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Abstract

The factors controlling estuarine phytoplankton diversity and production are relatively well known in temperate systems. Less however is known about the factors affecting phytoplankton community distribution in tropical estuaries. This is surprising given the economic and ecological importance of these large, deltaic ecosystems, such as are found in South East Asia. Here we present the results from an investigation into the factors controlling phytoplankton distribution and phytoplankton-bacterial coupling in the Bach Dang Estuary, a sub-estuary of the Red River system, in Northern Vietnam. Phytoplankton diversity and primary and bacterial production, nutrients and metallic contaminants (mercury and organotin) were measured during two seasons: wet (July 2008) and dry (March 2009). Phytoplankton community composition differed between the two seasons with only a 2% similarity between July and March. The large spatial extent and complexity of defining the freshwater sources meant that simple mixing diagrams could not be used in this system. We therefore employed multivariate analyses to determine the factors influencing phytoplankton community structure. Salinity and suspended particulate matter were important factors in determining phytoplankton distribution, particularly during the wet season. We also show that phytoplankton community structure is probably influenced by the concentrations of mercury species (inorganic mercury and methyl mercury in both the particulate and dissolved phases) and of tri-, di, and mono-butyl tin species found in this system. Freshwater phytoplankton community composition was associated with dissolved methyl mercury and particulate inorganic mercury concentrations during the wet season, whereas, during the dry season, dissolved methyl mercury and particulate butyl tin species were important factors for the discrimination of the phytoplankton community structure. Phytoplankton-bacterioplankton coupling was also investigated during both seasons. In the inshore, riverine stations the ratio between bacterial production and dissolved primary production was high supporting the hypothesis that bacterial carbon demand is supported by allochthonous riverine carbon sources. The inverse was true in the offshore stations,

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where BP:DPP values were less than 1, potentially reflecting differences in primary production due to shifting phytoplankton community diversity.

1 Introduction

Determining the factors that control diversity and function in an ecosystem is of fundamental importance if we wish to understand how ecosystems respond to climate and man-induced change. This is of particular importance in coastal ecosystems because despite their relatively small total area as compared to that of the global ocean, they play an important role in the aquatic carbon cycle (e.g., Borges et al., 2005). Moreover, with a large percentage of the world's population living within 100 km of the coast (Halpern et al., 2008), the impact of mans' activities on aquatic biodiversity and function cannot not be ignored.

Coastal seas and estuaries are ecosystems where the mixing of fresh and marine waters exerts considerable changes in physico-chemical properties and biological processes. Overlain with this are the impacts of waste water and other effluents from industrial and urban activities. All of which can exert a non-negligible impact on the structure and function of planktonic communities. For example, differences in phytoplankton and bacterioplankton salinity and nutrient tolerances can induce marked shifts in community diversity along estuarine salinity gradients. In a comparison of 9 European estuaries, Lemaire et al. (2002) found large changes in phytoplankton diversity along the salinity gradients. Similarly, Muylaert et al. (2009), report that in the Scheldt Estuary few taxa are present along the entire salinity gradient. Bacterial community composition also changes along salinity gradients. In the Choptank Estuary, del Giorgio and Bouvier (2002) demonstrated clear differences in community composition between the freshwater end of the estuary where the β -proteobacteria dominated and the higher salinity end where the α -proteobacteria were more dominant. Moreover, recent work from an estuary in India has shown that bacteria shift preferences in carbon source along salinity gradients (Thottathil et al., 2008).

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Shifting community diversity also exerts an effect on biogeochemical processes and carbon fluxes. Variations in primary production, respiration and $p\text{CO}_2$ flux along estuarine salinity gradients are probably related to the shifting community diversity, available nutrients and organic carbon and turbidity (Smith and Kemp, 2001; Fisher et al., 1988, 1998). It is therefore probable that estuarine metabolic balance is intimately linked to that of biological diversity (Borges et al., 2006). Indeed, in Chesapeake Bay, Smith and Kemp (2001) proposed that the shifts observed in the ratio of photosynthesis to respiration (P:R), a measure ecosystem metabolism, were due to changes in the phytoplankton populations present. This, combined with changes in bacterial community composition and cell activity levels (Bouvier and del Giorgio, 2002) and DOM concentration and bioavailability (Raymond and Bauer, 2000; Rochelle-Newall et al., 2007) all point towards the importance of understanding the factors that control community composition in estuarine and coastal waters.

Although several studies have examined the links between the factors influencing phytoplankton diversity and the relationship between primary production and respiration in estuarine and coastal systems in temperate ecosystems (e.g., Chesapeake Bay, Columbia River Estuary), less research has been focused on the factors that control phytoplankton diversity in tropical coastal ecosystems. Nutrient concentration and availability is an obvious factor controlling phytoplankton biomass (Ferguson et al., 2004; Jacquet et al., 2006), particularly in estuaries, however, other factors such as heavy metal contamination can also be important in sensitive coastal ecosystems (see review of Peters et al., 1997). The high toxicity of mercury and methyl-mercury to humans is well known and this has spurred many of the investigations of the role and bioaccumulation of this metal in aquatic food webs (e.g., Duarte et al., 2007; Ullrich et al., 2001; Downs et al., 1998). However, few studies have examined the impact of mercury on phytoplankton community structure and production in tropical systems. Other metals, such as the organo-tin compounds (tri-butyl tin and its derivatives) can also reach high concentrations in coastal systems, particularly around ports (Nhan et al., 2005; Oliveira and Santelli, 2010). Again, many studies on the impact of

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organo-tin compounds have focused on invertebrates and some have pointed out the negative impact of TBT (tributyltin) on phytoplankton populations (Sargian et al., 2005). However, few have looked at the role of these and other contaminants in determining phytoplankton community structure and microbial carbon flow in tropical estuaries. In the southwest lagoon of New Caledonia, an oligotrophic coral reef lagoon, it has been recently shown that elevated heavy metal concentrations can influence phytoplankton community structure, particularly in sites that had no prior exposure to elevated zinc and nickel concentrations (Rochelle-Newall et al., 2008a). However, the impact of other heavy metals, such as mercury and organo-tin on the lower levels of the food web of tropical, eutrophic coastal ecosystems has largely been ignored despite their ecological and biogeochemical importance in terms of coastal carbon fluxes (Borges, 2005).

Here we present an investigation into some of the factors potentially controlling phytoplankton diversity during two seasons in a turbid, tropical estuarine system (Bach Dang River Estuary, North Vietnam). We then link this to primary and bacterial production in an attempt to determine the factors controlling the carbon cycle within a South-East Asian estuary. The objective of this work was therefore to determine the factors controlling phytoplankton diversity and to determine if these shifts then manifest in an alteration in biogeochemical processes.

2 Materials and methods

The study site is located in the Bach Dang Estuary, North Vietnam. This site is a large estuary (20° N, 106° E), covering approximately 325 km² and forming the northeastern part of the Red River Delta complex (Fig. 1). Bach Dang, Cam and Lach Tray rivers are main tributaries of the Red-Thai Binh river system. The site is subject to a sub-tropical climate with a wet season (May–September) associated with the south monsoon and a dryer, cooler season (October–April) associated with the northeast monsoon. Samples were collected along three axial transects during two seasons (9–11 July 2008 and 12–15 March 2009) covering a range of salinities. During each season 9 stations

were sampled for phytoplankton diversity and abundance, organic and inorganic nutrients and carbon and the concentration of organotin (mono-, di-, and tri-butyl tin) and methyl mercury in both the particulate and dissolved fractions.

The locations of the stations are given in Table 1. At each sampling station, a CTD profiler (SeaBird SBE19) was deployed to measure temperature, salinity, photosynthetically active radiation (PAR) and in vivo fluorescence profiles. Turbidity (in Formazin Turbidity Units, FTU) was also measured with a Seapoint turbidity meter attached to the CTD package.

2.1 Nutrients and dissolved organic carbon

Inorganic nutrients were measured using standard fluorometric and spectrophotometric techniques after filtration (Whatman GF/F). Dissolved organic carbon (DOC) analyses were performed on filtered (Whatman GF/F) samples, collected in 40 mL pre-combusted (450 °C, overnight) glass tubes, sealed with a Teflon lined cap, after preservation with 36 µL 85% phosphoric acid (H₃PO₄). DOC concentration was measured on a Shimadzu TOC VCPH analyzer, using potassium phthalate calibration standards over the measurement range (0 to 450 µmol C L⁻¹). Certified reference materials (Hansell Laboratory, University of Miami) were used to assess the performance of the instrument on and between measurement days. The machine blank was between 3 and 5 µmol C L⁻¹ for the measurement days and the coefficient of variation (CV) of the measurement was always less than 2% of the mean of triplicate injections of duplicate samples.

2.2 Phytoplankton and bacterial abundance and activity

Chlorophyll-*a* (Chl-*a*) was measured on samples collected on GF/F filters using the method of Holm-Hansen et al. (1965). Phytoplankton samples were collected with a 20 µm plankton net or a 5 L Niskin bottle, following the methods described by Sournia (1978). Upon collection, samples were immediately fixed with Lugols solution (3 ml L⁻¹)

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and stored in the dark until return to the laboratory. Phytoplankton community composition was determined by epifluorescence microscopy (Olympus BX51) and a digital camera (Olympus DP12). Cell density was determined using an inverted microscope (Leica DMIL) and a Sedgewick Rafter Chamber. Phytoplankton were identified using standard references (Balech, 1995; Fukuyo et al., 1990; Taylor, 1976; Tomas, 1997; Truong, 1993; Yamagishi, 1992).

Subsamples for nano- and picophytoplankton, cyanobacteria and total bacterial abundance were fixed with buffered formalin (2% v/v) and stored immediately in liquid nitrogen until analysis by flow cytometry. Nano- (20 μm) and picophytoplankton (<2 μm) cells were detected and counted as described previously (Troussellier et al., 1993; Crosbie et al., 2003; Campbell et al., 1994) using a FACSCalibur flow cytometer (Becton Dickinson, San Jose, CA) with an air-cooled argon laser (488 nm, 15 mW). Cells excited at 488 nm were detected and counted using their right-angle light scattering (RALS) properties and their orange (585 nm filter) and red (465 nm filter) fluorescence from phycoerythrin and chlorophyll pigments, respectively. For each analysis, fluorescent beads (1, 2, 6, 10, 20 μm , Polysciences Inc., Warrington, PA) were systematically added to each sample to standardize the flow cytometer settings. Cell abundances were estimated by adding a known volume of fluorescent beads (True-Counts, Becton Dickinson) with known concentration.

Bacterial abundance was determined after staining with SYBR-Green I (Molecular Probes, OR, USA) as described by Marie et al. (1997) and sonication during 10 mn (Ultrasonik 300 Ney). The stained bacterial cells, excited at 488 nm, were enumerated using right-angle light scatter (RALS) and green fluorescence (FL1) at 530 nm. Fluorescent beads (0.96 μm and 2 μm , Polysciences Inc., Warrington, PA, USA) were added to each sample as an external standard. True count beads (Becton Dickinson, San Jose, Ca) were added to determine the volume analyzed.

Primary production (Dissolved Primary Production, DPP and Particulate Primary Production, PPP) was measured using $\text{NaH}^{14}\text{CO}_3$ following the method of Rochelle-Newall et al (2008b). Briefly, 39 mL water samples were inoculated with 1.2 MBq of

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NaH¹⁴CO₃ (Perkin-Elmer) and incubated in a semi-continuous flowing seawater bath under neutral density screening (50, 25, 12.5, 6% incident sunlight). After 4 h of incubation, samples were carefully filtered at low vacuum pressure onto 0.4 μm polycarbonate filters (Whatman Cyclopore). After acidification and drying of the filters, 5 mL of scintillation cocktail (Ultima Gold, Packard Instruments) was added. The amount of ¹⁴C incorporated into the particulate phase (PPP) was calculated using an inorganic carbon concentration of 25 700 μg C L⁻¹ (Marañón et al., 2004). For the DPP measurement, duplicate 5 mL of filtrate were collected, acidified with 100 μL of 5 mol L⁻¹ HCl and left for 24 h on a horizontal agitator table. After agitation, 15 mL of scintillation cocktail (Ultima Gold XR, Packard Instruments) was added and the samples counted using a Beckman Coulter LS 6500 Multi Purpose scintillation counter. For the radioactivity measurements, we consider that in order for the production rate to be significant there should be a ratio of at least 3 between the sample and the dark blank. Total Primary Production (TPP) represents the sum of DPP and PPP. An estimation of depth integrated primary productivity was determined using the water column light penetration values obtained from the PAR profiles of the CTD and calculated using the trapezoidal method.

Bacterial production (BP) was measured using ³H-leucine, following the method of Smith and Azam (1992) and following the protocol detailed in Rochelle-Newall et al. (2008a). The only differences were that we used 40 nM (final concentration) high specific activity ³H-leucine (Perkin Elmer) and the incubations were conducted in the dark and at in situ temperature. Leucine uptake was converted to carbon using the conversion factor 1.55 kg C mol⁻¹ leu (Kirchman, 2001).

2.3 Concentration of metallic species

Water samples were collected from the sub-surface by hand. In order to determine the particulate and dissolved concentrations, each water sample (0.3–1.5 L) was filtered through a pre-cleaned and labelled Durapore® PVDF filter membrane (0.45 μm,

Millipore, Bedford, MA, USA). Water samples were acidified (0.1% HCl w/v, Traceselect grade, Sigma Aldrich) and stored in pre-cleaned Teflon PFA bottles (Nalgene) at 4 °C until analysis. Each filter was rinsed with ultrapure water and immediately stored at -20 °C until analysis. Daily filtration blanks were also performed.

Dissolved and particulate speciation analysis was carried out as previously described in Monperrus et al. (2005), Rodríguez-González et al. (2005) and Martín-Doimeadios et al. (2003), using gas chromatography-inductively coupled plasma-mass spectrometry (GC-ICP-MS, Thermo Fisher) combined with isotope dilution, obtaining precise and accurate data at low concentration levels present in the samples.

2.4 Export of nutrients

The export of carbon and nutrients (organic and inorganic) was determined using the flow volumes of the respective tributaries and the average low tide nutrient concentrations in the river. River discharge was determined from cross-sections of velocity profiles that were measured nine times per tidal cycle using an Acoustic Doppler Currentmeter Profiler RDI Workhouse 1200 kHz (Vu et al., 2011). The tide is diurnal in this area. All of the samples for phytoplankton diversity and productivity within Haiphong Bay were collected during the neap tide, when the tidal range was a few tens of centimetres as compared to 4 m at spring tide. This sampling method was chosen to minimise the impact of the tidal regime on the spatial distribution of biological parameters.

2.5 Statistical analyses

In order to estimate the similarity between two phytoplankton communities, the Whitaker similarity index (W) was calculated using the following equation.

$$W = 1 - \sum_{i=1}^n \left(\frac{|a_{i1} - a_{i2}|}{2} \right)$$

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where a_1 and a_2 are relative abundance in sample 1 and 2, respectively. Since this index takes into account relative abundances, it provides a better estimate of the similarity between two communities (Hewson and Fuhrman, 2006). Spatial variation of phytoplankton community structure was assessed by Correspondence Analysis (CA).

The extent of the correlation of diversity with environmental factors was assessed by Canonical Correspondence Analysis (CCA) according to the procedure described by Fourçans et al. (2006). CA and CCA were performed with MVSP v3.12d software (Kovach Computing Service, Anglesey Wales). Relative abundances of phytoplankton species were transformed with $\arcsin(x^{0.5})$ according to Legendre and Legendre (1998) to normalize the distribution of the data as it is a condition required before applying multivariate statistical analysis (Dollhopf et al., 2001).

3 Results

3.1 Physics and meteorology

The meteorology and physical conditions of the two sample periods differed considerably (Table 1, Fig. 2). In July, temperatures were higher (28.5–31.1 °C and 18.5–23.1 °C, for July and March, respectively) and river discharge was higher, reflecting the higher precipitation rates observed during the wet season (Table 1). For example, at Station 4, river outflow was $988 \text{ m}^3 \text{ s}^{-1}$ in July as compared to $175 \text{ m}^3 \text{ s}^{-1}$ in the dry season (March) (Vu et al., 2011). As a consequence of this higher discharge, surface salinity was lower and turbidity was an order of magnitude higher during July (Table 1). Reflecting the higher riverine inputs, clear gradients of salinity and nutrients were observed along the estuarine gradient during the wet season. In contrast, during the dry season (March), when river flow was lower, salinity and nutrients were relatively homogenous along the estuary.

The large physical dispersion of the stations meant that a large range of salinities was covered: 0.11 to 27.7 and 11.3 to 31.1 for July and March, respectively (Table 1). In

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general, the highest salinities were observed at Stn. 28 and the lowest were observed in Stns. 4 and 10. However, there was a difference of over 11 salinity units at Stn. 4, the river station between the two seasons (0.11 and 11.3 for the wet and dry seasons, respectively).

3.2 Nutrient measurements

The concentrations of DIN, DIP, DOC, SiO₄ and Chl-*a* were all significantly higher during July than March (t-test, $p < 0.05$). Positive net seaward flows of nutrients and DOC and a general trend of decreasing nutrients with increasing salinity was observed for both sample periods, indicating their freshwater origin. In contrast to the nutrient fluxes, Chl *a* flux was negative, indicating a marine or estuarine, rather than freshwater, source. This was the case for both sample periods, with the net flux almost a factor of 10 higher in July (−173.92) than in March (−22.93) at the confluence station (Table 2).

3.3 Metallic species concentrations (Hg, Butyl-Sn)

Concentrations of tin and mercury species varied between stations and season and fell within the range of concentrations observed in temperate estuaries. In general, concentrations were higher during the wet season for both metals species. At Stn. 26, a mid-channel station (Fig. 1), there was a factor of 10 difference between the concentrations of particulate tributyltin (TBTp) between July and March (0.59 ng L^{−1} as compared to 0.05 ng L^{−1}, Table 3). The seasonal differences between mono- and di-butyl tin concentrations in the particulate phase at each station were generally much less marked, with values being in the same range for both seasons at any given station. This was not the case for the dissolved fraction, where concentrations of up to a factor of ten higher of di-butyl tin (DBTd) were observed during the dry season as compared to the wet season. For example, at Stn. 26, concentrations of DBTd were 1.37 ng L^{−1} as compared to 10.05 ng L^{−1} for July and March, respectively (Table 3).

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The concentrations of mercury species also varied between station and between season. There was a 3-fold increase in dissolved and particulate inorganic mercury concentrations in the wet season as compared to the dry season (Table 3). Conversely, methyl-mercury (MeHg) concentrations in the particulate phase were higher during the dry season. During the dry season the relationship between dissolved inorganic mercury and salinity was less clear, with relatively high values found in the mid salinity regions and lower concentrations observed at lower and higher salinity stations. The observed distribution suggests that Hg and organotin species were directly transported seaward during the wet season, while, during the dry season, they undergo various biogeochemical transformations within the estuary (Navarro et al., 2011).

3.4 Phytoplankton abundance and diversity

Differences in phytoplankton biomass and diversity, as determined by microscopy were also evident between the two sampling periods (Table 1, Fig. 3), with Chl-*a* concentrations being higher during the wet season. In July, diatoms dominated at all stations, the only exceptions were Stns. 29 and 23 sampled on the 9 and 10 July, respectively. In all the other cases, the diatom group represented between 43 to 99% of the phytoplankton community, with the dinoflagellates, chlorophytes and cyanobacteria making up the bulk of the rest of the community. In Stns. 29 and 23, dinoflagellates dominated the phytoplankton community (76 and 54%, respectively), with the diatom group being the second most dominant. In general, very few euglenophytes and silico-flagellates were observed across the estuary. Little variation in terms of group dominance was observed along the salinity gradient during this season. The only exceptions were at the lowest salinity sites (<7.6 salinity), where relatively high abundances of chlorophytes and in the case of Stn. 15, dinoflagellates were observed. In contrast to the microphytoplankton, the abundance of cyanobacteria cells (<3 μm) as determined by flow cytometry, varied over three orders of magnitude (Table 1). Abundances were particularly low at the riverine stations (0.14×10^3 cell mL⁻¹ and 0.16×10^3 cell mL⁻¹) with the highest abundances observed at Stn. 28 and 30 (322×10^3 cell mL⁻¹ and 371×10^3 cell mL⁻¹,

respectively). Pico- and nanophytoplankton abundances varied by less than one order of magnitude along the salinity gradient, with the highest abundances occurring in the offshore, higher salinity stations (Table 1).

During the dry season, and similar to the situation in July, diatoms dominated the community with abundances of over 65–99% of total. The only exceptions were Stns. 30, 15 and 28, when the diatoms and the dinoflagellates represented almost equal parts of the community. During the dry season, very few phytoplankton cells from the other groups were found and there was little clear evidence of a distribution varying along the salinity gradient. In sharp contrast to the situation during the wet season, there was little variability in cyanobacterial abundance along the salinity gradient. There was at most a factor of four difference between Stn 4 and Stn. 28 (8.2×10^3 cell mL⁻¹ and 56.8×10^3 cell mL⁻¹, respectively, Table 1). This relative stability was also reflected in the pico- and nanophytoplankton abundances, despite a factor of 5 increase in picoplankton abundance relative to that of the wet season.

Despite the general dominance of the diatom group between the two seasons, the actual phytoplankton species present differed considerably between the two sampling periods (Fig. 3). Indeed, the percentage similarities in diversity between July and March were very low and never exceeded 2% (Fig. 3; Table 1 Supplement). For example, the diatoms *Chaetoceros subtilis*, *Skeletonema costatum*, *Melosira granulata* and *M. granulata v. angustissima* dominated in July. At the two stations (23 and 29) where dinoflagellates dominated the community, *Protoperidinium c.f. thorianum* was the dominant species in terms of abundance. In contrast, during the dry season, *Thalassiosira spp.* dominated the phytoplankton assemblage. At Stns. 15 and 30, *Thalassiosira spp.* remained the dominant diatom, however the community assemblage was also made up of the dinoflagellates *Goniodoma polyedra*, *Certium trichoceros*, and *Protoperidium spp* at Stn. 30, and of *Dinophysis caudata* and *Prorocentrum micans* at Stn. 15. At Stn. 23, the dominant dinoflagellates were *Ceratium spp.* with the diatom *Pseudonitzschia spp.* making up the rest of the community.

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3.5 Primary and bacterial production

The rates of depth integrated dissolved and particulate primary production (DPP and PPP, respectively) and bacterial total abundance and production varied between season and showed some pronounced differences between stations (Table 1). Bacterial abundance and activity were higher during the wet season, with the highest abundances found at the offshore Stn. 28 and 30 concurrent with the highest Chl-*a* concentrations. In general, BP tended to increase with increasing turbidity however the relationship was not significant ($p > 0.05$). Indeed, the highest BP were found at the two “endmembers” of the estuary, Stn. 28 and Stn. 4 during this season. The ratio between BP and DPP, an indicator of the degree of coupling between the autotrophic and heterotrophic processes, varied considerably and exceeded 1 in some stations. The highest values of BP:DPP (30.9 and 12.07) were observed at Stn. 4 and 10, respectively. These two stations are characterized by high turbidities, low salinities and relatively elevated DOC concentrations. At the other stations, the ratio between BP and DPP was lower, with most stations displaying BP:DPP ratios lower than 1. BP was not significantly correlated with DPP ($p > 0.05$; Fig. 4).

BA was lower and little variation was observed along the salinity gradient (Table 1) during March (dry season). Although BP exhibited the same general trend of increasing BP with increasing turbidity, the rates of bacterial production were lower than during the wet season. However, and in contrast to the wet season, during March BP was correlated with DPP ($r^2 = 0.53$, $p < 0.05$; Fig. 4). Interestingly, and in contrast to the situation observed in July, the ratio between BP and DPP varied little over the salinity gradient with almost all values being lower than 1. The only exceptions were in higher salinity stations with ratios of 8.3 and 3.9 observed for Stns. 18 and 26, respectively.

In order to determine what factors were potentially controlling phytoplankton community composition in the estuary, we applied a canonical correspondence analysis (CCA) to the data for both sampling periods (Figs. 5 and 6 for July 2008 and March 2009, respectively). The variance explained by the two first axes was 61.5% and 54.2%

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for July 2008 and March 2009, respectively. In each CCA, nutrients (inorganic and organic) were excluded due to the strong correlation observed with salinity because of the dilution effect (Troussellier et al., 2002). During the wet season, the stations were separated into three groups (Fig. 5) reflecting the differences in community composition (Fig. 3). The first group, comprising of the lower salinity stations (4, 10, and 15), was grouped with the dissolved and particulate methylmercury species (MeHg_d and p), and with particulate inorganic mercury (Hg_p) although the real influence of the latter is difficult to assess since its inflation factor (IF) equals 0 (see Table 4). In contrast, at the other end of the salinity gradient, the stations 23 and 29 were related to the dissolved organic mercury (Hg_d) and mono-butyl tin (MBT_d). The third group, the mid salinity stations (Stns. 18, 26, 28 and 30), were all closely grouped, reflecting their similar phytoplankton community compositions (Fig. 3). These four stations were located along the first axis, in between DBT_d and DOC. The inflation factors (Table 4) from the CCA show that during this season both salinity and SPM can be considered as structural factors; however their large IF values indicate redundancy with other environmental variables. In addition, co-linearity between some of the butyltin group of species was also observed during the wet season.

During the dry season the distribution of stations in the CCA was more widespread, with the stations grouped in the left quadrants. The only exceptions were the two high salinity stations (Stn. 28, 30). The lower salinity stations, except for Stn. 4, were located in the same quadrant together with the stations from the same transect (23 and 15) as well as those from Cat Ba Island (18 and 26). The community diversity of this group was positively structured by MeHg_d and, to a less extent, by DOC. Particulate tri-, di-, and mono-butyl species also exerted a positive structural effect on phytoplankton community structure at Stns. 4 and 29. Interestingly, both marine stations (28 and 30) were clearly separated and negatively structured by MeHg_d in the case of station 28 whereas a positive influence of salinity and DBT_d was observed for Stn. 30, although the exact role of salinity is difficult to quantify due the colinearity observed (IF = 0, Table 4).

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Comparing the inflation factors for both periods (Table 4), the role of salinity in determining the distributions along the estuary during the wet season is clear, as is that of SPM. However, the large values observed indicate that these two factors are redundant when other variables taken into account in the analysis. During the dry season, despite the importance of riverine flow as a source of organic and inorganic matter in this estuary, the exact role of SPM and salinity could not be assessed due to the co-linearity observed. The inflation factors also underline the shifting importance of the different metal species along the estuarine gradient. Interestingly, DOC concentration, a variable strongly influenced by riverine inputs had a relatively constant IF between the two sampling periods.

4 Discussion

The factors controlling biological distributions in aquatic systems are myriad and it is probable that no single factor is responsible. This is particularly important in estuarine and coastal systems where water of terrestrial origin mixes with marine water, leading to complex gradients of inorganic and organic components. Despite this evident complexity, estuaries are generally considered as being linear systems where organic and inorganic carbon and nutrients from the freshwater and marine endmembers mix in a defined manner, such as is found in the classical mixing diagrams (Officer, 1979; Officer and Lynch, 1981). This two point mixing model is appealing in its simplicity and has permitted the estimation of the role of physico-chemistry and biology in controlling the distributions of various parameters along the salinity gradient by determining if the parameter examined exhibited conservative and non-conservative mixing. This has been particularly useful for understanding organic carbon production and removal, phytoplankton biomass and diversity as well as other biological parameters (e.g., Rochelle-Newall and Fisher, 2002; Fisher et al., 1998). However, the use of mixing diagrams is fundamentally based on the assumption of the presence of two, easily defined endmembers: one riverine and one marine. Yet, in many estuaries, particularly those

that are found in deltaic regions, it is often difficult to accurately determine these two endmembers. This therefore presents a problem if we wish to understand how and why biological parameters vary over spatial distances, particularly in systems, such as this one, that are characterized by complex freshwater inputs and hydrology during the wet and the dry seasons (Vu et al., 2011). Using multivariate analyses (canonical correspondence analysis) we tried to unravel some of the complex factors that control biological processes in a subtropical, estuarine ecosystem.

4.1 Phytoplankton diversity and activity

We observed large differences in phytoplankton distributions between wet and dry seasons (Fig. 3) as determined by microscopy or by flow cytometry. During the wet season large shifts in phytoplankton community structure between Stns. 4, 10 and 15 and Stn. 23 were observed, despite their being axially aligned. The large difference in salinity between the first two stations (<1 salinity units) and the later station (25 salinity units) probably explains the differences between the stations as salinity was determined to be one of the important factors controlling phytoplankton diversity during this season. Salinity is a well known controlling factor of phytoplankton activity and diversity in estuarine systems (e.g., Fisher et al., 1988; Quinlan and Philips, 2007). It is therefore not surprising that osmotic stress combined with dilution of nutrient rich riverine water by higher salinity, more oligotrophic marine water played some role in determining community structure. Indeed, as recently noted by Bettarel et al. (2011) in the same estuary, viral diversity and life strategy also appear to vary along the salinity gradient following the distributions of their potential hosts.

Heavy metals are also known to have a negative impact on phytoplankton diversity (e.g., Paulsson et al., 2000; Singh and Rai, 1991). Tri-butyl tin can reduce the fluorescence yield of phytoplankton photosynthesis, probably through its action on the thylakoid membranes of the photosynthetic apparatus (Sargian et al., 2005; Yoo et al., 2007) and recent evidence from shallow freshwater systems has highlighted the role of TBT in inducing shifts in phytoplankton community structure (Sayer et al.,

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in the San Francisco Bay Estuary, a site considered to be contaminated by mercury (Conaway et al., 2003). The relationships observed between the different mercury species and phytoplankton diversity may well be due more to the uptake capacity of the phytoplankton species and ambient DOC concentrations, rather than any negative impact of mercury on phytoplankton communities. Methyl mercury uptake in phytoplankton is known to vary with cell size, cell number and with DOC concentration (Pickhardt and Fisher, 2007). During the wet season, phytoplankton community diversity at the lower salinity stations where mercury concentrations were highest differed greatly from that of the other stations (Fig. 3). The presence of small cryptophytes and chlorophytes at these stations (Stns. 4, 10 and 15), with higher surface to volume ratios than that of the diatom cells dominating at the other, more offshore stations seems to support this hypothesis. Indeed, these three stations were tightly related to particulate inorganic mercury and methyl mercury concentrations, potentially reflecting the “uptake”, either through biotic or abiotic processes, of Hg by these phytoplankton species. The community composition of Stns. 15, 23 and 26 were also related. However, whether these organo-metal species (Hg and Sn) play some role in structuring phytoplankton diversity at these stations or whether the organo-metals are directly influenced through their partition and transformation by the plankton community structure is difficult to determine with this dataset.

4.2 Phytoplankton-bacterioplankton coupling

Phytoplankton and bacterioplankton production varied between the two seasons and along the transects. At the lower salinity stations, BP greatly exceeded DPP during the wet season, indicating that the DOC fueling BP originated from other sources than the immediately adjacent phytoplankton production. Indeed, the relatively high particulate associated bacterial production rates (data not shown) point towards a particulate organic carbon source. High particle attached bacterial production is a common feature of estuaries, particularly those with high sediment loads (e.g., Crump and Baross, 1996; Crump et al., 1998) such as was observed in this work during the wet season. In

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contrast, at the higher salinity, offshore stations during the wet season and at almost all of the stations during the dry season, the BP:DPP ratio was much lower (Fig. 4). This means that more DOC was being produced during primary production than was required for bacterial biomass production, potentially leading to an accumulation of DOC in the water column. This may well explain the relatively high concentrations of DOC observed in the higher salinity stations, further underlining the difficulty of using mixing diagrams to study distributions in this system.

There are many potential explanations as to why we observed accumulations of DOC in these sites. Low bioavailability of the freshly produced DOC (e.g., Renaud et al., 2005) or limitation of bacterial production by another parameter, such as nutrient limitation (Thingstad et al., 1997) or heavy metal contamination (Fisher and Reinfelder, 1995) are a few of them. Given the relatively high nutrient concentrations observed, it is unlikely that inorganic nutrients were limiting bacterial production at this site. Heavy metals are known to induce shifts in the BP:TPP ratio (e.g., Rochelle-Newall et al., 2008a), however, it is unclear whether or not they played a role in altering the bioavailability of DOM to the bacterial communities present. It has already been shown that the chemical composition of the DOC released by different phytoplankton differs as a function of the species or even strains present (Biersmith and Benner, 1998; Ozturk and Aslim, 2010) and that the DOM released during photosynthesis can vary in bioavailability to the bacterial communities present as a function of the growth stage (Renaud et al., 2005). It is therefore probable that the chemical quality of the DOM released during photosynthesis by the communities present at the outer most stations was different from that released during photosynthesis at the other stations. Indeed, the community composition at the two stations with the lowest BP:DPP rates during the wet season (Stns. 28 and 30) was characterized by very high abundances of cyanobacteria. Cyanobacteria are known to release metabolic products that limit bacterial production (Nausch, 1996; Renaud et al., 2005) and may have contributed towards reducing the bioavailability of the organic matter released during photosynthesis available for bacterial production.

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Of course, overlain with the biological and chemical interactions, physical processes such as wind direction and water circulation also play an important role in determining particle distributions. Stations 29 and 23, sampled on the 9 and 10 July 2008 have very similar community distributions, despite their relative separation and their difference to the other surrounding stations (Fig. 3). During the sampling period, and typical of the south monsoon during the wet season, the wind direction was from the south (180–200°) potentially confining the phytoplankton communities to the bay areas instead of their being dispersed into the higher salinity, off-shore waters as might be expected through water mixing. Furthermore, recent work on the water circulation during the tidal cycle in this area (Dinh and Ha, 2008) has shown that water out-flow during the tidal cycle shifts between channels. During low tide, water exits from the Bach Dang River (Stns. 4, 10, 15, 23) and enters via the channel adjacent to Cat Ba (Stns. 18, 26, 29). This cycle is reversed during high tide with the corresponding lateral transport of the water masses between these two channels. In this estuary, the prevailing wind direction combined with tidal influence (here reduced because sampling was performed during neap tides) may also explain the distribution of phytoplankton at these two stations.

5 Conclusions

Here we present some of the factors that potentially control phytoplankton distribution and phytoplankton-bacterioplankton coupling in a subtropical estuary. It is clear that in complex estuarine systems such as the Bach Dang Estuary, simple, endmember calculations at best miss a large part of the processes and at worst, ignore potentially important factors. It is already known that heavy metals can play an important role in determining phytoplankton community structure in coastal ecosystems. Here we further highlight the importance of some ecotoxicologically targeted organometallic species (MeHg, Butyl-Sn), their potential role in a complex, tropical estuarine system and suggest that their impact may well extend to the carbon cycle by influencing the transfer of carbon from the autotrophic to the heterotrophic compartments. Clearly,

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given the almost ubiquitous presence of heavy metals in the industrialised coastal ecosystems, the impact of these ecotoxicologically important organometallic species on coastal carbon cycling needs to be taken into account more frequently.

Supplementary material related to this article is available online at:

<http://www.biogeosciences-discuss.net/8/487/2011/bgd-8-487-2011-supplement.pdf>

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Table 1. Primary production and nutrient concentrations for both sampling periods. Salinity (Sal), Turbidity (Turb), and the concentrations of inorganic nutrients (dissolved inorganic nitrogen, DIN (NO_x, NH₄)), dissolved inorganic phosphorus (DIP), dissolved organic carbon (DOC) and chlorophyll-*a* (Chl-*a*) and dissolved (DPP) and depth integrated particulate primary production (PPP); bacterial abundance (BA) and production (BP), picophytoplankton (pico), nanoplankton (nano) and cyanobacterial (cyano) abundance are also noted.

Date	Stat.	Depth (m)	Sal.	Turb (FTU)	DIP (μM)	SiO ₄ (μM)	DOC (μM)	DIN (μM)	Chl- <i>a</i> (μg L ⁻¹)	BP (mmol C m ² h ⁻¹)	DPP (mmol C m ² h ⁻¹)	PPP (mmol C m ² h ⁻¹)	BA (×10 ⁶ ml ⁻¹)	Pico (×10 ³ ml ⁻¹)	Cyano (×10 ³ ml ⁻¹)	Nano (×10 ³ ml ⁻¹)
09 Jul 2008	18	12.0	10.03	30	1.4	104.0	144	14.1	3.06	1.32	0.17	0.82	4.60	7.01	82.21	0.90
09 Jul 2008	26	6.5	20.01	15	0.8	76.7	131	8.4	3.28	1.00	0.20	1.58	5.40	11.03	88.47	1.88
09 Jul 2008	29	8.8	19.95	16	0.6	50.4	125	6.9	4.04	0.55	0.91	2.89	3.37	6.37	105.19	0.74
09 Jul 2008	30	14.8	20.75	7	0.8	58.8	122	5.7	2.87	0.63	1.53	3.41	3.54	1.11	38.55	1.10
09 Jul 2008	28	9.3	23.92	2	0.2	55.0	169	5.3	1.59	1.35	1.77	1.83	6.70	5.92	322.03	2.17
10 Jul 2008	4	7.5	0.11	209	2.0	140.7	134	14.7	2.71	2.92	0.09	0.70	2.31	9.25	0.16	1.34
10 Jul 2008	10	5.0	0.78	168	2.2	139.5	133	15.0	2.60	1.57	0.13	0.17	2.36	8.03	0.14	1.77
10 Jul 2008	15	4.5	7.53	75	2.7	115.5	130	15.7	2.52	0.53	0.27	0.11	2.54	4.97	6.56	1.77
10 Jul 2008	23	5.8	25.40	19	1.2	39.2	99	3.9	2.28	0.44	3.33	2.16	3.90	13.25	50.84	1.56
10 Jul 2008	28	7.5	19.93	11	0.3	63.1	135	8.5	15.52	2.16	4.18	6.00	5.07	13.30	225.75	3.73
11 Jul 2008	30	8.5	9.75	13	1.9	104.2	140	8.1	5.49	1.30	17.55	3.81	17.26	3.51	371.66	0.95
11 Jul 2008	28	4.3	27.70	5	0.1	36.9	135	5.4	22.88	1.11	13.43	12.12	14.55	2.18	70.92	0.46
12 Mar 2009	18	12	27.1	24.3	1.23	62.69	117	21.28	2.616	0.40	0.05	0.12	2.61	52.3	38.7	1.5
12 Mar 2009	26	9	27.6	20.2	1.15	51.23	122	17.38	2.170	0.20	0.05	0.19	2.17	28.3	39.4	1.2
12 Mar 2009	29	8	29.3	5.8	0.55	48.38	109	16.02	1.667	0.16	0.22	0.59	1.94	25.0	33.7	1.3
12 Mar 2009	30	14	31.1	2.3	0.58	37.77	97	14.65	0.795	0.14	2.14	0.44	1.72	11.0	18.7	0.6
12 Mar 2009	28	9	29.4	7.6	0.94	42.65	93	16.39	1.571	0.17	0.14	0.05	2.90	30.7	56.8	1.7
15 Mar 2009	4	9	11.3	31.8	0.89	119.94	120	20.64	2.037	0.78	3.15	0.58	2.33	25.4	8.2	1.3
15 Mar 2009	10	15	18.8	16.1	0.70	95.27	115	20.13	2.776	0.75	4.68	2.06	3.19	22.1	17.2	1.5
15 Mar 2009	15	10	24.8	21.8	0.53	73.83	105	15.86	3.353	0.34	1.74	1.42	2.90	19.0	31.3	1.3
15 Mar 2009	23	5	30.3	27.5	0.38	48.56	111	11.08	2.110	0.13	1.06	1.58	2.88	29.0	35.3	1.2
15 Mar 2009	28	3	30.6	5.3	0.36	41.15	110	11.29	1.397	0.14	0.96	3.00	1.75	29.8	25.8	0.5

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Table 2. Fluxes of dissolved organic carbon (DOC), inorganic nutrients in tons of C, N, Si and P per day and chlorophyll-*a* in kg per day at the Confluence station. This station is located at the confluence of the Cam and Bach Dang Rivers, 300 m upstream of Stn. 4 (see Fig. 1). Positive values mean that the net flow is in the seaward direction and negative values indicate a net flow in the landward direction. Total flow is calculated from the sum of the river and marine flows.

Sample period	Flux	DOC (tons C/day)	Chl- <i>a</i> (kg/day)	DIN (t N/day)	SiO ₃ (t Si/day)	PO ₄ (t P/day)
Jul 2008	Total	199.42	-173.92	14.06	345.16	9.74
	Incoming (marine)	-175.53	-306.10	-18.15	-317.13	-10.98
	Outgoing (river)	374.95	132.18	32.21	662.30	20.72
Mar 2009	Total	23.85	-22.93	9.68	89.23	0.57
	Incoming (marine)	-120.79	-208.13	-31.58	-335.23	-2.63
	Outgoing (river)	144.64	185.21	41.25	424.45	3.20

Table 3. Metal concentrations measured at each station during both sample periods. TBT, DBT, MBT: tri, di and mono-butyl tin in the particulate (p) and dissolved (d) fractions. MeHg: methyl mercury in the particulate (p) and dissolved fractions (d); IHgP : inorganic mercury in the particulate (p) and dissolved (d) fractions.

Date	Station	TBTp ng L ⁻¹	DBTp ng L ⁻¹	MBTp ng L ⁻¹	TBTd ng L ⁻¹	DBTd ng L ⁻¹	MBTd ng L ⁻¹	MeHg _p ng L ⁻¹	IHg _p ng L ⁻¹	MeHg _d ng L ⁻¹	IHg _d ng L ⁻¹
09 Jul 2008	18				0.77	1.29	0.16	0.01	1.07	0.01	1.22
09 Jul 2008	26	0.59	0.94	0.42	1.64	1.37	0.19	0.00	0.62	0.01	0.85
09 Jul 2008	29	0.54	0.85	0.36	1.38	1.69	0.37	0.01	0.65	0.01	1.56
09 Jul 2008	30	0.73	0.78	0.28	2.26	1.52	0.24	0.00	0.75	0.02	0.55
09 Jul 2008	28	0.51	0.81	0.33	1.16	1.52	0.22	0.01	0.65	0.01	0.54
10 Jul 2008	4	0.98	2.23	0.92	1.39	1.11	0.21	0.02	4.45	0.02	0.39
10 Jul 2008	10	0.84	2.08	0.92	1.27	0.88	0.14	0.03	5.67	0.02	0.59
10 Jul 2008	15	0.78	1.77	0.71	1.68	1.29	0.20	0.07	4.45	0.02	1.26
10 Jul 2008	23	1.11	1.97	0.86	0.97	1.05	0.17	0.01	1.52	0.01	0.40
10 Jul 2008	28	0.95	1.08	0.46	1.54	0.99	0.16	0.01	0.83	0.01	0.18
11 Jul 2008	30	0.44	0.60	0.26	1.71	1.69	0.22	0.00	0.61	0.01	0.99
11 Jul 2008	28	0.54	0.68	0.29	0.83	1.70	0.20	0.01	0.74	0.01	0.49
12 Mar 2009	18	0.09	1.72	0.58	0.34	9.02	0.25	0.02	0.96	0.02	0.38
12 Mar 2009	26	0.05	1.24	0.44	0.21	10.05	0.19	0.03	0.80	0.01	0.29
12 Mar 2009	29	0.03	0.87	0.93	0.22	9.85	0.36	0.01	0.38	0.02	0.31
12 Mar 2009	30	0.03	0.54	0.30	0.13	9.03	0.42	0.01	0.22	0.01	0.25
12 Mar 2009	28	0.05	0.77	0.40	0.32	10.76	0.49	0.01	0.46	0.01	0.27
15 Mar 2009	4	0.05	4.67	1.33	1.00	1.07	0.08	0.04	1.17	0.01	0.28
15 Mar 2009	10	0.05	1.74	0.49	0.91	1.35	0.11	0.02	0.71	0.01	0.26
15 Mar 2009	15	0.03	1.83	0.51	0.32	9.49	0.27	0.02	0.64	0.02	0.23
15 Mar 2009	23	0.02	1.52	0.31	0.21	10.23	0.24	0.01	0.68	0.02	0.44
15 Mar 2009	28	0.03	0.50	0.18	0.18	9.09	0.23	0.00	0.26	0.01	0.28

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Table 4. Inflation factors of CCA for both months. A value of 0 indicates a co-linearity between factors.

Variable	Inflation factor Jul	Inflation factor Mar
Salinity	28.588	0.000
SPM	38.223	0.000
DOC	2.549	2.707
TBTp	0.000	1.515
DBTp	0.000	5.579
MBTp	9.525	3.111
TBTd	2.729	19.983
DBTd	0.000	14.463
MBTd	6.003	0.000
MeHgp	4.962	0.000
Hgp	0.000	0.000
MeHgd	0.000	3.995
Hgd	12.544	2.179

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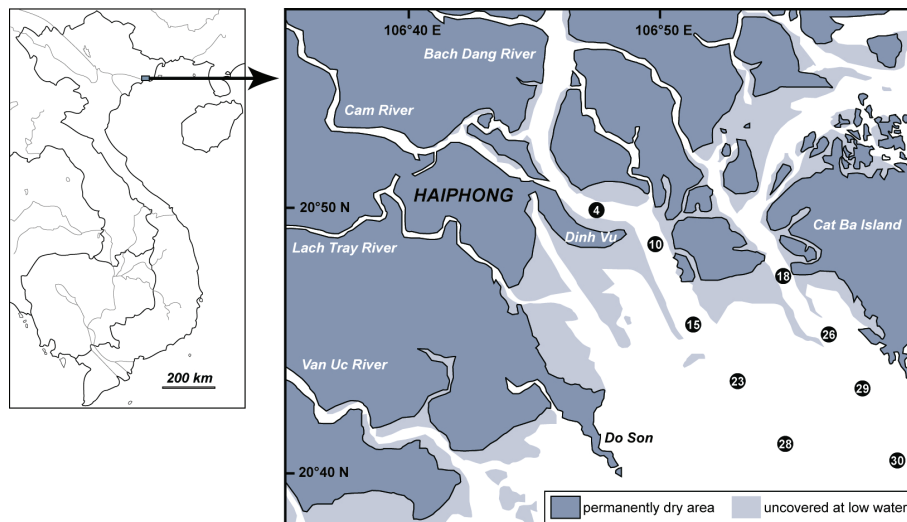


Fig. 1. Map of the sample stations measured during each cruise.

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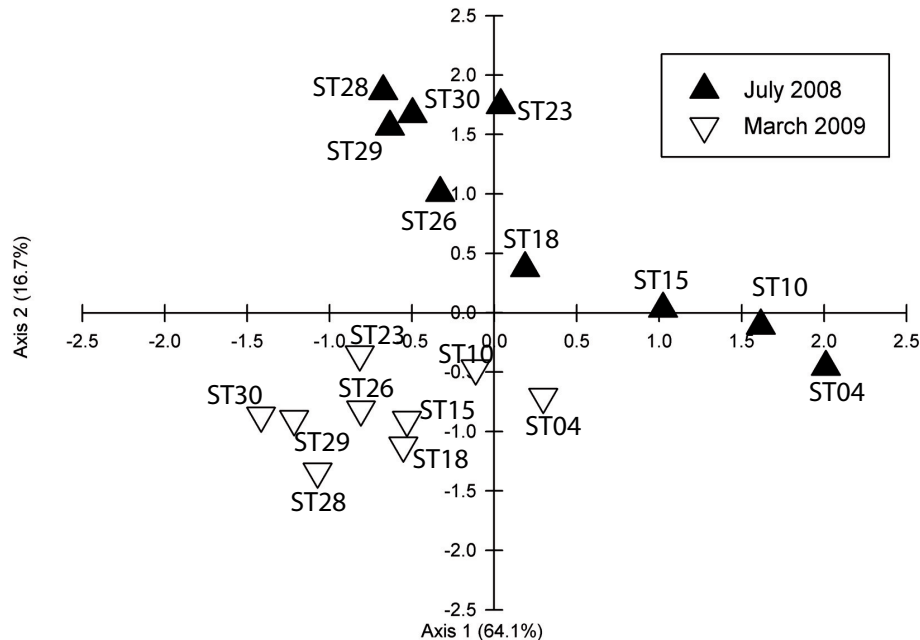


Fig. 2. Correspondence analysis (CA) of the environmental variables for each station and for July (wet season) and March (dry season). Black triangles (July) and white triangles (March).

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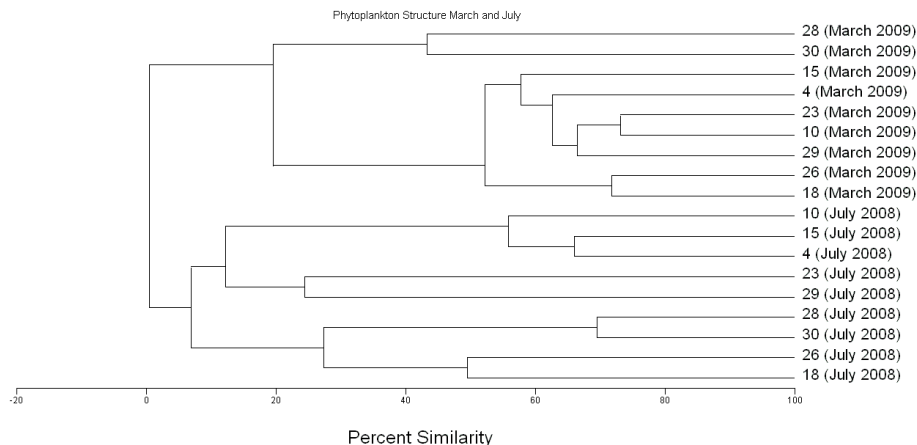


Fig. 3. Dendrogram of phytoplankton diversity for July (wet season) and March (dry season).

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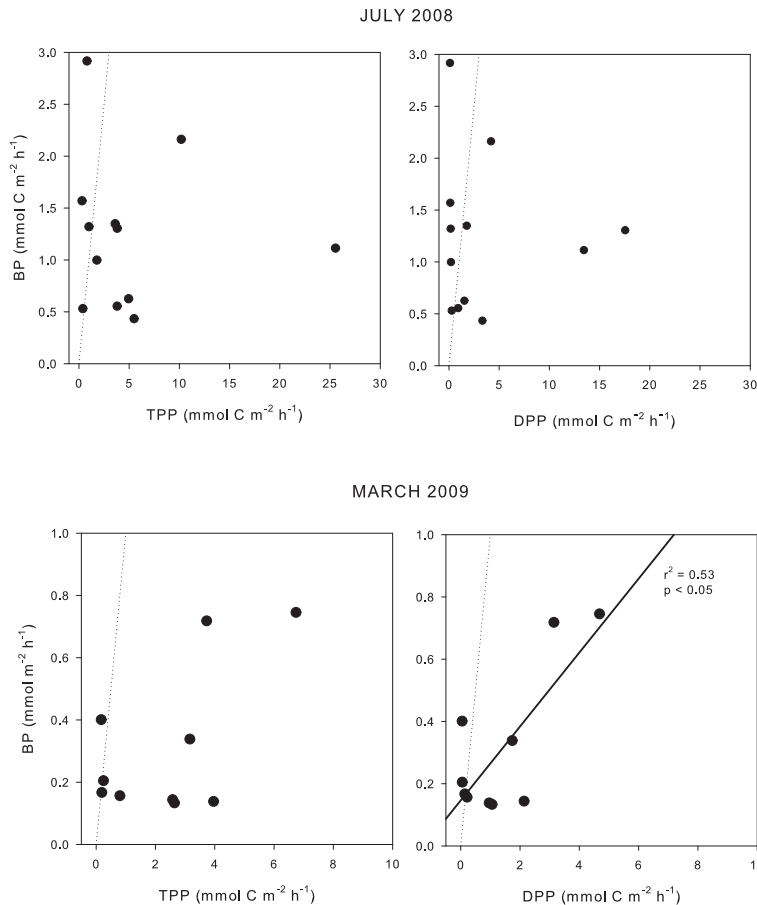


Fig. 4. Relationship between depth-integrated dissolved (DPP) and total primary production (TPP, dissolved+particulate primary production) and depth integrated bacterial production for both seasons. The dotted line represents the 1:1 line.

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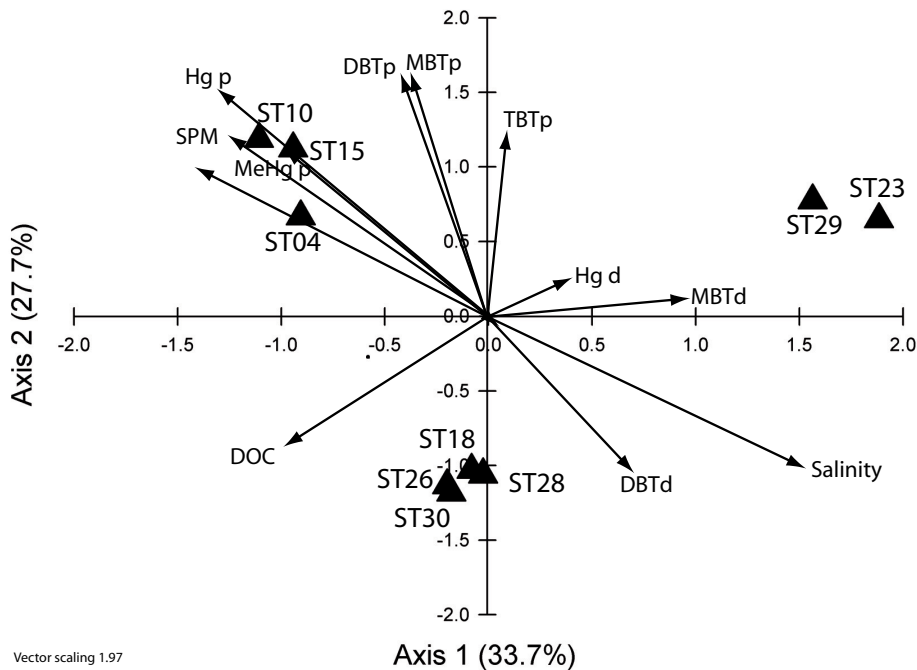
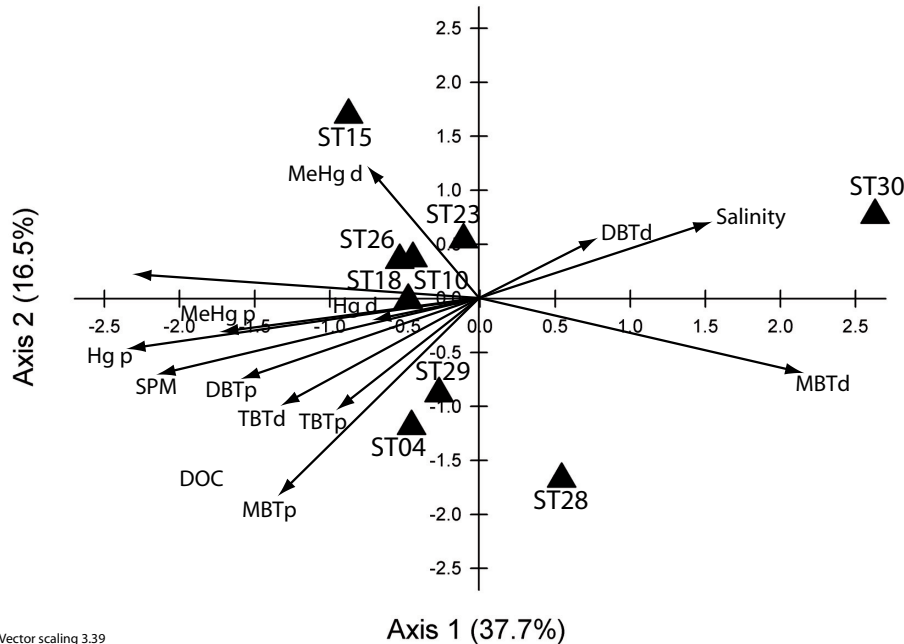


Fig. 5. Canonical correspondence analysis (CCA) of phytoplankton distribution and environmental factors for July. The inflation factors for the analysis are given in Table 4.

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Vector scaling 3.39

Fig. 6. CCA of phytoplankton distribution and environmental factors for March. The inflation factors for the analysis are given in Table 4.

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