74 µM) (Student's *t*-test: $t = 8.57$, $df = 32$, $p < 0.001$).

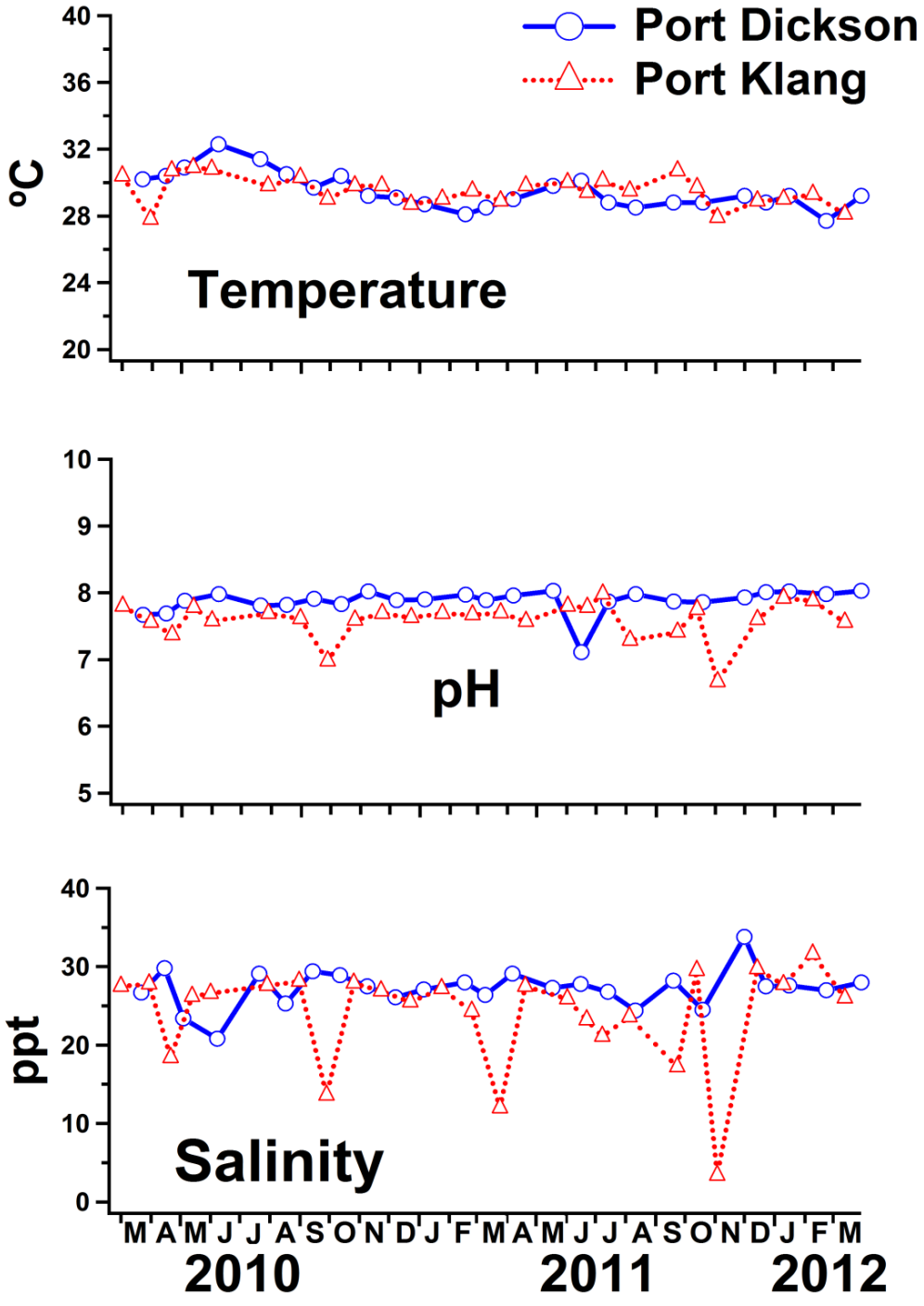

Figure 3.1: Temporal variation of seawater temperature, pH and salinity observed at PD and PK.

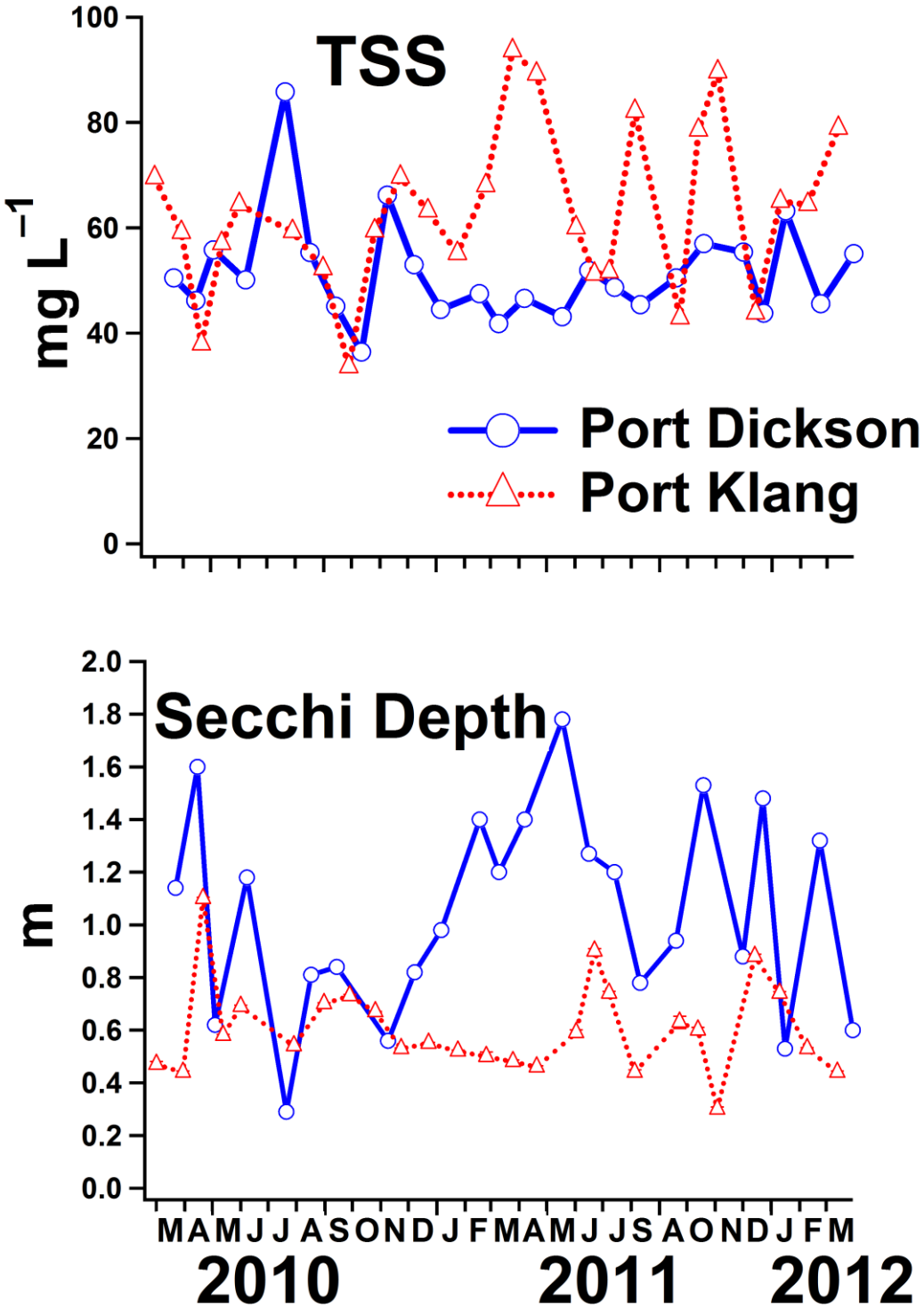

Figure 3.2: Temporal variation of TSS and Secchi depth at PD and PK.

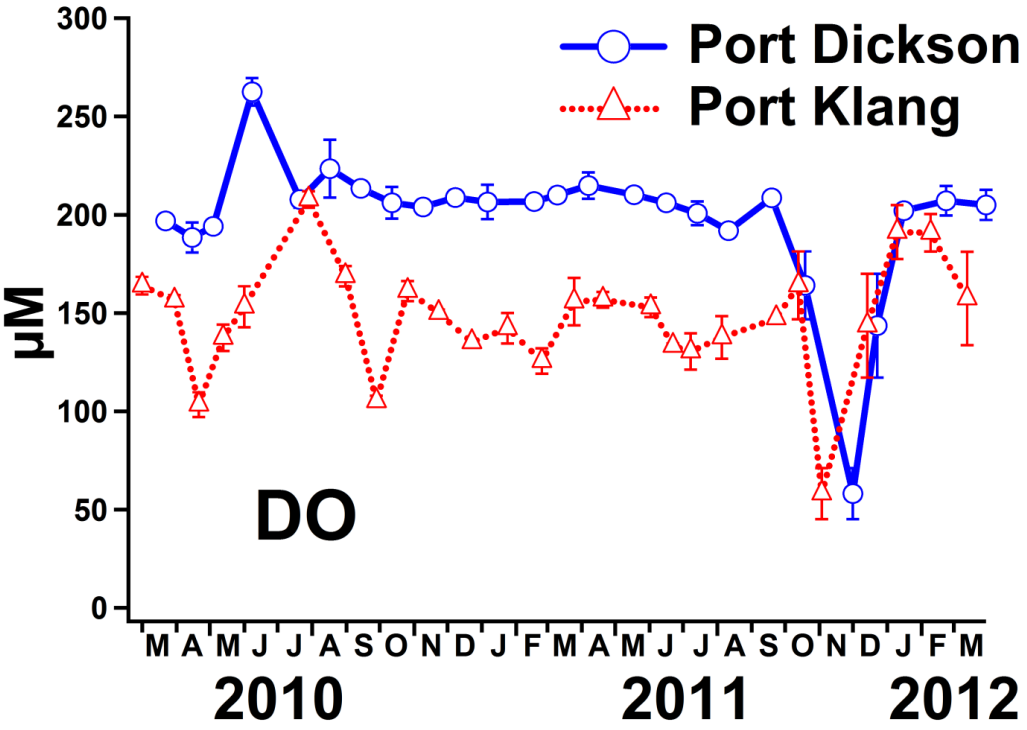

Figure 3.3: Temporal variation of dissolved oxygen (DO) concentration at PD and PK. DO were measured in triplicates. The error bars indicated the S.D. except when the error bar was smaller than the symbol

For Chl *a* variation (**Figure 3.4**), there was no significant difference between PK (range of 0.20 to 30.00 µg L^{-1}) and PD (range of 0.10 to 2.50 µg L^{-1}) (Student's *t*-test: $p < 0.05$). PK showed higher fluctuation of Chl *a* concentration ($CV > 85$ %) than PD. Two peaks were observed at PK on September and November 2011 at 26.31 and 11.97 µg L^{-1}, respectively. Compared to PK (range of 0.20 to 30.00 µg L^{-1}), PD was more stable ($CV < 45$ %) (range of 0.10 to 2.50 µg L^{-1}). Highest Chl *a* concentration at PD was recorded on September 2011 (2.54 ± 0.51 µg L^{-1}) which was around the same time as one of the Chl *a* peaks at PK.

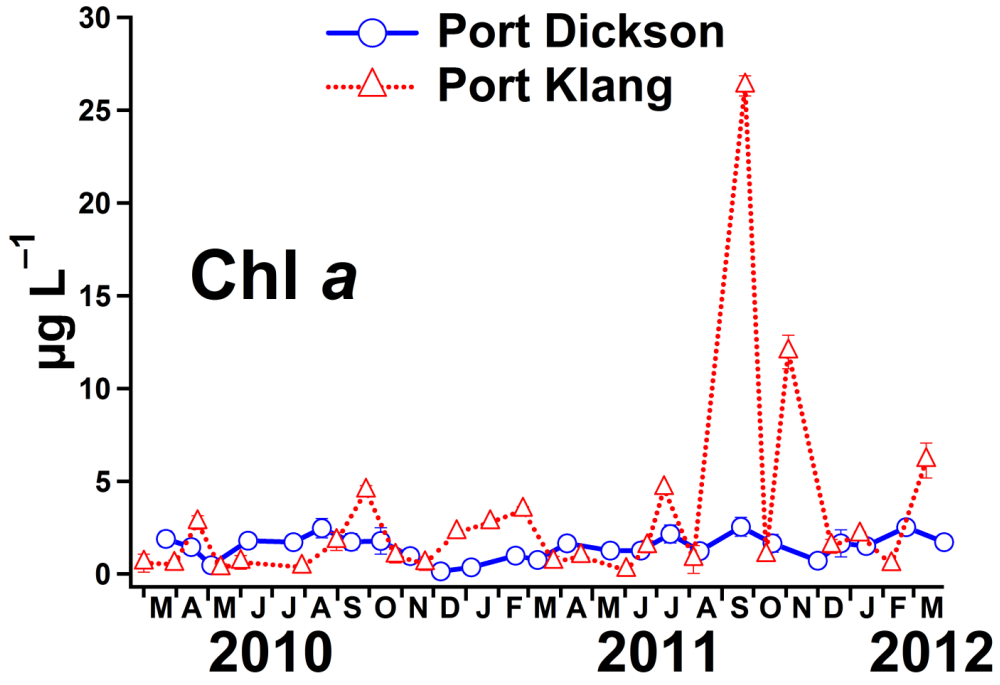

Figure 3.4: Temporal variation of Chl *a* concentration at PD and PK. All measurements were in triplicates. The error bars indicated the S.D. except when the error bar was smaller than the symbol.

3.2 *Dissolved inorganic nutrients*

Ammonium (NH_4), nitrate (NO_3), nitrite (NO_2), and silicate (SiO_4) concentrations recorded were significantly higher at PK compare to PD. Phosphate (PO_4) (**Figure 3.5**) showed no difference ranging from 0.55 to 2.48 µM in PK and 0.84 ± 0.83 µM in PD ($p > 0.05$). As for SiO_4 (**Figure 3.5**), PK (21.73 ± 14.71 µM) showed higher concentrations compare to PD (8.78 ± 5.36 µM) (Student's *t*-test: $t = 3.49$, $df = 22$, $p < 0.005$). Highest SiO_4 concentration was recorded at PK during September 2010.

NH_4 served as the main component of dissolved inorganic nitrogen (DIN) at PK and PD, accounting for 52 % and 56 % of DIN, respectively. **Figure 3.6** shows that NO_2 (Student's *t*-test: $t = -8.70$, $df = 25$, $p < 0.001$), NO_3 (Student's *t*-test: $t = -8.12$, $df = 30$, $p < 0.001$) and NH_4 (Student's *t*-test: $t = -3.51$, $df = 25$, $p < 0.01$) were significantly lower at PD as compared to PK. NO_2 ranged from 1.06 to 6.65 µM and 0.01 to 0.43 µM at PK and PD, respectively. Relatively higher range of NO_3 were observed at both PK (1.23 to 11.11 µM) and PD (0.04 to 2.91 µM). As for NH_4, peaks were recorded in the month of September at PK with a concentration of 61.92 µM and 78.04 µM in 2010 and 2011, respectively. Average concentration of NH_4 at PK (range of 0.14 to 78.04 µM) was higher than PD (range of 0.16 to 3.65 µM).

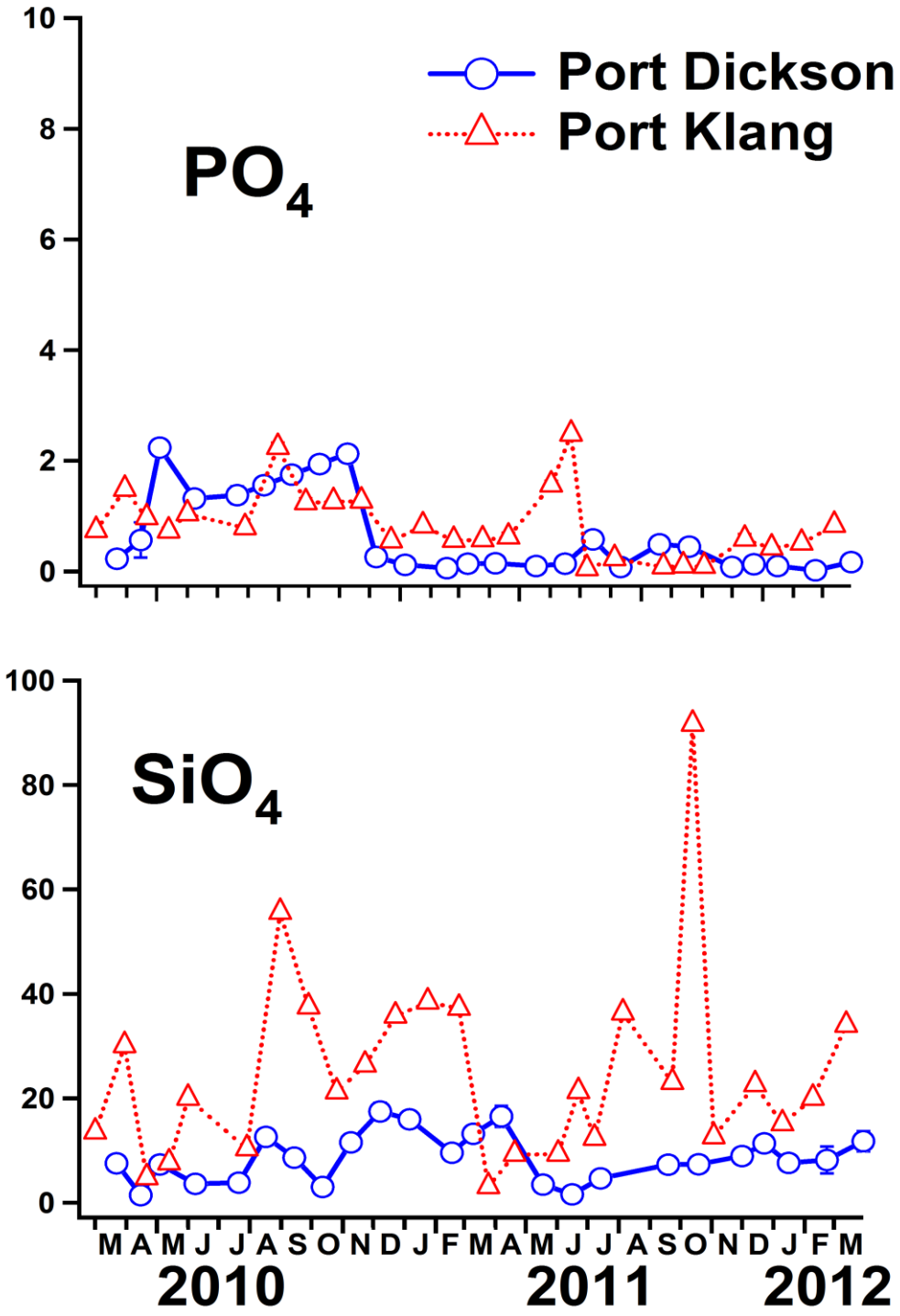

Figure 3.5: Temporal variation of phosphate (PO_4) and silicate (SiO_4) measured at PD and PK. All measurements were in triplicates. The error bars indicated the S.D. except when the error bar was smaller than the symbol.

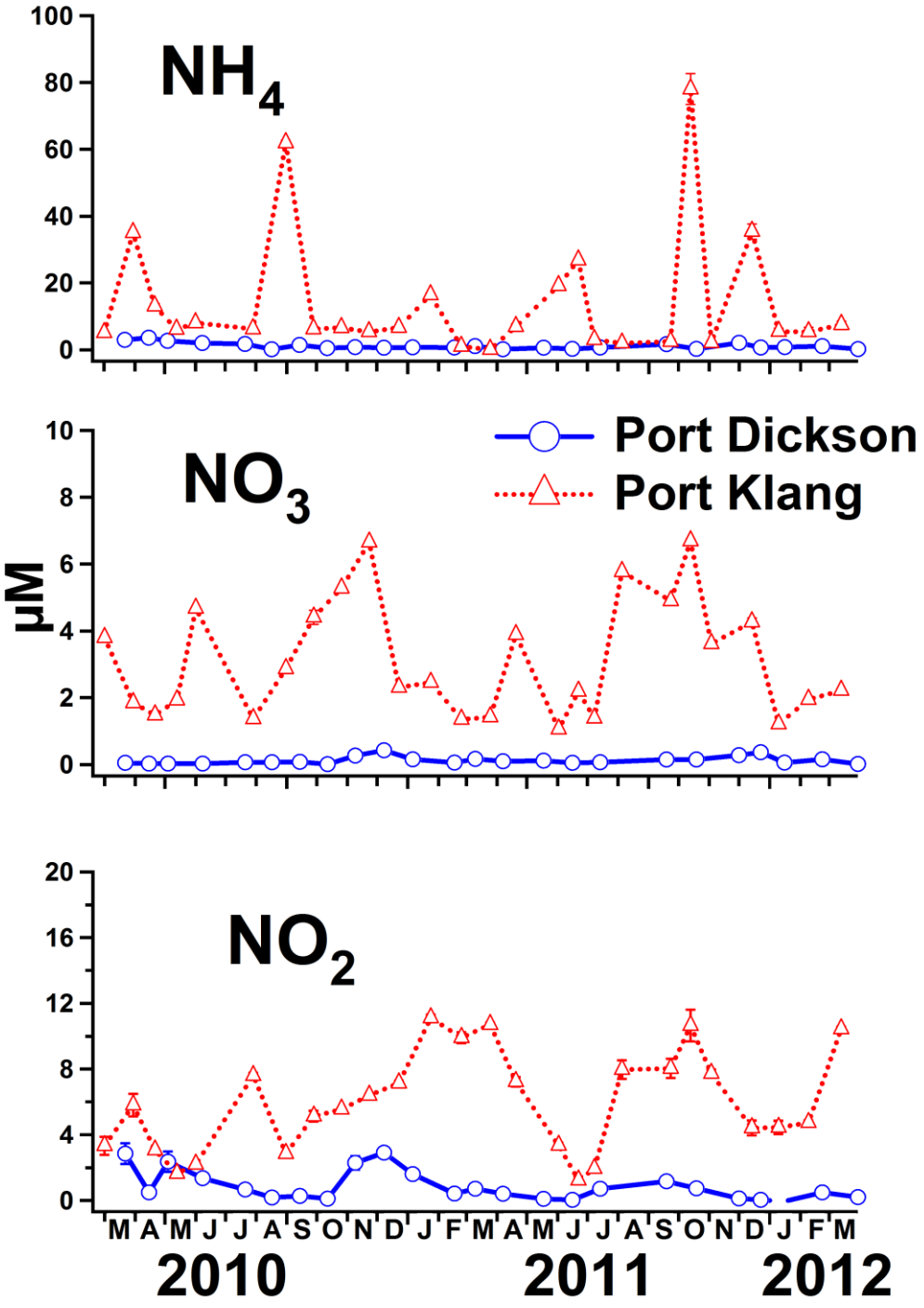

Figure 3.6: Temporal variation of nitrite (NO_2), nitrate (NO_3) and ammonium (NH_4) observed at PD and PK. All measurements were in triplicates. The error bars indicated the S.D. except when the error bar was smaller than the symbol.

3.3 *Temporal variation of bacteria*

Port Klang showed significantly higher bacterial abundance (**Figure 3.7**) as compared to PD (Student's *t*-test: $t = -5.30$, $df = 47$, $p < 0.001$) with an average of 2.78 ± 1.58 × 10^6 and 1.39 ± 0.49 × 10^6 cell ml^{-1}, respectively. Bacterial abundance at PK ranged from 1.17 × 10^6 to 7.92 × 10^6 cell ml^{-1} whereas at PD, bacterial abundance ranged from 0.42 × 10^6 to 2.80 × 10^6 cell ml^{-1}.

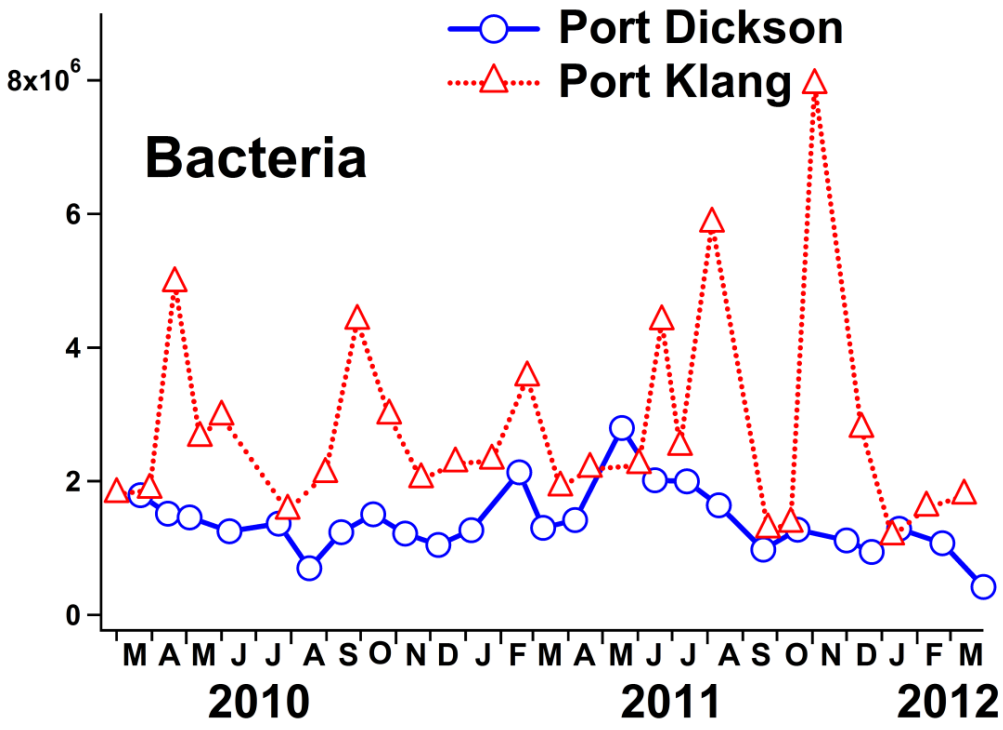

Figure 3.7: Temporal variation of bacterial abundance at PD and PK.

3.4 *Temporal variation of picocyanobacteria*

Picocyanobacteria abundances (**Figure 3.8**) were significantly different at both sampling sites with a higher average count recorded at PD (Student's *t*-test: $t = 10.44$, $df = 30$, $p < 0.001$). Abundance of picocyanobacteria at PD averaged at $1.33 \pm 0.47 \times 10^5$ cell ml^{-1} as compared to PK ($0.28 \pm 0.17 \times 10^5$ cell ml^{-1}). At PD, highest count for 2010 and 2011 (2.35×10^5 and 2.66×10^5 cell ml^{-1}, respectively) was observed in the same month (May 2010 and May 2011) whereas lowest counts for both years (0.78×10^5 and 0.30×10^5 cell ml^{-1}, respectively) was also recorded around the same period of time (October 2010 and October 2011). Similar trend was observed at PK. Counts obtained in June 2010 and June 2011 were highest for both year (0.71×10^5 and 0.62×10^5 cell ml^{-1}, respectively) whereas lower counts were observed around September for both year 2010 (0.09×10^5 cell ml^{-1}) and 2011 (0.03×10^5 cell ml^{-1}).

Picocyanobacterial biovolume from both sites were measured and found to range from 0.478 to 4.780 μm^3. Picocyanobacterial biovolume did not show any temporal variation at both PD ($p > 0.05$) and PK ($p > 0.15$). However the picocyanobacterial biovolume at PK was significantly larger than at PD (Student's *t*-test: $t = -3.72$, $df = 10$, $p < 0.01$). Carbon conversion factors at PK and PD were calculated at 257 ± 47 and 175 ± 32 fg $cell^{-1}$, respectively.

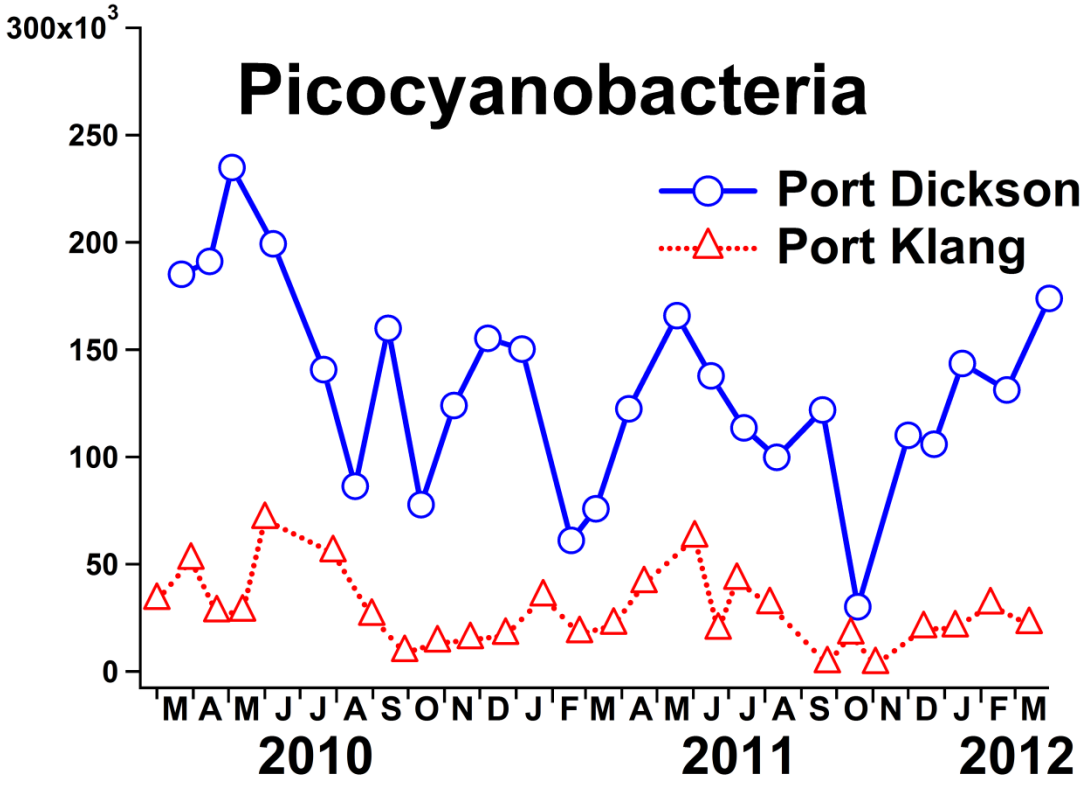

Figure 3.8: Temporal variation of picocyanobacterial abundance at PD and PK.

3.5 *Production (μ) and loss rates (g) of picocyanobacteria*

Production (μ) and loss (g) rates of picocyanobacteria (**Figure 3.9**) showed no significant differences between both sampling sites. However, fluctuation was found to be higher for μ at PK (CV = 55%) compared to PD (CV = 28%). Port Klang recorded μ ranging from – 0.03 to 1.57 d^{-1} and g ranging from 0.12 to 1.80 d^{-1}. Highest μ was recorded on December 2011 whereas highest g was detected on June 2011 (1.57 and 1.80 d^{-1}, respectively). At PD, μ and g averaged at 0.99 ± 0.28 d^{-1} and 0.83 ± 0.42 d^{-1}, respectively. Highest μ was detected in March 2011 (1.77 d^{-1}) whereas highest g was recorded in June 2011 (1.76 d^{-1}). g :μ ratio was similar at both sites ($p > 0.05$).

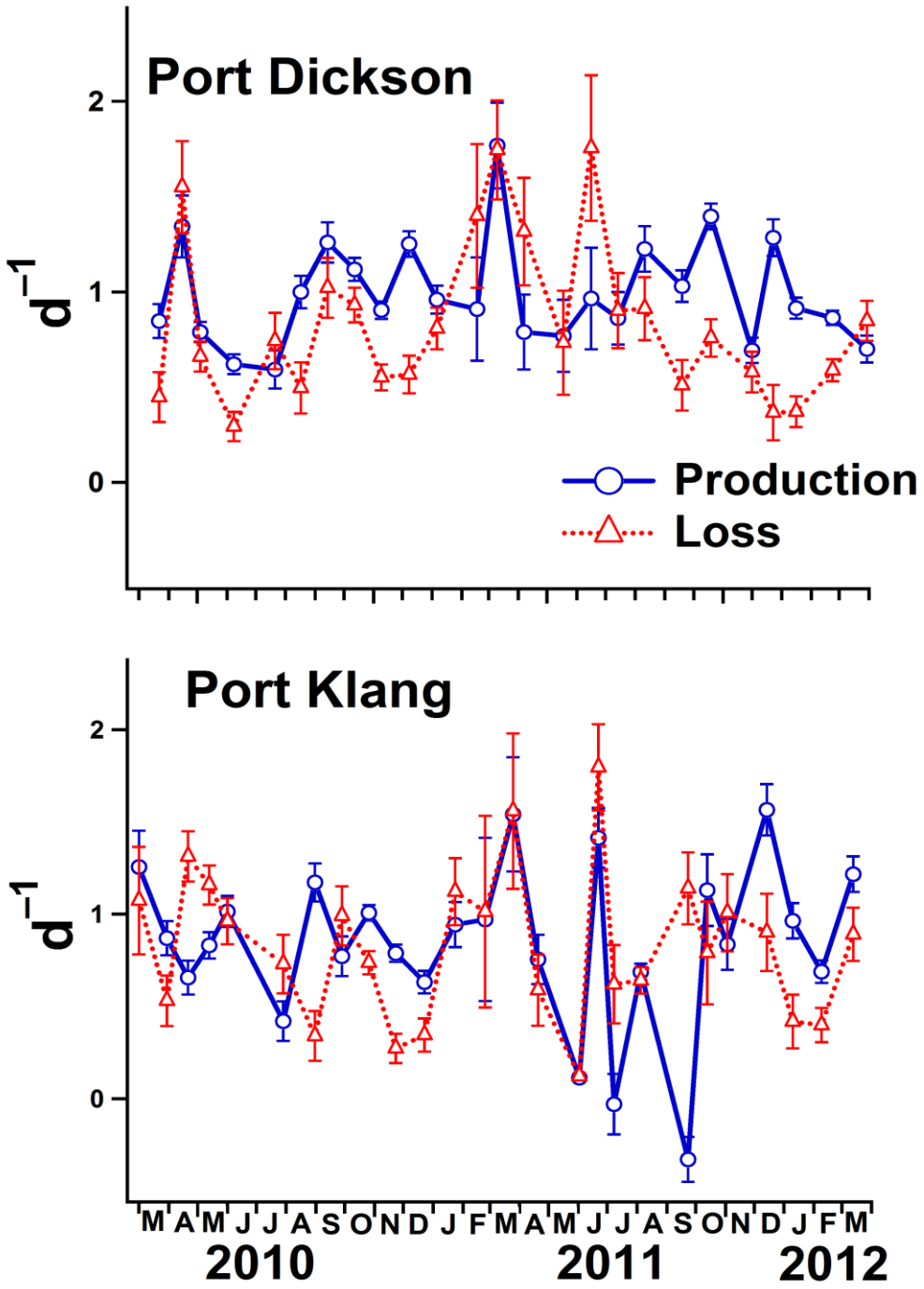

Figure 3.9: Production (μ) and loss (g) rates of picocyanobacteria measured at PD and PK. The error bars indicated the S.E. except when the error bar was smaller than the symbol.

3.6 *Two-factorial experiment*

In the two-factorial experiment, nutrient-enriched microcosms were no different from control at both PD and PK, and on most occasions exhibited μ rates (**Table 3.1**) that were lower than control (0.447 to 0.786 d^{-1}). Similarly, higher light intensity did not affect production at PK. However control microcosms from PD incubated under higher light intensity of 340 µmol m^{-2} s^{-1} showed significantly higher production (0.69 to 1.28 d^{-1}) than when incubated at 100 µmol m^{-2} s^{-1} (–0.25 to 1.01 d^{-1}) ($F = 5.942$, $df = 27$, $p < 0.05$). At PD, no significant difference was found for nutrient-enriched samples. As for *g* (**Table 3.2**), there was no difference among all treatments.

Table 3.1: μ (d^{-1}) measured in two-factorial experiment at PD and PK. Values shown are average ± S.D. Same superscripted alphabet showed significant difference. [a] Two-way ANOVA: F = 5.942, df = 27, $p < 0.05$

Irradiance	**Port Klang**		**Port Dickson**	
	Control	**Nutrient-enriched**	**Control**	**Nutrient-enriched**
340 µmol m^{-2} s^{-1}	1.055 ± 0.345 (0.689 – 1.566)	0.546 ± 0.351 (0.094 – 0.929)	0.935 ± 0.235[a] (0.693 – 1.285)	0.786 ± 0.382 (0.309 – 1.227)
100 µmol m^{-2} s^{-1}	0.518 ± 0.641 (– 0.317 – 1.023)	0.447± 0.564 (– 0.285 – 0.963)	0.456 ± 0.496[a] (– 0.247 – 1.028)	0.493± 0.505 (– 0.056– 1.187)

Table 3.2: g (d^{-1}) measured in two-factorial experiment at PD and PK. Values shown are average ± S.D.

Irradiance	**Port Klang**		**Port Dickson**	
	Control	**Nutrient-enriched**	**Control**	**Nutrient-enriched**
340 µmol m^{-2} s^{-1}	0.723 ± 0.291	0.573 ± 0.159	0.652 ± 0.237	0.697 ± 0.575
	(0.400 – 1.007)	(0.363 – 0.796)	(0.367 – 0.911)	(0.149 – 0.985)
100 µmol m^{-2} s^{-1}	0.622 ± 0.227	0.583 ± 0.204	0.505 ± 0.268	0.599 ± 0.252
	(0.235 – 0.798)	(0.332 – 0.878)	(0.250 – 1.906)	(0.291 – 1.022)

DISCUSSION

4.1 *Environmental characteristics*

Relatively high and stable seawater temperature observed in this study was typical of tropical waters (Lee and Bong, 2008). As expected, higher fluctuation of salinity at PK was due to the influx of river water from Klang Rivers. Higher concentration of dissolve inorganic nutrients and lower euphotic depth suggested that level of eutrophication was higher at PK than PD. Environmental characteristics observed at PK was similar to previous studies (Lee and Bong, 2008; Lee *et al.*, 2009). TSS and Secchi depth were measured to reflect water transparency and they were inversely correlated (**Figure 4.1**: $R^2 = -0.70$, $df = 45$, $p < 0.001$). This showed that water transparency or light penetration was poorer at PK as compared to PD.

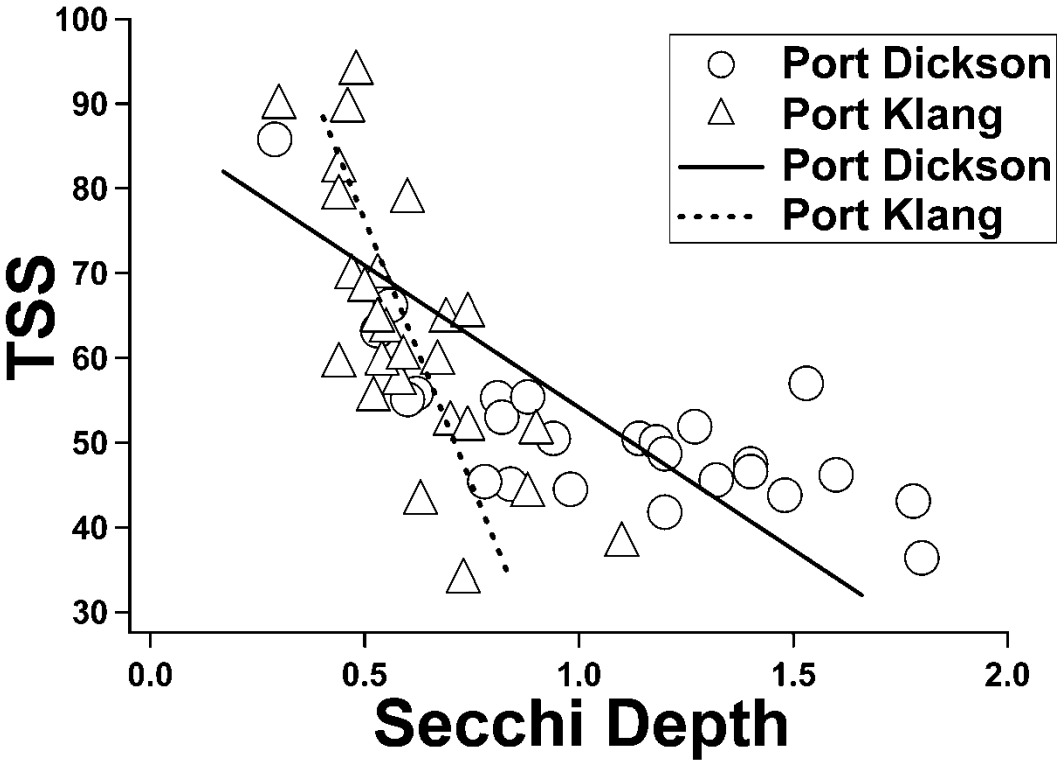

Figure 4.1: Correlation between TSS (mg L^{-1}) and Secchi Depth (m) at both sites. Linear regression slope for each site is also shown.

4.2 *Temporal variation of Chl a concentrations and heterotrophic bacteria*

Chl *a* concentration had similar range with those recorded in previous studies carried out in this region (Lee and Bong, 2008; Lee *et al*., 2009). Wider variation of Chl *a* concentrations at PK is believed to be caused by episodic nutrient input at the estuary (Lee and Bong, 2008). Two possible phytoplankton blooms that were observed in September and November 2011, were similar to the bloom observed in previous study (Lee and Bong, 2008) and was believed to be due to the increase in rainfall during inter-monsoon period that would lead to increase in surface run off and thus, contribute to higher inorganic nutrient concentrations in the river (Lim *et al.*, submitted). Abundance of picocyanobacteria did not seem to share the same peaks with the two phytoplankton blooms detected at PK. Thus, these blooms were caused by larger phytoplankton (unpublished data), and not by picocyanobacteria. This was also supported by the negative correlation between picocyanobacteria and Chl *a* in this study (**Figure 4.2**: $R^2 = -0.70$, $df = 45$, $p < 0.001$). The inverse relationship suggested separate ecological niches occupied by picocyanobacteria and phytoplankton that allowed predominance of picocyanobacteria when phytoplankton activity is limited (i.e. at lower Chl *a* concentration) (Agawin *et al.*, 2000a). Heterotrophic bacterial abundance showed similar range with other studies in tropical waters (Alongi *et al*., 2003; Lee and Bong, 2008; Lee *et al.*, 2009). Bacteria was also found to be tightly coupled with Chl *a* in this study (**Table 4.1) (**$R^2 = 0.55$, $df = 45$, $p < 0.001$) and this bacteria-phytoplankton coupling was similar to previous study (Lee and Bong, 2008).

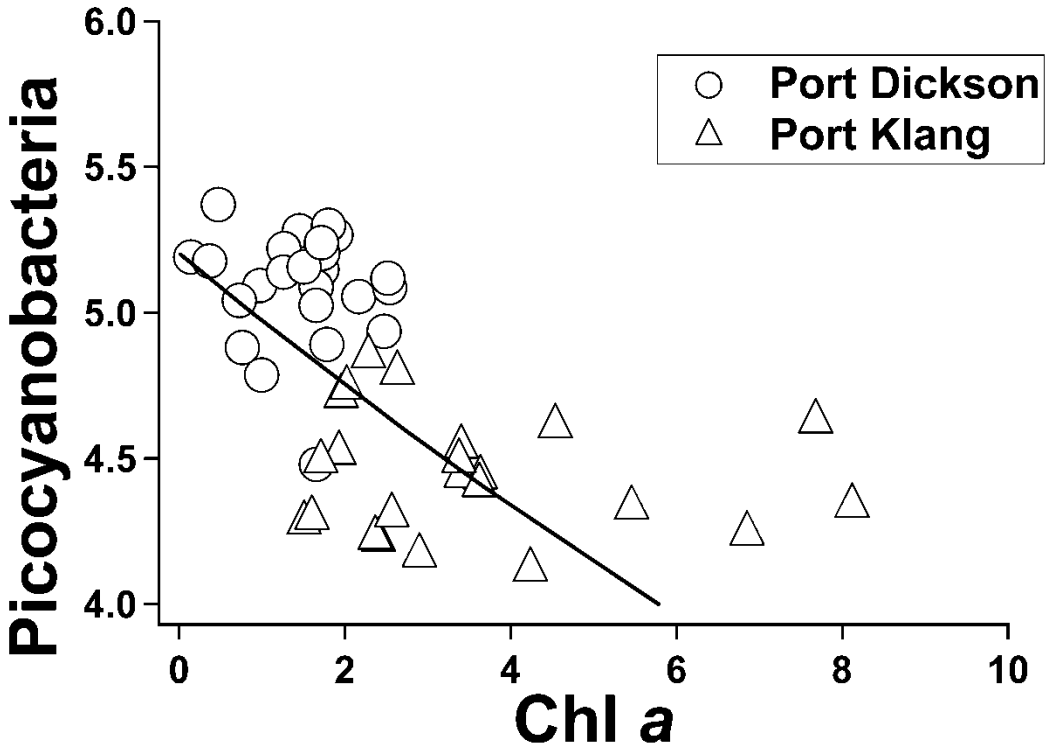

Figure 4.2: Relationship between picocyanobacterial abundance (log of cell ml^{-1}) and Chl *a* (µg L^{-1}) at PK and PD. Linear regression slope is also shown.

4.3 *Temporal variation of picocyanobacteria*

In this study, majority of autofluorescing cells in this study were yellow-fluorescing cells, which were presumed to be *Synechococcus.*. Due to low chlorophyll fluorescence of *Prochlorococcus*, especially for surface sample, it is almost impossible to obtain an accurate cell count via normal epifluorescence microscopy (Campbell *et al.*, 1994). In addition, *Synechococcus* dominates over *Prochlorococcus* as trophic status shifted from oligotrophy to mesotrophy and *Synechococcus* is found to be most dominant at coastal and continental shelf zones where temperature is high (22 – 28 °C) (Choi *et al.*, 2011). Thus, due to the methodology adopted, it is assumed that the cell counts obtained in this study were *Synechococcus.*

The main environmental factors that affect the distribution and abundance of picocyanobacteria are water temperature (Agawin *et al.*, 1998; Chang *et al*., 2003; Liu *et al*., 2007; Chen *et al.*, 2011), nutrient levels and light (Mackey *et al.*, 2009). In our study however, temperature variation did not correlate to the temporal changes of picocyanobacteria ($p > 0.05$). Picocyanobacterial abundance varied over two-orders but within a narrow 4 °C range (28 – 32 °C) in our study. In tropical waters where temperature is relatively optimum for most organisms (Pomeroy and Wiebe, 2001), the effects of temperature may not be significant.

We then compared with other studies to see whether similar observations could be made. When we analyzed data from subtropical (25 – 44 °N/S) (n = 84), and temperate waters (> 45 °N/S) (n = 31), picocyanobacterial abundance correlated significantly with seawater temperature (Appendix A) (**Figure 4.3**: $R^2 = 0.43$, $df = 114$, $p < 0.001$). However, when data from tropical waters (< 25 °N/S, including present study) were included (n = 124), picocyanobacterial abundance seemed to reach a plateau. Although correlation analysis was still significant, the correlation index was

substantially lower (R^2 = 0.10, *df* = 238, $p < 0.001$), and suggested that temperature played a lesser role in explaining picocyanobacterial distribution in tropical waters.

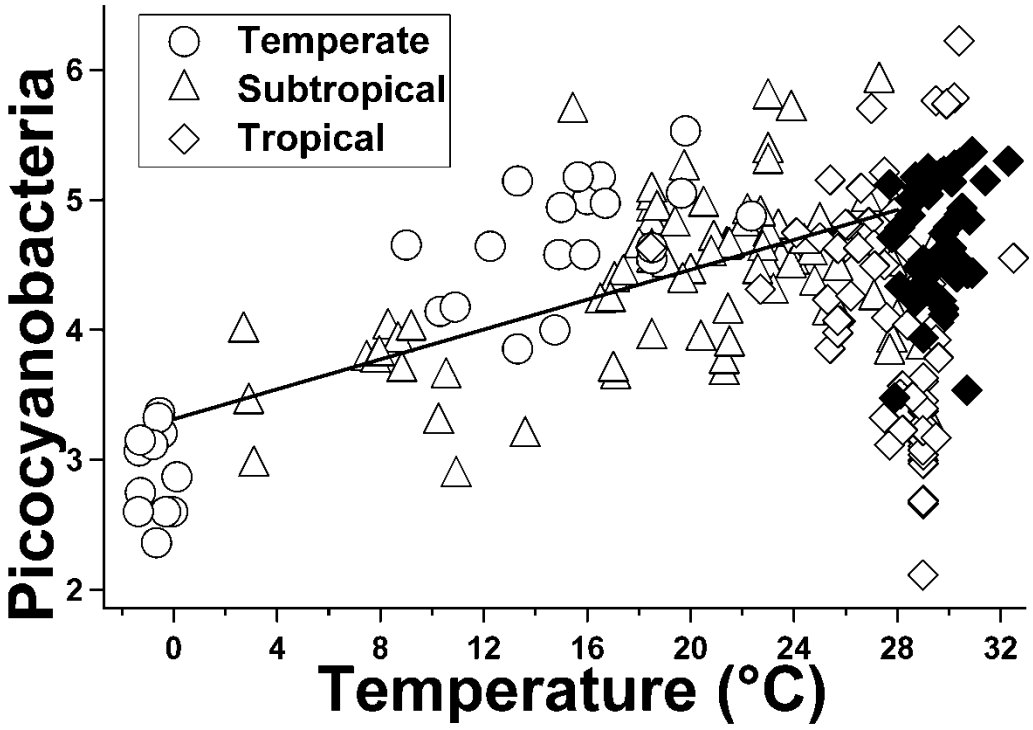

Figure 4.3: Relationship between picocyanobacterial abundance (log cell ml^{-1}) and temperature (°C) from tropical to temperate region (refer to appendix A) (filled symbols are from present study). Linear regression shown only involves data from subtropical and temperate studies.

When temperature limitation is weak, other factor such as nutrients and light availability could become the more important regulator (Chang *et al.*, 2003). Effect of nutrients and light availability on picocyanobacteria have been studied in different climates (Agawin *et al.*, 2002; Mackey *et al.*, 2009). Inverse correlation between NH_4, NO_3 and NO_2 with picocyanobacterial abundance were found in this study (**NH_4**: $R^2 = -0.37$, $df = 45$, $p < 0.05$; NO_3: $R^2 = -0.75$, $df = 45$, $p < 0.001$; NO_2: $R^2 = -0.66$, $df = 45$, $p < 0.001$). With their small size and high surface to volume ratio, small phytoplankton e.g. picocyanobacteria can thrive better in low nutrient environment (Raven, 1998). However, when nutrients are not limiting, larger phytoplankton prevails (Agawin *et al*., 2000a).

With reference to light availability which was not determined in this study, we measured Secchi disc depth as its proxy. There was tight coupling between Secchi depth and abundance of picocyanobacteria at both sites ($R^2 = 0.43$, $df = 45$, $p < 0.01$) , and indicated that light availability played an important role in regulating picocyanobacteria community. Picocyanobacterial abundance also decreased with increasing TSS ($R^2 = -0.70$, $df = 45$, $p < 0.01$) as higher TSS reduced water transparency which in turn can reduce photosynthesis (Schubert *et al.*, 2001). Environmental conditions at PK were showed to be more unfavourable to support picocyanobacteria populations as compared to PD. Relative to temperature, nutrient and light availability were found as important environmental factors that affect the distribution of picocyanobacteria.

4.4 *Production (μ) and loss rates (g) of picocyanobacteria*

A search on available literature revealed a lack of studies that measured both picocyanobacterial production and loss. Of the 57 studies that reported picocyanobacterial distribution (Appendix A), there were only 45 data points available for concurrent picocyanobacterial production and loss. Therefore our study helps fill the data gap. Production rates recorded in this study were significantly higher ($F = 15.57$, $df = 102$, $p < 0.001$) than those at subtropical waters ($q > 4.83$, $p < 0.01$). Previous study had noted that *Synechococcus* showed nutrient-saturated growth at ambient DIN concentration of 0.25 μM and suppressed growth rates under higher concentration of DIN (> 8 μM) (Agawin *et al.*, 2000b). Similar results were observed in this study. At our study sites where DIN concentration was constantly > 0.25 μM, it was believed that nutrient limitation was absent and this could explain the absence of correlation between DIN concentration and production rate.

In this study, we measured picocyanobacterial biovolume in order to estimate their carbon content, and to be able to express both production and loss in carbon terms. We found significant differences in the picocyanobacterial carbon content between the two stations and showed the importance of measuring the picocyanobacterial carbon content at different sites. By using the carbon conversion factors for each site, annual picocyanobacterial production at PK ranged from 2.8 to 4.0 g C m^{-2} y^{-1} and from 21 to 25 g C m^{-2} y^{-1} at PD.

Picocyanobacteria accounted for 2.30 % and 0.63 % of total primary production for each year of sampling at PK whereas at PD, picocyanobacterial production only accounted for 10.68 % and 8.57 % for each year of sampling. These results suggested that contribution of picocyanobacteria (*Synechococcus* sp.) to total primary production (based on total Chl *a* concentrations) diminishes with increasing concentration of

nutrients (Agawin *et al*., 2000a). This was similar to previous study done in tropical coastal waters where picocyanobacteria contribute 0.03 % to 16 % of primary production (Agawin *et al.*, 2003).

As for loss rates, significantly higher range ($F = 13.01$, $df = 84$, $p < 0.001$) were detected as compared to both subtropical and temperate waters ($q > 5.67$, $p < 0.001$; $q > 5.19$, $p < 0.01$) but our results were within the range of grazing rates measured in other studies (e.g. Brown *et al*., 1999; Hirose *et al.*, 2008; Chen *et al.*, 2009). The relatively higher temperature could contribute towards higher loss rates at tropical waters as temperature was found to exert influence on protist grazing over picocyanobacteria (Guo *et al.*, 2013).

Production and loss rate of picocyanobacteria measured at both sampling sites were tightly coupled (**Figure 4.4**: $R^2 = 0.47$, $df = 459$, $p < 0.001$). Our results showed that between 60 to 68 % (average = 64 %) of picocyanobacterial production were grazed. In order to ascertain if the degree of grazing pressure measured here is similar elsewhere, we compared our results with data from available literature. Linear regression slope comparison was then carried out according to Zar (1999). Average grazing pressure from other studies was 88 %, and was significantly higher than the grazing pressure in our study (Student's *t*-test: $t = 2.52$, $df = 82$, $p < 0.05$). The lower grazing pressure shown in this study could be due to the consumption of microzooplankton by mesozooplankton, which we did not remove by prefiltering our samples (< 200 µm) (Paterson *et al.*, 2008). By analysing all available data (including this study), we observed a 'global trend' where 90 % of picocyanobacterial production was grazed ($F = 80.3$, $df = 85$, $p < 0.001$). As a large amount of picocyanobacterial production is grazed and transferred onto higher trophic levels, the coupling between picocyanobacterial production and grazing becomes an important energy and carbon pathway especially in environments where picocyanobacteria thrive.

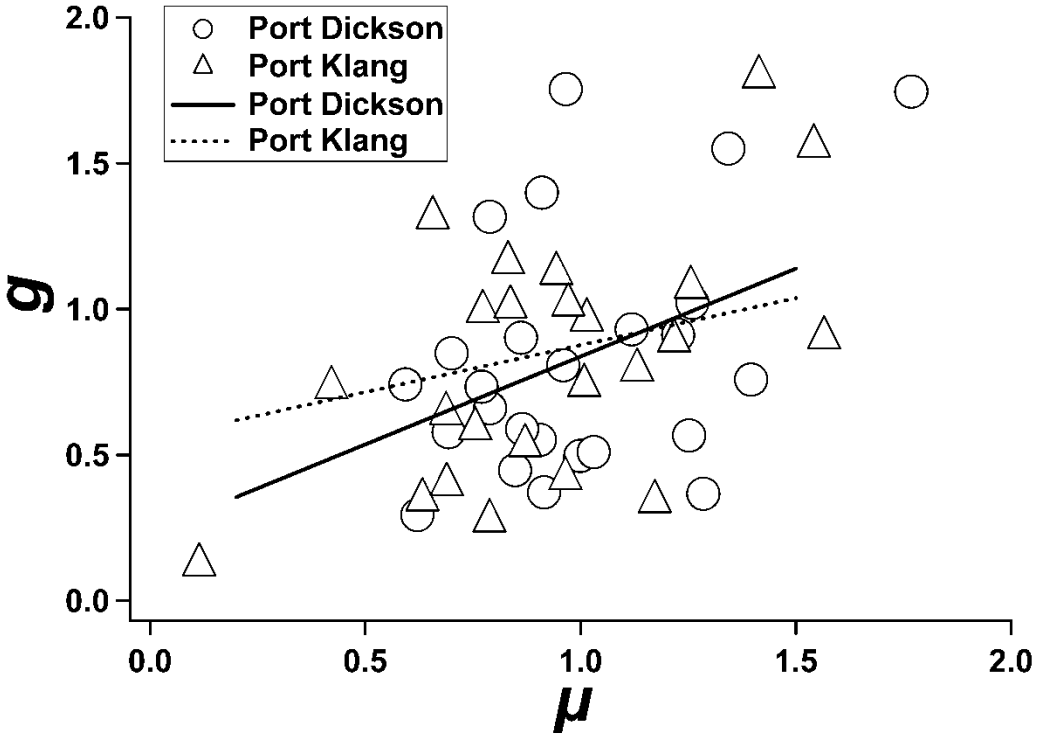

Figure 4.4: Correlation between μ (d^{-1}) and g (d^{-1}) in this study. Linear regression slope for each site is also shown.

Secchi depths were used to estimate the euphotic depth at both sampling sites (Welch, 1948) and the euphotic integrated primary production by picocyanobacteria were calculated. Euphotic-depth integrated primary production for PK ranged from 13.23 – 133.86 × 10^9 cell m^{-2} d^{-1} in first year of sampling and – 2.48 – 73.55 × 10^9 cell m^{-2} d^{-1} in second year of sampling whereas at PD, it ranged from 65.46 – 1111.41 × 10^9 cell m^{-2} d^{-1} and 174.23 – 614.79 × 10^9 cell m^{-2} d^{-1}, respectively. After taking into account the integrated loss rates, we determined if our sampling sites were net production or net loss for picocyanobacteria. Net integrated primary production recorded at PD were 277.72 and 158.19 × 10^{11} cell m^{-2} y^{-1} in first year and second year of sampling whereas at PK, net integrated primary production were 3.12 and 13.47 × 10^{11} cell m^{-2} y^{-1} respectively. The higher net production observed at PD during two years of sampling as compared to PK could explain the difference in picocyanobacterial abundance between both sites.

In the two-factorial experiments carried out, we did not observe any difference in picocyanobacterial production between control and nutrient enriched microcosms. As picocyanobacteria exhibits nutrient-saturated growth at 0.25 μM DIN (Agawin *et al.*, 2000b), the lack of response in our study could be due to the saturated growth already experienced by the picocyanobacteria as ambient DIN concentrations at both PK and PD were > 0.25 μM. In contrast, picocyanobacterial production was higher when incubated under stronger light intensity especially for PD but not PK samples. As picocyanobacteria do not adapt rapidly to light intensity (Palenik, 2001), picocyanobacteria in PK were already adapted to ambient low light conditions, and probably were not able to elicit a response to the sudden increase in light intensity. As for loss rates, there was no difference among all treatments, and loss rates were probably independent of nutrients or light intensity.

CONCLUSION

As compared to PD, environment at PK where level of eutrophication is higher, is less favourable for picocyanobacteria. Picocyanobacteria thrive in low nutrient conditions, ample light availability and to a certain extent, warmer waters. Although the contribution of picocyanobacteria towards total primary production was low (< 11 %), the tight coupling with grazing loss ensured that 60 to 68 % of picocyanobacterial production in this study was channelled up higher trophic levels. In conclusion, our study of tropical coastal waters with different trophic status revealed how picocyanobacteria and phytoplankton seemed to occupy separate ecological niches. Globally, up to 90% of picocyanobacterial production was grazed, and the magnitude suggested the importance of picocyanobacteria in aquatic environments. In the context of increasing eutrophication worldwide, the role of picocyanobacteria would probably be reduced whereas warming waters would not have much effect in tropical waters.

REFERENCES

Affronti, L. F. J., & Marshall, H. G. (1994). Using frequency of dividing cells in estimating autotrophic picoplankton growth and productivity in the Chesapeake Bay. *Hydrobiologia, 284* (3), 193-203.

Agawin, N. S. R., & Agustí, S. (1997). Abundance, frequency of dividing cells and growth rates of *Synechococcus* sp. (cyanobacteria) in the stratified Northwest Mediterranean Sea. *Journal of Plankton Research, 19* (11), 1599-1615.

Agawin, N. S. R., Agustí, S., & Duarte, C. M. (2002). Abundance of Antarctic picophytoplankton and their response to light and nutrient manipulation. *Aquatic Microbial Ecology, 29*, 161-172.

Agawin, N. S. R., Duarte, C. M., & Agustí, S. (1998). Growth and abundance of *Synechococcus* sp. in a Mediterranean Bay: seasonality and relationship with temperature. *Marine Ecology Progress Series, 170*, 45-53.

Agawin, N. S. R., Duarte, C. M., & Agustí, S. (2000a). Nutrient and temperature control of picoplankton to phytoplankton biomass and production. *Limnology and Oceanography, 45*, 591-600.

Agawin, N. S. R., Duarte, C. M., & Agustí, S. (2000b). Response of Mediterranean *Synechococcus* growth and loss to experimental nutrient inputs. *Marine Ecology Progress Series, 206*, 97-106.

Agawin, N. S. R., Duarte, C. M., Agustí, S., & Macmanus, L. (2003). Abundance, biomass and growth rates of *Synechococcus* sp. in a tropical coastal ecosystem (Philippines, South China Sea). *Estuarine, Coastal and Shelf Science, 56*, 493-502.

Alongi, D. M., Chong, V. C., Dixon, P., Sasekumar, A., & Tirendi, F. (2003). The influence of fish cage aquaculture on pelagic carbon flow and water chemistry

in tidally dominated mangrove estuaries of peninsular Malaysia. *Marine Environment Research, 55*, 313-333.

André, J. M., Navaratte, C., Blanchot, J., & Radenae, M. H. (1999). Picophytoplankton dynamics in the equatorial Pacific: growth and grazing rates from cytometric counts. *Journal of Geophysical Research, 10* (4), 3369-3380.

Ayukai, T. (1992). Picoplankton dynamics in Davies Reef Lagoon, the Great Barrier Reef, Australia. *Journal of Plankton Research, 14*, 1593-1606.

Berninger, U. G., & Wickham, S. A. (2005). Response of the microbial food web to manipulation of nutrients and grazers in the oligotrophic Gulf of Aqaba and northern Red Sea. *Marine Biology, 147*, 1017-1032.

Blanchot, J., André, J. M., Navarette, C., Neveux, J., & Radenac, M. H. (2001). Picophytoplankton in the equatorial Pacific: vertical distributions in the warm pool and in the high nutrient low chlorophyll conditions. *Deep-Sea Research I, 48*, 297-314.

Brown, S. L., Landry, M. R., Barber, R. T., Campbell, L., Garrison, D. L., & Gowing, M. M. (1999). Picophytoplankton dynamics and production in the Arabian Sea during the 1995 Southwest monsoon. *Deep-Sea Research II, 46*, 1745-1768.

Campbell, L., Nolla, H. and Vaulot, D. (1994). Importance of *Prochlorococcus* to community structure in the central North Pacific Ocean. *Limnology and Oceanography, 39* (4), 954-961.

Chang, J., Lin, K. H., Chen, K. M., Gong, G. C., & Chiang, K. P. (2003). *Synechococcus* growth and mortality rates in the East China Sea: range of variations and correlation with environmental factors. *Deep-Sea Research II, 50*, 1265-1278.

Charpy, L., & Blanchot, J. (1998). Photosynthetic picoplankton in French Polynesian atoll lagoons: estimation of taxa contribution to biomass and production by flow cytometry. *Marine Ecology Progress Series, 162*, 57-70.

Chen, B. Z., Liu, H. B., Landry, M. R., Dai, M. H., Huang, B. Q., & Sun, J. (2009). Close coupling between phytoplankton growth and microzooplankton grazing in the western South China Sea. *Limnology and Oceanography, 54* (4), 1084-1097.

Chen, B. Z., Wang, L., Song, S. Q., Huang, B. Q., Sun, J., & Liu, H. B. (2011). Comparisons of picophytoplankton abundance, size, and fluorescence between summer and winter in northern South China Sea. *Continental Shelf Research, 31*, 1527-1540.

Choi, D. H., Noh, J. H., Hahm, M. S., & Lee, C. M. (2011). Picocyanobacterial abundances and diversity in surface water of the northwestern Pacific Ocean. *Ocean Science Journal, 46* (4), 265-271.

Flombaum, P., Gallegos, J. L., Gordillo, R. A., Rincón, J., Zabala, L. L., Jiao, N. Z., … Martiny, A. C. (2013). Present and future global distributions of the marine Cyanobacteria *Prochlorococcus* and *Synechococcus*. *Proceedings of the National Academy of Sciences, 110* (24), 9824-9829.

Gin, K. Y. H., Zhang, S., & Lee, Y. K. (2003). Phytoplankton community structure in Singapore's coastal waters using HPLC pigment analysis and flow cytometry. *Journal of Plankton Research, 25* (12), 1507-1519.

Grasshoff, K., Kremling, K., & Ehrhardt, M. (1999). *Methods of seawater analysis* (3rd ed.). Weinheim, Germany: Wiley-VCH.

Guo, C., Liu, H., Zheng, L., Song, S., Chen, B., & Huang, B. (2013). Bottom-up and top-down controls on picoplankton in the East China Sea. *Biogeosciences Discussions, 10*, 8203-8245.

Hamasaki, K., Satoh, F., Kikuchi, T., Toda, T., & Taguchi, S. (1999). Biomass and production of cyanobacteria in a coastal water of Sagami Bay, Japan. *Journal of Plankton Research, 21* (8), 1583-1591.

Hammer, Ø., Harper, D. A. T., & Ryan, P. D. (2001). PAST: Paleontological Statistics Software Package for Education and Data Analysis. *Palaeontologia Electronica*, 4-9.

Hirose, M., Katano, T., & Nakano, S. I. (2008). Growth and grazing mortality rates of *Prochlorococcus*, *Synechococcus* and eukaryotic picophytoplankton in a bay of the Uwa Sea, Japan. *Journal of Plankton Research, 30* (3), 241-250.

Jacquet, S., Lennon, J. F., Marie, D., & Vaulot, D. (1998). Picoplankton population dynamics in coastal waters of the northwestern Mediterranean Sea. *Limnology and Oceanography, 43* (8), 1916-1931.

Johnson, P. W., & Sieburth, J. M. (1982). *Insitu* morphology and occurrence of eukaryotic phototrophs of bacterial size in the picoplankton of estuarine and oceanic waters. *Journal of Phycology, 18*, 318-327.

Kana, T. M., & Gilbert, P. M. (1987). Effect of irradiances up to 2000 μE m–2 sec–1 on marine *Synechococcus* WH7803-I. Growth, pigmentation, and cell composition. *Deep-Sea Research, 34*, 479-495.

Kepner, R.L., Jr., Pratt, J.R., 1994. Use of fluorochromes for direct enumeration of total bacteria in environmental samples: Past and present. *Microbiological Reviews 58*, 603-615.

Kuipers, B., Witte, H., Noort, G. V., & Gonzalez, S. (2003). Grazing loss rates in pico- and nanoplankton in the Faroe-Shetland Channel and their different relations with prey density. *Journal of Sea Research, 50*, 1-9.

Landry, M. R., & Hassett, R. P. (1982) Estimating the grazing impact of marine micro-zooplankton. *Marine Biology, 67*, 283-288.

Lee, C. W., & Bong, C. W. (2012). Relative contribution of viral lysis and grazing to bacterial mortality in tropical coastal waters of Peninsular Malaysia. *Bulletin of Marine Science. 88* (1), 1-14.

Lee, C. W., & Bong, C. W. (2008). Bacterial abundance and production, and their relation to primary production in tropical coastal waters of Peninsular Malaysia. *Marine and Freshwater Research, 59,* 10-21.

Lee, C. W., Bong, C. W., & Hii, Y. S. (2009). Temporal variation of bacterial respiration and growth efficiency in tropical coastal waters. *Applied and Environmental Microbiology, 75*, 7594-7601.

Lee, C. W., Bong, C. W., NG, C. C., & Siti-Aisyah Alias. (2006). Factors affecting variability of heterotrophic and phototrophic microorganisms at high water in a mangrove forest at Cape Rachado, Malaysia. *Malaysian Journal of Science, 25* (2), 55-66.

Lee, C. W., Lim, J. H., & Heng, P. L. (2013). Investigating the spatial distribution of phototrophic picoplankton in a tropical estuary. *Environmental Monitoring and Assessment, 185*, 9697-9704.

Li, W. K. W. (1998). Annual average abundance of heterotrophic bacteria and *Synechococcus* in surface ocean waters. *Limnology and Oceanography, 43* (7), 1746-1753.

Lim, J. H., Lee, C. W., & Kudo, I. (2014). Temporal variation in phytoplankton growth and grazing loss in tropical coastal waters. *Submitted.*

Liu, H. B., Campbell, L., & Landry, M. R. (1995). Growth and mortality rates of *Prochlorococcus* and *Synechococcus* measured with a selective inhibitor technique. *Marine Ecology Progress Series, 116,* 277-287.

Liu, H. B., Chang, J., Tseng, C. M., Wen, L. S., & Liu, K. K. (2007). Seasonal variability of picoplankton in the Northern South China Sea at the SEATS station. *Deep-Sea Research II, 54*, 1602-1616.

Mackey, K. R. M., Rivlin, T., Grossman, A. R., Post, A. F., & Paytan, A. (2009). Picophytoplankton responses to changing nutrient and light regimes during a bloom. *Marine Biology, 156*, 1531-1546.

Martin, A. P., Zubkov, M. V., Burkill, P. H., & Holland, R. J. (2005). Extreme spatial variability in marine picoplankton and its consequences for interpreting Eulerian-time series. *Biology Letters, 1*, 366-369.

McDaniel, L., & Paul, J. H. (2005). Effect of nutrient addition and environmental factors on prophage induction in natural populations of marine *Synechococcus* species. *Applied and Environmental Microbiology, 71* (2), 842-850.

Morán, X. A. G., López-Urrutia, A., Calvo-Díaz, A., & Li, W. K. W. (2010). Increasing importance of small phytoplankton in a warmer ocean. *Global Change Biology, 16*, 1137-1144.

Morel, A., Ahn,Y. W., Partensky, F., Vaulot, D., & Claustre, H. (1993). *Prochlorococcus* and *Synechococcus*: a comparative study of their optical properties in relation to their size and pigmentation. *Journal of Marine Research, 51*, 617-649.

Nakamura, Y., Sasaki, S., Hiromi, J., & Fukami, K. (1993). Dynamics of picocyanobacteria in the Seto Inland Sea (Japan) during summer. *Marine Ecology Progress Series, 96*, 117-124.

Ning, X. R., Cloern, J. E., & Cole, B. E. (2000). Spatial and temporal variability of picocyanobacteria *Synechococcus* sp. in San Francisco Bay. *Limnology and Oceanography, 45* (3), 695-702.

Palenik, B. (2001). Chromatic adaptation in marine *Synechococcus* strains. *Applied and Environmental Microbiology, 67* (2), 991-994.

Parsons, T. R., Maita, Y., & Lalli, C. M. (1984). *A manual of chemical and biological methods for seawater analysis*. Permagon, Oxford.

Partensky, F., Hess, W. R., & Vaulot, D. (1999). *Prochlorococcus*, a marine photosynthetic prokaryote of global significance. *Microbiology and Molecular Biology Reviews, 63* (1), 106-127.

Paterson, H. L., Knott, B., Koslow, A. J., & Waite, A. M. (2008). The grazing impact of microzooplankton off southwest Western Australia: as measured by the dilution technique. *Journal of Plankton Research, 30* (4), 379-392.

Pomeroy, L. R., & Wiebe, W. J. (2001). Temperature and substrates as interactive limiting factors for marine heterotrophic bacteria. *Aquatic Microbial Ecology, 23*, 187-204.

Proctor, L. M. and Fuhrman, J. A. (1990). Viral mortality of marine bacteria and cyanobacteria. Nature. Vol. 343: 60 – 62.

Raven, J. A. (1998). Small is beautiful: the picophytoplankton. *Functional Ecology, 12*, 503-513.

Reckermann, M., & Veldhuis, M. J. W. (1997). Trophic interactions between picophytoplankton and micro- and nanozooplankton in the western Arabian Sea during the NE monsoon 1993. *Aquatic Microbial Ecology, 12*, 263-273.

Scanlan, D. J., Ostrowski, M., Dufresne, A., Garczarek, L., Hess, W. R., Post, A. F., …Partensky, F. (2009). Ecological genomics of marine picocyanobacteria. *Microbiology and Molecular Biology Reviews, 73* (2), 249-299.

Schubert, H., Sagert, S., & Forster, R. M. (2001). Evaluation of the different levels of variability in the underwater light field of a shallow estuary. *Helgoland Marine Research, 55*, 12-22.

Toledo, G., & Palenik, B. (2003). A *Synechococcus* serotype is found preferentially in surface marine waters. *Limnology and Oceanography, 48,* 1744-1755.

Uysal, Z. (2001). Chrococcoid cyanobacteria *Synechococcus* spp. in the Black Sea: pigments, size, distribution, growth and diurnal variability. *Journal of Plankton Research, 23* (2), 175-189.

Veldhuis, M. J. W., Timmermans, K. R., Croot, P., & Wagt, B. V. D. (2005). Picophytoplankton; a compartive study of their biochemical composition and photosynthetic properties. *Journal of Sea Research, 53,* 7-24.

Vidal, L., Rodríquez-Gallego, L., Conde, D., Martínez-López, W., & Bonilla, S. (2007). Biomass of autotrophic picoplankton in subtropical coastal lagoons: is it relevant? *Limnetica, 26* (2), 441-452.

Welch, P. S. (1948). *Limnological methods*. Philadelphia: Blakiston.

West, N. J., & Scanlan, D. J. (1999). Niche-partitioning of *Prochlorococcus* populations in a stratified water column in the eastern North Atlantic Ocean. *Applied and Environmental Microbiology, 65*(6), 2585-2591.

Winder, M. (2009). Photosynthetic picoplankton dynamics in Lake Tahoe: temporal and spatial niche partitioning among prokaryotic and eukaryotic cells. *Journal of Plankton Research, 31* (11), 1307-1320.

Worden, A. Z., & Binder, B. J. (2003). Application of dilution experiments for measuring growth and mortality rates among *Prochlorococcus* and *Synecococcus* populations in oligotrophic environments. *Aquatic Microbial Ecology, 30,* 159-174.

Worden, A. Z., Nolan, J. K., & Palenik, B. (2004). Assessing the dynamics and ecology of marine picophytoplankton: the importance of the eukaryotic component. *Limnology and Oceanography, 49* (1), 168-179.

Zar, J.H. (1999). *Biostatistical Analysis* (4th ed.). Upper Saddle River, NJ: Prentice Hall.

Zhang, Y., Jiao, N. Z., & Hong, N. (2008). Comparative study of picoplankton biomass and community structure in different provinces from subarctic to subtropical oceans. *Deep-Sea Research II, 55,* 1605-1614.

Zhao, Y., Zhao, L., Xiao, T., Liu, C. G., Sun, J., Zhou, F., … Huang, L. F. (2013). Temporal variation of picoplankton in the spring bloom of Yellow Sea, China. *Deep Sea Research II*. http://dx.doi.org/10.1016/j.dsr2.2013.05.015

Zwirglmaier, K., Jardillier, L., Ostrowski, M., Mazard, S., Garczarek, L., Vaulot, D., … Scanlan, D. J. (2008). Global phylogeography of marine *Synechococcus* and *Prochlorococcus* reveals a distinct partitioning of lineages among oceanic biomes. *Environmental Microbiology, 10* (1), 147-161.

Appendix A

Cross-latitudinal analysis from a total of 58 studies (including present study). This analysis comprised of 21 studies from tropical waters (including present studies), 22 studies from subtropical waters, 13 studies from temperate waters and 2 cross-latitudinal studies.

Reference	Location	Climate	Latitude	Temperature (°C)	Abundance (x 10^3cell ml^{-1})	μ (d^{-1})	g (d^{-1})
Present study	Strait of Malacca	Tropical	2.29°N	29.5 ± 1.1	133 ± 47	0.99 ± 0.28	0.83 ± 0.42
		Tropical	3°N	29.5 ± 0.9	28 ± 17	0.84 ± 0.44	0.83 ± 0.43
Odate & Fukuchi, 1994	South East Asia	Tropical	10°S - 5°N	NA	74 ± 56	--	--
	Western North Pacific Ocean	Tropical	5 - 20°N	NA	1.4 ± 0.51	--	--
	Eastern Indian Ocean	Tropical	22 - 10°S	NA	3.6 ± 2.0	--	--
Liu *et al*., 1995	Central Pacific Ocean	Tropical	22°45'N	NA	10 -100	0.70 ± 0.04	0.39 ± 0.05
					--	0.58 ± 0.21	0.22 ± 0.07
					--	0.54 ± 0.08	0.2 ± 0.14
Ayukai, 1996	Davies & Myrmidon Reef	Tropical	18°49'N	25.7	42.3	--	--
			18°49'N	26.1	63.5	--	--
			18°49'N	26	64.7	--	--
			18°49'N	27.5	31.7	--	--
			18°16'N	25.3	17.1	--	--
			18°16'N	25.4	7.2	--	--
			18°16'N	25.8	11.7	--	--
			18°16'N	26.2	18.2	--	--
			18°16'N	25.7	9.5	--	--
			18°16'N	25.7	11.8	--	--

Appendix A, continued.

Reference	Location	Climate	Latitude	Temperature (°C)	Abundance (x 10^3cell ml^{-1})	μ (d^{-1})	g (d^{-1})
Buck *et al*., 1996	North Atlantic Ocean	Tropical	5°S	--	2.4	--	--
			0		40.8	--	--
			5°N		6.6	--	--
			10°N		11.7	--	--
			15°N		25.1	--	--
			20°N		3.5	--	--
		Subtropical	25°N		4.1	--	--
			30°N		3.9	--	--
			35°N		9	--	--
			40°N		8.6	--	--
		Temperate	45°N		15	--	--
			50°N		27.5	--	--
			55°N		31.8	--	--
			60°N		59	--	--
Reckermann & Veldhuis, 1997	Western Arabic Sea	Tropical	4°N	26.9	67.18	0.653	0.195
			7°N	26.7	51.15	0.595	0.042
			10°N	26	43.69	0.52	0.201
			12°N	26	53.58	0.928	0.503
			12°N	25.9		0.399	0.148
			12°N	25.4	142.23	0.742	0.708
			14°N	25.7		1.123	1.187
			16°N	25.8	47.25	0.681	0.383

Appendix A, continued.

Reference	Location	Climate	Latitude	Temperature (°C)	Abundance (x 10^3cell ml^{-1})	μ (d^{-1})	g (d^{-1})
Charpy & Blanchot, 1998	South Pacific Ocean	Tropical	17°28'S	--	190 ± 2.7	--	--
			17°28'S		369.7 ± 87.6	--	--
			17°35'S		43.8 ± 7.6	--	--
			17°35'S		17.3 ± 3.6	--	--
			16°43'S		66.3 ± 4	--	--
			16°43'S		86.6 ± 8.1	--	--
			15°50'S		32.1 ± 4.8	--	--
			15°50'S		43.8 ± 4.3	--	--
			18°03'S		39.1 ± 4.8	--	--
			18°03'S		79 ± 8.5	--	--
			16°41'S		49.7 ± 6.2	--	--
			16°41'S		50 ± 3.3	--	--
			16°50'S		7.1 ± 1	--	--
			16°50'S		0.5 ± 0.1	--	--
			15°45'S		44.2 ± 2.3	--	--
			15°45'S		32.1 ± 5.9	--	--
			14°30'S		71.5 ± 3.5	--	--
			17°19'S		0.1	--	--
			17°19'S		0.1	--	--
			16°49'S		277.7 ± 16.6	--	--
			16°49'S		25.7 ± 4.2	--	--

Appendix A, continued.

Reference	Location	Climate	Latitude	Temperature (°C)	Abundance (x 10^3cell ml^{-1})	μ (d^{-1})	g (d^{-1})
Brown *et al*., 1999	Arabian Sea	Tropical	19°N	27.9	80	1.12	0.72
			10°N	27.8	52	0.46	0.34
			14.5°N	27.1	45	0.58	0.50
			16°N	26.6	123	0.84	0.73
			17°N	25	51	0.58	0.33
			18°N	24.1	56	0.66	0.33
André *et al*., 1999	Equatorial Pacific	Tropical	0°	--	9	0.20 - 0.90	0.20 - 0.90
Blanchot *et al*., 2001	Equatorial Pacific	Tropical	0°	29 - 30	1.48 ± 0.07	--	--
		Tropical			8.35 ± 0.97	--	--
Huang *et al*., 2002	South China Sea	Tropical	20 - 22°N	27 - 28	13.8 - 150	--	--
Agawin *et al*., 2003	South China Sea	Tropical	16°08'N	26 - 32°C	4 ± 2.7	--	--
			16°14'N		2.86 ± 0.41	--	--
			16°16'N		1.84 ± 0.21	--	--
			16°21'N		2.36 ± 1	--	--
			16°26'N		1.39 ± 0.08	--	--
			16°24'N		1.27 ± 0.15	--	--
			16°26'N		1.69 ± 0.36	--	--
			16°42'N		0.99 ± 0.01	--	--
			16°54'N		0.99 ± 0.12	--	--
			16°50'N		1.47	--	--
			16.44°N		0.49 ± 0.01	--	--

Appendix A, continued.

Reference	Location	Climate	Latitude	Temperature (°C)	Abundance (x 10^3cell ml^{-1})	μ (d^{-1})	g (d^{-1})
Agawin *et al*., 2003	South China Sea	Tropical	16.44°N		1.88 ± 0.22	--	--
(continued)			16.43°N		1.63 ± 0.09	--	--
			16.43°N		2.48 ± 0.25	--	--
			16.42°N		0.94 ± 0.02	--	--
			16.42°N		0.13	--	--
			16.40°N		1.42 ± 0.02	--	--
			16.38°N		0.46	--	--
			16.34°N		1.09 ± 0.21	--	--
			16.44°N		1.19 ± 0.02	--	--
			16.39°N		0.48 ± 0.05	0.2 - 1.28	--
			16.35°N		4.26 ± 0.09	--	--
			16.37°N		1.67 ± 0.01	--	--
Gin *et al*., 2003	Singapore Strait	Tropical	1°10' - 1°20'N	--	53.6 - 115.2	--	--
	Johor Strait	Tropical	1°20' - 1°30'N	--	12.4 - 27.1	--	--
Yang & Jiao, 2004	Nansha Island (SCS)	Tropical	6 - 12°N	27.7 - 29.5	0.40 - 5.70	--	--
Campbell *et al*., 2005	Southwestern Pacific Ocean	Tropical	13 - 13.9°S	27.13 ± 3.17	2 - 60	--	--
Lee *et al*., 2006	Cape Rachado, Malaysia	Tropical	2.40° N	29.5	580 ± 420	--	--
		Tropical	2.40° N	30.2	610 ± 440	--	--
		Tropical	2.40° N	29.9	550 ± 100	--	--
Liu *et al*., 2007	Northern South China Sea	Tropical	18°N	23 - 31	100 - 1000	--	--
Chen *et al*., 2009	Western South China Sea	Tropical	11 - 15.75°N	18.46	43 ± 46	0.14 - 1.83	0 - 1.04

Appendix A, continued.

Reference	Location	Climate	Latitude	Temperature (°C)	Abundance (x 10^3cell ml^{-1})	μ (d^{-1})	g (d^{-1})
Chen *et al*., 2011	Northern South China Sea	Tropical	18 - 24°N	29.6	6.1	--	--
		Tropical		22.7	20.5	--	--
Choi *et al*., 2011	Northwestern Pacific Ocean	Tropical	5 - 45°N	22.1 - 30.9	1 - 84	--	--
Jing & Liu, 2012	South China Sea	Tropical	14.25°N	27.6	12.4	--	--
			14.75°N	28.9	23.8	--	--
			15.75°N	29.4	18.6	--	--
			12.50°N	28.4	120	--	--
			11.50°N	28.4	10.9	--	--
			12.50°N	29.3	22.4	--	--
			11.50°N	32.5	35.6	--	--
			18.30°N	29.6	11.4	--	--
Lee *et al*., 2013	Strait of Malacca	Tropical	2.40° - 3.00°N	30.4 ± 0.3	1.67 ± 0.35	--	--
Nakamura *et al*., 1993	Seto Inland Sea	Subtropical	34°40'N	24	7.00 - 57.00	0.585	--
Affronti & Marshall, 1994	Chesapeake Bay	Subtropical	36°58'N	4.62 - 26.25	7.36 - 928	0.62	--
Chang *et al*., 1996	Western Pacific	Subtropical	25°09'N	28	90	--	--
Modigh *et al*., 1996	Mediterranean sea	Subtropical	40.74°N	13.98 - 27.8	14.38 ± 13.54	--	--
Caron *et al*., 1999	Sargasso Sea	Subtropical	32°N	19 - 27	10 - 50	--	--
Hamasaki *et al*., 1999	Sagami Bay	Subtropical	35°09'N	24.8	22	0.84	--
				25.3	13	1.9	--
				22.2	78	0.84	--
				17.1	4.2	--	--

Appendix A, continued.

Reference	Location	Climate	Latitude	Temperature (°C)	Abundance (x 10^3cell ml^{-1})	μ (d^{-1})	g (d^{-1})
Ning *et al*., 2000	San Francisco Bay	Subtropical	38°N	13 - 24	114	--	--
Jacquet *et al*., 2002	Western Mediterranean	Subtropical	35 - 38°N	15 - 18	16	--	--
Chang *et al*., 2003	East China Sea	Subtropical	25 - 32°N	12 - 26	--	0.42	0.21
Collier & Palenik, 2003	Southern California Bight	Subtropical	29 - 35°N	17.05 ± 1.89	23.26 ± 27.06	--	--
Worden & Binder, 2003	Sargasso Sea	Subtropical	26°00'N	--	7	0.45	0.49
			31°40'N		15	--	--
			35°54'N		9	0.42	0.09
			38°25'N		42	0.69	0.37
			32°30'N		9.5	0.68	0.3
			30°10'N		22	0.63	0.46
Worden *et al*., 2004	Pacific Ocean	Subtropical	32°53'N	13 - 24	33	0.52	0.27
			32°53'N		82	0.86	0.31
			32°53'N		100	0.69	0.39
			32°53'N		38	0.77	0.15
			32°53'N		65	0.56	0.22
			32°53'N		42	0.58	0.4
Berninger & Wickham, 2005	Gulf of Aqaba	Subtropical	29°30'N	21.3	4.5 ± 0.5	-0.49	-1.06
			29°30'N	21.3	5.5 ± 1.1	0.53	-0.42
			28°30'N	21.4	43.5 ± 9.4	0.56	0.11
			27°30'N	22.7	27.5 ± 2.7	-2.74	-2.78
			27°30'N	21.5	7.5 ± 3.3	0.5	0.19

Appendix A, continued.

Reference	Location	Climate	Latitude	Temperature (°C)	Abundance (x 10^3cell ml^{-1})	μ (d^{-1})	g (d^{-1})
Fuller *et al*., 2005	Gulf of Aqaba	Subtropical	29°28'N	22 - 25	30 - 39	--	--
Jiao *et al*., 2005	East China Sea	Subtropical	32°N	27.8	31	--	--
				28	12	--	--
				13.6	1.5	--	--
		Subtropical	28 - 31.5°N	29.2	2	--	--
				20.4	8.3	--	--
Pan *et al*., 2005	East China Sea	Subtropical	28 - 31°N	21 - 25	59.7 ± 98	--	--
			31°N		190.2 ± 527.7	--	--
Vidal et al., 2007	Atlantic Ocean	Subtropical	34°20' - 34°54'S	19.75	170	--	--
Hirose et al., 2008	Uwa sea, Japan	Subtropical	33°2'N	18 - 28	1.2 - 460	0.25 - 1.39	0.62 - 1.54
Cai et al., 2010	Chesapeake Bay	Subtropical	37°N	3.1	0.89	--	--
				26.8	96.3	--	--
			38°N	2.7	9.6	--	--
				27.3	798	--	--
			39°N	2.9	2.7	--	--
				23.9	484	--	--

Appendix A, continued.

Reference	Location	Climate	Latitude	Temperature (°C)	Abundance (x 10^3cell ml^{-1})	μ (d^{-1})	g (d^{-1})
Michelou *et al*., 2007	North Atlantic Ocean	Subtropical	29.3°N	NA	9 ± 19	--	--
			29.8		14 ± 24	--	--
			30.1		12 ± 22	--	--
			31.7		25 ± 43.4	--	--
			32.6		19 ± 29	--	--
			33.3		36 ± 45	--	--
			34.1		13 ± 17	--	--
			34.8		30 ± 52	--	--
			35.3		16 ± 24	--	--
			35.8		59 ± 83	--	--
			36.3		41 ± 52	--	--
			36.7		25 ± 22	--	--
			37.1		12 ± 14	--	--
			37.4		11 ± 13	--	--
			37.6		10 ± 12	--	--
			37.8		23 ± 32	--	--
			37.9		12 ± 14	--	--
			37.9		28 ± 39	--	--
			37.9		3 ± 4	--	--
		Temperate	45		29 ± 26	--	--
			48		84 ± 80	--	--
			50		152 ± 150	--	--
			52.9		63 ± 42	--	--

Appendix A, continued.

Reference	Location	Climate	Latitude	Temperature (°C)	Abundance (x 10^3cell ml^{-1})	μ (d^{-1})	g (d^{-1})
Michelou *et al*., 2007	North Atlantic Ocean	Temperate	54.1		35 ± 21	--	--
(continued)			57		54 ± 30	--	--
			58		18 ± 10	--	--
			61.1		41 ± 34	--	--
			64.5		9 ± 40	--	--
Guo *et al*., 2013	East China Sea	Subtropical	25 - 32°N	28.32	97.63	1.08	1.11
				29.54	46.12	0.74	0.41
				28.92	7.04	0.55	0.37
				10.93	0.74	1.04	0.44
				17.01	4.77	0.67	0.29
				21.45	13.44	0.39	0.31
Tsai *et al*., 2013	East China Sea	Subtropical	25 - 32°N	24.8	6 - 92	--	--
				24.7	5 - 70	--	--
				25.7	3 - 54	--	--
				27.1	3 - 31	--	--
				27.8	4 - 12	--	--
				27.7	1 - 12	--	--
				25	19 - 114	--	--
				23.4	7 - 112	--	--
				22.1	3 - 123	--	--
				19.1	2 - 59	--	--

Appendix A, continued.

Reference	Location	Climate	Latitude	Temperature (°C)	Abundance (x 10^3cell ml^{-1})	μ (d^{-1})	g (d^{-1})
Tsai *et al*., 2013	East China Sea	Subtropical	25 - 32°N	18.7	2 - 163	--	--
(continued)				23.2	14 - 24	--	--
				23	19 - 86	--	--
				23.9	17 - 43	--	--
				25.7	7 - 19	--	--
				24.4	8 - 84	--	--
				22.8	3 - 78	--	--
				21.4	4 - 79	--	--
				20.9	3 - 94	--	--
				20	3 - 51	--	--
				17.4	4 - 49	--	--
				16.9	3 - 30	--	--
				18.5	2 - 15	--	--
				18	2 - 84	--	--
				19.4	3 - 126	--	--
				20.5	5 - 173	--	--
				22.6	5 - 49	--	--
				22.7	5 - 145	--	--
				21.5	5 - 81	--	--
				20.8	3 - 71	--	--
				19.7	4 - 42	--	--

Appendix A, continued.

Reference	Location	Climate	Latitude	Temperature (°C)	Abundance (x 10^3cell ml^{-1})	μ (d^{-1})	g (d^{-1})
Zhao *et al*., 2013	Yellow Sea, China	Subtropical	33.5 - 37.5 °N	8.29	10.17	--	--
				10.24	1.9	--	--
				10.54	4.26	--	--
		Subtropical	35.8 - 37 °N	8.68	8.04	--	--
				8.83	4.76	--	--
				9.19	9.9	--	--
		Subtropical	35 - 35.58 °N	7.46	5.77	--	--
				7.81	5.58	--	--
				7.95	6.31	--	--
Vanucci *et al*., 1994	Northern Adriatic Sea	Temperate	44°N	4.5 - 25.3	38	--	--
				6.5 - 25.3	38	--	--
Albertano et al., 1997	Central Baltic Sea	Temperate	55°45'N	13.3	140	--	--
				19.8	340	--	--
Agawin *et al*., 1998	Mediterranean Bay	Temperate	41°40'N	11 - 26	5 - 70	0.2 - 1.5	--
Jacquet *et al*., 1998	Northwestern Mediterranean Sea	Temperate	43°41'N	11 - 26	43	0.69 - 1.25	0.50 - 1.00
Lee *et al*., 2001	Funka Bay	Temperate	42°16.2'N	13 - 20	150.5	--	--
Zubkov *et al*., 2000	Atlantic Ocean	Temperate	51°21'S - 9°47'N	2 - 30	100.5	--	--
Uysal, 2001	Black Sea	Temperate	41° - 45°N	7 - 11	45	--	--
Kuipers *et al*., 2003	Faroe-Shetland Channel	Temperate	60 - 62°N	10.89	5 - 25	--	0.075 - 0.275
Martin *et al*., 2005	Celtic Sea	Temperate	50°45'N	15	25 - 150	--	--

Appendix A, continued.

Reference	Location	Climate	Latitude	Temperature (°C)	Abundance (x 10^3cell ml^{-1})	μ (d^{-1})	g (d^{-1})
Agawin & Agustí, 1997	Northwest Mediterranean Sea	Temperate	41°21'N	--	7.18 ± 0.84	1.05	1.65 ± 0.08
			41°21'N		4.03 ± 0.19	0.23	--
			41°7.5'N		2.15 ± 1.03	1.79	--
			40°21'N		1.7 ± 0.44	1.49	--
			41°31'N		4.44 ± 0.45	0.76	--
			41°21'N		4.57 ± 1.07	--	--
			41°16'N		9.15 ± 2.4	--	--
			41°07'N		4.0 ± 2.25	--	--
			41°37'N		12.94 ± 0.15	1.07	--
Paoli *et al*., 2007	Adriatic Sea	Temperate	45°40' - 45°45'N	20.1-24.6	0.3 - 150	--	--
			45°40' - 45°45'N	5.3-21.3	0.3 - 14	--	--
			45°15' - 45°31'N	13-26.3	2 - 223	--	--
			44°31'N	12.2 - 12.3	8 - 80	--	--
			41°23'N	8.2 - 23.1	0.4 - 300	--	--
			40°42'N	11.7 17.8	1 - 19	--	--
			40°19'N	12.7 - 20.7	55 - 134	--	--

Appendix A, continued.

Reference	Location	Climate	Latitude	Temperature (°C)	Abundance (x 10^3cell ml^{-1})	μ (d^{-1})	g (d^{-1})
Waleron *et al*., 2007	Arctic ocean	Temperate	69°30'N	-0.04	0.4	--	--
			69°31'N	-0.54	2.31	--	--
			70°9'N	-0.32	0.4	--	--
			70°9'N	-0.45	1.6	--	--
			70°51'N	-0.61	2.11	--	--
			71°16'N	-1.34	1.17	--	--
			70°47'N	0.12	0.74	--	--
			71°32'N	-1.29	0.56	--	--
			71°35'N	-1.37	0.4	--	--
			71°27'N	-0.67	0.23	--	--
			68°23'N	-0.81	1.31	--	--
			69°50'N	-1.3	1.42	--	--
Zhang *et al*., 2008	North pacific Ocean	Temperate	47°N	10.3 ± 0.5	13.9	--	--
		Tropical	18°N	27.7	1.3	--	--
			18°N	28.2	1.7	--	--
			10°N	27.5	2.1	--	--
			10°N	28.2	3.7	--	--
			10°N	28.1	3.2	--	--
			10°N	28.5 ± 0.03	41	--	--

References used in Appendix A

Affronti, L. F. J., & Marshall, H. G. (1994). Using frequency of dividing cells in estimating autotrophic picoplankton growth and productivity in the Chesapeake Bay. *Hydrobiologia, 284* (3), 193-203.

Agawin, N. S. R., & Agustí, S. (1997). Abundance, frequency of dividing cells and growth rates of *Synechococcus* sp. (cyanobacteria) in the stratified Northwest Mediterranean Sea. *Journal of Plankton Research, 19* (11), 1599-1615.

Agawin, N. S. R., Duarte, C. M., & Agustí, S. (1998). Growth and abundance of *Synechococcus* sp. in a Mediterranean Bay: seasonality and relationship with temperature. *Marine Ecology Progress Series, 170*, 45-53.

Agawin, N. S. R., Duarte, C. M., Agustí, S., & Macmanus, L. (2003). Abundance, biomass and growth rates of *Synechococcus* sp. in a tropical coastal ecosystem (Philippines, South China Sea). *Estuarine, Coastal and Shelf Science, 56*, 493-502.

Albertano, P., Somma, D. D., & Capucci, E. (1997). Cyanobacterial picoplankton from the central Baltic Sea: cell size classification by image-analysed fluorescence microscopy. *Journal of Plankton Research, 19* (10), 1405-1416.

André, J. M., Navaratte, C., Blanchot, J., & Radenae, M. H. (1999). Picophytoplankton dynamics in the equatorial Pacific: growth and grazing rates from cytometric counts. *Journal of Geophysical Research, 10* (4), 3369-3380.

Ayukai, T. (1996). Possible limitation of the dilution technique for estimating growth and grazing mortality rates of picoplanktonic cyanobacteria in oligotrophic tropical waters. *Journal of Experimental Marine Biology and Ecology, 198*, 101-111.

Berninger, U. G., & Wickham, S. A. (2005). Response of the microbial food web to manipulation of nutrients and grazers in the oligotrophic Gulf of Aqaba and northern Red Sea. *Marine Biology, 147*, 1017-1032.

Blanchot, J., André, J. M., Navarette, C., Neveux, J., & Radenac, M. H. (2001). Picophytoplankton in the equatorial Pacific: vertical distributions in the warm pool and in the high nutrient low chlorophyll conditions. *Deep-Sea Research I, 48*, 297-314.

Brown, S. L., Landry, M. R., Barber, R. T., Campbell, L., Garrison, D. L., & Gowing, M. M. (1999). Picophytoplankton dynamics and production in the Arabian Sea during the 1995 Southwest monsoon. *Deep-Sea Research II, 46*, 1745-1768.

Buck, K. R., Chavez, F. P., & Campbell, L. (1996). Basin-wide distributions of living carbon components and the inverted trophic pyramid of the central gyre of the North Atlantic Ocean, summer 1993. *Aquatic Microbial Ecology, 10*, 283-298.

Cai, H. Y., Wang, K., Huang, S. J., Jiao, N. Z., & Chen, F. (2010). Distinct patterns of picocyanobacterial communities in winter and summer in the Chesapeake Bay. *Applied and Environmental Microbiology, 76* (9), 2955-2960.

Campbell, L., Carpenter, E. J., Montoya, J. P., Kustka, A. B., & Capone, D. G. (2005). Picoplankton community structure within and outside a Trichodesmium bloom in the Southwestern Pacific Ocean. *Vie Et Milieu, 55* (3-4), 185-195.

Caron, D. A., Peele, E. R., Lim, E. L., & Dennett, M. R. (1999). Picoplankton and nanoplankton and their trophic coupling in surface waters of the Sargasso Sea south of Bermuda. *Limnology and Oceanography, 44* (2), 259-272.

Chang, J., Chung, C. C., & Gong, G. C. (1996). Influences of cyclones on Chl *a* concentration and *Synechococcus* abundance in a subtropical Western Pacific coastal ecosystem. *Marine Ecology Progress Series, 140*, 199-205.

Chang, J., Lin, K. H., Chen, K. M., Gong, G. C., & Chiang, K. P. (2003). *Synechococcus* growth and mortality rates in the East China Sea: range of variations and correlation with environmental factors. *Deep-Sea Research II, 50*, 1265-1278.

Charpy, L., & Blanchot, J. (1998). Photosynthetic picoplankton in French Polynesian atoll lagoons: estimation of taxa contribution to biomass and production by flow cytometry. *Marine Ecology Progress Series, 162*, 57-70.

Chen, B. Z., Liu, H. B., Landry, M. R., Dai, M. H., Huang, B. Q., & Sun, J. (2009). Close coupling between phytoplankton growth and microzooplankton grazing in the western South China Sea. *Limnology and Oceanography, 54* (4), 1084-1097.

Chen, B. Z., Wang, L., Song, S. Q., Huang, B. Q., Sun, J., & Liu, H. B. (2011). Comparisons of picophytoplankton abundance, size, and fluorescence between summer and winter in northern South China Sea. *Continental Shelf Research, 31*, 1527-1540.

Choi, D. H., Noh, J. H., Hahm, M. S., & Lee, C. M. (2011). Picocyanobacterial abundances and diversity in surface water of the northwestern Pacific Ocean. *Ocean Science Journal, 46* (4), 265-271.

Collier, J. L., & Palenik, B. (2003). Phycoerythrin-containing picoplankton in the Southern California Bight. *Deep-Sea Research II, 50*, 207-219.

Fuller, N. J., West, N. J., Marie, D., Yallop, M., Rivlin, T., Post, A. F., & Scanlan, D. J. (2005). Dynamics of community structure and phosphate status of picocyanobacterial populations in the Gulf of Aqaba, Red Sea. *Limnology and Oceanography, 50* (1), 363-375.

Gin, K. Y. H., Zhang, S., & Lee, Y. K. (2003). Phytoplankton community structure in Singapore's coastal waters using HPLC pigment analysis and flow cytometry. *Journal of Plankton Research, 25* (12), 1507-1519.

Guo, C., Liu, H., Zheng, L., Song, S., Chen, B., & Huang, B. (2013). Bottom-up and top-down controls on picoplankton in the East China Sea. *Biogeosciences Discussions, 10*, 8203-8245.

Hamasaki, K., Satoh, F., Kikuchi, T., Toda, T., & Taguchi, S. (1999). Biomass and production of cyanobacteria in a coastal water of Sagami Bay, Japan. *Journal of Plankton Research, 21* (8), 1583-1591.

Hirose, M., Katano, T., & Nakano, S. I. (2008). Growth and grazing mortality rates of *Prochlorococcus, Synechococcus* and eukaryotic picophytoplankton in a bay of the Uwa Sea, Japan. *Journal of Plankton Research, 30* (3), 241-250.

Huang, B. Q., Lin, X. J., Liu, Y., Dai, M. H., Hong, H. S., & Li, W. K. K. (2002). Ecological study of picoplankton in Northern South China Sea. *Chinese Journal of Oceanology and Limnology, 20*, 22-32.

Jacquet, S., Lennon, J. F., Marie, D., & Vaulot, D. (1998). Picoplankton population dynamics in coastal waters of the northwestern Mediterranean Sea. *Limnology and Oceanography, 43* (8), 1916-1931.

Jacquet, S., Prieur, L., Jacquet, C. A., Lennon, J. F., & Vaulot, D. (2002). Short-timescale variability of picophytoplankton abundance and cellular parameters in surface waters of the Alboran Sea (western Mediterranean). *Journal of Plankton Research, 24* (7), 635-651.

Jiao, N. Z., Yang, Y. H., Hong, N., Ma, Y., Harada, S., Koshikawa, H., & Watanabe, M. (2005). Dynamics of autotrophic picoplankton and heterotrophic bacteria in the East China Sea. *Continental Shelf Research, 25*, 1265-1279.

Jing, H. M., & Liu, H. B. (2012). Phylogenetic composition of *Prochlorococcus* and *Synechococcus* in cold eddies of the South China Sea. *Aquatic Microbial Ecology*, *65*, 207-219.

Kuipers, B., Witte, H., Noort, G. V., & Gonzalez, S. (2003). Grazing loss rates in pico- and nanoplankton in the Faroe-Shetland Channel and their different relations with prey density. *Journal of Sea Research, 50*, 1-9.

Lee, C. W., Bong, C. W., NG, C. C., & Siti-Aisyah Alias. (2006). Factors affecting variability of heterotrophic and phototrophic microorganisms at high water in a mangrove forest at Cape Rachado, Malaysia. *Malaysian Journal of Science, 25* (2), 55-66.

Lee, C. W., Kudo, I., Yanada, M., & Maita, Y. (2001). Bacterial abundance and production and their relation to primary production in Funka Bay. *Plankton Biology and Ecology, 48* (1), 1-9.

Lee, C. W., Lim, J. H., & Heng, P. L. (2013). Investigating the spatial distribution of phototrophic picoplankton in a tropical estuary. *Environmental Monitoring and Assessment, 185*, 9697-9704.

Liu, H. B., Campbell, L., & Landry, M. R. (1995). Growth and mortality rates of *Prochlorococcus* and *Synechococcus* measured with a selective inhibitor technique. *Marine Ecology Progress Series, 116*, 277-287.

Liu, H. B., Chang, J., Tseng, C. M., Wen, L. S., & Liu, K. K. (2007). Seasonal variability of picoplankton in the Northern South China Sea at the SEATS station. *Deep-Sea Research II, 54*, 1602-1616.

Martin, A. P., Zubkov, M. V., Burkill, P. H., & Holland, R. J. (2005). Extreme spatial variability in marine picoplankton and its consequences for interpreting Eulerian – time series. *Biology Letters, 1*, 366-369.

Michelou, V. K., Cottrell, M. T., & Kirchman, D. L. (2007). Light-stimulated bacterial production and amino acid assimilation by cyanobacteria and other microbes in the North Atlantic Ocean. *Applied and Environmental Microbiology, 73* (17), 5539-5546.

Modigh, M., Saggiomo, V., & d'Alcalà, M. R. (1996). Conservative features of picoplankton in a Mediterranean eutrophic area, the Bay of Naples. *Journal of Plankton Research, 18*(1), 87-95.

Nakamura, Y., Sasaki, S., Hiromi, J., & Fukami, K. (1993). Dynamics of picocyanobacteria in the Seto Inland Sea (Japan) during summer. *Marine Ecology Progress Series, 96*, 117-124.

Ning, X. R., Cloern, J. E., & Cole, B. E. (2000). Spatial and temporal variability of picocyanobacteria *Synechococcus* sp. in San Francisco Bay. *Limnology and Oceanography, 45* (3), 695-702.

Odate, T., & Fukuchi, M. (1994). Surface distribution of picophytoplankton along the first leg of the JARE- 3 cruise, from Tokyo to Fremantle, Australia. *Bulletin of Plankton Society of Japan, 41* (2), 93-104.

Pan, L. A., Zhang, L. H., Zhang, J., Gasol, J. M., & Chao, M. (2005). On-board flow cytometric observation of picoplankton community structure in the East China Sea during the fall of different years. *FEMS Microbiology Ecology, 52*, 243-253.

Paoli, A., Celussi, M., Valeri, A., Larato, C., Bussani, A., Umani, S. F., Vadrucci, M. R., Mazziotti, C., & Negro, P. D. (2007). Picocyanobacteria in Adriatic transitional environments. *Estuarine, Coastal and Shelf Science, 75*, 13-20.

Reckermann, M., & Veldhuis, M. J. W. (1997). Trophic interactions between picophytoplankton and micro- and nanozooplankton in the western Arabian Sea during the NE monsoon 1993. *Aquatic Microbial Ecology, 12*, 263-273.

Tsai, A. Y., Gong, G. C., Sanders, R. W., & Chiang, K. P. (2013). Relationship of *Synechococcus* abundance to seasonal ocean temperature ranges. *Terrestrial Atmospheric and Oceanic Sciences, 24* (5), 925-932.

Uysal, Z. (2001). Chrococcoid cyanobacteria *Synechococcus* spp. in the Black Sea: pigments, size, distribution, growth and diurnal variability. *Journal of Plankton Research, 23* (2), 175-189.

Vanucci, S., Pomar, M. L. C. A., & Maugeri, T. L. (1994). Seasonal pattern of phototrophic picoplankton in the eutrophic coastal waters of the northern Adriatic Sea. *Botanica Marina, 37*, 57-66.

Vidal, L., Rodríquez-Gallego, L., Conde, D., Martínez-López, W., & Bonilla, S. (2007). Biomass of autotrophic picoplankton in subtropical coastal lagoons: is it relevant? *Limnetica, 26* (2), 441-452.

Waleron, M., Waleron, K., Vincent, W. F., & Wilmotte, A. (2007). Allochthonous inputs of riverine picocyanobacteria to coastal waters in the Arctic Ocean. *FEMS Microbial Ecology, 59*, 356-365.

Worden, A. Z., & Binder, B. J. (2003). Application of dilution experiments for measuring growth and mortality rates among *Prochlorococcus* and *Synecococcus* populations in oligotrophic environments. *Aquatic Microbial Ecology, 30*, 159-174.

Worden, A. Z., Nolan, J. K., & Palenik, B. (2004). Assessing the dynamics and ecology of marine picophytoplankton: the importance of the eukaryotic component. *Limnology and Oceanography, 49* (1), 168-179.

Yang, Y. H., & Jiao, N. Z. (2004). Dynamics of picoplankton in the Nansha Islands area of the South China Sea. *Acta Oceanologica Sica, 23* (3), 493-504.

Zhang, Y., Jiao, N. Z., & Hong, N. (2008). Comparative study of picoplankton biomass and community structure in different provinces from subarctic to subtropical oceans. *Deep-Sea Research II, 55*, 1605-1614.

Zhao, Y., Zhao, L., Xiao, T., Liu, C. G., Sun, J., Zhou, F., … Huang, L. F. (2013). Temporal variation of picoplankton in the spring bloom of Yellow Sea, China. *Deep Sea Research II*. http://dx.doi.org/10.1016/j.dsr2.2013.05.015

Zubkov, M. V., Sleigh, M. A., & Burkill, P. H. (2000). Assaying picoplankton distribution by flow cytometry of underway samples collected along a meridional transect across the Atlantic Ocean. *Aquatic Microbial Ecology, 21*, 13-20.