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Phytoplankton distribution and productivity in a highly turbid, tropical coastal system (Bach Dang Estuary, Vietnam)

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ABSTRACT

Phytoplankton diversity, primary and bacterial production, nutrients and metallic contaminants were measured during the wet season (July) and dry season (March) in the Bach Dang Estuary, a sub-estuary of the Red River system, Northern Vietnam. Using canonical correspondence analysis we show that phytoplankton community structure is potentially influenced by both organometallic species (Hg and Sn) and inorganic metal (Hg) concentrations. During March, dissolved methylmercury and inorganic mercury were important factors for determining phytoplankton community composition at most of the stations. In contrast, during July, low salinity phytoplankton community composition was associated with particulate methylmercury concentrations, whereas phytoplankton community composition in the higher salinity stations was more related to dissolved inorganic mercury and dissolved mono and tributyltin concentrations. These results highlight the importance of taking into account factors other than light and nutrients, such as eco-toxic heavy metals, in understanding phytoplankton diversity and activity in estuarine ecosystems.

1. Introduction

Determining the factors that control diversity and function 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 human activities on aquatic biodiversity and function cannot 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, such as organometallic species, from industrial and urban activities. All of which can exert a non-negligible impact on the structure and function of planktonic communities. Differences in phytoplankton and bacterioplankton salinity and nutrient tolerances can induce marked shifts in community diversity along estuarine salinity gradients (del Giorgio and Bouvier, 2002; Lemaire et al., 2002; Muylaert et al., 2009), in resource utilization (Thottathil et al., 2008) and can alter water quality through its control on nutrient export or stockage (Cardinale, 2011).

Shifting community diversity also impacts biogeochemical processes and carbon fluxes. Variations in primary production, respiration and pCO_2 flux along estuarine salinity gradients are related to shifting community diversity, nutrient and organic carbon availability and turbidity (Fisher et al., 1988, 1999; Smith and Kemp, 2001). It is therefore probable that estuarine metabolic balance is intimately linked to that of biological diversity (Borges et al., 2006; Smith and Kemp, 2001). These factors combined DOM concentration and bioavailability (Raymond and Bauer, 2000; Rochelle-Newall et al., 2007) all underline the importance of understanding the factors that control community composition in estuarine and coastal waters.

Although many studies such as those cited above have examined the links between the factors influencing phytoplankton

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diversity and community composition 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, nor has the role of organometallic contaminants been examined. Nutrient concentration and availability are obvious factors controlling phytoplankton diversity and production (Ferguson et al., 2004; Jacquet et al., 2006), particularly in estuaries, and other factors such as heavy metal contamination are also known to be important in sensitive coastal ecosystems (see review of Peters et al., 1997). The high toxicity of mercury and methylmercury 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. Downs et al., 1998; Duarte et al., 2007; Ullrich et al., 2001). However, few studies have examined the impact of mercury on phytoplankton community structure and production in tropical estuarine systems. Other metals, such as organotin compounds (tributyltin and its derivatives) can also reach high concentrations in coastal systems, particularly around ports (Nhan et al., 2005; Oliveira and Santelli, 2010). Many of the studies on the impact of these compounds on aquatic ecosystems have focused on invertebrates and some have pointed out the negative impact of TBT (tributyltin) on phytoplankton populations in temperate systems (Petersen and Gustavson, 2000; Sargian et al., 2005; Sayer et al., 2006). 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 organotin on the lower levels of the food web of tropical, eutrophic coastal ecosystems has largely been ignored despite the ecological and biogeochemical importance of these ecosystems in terms of coastal carbon fluxes (Borges, 2005).

Here we present an investigation into some of the factors potentially controlling phytoplankton community composition and activity during two seasons in a turbid, tropical estuarine system (Bach Dang River Estuary, North Vietnam). Although many studies have examined the impact of various parameters on phytoplankton diversity (nutrients, light, ecotoxic substances), most work, particularly those involving ecotoxicology have relied upon experimental based studies. Here, we apply multivariate statistical analysis to field data to tease apart the factors that are potentially influencing phytoplankton community diversity in a complex, multi-source, tropical estuarine system. The objective of this work was therefore to identify the factors controlling phytoplankton diversity and to determine if these shifts then manifest in an alteration in the coupling observed between phytoplankton primary production and bacterial secondary production.

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 km^2 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 estuary 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 tributyltin) and methylmercury 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. All samples, unless otherwise noted, were collected from the surface (1 m depth) with a Niskin sampler.

2.1. Nutrients and dissolved organic carbon

Dissolved inorganic nitrogen (DIN = ammonium + nitrate + nitrite) was measured by the indophenol method of Eaton et al.

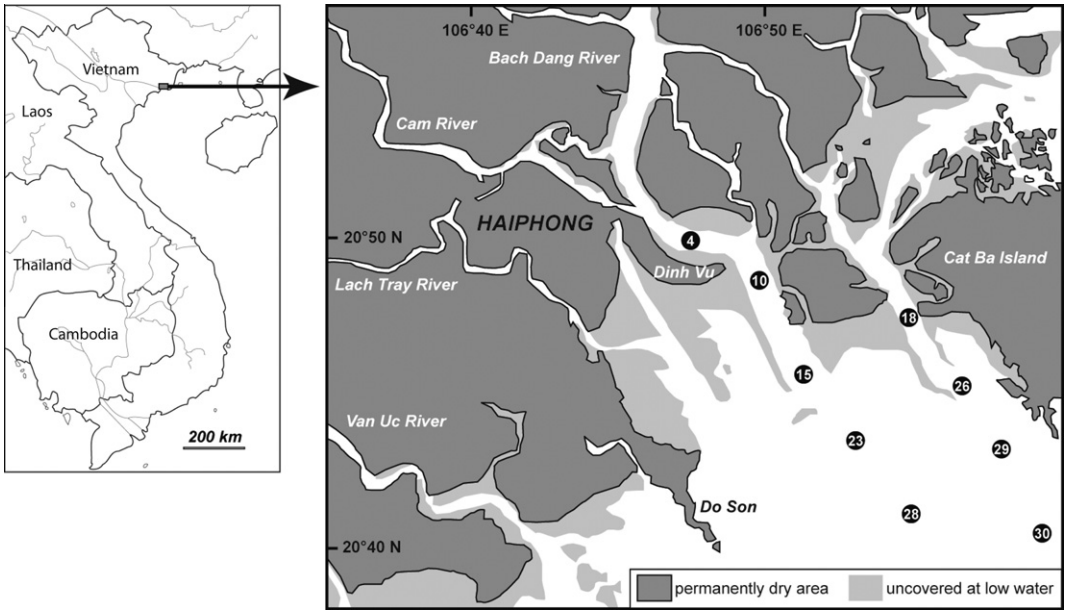

Fig. 1. Map of the sample stations measured during each cruise.

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 (NOx, NH_4), 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	Stn.	Depth (m)	Sal.	K_d	Turb (FTU)	DIP (μM)	SiO_4 (μM)	DOC (μM)	DIN (μM)	Chl *a* (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 ($\times 10^6$ mL^{-1})	Pico ($\times 10^3$ mL^{-1})	Cyano ($\times 10^3$ mL^{-1})	Nano ($\times 10^3$ mL^{-1})
09/07/08	18	12.0	10.0	1.84	30	1.45	104.0	144	14.1	3.06	1.32	0.17	0.82	4.60	7.01	82.21	0.90
09/07/08	26	9.0	20.0	1.31	15	0.82	76.7	131	8.4	3.28	1.00	0.20	1.58	5.40	11.03	88.47	1.88
09/07/08	29	8.8	20.0	0.44	16	0.56	50.4	125	6.9	4.04	0.55	0.91	2.89	3.37	6.37	105.19	0.74
09/07/08	30	14.8	20.8	0.24	7	0.80	58.8	122	5.7	2.87	0.63	1.53	3.41	3.54	1.11	38.55	1.10
09/07/08	28	9.3	23.9	0.27	2	0.23	55.0	169	5.3	1.59	1.35	1.77	1.83	6.70	5.92	322.03	2.17
10/07/08	4	7.5	0.1	5.01	209	2.02	140.7	134	14.7	2.71	2.92	0.09	0.70	2.31	9.25	0.16	1.34
10/07/08	10	8.0	0.8	5.76	168	2.18	139.5	133	15.0	2.60	1.57	0.13	0.17	2.36	8.03	0.14	1.77
10/07/08	15	4.5	7.5	4.1	75	2.70	115.5	130	15.7	2.52	0.53	0.27	0.11	2.54	4.97	6.56	1.77
10/07/08	23	4.3	25.4	1.57	19	1.21	39.2	99	3.9	2.28	0.44	3.33	2.16	3.90	13.25	50.84	1.56
10/07/08	28	9.0	19.9	1.79	11	0.33	63.1	135	8.5	15.52	2.16	4.18	6.00	5.07	13.30	225.75	3.73
11/07/08	30	15	9.8	1.56	13	1.85	104.2	140	8.1	5.49	1.30	17.55	3.81	17.26	3.51	371.66	0.95
11/07/08	28	10	27.7	1.32	5	0.11	36.9	135	5.4	22.88	1.11	13.43	12.12	14.55	2.18	70.92	0.46
12/03/09	18	12.0	27.1	1.67	24	1.23	62.7	117	21.3	2.62	0.40	0.05	0.12	2.61	52.26	38.65	1.53
12/03/09	26	6.5	27.6	1.53	20	1.15	51.2	122	17.4	2.17	0.20	0.05	0.19	2.17	28.30	39.42	1.17
12/03/09	29	8.8	29.3	0.70	6	0.55	48.1	109	16.0	1.67	0.16	0.22	0.59	1.94	25.01	33.68	1.32
12/03/09	30	14.8	31.1	0.21	2	0.58	37.8	97	14.6	0.80	0.14	2.14	0.44	1.72	11.04	18.73	0.58
12/03/09	28	9.3	29.4	0.76	8	0.94	42.7	93	16.4	1.57	0.17	0.14	0.05	2.90	30.72	56.84	1.74
15/03/09	4	7.5	11.3	2.31	32	0.89	119.9	120	20.6	2.04	0.78	3.15	0.58	2.33	25.40	8.25	1.28
15/03/09	10	8.5	18.8	2.26	16	0.70	95.3	115	20.1	2.78	0.75	4.68	2.06	3.19	22.07	17.16	1.51
15/03/09	15	4.3	24.8	1.45	22	0.53	73.8	105	15.9	3.35	0.34	1.74	1.42	2.90	18.99	31.34	1.33
15/03/09	23	3.8	30.3	2.43	28	0.38	48.6	111	11.1	2.11	0.13	1.06	1.58	2.88	29.04	35.30	1.17
15/03/09	28	9.3	30.6	0.50	5	0.36	41.2	110	11.3	1.40	0.14	0.96	3.00	1.75	29.76	25.81	0.53

(1995) and Raimbault et al. (1990) for ammonium and nitrate and nitrite, respectively after filtration (Whatman GF/F). Phosphates (DIP) and silicates (SiO_4) were measured following the methods of Grasshoff et al. (1983), after filtration (GF/F Whatman for DIP and 0.2 μm Nuclepore membrane for SiO_4). Dissolved organic carbon (DOC) analyses were performed on filtered (Whatman GF/F) 30 mL samples, collected in pre-combusted (450 °C, overnight) glass tubes, sealed with a Teflon lined cap, after preservation with 36 μL 85% phosphoric acid (H_3PO_4). Samples were stored at ambient temperature and in the dark until measurement. DOC concentration was measured on a Shimadzu TOC VCPH analyser, using potassium phthalate calibration standards over the measurement range (0–450 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). Samples for phytoplankton diversity were collected with a 5 L Niskin bottle, following the methods described by Sournia (1978). Upon collection, samples were immediately fixed with Lugol's solution (3 mL L^{-1}) 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 was 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- (2–20 μm) and picophytoplankton (<2 μm) cells were detected and counted as described previously (Campbell et al., 1994; Crosbie et al., 2003; Troussellier et al., 1993) 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 measured by 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 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 analysed.

Primary production (dissolved primary production, DPP and particulate primary production, PPP) was measured following Rochelle-Newall et al. (2008b). Briefly, 39 mL water samples were inoculated with 1.2 MBq of $NaH^{14}CO_3$ (Perkin–Elmer) and incubated in a semi-continuous flowing seawater bath under neutral density screening (100%, 50%, 25%, 12.5%, 6%, 0% 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 ^{14}C incorporated into the particulate phase (PPP) was calculated using an inorganic carbon concentration of 25700 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, the production rate of a sample was considered to be significant when the scintillation count of the sample was at least 3 times that of the blank. Total primary production (TPP) represents the sum of DPP and PPP. Depth integrated primary production was calculated using the primary production measurements obtained in the simulated *in situ* incubations using light screens (100%, 50%, 25%, 12.5% and 6.25%, 0%). These rates were then related to the corresponding light levels and hence depths in the water column as obtained from the CTD light profiles. 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). As bacterial production was only measured at two depths, the same method (trapezoidal method) was used, only with two depth sections.

2.3. Concentration of metallic species

All the vessels used for collection and filtration were first washed with detergent then rinsed with MQ water. They were then cleaned in successive acid baths (nitric acid and hydrochloric acid) and rinsed with Milli-Q water before drying in a laminar flow hood. All containers were then stored in double sealed polyethylene bags until use. Samples were collected by hand from the sub-surface in pre-cleaned 2 L PFA bottles (Nalgene). New shoulder length, polyethylene gloves were used for each sampling to avoid any contamination. In order to determine the particulate and dissolved concentration of metallic species, 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), Rodriguez-Gonzalez et al. (2005) and Martin-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. For quality assurance, the certified reference materials (CRM) estuarine sediment IAEA-405 (International Atomic Energy Agency, Monaco) for methylmercury and total mercury analysis and the marine sediment PACS-2 (National Research Council Canada, Ottawa, Canada) for butyltin species were used. For isotope dilution, the species used were IHg enriched in ^{199}Hg (91%), MeHg enriched in ^{201}Hg (96.5%) and a mix of MBT, DBT and TBT enriched in ^{119}Sn (82.4%; ISC Science, Oviedo, Spain). Procedural blanks were performed with each batch of analyses to control for contamination during sample preparation.

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 Döppler Currentmeter Profiler RDI Workhouse 1200 kHz (Lefebvre et al., in review). Total net flow is calculated from riverine outflow and marine inflow as calculated from transversal current measurements (measured from bank to back across the channel during the diurnal tidal cycle) conducted during 24 h cycles on the river. Thus, if a value is positive, i.e. the netflow is going in the seaward direction, it is considered that the source is riverine. Conversely, if the net value is negative, that is, it is going upstream, it is considered that the source is marine or estuarine. All samples 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 Whittaker similarity index (W) was calculated using the following equation.

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

where a_1 and a_2 are relative abundance in samples 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, and hence diversity, at each station was assessed by correspondence analysis (CA). The extent of the correlation of phytoplankton species diversity at each station with the corresponding 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 ($\times^{0.5}$) 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. Environmental conditions

The environmental conditions of the two sample periods differed considerably (Table 1, Fig. 2). In July, temperatures were higher (28.5–31.1 and 18.5–23.1 °C, for July and March, respectively) and river discharge was higher, reflecting the higher precipitation rates observed during July (Table 2). For example, at St. 4, river outflow was 988 $m^3\ s^{-1}$ in July as compared to 175 $m^3\ s^{-1}$ in March (Lefebvre et al., in review). Consequently, surface salinity was lower and turbidity was up to 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 July. In contrast, during 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: 0.11–27.7 and 11.3–31.1 for July and March, respectively (Table 1). In 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 July and March, respectively).

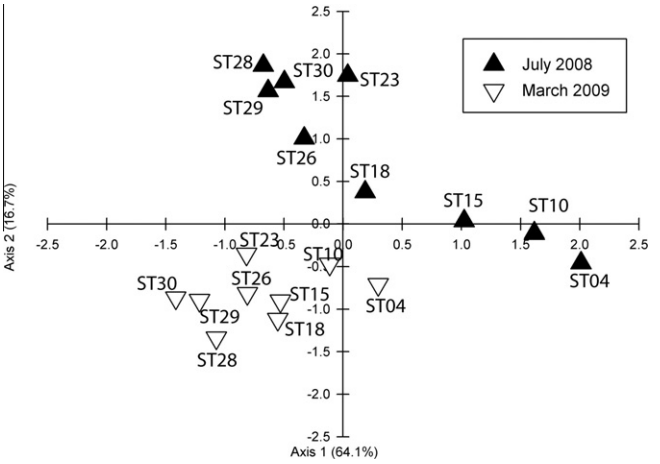

Fig. 2. Correspondence analysis (CA) of the environmental variables for each station and for July (wet season) and March (dry season). Black triangles (July) and white triangles (March).

3.2. Nutrient concentrations

DIN concentrations ranged from 3.9 to 15.7 μM N and 11.1 and 21.3 μM N for July and March, respectively, with the concentrations being significantly higher during March (t-test, $p < 0.05$), Table 1. In general, the highest concentrations were observed in the low salinity stations (Stns. 4 and 10) and the lowest in the high salinity stations (Stns. 23 and 28). There was no significant difference between either DIP or SiO_4 concentrations between the two samplings. Concentrations of DIP varied between 0.11 and 2.7 μM PO_4 and 0.36 and 1.23 μM PO_4 for July and March, respectively. During July, the highest concentrations were observed in the lower salinity stations (2.02–2.70 μM PO4, for Stns. 4, 10 and 15; Table 1). In contrast, during March at was at Stns. 18 and 26 that the highest values were observed (1.23 and 1.15 μM PO_4 for Stns. 18 and 26, respectively. In contrast, silicate concentrations were always highest in the low salinity stations (Stns. 4 and 10) during both seasons and lowest in the high salinity waters. Concentrations ranged from 39.9 to 140.7 μM SiO_4 and 37.8 to 119.9 μM SiO_4 for July and March, respectively and were higher during July. The general positive net seaward flows of nutrients and DOC (Table 2) 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 (butyl-Sn) and mercury species varied between stations and season and fell within the range of those observed in temperate estuaries. For tributyltin (TBT), the concentrations of both particulate and dissolved forms were higher in July than in March; no significant difference of mono-butyl tin concentration was found between the two seasons when the entire dataset was compared. There were however some differences between specific stations over the two seasons. For example, at Stn.

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. Measurements were made 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^{-1})	Chl *a* (kg day^{-1})	DIN (tons N day^{-1})	SiO_3 (tons Si day^{-1})	PO_4 (tons P day^{-1})
July 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
March 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-butyltin in the particulate (p) and dissolved (d) fractions. MeHg: methylmercury 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})	MeHgp (ng L^{-1})	IHgp (ng L^{-1})	MeHgd (ng L^{-1})	IHgd (ng L^{-1})
09/07/08	18	–	–	–	0.768	1.294	0.164	0.0094	1.0705	0.0139	1.2201
09/07/08	26	0.593	0.940	0.417	1.641	1.370	0.186	0.0031	0.6187	0.0133	0.8448
09/07/08	29	0.541	0.845	0.355	1.381	1.688	0.373	0.0055	0.6515	0.0133	1.5614
09/07/08	30	0.726	0.776	0.278	2.257	1.519	0.241	0.0034	0.7455	0.0174	0.5540
09/07/08	28	0.509	0.809	0.332	1.164	1.519	0.215	0.0096	0.6462	0.0089	0.5441
10/07/08	4	0.976	2.233	0.916	1.386	1.110	0.205	0.0150	4.4525	0.0230	0.3892
10/07/08	10	0.838	2.084	0.923	1.272	0.875	0.142	0.0283	5.6659	0.0213	0.5854
10/07/08	15	0.777	1.771	0.709	1.684	1.285	0.199	0.0701	4.4514	0.0184	1.2623
10/07/08	23	1.105	1.973	0.855	0.972	1.046	0.165	0.0073	1.5198	0.0108	0.4032
10/07/08	28	0.946	1.083	0.460	1.538	0.985	0.156	0.0098	0.8324	0.0099	0.1828
11/07/08	30	0.443	0.599	0.258	1.711	1.689	0.224	0.0016	0.6111	0.0120	0.9869
11/07/08	28	0.536	0.679	0.294	0.828	1.695	0.204	0.0087	0.7436	0.0092	0.4876
12/03/09	18	0.087	1.716	0.575	0.341	9.022	0.254	0.0154	0.9553	0.0187	0.3763
12/03/09	26	0.046	1.238	0.442	0.207	10.046	0.186	0.0284	0.7971	0.0148	0.2885
12/03/09	29	0.033	0.875	0.927	0.215	9.852	0.359	0.0067	0.3845	0.0181	0.3074
12/03/09	30	0.027	0.538	0.296	0.129	9.030	0.415	0.0076	0.2200	0.0142	0.2497
12/03/09	28	0.051	0.770	0.402	0.324	10.759	0.486	0.0118	0.4646	0.0116	0.2687
15/03/09	4	0.052	4.666	1.333	0.997	1.066	0.083	0.0368	1.1710	0.0108	0.2768
15/03/09	10	0.048	1.740	0.487	0.908	1.349	0.109	0.0176	0.7066	0.0120	0.2563
15/03/09	15	0.034	1.832	0.512	0.320	9.493	0.266	0.0199	0.6435	0.0156	0.2298
15/03/09	23	0.020	1.522	0.308	0.211	10.229	0.235	0.0108	0.6808	0.0174	0.4432
15/03/09	28	0.033	0.500	0.181	0.172	9.093	0.225	0.0046	0.2629	0.0097	0.2803

– : Not determined.

4, the concentration of particulate MBT was 0.916 ng L^{-1} compared to 1.333 ng L^{-1} for July and March, respectively (Table 3). For di-butyl tin, although the concentration of the particulate form, despite small scale variability, did not vary significantly between season at each station, the concentrations of the dissolved form were significantly higher in March for almost all the stations (Table 3). 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.

The concentrations of mercury species (dissolved and particulate inorganic and methylmercury) also varied between station and between season. In general, inorganic mercury concentrations (both particulate and dissolved forms) were higher in July (0.61–5.67 and 0.18–1.56 ng L^{-1} for particulate and dissolved IHg, respectively), than during March (0.22–1.17 and 0.22–0.44 ng L^{-1} for particulate and dissolved IHg respectively; Table 3). Particulate methylmercury (MeHgp) concentrations were generally low (0.003–0.071 ng L^{-1}) during both July and March. At some stations the concentrations of the particulate form of MeHg were higher during July (0.028 and 0.070 ng L^{-1}) than during March (0.018 and 0.02 ng L^{-1}) at Stns. 10 and 15, respectively. Whereas at other stations (e.g. 18, 26) concentrations were higher during March (0.015 and 0.028 ng L^{-1}) than during July (0.009 and 0.0031 ng L^{-1}; Table 3). Dissolved methylmercury (MeHgd) was also low and varied between 0.0089 and 0.0230 ng L^{-1} for July and 0.0097–0.0187 ng L^{-1} for March. The highest concentrations were observed in the lower salinity stations (Stns. 10 and 15) during July and in the more offshore stations during March (Stns. 18, 23, and 30).

3.4. Phytoplankton abundance and diversity

Differences in phytoplankton abundance and diversity, as determined by microscopy were also evident between the two sampling periods (Tables 1 and 4, Fig. 3, Table 1 Supplementary materials), with total Chl *a* concentrations being higher during July. In July, diatoms dominated at all stations, the only exceptions were Stns. 29 and 23 sampled on the 9th and 10th July, respectively. In all the other cases, the diatom group (Bacillariophyceae) represented between 43% and 99% of the phytoplankton community, with the dinoflagellates (Dinophyceae), chlorophytes (Chlorophyceae) 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 (Euglenophyceae) and silico-flagellates (Dictyochophyceae) 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

Table 4
Relative percentage (%) contributions of each phytoplankton group as determined from the microscope counts.

Date	Station	Chlorophyceae	Cyanobacteria	Bacillariophyceae	Dinophyceae	Euglenophyceae	Dictyochophyceae
09/07/08	18	0.2	2.8	92.4	5.6	0	0
09/07/08	26	0.2	0	98.7	1.1	0	0
09/07/08	29	0	1.0	23.1	75.9	0	0
09/07/08	30	0	0	98.6	1.4	0	0
09/07/08	28	0	0	98.0	2.0	0	00
10/07/08	4	15.2	19.5	61.3	3.1	0	0
10/07/08	10	22.6	7.5	63.5	3.1	0.8	0.6
10/07/08	15	13.7	22.3	43.6	19.9	2.5	0
10/07/08	23	0.8	1.6	44.2	53.5	0.5	0
10/07/08	28	0	0.1	95.5	3.1	0	0
11/07/08	30	0.1	0.1	94.4	4.7	0	0
11/07/08	28	–	–	–	–	–	–
12/03/09	18	0	0	79.8	19.4	0	0.8
12/03/09	26	0	0	65.4	34.6	0	0
12/03/09	29	0	0	86.9	13.1	0	0
12/03/09	30	0	0	46.4	53.6	0	0
12/03/09	28	0	0	65.5	30.9	0	3.6
15/03/09	4	0	0	94.2	5.84	0	0
15/03/09	10	0	0	76.5	23.5	0	0
15/03/09	15	0	0	50.7	49.3	0	0
15/03/09	23	0	0	64.9	35.1	0	0
15/03/09	28	0	0	51.9	48.1	0	0

– : Not determined.

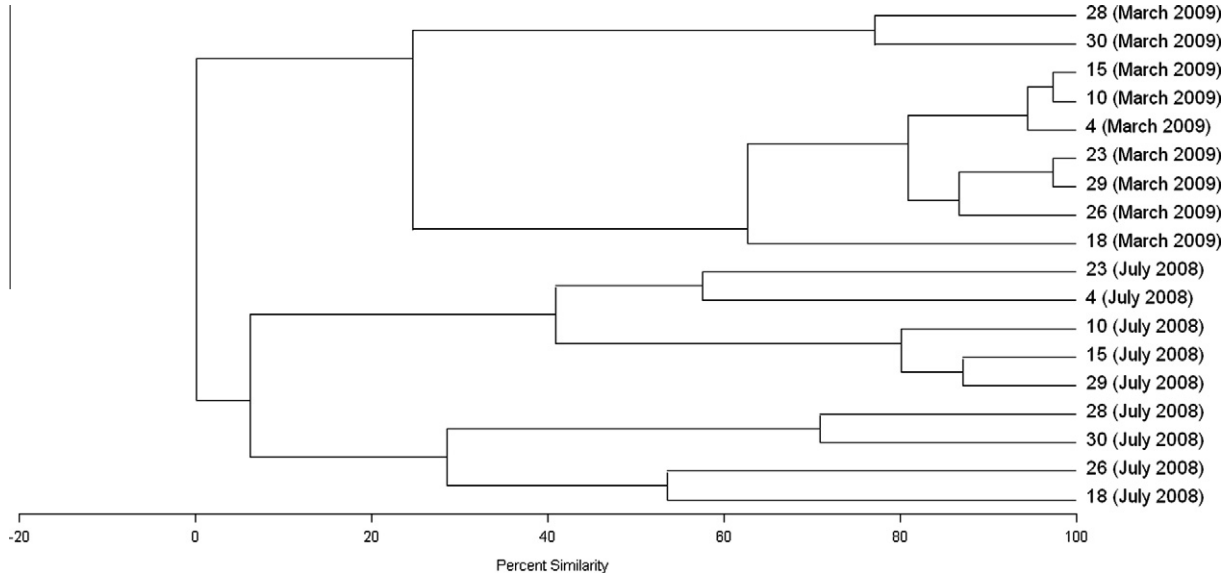

Fig. 3. Dendrogram of phytoplankton diversity for July and March. The numbers correspond to the sampling stations.

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 and 0.16×10^3 cell mL^{-1}) with the highest abundances observed at Stns. 28 and 30 (322×10^3 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 March, and similar to the situation in July, diatoms dominated the community with abundances of over 65–99% of total (Table 4). The only exceptions were Stns. 30, 15 and 28, when the diatoms and the dinoflagellates represented almost equal parts of the community. During March, 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 July, 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 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 July.

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 Supplementary materials). 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 March, *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*, *Ceratium trichoceros*, and *Protoperidinium* 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 *Pseudo-nitzschia* spp. making up the rest of the community.

3.5. Primary and bacterial abundance and production

The rates of depth integrated dissolved and particulate primary production (DPP and PPP, respectively) and bacterial total abundance and production varied between seasons and showed some pronounced differences between stations (Table 1). Bacterial abundance and activity were higher during July, with the highest abundances found at the offshore Stns. 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, Stns. 28 and 4 during this season.

The ratio between BP and DPP is an indicator of the degree of coupling between the autotrophic and heterotrophic processes (Marañón et al., 2004; Rochelle-Newall et al., 2008b). High values (i.e. >1) of BP:DPP indicate that the amount of carbon being incorporated into the bacterial pool is higher than that produced by primary production (DPP) and point towards an additional carbon source. Conversely, low values of BP:DPP (i.e. <1) indicate that primary production is sufficiently high to support bacterial carbon demand. During July values of BP:DPP varied considerably and exceeded 1 in some stations. The highest values of BP:DPP (30.9 and 12.07) were observed at Stns. 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).

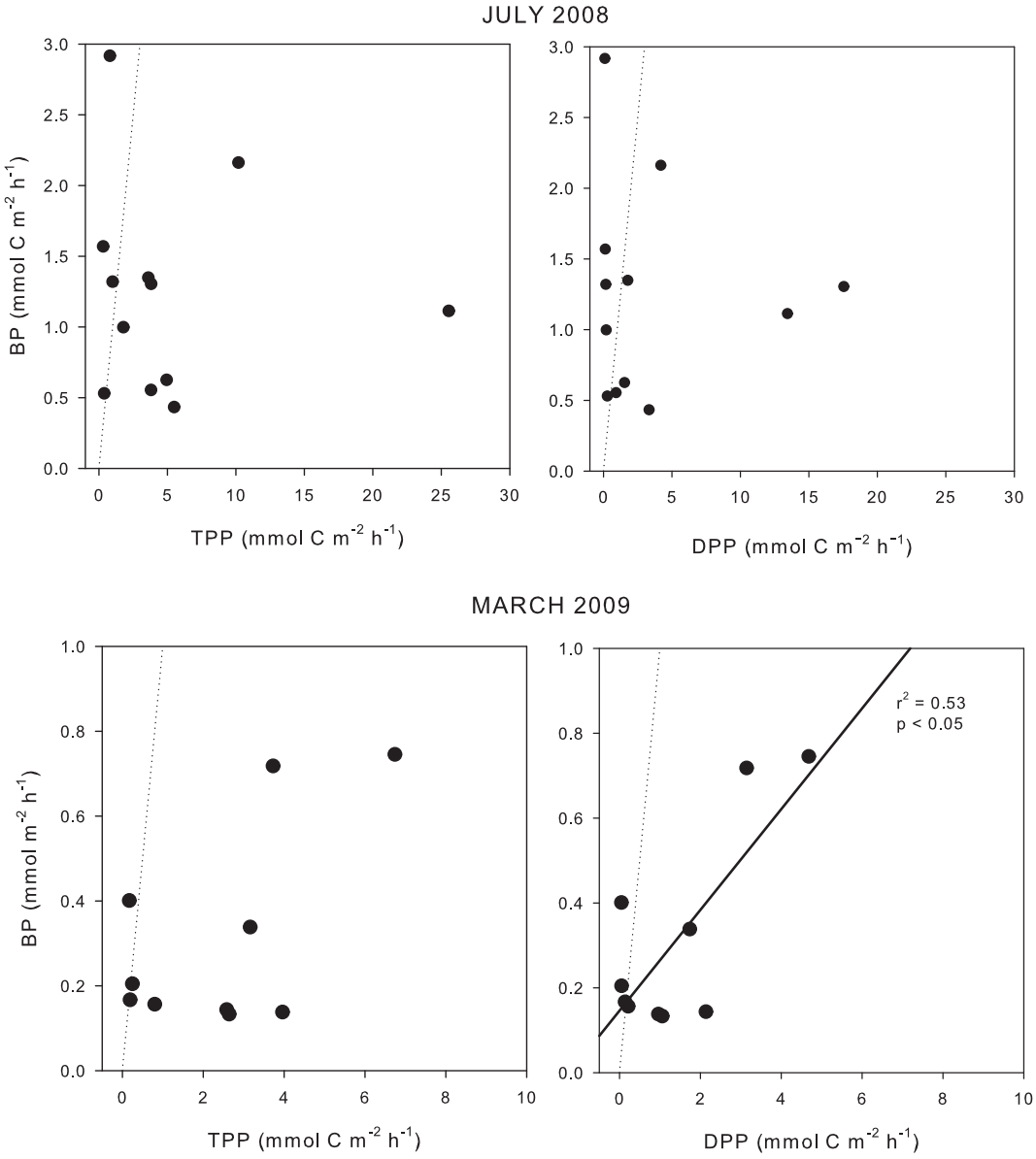

Fig. 4. Relationship between depth-integrated dissolved (DPP) and total primary production (TPP, dissolved + particulate primary production) and depth integrated bacterial production for both seasons. The dotted line represents the 1:1 line.

During March, BA was lower and little variation was observed along the salinity gradient (Table 1). Although BP exhibited the same general trend of increasing BP with increasing turbidity, rates were lower than during July. However, and in contrast to July, BP was correlated with DPP ($r^2 = 0.53$, $p < 0.05$; Fig. 4) during March. Interestingly, the ratio between BP and DPP varied little over the salinity gradient and almost all values were 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 61.4% and 50.2% for July 2008 and March 2009, respectively. All of the data were included in the CCA and the inflation factors (IF) are given in Table 5. Only the data that do not show multicolinearity (an infinite value of IF) were shown on the graphs for clarity. During July, the stations were separated into three groups (Fig. 5) reflecting the differences in community composition (Fig. 3) and the distribution along the salinity gradient. The first group, comprising of the lower salinity stations (4, 10, and 15), was grouped with particulate methylmercury (MeHgp) and particulate mono-butyl tin (MBTp)

Table 5
Inflation factor of CCA analysis for both campaigns. A value close to 1 indicates no redundancy with other variables. mc: multicolinearity between variables. When multicolinearity was detected, variables were not taken into account in the analysis and the graphic presentation (Figs. 5 and 6).

Variable	July 2008	March 2009
	Inflation factor	
Salinity	28.588	mc
SPM	38.223	mc
DOC	2.549	3.810
POC	mc	7.914
PON	mc	mc
NO_3	mc	1.42
NH_4	mc	mc
PO_4	mc	mc
SiO_3	mc	mc
TBTp	mc	mc
DBTp	mc	13.182
MBTp	9.525	9.450
TBTd	2.729	mc
DBTd	mc	5.353
MBTd	6.003	mc
MeHg p	4.962	mc
Hg p	mc	mc
MeHg d	mc	5.505
Hg d	12.544	2.227

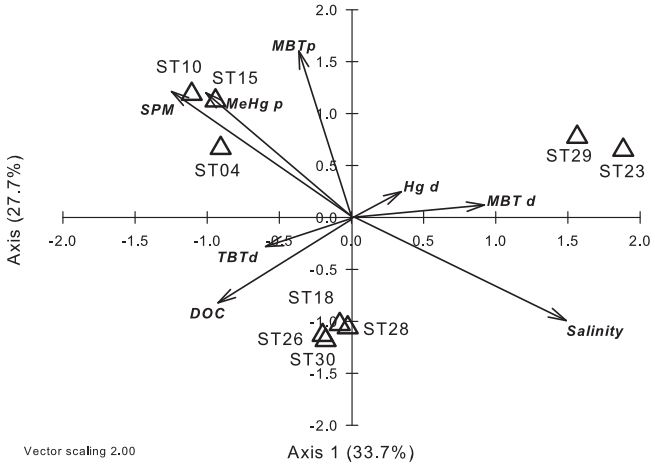

Fig. 5. Canonical correspondence analysis (CCA) of phytoplankton distribution and environmental factors for July. The inflation factors for the analysis are given in Table 5.

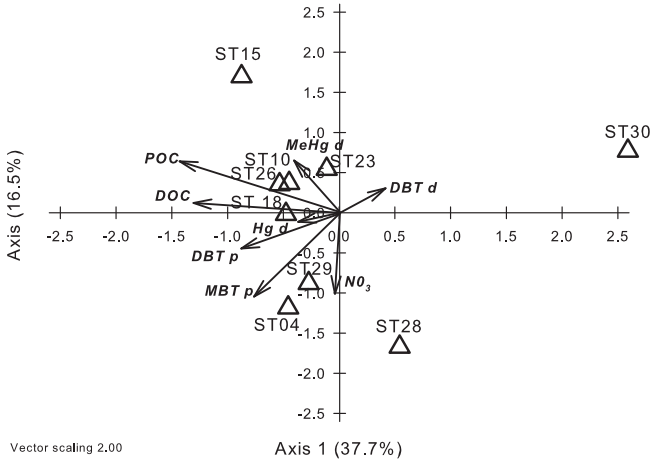

Fig. 6. CCA of phytoplankton distribution and environmental factors for March. The inflation factors for the analysis are given in Table 5.

and SPM concentrations. In contrast, at the other end of the salinity gradient, the Stns. 23 and 29 were related to the dissolved organic mercury (Hgd) and mono-butyl tin (MBTd) concentration. 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). The phytoplankton diversity of this group was partly positively related to the concentration of DOC and of TBTd and negatively related to the concentration of dissolved mercury (Hgd). The inflation factors (Table 5) from the CCA show that during July both salinity and SPM can be considered as structural factors, as well as the concentration of dissolved mercury and particulate MBT. During this season, and probably as a consequence of the high riverine flow rates, multicolinearity was observed for all of the nutrients.

During March the distribution of stations in the CCA was more widespread, with most of the stations grouped in the left quadrants (Fig. 6). The community diversity of this group (Stns. 10, 15, 18, 23, 26) was positively structured by MeHgd concentration as well as by DOC and POC. Particulate di, and mono-butyl species and dissolved mercury also exerted a positive structural effect on phytoplankton community structure at Stns. 4 and 29, despite their opposing salinities. Interestingly, both high salinity stations (28 and 30) were clearly separated, indicating that different factors were controlling phytoplankton diversity and this despite their relatively similar salinities (29.4 and 31.1 for Stns. 28 and 30, respectively). The phytoplankton diversity of Stn 28 was positively related to NO_3 concentration, whereas the community structure observed at Stn. 30 was positively correlated with DBTd concentration.

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. Fisher et al., 1998; Rochelle-Newall and Fisher, 2002). 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 dry seasons (Lefebvre et al., in review). The lack of a simple relationship between salinity and many of the nutrients and DOC in this estuary further underlines the complexity of using simple dilution models to understand the distribution of biological and chemical parameters along estuarine salinity gradients (Troussellier et al., 2002). In contrast, multivariate analyses such as CCA provide the possibility of identifying the multiple factors controlling biological processes in this type of complex ecosystem.

4.1. Phytoplankton diversity and activity

We observed large differences in phytoplankton species distributions between wet (July) and dry (March) seasons (Fig. 3). During July 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 unit) 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 (Fig. 5, Table 5). Salinity is a well known controlling factor of phytoplankton activity and diversity in estuarine systems (e.g. Fisher et al., 1988; Quinlan and Phlips, 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.

Although we included the entire dataset in the CCA, we selected to only present the data that did not show multicolinearity in the graphs. Indeed, it is here that one of the limits of the CCA is evident as co-linearity means that we cannot determine the importance of each factor separately. During July, and probably as a consequence of the high riverine inputs, salinity and SPM are strong determinant factors for phytoplankton distributions. It is well known that light, and thus SPM concentration and nutrients are important factors in determining phytoplankton distributions in estuaries (e.g. Fisher et al., 1988), however, the multicolinearity of all of the nutrients (DIN, DIP, SiO_4) during July meant that we were unable to determine which nutrient, if any, was influencing phytoplankton diversity. In contrast, during March, the strong structural impact of salinity observed in July was not evident and salinity and SPM were found to be co-linear (Table 5). Moreover, during this season, although the structural impact of most of the inorganic nutrients was also co-linear, nitrate was found to be a structural factor of phytoplankton diversity, albeit with a relatively low inflation factor (1.42). Interestingly, during both seasons the inflation factors relating to the influence of heavy metal species on phytoplankton diversity point towards the potential structural role of these metals in this estuary. This was particularly evident for dissolved and particulate MBT concentration during July (IF = 9.52 and 6.0 for MBTp and MBTd, respectively), and for DBTp and MBTp during March (13.1 and 9.45 for DBTp and MBTp, respectively).

Heavy metals are also known to have a negative impact on phytoplankton diversity (e.g. Paulsson et al., 2000; Singh and Rai, 1991). Tributyltin 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., 2006). Through their action on photosynthetic pathways and hence on primary production, heavy metals can also negatively impact biogeochemical cycles even at the low concentrations of TBT similar to those found in the Bach Dang Estuary. Sidharthan et al. (2002) found significant reductions in growth rate of a marine microalgae *Nannochloropsis oculata* at concentrations of TBT as low as 0.0625 nM (ca. 7.5 ng/l (as Sn)), similar to the concentrations observed in this work. The impact of varying salinity and pH on the toxicity of butyltin species and their impacts on phytoplankton community diversity has not been widely tested, however the LC50's of the species that have been tested in culture differ by over a factor of 50 (Sidharthan et al., 2002), pointing towards differences in tolerance to TBT. Similarly, Petersen and Gustavson (2000), working in a Danish coastal system observed large differences in the tolerance of pico, nano- and microphytoplankton to TBT. MBT along with DBT are the degradation products of TBT and considered less toxic than TBT to aquatic phytoplankton (Maguire et al., 1983), although some microorganisms may also exhibit significant sensitivity to DBT and MBT (Gadd, 2000; Lascourrèges et al., 2000).

Moreover, it is known that, at least in culture, that certain species of phytoplankton such as *Chlorella* sp. can degrade TBT to DBT and MBT (StLouis et al., 1997; Tsang et al., 1999) and this may well in part explain some of the relationships between the metallic species and phytoplankton community structure. Particularly during March where significant degradation of TBT, as indicated by the higher relative concentrations of MBT to the total butyl-tin concentrations, may have been occurring (Table 3). Indeed the observed distributions of the different butyl-tin species suggests that during July organotin species were directly transported seaward, while, during March, various biogeochemical transformations were taking place within the estuary, resulting in higher relative concentrations of DBT and MBT. Thus it is probable that TBT and its degradation products induce shifts in phytoplankton community diversity and productivity in coastal systems.

The impact of mercury on phytoplankton diversity and production is less clear. Although, high concentrations of mercury species are considered to be toxic to aquatic organisms, the lower concentrations observed in this work are not generally found to negatively impact phytoplankton growth or production in cultures (e.g. Fisher et al., 1984; Pickhardt and Fisher, 2007). Indeed, the concentrations observed here of methylmercury and inorganic mercury are up to a factor of 10 lower than those found 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 or absorption capacity of the phytoplankton species and ambient DOC concentrations, rather than any negative impact of mercury on phytoplankton communities. Methylmercury uptake in phytoplankton is known to vary with cell size, cell number and with DOC concentration (Pickhardt and Fisher, 2007). During July, phytoplankton community diversity at the lower salinity stations where DOC and mercury concentrations were highest differed greatly from that of the other stations (Fig. 3, Tables 1 and

2). 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 (Table 4). Indeed, phytoplankton diversity at these three stations was correlated with particulate methylmercury concentration (MeHgp). We were unable to determine if this relationship was due to biotic or abiotic processes as we did not determine if Hg was being actively taken up by these phytoplankton species or if the accumulation of MeHgp was due simply to the association of this organometal with the biogenic particles. Similarly, we observed a relationship between the dissolved fractions of MeHg and Hg during March for several of the stations. However, whether these organo-metal species (Hg and Sn) play a direct role in structuring phytoplankton diversity at these stations or whether the distribution of organometal species is directly influenced through their partition and transformation by the plankton community structure is difficult to determine with this dataset. Indeed, to answer this question experiments designed at comparing the direct impact of these metals on complex communities and the impact of phytoplankton communities on organometallic species are necessary.

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 July, indicating that the DOC fuelling BP originated from other sources than the immediately adjacent phytoplankton production. Indeed, the relatively high particle 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 July. In contrast, at the higher salinity, offshore stations during July and at almost all of the stations during March, 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 difficultly 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 (e.g. Justic et al., 1995). 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 July (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.

4.3. Influence of hydrodynamics

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 and biological activities, particularly in tropical systems (Mari et al., 2007; Torréton et al., 2007, 2010). Over and above the clear seasonal differences in phytoplankton community composition, we also observed a relatively high degree of daily variability at the two offshore stations (28 and 30) during July. This was due to a shift in wind direction (westerly) and intensity over the three day sampling period. It is probable that this increase in wind direction pushed some of the higher turbidity waters from the Van Uc system into the less turbid Haiphong Bay system and this is supported by the higher nutrient and turbidity and lower salinity measurements observed. At the beginning of the sampling period and typical of the south monsoon during July, the wind direction was from the south (180–200°). This would have confined the phytoplankton communities to the bay areas instead and reduced their dispersal into the higher salinity, off-shore waters as would be expected through classical water mixing. The short term shift in wind direction released this control and allowed the high nutrient, high biomass waters to move out towards the higher salinity stations. Wind direction may well also explain in part the similarities in both phytoplankton composition and in the factors controlling that composition during July for the three groups observed in Fig. 5. Furthermore, recent work on the water circulation during the tidal cycle in this area (Dinh and Ha, 2008) has shown that water outflow 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. Thus, in this estuary, the prevailing wind direction combined with tidal influence (here reduced because sampling was performed during neap tides) may also play an important role the distribution of phytoplankton.

5. Conclusion

Here we present some of the factors that potentially control phytoplankton distribution 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, using multivariate statistics applied to field data, 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. Undoubtedly, these hypotheses need to be more rigorously tested using experimental approaches to complete the results gained from this field study in order to better rank the relative impact of all the potential factors (e.g. nutrient concentrations, turbidity, metal concentrations) that control phytoplankton diversity. Nevertheless, and given the almost ubiquitous presence

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

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.marpolbul.2011.08.044.

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