Tropical Ecology **58**(2): 251–269, 2017 ISSN 0564-3295

www.tropecol.com

Spatio-temporal variation in phytoplankton communities along a salinity and pH gradient in a tropical estuary (Brunei, Borneo, South East Asia)

ROKSANA MAJEWSKA[1,2,3*], AIMIMULIANI ADAM[4,5], NORMAWATY MOHAMMAD-NOOR[5], PETER CONVEY[6], MARIO DE STEFANO[3], DAVID J. MARSHALL[4]

[1]*Unit for Environmental Sciences and Management, School of Biological Sciences, North-West University, Private Bag X6001, Potchefstroom 2520, South Africa*

[2]*South African Institute for Aquatic Biodiversity (SAIAB), Private Bag 1015, Grahamstown 6140, South Africa*

[3]*Department of Environmental, Biological and Pharmaceutical Sciences and Technologies, University of Campania "Luigi Vanvitelli", via Vivaldi 43, 81188 Caserta, Italy*

[4]*Biology Department, Faculty of Science, Universiti Brunei Darussalam, Jalan Tungku Link, BE1410 Gadong, Brunei Darussalam*

[5]*Department of Marine Science, Kulliyyah of Science, International Islamic University Malasia, 25200 Kuantan, Pahang, Malaysia*

[6]*British Antarctic Survey, Natural Environment Research Council, High Cross, Madingley Road, Cambridge CB3 0ET United Kingdom*

Abstract: Tropical estuaries often have a low buffering capacity and may experience acidification, both naturally through microbial degradation and run-off from acid sulphate soils (ASS), or from various anthropogenic sources. Here, we describe phytoplankton communities from the turbid, acidified, and eutrophic Sungai Brunei and Brunei Bay estuarine system. Four sampling stations were selected, representing the full spectrum of the salinity (0.4–28.5 PSU) and pH (5.87–8.06) gradients associated with this system. A total of 25 microalgal families of phytoplankton (including 22 diatom and seven dinoflagellate genera) and one of ciliates were recorded in the survey, which was carried out between August 2011 and June 2012. Phytoplankton density ranged from 7 to 9107 cells ml^{-1}. Diatoms were a dominant component of the communities, with *Nitzschia* spp., *Rhizosolenia* spp., and *Leptocylindrus* sp. reaching the highest abundances. Phytoplankton communities present at the four sampling stations differed significantly in terms of both algal abundance and composition and were strongly influenced by the effect of season (30% of the total variance). The interactive effects of pH and salinity, and of pH and temperature, explained 16.7% and 17.5% of the total observed variation, respectively. A positive correlation between pH and the number of taxa found was detected. The functional diversity observed in phytoplankton from the Brunei River estuary was generally low with few taxa adapted well to the chronically low pH conditions. This study provides baseline data about structural and compositional changes in a tropical estuarine phytoplankton community associated with various levels of acidification of both natural and anthropogenic origins.

[*]*Corresponding Author*; e-mail: roksana.majewska@unina2.it
Present Address: Department of Environmental, Biological and Pharmaceutical Sciences and Technologies Seconda Universita degli Studi di Napoli, Via Vivaldi 43, 81100 Caserta (CE), Italy

Key words: Acidification, diatom, eutrophic, monsoon, plankton, Sungai Brunei, turbid.

Handling Editor: Donna Marie Bilkovic

Introduction

Marine phytoplankton contribute to up to half of global primary production, providing organic matter for the great majority of marine life and being crucial to the global carbon cycle (Cassar *et al*. 2003; Falkowski 2012; Longhurst *et al.* 1995). Estuarine phytoplankton production amounts to 256 g C m^{-2} per year, which places estuarine systems among the most productive globally (Boynton *et al.* 1982; Costanza *et al.* 1997). Productivity and biodiversity are not necessarily connected, and high biodiversity does not seem to be an *a priori* condition required for successful functioning of estuarine ecosystems (Elliot & Quintino 2007). As with other transitional waters, and transitional zones in general, estuaries provide highly variable, naturally stressed habitats that are also commonly exposed to anthropogenic disturbance (de Jonge *et al.* 2002; Hu & Cai 2013; Nirmal Kumar *et al.* 2013; Pradhan *et al.* 2009). Indeed, estuaries are considered to be amongst the most threatened biological systems, and it is also expected that negative impacts of the many anthropogenic perturbations will be more severe in tropical than temperate regions due to such factors as the generally weak nitrogen retention by tropical forests and its extensive leaching by tropical rains (Downing *et al*. 1999; Su *et al.* 2004).

Although estuaries have long been considered living "macro-laboratories" for studying the effects of various stressors (e.g. highly variable salinity, temperature, turbidity, high organic matter and nutrient concentrations, heavy metals), the majority of studies to date have focused on benthic organisms (Alfaro 2006; Green & Barnes 2010; Hopkinson *et al.* 1999; Hossain & Marshall 2014; Majewska *et al.* 2012; Miller *et al.* 2009; Morrisey *et al.* 2003; Widdicombe & Spicer 2008). Given heightened concerns about the ecological consequences of increase in global atmospheric CO_2 concentration, it is remarkable that to date little consideration has been given to estuarine acidification and ecological responses thereto. Various hydrographic surveys, time series data and models for open seas indicate clearly that absorption of anthropogenic CO_2 across the ocean surface is, and will be, followed by decrease of seawater pH (Caldeira & Wickett 2003; Duarte *et al.* 2013; Fabry *et al.* 2008; Martin *et al.* 2008; Orr *et al.* 2005; Widdicombe & Spicer 2008). In contrast, the carbonate system of many estuaries is characterized by supersaturated water pCO_2 levels at their highest reaches, derived from natural heterotrophic microbial decomposition, such that these estuaries are sources rather than sinks for atmospheric CO_2 (Raymond *et al.* 2000; Sarma *et al.* 2001; Thottathil *et al.* 2008). Additionally, in many regions across the globe, particular geological formations result in acid (iron) sulphate soils, and consequently in highly acidic freshwater runoff from these soils (Cook *et al.* 2000; Grealish & Fitzpatrick 2013; Marshall *et al.* 2008). Dissociation products of strong acids (HNO_3 and H_2SO_4) are also derived from industry and agriculture, and further contribute to acidification. Although, on a global scale, the contribution of such acids to anthropogenic ocean acidification may be minor, they can be far more significant in coastal waters and estuaries, where they impact local fisheries, industries, coastal centres and communities (Doney *et al.* 2009; Feely *et al.* 2010; Hu & Cai 2013). Acidification in estuaries is further aggravated by low salinity and, hence, poor buffering capabilities (Miller *et al.* 2009). Furthermore, recent studies have linked acidification to natural and artificial eutrophication through raising primary production (Sunda & Cai 2012; Wallace *et al.* 2014).

Although some important aspects of the responses of marine biota to lowered pH have been addressed, studies have largely focused on shell-forming calcifying organisms, as such organisms are considered likely to experience increased shell dissolution (Dove & Sammut 2007; Gazeau *et al.* 2013; Green & Barnes 2010; Hossain & Marshall 2014; Marshall *et al.* 2008; Miller *et al.* 2009; Waldbusser *et al.* 2011). A number of studies have attempted to assess acidification effects on phytoplankton under controlled laboratory conditions (e.g. Berge *et al.* 2010, Brading *et al.* 2011; Hansen 2002; Hinga 2002; Lohbeck *et al.* 2012; Low-Décarie *et al.* 2011), but data on *in situ* microalgal responses to lowered pH are scarce (e.g. Geelen & Leuven 1986). Acidification gradients in

open waters are uncommon, and studies on naturally occurring CO_2-driven pH gradients, such as those associated with the volcanic vents, almost exclusively describe benthic communities (Hall-Spencer *et al.* 2008; Johnson *et al.* 2012; Porzio *et al.* 2013; Turnicliffe *et al.* 2009). The most comprehensive recent work on acid sulphate estuaries has been carried out in the temperate region of Sydney, Australia (Amaral *et al.* 2011a,b; 2012a,b)

The Brunei River estuary, an eutrophic, acidified, highly turbid aquatic system, offers an opportunity to investigate tropical phytoplankton communities along a relatively steep gradient of salinity and pH. By investigating this estuarine system, we aimed to characterize the spatial and temporal patterns for microalgal communities, and assess potential effects of variable environmental conditions on their densities and composition.

Materials and methods

Sampling area

The Brunei River estuary system occupies an area of ca. 1380 km^2 of Brunei Bay, including the Inner Brunei Bay. Three main rivers (Sungai Limbang, Sungai Temburong, and Sungai Brunei) have a major freshwater input into the estuary. The area is divided into Brunei Channel and Temburong Channel (Currie 1979). Along most of the banks, the system is fringed with extensive *Rhizophora* mangrove stands (Chua *et al.* 1987). Due to the equatorial tropical climate the region is characterized by seasonal heavy rainfalls and high temperatures throughout the year. The local tides are diurnal or semi-diurnal, with maximum daily amplitudes of around 2 m in the vicinity of the estuary's mouths (Hossain & Marshall 2014; Hossain *et al.* 2014). Brunei's capital and largest city, Bandar Seri Begawan (BSB; population = 200,000) is located on the banks of the Sungai Brunei estuary. A large fraction of the city's domestic waste as well as treated and untreated sewage effluent is discharged into the estuary (Marshall *et al.* 2008; Yau 1991). Furthermore, a large traditional water village (Kampong Ayer, current population c. 15,000) is located on the estuarine water near BSB. The Sungai Brunei estuary is distinctly brown and highly turbid, and receives a significant organic load from both mangroves and urban centres. In addition, the system is affected by eutrophication, acidic sulphate groundwater inflows, and heterotrophic metabolism. As a result, steep pH and salinity gradients extend along the estuary. Due to local dynamics, both pH and salinity are highly variable and range between 4.0–8.0 and 0–34 PSU, respectively (Bolhuis *et al.* 2014).

Phytoplankton sampling

Phytoplankton samples were collected at four stations located along the Brunei River estuary, Chermin Island (S1; 4.927650°N, 115.020406°E), Sungai Bunga (S2; 4.900956°N, 114.998353°E), Pintu Malim (S3; 4.888897°N, 114.979133°E), and Kedayan River-Kiulap (S4; 4.886733°N, 114.936767°E). These were selected to represent a strong gradient of pH and salinity, both decreasing in an upstream direction from S1 to S4 (Fig. 1). Samples were taken at 1- to 3-week intervals between August 2011 and June 2012. Heavy rainfall associated with the Asian monsoons (north-eastern monsoon, NEM, from November to March; south-western monsoon, SWM, from May to September), as well as the dry inter-monsoon periods (April and October), significantly affect the local conditions, bringing droughts and flooding alternately (Adam A., personal observations).

Phytoplankton samples were collected by towing a plankton net (20 μm mesh) behind a small boat ca. 0.5 m under the water surface. On each sampling occasion (18 in total), the same procedure was carried out for 3 min at a constant speed (2 ms^{-1}). Subsequently, collected material was concentrated, transferred into 100 ml containers, and preserved with Lugol's iodine solution. All sampling took place during the daytime, at the same tidal level (just after high tide). Tidal levels were estimated using TideComp v.7.04 (Pangolin, Bristol, UK) software.

Sedgewick-Rafter counting slides were used for phytoplankton identification and quantification. Prior to observation the samples were homogenised by gently agitating the sample bottle. From a well-mixed sample, 1 ml was dispensed into the counting cell and viewed under a compound microscope at 40x and 100x magnifications. This procedure was repeated three times and the final result was expressed as the mean value of the three counts. Collected phytoplankton were identified to the lowest taxonomic level possible using relevant references (e.g. Ehrenberg 1844; Lauder 1864; Schmidt 1874, 1878, 1888, 1890, 1892, 1893; Shirota 1966; Tomas 1997). Scanning electron microscopy of phytoplankton samples was undertaken in Italy (II University of

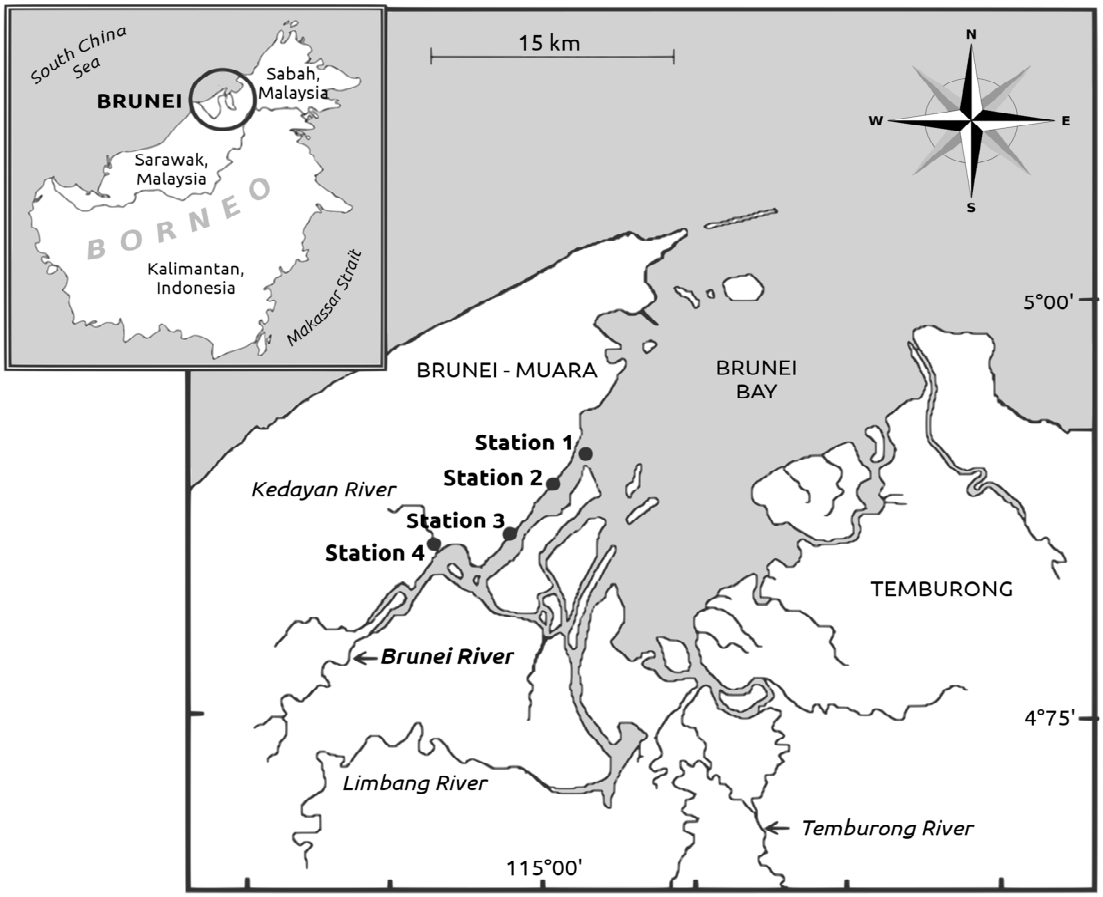

Fig. 1. Sampling stations along Brunei River estuary. Salinity and pH decrease in an upstream direction from station 1 to station 4.

Naples). Samples were shipped in Lugol's solution. In order to remove all organic matter, material was digested following a slight modification of the method of von Stosch (Hasle & Syvertsen 1997) using a mixture of boiling concentrated acid (64% nitric acid and 97% sulphuric acid added at a 1:3 volume ratio). Following digestion and centrifuging (1200 rpm), cleaned material was rinsed and diluted with deionized water. Subsequently, the oxidized suspension was filtered using 25 mm discs of a 3-µm Whatman™ polycarbonate membrane filter that then were attached to the aluminum stubs by carbon tape. The stubs were sputter-coated with gold-palladium and examined in a ZEISS Supra 40 SEM microscope at 5 kV (Centro Grandi Apparecchiature, II University of Campania "Luigi Vanvitelli", Naples, Italy).

Hydrological parameters

Physicochemical properties of water (salinity, temperature and pH) were measured *in situ* at 0.5 m depth at the time of sampling using a calibrated pH and salinity meter (Hanna Instruments, USA, two points calibration, used from August 2011 to January 2012; YSI Model 63, Yellow Springs Instrument Co., three points calibration, used from February to June 2012). For dissolved oxygen (DO) measurements, a 1l seawater sample was collected at each site on every sampling occasion and kept in an airtight polyethylene jar. Subsequently, the sample was taken to the laboratory and the DO level was measured using a NexSens WQ-DO Sensor. The sensor was calibrated in a 100% oxygen saturated environment (air calibration). In order to ensure proper polarization of the electrodes, the probe was warmed up for 15–30 min before calibration. Barometric pressure (745 mmHg) and salinity were set before the DO reading was taken. Correlations between environmental parameters (pH, salinity, temperature, and DO) and population density were analysed using SPSS v. 15.

Table 1. Plankton taxa found in the Brunei River Estuary.

Class	Family	Genera and Species
Bacillariophyceae	Achanthaceae	*Achnanthes* sp
	Amphipleuraceae	*Amphiprora* sp.
	Bacillariaceae	*Nitzschia longissima* (Brébisson) Ralfs
		Nitzschia spp.
		Pseudo-nitzschia sp.
	Biddulphiaceae	*Biddulphia mobiliensis* (J.W. Bailey) Grunow
		Biddulphia sinensis Greville
	Chaetocerotaceae	*Bacteriastrum varians* Lauder
		Bacteriastrum spp.
		Chaetoceros danicus Cleve
		Chaetoceros peruvianus Brightwell
		Chaetoceros rostratus Ralfs
		Chaetoceros spp.
	Coscinodiscaceae	*Coscinodiscus jonesianus* (Greville) Ostenfeld
		Coscinodiscus obscurus A. Schmidt
		Coscinodiscus radiatus Ehrenberg
		Coscinodiscus wailesii Gran & Angst
		Coscinodiscus spp.
	Hemiaulaceae	*Hemiaulus* sp.
	Leptocylindraceae	*Corethron* sp.
		Leptocylindrus sp.
	Lithodesmiaceae	*Ditylum sol* Cleve
	Melosiraceae	*Melosira* spp.
	Naviculaceae	*Navicula* spp.
	Pleurosigmataceae	*Pleurosigma* spp.
	Rhizosoleniaceae	*Guinardia striata* (Stolterfoth) Hasle
		Rhizosolenia alata Brightwell
		Rhizosolenia acuminate (H. Peragallo) Gran
		Rhizosolenia arafurensis Castracane
		Rhizosolenia bergonii H. Peragallo
		Rhizosolenia clevei Ostenfeld
		Rhizosolenia delicatula Cleve
		Rhizosolenia pungens Cleve-Euler
		Rhizosolenia stolterfothii H.Peragallo
	Stephanodiscaceae	*Cyclotella litoralis* Lange & Syvertsen
		Cyclotella meneghiniana Kützing
	Surirellaceae	*Surirella fastuosa* Ehrenberg
	Thalassionemaceae	*Thalassionema frauenfeldii* (Grunow) Tempère & Peragallo
		Thalassionema nitzchioides (Grunow) Mereschkowsky
	Thalassiosiraceae	*Thalassionema* sp.
		Thalassiosira nanolineata (A.Mann) Fryxell & Hasle
		Thalassiosira spp.
	Triceratiaceae	*Triceratium* sp.

Contd...

Table 1. Continued.

Class	Family	Genera and Species
Dinophyceae	Ceritiaceae	*Ceratium furca* (Ehrenberg) Claparède & Lachmann
		Ceratium fusus (Ehrenberg) Dujardin
		Ceratium trichoceros (Ehrenberg) Kofoid
		Ceratium tripos (O.F. Müller) Nitzsch
		Ceratium massiliense (Gourret) Karsten
	Dinophysiaceae	*Dinophysis acuta* Ehrenberg
	Diplopsaliaceae	*Dinophysis caudata* Saville-Kent
		Preperidinium meunieri (Pavillard) Elbrächter
	Gonyaulacaceae	*Pyrodinium bahamense* var. *compressum* (Böhm) Steidinger, Tester & F.J.R. Taylor
	Peridiniaceae	*Peridinium* spp.
	Prorocentraceae	*Prorocentrum micans* Ehrenberg
		Prorocentrum granile Schütt
	Protoperidiniaceae	*Protoperidinium pellucidum* Bergh
		Protoperidinium crassipes (Kofoid) Balech
Spirotrichea	Codonellidae	*Protoperidinium* sp.
		Codonella spp.

Statistical analyses

Statistical analyses were performed using PAST 2.17b (Hammer *et al.* 2001), PRIMER Ver. 5 (Clark & Warwick 2001), and Canoco 5 (ter Braak & Šmilauer 2012) software. Although taxa were identified to the lowest taxonomic level possible, further analyses were performed on generic-level data to avoid detection of false patterns due to potential misidentification. A similarity percentage analysis (SIMPER) was run to identify phytoplankton taxa responsible for the similarity within groups. To evaluate the relationships between the phytoplankton communities and measured environmental variables, a constrained ordination method was used. Prior to this analysis, an unconstrained unimodal ordination (detrended correspondence analysis, DCA) was performed and the lengths of its ordination axes were measured. On this basis (the longest axis = 2.4 turnover units) the linear method was selected as the most appropriate for the analysed dataset (Šmilauer & Lepš 2014). Subsequently, redundancy analysis (RDA) and partial RDA were performed on log-transformed abundance data. A Monte Carlo permutation test was used to test the significance of the axes (4999 permutations, $P < 0.05$). In order to select the best subset of the chosen environmental variables to summarize the variation in phytoplankton composition, interactive forward selection was performed. The conditional and simple effects of individual explanatory variables upon the compositional data were assessed using a variation partitioning procedure (Legendre 2007). To visualize the relation of number of taxa found to different pH levels measured at the sampling sites, a Generalized Additive Model (GAM) was used (Šmilauer & Lepš 2014). GAM is a flexible statistical method able to relatively precisely characterize nonlinear contributions of the selected predictor and thus help to better understand the interactive behaviour of different variables (Hastie & Tibshirani 1990).

Results

Phytoplankton community

A total of 25 microalgal families were found in the Sungai Brunei estuary area during the period of study, with 22 genera of diatoms, seven of dinoflagellates and one family of ciliates (Table 1, Figs. 2–3). In terms of phytoplankton abundance, the highest density was recorded at S3 (time-averaged mean: 1251 cells $ml^{-1} \pm 2544$), followed by S2 (1119 cells $ml^{-1} \pm 2197$), S1 (437 cells $ml^{-1} \pm 744$) and lastly S4 (118 cells $ml^{-1} \pm 265$). Generally, the highest phytoplankton densities (up to 9107 cells ml^{-1}, S3) were observed in August and October, which coincided with increases in pH, salinity, and

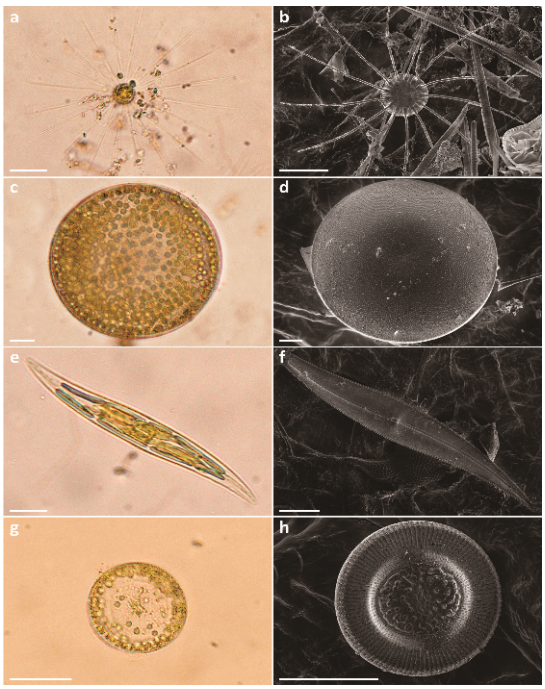

Fig. 2. LM and SEM images of selected diatoms found in Brunei River estuary. **a**, **b** *Bacteriastrum* sp. **c**, **d**. *Coscinodiscus* sp. **e**, **f**. *Pleurosigma* sp. **g**, **h**. *Cyclotella litoralis*. Scale bars: a, b, g, h = 20 µm, c–f = 10 µm.

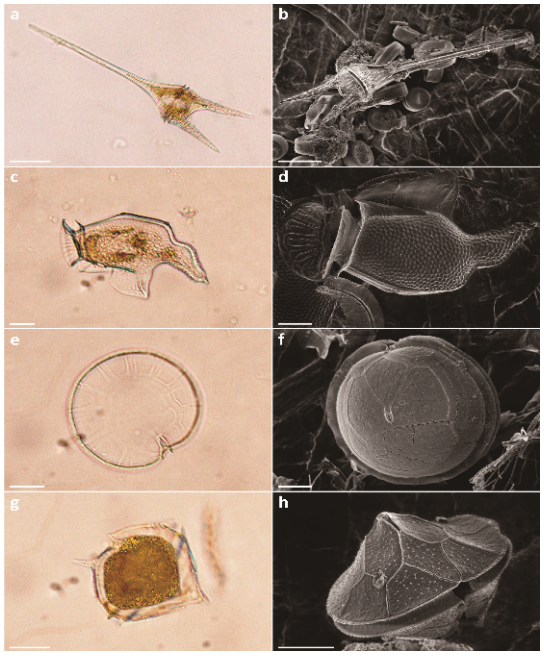

Fig. 3. LM and SEM images of selected dinoflagellates found in Brunei River estuary. **a**, **b**. *Ceratium furca*. **c**, **d**. *Dinophysis caudata*. **e**, **f**. *Preperidinium meunieri*, **g**, **h**. *Protoperidinium* sp. Scale bars: a, b = 20 µm, c–h = 10 µm.

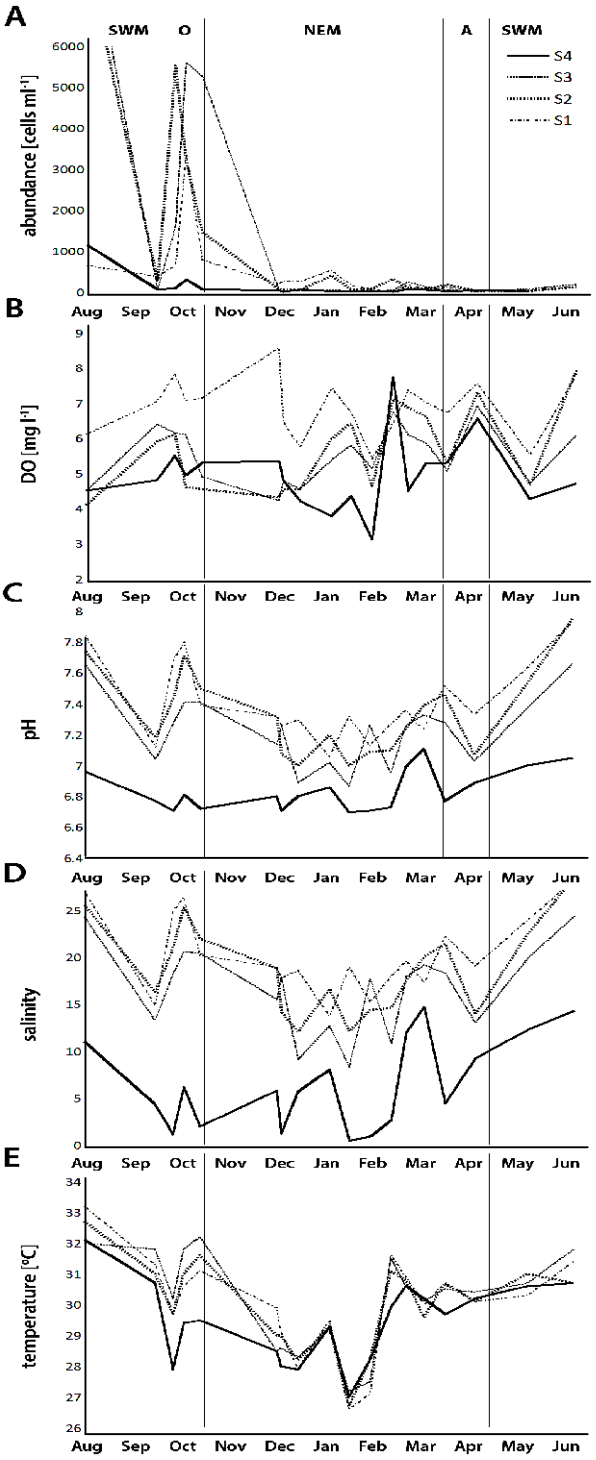

Fig. 4. Seasonal changes in phytoplankton density (A), dissolved oxygen concentration (B), pH (C), salinity (D), and temperature (E) from August 2011 to June 2012. SWM = south-western monsoon, NEM = north-eastern monsoon, A = April (dry inter-monsoon period), O = October (dry inter-monsoon period).

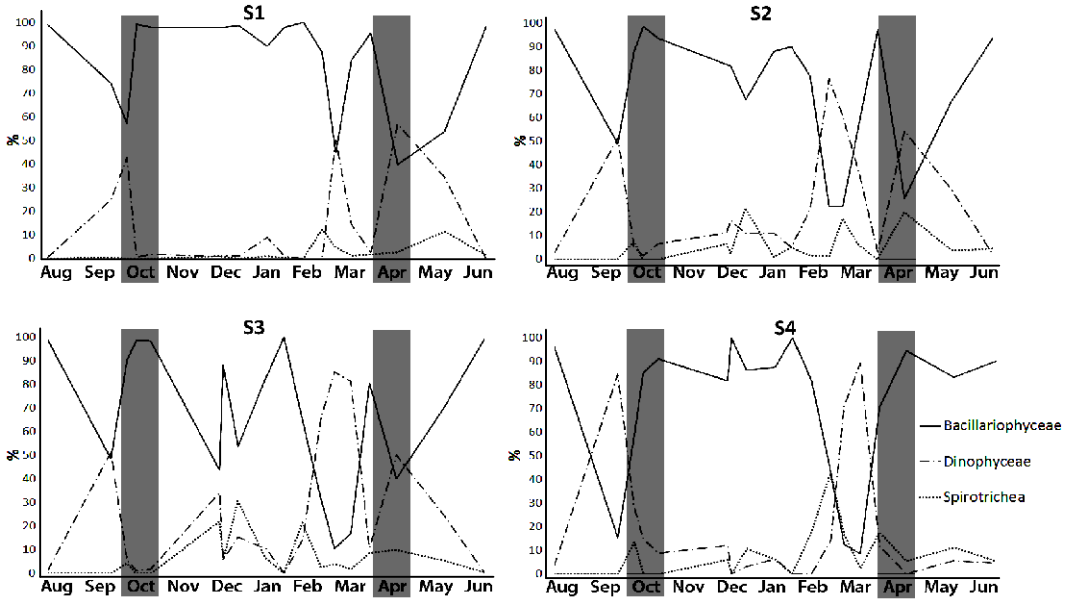

Fig. 5. Temporal trends of the relative abundances of the main algal and zooplankton classes found at the sampling sites (S1-S4). Grey areas indicate dry inter-monsoon periods.

temperature (Fig. 4). The differences in phytoplankton abundances observed among the four sampling stations during the study period were significant (Kruskal-Wallis, $P < 0.05$).

Comparing the communities, the highest average dissimilarity occurred between those found at S4 and S1 (69.2%), S4 and S2 (68.2%), and S4 and S3 (67.6%) while the most similar were the communities from S1 and S2 (59.8%). Diatoms constituted the main fraction of the observed microalgal communities (up to > 99%; Fig. 5), with *Nitzschia* (0–61.5% of the total cell number in the sample; 25.9% on average) being the dominant genus. This was followed by two other high-density genera, *Rhizosolenia* (0–52.4%; 24.1% on average) and *Leptocylindrus* (0–75.5%; 21.9% on average). According to the SIMPER analysis, these taxa together with *Chaetoceros* spp., *Thalassionema* spp., *Dinophysis* spp., and *Pleurosigma* spp. were responsible for more than 50% of the dissimilarity between the sampling stations (for further detail see Supplemental material: Tables SI–S6). Although diatoms dominated clearly throughout the greater part of the year, dinoflagellates attained dominance at the end of the monsoon seasons (Fig. 5).

Phytoplankton communities vs. environmental factors

Values of salinity, pH, and DO generally decreased landward, from S1 to S4 (Fig. 4), with the differences between the stations being statistically significant (Kruskal-Wallis, $P < 0.05$). However, the four sampling sites did not differ significantly in terms of water temperature (26.6–33.2 °C; mean temperature at the four sampling sites ranged from 29.4–30.2 °C). All of the environment variables (pH, temperature, salinity, DO) were significantly positively correlated with phytoplankton density (Table 2).

Differences among seasons

A partial RDA with supplementary variables was performed to test the effect of season on phytoplankton communities (Fig. 6A; Eigenvalues: 0.2871, 0.0073, 0.0066). Sampling site was used as a covariate and, therefore, its effect on phytoplankton communities was removed from the model. The explained variation accounted for 33.2% of the total variance in phytoplankton compo-

Table 2. Correlation coefficients between environmental factors and phytoplankton abundance DO = dissolved oxygen.

	pH	Temperature	Salinity	DO
Temperature	0.490*			
Salinity	0.811*	0.514*		
DO	0.520*	0.341**	0.286**	
Density	0.496*	0.461*	0. 563*	0.317**

*$P < 0.01$ **$P < 0.05$.

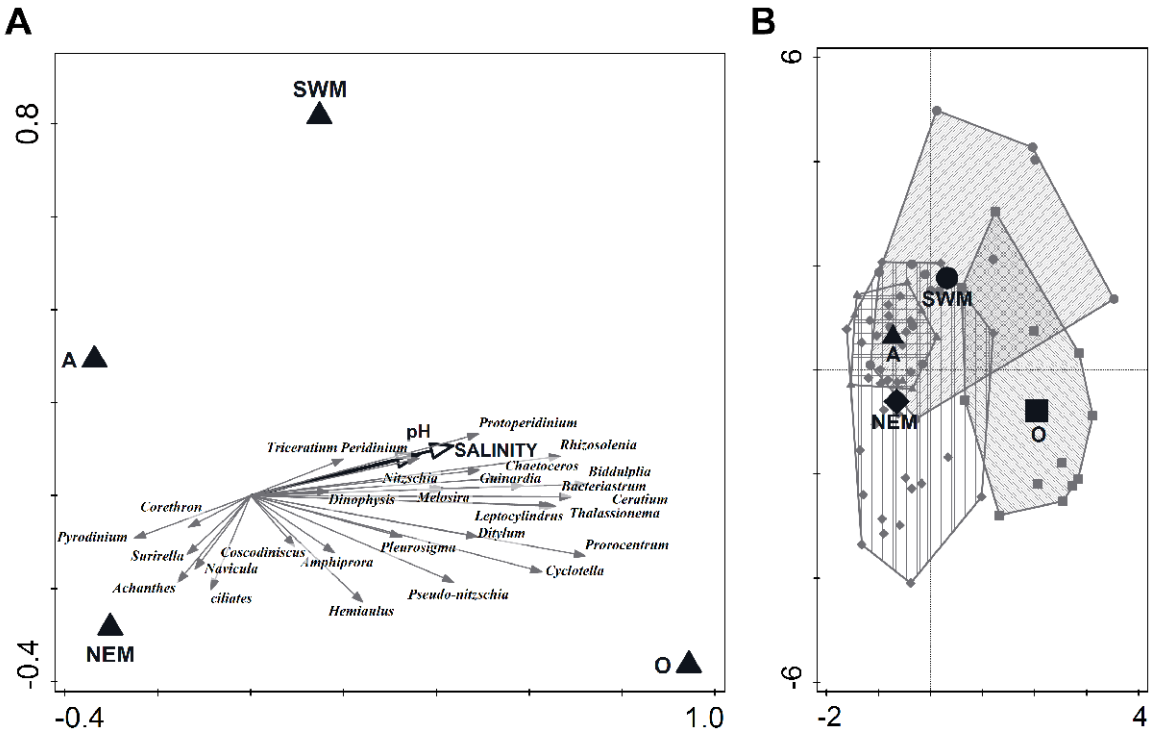

Fig. 6. The effect of sampling season on phytoplankton communities. A) Quadplot diagram from RDA summarizing the differences in phytoplankton communities caused by sampling season. Score scaling is focused on phytoplankton taxa scores (standardized), Empty arrows = environmental variables (supplementary) Solid arrows = phytoplankton taxa, Solid triangles = sampling season: SWM = south-western monsoon, NEM = north-eastern monsoon, A = April (dry inter-monsoon period), O = October (dry inter-monsoon period), B) Scatter of samples classified into 4 groups according to the season in which they were collected. The polygons are plotted in the space of the first two RDA axes. Score scaling is focused on phytoplankton taxa scores (standardized), SWM = south-western monsoon, NEM = north-eastern monsoon, A = April (dry inter-monsoon period), O = October (dry inter-monsoon period).

sitional data (adjusted explained variation = 30.0%). As confirmed by the Monte Carlo permutation test (P = 0.001) this effect was significant. Most of the algal taxa appeared to be correlated (either positively or negatively) with salinity and pH. The spring inter-monsoon period (April) was clearly the season in which the conditions were the least favourable for phytoplankton community development. Although both NEM and SWM samples created highly heterogeneous overlapping groups, samples collected during the two inter-monsoon periods (April and October) formed two distinct clusters, suggesting a substantial seasonal change in phytoplankton communities (Fig. 6B).

Differences among sites

A partial RDA was run to test the effect of sampling site on the algal communities (Fig. 7A; Eigenvalues: 0.0732, 0.0135, 0.0054). The sampling season was used as a covariate, allowing removal of its effect on algal assemblages from the final model. The explained variation accounted for 25.0% of the total variance in phytoplankton compositional data (adjusted explained variation = 20.5%) and the Monte Carlo permutation test (P = 0.001) again confirmed significance of the observed effect. All of the phytoplankton taxa responded negatively to the conditions of S4. Only the two benthic taxa (*Achnanthes* and *Navicula*) were more common in samples collected at S4 than in those collected at the other stations. The phytoplankton samples were classified into four groups according to the site from which they were collected (Fig. 7B). The overlapping clusters illustrate the spatial continuum throughout the sampling area. Nevertheless, samples collected at S1 and S4 were more floristically heterogeneous than those collected at the other two stations. In

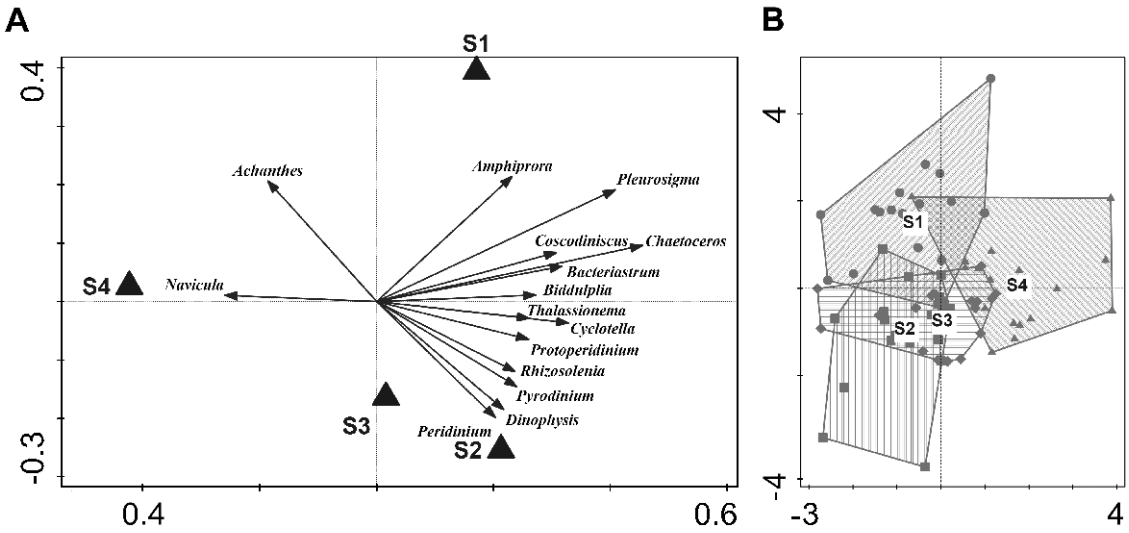

Fig. 7. The effect of sampling site on phytoplankton communities. (A) Biplot diagram from RDA summarizing the differences in phytoplankton communities caused by difference in sampling site. Score scaling is focused on phytoplankton taxa scores (standardized). Only the 15 best fitting taxa are shown (fit into the ordination space on both axes = 6.7%). Solid arrows = phytoplankton taxa, Solid triangles = sampling sites. (B) Scatter of samples classified into 4 groups according to the sampling site where they were collected. The polygons are plotted in the space of the first two RDA axes. Score scaling is focused on phytoplankton taxa scores (standardized).

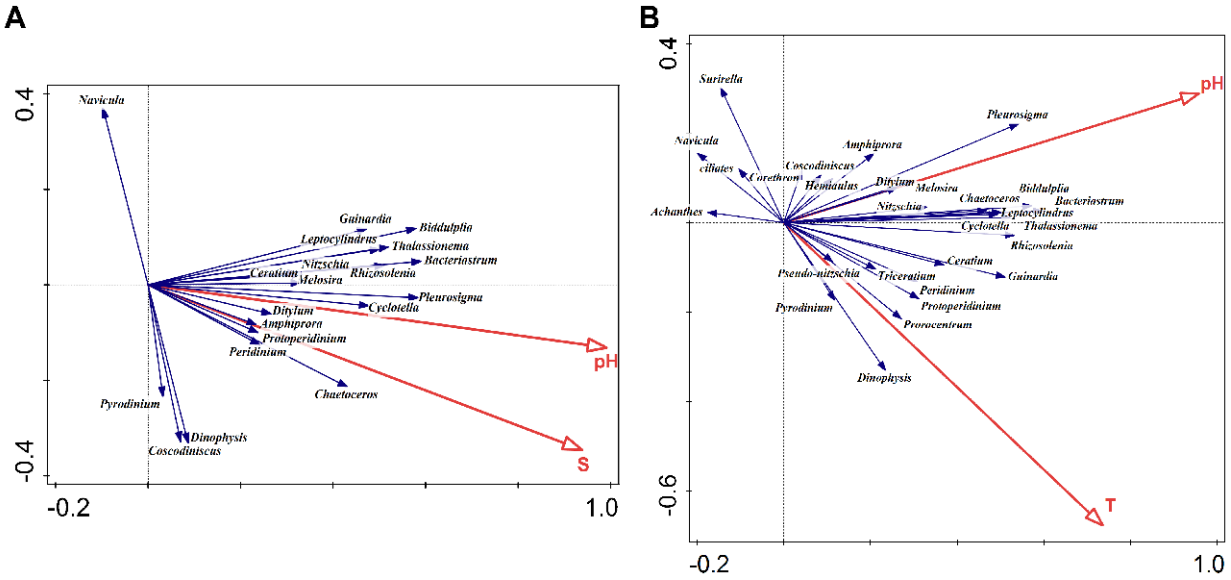

Fig. 8. Biplot diagrams from RDA visualizing the joint effect of A) pH and salinity and B) pH and temperature on phytoplankton communities. Score scaling is focused on alga scores (standardized). Only the 20 (A) and 28 (B) best fitting taxa are shown.

addition, samples collected at S4, in contrast with the other samples, tended to be placed towards one side of the first axis of the plot (Fig. 7B). This suggests a relatively high dissimilarity between the groups created by the S4 samples and those from the three remaining sample locations.

Influence of water physicochemical properties

Forward selection of explanatory variables allowed determination of the most important environmental variables affecting phytoplankton communities. The season of sampling along with

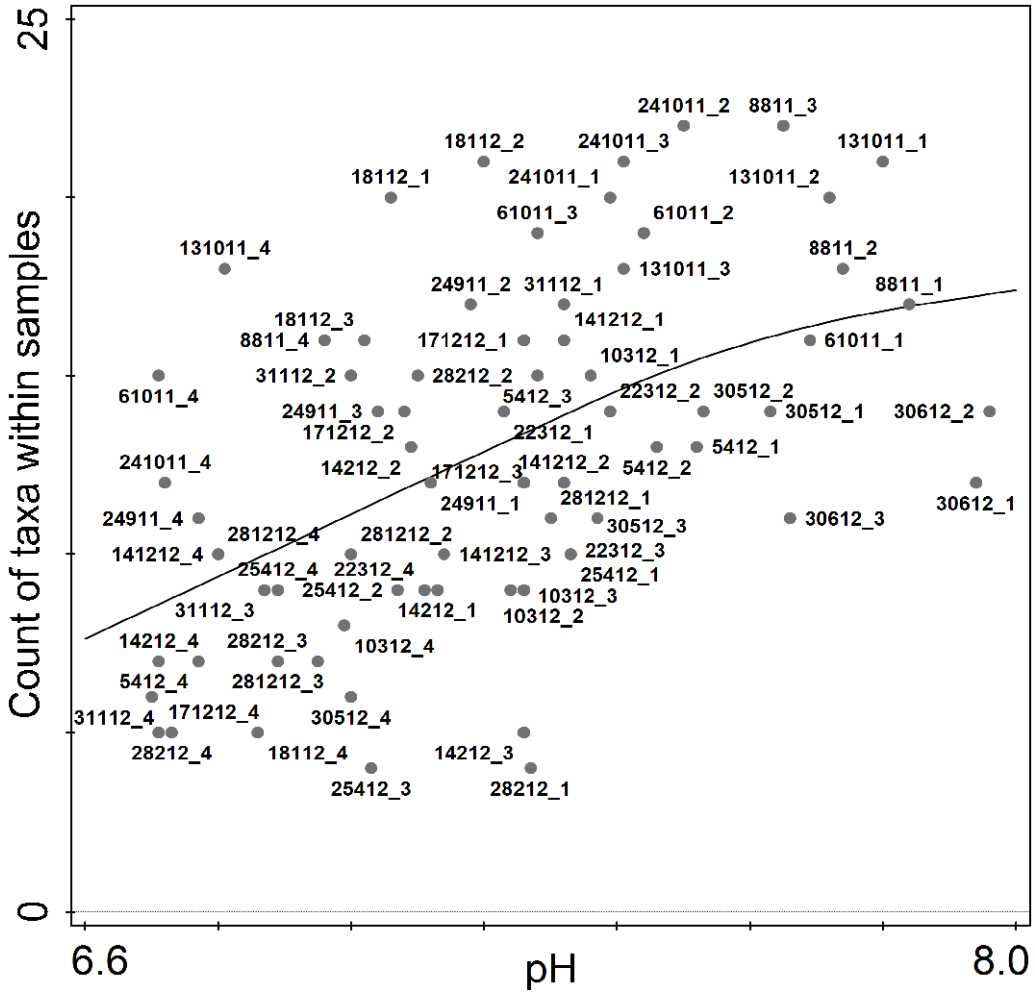

Fig. 9. GAM (Generalized Additive Model) plot of phytoplankton taxa number within samples against pH. The sample labels correspond to date of sampling (first 4-6 digits) and sampling site (last digit).

the sampling site were used as covariates. pH was indicated as the most effective predictor of the phytoplankton community composition ($P = 0.005$), followed by temperature ($P < 0.01$) and salinity ($P < 0.05$). Variation partitioning by partial constrained ordination (RDA) was then performed to quantify the effects (and their overlap) of pH and salinity as well as pH and temperature on the algal communities (Fig. 8). As pH and salinity are positively correlated, the overlap of their effects on phytoplankton community is expected (Fig. 8A). The amount of variation explained by the joint effect of the two environmental variables was 16.7% ($P = 0.001$). The sole (partial) effect of pH explained 2.5% ($P = 0.02$), while the sole effect of salinity accounted for 0.9% ($P > 0.05$) of the total variation (see Supplemental material, Figs. S1 and S2 for illustration of variation explained by pH or salinity alone). Similarly, the partitioning procedure indicated that the joint effect of pH and temperature explained 17.5% of the observed variability among samples ($P = 0.001$; Fig. 8B). The partial effects of pH or temperature alone represented 8.1% ($P = 0.001$) and 1.6% ($P > 0.05$) of the total variation, respectively (see Supplemental material, Figs. IIa and b for illustration of variations explained by pH or temperature alone). A clear correlation between the number of phytoplankton taxa recorded and pH ($P = 0.001$) was detected (Fig. 9). The number of taxa decreased with decreasing pH. That decline was especially evident below a pH of 7.5 (Fig. 9).

Discussion

The number of taxa found was low compared with some other studies conducted in tropical regions (e.g. Angsupanich & Rakkheaw 1997;

Lueangthuwapranit *et al.* 2011; Su *et al.* 2004). Su *et al.* (2004) reported 64 phytoplankton species (including diatoms, dinoflagellates, chlorophytes, and cyanobacteria) in their study of Tapong Bay (Taiwan) and Lueangthuwapranit *et al.* (2011) observed 74 genera from 6 divisions in samples taken from the Na Thap River estuary (Thailand). Although we cannot exclude these differences being influenced by methodology, we do not consider this is sufficient to explain the generally low functional diversity observed in phytoplankton from the Brunei River estuary. Whereas the phytoplankton communities present at the four sampling stations differed significantly in terms of both algal abundance and composition, the main differences were not caused by presence or absence of strictly stenohaline taxa, but rather by the dominance of the main common diatom and dinoflagellate taxa changing along the pH and salinity gradients.

As noted in several studies (Jacobsen & Andersen 1994; Loverde-Oliveira *et al.* 2009; Muylaert & Sabbe 1999), many dinoflagellate species may have mixo- or heterotrophic nutrition, which favours their survival in highly turbid conditions. Furthermore, it has been suggested that a higher carbon: chlorophyll ratio in dinoflagellates compared with diatoms may be responsible for their faster growth in conditions of lower pH and elevated CO_2 concentration (Low-Décarie *et al.* 2011; Tortell *et al.* 2008). Many diatom species are known to be relatively resistant to a moderate decrease in water pH (up to 0.3 pH units), but their numbers reduce drastically with any further decrease of pH (Geelen & Leuven 1986, and references therein; Majewska, personal observations). Also, species within some diatom genera (e.g. *Cyclotella*) are known to have clear heterotrophic capabilities, which enable them to survive in low-light conditions (Lylis & Trainer 1973).

The outcomes of several statistical models indicated that the communities investigated were strongly influenced by season (explaining ca. 30% of the total variance in phytoplankton compositional data) and by the set of factors specifically related to each sampling station (ca. 20% of the total variance). pH appeared to have a stronger influence on phytoplankton assemblages than salinity, temperature, or dissolved oxygen. Although pH is strongly correlated with salinity (Hansen 2002; Zhou & Rowland 1997), in many cases its effect when considered alone was opposite to that of salinity, i.e. taxa associated with lower salinity levels appeared to prefer higher pH conditions, and vice versa, when the shared effect of pH and salinity was excluded from the model. A large majority of the taxa had their local optima in stations characterized by relatively high pH values, while the GAM plot illustrates the positive correlation of pH and the number of taxa found in the samples. Other studies on tropical estuarine phytoplankton have reported similar trends. Madhu *et al.* (2007) observed that diversity of phytoplankton in Cochin backwaters (southwest India) decreased during the monsoon season when heavy rainfalls caused decrease in both salinity and pH, whereas Costa *et al.* (2001), who examined phytoplankton in the Paraiba do Sul River estuary (Brazil), observed that the highest biodiversity was associated with the highest pH and high water residence, and concluded that freshwater discharges controlled both species composition and their biomass.

Minima in phytoplankton diversity and abundance were found at the most landward station, S4, indicating the least favourable conditions for microalgal growth. Although most of the genera were present in different seasons and in various numbers at all four stations, genera such as *Corethron*, *Cyclotella*, *Guinardia,* and *Triceratium* were never present in samples from S4. A combination of extreme turbidity, resulting in severe light limitation, distinctly lower salinity, pH, and DO values in the proximity of large urban areas (e.g. Kiulap, Gadong, water villages along the estuary) could have affected local phytoplankton markedly more than at the more seaward locations. A relatively high contribution of typically benthic taxa (*Achnanthes* spp., *Navicula* spp.) to the phytoplankton found in S4 samples indicated significant exchange with the benthic communities and a high level of physical disturbance (turbidity, river runoff; Aquino *et al.* 2015), which might also limit the development of phytoplankton assemblages (Adesalu 2010; Cloern 1987; Muylaert & Sabbe 1999; Palleyi *et al.* 2008).

Sampling season proved to have a pronounced effect on the estuarine phytoplankton. The RDA models clearly indicated that the dry inter-monsoon period following NEM heavy rainfalls was the least suitable for the phytoplankton development. The two monsoon seasons resulted in changes in microalgal communities in qualitatively and quantitatively distinct ways. The highest abundances (up to 9107 cells ml^{-1}) were associated with the SWM season and not with NEM, in which a series of the heaviest rainfalls occurred.

Fluctuations in water parameters observed during the NEM season were an effect of turbulent events and high water mixing caused by both heavy rainfalls and north-eastern winds favouring marine water intrusions into the estuary. Wet season and other rainfall events may promote phytoplankton blooms through the leaching of biogenic substances from the adjacent land and the large influx of riverine water highly enriched in organic matter and nutrients (Angsupanich & Rakkheaw 1997; Gameiro *et al.* 2004; Jackson *et al.* 1987; Lueangthuwapranit *et al.* 2011; Mallin *et al.* 1993). In addition, algal growth is enhanced by lower tidal variation associated with the monsoon seasons (Palleyi *et al.* 2008), with tides being recognised as possibly one of the most important factors for local phytoplankton development (Lueangthuwapranit *et al.* 2011; Su *et al.* 2004; Wan Maznah *et al.* 2016). In some cases, however, extremely intense rainfall events may negatively affect phytoplankton communities by increasing turbidity (Mallin *et al.* 1993) and by introducing into the aquatic systems an excessive amount of acid drainage water and heavy metals that are easily leached from the soil in the low pH environment (Green & Barnes 2010; Macdonald *et al.* 2007; Russel & Helmke 2002). Strongly geologically originated ASS are found in the vicinity of the Sungai Brunei estuary (Bolhuis *et al.* 2014; Grealish & Fitzpatrick 2013) and the risk of these becoming increasingly damaging to the environment depends greatly on local management and land use practices. Appropriate management practices are not always well understood and it is highly probable that intense leaching during the wet seasons causes a serious threat to water quality and aquatic life (e.g. Dent & Pons 1995; Sammut *et al.* 1995). Adam *et al.* (2011) working in eastern Malaysia and Tan *et al.* (2006) in the eastern Malacca Straits reported that intense phytoplankton blooms were observed mainly during the NEM. The monsoon influence was associated with strong north-east winds responsible for extensive mixing of the water column. These studies, however, were focused on marine habitats that are only minimally influenced by freshwater and it is unclear how applicable their findings are to the estuarine environment.

A smaller peak in algal abundance was also observed during the dry period in October. The phenomenon was associated with an increase in DO, salinity, and pH and a decrease in temperature suggesting a higher inflow of saline water into the river. Similar changes in hydrological conditions were less pronounced in April when the heavy winter rains subsided, and this was not followed by an increase in plankton abundance. Costa *et al.* (2009) observed that a strong influence of marine waters on the Paraiba do Sul River estuary (Brazil) caused increase in both phytoplankton biomass and species richness and that a high river flow and turbidity supported mainly fast growing nanoplankton (< 20 μm) or some large diatom cells able to remain suspended even in shallow and turbulent waters. In our studies phytoplankton samples were collected with a 20 μm mesh and it is likely that any peak in nanoplankton abundance would not have been detected with the sampling methodology used.

Sampling season, site, and water pH explained ca. 60% of the observed variation in the phytoplankton data obtained. However, we recognise that variables not included in these analyses might also significantly affect the communities. This may be inferred from the low eigenvalue of the second axis of the RDA, which was weakly correlated with the selected environmental variables. For instance, high nutrient concentrations often found in the upper part of the estuary (Pintu Malim - Sungai Kedayan), might counter the negative effect of low pH (Jalal *et al.* 2011). In this region, nutrient levels are likely to be elevated through sewage treatment activities at Pintu Malim, release of organic materials from Kampong Ayer, small-scale aquaculture facilities, and the effect of Sungai Kedayan inflow, which carries water from a large urbanized area. According to Biswas *et al.* (2011) diatoms may benefit from elevated CO_2 concentrations in the estuarine water, but only if the nutrient levels are not a limiting factor. Amongst other potentially important influences, grazing and other biological factors (e.g. parasitism) have been suggested to play an important role in phytoplankton dynamics (Canter & Lund 1953; Muylaert & Sabbe 1999).

Although the acute temporal variability in acidification that coincides with seasonal flooding is assumed to relate largely to ASS inflows, finer temporal scale tidal fluctuations in pH during inter-monsoons are more likely to be associated with the combination of a lowered buffering capacity, microbial decomposition, and pCO_2 saturation in the upper estuarine reaches (Bolhuis *et al.* 2014; Hossain *et al.* 2014; Proum S. unpublished). As many of the processes involved in this complex acidification are natural, with potentially acid-generating geological formations and biological communities (mangroves) probably

dating back to the mid-Miocene (Hossain *et al.* 2014), we assume that the phytoplankton taxa living in the Brunei estuary are well adapted to thrive in an environment unsuitable for other planktonic species.

Low-Décarie *et al.* (2011) considered that changes in community dynamics in environments affected by elevated CO_2 concentrations and water acidification are based mostly on physiological differences between major taxa, while the response of species within each taxon is less distinct. If so, this suggests that large shifts in community composition may be predicted to a certain extent without a detailed knowledge of individual species' ecological preferences. However, most studies underlying such conclusions have been conducted under laboratory conditions, and examining a limited number of taxa (e.g. Low-Décarie *et al.* 2011; Richmond *et al.* 1982; Tortell 2000). It is likely that individual elements of natural phytoplankton communities may behave differently under the influence of the combined effects of multiple environmental factors, and further studies on individual species' autecology are required to explain phytoplankton dynamics and predict future changes within microalgal assemblages.

Estuaries are a very distinctive aquatic environment and the assessment of their potential degradation requires care. Estuarine ecosystems are considered to be naturally stressed due the extremely high variability in parameters such as salinity, pH, temperature, nutrients, dissolved oxygen, sediment dynamics, current speed and direction and light penetration. Nevertheless, while these may be stressful for strictly marine or freshwater organisms, for estuarine-adapted biota they represent the normal state. Indeed, the main characteristics of this natural stress often bear a close resemblance to those of the more recently imposed anthropogenic stressors. Because of that, it is particularly difficult to detect the effects of anthropogenic perturbations in estuaries and one should not rely exclusively on species diversity but rather on the system's functional characteristics, which are better reflected by higher taxa composition (Elliot & Quintino 2007; Wright 1982). The similarities between highly variable natural stressors and those under anthropogenic influence require further clarification.

Acknowledgements

We thank our colleagues: Teddy Chua, Sorya Proum, and Lukasz Polanski for their technical support. A visit to Brunei by Mario De Stefano, as well as field and laboratory work for this study, were funded through a Brunei Research Council grant to David J. Marshall (UBD/S&T/16). Aimi Adam received a Graduate Research Scholarship (GRS) from the Universiti Brunei Darussalam. Peter Convey was supported by NERC funding to the British Antarctic Survey's 'Biodiversity Evolution and Adaptation' Team.

References

Adam, A., N. Mohammad-Noor, A. Anton, E. Saleh, S. Saad & R. S. Muhd-Shaleh. 2011. Temporal and spatial distribution of harmful algal bloom (HAB) species in coastal waters of Kota Kinabalu, Sabah, Malaysia. *Harmful Algae* **10**: 495–502.

Adesalu, T. A. 2010. Phytoplankton dynamics of river Oli in Kainji Lake National Park, Nigeria during dry season. *International Journal of Botany* **6**: 112–116.

Alfaro, A. C. 2006. Benthic macro-invertebrate community composition within a mangrove/seagrass estuary in northern New Zealand. *Estuarine Coastal and Shelf Science* **66**: 97–110.

Amaral, V., H. N. Cabral, & M. J. Bishop. 2012a. Effects of estuarine acidification on predator-prey interactions. *Marine Ecology Progress Series* **445**: 117–127.

Amaral, V., E. L. Thompson, M. J. Bishop & D. A. Raftos. 2012b. The proteomes of Sydney rock oysters vary spatially according to exposure to acid sulfate runoff. *Marine and Freshwater Research* **63**: 361–369.

Amaral, V., H. N. Cabral & M. J. Bishop. 2011a. Effects of runoff from acid-sulfate soils on pneumatophores of the grey mangrove *Avicennia marina*. *Marine and Freshwater Research* **62**: 974–979.

Amaral, V., H. N. Cabral & M. J. Bishop. 2011b. Resistance among wild invertebrate populations to recurrent estuarine acidification. *Estuarine Coastal and Shelf Science* **93**: 460–467.

Angsupanich, S. & S. Rakkheaw. 1997. Seasonal variation of phytoplankton community in Thale Sap Songkhla, a lagoonal lake in Southern Thailand. *Netherlands Journal of Aquatic Ecology* **30**: 297–307.

Aquino, E. P., L. G. P. Figueiredo, G. C. P. Borges, L. C. Ferreira, J. Z. D. O. Passavante, M. D. Glória & G. D. Silva-Cunha. 2015. Seasonal and spatial variation in phytoplankton community structure of an estuary in Northeastern Brazil. *Tropical Ecology* **56**: 125–131.

Berge, T., N. Daugbjerg, B. B. Andersen & P. J. Hansen. 2010. Effect of lowered pH on marine phytoplankton growth rates. *Marine Ecology Progress Series* **416**: 79–91.

Biswas H., A. Cros, K. Yadav, V. V. Ramana, V. R. Prasad, T. Acharyya & P. V. R. Babu. 2011. The response of a natural phytoplankton community from the Godavari River Estuary to increasing CO_2 concentration during the pre-monsoon period. *Journal of Experimental Marine Biology and Ecology* **407**: 284–293.

Bolhuis, H., H. Schluepmann, J. Kristalijn, Z. Sulaiman & D. J. Marshall. 2014. Molecular analysis of bacterial diversity in mudflats along the salinity gradient of an acidified tropical Bornean estuary (South East Asia). *Aquatic Biosystems* **10**: 10. doi:10.1186/2046-9063-10-10.

Boynton, W. R., W. M. Kemp & C. W. Keefe. 1982. A comparative analysis of nutrients and other factors influencing estuarine phytoplankton production. pp. 69–90 *In*: V. S. Kennedy (ed.) *Estuarine Comparisons*. Academic Press, New York.

Brading, P., M. E. Warner, P. Davey, D. J. Smith, E. P. Achterberg & D. J. Suggett. 2011. Differential effects of ocean acidification on growth and photosynthesis among phylotypes of *Symbiodinium* (Dinophyceae). *Limnology and Oceanography* **56**: 927–938.

Canter, H. M. & J. W. G. Lund. 1953. Studies on plankton parasites: II. The parasitism of diatoms with special reference to lakes in the English Lake District. *Transactions of the British Mycological Society* **27**: 6–93.

Cai, W. J., X. Hu, W. J. Huang, M. C. Murrell, J. C. Lehrter, S. E. Lohrenz, W.-C. Chou, Z. Weidong, T. H. James, W. Yongchen *et al.* 2011. Acidification of subsurface coastal waters enhanced by eutrophication. *Nature Geoscience* **4**: 766–770.

Caldeira, K. & M. E. Wickett. 2003. Oceanography: anthropogenic carbon and ocean pH. *Nature* **425**: 365.

Cassar, N., E. A. Laws & R. R. Bidigare. 2003. Bicarbonate uptake by Southern Ocean phytoplankton. *Global Biogeochemical Cycles* **18** doi: 10.1029/2003GB002116

Chua, T.-E., L. M. Chou & M. S. M. Sadorra. 1987. The coastal environmental profile of Brunei Darusslam: resource assessment and management issues. *ICLARM Technical Report* **18**. Fisheries Department, Ministry of Development, Brunei Darussalam.

Cloern, J. E. 1987. Turbidity as a control on phytoplankton biomass and productivity in estuaries. *Continental Shelf Research* **7**: 1367–1381.

Cook, F. J., W. Hicks, E. A. Gardner, G. D. Carlin & D. W. Froggatt. 2000. Export of acidity in drainage water from acid sulphate soils. *Marine Pollution Bulletin* **41**: 319–326.

Costa, L. S., V. L. M. Huszar & A. R. Ovalle. 2009. Phytoplankton functional groups in a tropical estuary: hydrological control and nutrient limitation. *Estuaries and Coasts* **32**: 508–521.

Costanza, R., R. d'Arge, R. de Groot, S. Farber, M. Grasso, B. Hannon, K. Limburg, S. Naeem, R. V. Oneill, J. Paruelo *et al.* 1997. The value of the world's ecosystem services and natural capital. *Nature* **387**: 253–260.

Currie, D. 1979. Some aspects of the hydrology of the Brunei Estuary. *Brunei Museum Journal* **6**: 199–239.

de Jonge, V. N., M. Elliott & E. Orive. 2002. Causes, historical development, effects and future challenges of a common environmental problem: eutrophication. *Hydrobiologia* **475**: 1–19.

Dent, D. L. & L. J. Pons. 1995. A world perspective on acid sulfate soils. *Geoderma* **67**: 263–276.

Doney, S. C., V. J. Fabry, R. A. Feely & J. A. Kleypas. 2009. Ocean acidification: The other CO_2 problem. *Annual Review of Marine Science* **1**: 169–192.

Dove, M. C. & J. Sammut. 2007. Impacts of estuarine acidification on survival and growth of Sydney rock oysters *Saccostrea glomerata* (Gould 1850). *Journal of Shellfish Research* **26**: 519–527.

Downing, J. A., M. McClain, R. J. Twilley, M. Melack, J. Elser, N. N. Rabalais, W. M. Lewis,, R. E. Turner Jr., J. Corredor, D. Soto *et al.* 1999. The impact of accelerating land-use change on the N-cycle of tropical aquatic ecosystems: current conditions and projected changes. *Biogeochemistry* **46**: 109–148.

Duarte, C. M., I. E. Hendriks, T. M. Moore, Y. S. Olsen, A. Steckbauer, L. Ramajo, J. Carstensen, J. A. Trotter & M. McCulloch. 2013. Is Ocean acidification an open-ocean syndrome? Understanding anthropogenic impacts on seawater pH. *Estuaries and Coasts* **36**: 221–236.

Ehrenberg, C. G. 1844. Mittheilung über 2 neue lager von Gebirgsmassen aus infusorien als Meeres-Absatz in Nord Amerika und eine vergleichung derselben mit den organischen Kreide-Ge-bilden in Europa und Afrika. Bericht über die zur Bekanntmachung Geeigneten Verhandlungen der Königl. Preuss. *Akademie Der Wissenschaften zu Berlin* **1844:** 57–97.

Elliot, M. & V. Quintino. 2007. The Estuarine quality

paradox, environmental homeostasis and the difficulty of detecting anthropogenic stress in naturally stressed areas. *Marine Pollution Bulletin* **54**: 640–645.

Fabry, V. J., B. A. Seibel, R. A. Feely & J. C. Orr. 2008. Impacts of ocean acidification on marine fauna and ecosystem processes. *ICES Journal of Marine Science: Journal du Conseil* **65**: 414–432.

Falkowski, P. 2012. Ocean science: The power of plankton. *Nature* **483**: 17–20.

Feely, R. A., S. R. Alin, J. Newton, C. L. Sabine, M. Warner, A. Devol, C. Krembs & C. Maloy. 2010. The combined effects of ocean acidification, mixing, and respiration on pH and carbonate saturation in an urbanized estuary. *Estuarine Coastal and Shelf Science* **88**: 442–449.

Gameiro, C., P. Cartaxana, M. T. Cabrita & V. Brotas. 2004. Variability in chlorophyll and phytoplankton composition in an estuarine system. *Hydrobiologia* **525**: 113–124.

Gazeau, F., S. Alliouane, C. Bock, L. Bramanti, M. López Correa, M. Gentile, T. Hirse, H.-O. Pörtner & P. Ziveri. 2014. Impact of ocean acidification and warming on the Mediterranean mussel (*Mytilus galloprovincialis*). *Frontiers in Marine Science* **1**, doi: 10.3389/fmars.2014.00062.

Gazeau, F., L. M. Parker, S. Comeau, J.-P. Gattuso, W. A. O'Connor, S. Martin, H.-O. Pörtner & P. M. Ross. 2013. Impacts of ocean acidification on marine shelled molluscs. *Marine Biology* **160**: 2207–2245.

Geelen, J. F. M. & R. S. E. W. Leuven. 1986. Impact of acidification on phytoplankton and zooplankton communities. *Cellular and Molecular Life Sciences* **42**: 486–494.

Grealish, G. J. & R. W. Fitzpatrick. 2013. Acid sulphate soil characterization in Negara Brunei Darussalam: a case study to inform management decisions. *Soil Use Management* **29**: 432–444.

Green, T. J. & A. C. Barnes. 2010. Reduced salinity, but not estuarine acidification, is a cause of immune-suppression in the Sydney rock oyster *Saccostrea glomerata*. *Marine Ecology Progress Series* **402**: 161–170.

Hall-Spencer, J. M, R. Rodolfo-Metalpa, S. Martin, E. Ransome, M. Fine, S. M. Turner, S. J. Rowley, D. Tedesco & M. C. Buia. 2008. Volcanic carbon dioxide vents show ecosystem effects of ocean acidification. *Nature* **454**: 96–99.

Hammer, Ø., D. A. T. Harper & P. D. Ryan. 2001. PAST: Palaeontology statistical software package for education and data analysis. *Palaeontologia Electronica* **4**: 1–9.

Hansen, P. J., 2002. Effect of high pH on the growth and survival of marine phytoplankton: implications for species succession. *Aquatic Microbial Ecology* **28**: 279–288.

Hasle, G. R. & E. E. Syvertsen. 1997. Marine diatoms. *In*: C. R. Tomas (ed.) *Identifying Marine Phytoplankton*. Academic Press, San Diego, pp. 5–385.

Hasties, T. J. & R. J. Tibshirani. 1990. *Generalized Additive Models*. Chapman and Hall, New York.

Hinga, K. R. 2002. Effects of pH on coastal marine phytoplankton. *Marine Ecology Progress Series* **238**: 281–300.

Hopkinson, C. S., A. E. Giblin, J. Tucker & R. H. Garrit. 1999. Benthic metabolism and nutrient cycling along an estuarine salinity gradient. *Estuaries* **22**: 863–881.

Hossain, M. B. & D. J. Marshall. 2014. Benthic infaunal community structuring in an acidified tropical estuarine system. *Aquatic Biosystems* **10**: 11, doi: 10.1186/2046-9063-10-11.

Hossain, M. B., D. J. Marshall, & V. Senapathi. 2014. Sediment granulometry and organic matter content in the intertidal zone of the Sungai Brunei estuarine system, northwest coast of Borneo. *Carpathian Journal of Earth and Environmental Sciences* **9**: 231–239.

Hu, X. & W.-J. Cai. 2013. Estuarine acidification and minimum buffer zone - A conceptual study. *Geophysical Research Letters* **40**: 5176–5181.

Jalal, K. C. A., B. M. Ahmad Azfar, B. Akhbar John & Y. B. Kamaruzzaman. 2011. Spatial variation and community composition of phytoplankton along the Pahang estuary, Malaysia. *Asian Journal of Biological Sciences* **4**: 468–476.

Jackson, R., P. L. Williams & I. Joint. 1987. Freshwater phytoplankton in the low salinity region of the River Tamar Estuary. *Estuarine Coastal and Shelf Science* **25**: 299–311.

Jacobson, D. M. & R. A. Andersen. 1994. The discovery of mixotrophy in photosynthetic species of *Dinophysis* (Dinophyceae): ligh and electron microscopical observations of food vacuoles in *Dynophysis acuminata*, *D. norvegica* and two heterotrophic dinophysoid dinoflagellates. *Phycologia* **33**: 97–110.

Johnson, V. R., B. D. Russell, K. E. Fabricius, C. Brownlee & J. M. Hall-Spencer. 2012. Temperate and tropical brown macroalgae thrive, despite decalcification, along natural CO_2 gradients. *Global Change Biology* **18**: 2792–2803.

Lauder, H. S. 1864. Remarks on the marine Diatomaceae found at Hong Kong, with descriptions of new species. *Transactions of the Microscopical*

Society of London **12**: 75–79.

Legendre, P. 2007. Studying beta diversity: ecological variation partitioning by multiple regression and canonical analysis. *Journal of Plant Ecology* **1**: 3–8.

Lohbeck, K. T., U. Riebesell & T. B. Reusch. 2012. Adaptive evolution of a key phytoplankton species to ocean acidification. *Nature Geosciences* **5**: 346–351.

Longhurst, A., S. Sathyendranath, T. Platt & C. Caverhill. 1995. An estimate of global primary production in the ocean from satellite radiometer data. *Journal of Plankton Research* **17**: 1245–1271.

Loverde-Oliveira, S.M., V. L. Moraes Huszar, N. Mazzeo & M. Scheffer. 2009. Hydrology-driven regime shifts in a shallow tropical lake. *Ecosystems* **12**: 807–819.

Low-Décarie, E., G. F. Fussmann & G. Bell. 2011. The effect of elevated CO_2 on growth and competition in experimental phytoplankton communities. *Global Change Biology* **17**: 2525–2535.

Lueangthuwapranit, C., U. Sampantarak & S. Wongsai. 2011. Distribution and abundance of phytoplankton: Influence of salinity and turbidity gradient in the Na Thap River, Songkhla Province, Thailand. *Journal of Coastal Research* **27**: 585–594.

Lylis, J. C. & F. R. Trainor. 1973. The heterotrophic capabilities of *Cyclotella meneghiniana*. *Journal of Phycology* **9**: 365–369.

Macdonald, B. C. T., I. White, M. E. Astrom, A. F. Keene, M. D. Melville & J. K. Reynolds. 2007. Discharge of weathering products from acid sulfate soils after a rainfall event, Tweed River, eastern Australia. *Applied Geochemistry* **22**: 2695–2705.

Madhu, N. V., R. Jyothibabu, K. K. Balachandran, U. K. Honey, G. D. Martin, J. G. Vijay, C. A. Shiyas, G. V. M. Gupta & C. T. Achuthankutty. 2007. Monsoonal impact on planktonic standing stock and abundance in a tropical estuary (Cochin backwaters-India). *Estuarine Coastal and Shelf Science* **73**: 54–64.

Majewska, R., P. Lemke, P., A. Zgrundo & M. De Stefano. 2012. Benthic diatoms of the Vistula River estuary (Northern Poland) - seasonality, substrata preferences, and the influence of water chemistry. *Phycological Research* **60**: 1–19.

Mallin, M. A., H. W. Paerl, J. Rudek & P. W. Bates. 1993. Regulation of estuarine primary production by watershed rainfall and river flow. *Marine Ecology Progress Series* **93**: 199–203.

Marshall, D. J., J. H. Santos, K. M. Y. Leung & W. H. Chak. 2008. Correlations between gastropod shell dissolution and water chemical properties in a tropical estuary. *Marine Environmental Research* **66**: 422–429.

Martin, S., R. Rodolfo-Metalpa, E. Ransome, S. Rowley, M. C. Buia, J. P. Gattuso & J. Hall-Spencer. 2008. Effects of naturally acidified seawater on seagrass calcareous epibionts. *Biology Letters* **4**: 689–692.

Miller, A. W., A. C. Reynolds, C. Sobrino & G. F. Riedel. 2009. Shellfish face uncertain future in high CO_2 world: influence of acidification on oyster larvae calcification and growth in estuaries. *PLoS ONE* **4**: e5661, doi:10.1371/journal.pone.0005661.

Morrisey, D. J., S. J. Turner, G. N. Mills, R. B. Williamson & B. E. Wise. 2003. Factors affecting the distribution of benthic macrofauna in estuaries contaminated by urban runoff. *Marine Environmental Research* **55**: 113–136.

Muylaert, K. & K. Sabbe. 1999. Spring phytoplankton assemblages in and around the maximum turbidity zone of the estuaries of the Elbe (Germany), the Schelde (Belgium/The Netherlands) and the Gironde (France). *Journal of Marine Systems* **22**: 133–149.

Nirmal Kumar, J. I., S. R. Khan, R. N. Kumar & P. R. Sajish. 2013. Assessment of hydrochemical characters variations in relation to phytoplankton during pre-monsoon at J-point of Mahi Estuary, Gujarat, India. *Our Nature* **11**: 85–95.

Orr, J. C., V. J. Fabry, O. Aumont, L. Bopp, S. C. Doney, R. A. Feely, A. Gnanadesikan, N. Gruber, A. Ishida, F. Joos *et al.* 2005. Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. *Nature* **437**: 681–686.

Pelleyi, S., R. N. Kar & C. R. P. Panda. 2008. Seasonal variability of phytoplankton population in the Brahmani estuary of Orissa, India. *Journal of Applied Sciences and Environmental Management* **12**: 19–23.

Porzio, L., S. L. Garrard & M. C. Buia. 2013. The effect of ocean acidification on early algal colonization stages at natural CO_2 vents. *Marine Biology* **160**: 2247–2259.

Pradhan, U. K, P. V. Shirodkar & B. K. Sahu. 2009. Physicochemical characteristics of the coastal water of Devi Estuary, Orissa and evaluation of its seasonal changes using chemometric techniques. *Current Science* **96**: 1203–1204.

Raymond, P. A., J. E. Bauer, & J. J. Cole. 2000. Atmospheric CO_2 evasion, dissolved inorganic carbon production, and net heterotrophy in the York River estuary. *Limnology and Oceanography* **45**: 1707–1717.

Richmond, A., S. Karg & S. Boussiba. 1982. Effects of bicarbonate and carbon dioxide on the competition between *Chlorella vulgaris* and *Spirulina platensis*. *Plant Cell Physiology* **23**: 1411–1417.

Russell, D. J. & S. A. Helmke. 2002. Impacts of acid

leachate on water quality and fisheries resources of a coastal creek in northern Australia. *Marine and Freshwater Research* **53**: 19–33.

Sarma, V. V. S. S., M. Dileep Kumar & M. Manerikar. 2001. Emission of carbon dioxide from a tropical estuarine system, Goa, India. *Geophysical Research Letters* **28**: 1239–1242.

Sammut, J., M. D. Melville, R. B. Callinan & G. C. Fraser. 1995. Estuarine acidification: Impacts on aquatic biota of draining acid sulphate soils. *Australian Geographical Studies* **33**: 89–100.

Schmidt, A. 1874, 1878, 1888, 1890, 1892, 1893. *In*: A. Schmidt *et al*. *Atlas der Diatomaceenkunde*: Leipzig (O.R. Reisland).

Shirota, A. 1966. *The Plankton of South VietNam: Fresh water and marine plankton.* Overseas Technical Cooperation Agency, Japan.

Su, H.-M., H.-J. Lin & J.-J. Hung. 2004. Effects of tidal flushing on phytoplankton in a eutrophic tropical lagoon in Taiwan. *Estuarine Coastal and Shelf Science* **61**: 739–750.

Sunda, W. G. & W.-J. Cai. 2012. Eutrophication induced CO_2-acidification of subsurface coastal waters: Interactive effects of temperature, salinity, and atmospheric pCO_2. *Environmental Science and Technology* **46**: 10651–10659.

Šmilauer, P. & J. Lepš. 2014. *Multivariate Analysis of Ecological Data using Canono 5*. 2nd edition. Cambridge University Press, Cambridge.

Tan, C. K., J. Ishizaka, S. Matsumura, F. M. Yusoff & M. I. H. Mohamed. 2006. Seasonal variability of SeaWiFS chlorophyll a in the Malacca Straits in relation to Asian monsoon. *Continental Shelf Research* **26**: 168–178.

ter Braak, C. J. F. & P. Šmilauer. 2012. *Canoco Reference Manual and User's Guide: Software for Ordination (Version 5.0)*. Microcomputer Power, Ithaca.

Thottathil, S. D, K. K. Balachandran, G. V. M. Gupta, N. V. Madhu & S. Nair. 2008. Influence of allochthonous input on autotrophic-heterotrophic switch-over in shallow waters of a tropical estuary (Cochin Estuary), India. *Estuarine Coastal and Shelf Science* **78**: 551–562.

Tomas, C. R. 1997. *Identifying Marine Phytoplankton.* Academic Press.

Tortell, P. D. 2000. Evolutionary and ecological perspectives on carbon acquisition in phytoplankton. *Limnology and Oceanography* **45**: 744–750.

Tortell, P., C. Payne, Y. Li, S. Trimborn, B. Rost, W. O. Smith, C. Rieselman, R. D. Dunbar, P. Sedwick & G. R. DiTullio. 2008. CO_2 sensitivity of Southern Ocean phytoplankton. *Geophysical Research Letters* **35**: L04605, doi: 10.1029/2007GL032583.

Tunnicliffe, V., K. T. Davies, D. A. Butterfield, R. W. Embley, J. M. Rose & W. W. Chadwick Jr. 2009. Survival of mussels in extremely acidic waters on a submarine volcano. *Nature Geoscience* **2**: 344–348.

Waldbusser, G. G., E. P. Voight, H. Bergschneider, M. A. Green & R. I. E. Newell. 2011. *Estuaries and Coasts* **34**: 221–223.

Wallace, R. B., H. Baumann, J. S. Grear, R. C. Aller & C. J. Gobler. 2014. Coastal ocean acidification: The other eutrophication problem. *Estuarine Coastal and Shelf Science* **148**: 1–13.

Wan Maznah, W. O., S. Rahmah, C. C. Lim, W. P. Lee, K. Fatema & M. I. Mansor. 2016. Effects of tidal events on the composition and distribution of phytoplankton in Merbok river estuary Kedah, Malaysia. *Tropical Ecology* **57**: 213–229.

Widdicombe, S. & J. I. Spicer. 2008. Predicting the impact of ocean acidification on benthic biodiversity: what can animal physiology tell us? *Journal of Experimental Marine Biology and Ecology* **366**: 187–197.

Wright, S. 1982. Character change, speciation, and the higher taxa. *Evolution* **36**: 427–443.

Yau, K. H. 1991. Water quality of Brunei Bay and estuary. *ICLARM Conference Proceedings* **22**: 189–l93.

Zhou, J. L. & S. J. Rowland. 1997. Evaluation of the interactions between hydrophobic organic pollutants and suspended particles in estuarine waters. *Water Research* **31**: 1708–1718.

(Received on 11.02.2015 and accepted after revisions, on 19.03.2016)

Supporting Information

Additional Supporting information may be found in the online version of this article.

Table S1. Average of square-rooted abundances (cells ml^{-1}) of phytoplankton taxa found at station 1 and 4, and their contribution to the dissimilarity observed between the groups (SIMPER).

Table S2. Average of square-rooted abundances (cells ml^{-1}) of phytoplankton taxa found at station 1 and 3, and their contribution to the dissimilarity observed between the groups (SIMPER).

Table S3. Average of square-rooted abundances (cells ml^{-1}) of phytoplankton taxa found at station 1 and 2, and their contribution to the dissimilarity observed between the groups (SIMPER).

Table S4. Average of square-rooted abundances (cells ml^{-1}) of phytoplankton taxa found at station 2 and 3, and their contribution to the dissimilarity observed between the groups (SIMPER).

Table S5. Average of square-rooted abundances (cells ml^{-1}) of phytoplankton taxa found at station 2 and 4, and their contribution to the dissimilarity observed between the groups (SIMPER).

Table S6. Average of square-rooted abundances (cells ml^{-1}) of phytoplankton taxa found at station 3 and 4, and their contribution to the dissimilarity observed between the groups (SIMPER).

Fig. S1. Biplot diagrams from RDA visualizing the partial effect of a) pH (after subtraction of the shared effect of pH and salinity) and b) salinity (after subtraction of the shared effect of pH and salinity) on phytoplankton taxa.

Fig. S2. Biplot diagrams from RDA visualizing the partial effect of a) pH (after subtraction of the shared effect of pH and temperature) and b) temperature (after subtraction of the shared effect of pH and temperature) on phytoplankton taxa.