Long-term comparison of dissolved nitrogen species in tropical estuarine and coastal water systems

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Abstract Long-term observation of aquatic habitat is invaluable as it reveals trends that are not evident from shorter-term measurements. In this study, we assessed measurements of dissolved nitrogen species over a 12-year period (2004 – 2015) at Port Klang estuary and Port Dickson coastal water. Total suspended solids (TSS) was also measured, and increased at 6 – 9 mg L^{-1} yr^{-1}. In contrast, total dissolved nitrogen (TDN) did not increase but was higher at Port Klang (8 – 320 µM) relative to Port Dickson (2 − 30 µM). Chl *a* concentration was also higher at Port Klang (5.18 ± 7.79 µg L^{-1}) than at Port Dickson (2.27 ± 1.39 µg L^{-1}). Among the dissolved nitrogen species, we found that ammonium (NH_4) was predominant at Port Klang where dissolved inorganic nitrogen (DIN) contributed 71 ± 3% of TDN. At Port Dickson, dissolved organic nitrogen (DON) predominated, contributing 92 ± 7% of TDN. When we calculated the nitrogen loading at

Port Klang, dissolved nitrogen load increased over time (1.13 ± 0.29 kg d^{-1} for TDN, 1.11 ± 0.29 kg d^{-1} for DIN, and 0.15 ± 0.06 kg d^{-1} for DON). We showed that the nitrogen loading increase at Port Klang was not due to climatic forcing but was most probably due to urbanization occurring upstream that inevitably increased river discharge rates (4.04 ± 0.12 m^3 s^{-1} yr^{-1}).

Keywords: long-term; dissolved nitrogen; total suspended solids; nutrient loading; tropical waters

Introduction

Over the last few decades, the rapid increase in anthropogenic activities (e.g. effluent discharge, agriculture and land development) and changes in patterns of precipitation due to a changing climate are increasing the nutrient load to coastal habitats around the world (Strauch et al., 2015; Williams et al., 2015). Although nutrients are essential to sustain primary productivity, excessive nutrient input particularly nitrogen and phosphorus will lead to eutrophication which is generally detrimental to the ecosystem (Peyman et al., 2017). At present, most studies on the influence of excessive nutrient inputs towards eutrophication are from temperate waters even though tropical oceans cover about 40% of the global ocean. Therefore, it is necessary to study the distribution of different dissolved nitrogen species in the tropical waters of South-East Asia. The distribution of different phosphorus pool in these waters was published earlier in Lim et al. (2018).

In marine ecosystem, total dissolved nitrogen (TDN) can be divided into inorganic nitrogen (DIN) and organic nitrogen (DON) pools (Flynn, 2008; Voss et al., 2013). DIN comprises mainly of ammonium (NH_4), nitrate (NO_3) and nitrite (NO_2) which plays an important role in regulating autotrophic processes such as primary productivity. In contrast, DON is typically more important in regulating bacterial heterotrophic processes (Letscher et al., 2013). DIN and DON may come from both autochthonous processes and allochthonous input. The former includes natural nitrogen fixation by different biogeochemical and microbial processes in the nitrogen cycle (Gruber, 2008; Letscher et al., 2013; Voss et al., 2013) whereas the latter generally refers to nitrogen inputs from extraneous habitats. Estuaries in particular, receive a large supply of allochthonous material via riverine input (Flynn, 2008; Brandini et al., 2016).

Long-term observation of aquatic habitats is invaluable as it reveals trends otherwise hidden, and establish relationships with potential drivers for example anthropogenic pressure and climate change. However most of the published reports in South-East Asia focussed on different DIN pools over sampling periods of two years or less (e.g. Tanaka and Choo, 2000; Lee et al. 2009; Teoh et al., 2016; Peyman et al., 2017). These studies are not able to reveal long-term trends as the effects of anthropogenic pressure and climate change on many ecological processes vary over longer time frames (Burt et al., 2010, Dodds et al., 2012). At present, most of the long-term reports are from temperate regions. For example, the increasing trend of DIN and NO_3 observed over >10 years at Yellow River, China (Yue et al., 2017) and the coastal Arabian Sea (Godhe et al., 2015), respectively. There are also reports of decreasing trend over >15 years of total nitrogen and NH_4 at Chesapeake Bay (Lefcheck et al., 2018) and two estuaries in Australia (Larsson et al., 2016), respectively due to different remediation efforts.

There is an obvious knowledge gap concerning the long-term variation of dissolved nitrogen species in the South-East Asia region. Thus in this study, we complemented our current understanding of nitrogen pool distribution in coastal waters via long-term (12 years) analysis of DIN and DON species. We measured at stations with different levels of eutrophication: a mesotrophic coastal water station and a eutrophic estuarine station. The choice of these two stations was to reveal the typical range of nitrogen species for waters in Peninsular Malaysia whereas a comparison of these two stations could illustrate temporal trends not evident from shorter-term observation. In addition to TDN, total suspended solid (TSS) was also measured. TSS is a persistent problem in this region (Chua et al., 2000), and is an indicative of the general changes in water quality in these waters (Bong and Lee, 2008). TSS could also be a source of particulate organic matter (POM) which includes particulate organic nitrogen (PON) (e.g. Samantha and Anderson, 2008; Dong et al., 2010; Robinson et al., 2012). Although PON is not measured here, the changes in TSS could be suggestive of PON contribution.

Materials and Methods

Study site

We compiled data collected by this laboratory over a 12-year period (2004 − 2015) at Port Klang and Port Dickson. Data from the earlier years were previously published to briefly introduce the environmental conditions e.g. 2004 – 2005 from Lee and Bong (2008), 2007 – 2008 (Tan and Lee, 2014), 2008 – 2010 (Vijayan and Lee, 2014) and 2010 – 2011 (Lim et al., 2015). For this present study, we collected more data from 2013 – 2015. All of the seawater samples were collected from the surface (≈ 0.1 m), using bottles that were soaked in diluted hydrochloric acid for 24 h before rinsing three times with distilled water.

Geologically, Port Klang (3°00' N, 101°24' E) and Port Dickson (2°31' N, 101°47' E) (Fig. 1) are located on the west coast of Peninsular Malaysia along the Strait of Malacca. Port Klang is located on a coastal plain estuary where Klang River flows before dispersing into Klang Strait and subsequently to the Strait of Malacca (Sasekumar and Chong, 2005). Port Klang sampling station is located near the river mouth (± 300 m) and is regularly well-mixed, possibly due to strong current conditions (Chong et al., 2012). In contrast, our sampling station at Port Dickson is located at a sandy beach (about 270 m in length). The coastal water here has two prominent ridge and runnels that runs almost parallel to the prevailing waves and coastline (Sharifah Mastura, 1984). Both stations experience semi-diurnal tide and are generally influenced by short extreme intense rainfall yearly during the two inter-monsoon period from March to April and September to October (Syafrina et al., 2015).

Environmental parameters

In-situ measurements of temperature and salinity were carried out with a conductivity meter (YSI 30, USA) whereas dissolved oxygen (DO) was measured via Winkler titration method (Parsons et al., 1984). In the laboratory, the seawater sample was filtered through precombusted (500°C for 3 h) glass fibre filter (GF/F). One filter was kept frozen (–20°C) for chlorophyll *a* analysis (Chl *a*), whereas another filter was kept for total suspended solid (TSS). TSS was measured by gravimetry method where the filter was dried at 70°C for 7 days until a constant weight.

Dissolved nitrogen characterization

Water samples for TDN and DIN (NH_4, NO_3 and NO_2) analyses were initially filtered thorough pre-combusted GF/F (500°C for 3 h). TDN samples were analyzed by high-temperature combustion method using a total organic carbon analyzer with a total nitrogen measuring unit (Shimadzu TOC-L, Japan). For DIN, NH_4, NO_3 and NO_2 were characterized by the colorimetric method according to Parsons et al., (1984) using a spectrophotometer (Hitachi U–1900, Japan) with a 20 mm optical path length glass cell. Subsequently, DON was obtained as follows: DON = TDN – DIN (Saunders III et al., 2017).

Biological analyses

In this study, Chl *a* was used as a proxy for phytoplankton biomass and was extracted in ice-cold acetone (90% v/v) overnight at –20°C. Samples before 2011 were measured with a spectrophotometer (U-1900, Hitachi, Japan) and the concentration was determined via the trichromatic method (Parsons et al., 1984) whereas samples after 2011 were measured with a spectrofluorometer (LS-55, Perkin Elmer, USA). The fluorescence intensity after acetone extraction was read at the excitation/emission wavelength of 440/680 nm. Both approaches were standardized to a commercially available Chl *a* standard (Sigma-Aldrich, USA).

The abundance of bacteria and phototrophic picoplankton were measured via the direct count method using an epifluorescence microscope (Olympus BX-60, Japan). For bacteria, one ml of sample was filtered onto a black 0.2-µm pore size Isopore polycarbonate filter and stained with 4’,6-diamino-2-phenylindole (DAPI, 1 µg mL^{-1} final concentration) for seven minutes (Porter and Feig, 1980). Slides were kept frozen and enumerated within one week. A minimum of 500 bacterial cells were counted with a U-MWU filter cassette (excitor 330–385 nm, dichroic mirror 400 nm, barrier 420 nm). Correction for autofluorescing picoplankton was carried out by viewing the filter under the U-MWG2 filter cassette (excitor 510–550 nm, dichroic mirror 570 nm, barrier 590 nm). For phototrophic picoplankton, 10 ml of sample was filtered onto a black 0.2-µm pore size Isopore polycarbonate filter. Slides were kept frozen and a minimum of 300 phototrophic picoplankton cells were enumerated under the U-MWG2 filter cassette within three days.

Statistical analyses

For data analyses, we substituted the dissolved nutrient and Chl *a* concentration that were below detection limit ($NH_4 \leq 0.03$ µM; $NO_2 \leq 0.02$ µM; $NO_3 \leq 0.03$ µM; DON ≤ 0.02 µM, and Chl *a* ≤ 0.04 µg L^{-1}) with zero. Based on the linear regression model of the calibration curve, the detection limit was calculated as (standard error of the slope × 3) / coefficient of the slope (Shrivastava and Gupta, 2011). Bacterial and phototrophic picoplankton abundance were log-transformed before statistical analyses to meet parametric assumptions of equality of variance and normal distribution. Analysis of variance (ANOVA) was used to compare the temporal variation of different parameters measured whereas correlation analysis was used to show the relationship between different parameters. All statistical test was carried out with the software PAST (Hammer et al., 2001).

Results

Environmental parameters

Surface seawater temperatures measured at both Port Klang and Port Dickson were similar, and averaged 29.7 ± 1.0°C and 29.7 ± 1.3°C, respectively (Table 1). In contrast, average salinity at Port Klang (23.3 ± 7.5 ppt) was lower and varied over a wider range (coefficient of variation, CV = 32%) than Port Dickson (mean = 28.9 ± 3.0 ppt, CV= 10%) ($t = 6.48$, $df = 115$, $p < 0.001$). Similarly, average DO at Port Klang was lower and varied over a wider range (143 ± 50 µM, CV = 35%) than Port Dickson (219 ± 38 µM, CV= 17%) ($t = 11.20$, $df = 160$, $p < 0.001$).

TSS measurements in 2004 and 2005 revealed serious TSS pollution at both Port Klang and Port Dickson where TSS was elevated to 258 to 332 mg L^{-1} and 259 to 388 mg L^{-1}, respectively (Fig. 2). Continued monitoring showed TSS levels reverting to lower levels (< 150 mg L^{-1}) at both Port Klang and Port Dickson in 2007. However after 2007, TSS at both Port Klang and Port Dickson increased steadily at an average rate of 9 ± 2 mg L^{-1} yr^{-1} ($F = 26.19$, df = 70, $p < 0.001$) and 6 ± 1 mg L^{-1} yr^{-1} ($F = 25.17$, df = 79, $p < 0.001$), respectively.

Distribution of DIN and DON

TDN concentration at Port Klang generally fluctuated from 8 to 150 µM but increased to 316 µM in Feb 2014 (Fig. 3). In contrast, TDN concentration at Port Dickson was lower and did not increase beyond 30 µM ($t = 5.09$, df = 50, $p < 0.001$). Of the TDN, DIN ranged from 4 to 256 µM at Port Klang whereas at Port Dickson, DIN ranged from 0.16 to 11.6 µM. Among the DIN species, NH_4 was the main component at both Port Klang and Port Dickson and accounted for 99 ± 1% ($R^2 = 0.988$, df = 87, $p < 0.001$) and 90 ± 3% ($R^2 = 0.903$, df = 84, $p < 0.001$) of DIN, respectively (Fig. 4). At Port Klang, NH_4 concentration fluctuated from 0.14 to 90.6 µM (23.3 ± 23.6 µM) except on several occasions when it breached 100 µM levels (Nov 2008, Oct 2009, Sep 2013 and Feb 2014). Relative to Port Klang, NH_4 concentration at Port Dickson was lower (1.63 ± 2.21 µM) and did not increase beyond 11 µM ($t = 6.66$, df = 88, $p < 0.001$).

Similarly, NO_3 and NO_2 at Port Klang were higher than at Port Dickson (NO_3: $t = 11.24$, df = 92, $p < 0.001$; NO_2: $t = 12.07$, df = 88, $p < 0.001$). At Port Dickson, NO_3 and

NO_2 concentrations averaged 0.76 ± 0.71µM and 0.19 ± 0.17 µM, respectively. At Port Klang, the average NO_2 concentration was more than an order higher (2.95 ± 2.15 µM) whereas NO_3 varied over a wide range from 0.07 to 15.1 µM (4.87 ± 3.29 µM) and showed increase (0.37 ± 0.11 µM yr^{-1}) over the sampling period (F = 12.51, df = 84, $p < 0.001$).

For organic nitrogen, DON ranged from 0.19 to 35.1 µM at Port Klang except in Feb 2014, Mar 2015, Apr 2015 and Sep 2015 when its concentration increased above 50 µM (Fig. 3). In contrast to Port Klang, DON at Port Dickson was lower (t = 4.06, df = 63, $p < 0.001$) and varied from 0.15 to 17.9 µM except on two occasions in Jan 2010 and Feb 2011 when DON concentration increased > 20 µM. Average DON without the episodic spikes was 14.7 ±10.4 µM at Port Klang and 7.68 ± 4.69 µM at Port Dickson. In this study, most of the nitrogen species were recorded at higher concentrations at Port Klang. We also observed that DIN was predominant at Port Klang, contributing 71 ± 3% to TDN (R^2= 0.910, df= 48, $p< 0.001$) whereas DON was predominant at Port Dickson, contributing 92 ± 7% to TDN (R^2 = 0.814, df = 39, $p < 0.001$) (Fig. 5).

Biological variables

Chl *a* concentration fluctuated from 0.20 to 50.5 µg L^{-1} at Port Klang and from 0.07 to 9.70 µg L^{-1} at Port Dickson (Fig. 6), and was higher at Port Klang (t = 3.41, df = 90, $p < 0.001$). On four occasions (May 2005, Sep 2011, Sep 2013 and Oct 2013), Chl *a* peaked > 20 µg L^{-1} at Port Klang, at 32.8 µg L^{-1}, 26.3 µg L^{-1}, 32.6 µg L^{-1} and 50.5 µg L^{-1}, respectively. The average Chl *a* concentration at Port Klang (3.70 ± 3.43 µg L^{-1})

without these extreme events, was still statistically higher than Port Dickson (2.27 ± 1.39 μg L^{-1}) ($t = 3.49$, $df = 106$, $p < 0.001$).

Phototrophic picoplankton abundance was also different at both sites ($t = 16.68$, $df = 139$, $p < 0.001$) where counts recorded at Port Klang ($2.96 \pm 2.33\times10^4$ cells mL^{-1}) was about one order lower than Port Dickson ($1.45 \pm 0.96\times10^5$ cells mL^{-1}) (Fig. 6). In contrast, average bacterial abundance at Port Klang ($3.01 \pm 2.07\times10^6$ cells mL^{-1}) was about two times higher than at Port Dickson ($1.31 \pm 0.77\times10^6$ cells mL^{-1}) ($t = 8.47$, $df = 158$, $p < 0.001$) (Fig. 6), and fluctuated from 0.06 to 1.13×10^7 and 0.32 to 5.04×10^6 cells mL^{-1}, respectively. Of interest, the long-term observational period in this study revealed a decreasing trend in bacterial abundance at both Port Klang ($F = 29.71$, $df = 83$, $p < 0.001$) and Port Dickson ($F = 32.57$, $df = 82$, $p < 0.001$).

Discussion

Environmental parameters

Surface water temperature observed throughout the sampling period at both stations were relatively high and stable, typical of tropical waters (Tanaka and Choo, 2000; Lee et al. 2009; Teoh et al., 2016) (Table 1). Salinity was generally more variable at Port Klang estuary than at Port Dickson coastal water due to the influx of freshwater from Klang River (Lim et al., 2015). The lower DO concentration at Port Klang also reflected poorer water quality that prevailed there (Lim et al., 2018).

In this study, long-term measurements of TSS at Port Klang and Port Dickson revealed severe TSS pollution (> 250 mg L^{-1}) in 2004 and 2005. At Port Klang, TSS pollution was probably due to nearby land clearing activities and construction (Lee and Bong, 2006) whereas at Port Dickson, it was mainly due to a beach restoration project which included dredging, transport and placement of sand (Ghazali, 2005). Despite TSS reverting to lower levels after this period (< 150 mg L^{-1}), TSS increased steadily after 2007. The increasing TSS revealed how both habitats had deteriorated over time, concurring with reports that showed increasing trophic state index at both sites (Othman et al., 2012; Lim et al., 2018). We analyzed both PON and TSS data from the Matang mangrove estuary of Peninsular Malaysia (Alongi et al., 2003), and found that PON can attribute about 27 ± 3% of TSS ($F= 82.24$, $df= 18$, $p< 0.001$). Although the Matang mangrove estuary is undeniably a more productive habitat, the increasing TSS in this study did suggest an increasing contribution of PON towards the total nitrogen pool.

In contrast to TSS, long-term measurements of bacterial abundance showed an overall decreasing trend at both Port Klang and Port Dickson. One possible reason for the decreasing bacterial abundance could be due to the initiative taken by the federal government of Malaysia (Government of Malaysia, 2001) to upgrade and centralize the public sewerage treatment systems (Chan, 2012). Proper treatment of sewage and grey water before releasing the effluent into river systems could have improved the water quality and reduced bacterial abundance but a more thorough analysis is probably required to verify this.

Distribution of DIN and DON

In terms of concentration, DIN was higher than DON at Port Klang, and its DIN pool was comprised mostly of NH_4. Similar observations have been reported in Malaysia and other tropical regions where NH_4 can make up 28% - 92% of DIN (Tanaka and Choo, 2000; Teoh et al., 2016) and 70 - 100% of DIN (Pratihary et al., 2009; Brandini et al., 2016), respectively. Together with the lower DO levels, this pattern of nitrogen distribution is generally attributed to denitrification process (Lee et al., 2013; Teoh et al., 2016).

At Port Klang, NH_4 correlated with salinity ($R^2 = -0.528$, df = 83, $p < 0.001$), and further substantiated an earlier suggestion that freshwater river input from Klang River was an important allocthonous source of NH_4 to the Klang estuary (Lim et al., 2015). Increased precipitation during the rainy season (Syafrina et al., 2015) would likely increase nutrient supply into the estuary, and could explain the episodic peaks in NH_4 concentration (>100 µM). Although we were unable to pinpoint the exact source of NH_4 measured at Port Klang, observations from the nearby Langat River suggested it could be from the middle to the lower part of Klang River (Lee et al., 2013). We also effectively rule out the possible influence from port activities as Klang River is a known source of water pollution to Port Klang (e.g. Sasekumar and Chong, 2005; Chong et al., 2012; Sany et al., 2013). The higher concentration of NH_4 could have also driven biological processes at Port Klang as NH_4 correlated with both Chl *a* ($R^2 = 0.329$, df = 79, $p < 0.001$) and bacterial abundance ($R^2 = 0.136$, df = 78, $p < 0.001$) (Fig. 7). These relationships suggested NH_4 driven primary productivity and bacterial ammonification processes that are well documented for various aquatic ecosystems (Bettarel et al., 2011; Lim et al., 2015).

At Port Klang, only NO_3 increased over the 12-year observation period albeit at a slow rate (Martin et al., 2011; Godhe et al., 2015). As the +V oxidative state of NO_3 is favorable to an oxic marine environment (Gruber, 2008; Voss et al., 2013) and is reflective of well aerated or cleaner waters (Wang et al., 2018), the increasing NO_3 over our observation period could also be a result of the governmental arrangements to upgrade sewerage treatment systems (Chan, 2012).

At Port Dickson, TDN, DIN and DON concentrations were strikingly lower compared to Port Klang. In contrast to Port Klang where DIN was the predominant dissolved nitrogen, DON was predominant at Port Dickson. DON at Port Dickson was inversely correlated with TSS ($R^2 = 0.661$, df = 39, $p < 0.001$) (Fig. S1). As increasing TSS could have reduced transparency and therefore photosynthetic activity, DON would be negatively affected by TSS. DON at Port Dickson was also correlated with bacterial abundance ($R^2 = 0.190$, df = 38, $p < 0.01$) and could suggest that microbial conversion was an important mineralization process (Lee et al., 2009; Lim et al., 2018).

Although DIN was a minor component at Port Dickson, the trend where NH_4 predominated was similar to Port Klang. The proportionally higher concentration of NH_4 is reflective of the coastal water conditions in this region (Lee et al., 2013; Teoh et al., 2016). However as the DO concentration at Port Dickson was consistently > 200 µM, it would explain the generally lower NH_4 concentrations at Port Dickson relative to Port Klang even though there was not enough evidence from this study to identify whether nitrification or denitrification was more important at Port Dickson.

Nitrogen loading at Port Klang estuary

As estuaries receive a large supply of allochthonous material via riverine input (Flynn, 2008; Brandini et al., 2016), we estimated the nitrogen loading at Port Klang estuary with available Klang River discharge rate data from Malaysia Drainage and Irrigation Department. Daily average Klang River discharge rate fluctuated over three orders, from 0.1 to 540.4 $m^3\ s^{-1}$ and showed an overall increasing trend by 4.04 ± 0.12 $m^3\ s^{-1}\ yr^{-1}$ throughout the sampling period ($R^2 = 0.218$, df = 4382, $p < 0.001$) (Fig. 8A). Due to the increasing Klang River discharge rate, all of the dissolved nitrogen load calculated increased significantly by 1.13 ± 0.29 kg d^{-1} for TDN, 1.11 ± 0.29 kg d^{-1} for DIN, and 0.15 ± 0.06 kg d^{-1} for DON (Fig. S2). Other estuaries have also reported higher nutrient loadings due to increasing river discharge rate (e.g. Seitzinger et al., 2010; Huang et al., 2014).

In this study, we considered two main factors that could have led to an increase in river discharge rate i.e. increase in precipitation level and increase in urbanization (Voss et al., 2013; Strauch et al., 2015; Williams et al., 2015). As warmer conditions hold more water vapour and have higher evaporation rates, global warming is expected to cause more intense precipitation in parts of the tropical region (Wang et al., 2017). However daily precipitation data at Port Klang available from Malaysia Meteorological Department was relatively constant (< 110.3 mm d^{-1}) throughout the 12-year period (Fig. 8B), and we were not able to discern any pattern of increase in precipitation within this period.

As to whether urbanization caused an increase in river discharge rate, we collected population statistics from the Department of Statistics, Malaysia for the states of Selangor and Kuala Lumpur, which are included in the Klang River catchment basin (Sasekumar and Chong, 2005). Human population is used in this study as a proxy for urbanization, and data obtained showed that the population in these two states increased at an average of 164,000 individuals yr^{-1} ($R^2 = 0.997$, df = 11, $p < 0.001$) (Fig. 8C). Correlation analysis of the Klang River discharge rate versus the annual population increase was significant ($R^2 = 0.555$, df = 11, $p < 0.01$) (Fig. S3). Although human population does not produce water, the diversion of water resources from other source is essential to meet the increasing demand (McDonald et al., 2014). Increasing deforestation and the increase in impervious surface area from man-made structure (e.g. rooftops, sidewalks and parking lots) (Guzha et al., 2018; Levy et al., 2018) could have also increased the amount of surface runoff and hence increased the Klang River discharge rate (Burns et al., 2005; Zhao et al., 2016).

Summary

Long-term observation at both Port Klang and Port Dickson revealed an episode of severe TSS pollution in 2004 – 2005. Although the source of pollution was removed after 2005, observations over the 12-year period revealed steadily increasing TSS at both Port Klang and Port Dickson. We also showed that among the dissolved nitrogen pool, only NO_3 at Port Klang showed an increasing trend over the monitoring period. These observations would not have been possible if this study was carried out within a shorter time frame. Comparison of both Port Klang and Port Dickson also revealed the differences in the contribution of both DIN and DON towards TDN. Among the dissolved nitrogen pool, we found that DIN consisted mainly of NH_4 was the predominant nitrogen pool at Port Klang, and from correlation analysis, it drives both phototrophic and bacteria processes. In contrast, we showed that DON predominated the dissolved nitrogen pool at Port Dickson and microbial conversion could be an important mineralization process. In addition, we also showed that the increase in nitrogen loading at Port Klang was due to the increasing urbanization upstream that increased river discharge rates and not climatic forcing.

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Fig. 1 Location of Port Klang estuary (filled circle: 3°00’N, 101°24’E) and Port Dickson coastal water (filled triangle: 2°31’N, 101°47’E) sampling stations.

Fig. 2 Temporal variation of TSS (mg L^{-1}) at Port Klang and Port Dickson over a 12-year period. The solid lines represent increase in TSS concentration over the monitoring period.

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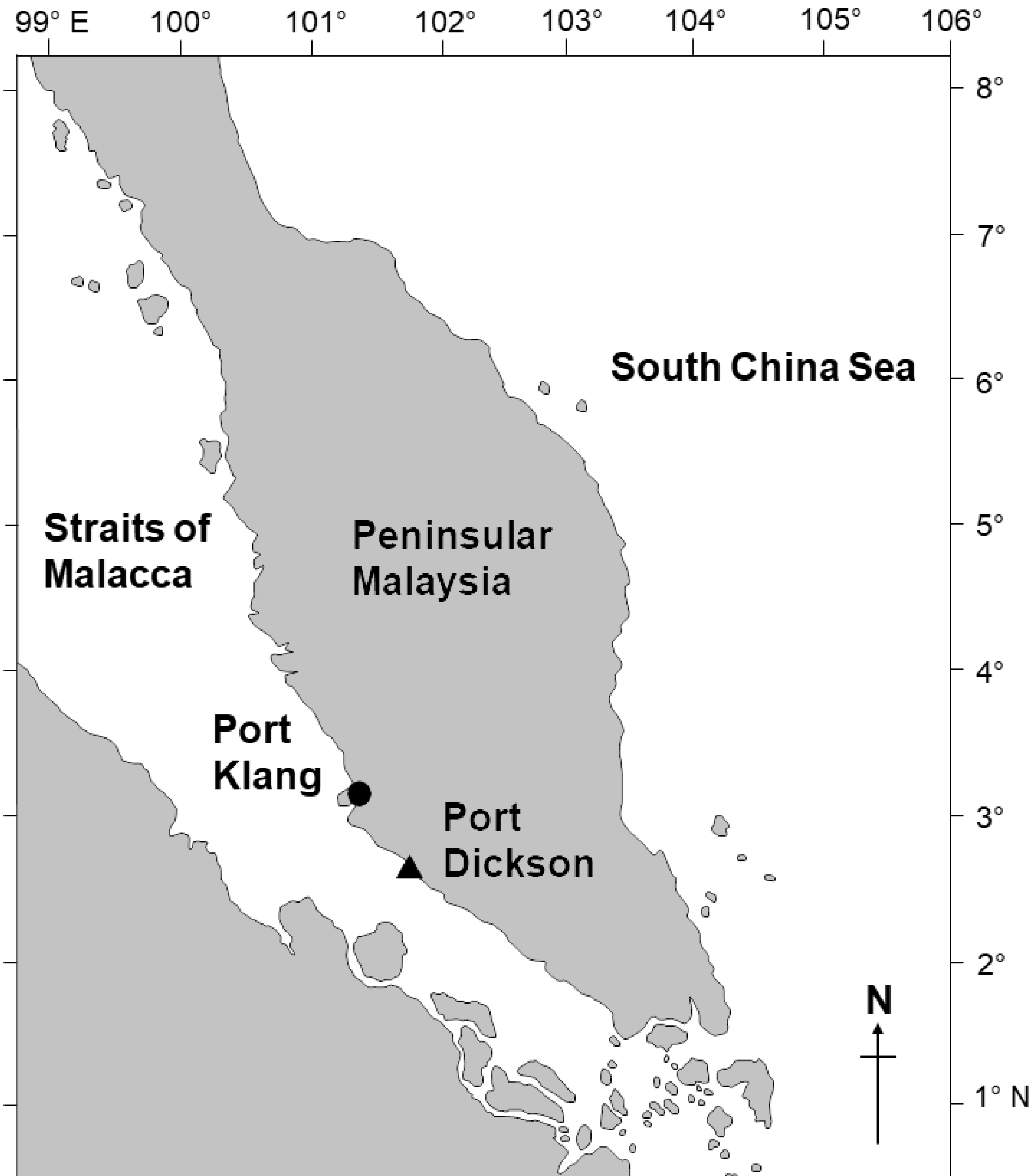
Fig. 5 Scatter plot of DIN (µM) and DON (µM) relative to TDN (µM) at Port Klang and Port Dickson. Linear regression is shown when significant.

Fig. 6 Temporal variation of Chl *a* (µg L^{-1}), phototrophic picoplankton abundance (10^5 cells mL^{-1}), and bacterial abundance (10^6 cells mL^{-1}) at Port Klang and Port Dickson over a 12-year period. The solid lines represent significant trend over the monitoring period.

Fig. 7 Correlation analyses between Chl *a* (µg L^{-1}) on the left y-axis and bacterial abundance (log cells mL^{-1}) on the right y-axis against NH_4 (µM) at Port Klang. Linear regression is shown when significant (dashed line for Chl *a*–NH_4 and solid line for

Bacteria−NH_4). Filled symbols represent outliers and are not included in the correlation analyses.

Fig. 8 A) Long-term monitoring of Klang River discharge rate (m^3 s^{-1}) from Malaysia Drainage and Irrigation Department (www.water.gov.my). The solid line shows significant increase over the 12-year observation period. B) Bar charts showing the daily precipitation data (mm d^{-1}) at Port Klang from the Malaysia Meteorological Department (www.met.gov.my). C) Estimated annual population for the states Kuala Lumpur and Selangor where Klang River flows through (Malaysia Meteorological Department, www.met.gov.my).


99° E
100°
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102°
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106°
8°
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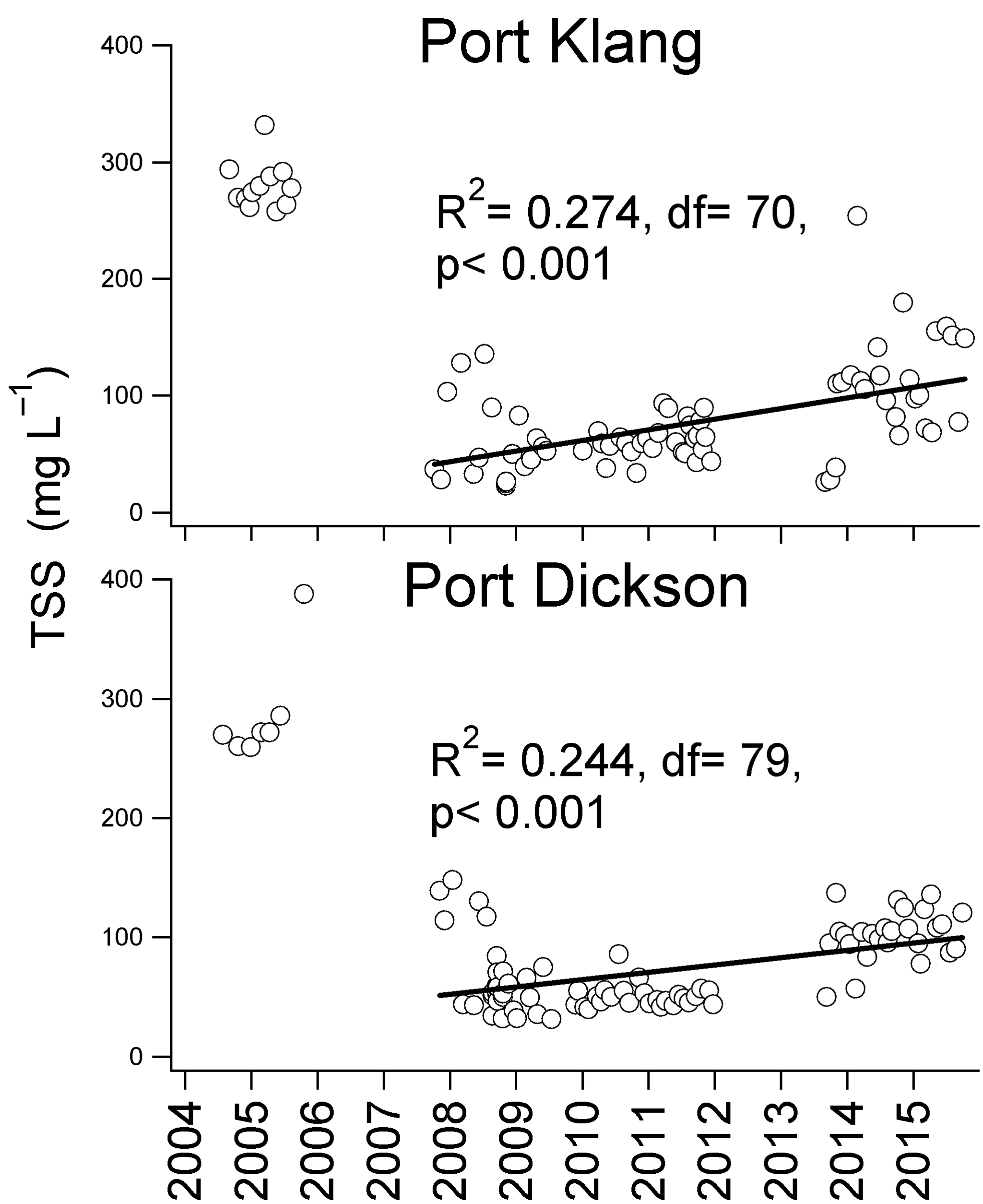
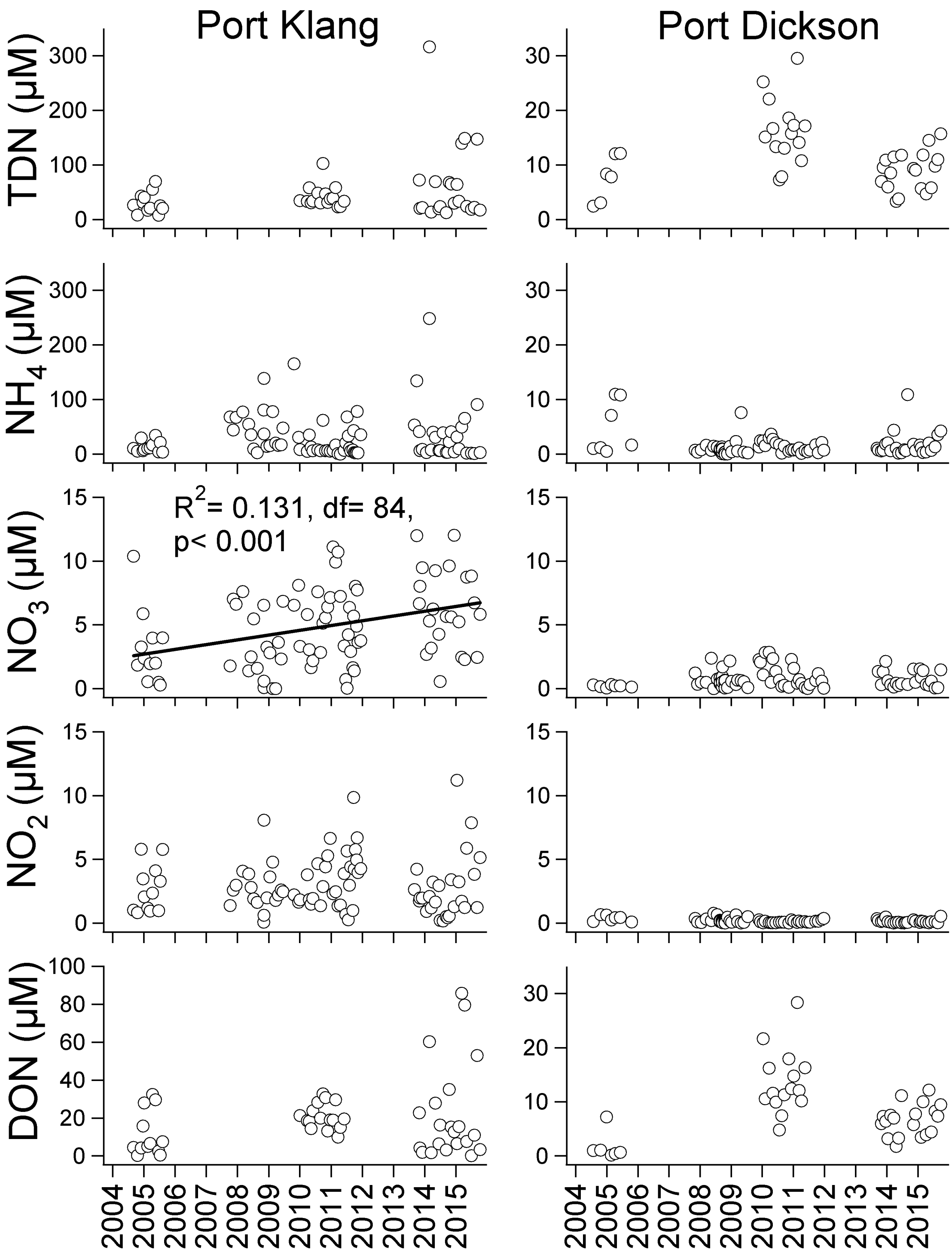
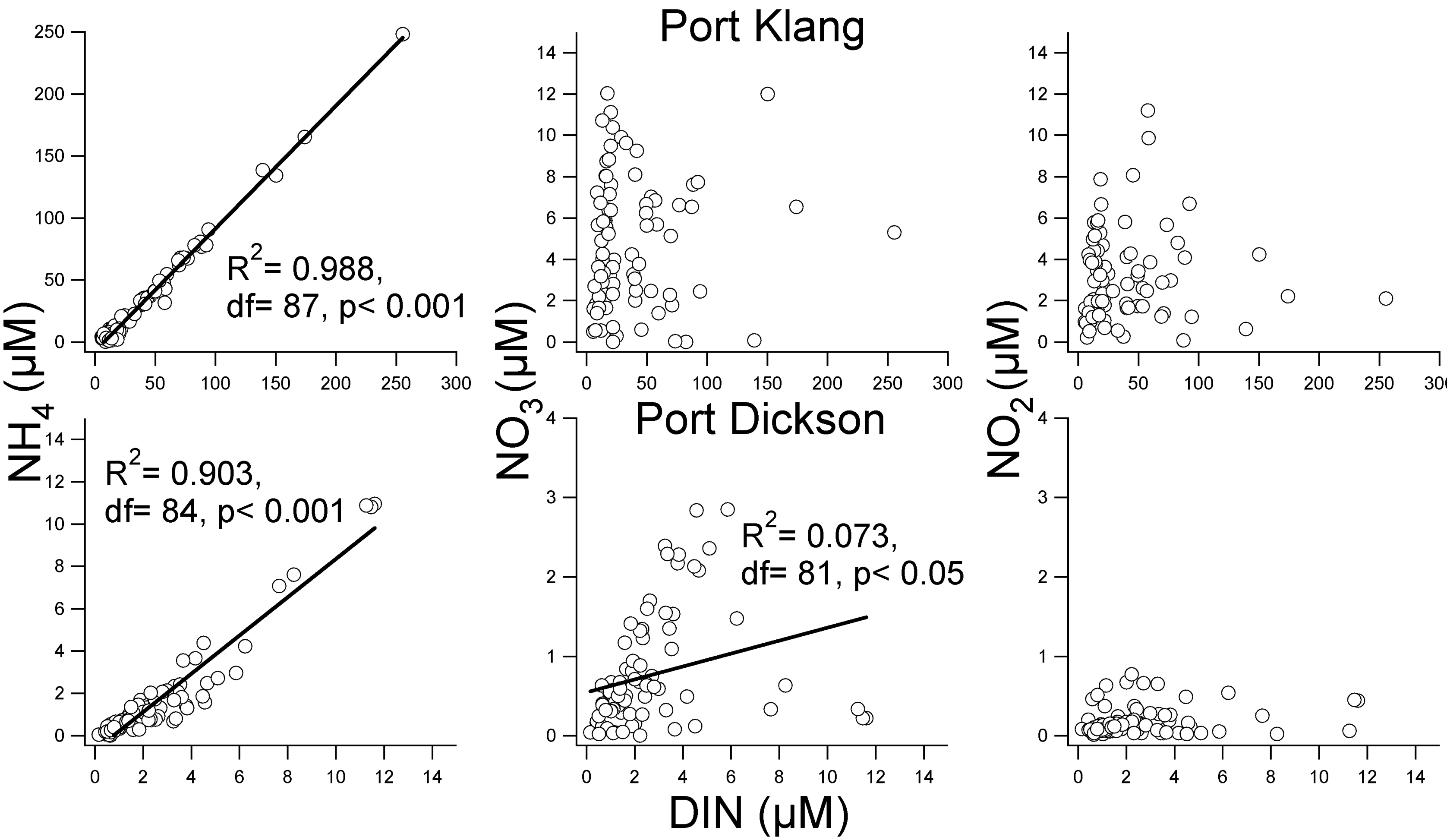
4°
3°
2°
1° N
South China Sea
Straits of Malacca
Peninsular Malaysia
Port Klang
Port Dickson
N



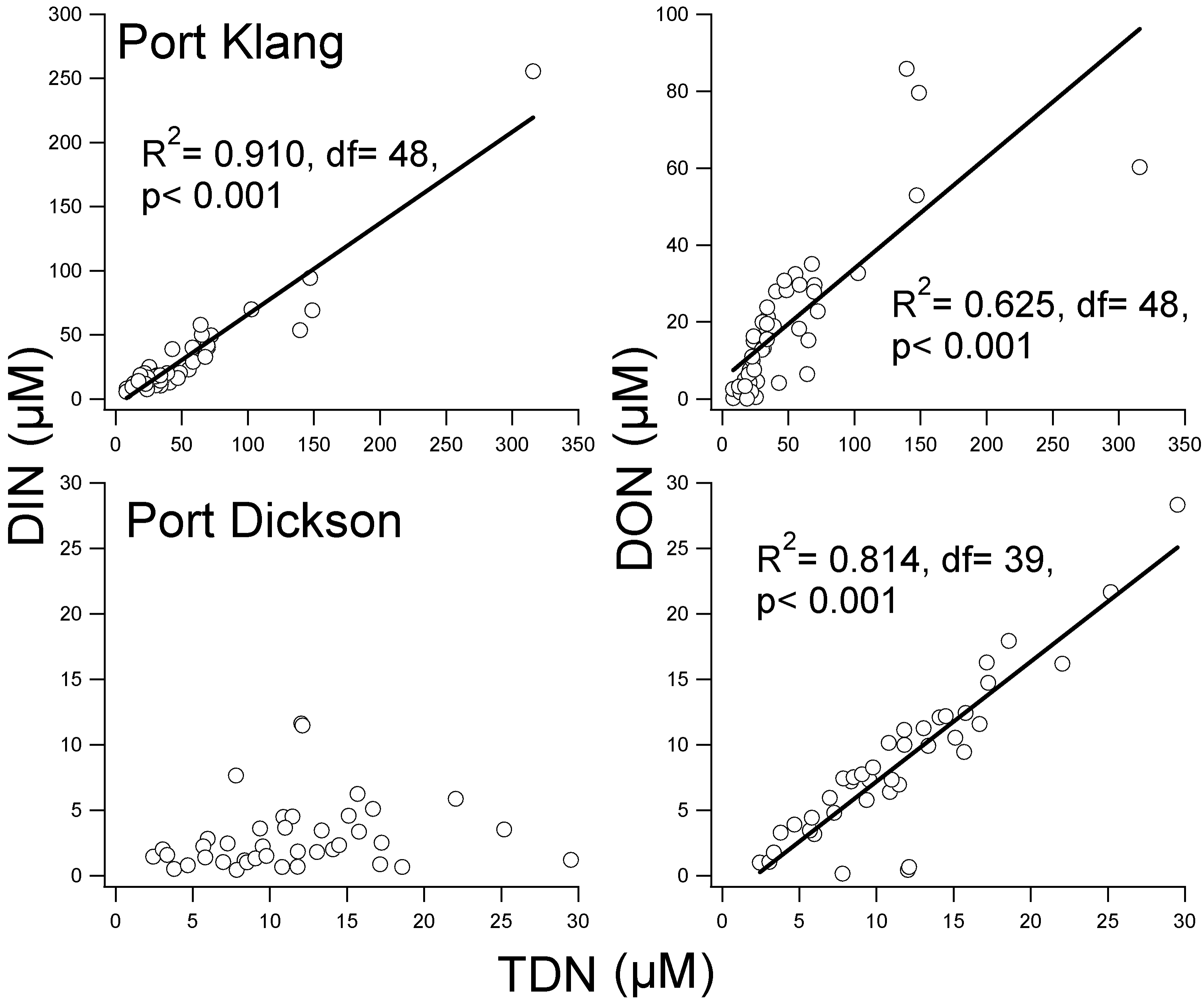
Port Klang
R2= 0.274, df= 70,
p< 0.001
Port Dickson
R2= 0.244, df= 79,
p< 0.001
TSS (mg L−1)
400
300
200
100
0
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015



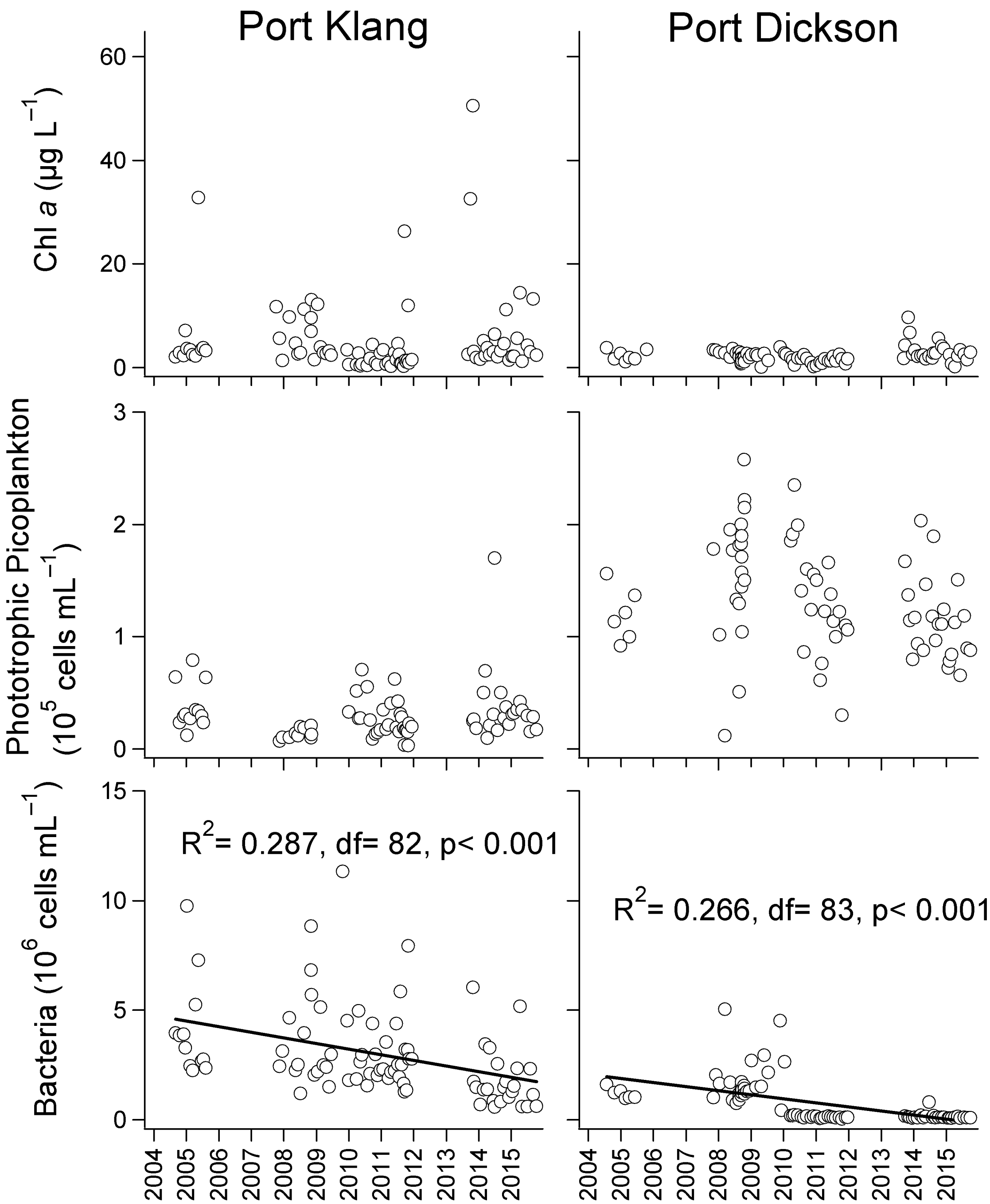
Port Klang
Port Dickson
TDN (µM)
NH4 (µM)
NO3 (µM)
NO2 (µM)
DON (µM)
R2= 0.131, df= 84,
p< 0.001
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015

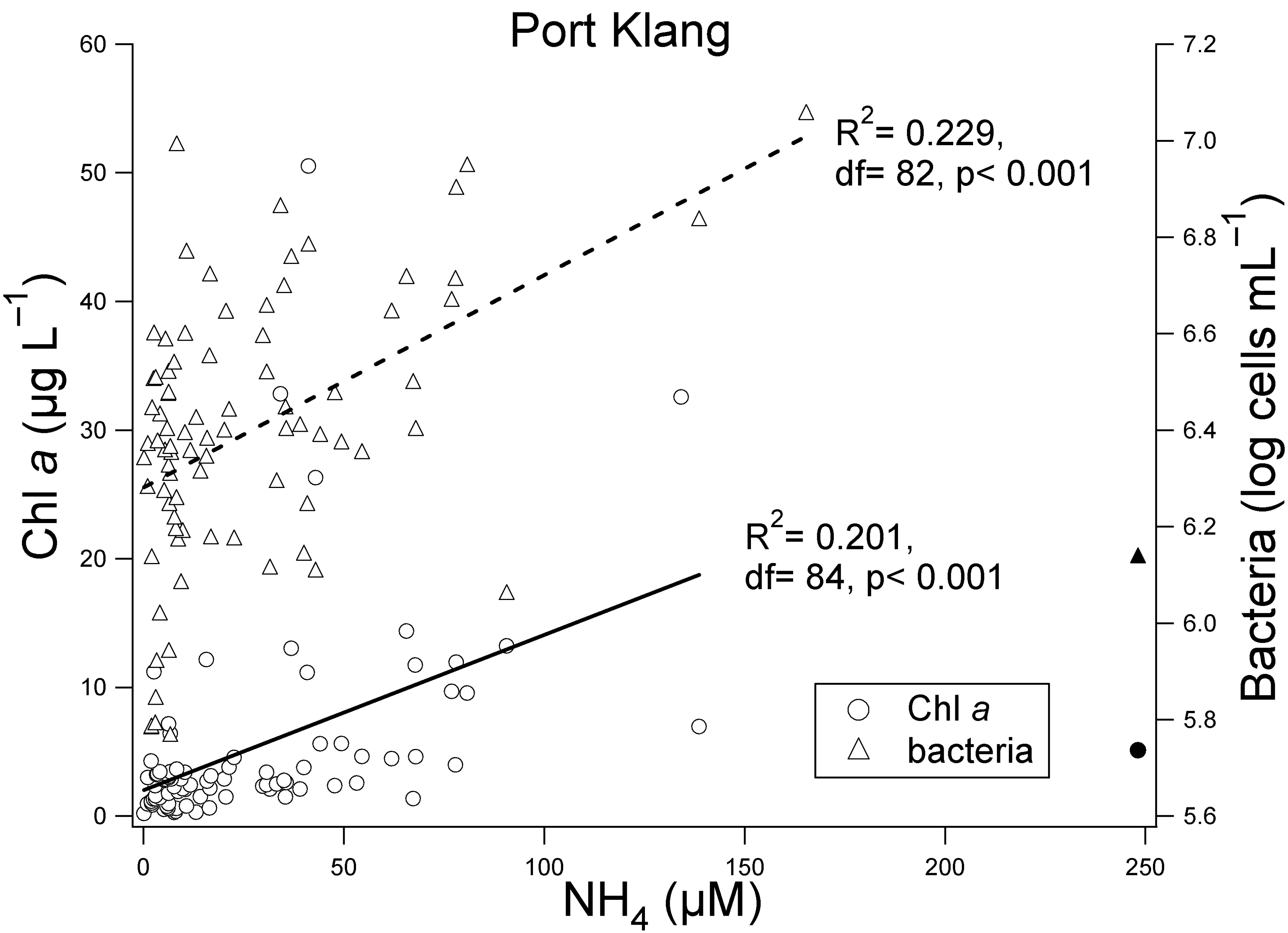


Port Klang
Port Dickson
R^2= 0.988,
df= 87, p< 0.001
R^2= 0.903,
df= 84, p< 0.001
R^2= 0.073,
df= 81, p< 0.05
NH_4 (µM)
NO_3 (µM)
NO_2 (µM)
DIN (µM)

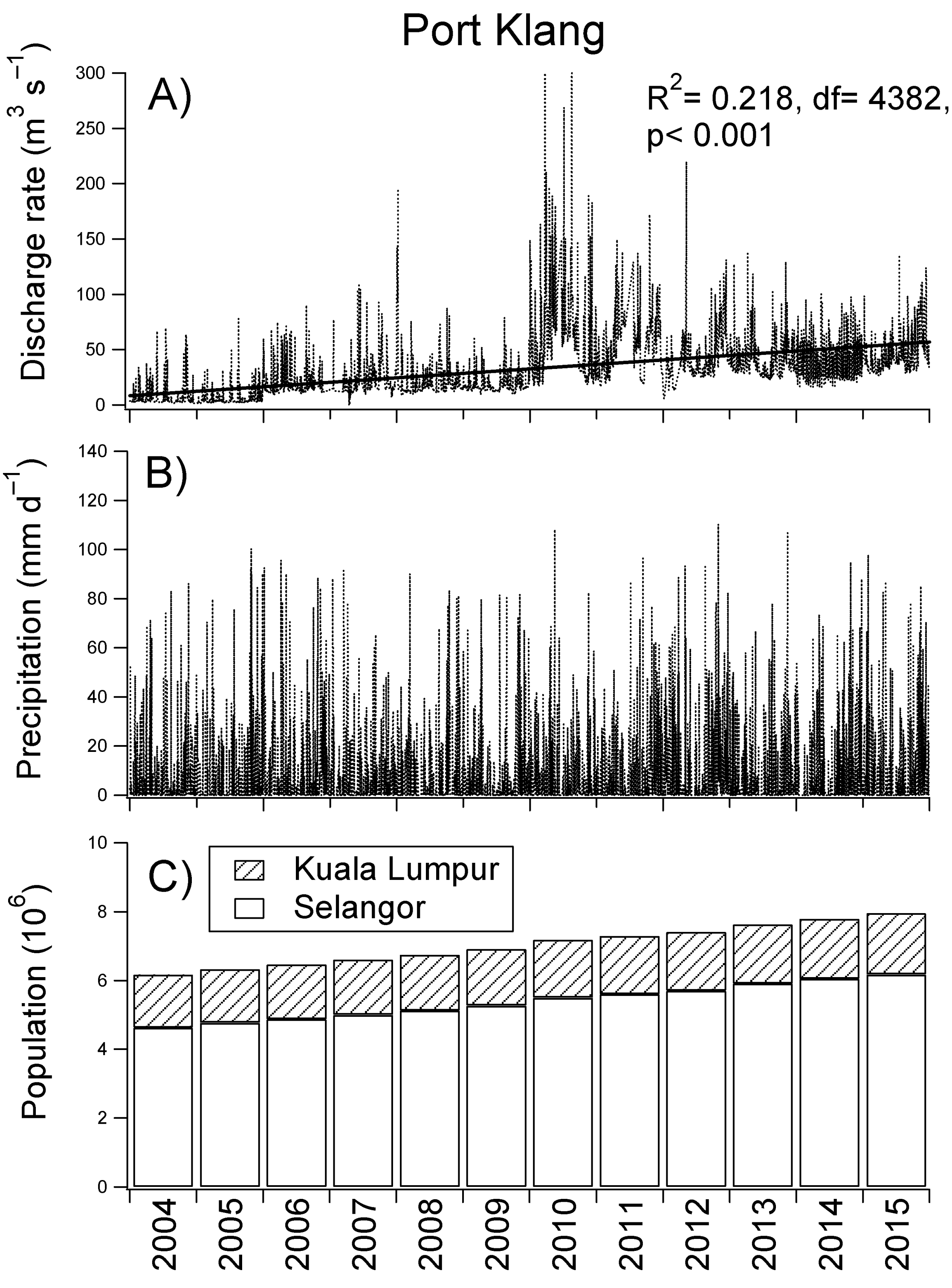


Port Klang
R^2= 0.910, df= 48,
p< 0.001
R^2= 0.625, df= 48,
p< 0.001
Port Dickson
R^2= 0.814, df= 39,
p< 0.001
DIN (μM)
DON (μM)
TDN (μM)


Port Klang
Port Dickson
Chl a (µg L−1)
Phototrophic Picoplankton (10^5 cells mL−1)
Bacteria (10^6 cells mL−1)
R2= 0.287, df= 82, p< 0.001
R2= 0.266, df= 83, p< 0.001
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015



Port Klang
Chl a (µg L⁻¹)
Bacteria (log cells mL⁻¹)
NH4 (µM)
R2= 0.229,
df= 82, p< 0.001
R2= 0.201,
df= 84, p< 0.001
Chl a
bacteria
0
10
20
30
40
50
60
5.6
5.8
6.0
6.2
6.4
6.6
6.8
7.0
7.2
0
50
100
150
200
250



Port Klang
A)
R^2= 0.218, df= 4382, p< 0.001
Discharge rate (m3 s-1)
0
50
100
150
200
250
300
B)
Precipitation (mm d-1)
0
20
40
60
80
100
120
140
C)
Kuala Lumpur
Selangor
Population (106)
0
2
4
6
8
10
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015

Table 1: Different environmental conditions measured at Port Klang and Port Dickson (mean ± S. D.). Student's *t* test was carried out to compare the values from both stations, and significance of differences between the sites is as follows: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015
<u>Port Klang</u>												
Temperature (°C)	29.5 ± 0.3	30.3 ± 0.8	--	29.0 ± 0.7	30.5 ± 0.8	30.4 ± 0.8	29.8 ± 1.0	29.3 ± 0.7	--	30.1 ± 1.6	29.3 ± 1.4	28.5 ± 3.8
Salinity (‰) ***	24.8 ± 4.9	27.3 ± 5.2	--	15.7 ± 5.3	21.6 ± 7.9	26.1 ± 2.8	25.3 ± 4.8	23.4 ± 6.8	--	22.5 ± 7.5	26.9 ± 6.3	20.1 ± 10.9
Dissolved Oxygen (μM) ***	170 ± 40	140 ± 50	--	110 ± 20	200 ± 80	190 ± 20	150 ± 30	150 ± 30	--	140 ± 70	145 ± 80	105 ± 50
<u>Port Dickson</u>												
Temperature (°C)	30.4 ± 1.8	29.8 ± 0.4	--	30.9 ± 1.6	30.3 ± 1.4	30.3 ± 1.3	30.3 ± 1.1	28.9 ± 0.7	--	28.9 ± 2.8	29.4 ± 1.0	29.4 ± 1.3
Salinity (‰) ***	30.1 ± 0.1	31.3 ± 1.0	--	28.7 ± 1.3	28.8 ± 0.6	28.2 ± 2.1	26.4 ± 2.8	27.6 ± 2.4	--	31.6 ± 0.8	31.0 ± 5.6	28.8 ± 2.1
Dissolved Oxygen (μM) ***	240 ± 40	190 ± 30	--	265 ± 60	230 ± 30	220 ± 40	220 ± 40	210 ± 10	--	210 ± 41	210 ± 62	210 ± 15

-- = no data available