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Temporal variation of phytoplankton growth and grazing loss in the west coast of Peninsular Malaysia

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Abstract   Phytoplankton growth (μ) and grazing loss (g) rates were measured monthly by the Landry-Hassett dilution method over a two-year period at both estuarine (Klang)

15   and coastal water (Port Dickson) systems along the Straits of Malacca. Chl a concentration ranged from 0.20 to 4.47 µg L^{-1} at Klang except on two occasions when Chl a spiked above 10 µg L^{-1}. In contrast, Chl a concentrations were relatively stable at Port Dickson (0.14 to 2.76 µg L^{-1}). From the rate measurements, μ was higher (t = 2.01, df = 43, p < 0.05) at Klang (0.30 to 2.26 d^{-1}) than Port Dickson (0.18 to 1.66 d^{-1}) but g was not significantly different (p > 0.80). g

20   ranged from 0.30 to 1.50 d^{-1} and 0.21 to 1.51 d^{-1} at Klang and Port Dickson, respectively. In this study, grazing loss was coupled to phytoplankton growth and the ratio of g : μ or grazing pressure which estimates the proportion of primary production grazed was 50% at Klang and lower than at Port Dickson (68%) (t = 2.213, df = 36, p < 0.05). We found that the higher growth

rates in an eutrophic system i.e. Klang were not matched by higher grazing loss, and this may have implications for the biogeochemical cycling in coastal waters.

Introduction

10   Marine phytoplankton accounts for nearly half of the global primary production (Field et al. 1998), and plays an essential role in marine food webs (Falkowski et al. 1998). The fate of phytoplankton production follows either the classical or microbial pathways (Calbet and Landry 2004, Schmoker et al. 2013). In the classical pathway, phytoplankton production is transferred to higher level via grazing, and subsequently affects fishery yield (Mann 1993) whereas in the

15   microbial pathway, phytoplankton production is coupled to bacterial consumption via the dissolved organic matter pool (Azam et al. 1983, del Giorgio et al. 1997, Cole 1999). Although the microbial pathway is relatively well-studied in the coastal waters of Peninsular Malaysia (Lee and Bong 2008, Lee et al. 2009, Bong and Lee 2011, Lee and Bong 2012), the classical pathway remains poorly characterized.

20   Grazing loss is an important process in the classical pathway as the fate of phytoplankton production not grazed is either through horizontal export into adjacent waters or sinking (Cloern et al. 1985, Turner 2002, Stukel et al. 2011). From a cross-compilation of 66 studies, Calbet and Landry (2004) revealed that grazing loss accounts for 60 – 75% of daily phytoplankton

production, and is higher in estuarine and coastal waters than in oceanic waters. Although it is generally accepted that grazing loss is higher in productive waters (Schmoker et al. 2013), the impact of grazing loss is still not clear as grazing loss rates are extremely variable, are site-specific and can be affected by multiple environmental drivers e.g. temperature (Irwin and Oliver

5   2009, Boyce et al. 2010).

The review by Calbet and Landry (2004) was also constrained by the lack of data from tropical waters where nine was from tropical waters and only a single study was from tropical coastal waters. Other than filling an obvious data gap (Schmoker et al. 2013), it is vital to investigate the phytoplankton production and grazing loss in tropical waters in view of the

10 current global climate change (IPCC 2007). Ocean warming is expected to have the strongest effect in tropical waters where most organisms are already functioning at or near optimum temperatures (Pomeroy and Wiebe 2001). Rising ocean temperatures also enhances grazing loss (Irwin and Oliver 2009, Boyce et al. 2010, Chen et al. 2012). However in the absence of baseline data or previous studies, it will be difficult to ascertain the effects of climate change in the

15 coastal waters of Peninsular Malaysia.

In this study, we measured both phytoplankton production and grazing loss over a two-year period in tropical waters along the Straits of Malacca, using the Landry and Hassett dilution method (Landry and Hassett 1982). In this method, the rate of encounter between predator and prey is decreased by diluting the seawater (Kuipers and Witte 1999), hence encouraging the

20 growth of primary producers (Kirchman et al. 1984, Landry et al. 1984). We sampled two sites with different levels of productivity (Lee and Bong 2008, Lee et al. 2009), as process interactions and relationships in the classical food web may differ between them (Pagano et al. 2006, Yoshiyama and Sharp 2006, Ma et al. 2014).

Therefore in this study, we hope to better understand trophic transfers in the classical

pathway, and also provide a reference point for future studies. We aim to address the following

questions: What is the growth and loss rate of primary producers in tropical coastal waters, and

differences in phytoplankton growth and grazing loss between sites of different productivity?

5

Materials and methods

Sampling

10   Surface seawater samples (at about 0.1 m depth) were collected monthly at both Klang

estuary (3°0'N, 101°24'E) and coastal water of Port Dickson (2°31'N, 101°47'E) from January

2010 to December 2011 (n = 24) (Figure 1). In-situ seawater temperature and salinity were

measured with a salinity probe (YSI–30, USA) whereas dissolved oxygen (DO) was measured

via the Winkler titration method (Grasshoff et al. 1999). Secchi disc depth was also measured to

15   estimate the extent of the euphotic zone (Welch 1948).

In the laboratory, seawater samples were filtered through pre-combusted (500°C for 3h)

glass fibre filters (GF/F). The filtrate was kept frozen at –20°C for inorganic nutrient analysis

whereas the filters were kept for chlorophyll a (Chl a) and total suspended solids (TSS)

measurements. Chl a was extracted in ice-cold acetone (90% v/v) at –20°C overnight, and

20   determined via the trichromatic method (Parson et al. 1984) whereas the net weight increase of

the filter after drying (50°C for 7 d) was calculated as TSS. In this study ammonium (NH_4),

nitrite + nitrate (NO_2+NO_3), silicic acid (SiO_4) and phosphate (PO_4) were measured with a

QuAAtro (Bran+Luebbe) according to Grasshoff et al. (1999).

Phytoplankton growth and loss rates measurement

The Landry and Hassett dilution method was used to determine concurrent growth

5   (Landry et al. 1984) and grazing loss rate (Landry and Hassett 1982). Seawater from the same

station was filtered through 0.2 µm membrane filter to generate particle-free seawater, and then

diluted with seawater sample to the following fractions: 0.2, 0.4, 0.6, 0.8 and 1.0 (undiluted).

Each experimental setup was completed in < 4 h, and every fraction was subsequently incubated

in an incubator (INNOVA 4900, USA) at in-situ temperature under light condition (340 µ mol

10   $m^{-2} s^{-1}$) for 16 h. Each treatment was duplicated and the phytoplankton growth rate in each

fraction was determined by the following equation: ln final Chl a – ln initial Chl a. The growth

rate in each fraction was plotted against dilution, and the potential growth rate (µ) was

determined from the y-intercept. In order for comparison with other studies, we performed

concurrent incubation with the standardized method of 12 L: 12 D incubation period (Figure 2)

15   and corrected our data, accordingly. The negative relationship observed for g could be due to the

fact that the irradiance affected µ more than g, and in this case reduced the grazing activity

(Gutiérrez-Rodríguez et al. 2009). However the sampling size here is small, and further study is

required. We also corrected for the lower light transmittance at Klang (120 µ mol $m^{-2} s^{-1}$).

In this study, grazing loss (g) was determined from the linear regression slope in the

20   growth rate against dilution graph. g was total grazing as we did not distinguish between

microzooplankton and mesozooplankton but it is known that microzooplankton is the most

important factor for primary production loss (Calbet and Landry 2004, Schmoker et al. 2013).

Apparent production and grazing rate was subsequently calculated by multiplying µ and g with

the initial Chl *a* concentration. For unit conversion into carbon, we measured the total biovolume

of phytoplankton cells on 37 occasions, and converted the biovolume to C units (Moal et al.

1987). The phytoplankton community at Klang and Port Dickson was different, and was

predominantly *Skeletonema* sp. and *Chaetoceros* sp., respectively. The C biomass and concurrent

5   Chl *a* concentration were then used to determine the C: Chl *a* ratio which fluctuated arbitrarily,

and averaged 86 at Klang and 148 at Port Dickson (*submitted*).

Statistical analyses

10   All values were reported as mean ± standard deviation (S.D.) unless otherwise mentioned.

For each dilution set-up, the least-square linear regression test was carried out. Student's *t*-test

was used to compare the different parameters between Klang and Port Dickson whereas

correlation analysis was used to show relationships between the different parameters measured.

Outliers in the correlation analyses were determined as outside the range of mean ± 2 S.D. All

15   statistical tests were carried out with the software PAST (Hammer et al. 2001).

Results

Environmental condition

20

Surface seawater temperatures measured at both Klang and Port Dickson were similar,

and ranged from 28 to 32°C (Table 1). In contrast, salinity was lower at Klang (t = 2.45, df = 30,

p < 0.05), and varied over a wider range (3 − 30 ppt). At Klang, both DO (t = 10.02, df = 32, p <

0.001) and Secchi disc depth (t = 6.22, df = 31, p < 0.001) were lower whereas TSS was higher (t = 3.08, df = 36, p < 0.001). In this study, dissolved inorganic nutrients (NH_4, NO_2+NO_3 and SiO_4) were also found to be higher at Klang (t > 3.92, p < 0.001), with the exception of PO_4.

Chl *a* was measured as a proxy for phytoplankton biomass, and ranged from 0.20 to 4.47 µg L^{-1} at Klang, except in Sep and Nov 2011 when Chl *a* concentration increased above 10 µg L^{-1} (Figure 3). In contrast, Chl *a* concentration at Port Dickson was relatively constant (1.49 ± 0.73 µg L^{-1}), and did not increase beyond 2.76 µg L^{-1}.

Phytoplankton growth and loss rates

In this study, we used the Landry and Hassett dilution method to estimate both µ and g, and obtained statistically significant regression slopes on all occasions (Table 2). At Klang, µ ranged from 0.30 to 2.26 d^{-1} whereas at Port Dickson, µ ranged from 0.18 to 1.66 d^{-1}. µ was slightly elevated at Klang during the Apr – May period in both 2010 and 2011. Although we also detected an increase in µ during the same period (Apr – May 2010) at Port Dickson, a steeper increase was observed during the year-end period (Dec 2010 – Jan 2011). For grazing loss, g ranged from 0.30 to 1.50 d^{-1} and 0.21 to 1.51 d^{-1} at Klang and Port Dickson, respectively. g generally changed in tandem with µ at Port Dickson but at Klang, g did not respond to an increase in µ in Apr – May 2010. The ratio of g : µ or grazing pressure measures the portion of phytoplankton growth that is grazed, and ranged from 0.21 to 1.70 (0.88 ± 0.11) at Klang and from 0.59 to 1.67 (1.47 ± 0.34) at Port Dickson.

When converted to carbon units, primary production ranged 0.03 – 1.38 g C m^{-3} d^{-1} at Klang and 0.03 − 0.20 g C m^{-3} d^{-1} at Port Dickson whereas grazing rate ranged 0.02 – 2.11 g C

m^{-3} d^{-1} and $0.03 - 0.23$ g C m^{-3} d^{-1} at Klang and Port Dickson, respectively. Over the euphotic

zone, integrated primary production at Klang ranged $42 - 400$ mg C m^{-2} d^{-1} in 2010 but varied

over a wider range in 2011 ($42 - 2120$ mg C m^{-2} d^{-1}) (Figure 4). This was primarily due to the

spike in primary production in Sep 2011. At Port Dickson, integrated primary production was

5   relatively stable, and ranged $52 - 344$ mg C m^{-2} d^{-1} in 2010 and $63 - 782$ mg C m^{-2} d^{-1} in 2011.

Discussion

Environmental condition

10

       Surface seawater temperature observed was relatively high and stable, and is typical of

tropical coastal waters (Lee and Bong 2008). Salinity was more variable at the Klang estuary due

to the Klang River influx. From the mixing graph (Figure 5), the decreasing nutrient

concentrations as salinity increased seemed to suggest river inflow as a source that supplied

15   inorganic nutrients especially NH_4 and SiO_4 to the Klang estuary. Relative to Port Dickson,

Klang waters exhibited lower DO and transparency (i.e. higher TSS levels and lower Secchi disc

depth). These observations reflected the poorer water quality that prevails at Klang (Lee et al.

2009).

       Chl *a* at Port Dickson was relatively constant throughout the two-year sampling period

20   whereas at Klang, there were episodic spikes in Chl *a* concentration. The spikes in Chl *a*

concentration were detected at Klang in Sep and Nov 2011 and reached 26.31 and 11.97 µg L^{-1},

respectively. Without these episodes, the average Chl *a* concentration at Klang was 1.56 ± 1.35

µg L^{-1}, and not different from Port Dickson ($p > 0.30$). Chl *a* concentration at Port Dickson was

independent of any of the inorganic nutrients measured whereas at Klang, Chl *a* increased with inorganic nutrients (NH$_4$: $R^2 = 0.319$, df = 22, $p < 0.01$ and SiO$_4$: $R^2 = 0.276$, df = 22, $p < 0.01$). As both Chl *a* and inorganic nutrient concentrations were also correlated with salinity albeit inversely (NH$_4$: $R^2 = -0.491$, df = 22, $p < 0.001$; SiO$_4$: $R^2 = -0.590$, df = 22, $p < 0.001$; Chl *a*: $R^2 = -0.275$, df = 22, $p < 0.01$), the correlations observed between Chl *a* and inorganic nutrients were more likely a product of collinearity. Although the higher concentrations of inorganic nutrients could have stimulated phytoplankton growth and subsequently increased Chl *a*, our results had shown that Chl *a* concentration was independent of phytoplankton growth (μ) at Klang ($p > 0.10$). Therefore a more likely explanation for the Chl *a* peaks at Klang was the transport of both inorganic nutrients and Chl *a* by the Klang River into the estuary, which concurs with observations from the nearby Langat River (Lee et al. 2013).

Phytoplankton growth and loss rates

The phytoplankton growth rate or μ at Klang (0.70 ± 0.08 d^{-1}) was slightly higher than Port Dickson (0.54 ± 0.06 d^{-1}) (t = 2.01, df = 43, $p < 0.05$). The higher inorganic nutrient concentrations at Klang could have supported the higher μ. However μ was independent of any inorganic nutrients measured at Klang whereas at Port Dickson, μ correlated with both NO$_2$+NO$_3$ ($R^2 = 0.248$, df = 20, $p < 0.05$) and SiO$_4$ ($R^2 = 0.258$, df = 20, $p < 0.05$). When looking at the data from both stations, there was a possible threshold concentration for NO$_2$+NO$_3$ (≈ 4 μM) and SiO$_4$ (≈ 20 μM) beyond which the distribution of μ was independent of the inorganic nutrients (Figure 6). As both Klang and Port Dickson could represent a trophic

gradient, this threshold may also exist at other locations. Sampling at other locations will allow us to see if this observation holds true.

The range of primary production rates measured in this study was similar to those obtained using the ^{14}C uptake method (Alongi et al. 2003) and light-dark dissolved oxygen method (Lee and Bong 2008). Phytoplankton production rate from the dilution method has also been shown to be related and comparable to the more established ^{14}C uptake method (Calbet and Landry 2004). However the dilution method has known limitations e.g. non-linear response (Paterson et al. 2008, York et al. 2010) or nutrient depletion (Landry and Hassett 1982). Non-linear responses were not observed in this study, and the higher nutrient concentrations in our samples as well as the shorter incubation time enabled us to complete the dilution experiments before nutrient depletion sets in. Changes in the cellular chlorophyll content of primary producers during incubation may also affect the estimation of phytoplankton growth rates but the overall effect is reported to be small (Liu et al. 2009).

In contrast to the higher phytoplankton growth rate observed at Klang, grazing loss rates were not different between the two stations ($p > 0.80$). Grazing loss was however coupled to phytoplankton growth at Klang ($R^2 = 0.267$, df = 17, $p < 0.05$) and Port Dickson ($R^2 = 0.570$, df = 19, $p < 0.001$) (Figure 7). Although causality cannot be inferred from correlation analysis, the correlation observed between grazing loss and phytoplankton growth was probably an indication of the coupling between phytoplankton growth and microzooplankton grazing that is well documented for a wide range of ecosystems (Calbert 2001, Calbert and Landry 2004).

The relationship between grazing loss and phytoplankton growth at both Klang and Port Dickson could be expressed via the following linear regression equation: Klang: $g = 0.499\mu + 0.237$ (F = 6.18, df = 18, $p < 0.05$); Port Dickson: $g = 0.679\mu + 0.182$ (F = 25.2, df = 20, $p <$

0.001). From the equation, g : μ or grazing pressure at both Klang and Port Dickson accounted for 50% and 68% of phytoplankton production, respectively. When we compared the regression slopes (Zar 1999), grazing pressure was higher at Port Dickson than Klang (t = 2.213, df = 36, p < 0.05). In eutrophic ecosystems e.g. Klang, more trophic levels and a higher amount of biomass are usually available (Pagano et al. 2006, Lee and Bong 2008). In this situation, predator – prey coupling is sometimes less obvious or uncoupling sometimes occur as other resources or prey are available. When we extended the linear regression relationship until μ = 0 or when there is no phytoplankton production, grazing activity was still supported at 0.24 d^{-1} at Klang which was higher than at Port Dickson (0.18 d^{-1}). This would only be possible if grazing was supported by other prey.

Although phytoplankton production at Klang was higher, the amount transferred to higher trophic levels via grazing was lower which could imply a higher carbon export either though sinking (Cloern et al. 1985) or lateral transport away from the estuary (Stukel et al. 2011). However the balance between production and grazing could only be adequately assessed if a more thorough carbon budget calculation were carried out. We summed phytoplankton growth and loss data from both stations to estimate the average grazing pressure across a trophic gradient in the coastal waters of Peninsular Malaysia. Phytoplankton production was coupled to grazing, and could be represented by the following: g = 0.596μ + 0.202 (F = 29.1, df = 39, p < 0.001). Average grazing pressure across a trophic gradient in our coastal waters was about 60%. Although grazing loss could be underestimated if grazers also carry out mixotrophy which will result in a lower reduction of Chl *a*, our results showed close similarity to the grazing pressure from the cross-compilation study by Calbet and Landry (2004).

The simplifications used in the dilution method do not approach the complexity of an actual food web (Pomeroy 2001) where composition and population dynamics of both prey and grazer may influence the rates measured (Modigh and Franzé 2009). However dilution experiments done in large numbers can be used to describe the process interactions and

5   relationships in natural systems (Landry 2014). This study shows strikingly different responses in predator-prey coupling between eutrophic and oligotrophic systems. We found that the higher growth rates were not matched by higher grazing loss in the eutrophic system. This study filled an obvious data gap, and provided valuable baseline data for future studies. Our findings will also contribute to our understanding of ecosystem dynamics and biogeochemical cycling in

10   coastal waters.

Acknowledgements

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List of Figures

Figure 1. Location of Klang (3°0'N, 101°24'E) and Port Dickson (2°31'N, 101°47'E) sampling stations.

5 **Figure 2**. Comparison of μ and g from Landry and Hassett dilution experiments carried out in 12 h light and 12 h dark (12 L: 12 D) incubation versus 16 h light (16 L) incubation period. Correlation analyses are shown, and solid lines show the linear regression.

Figure 3. Temporal variation of Chl *a*, μ, g and g : u (grazing pressure) at both Klang and Port Dickson. For Chl *a*, error bars indicate the S.D. whereas for μ and g, error bars indicate the S.E.

10 Error bars are shown except when smaller than the symbol. For grazing pressure, dashed lines at g : μ = 1.0 are also shown.

Figure 4. Temporal variation of the integrated primary production in the euphotic layer (mg C $m^{-2} d^{-1}$) at Klang and Port Dickson. Error bars indicate the S.E and are shown except when smaller than the symbol.
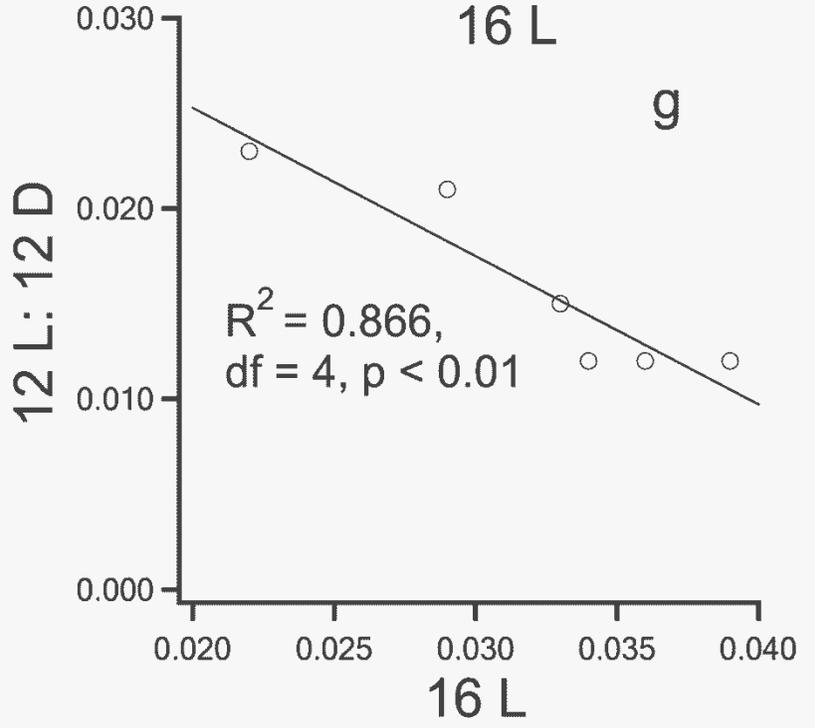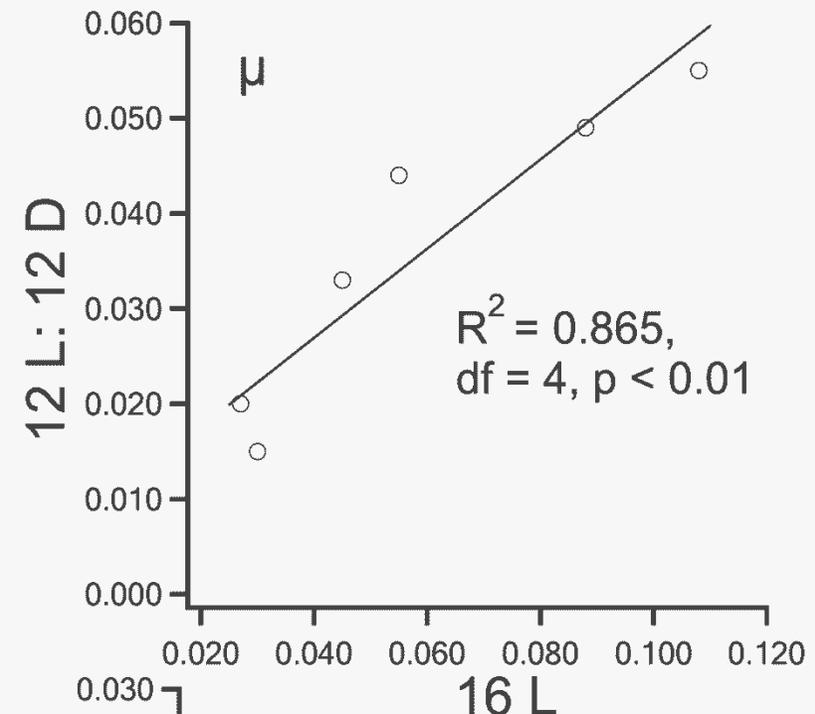
15 **Figure 5**. Mixing graph showing the distribution of dissolved inorganic nutrients across a salinity gradient. Correlation analyses that are significant are shown. Solid line shows the linear regression for salinity versus ammonium (NH_4) whereas dashed line shows the linear regression for salinity versus silicic acid (SiO_4).
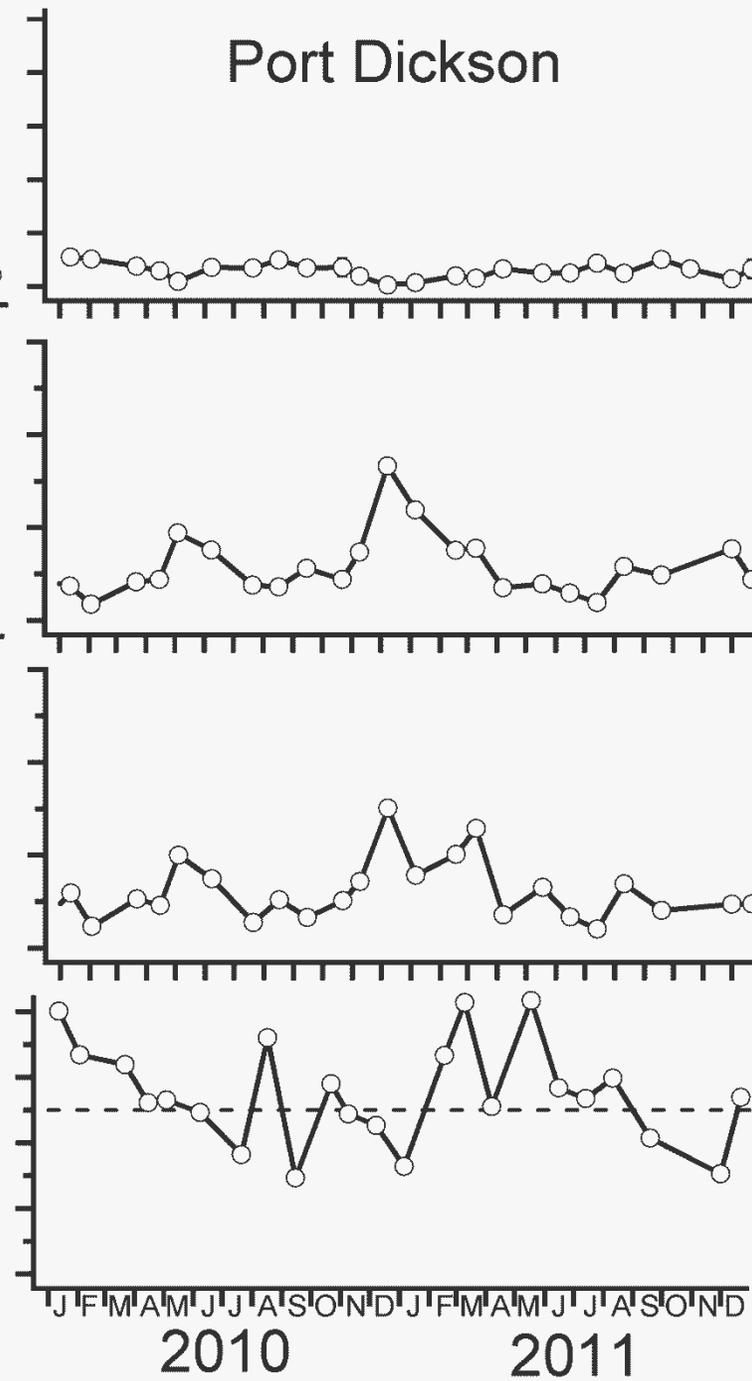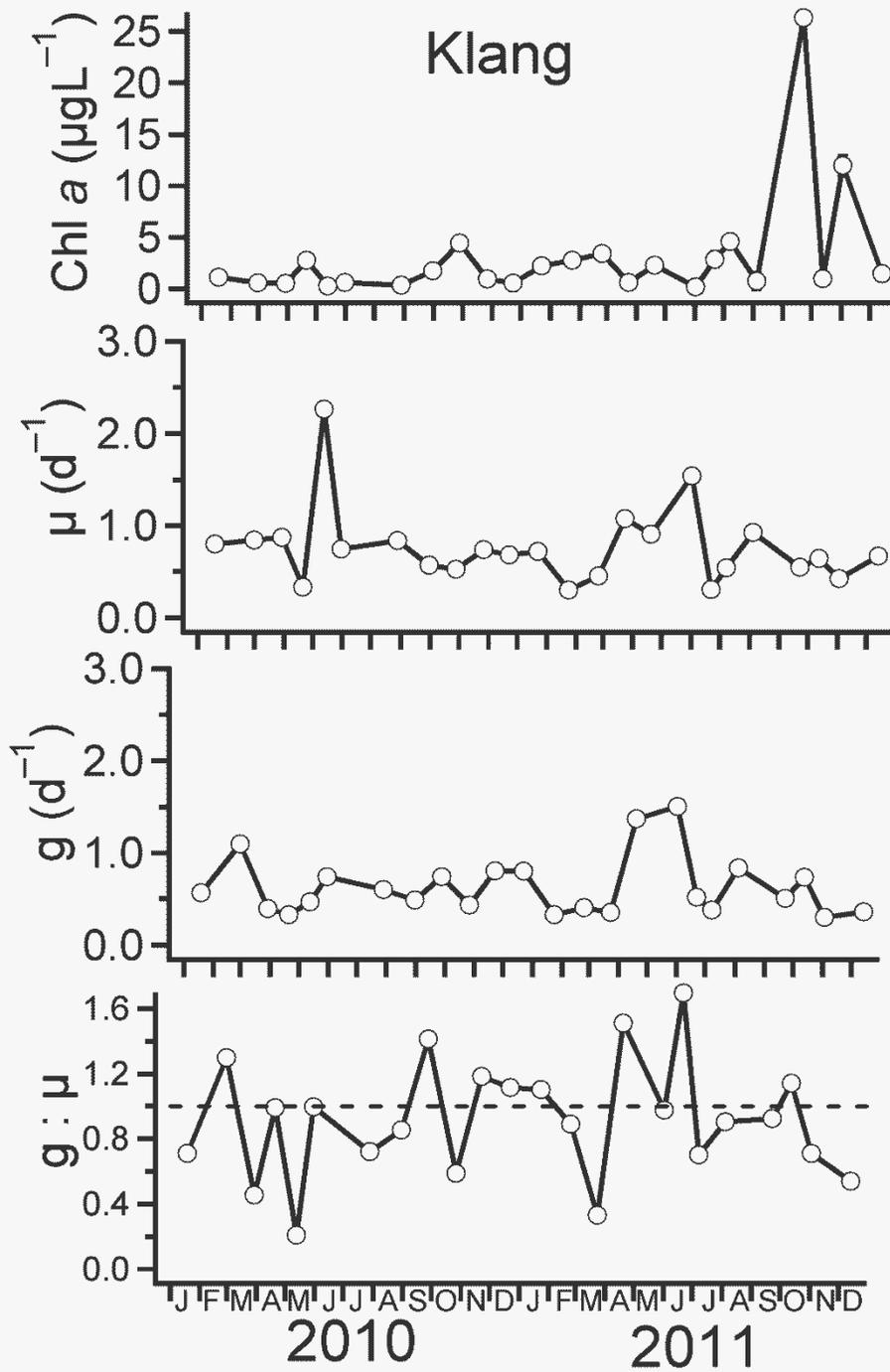
Figure 6. Correlation analysis between (A) μ and NO_2+NO_3 and (B) μ and SiO_4. Correlation

20 analysis was only significant for Port Dickson data. Solid lines show the linear regression for Port Dickson data.
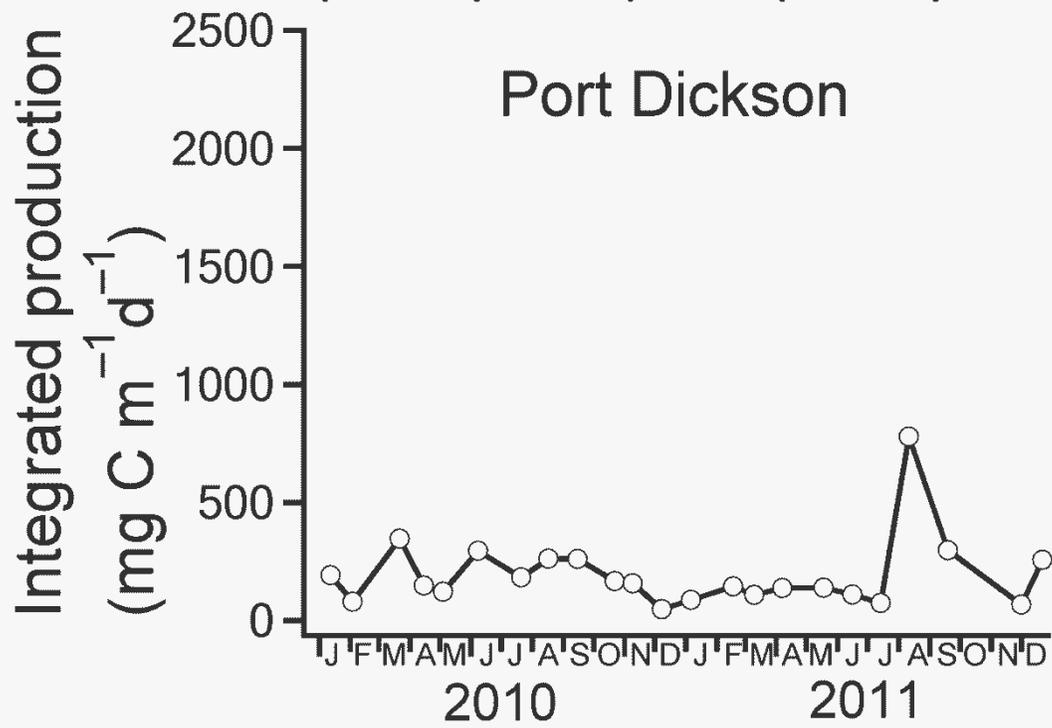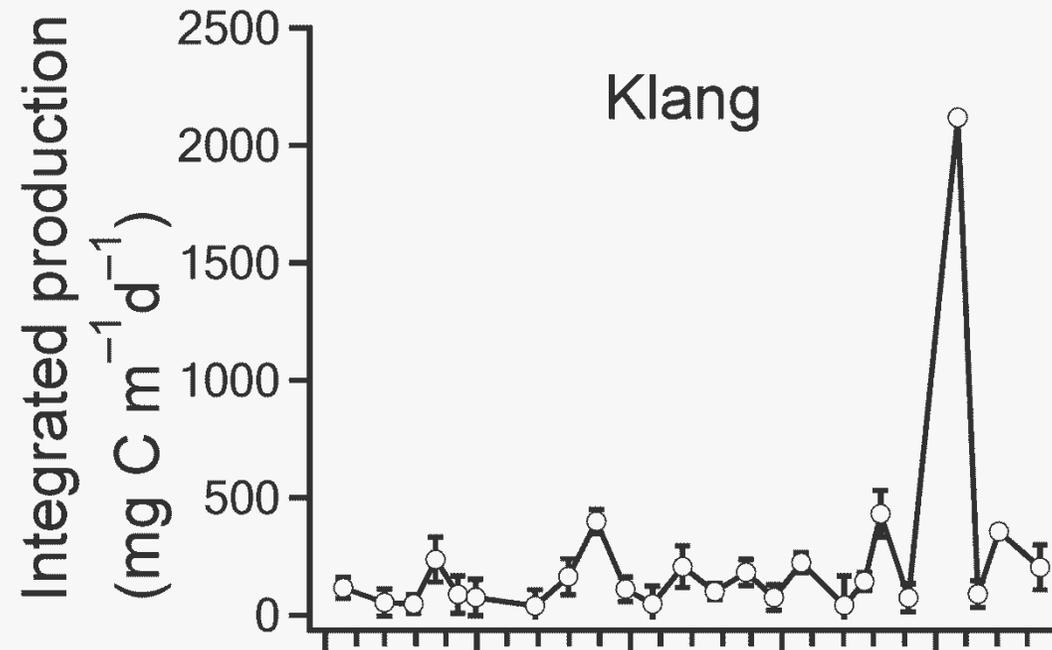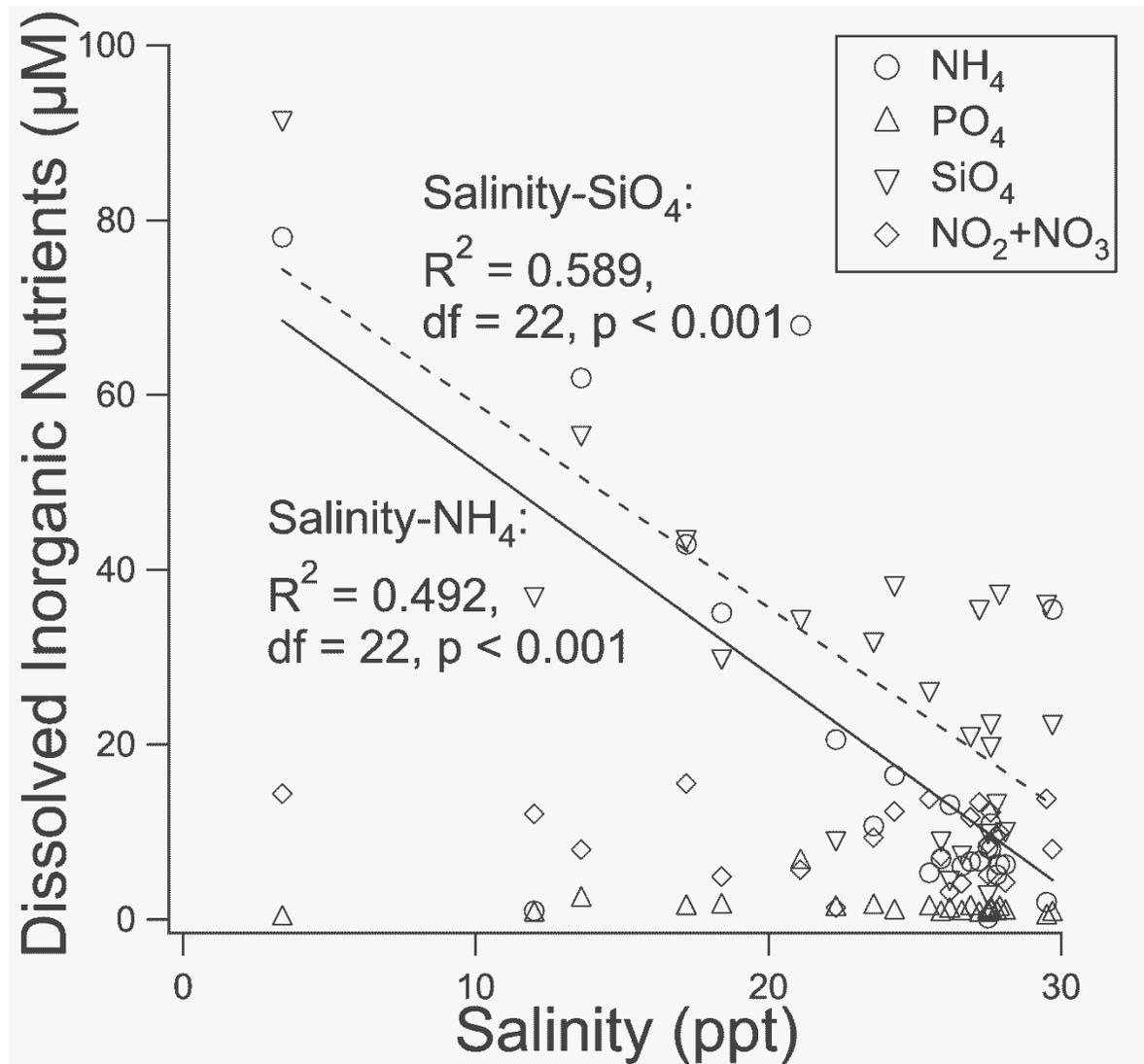
Figure 7. Correlation analysis between g and μ for both Klang and Port Dickson (PD). Solid line shows the linear regression for Port Dickson data whereas dashed line shows the linear regression for Klang data. Filled symbols represent outliers.
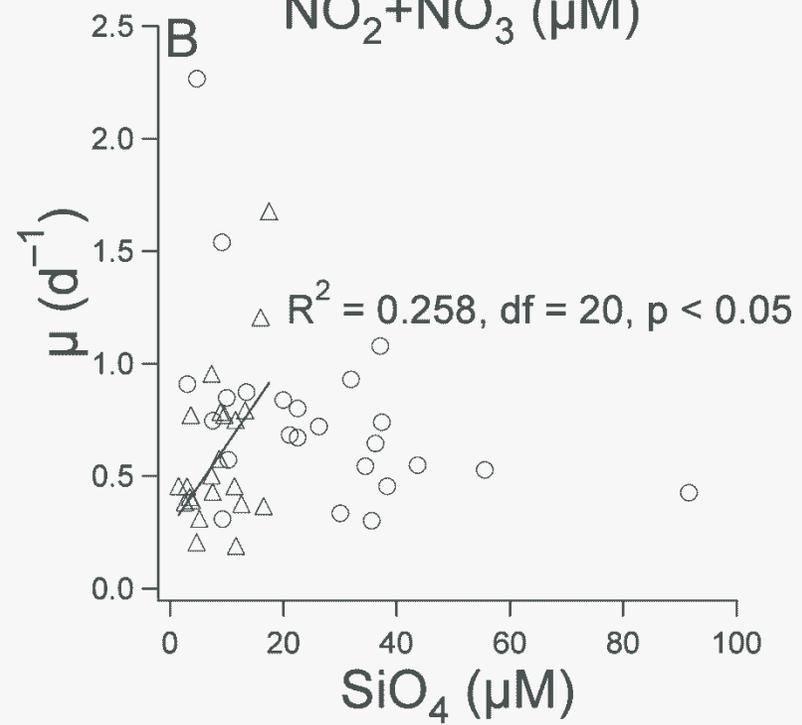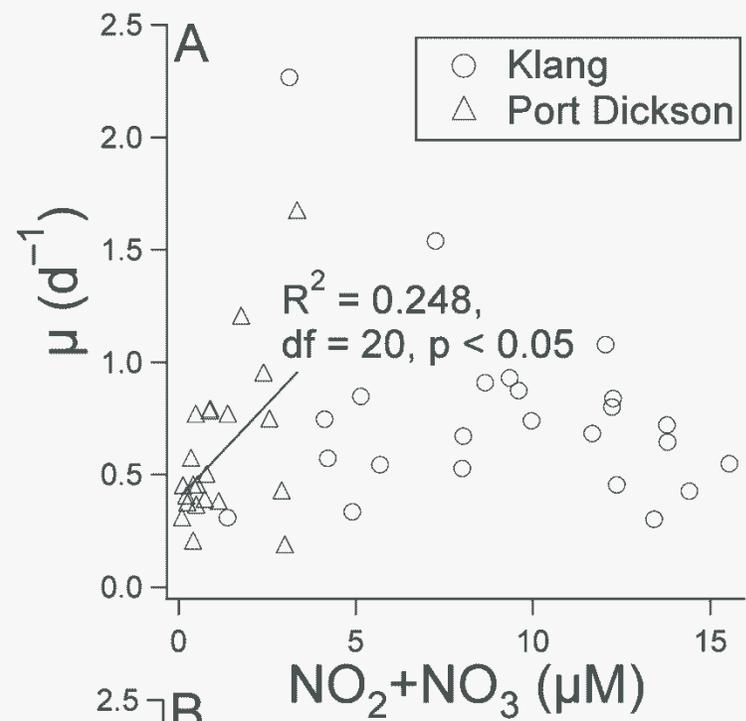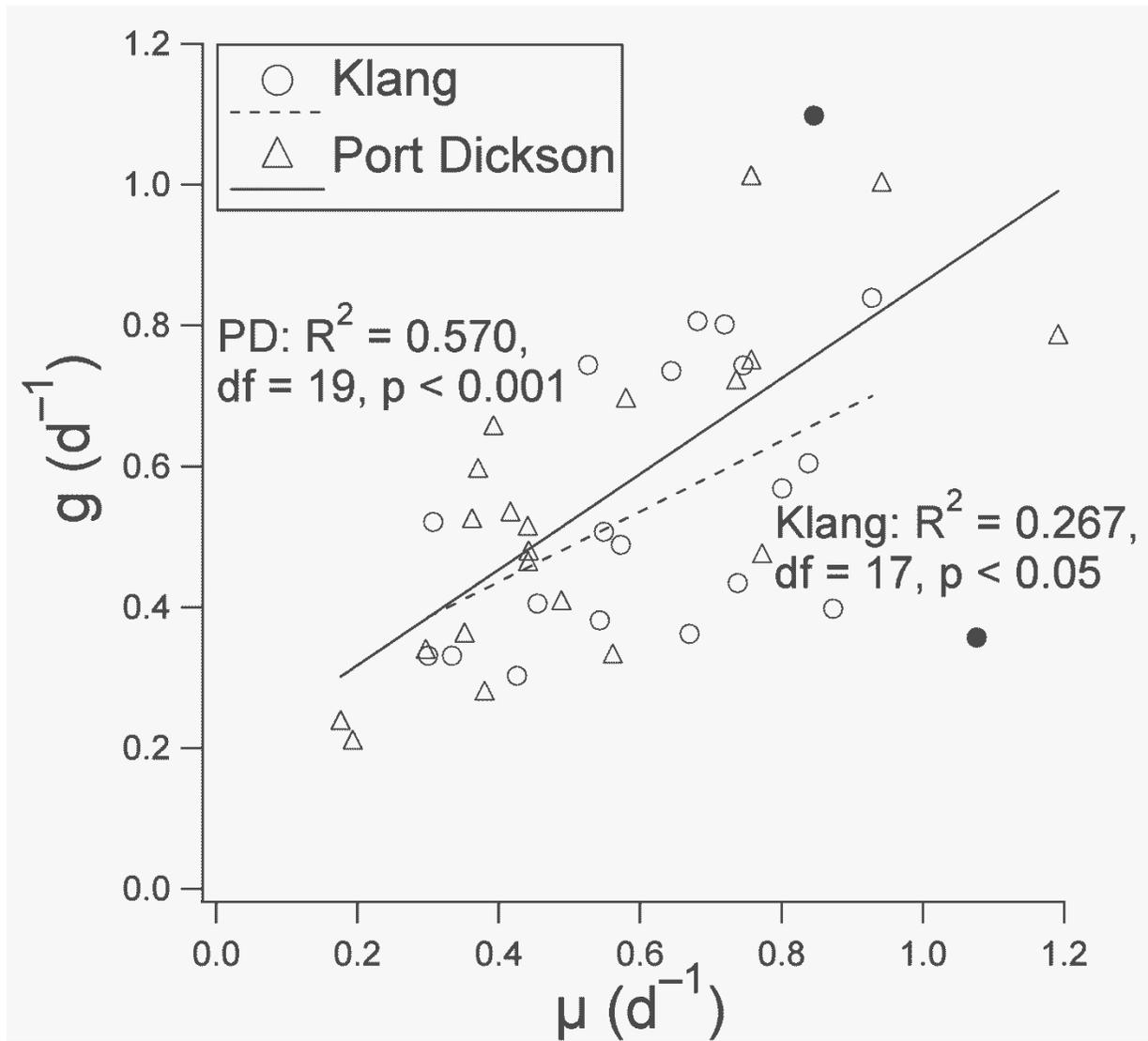
μ

$R^2 = 0.865$,
df = 4, p < 0.01

g

$R^2 = 0.866$,
df = 4, p < 0.01

A

μ (d⁻¹) vs NO₂+NO₃ (μM)

$R^2 = 0.248$, df = 20, $p < 0.05$

Legend: ○ Klang, △ Port Dickson

B

μ (d⁻¹) vs SiO₄ (μM)

$R^2 = 0.258$, df = 20, $p < 0.05$
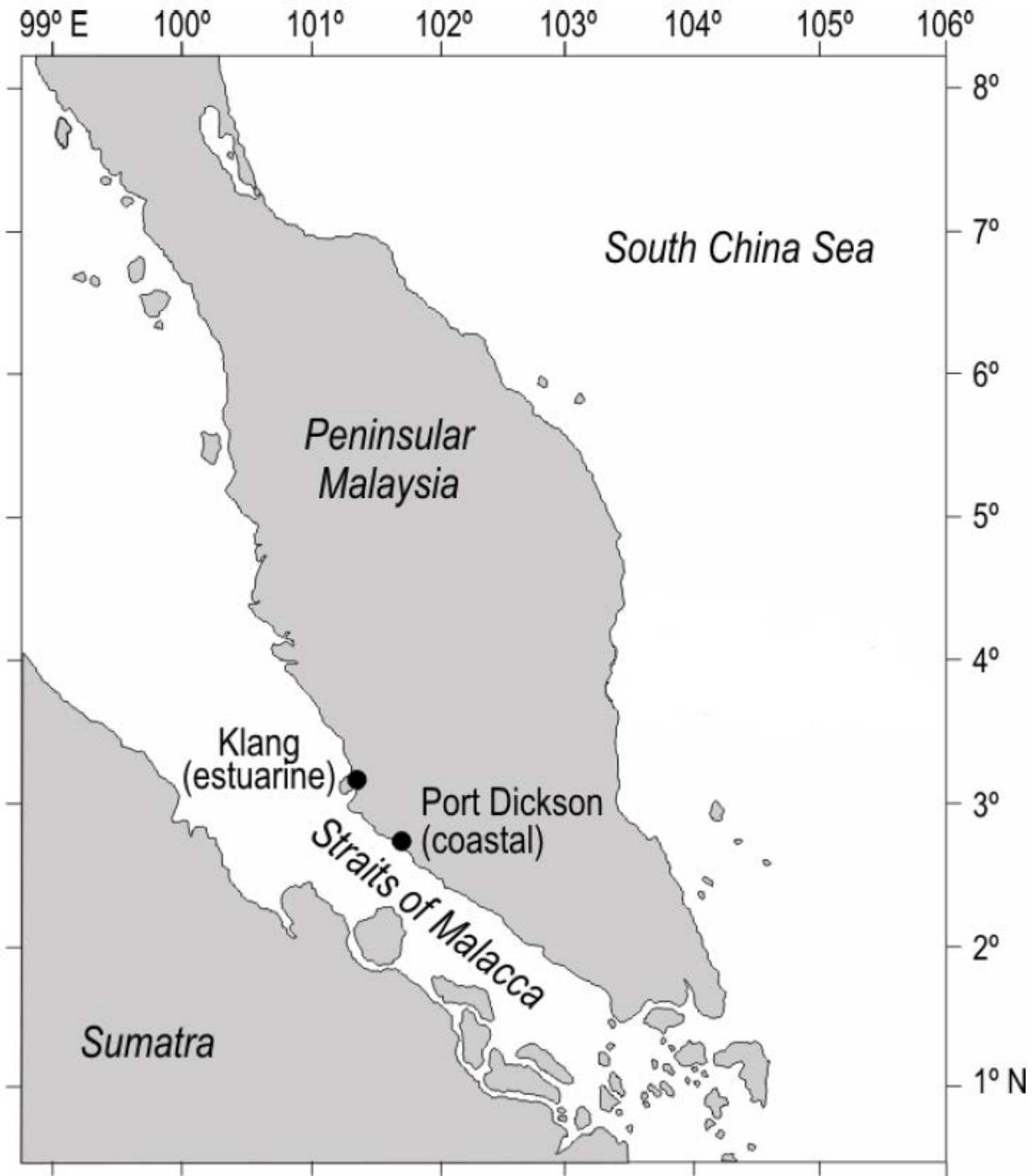
1 **Table 1**. Environmental conditions at Klang and Port Dickson (mean ± S.D). Student's t-tests

2 were carried out to compare values from Klang and Port Dickson, and significance of

3 differences between the sampling sites are as follows: *, $P < 0.05$; **, $P < 0.01$; ***, $P <$

4 0.001.

	Klang	Port Dickson
Temperature (°C)	29.6 ± 0.9	29.5 ± 1.0
Salinity (ppt)*	23.6 ± 6.5	26.6 ± 3.7
DO (µM)*	140 ± 30	210 ± 10
TSS (mg L^{-1})*	62 ± 16	50 ± 10
Secchi Depth (m)*	0.60 ± 0.16	1.13 ± 0.39
NH$_4$ (µM)*	19.25 ± 22.38	1.31 ± 0.97
NO$_2$+NO$_3$ (µM)*	9.21 ± 4.03	1.15 ± 1.01
SiO$_4$ (µM)*	27.22 ± 19.57	8.48 ± 4.69
PO$_4$ (µM)	1.16 ± 1.24	0.70 ± 0.75

Table 2. Corrected data of $\mu \pm SE$ (d^{-1}) and g $\pm$ SE (d^{-1}) for Klang and Port Dickson. Correlation index and p values are also shown.

Klang Date	Growth rate (μ)	Grazing rate (g)	R^2	p	Port Dickson Date	Growth rate (μ)	Grazing rate (g)	R^2	p
19-Jan-10	0.8007 ± 0.0547	0.5689 ± 0.1422	0.55	0.001	12-Jan-10	0.3704 ± 0.0310	0.5933 ± 0.0756	0.71	0.01
1-Mar-10	0.8459 ± 0.0750	1.0980 ± 0.2350	0.56	0.001	3-Feb-10	0.1759 ± 0.0413	0.2353 ± 0.1034	0.22	0.05
30-Mar-10	0.8727 ± 0.0434	0.3975 ± 0.1094	0.34	0.001	22-Mar-10	0.4164 ± 0.0317	0.5315 ± 0.0820	0.64	0.001
21-Apr-10	0.3337 ± 0.0511	0.3307 ± 0.1507	0.21	0.05	15-Apr-10	0.4412 ± 0.0421	0.4608 ± 0.1042	0.42	0.001
13-May-10	2.2647 ± 0.0705	0.4709 ± 0.1778	0.23	0.01	4-May-10	0.9418 ± 0.0764	0.9997 ± 0.1980	0.54	0.001
31-May-10	0.7457 ± 0.0582	0.7430 ± 0.1567	0.48	0.001	8-Jun-10	0.7572 ± 0.1192	0.7470 ± 0.3100	0.17	0.05
29-Jul-10	0.8381 ± 0.0637	0.6044 ± 0.1728	0.33	0.01	21-Jul-10	0.3798 ± 0.0477	0.2768 ± 0.1273	0.16	0.05
31-Aug-10	0.5723 ± 0.0560	0.4887 ± 0.1554	0.29	0.01	17-Aug-10	0.3621 ± 0.0479	0.5216 ± 0.1275	0.46	0.001
28-Sep-10	0.5262 ± 0.0342	0.7439 ± 0.0888	0.71	0.001	15-Sep-10	0.5616 ± 0.0427	0.3296 ± 0.1111	0.24	0.01
27-Oct-10	0.7383 ± 0.0396	0.4343 ± 0.1018	0.42	0.001	22-Oct-10	0.4406 ± 0.0418	0.5111 ± 0.1035	0.65	0.001
23-Nov-10	0.6813 ± 0.0714	0.8061 ± 0.1917	0.40	0.001	9-Nov-10	0.7361 ± 0.0514	0.7187 ± 0.1338	0.51	0.001
23-Dec-10	0.7193 ± 0.0874	0.8017 ± 0.2394	0.34	0.01	8-Dec-10	1.6624 ± 0.1232	1.5085 ± 0.3194	0.45	0.001
24-Jan-11	0.3000 ± 0.0376	0.3309 ± 0.1102	0.31	0.01	6-Jan-11	1.1913 ± 0.0682	0.7828 ± 0.1773	0.41	0.001
24-Feb-11	0.4549 ± 0.0576	0.4050 ± 0.1479	0.24	0.01	17-Feb-11	0.7570 ± 0.0737	1.0084 ± 0.1957	0.60	0.001
24-Mar-11	1.0761 ± 0.0602	0.3570 ± 0.1545	0.20	0.05	10-Mar-11	0.7792 ± 0.0994	1.2902 ± 0.2883	0.47	0.001
20-Apr-11	0.9083 ± 0.0512	1.3727 ± 0.1464	0.81	0.001	7-Apr-11	0.3516 ± 0.0525	0.3594 ± 0.1484	0.23	0.05
2-Jun-11	1.5391 ± 0.1047	1.5046 ± 0.2684	0.55	0.001	18-May-11	0.3924 ± 0.0415	0.6542 ± 0.1308	0.57	0.001
22-Jun-11	0.3071 ± 0.0298	0.5210 ± 0.0870	0.63	0.001	16-Jun-11	0.2965 ± 0.0376	0.3363 ± 0.1238	0.36	0.05
7-Aug-11	0.5430 ± 0.0714	0.3811 ± 0.1783	0.17	0.05	14-Jul-11	0.1933 ± 0.0305	0.2069 ± 0.0868	0.23	0.05
8-May-11	0.9280 ± 0.0664	0.8396 ± 0.1767	0.46	0.001	8-Nov-11	0.5801 ± 0.0426	0.6928 ± 0.1106	0.62	0.001
23-Sep-11	0.5490 ± 0.0181	0.5073 ± 0.0469	0.81	0.001	19-Sep-11	0.4887 ± 0.0410	0.4053 ± 0.1066	0.34	0.001
13-Oct-11	0.6437 ± 0.0511	0.7355 ± 0.1449	0.54	0.001	1-Dec-11	0.7724 ± 0.0758	0.4722 ± 0.2030	0.18	0.05
11-Mar-11	0.4254 ± 0.0236	0.3022 ± 0.0615	0.46	0.001	22-Dec-11	0.4418 ± 0.0585	0.4759 ± 0.1741	0.33	0.05
14-Dec-11	0.6700 ± 0.0613	0.3621 ± 0.1552	0.19	0.05					