

## The Mekong River plume fuels nitrogen fixation and determines phytoplankton species distribution in the South China Sea during low- and high-discharge season

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### Abstract

The influence of the Mekong River (South China Sea) on N<sub>2</sub> fixation and phytoplankton distribution was investigated during the lowest- and highest-discharge seasons (April 2007 and September 2008, respectively). The river plays an essential role in providing nutrients (nitrate, phosphate, silicate) for the adjacent sea and creates different salinity and nutrient gradients over different seasons. River water (salinity 0), mesohaline waters (salinity 14–32), a transition zone with salinities between 32 and 33.5, and marine waters (salinity above 33.5) were sampled at different spatial resolutions in both cruises. High N<sub>2</sub> fixation rates were measured during both seasons, with rates of up to 5.05 nmol N L<sup>-1</sup> h<sup>-1</sup> in surface waters under nitrogen-replete conditions, increasing to 22.77 nmol N L<sup>-1</sup> h<sup>-1</sup> in nitrogen-limited waters. Asymbiotic diatoms were found only close to the river mouth, and symbiotic diatoms, which potentially hosted diazotrophs, were most abundant in waters where N<sub>2</sub> fixation rates were highest, nitrate concentrations were at the detection limit, and phosphate and silicate were still available. Filamentous cyanobacteria like *Trichodesmium* were present only in marine waters with salinities above 33.5. Overall, N<sub>2</sub> fixation accounts for 1–47% of the nitrogen demand of primary production.

Dinitrogen (N<sub>2</sub>) fixation plays an important role in fueling the oligotrophic ocean with nitrogen (N) (Mulholland 2007). Recent studies propose that N<sub>2</sub> fixation may also be significant in coastal and other nutrient rich environments (Deutsch et al. 2007; Bonnet et al. 2009). In this context, river plumes have been suggested to host other groups of N<sub>2</sub>-fixing organisms than the typical open-ocean diazotrophs *Trichodesmium* and unicellular cyanobacteria (Capone et al. 1997; Church et al. 2005a); for example, in the Amazon River plume, diatom–diazotroph associations (DDAs) can displace *Trichodesmium* as the dominant diazotroph of the region, and rates of N<sub>2</sub> fixation in DDA blooms exceed vertical nitrate fluxes (Carpenter et al. 1999; Subramaniam et al. 2008). Diatoms hosting diazotroph symbionts come from various genera. *Hemiaulus*, *Rhizosolenia*, *Guinardia*, and *Bacteriastrum* associate with the heterocystous cyanobacterium *Richelia intracellularis*, while diatoms of the genus *Chaetoceros* are typically found in symbiosis with *Calothrix rhizosoleniae*, another diazotroph cyanobacterium that is closely related to *Richelia* (Carpenter 2002; Foster and Zehr 2006). The trichomes of *Richelia intracellularis* reside as an endosymbiont between the plasmalemma and the frustule of the diatom in *Hemiaulus*, *Rhizosolenia*, and *Guinardia* (Villareal 1992). In the DDAs *Bacteriastrum-Richelia intracellularis* and *Chaetoceros-Calothrix rhizosoleniae*, the symbiont resides as an epiphyte on the spines of the diatom (Carpenter 2002; Foster and O'Mullan 2008).

Studies by Foster and others (2007) later confirmed that DDAs (*Hemiaulus-Richelia*) dominate in the Amazon River

plume waters with measurable concentrations of nitrate (NO<sub>3</sub>) (< 0.01–0.13 μmol L<sup>-1</sup>), phosphate (PO<sub>4</sub>) (< 0.02–0.13 μmol L<sup>-1</sup>), and silicate (Si[OH]<sub>4</sub>) (0.3–11.3 μmol L<sup>-1</sup>), while *Trichodesmium* and different groups of free-living unicellular cyanobacteria were more abundant in oceanic waters outside the river plume, where nutrients were below the detection limit (Foster et al. 2007; Subramaniam et al. 2008). Also in the Congo River plume in the Eastern Equatorial Atlantic, conditions seem to be favorable for DDAs, even though *Trichodesmium* dominated the diazotroph community (Foster et al. 2008). The DDAs are thought to have a growth advantage in river plumes where nitrogen is limiting but phosphorus and silicate are still available, while nitrogen is supplied through N<sub>2</sub> fixation by the diazotroph symbiont (Janson et al. 1999; Carpenter 2002). Furthermore, tropical rivers provide trace metals such as iron (Nittrouer et al. 1995; Bergquist and Boyle 2006), which is an essential component of the dinitrogenase enzyme complex of all diazotrophs. All these findings suggest that enhanced N<sub>2</sub> fixation in tropical river plumes might be a global phenomenon and would thus contribute substantially to oceanic N<sub>2</sub> fixation.

Like other tropical rivers, the Mekong River discharges relatively high concentrations of nutrients, with over 40 μmol L<sup>-1</sup> of nitrate plus nitrite (NO<sub>3</sub>+NO<sub>2</sub>) and about 1 μmol L<sup>-1</sup> of PO<sub>4</sub> and over 100 μmol Si(OH)<sub>4</sub> (<http://www.gemstat.org>). These nutrients foster new production in the South China Sea (SCS), into which the Mekong River discharges. Previous results from the SCS show that the Mekong River plume is linked to elevated rates of N<sub>2</sub> fixation as far as 200 km northeast of the river mouth (Voss et al. 2006), which suggests a scenario similar to the one in the Amazon River plume. The study by Voss et al. (2006) investigated the Vietnamese upwelling area and the adjacent offshore oligotrophic region, where diazotroph

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species were possibly influenced by both riverine and initially upwelled nutrients. In contrast to the study by Voss et al. (2006), we carried out a detailed sampling closer to the river mouth, where the changes in salinity and nutrient concentrations are expected to have the greatest influence on species composition and rates of N<sub>2</sub> fixation. The diazotroph community in the SCS includes DDAs, *Trichodesmium*, and unicellular diazotrophs (< 10 μm), comprising coccoid cyanobacteria and proteobacteria (Moisander et al. 2008), and it can be expected that the Mekong River plume triggers unequal distribution patterns of these different groups. In the present study, we measured rates of N<sub>2</sub> fixation and determined phytoplankton species composition in SCS surface waters off the Mekong river mouth. The study compares two field investigations, one conducted in April 2007, coinciding with intermonsoon and the river's lowest annual outflow, and a second investigation conducted in September 2008 during southwest (SW) monsoon and the river's highest annual outflow (Hoa et al. 2007). The goals of this study are to describe and compare the biogeochemical settings of the river plume during both seasons and to assess how these settings are related to N<sub>2</sub> fixation and to the distribution of phytoplankton, including asymbiotic diatoms, *Trichodesmium* spp., and different diatom species that potentially host diazotroph symbionts.

## Methods

**Study area**—The Mekong Delta lies between 9.0°N and 10.5°N in the Southeast Asian monsoon region. The SW monsoon between June and September coincides with the rainy season and the rivers largest annual outflow (40,000 m<sup>3</sup> s<sup>-1</sup>; Hoa et al. 2007). During this time of the year, the winds have an approximate speed of 6 m s<sup>-1</sup> (Hellerman and Rosenstein 1983) and deflect the river plume northeast along the coast before it turns east into the open SCS. The northeast (NE) monsoon appears between November and March, coinciding with the dry season, and the prevailing winds have an average velocity of 9 m s<sup>-1</sup> (Hellerman and Rosenstein 1983). The intermonsoon during April and May and during October and early November marks the transition between monsoon seasons (Fang et al. 2002). In April, at the end of the dry season, the river outflow reaches its annual low with an outflow of about 2100 m<sup>3</sup> s<sup>-1</sup> (Hoa et al. 2007), resulting in a relatively small river plume (covering approximately 37,000 km<sup>2</sup> of the SCS), which is deflected southward. The total freshwater discharge of the Mekong River is about 500 km<sup>3</sup> yr<sup>-1</sup>, with 15% being discharged during the dry season and 85% during the rainy season (Hoa et al. 2007). Compared to the Amazon and Congo Rivers with 6900 and 1300 km<sup>3</sup> yr<sup>-1</sup>, respectively (Korzun 1978; World Hydrology Cycle Observing System, <http://www.whycos.org>), the discharge by the Mekong River is low, but it is the largest river draining into the SCS.

Annual loads of total nitrogen (TN) and total phosphorus (TP) are modeled for the river mouth reaching  $2.7 \times 10^4$  t TN yr<sup>-1</sup> and  $9.0 \times 10^2$  t TP yr<sup>-1</sup> (Yoshimura and Takeuchi 2007). The model also predicts a discharge of 0.1 kg TN s<sup>-1</sup> during the dry season (December through

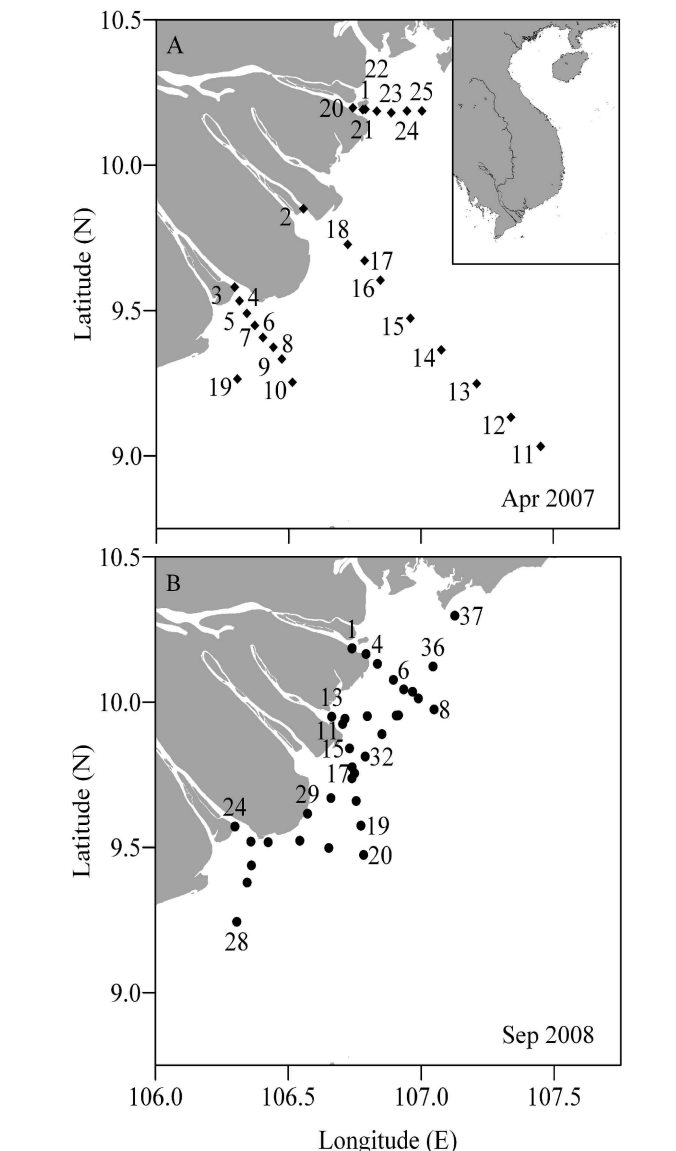


Fig. 1. Map of the Mekong River Estuary with all CTD stations of (A) April 2007 and (B) September 2008. The map was created with the Surfer® 8.09 (Golden Software).

May) and 10 kg TN s<sup>-1</sup> during the rainy season (May through November) (Yoshimura and Takeuchi 2007).

**Sampling**—Samples were collected aboard a Vietnamese monitoring vessel, BTh-0666 KN, between 15 April and 20 April 2007 (Fig. 1A) and between 18 September and 22 September 2008 (Fig. 1B). About 40% of the total water volume is discharged from the southernmost river arm (Sta. 3 in April 2007 and Sta. 24 in September 2008), whereas the other river arms represented by Sta. 20 and Sta. 2 (April 2007) and Sta. 1 and Sta. 12 (September 2008) discharge ~ 30% and 13%, respectively (Nguyen et al. 2008).

Northeasterly winds of 10 to 12 m s<sup>-1</sup> occurred during a 2-week period before the cruise in April 2007 and mixed the entire water column. Throughout the first 4 d of the cruise in April 2007 (Sta. 1–20), winds did not restrict sampling,

but on the northernmost transect (Sta. 21–25), winds of  $6 \text{ m s}^{-1}$  allowed sampling only for nutrient concentrations and phytoplankton composition. Three transects were sampled; each started in a river arm and was directed out to the open sea. We planned to sample the same stations in September 2008, but because of constant winds between 5 and  $10 \text{ m s}^{-1}$ , sampling farther offshore was impossible and was kept close to the coast.

At all stations, the entire water column was measured for conductivity, temperature and depth (CTD) with Seabird sensors (SEB19 plus), and turbidity with a Seapoint Turbidity Meter (recording Nephelometric Turbidity Units [NTU], waters below 10 NTU have moderate plant and animal life; waters below 1 NTU represent oligotrophic conditions; CWT 2004). A total of 25 (April 2007) and 35 (September 2008) surface samples (0–1 m) were taken with a 10-L Niskin bottle. A 24-h time-series station (Sta. 19; Fig. 1A) was sampled between 05:30 h on 18 April 2007 and 07:00 h on 19 April 2007. The time-series station was sampled hourly with the CTD and provided a total of seven sampling points (SP) for nutrients, chlorophyll, phytoplankton,  $\text{N}_2$  fixation, and primary production (sampling every 3 h during the day, every 6 h during the night). The same station (Sta. 28; Fig. 1B) was sampled again between 11:40 h on 20 September 2008 and 11:40 h on 21 September 2008, providing hourly measurements with a CTD between 11:40 h and 17:40 h (20 September 2008) as well as 05:40 h and 11:40 h (21 September 2008) and a total of four SP for chlorophyll,  $\text{N}_2$  fixation, primary production, and phytoplankton (sampling every 6 h) as well as six SP for nutrients (sampling every 3 h). The geographical position of this time-series station was chosen in order to enable sampling of the hydrographic situation of the Mekong River plume throughout a complete tidal cycle.

*Analysis*—Water samples for the determination of  $\text{NO}_3$ ,  $\text{NO}_2$ ,  $\text{PO}_4$ , and  $\text{Si}(\text{OH})_4$  were taken, filtered through WHATMAN GF/F glass-fiber filters ( $0.7\text{-}\mu\text{m}$  pore size), and stored frozen onboard before they were analyzed at the Institute of Oceanography, Nha Trang. Standard colorimetric methods (Grasshoff et al. 1983) were used. These methods usually reach a precision of  $0.05 \mu\text{mol L}^{-1}$  for  $\text{NO}_3$ ,  $0.01 \mu\text{mol L}^{-1}$  for  $\text{NO}_2$ ,  $0.01 \mu\text{mol L}^{-1}$  for  $\text{PO}_4$ , and  $0.1 \mu\text{mol L}^{-1}$  for  $\text{Si}(\text{OH})_4$ . However, because of the local circumstances (nutrient traces in the only available distilled water), we could not achieve a precision better than  $0.05 \mu\text{mol L}^{-1}$  for  $\text{NO}_3$ ,  $\text{NO}_2$ , and  $\text{PO}_4$ . Nutrient ratios (N:P, Si:N) were therefore not calculated for samples having unreliable concentrations  $\leq 0.1 \mu\text{mol L}^{-1}$ .

Chlorophyll *a* (Chl *a*) was measured at 12 stations in April 2007 and at 15 stations in September 2008. A water volume between 100 and 2000 mL was used to cover a Whatman GF/F glass-fiber filter appropriately with organic material. Chlorophyll samples were stored frozen at  $-80^\circ\text{C}$  until analyzed. Chlorophyll was extracted in ethanol and measured with a Turner 10-AU-005 fluorometer (Wasmund et al. 2006).

Samples for phytoplankton identification (1.5 liters) were taken at 21 stations (April 2007) and 13 stations (September 2008) and preserved with 0.5 mL formaldehyde

(33%) and 10 mL of Lugol solution, according to the Intergovernmental Oceanographic Commission protocol (Hallegraeff 1995). Phytoplankton identification and counting by light microscopy included only the size fraction  $> 10 \mu\text{m}$  and was carried out using standard protocols after Utermöhl (1958). A fluorescence microscope for the detection of intracellular symbionts was not available.

Rates of  $\text{N}_2$  fixation and primary production were measured using the tracer assay described by Montoya et al. (1996). Surface water was taken with Niskin bottles at nine stations in April 2007 and at 13 stations in September 2008. The water was filled into three 2.3-liter Nalgene bottles at each station. The Nalgene bottles were equipped with septum caps made of Teflon-lined butyl rubber. Subsequently, both  $^{15}\text{N}_2$ -gas (2 mL; Sercon, 99 atom%) and  $0.1 \mu\text{mol L}^{-1}$   $\text{NaH}^{13}\text{CO}_3$  solution (0.5 mL; Sigma Aldrich, 98 atom%) were added with syringes. The bottles were incubated on deck and cooled with surface seawater. During the day, the incubations lasted for 6 h, and the incubators were covered with neutral-density screening (50%), which correspond to the light intensities of surface waters of mesotrophic waters or 10-m depth in oligotrophic waters. At night, the incubations lasted about 12 h to ensure a  $^{15}\text{N}_2$  uptake above the detection limit. Because of the limited cruise time during both investigations,  $\text{N}_2$  fixation and primary production incubations were always initiated immediately after sampling so that most day incubations lasted either from 06:00 h to 12:00 h or from 12:00 h to 18:00 h. Incubations during the night covered the entire dark period (Table 1).

The incubations were terminated by gentle vacuum filtration. In April 2007,  $\text{N}_2$  fixation was measured in two size fractions ( $<$  and  $> 10 \mu\text{m}$ ), and the total  $\text{N}_2$  fixation rates were calculated by adding the respective rates. Samples were initially filtered through acid-washed gauze ( $10\text{-}\mu\text{m}$  pore size). The filamentous cyanobacteria and diatoms ( $> 10 \mu\text{m}$ ) were then rinsed onto precombusted Whatman GF/F glass-fiber filters (4 h at  $500^\circ\text{C}$ ,  $0.7\text{-}\mu\text{m}$  pore size). The particles  $< 10 \mu\text{m}$  in the filtrate were collected over additional glass-fiber filter. In September 2008 the size fractions were not separated, and only total  $\text{N}_2$  fixation rates were measured. All filters were stored frozen at  $-20^\circ\text{C}$ . In the laboratory, the filters were acidified over fuming hydrochloric acid (37%) for 6 h, subsequently dried ( $40^\circ\text{C}$ ; 12 h), packed into tin cups, and pelletized. Isotopic measurements of the filters were done with a Delta S (Thermo) isotopic ratio mass spectrometer connected to an elemental analyzer CE1108 via open split interface. Rates of  $\text{N}_2$  fixation and primary production were calculated as described by Montoya et al. (1996).

## Results

*Hydrographic conditions and nutrient distributions*—The coastal waters of the SCS are characterized by strong tides of up to 4.5 m (Hoa et al. 2007), which cause a deep intrusion of ocean waters upstream into the Mekong River during the intermonsoon, the low-discharge season. In April 2007, waters with a salinity of 0 were therefore found

Table 1. Total N<sub>2</sub> fixation rates and percentage of each size fraction on total N<sub>2</sub> fixation for April 2007 and total N<sub>2</sub> fixation rates for September 2008. N<sub>2</sub> fixation incubations occurred during day hours (sunrise to sunset), and incubations lasted 6 h; underlined values indicate incubations lasting from 06:00 h to 12:00 h, and bold values indicate incubations lasting from 12:00 h to 18:00 h; during night hours (sunset to sunrise) incubations lasted 12 h and are italic; all other incubations had two time periods overlapping.

April 2007					September 2008		
Sta.	Total N <sub>2</sub> fixation (nmol N L <sup>-1</sup> h <sup>-1</sup> )	% of total N <sub>2</sub> fixation		Sta.	Total N <sub>2</sub> fixation (nmol N L <sup>-1</sup> h <sup>-1</sup> )		
		(<10 μm)	(>10 μm)				
<i>1</i>	<i>Night</i>	<i>0.11</i>	<i>68.96</i>	<i>31.04</i>	<u>1</u>	<u>Day</u>	<u>0.69</u>
<u>2</u>	<u>Day</u>	<u>1.01</u>	78.36	21.64	<u>4</u>	<u>Day</u>	0.06
<b><u>3</u></b>	<b><u>Day</u></b>	<b><u>1.07</u></b>	<b>70.55</b>	<b>29.45</b>	<b><u>6</u></b>	<b><u>Day</u></b>	<b><u>3.06</u></b>
8	Day	6.13	67.26	32.74	8	Day	4.22
<i>10</i>	<i>Night</i>	<i>3.95</i>	<i>74.83</i>	<i>25.17</i>	<i>11</i>	<i>Night</i>	<i>0.12</i>
<u>11</u>	<u>Day</u>	<u>11.36</u>	95.15	4.85	<u>13</u>	<u>Day</u>	<u>0.13</u>
<b><u>15</u></b>	<b><u>Day</u></b>	<b><u>16.36</u></b>	<b>6.56</b>	<b>93.44</b>	<u>15</u>	<u>Day</u>	<u>3.51</u>
20SP1	Day and night	0.60	75.51	24.49	17	Day	0.90
20SP2	Day	2.86	55.01	44.99	20	Day	5.05
<u>19SP1</u>	<u>Day</u>	<u>15.54</u>	46.77	53.23	<i>20</i>	<i>Night</i>	<i>2.34</i>
<u>19SP2</u>	<u>Day</u>	<u>12.90</u>	73.78	26.22	<u>24</u>	<u>Day</u>	<u>0.18</u>
<b><u>19SP3</u></b>	<b><u>Day</u></b>	<b><u>22.77</u></b>	<b>63.06</b>	<b>36.94</b>	<u>29</u>	<u>Day</u>	<u>1.66</u>
19SP4	Day	12.52	21.02	78.98	<u>36</u>	<u>Day</u>	<u>0.74</u>
<i>19SP5</i>	<i>Night</i>	<i>14.62</i>	26.83	73.17	<b><u>28SP1</u></b>	<b><u>Day</u></b>	<b><u>0.70</u></b>
19SP6	Day and night	18.99	23.80	76.20	<i>28SP2</i>	<i>Night</i>	<i>0.10</i>
<u>19SP7</u>	<u>Day</u>	<u>15.70</u>	35.59	64.41	<u>28SP3</u>	<u>Day</u>	<u>1.02</u>
					<u>28SP4</u>	<u>Day and night</u>	<u>0.64</u>

approximately 30 km upstream (H. Hein unpubl.). A sample of Mekong River freshwater was taken at the station My Tho, which lies ~ 50 km upstream. The lowest salinity at sampling stations in the River mouth was 14.3 (Sta. 3) and from there, salinities gradually increased to maximal values of around 34 at stations farthest offshore (Fig. 2A). During SW monsoon in September 2008, the outflow was much higher so that freshwater was found in the river arms at Sta. 1 and 24 (Fig. 2B). The plume extended much farther offshore during that cruise; however, because of considerable swell, it was impossible for our small vessel to reach sampling stations as far offshore as in April 2007. Salinities above 30 were measured at outer Sta. 8 and 20, and the maximal salinities were found at Sta. 28, ranging between 31.9 and 32.0.

Based on surface salinity and nutrient distributions, we distinguish three station categories, which will be referred to throughout this article: (1) mesohaline (14.3–32.0), which was sampled during both cruises; (2) transitional (> 32.0–33.5); and (3) oceanic (> 33.5), whereby the latter salinities are typical for open sea water as defined by Dippner et al. (2007) for the SCS off Vietnam. Transitional and oceanic salinities were sampled only in April 2007, and, as mentioned, salinities < 14.0 (0.1–8.9) were found only in September 2008. In order to clearly show the differences between the observed salinity gradients during lowest and highest river discharge, the salinity isoline of 32 is highlighted in all figures showing surface distributions. The salinity of 32 was also chosen because it was previously defined as a lower boundary for the occurrence of *Trichodesmium* (Jones et al. 1982; Revelante and Gilmartin 1982).

Overall, concentrations of all nutrients showed a strong offshore gradient and were negatively correlated with

salinity in April 2007; in September 2008, only NO<sub>3</sub>+NO<sub>2</sub> and Si(OH)<sub>4</sub> showed significantly negative correlations with salinity (Fig. 2C–H; Table 2). The highest concentrations of NO<sub>3</sub>+NO<sub>2</sub>, PO<sub>4</sub>, and Si(OH)<sub>4</sub> were encountered within the river arms, at Sta. 2 and 3 in April 2007, and at Sta. 1 in September 2008 (Fig. 2C–H). Interestingly, concentrations of 4.2–19.6 μmol L<sup>-1</sup> NO<sub>3</sub>+NO<sub>2</sub> and 0.4–1.0 μmol L<sup>-1</sup> PO<sub>4</sub> in mesohaline waters in April 2007 did not differ much from concentrations in waters with much lower salinity (≤ 8.9) in September 2008 (17.3–22.3 μmol L<sup>-1</sup> NO<sub>3</sub>+NO<sub>2</sub> and 0.6–0.9 μmol L<sup>-1</sup> PO<sub>4</sub>); however, for concentrations of Si(OH)<sub>4</sub>, there was indeed a clear difference (18.8–42.6 μmol L<sup>-1</sup> in mesohaline waters in April 2007 vs. 115–176 μmol L<sup>-1</sup> at salinities ≤ 8.9 in September 2008) (Table 3). Mesohaline waters had overall similar nutrient concentrations during both investigations (Table 3). Transitional waters, which were only sampled in April 2007, had NO<sub>3</sub>+NO<sub>2</sub> concentrations of ≤ 0.9 μmol L<sup>-1</sup>, PO<sub>4</sub> of ≤ 0.3 μmol L<sup>-1</sup>, and Si(OH)<sub>4</sub> concentrations of ≤ 10.1 μmol L<sup>-1</sup>. At oceanic stations, concentrations of PO<sub>4</sub> were below detection, concentrations of NO<sub>3</sub>+NO<sub>2</sub> were mostly ≤ 0.3 μmol L<sup>-1</sup> besides one higher value at Sta. 14 (1.0 μmol L<sup>-1</sup>), and concentrations of Si(OH)<sub>4</sub> were ≤ 3.6 μmol L<sup>-1</sup>.

Ratios of NO<sub>3</sub>+NO<sub>2</sub> to PO<sub>4</sub> (N:P) generally decreased with increasing salinity during both investigations (Fig. 3; Table 3). N:P ratios lower than the Redfield ratio of 16:1 were confined largely to transitional waters during April 2007 (only mesohaline Sta. 6 had an N:P ratio of 11). In September 2008, N:P ratios in mesohaline waters were more variable but below 16 at 13 out of 17 stations. In waters with salinities below 14, N:P ratios were always above 20 (Fig. 3; Table 3). Ratios of Si(OH)<sub>4</sub> to NO<sub>3</sub>+NO<sub>2</sub> (Si:N) tended to increase with increasing salinity, although

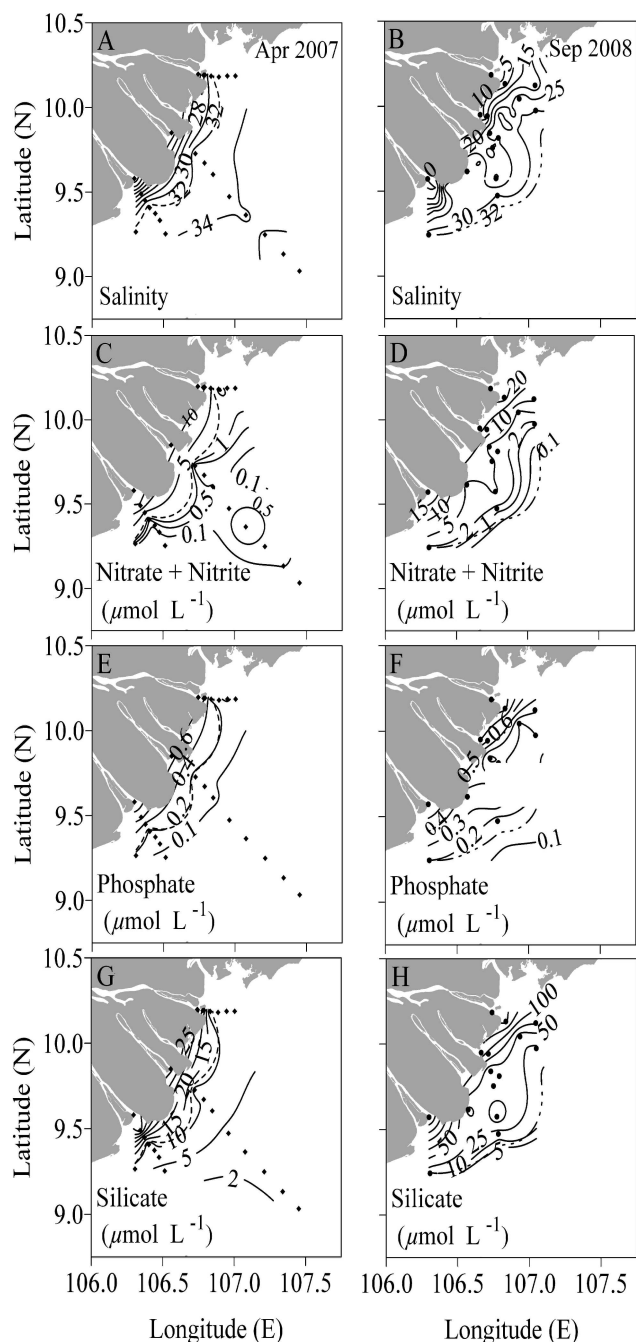


Fig. 2. Salinity and nutrient distribution in the investigation area in the first column are the results from April 2007: (A) salinity, (C)  $\text{NO}_3$  concentrations, (E)  $\text{PO}_4$  concentrations, (G)  $\text{Si(OH)}_4$  concentrations. September 2008 data are in the second column: (B) salinity, (D)  $\text{NO}_3$  concentrations, (F)  $\text{PO}_4$  concentrations, (H)  $\text{Si(OH)}_4$  concentrations.  $2.0 \mu\text{mol L}^{-1}$   $\text{Si(OH)}_4$  marks the limit for diatom growth.  $0.1 \mu\text{mol L}^{-1}$  denotes the detection limit for all nutrients. The dashed lines denote the salinity boundary of 32.

this increase was not as obvious in September 2008 as in April 2007 (Table 3).

*N<sub>2</sub> fixation and primary production*—During both investigations,  $\text{N}_2$  fixation was detectable at all stations (Fig. 4). In

Table 2. Pearson correlation matrix comparing changes in salinity, turbidity, and nutrient concentrations.  $p$ -values  $\leq 0.01$  are considered significant.

	April 2007		September 2008	
	Salinity	$p$ -value	Salinity	$p$ -value
Salinity	1.000		1.000	
Turbidity	-0.723	$\leq 0.001$	-0.720	0.0125
Nitrate+nitrite	-0.944	$\leq 0.001$	-0.978	$\leq 0.001$
Phosphate	-0.916	$\leq 0.001$	-0.783	$\leq 0.001$
Silicate	-0.933	$\leq 0.001$	-0.989	$\leq 0.001$

April 2007, total  $\text{N}_2$  fixation rates in mesohaline surface waters ranged between  $0.11$  and  $2.86 \text{ nmol N L}^{-1} \text{ h}^{-1}$  (mean  $1.13 \pm 1.04 \text{ nmol N L}^{-1} \text{ h}^{-1}$ ) and increased to very high values in transitional waters ( $5.76$ – $22.77 \text{ nmol N L}^{-1} \text{ h}^{-1}$ , mean:  $14.33 \pm 5.08 \text{ nmol N L}^{-1} \text{ h}^{-1}$ ) and oceanic waters ( $4.00$ – $16.40 \text{ nmol N L}^{-1} \text{ h}^{-1}$ , mean:  $10.55 \pm 6.24 \text{ nmol N L}^{-1} \text{ h}^{-1}$ ). In September 2008,  $\text{N}_2$  fixation rates ranged from  $0.10$  to  $5.05 \text{ nmol N L}^{-1} \text{ h}^{-1}$  in mesohaline waters (mean:  $1.81 \pm 1.7 \text{ nmol N L}^{-1} \text{ h}^{-1}$ ) and from  $0.06$ – $0.69 \text{ nmol N L}^{-1} \text{ h}^{-1}$  in waters having salinities below 14 (mean:  $0.26 \pm 0.29 \text{ nmol N L}^{-1} \text{ h}^{-1}$ ). Thus, the rates found in mesohaline waters were clearly higher in September 2008 and are comparable to the lower range of values from transitional waters in April 2007 (Fig. 4; Table 3).

During each investigation, we included three stations at which incubation periods covered the entire dark period (sunset to sunrise). In April 2007, these  $\text{N}_2$  fixation rates were  $0.11 \text{ nmol N L}^{-1} \text{ h}^{-1}$  (mesohaline waters) and  $3.95$  and  $14.62 \text{ nmol N L}^{-1} \text{ h}^{-1}$  (transitional waters). In September 2008,  $\text{N}_2$  fixation rates were comparable to the lower range of the April 2007 rates with  $0.10$ ,  $0.12$ , and  $2.34 \text{ nmol N L}^{-1} \text{ h}^{-1}$ . Furthermore, the  $\text{N}_2$  fixation rates measured at night were of the same magnitude as the  $\text{N}_2$  fixation rates from daytime incubations at nearby stations. In April 2007, the highest  $\text{N}_2$  fixation rates of up to  $22.77 \text{ nmol N L}^{-1} \text{ h}^{-1}$  were measured at the time-series Sta. 19 (transitional waters) and were similarly high during day and night. In September 2008, the highest  $\text{N}_2$  fixation rates for day and night measurements were  $5.05$  and

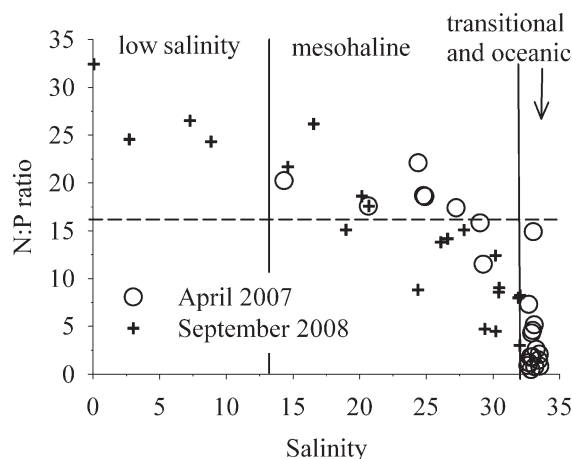


Fig. 3. N:P ratios in the different salinity ranges for both cruises. The dashed line marks the Redfield ratio of 16:1.

Table 3. Ranges, means, and standard deviations (SD) for variables for both investigations in April 2007 and September 2008, within different salinity ranges.

	April 2007						September 2008								
	Mesohaline (salinity 14.0–32.0)			Transitional (salinity 32.0–33.5)†			Oceanic (salinity >33.5)			Salinity <14.0			Mesohaline (salinity 14.0–32.0)‡		
	Range (mean±SD)	n		Range (mean±SD)	n		Range (mean±SD)	n		Range (mean±SD)	n		Range (mean±SD)	n	
Salinity	14.3–31.4 (24.4±5.6)	10		32.6–33.5 (33.0±0.3)	16		33.5–34.0 (33.8±0.2)	6		0.1–8.9 (4.2±3.9)	11		15.8–32.0 (25.7±5.0)	49	
Turbidity (NTU)	11.2–250.0 (68.8±68.4)	10		0.4–20.9 (6.2±5.5)	16		0.0–1.8 (0.5±0.6)	6		37.2–296.0 (194±107.7)	6		2.0–185.9 (26.3±35.0)	42	
Temperature (°C)	29.0–31.5 (30.1±0.7)	10		29.4–30.6 (29.9±0.4)	16		28.5–30.5 (29.4±0.7)	6		27.9–29.2 (28.4±0.3)	11		28.5–30.2 (28.9±0.4)	49	
Nitrate+nitrite ( $\mu\text{mol L}^{-1}$ )	4.2–19.6 (12.4±4.4)	8		0.0–1.0* (0.3±0.3)	13		0.0–1.0 (0.3±0.4)	6		17.3–22.3 (20.4±2.3)	4		1.1–13.0 (5.1±4.3)	17	
Phosphate ( $\mu\text{mol L}^{-1}$ )	0.4–1.0 (0.7±0.2)	8		0.1–0.2* (0.15±0.03)	13		Not detectable	6		0.6–0.9 (0.8±0.1)	4		0.1–0.7 (0.4±0.2)	17	
Silicate ( $\mu\text{mol L}^{-1}$ )	18.8–42.6 (32.0±9.2)	8		4.8–10.1* (7.7±1.7)	13		2.3–7.5 (3.5±1.9)	6		115.5–176.5 (151.9±259.0)	4		8.6–92.0 (39.4±27.1)	17	
N:P	11.5–22.1 (17.7±3.2)	8		0.8–5.2* (2.3±1.6)	12		—	—		24.3–32.4 (26.9±3.8)	4		3.0–26.2 (12.3±6.4)	17	
Si:N	2.1–4.5 (2.8±0.8)	8		4.0–49.3* (32.2±15.0)	13		2.8–49.3 (18.5±18.6)	4		6.7–8.9 (7.4±1.0)	4		4.6–17.2 (9.1±3.4)	17	
Chlorophyll <i>a</i> ( $\mu\text{g L}^{-1}$ )	2.2–3.9 (3.2±0.7)	5		1.4–3.0 (2.1±0.6)	9		0.2–0.4 (0.3±0.1)	4		0.3–1.0 (0.6±0.3)	4		0.2–1.3 (0.5±0.4)	15	
<i>N<sub>2</sub> fixation</i> ( $\text{nmol N L}^{-1} \text{h}^{-1}$ )															
<10 $\mu\text{m}$	0.08–1.57 (0.73±0.44)	5		2.63–14.36 (6.28±3.95)	8		1.07–10.80 (4.95±5.16)	3		—	—		—	—	
>10 $\mu\text{m}$	0.03–1.29 (0.40±0.51)	5		1.89–14.47† (8.06±4.00)	8		0.55–15.28 (5.61±8.38)	3		—	—		—	—	
Total	0.11–2.86 (1.13±1.04)	5		5.76–22.77† (14.33±5.08)	8		4.00–16.40 (10.55±6.24)	3		0.06–0.69 (0.26±0.29)	4		0.10–5.05 (1.81±1.70)	12	
Primary production ( $\mu\text{mol C L}^{-1} \text{h}^{-1}$ )															
<10 $\mu\text{m}$	0.01–0.07 (0.04±0.03)	4		0.01–0.25 (0.11±0.09)	6		0.01–0.04	2		—	—		—	—	
>10 $\mu\text{m}$	0.01–0.11 (0.06±0.04)	4		0.04–0.45 (0.24±0.19)	6		0.005–0.01	2		—	—		—	—	
Total	0.02–0.14 (0.11±0.06)	4		0.05–0.64 (0.34±0.28)	6		0.01–0.05	2		0.03–0.15 (0.07±0.06)	4		0.06–0.68 (0.23±0.17)	10	

\* Sta. 23, 24, and 25 (April 2007) are not included because of a complete change in weather conditions.

† Includes individual measurements at time-series Sta. 19.

‡ Includes individual measurements at time-series Sta. 28.

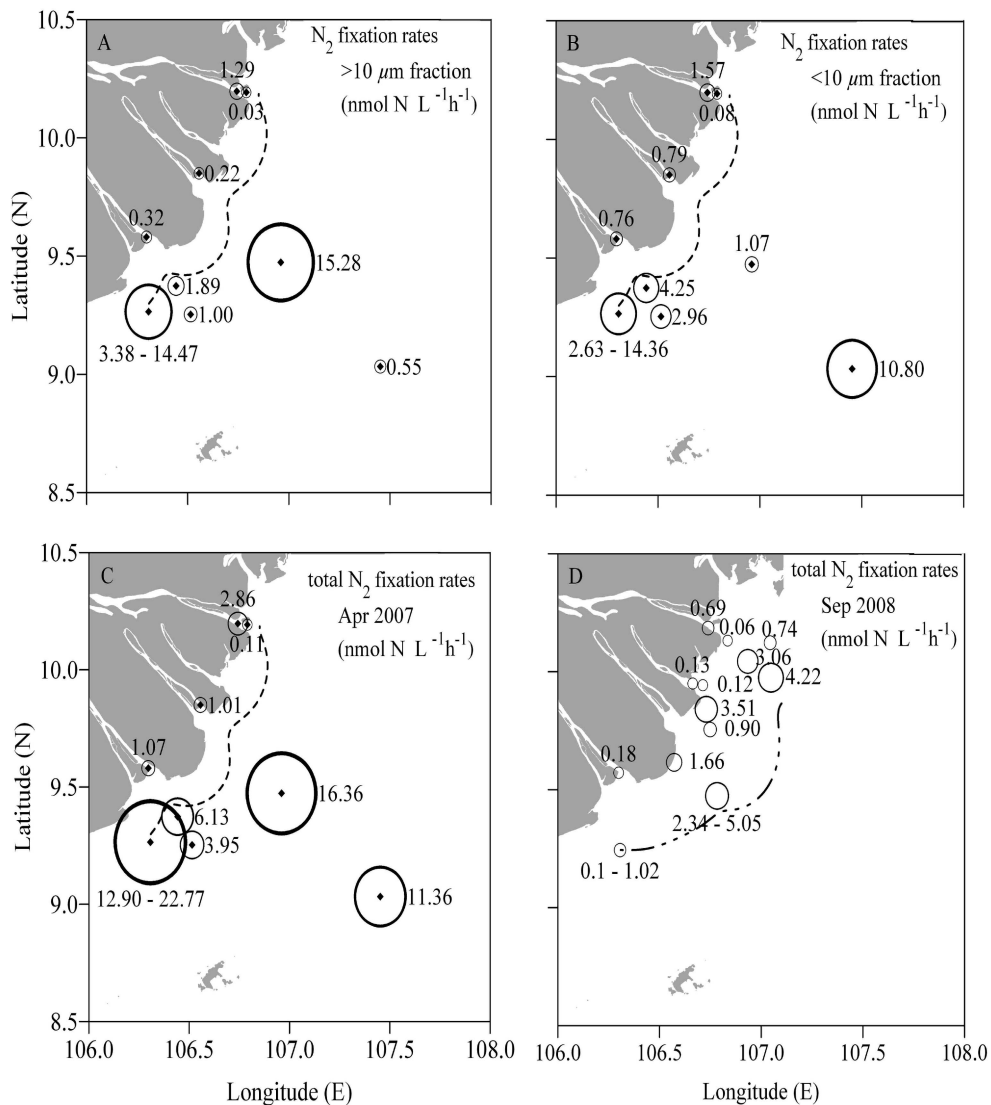


Fig. 4.  $N_2$  fixation rates for April 2007: (A)  $> 10\text{-}\mu\text{m}$  size fraction, (B)  $< 10\text{-}\mu\text{m}$  size fraction, and (C) total rates calculated by adding. (D) Total  $N_2$  fixation rates for September 2008. Symbols are scaled linearly proportional to the measured values. Rates at Sta. 19 (April 2007), 20, and 28 (September 2008) show the measured range; circle size corresponds to the mean rate. The dashed lines show the salinity boundary of 32.

$2.34 \text{ nmol N L}^{-1} \text{ h}^{-1}$ , respectively (both Sta. 20, mesohaline waters).

In April 2007 we conducted size fractionation to distinguish between unicellular diazotrophs and larger diazotrophs. Both size fractions contributed different fractions to the total  $N_2$  fixation, but averaged over the entire investigation area, the contributions were about equal (Table 3). For example, at Sta. 19,  $N_2$  fixation rates in the  $< 10\text{-}\mu\text{m}$  size fraction ranged between 2.63 and  $14.36 \text{ nmol N L}^{-1} \text{ h}^{-1}$  and between 3.38 and  $14.47 \text{ nmol N L}^{-1} \text{ h}^{-1}$  in the  $> 10\text{-}\mu\text{m}$  size fraction (Fig. 5).

We evaluated the effect of light or nutrient ratios on  $N_2$  fixation rates by plotting  $N_2$  fixation rates against turbidity and the ratio of  $\text{NO}_3 + \text{NO}_2$  and  $\text{PO}_4$  (N:P) (Fig. 6). In April 2007, the two water bodies with salinity either above (transitional and oceanic waters) or below 32 (mesohaline

waters) formed two distinctive clusters in terms of  $N_2$  fixation rates and abiotic variables. Low  $N_2$  fixation rates ( $< 3 \text{ nmol N L}^{-1} \text{ h}^{-1}$ ) co-occurred with salinities below 32, a turbidity above 10 NTU and N:P ratios above 10. Higher  $N_2$  fixation ( $> 3 \text{ nmol N L}^{-1} \text{ h}^{-1}$ ) rates were observed at salinities above 32, turbidities below 10 NTU, and N:P ratios below 10. In September 2008,  $N_2$  fixation rates corresponded similar to turbidity, and N:P ratios but fell in between the two clusters from April 2007 (Fig. 6).

Primary production rates in April 2007 were lower in waters with salinity below 32, whereas primary production in waters with a salinity above 32 was higher and more variable (Table 3). Similar to the  $N_2$  fixation rates, the time-series station exhibited the highest rates in primary production. In September 2008, primary production rates covered the entire range measured in April 2007.

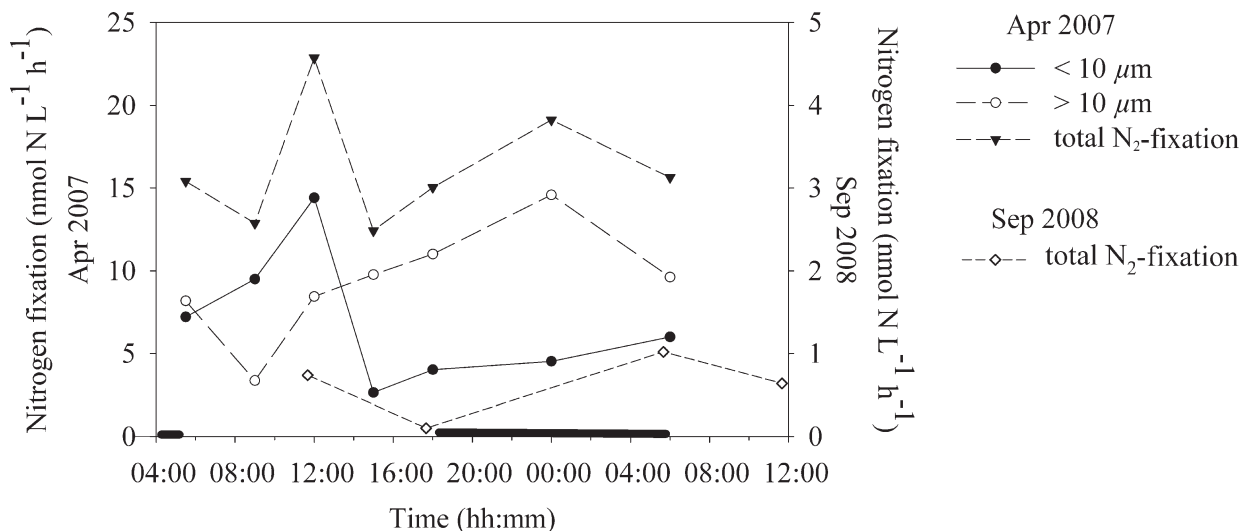


Fig. 5. Course of N<sub>2</sub> fixation (total and within the different size fractions) at time-series Sta. 19 (April 2007) and Sta. 28 (September 2008). Black bars indicate night hours.

*Phytoplankton species distribution*—For clarity, we classified the detected phytoplankton species into three different groups, that is, potentially symbiotic diatoms, asymbiotic diatoms, and filamentous cyanobacteria. Potentially symbiotic diatoms summarize the most common diatoms capable of hosting *Richelia intracellularis* and *Calothrix rhizosoleniae* (Carpenter 2002) and comprise *Rhizosolenia* spp., *Chaetoceros* spp., *Guinardia* spp., *Hemiaulus* spp., and *Bacteriastrium* spp. We did not microscopically investigate the presence of *Richelia intracellularis* or *Calothrix rhizosoleniae*, but nitrogenase (*nifH*) gene analysis confirmed the presence of certain heterocystous symbiotic cyanobacteria in the area (D. Bombar unpubl.), and here we rather show the distribution and abundance of diatoms that potentially hosted N<sub>2</sub>-fixing symbionts. All other detected diatom species were grouped as asymbiotic diatoms. Filamentous cyanobacteria included the diazotrophs *Trichodesmium eurythraeum* and *T. thiebautii*.

Diatoms (Bacillariophyceae) were the most abundant group at all stations during both investigations, but the dominating genera differed. In April 2007, *Skeletonema* sp., *Thalassionema* spp., and *Asterionella* sp. were the dominant asymbiotic diatoms. Highest abundances (reaching 30,000–55,000 cells L<sup>-1</sup>) were observed in mesohaline and transitional waters (at coastal Sta. 8, 18, 19, and 21) and decreased toward the open sea to below 1000 cells L<sup>-1</sup> (Sta. 11–15; Fig. 7A). In September 2008 the most abundant asymbiotic diatoms were the genera *Coscinodiscus*, *Thalassiosira*, and *Skeletonema*. The overall abundances of asymbiotic diatoms were lower and stayed below 10,000 cells L<sup>-1</sup> (except for Sta. 27–29, 35, and 37, where numbers ranged between 11,000 and 30,000 cells L<sup>-1</sup>). There was no offshore gradient visible as in April 2007, but numbers increased toward the southernmost Sta. 27 and 28. Overall, abundances of asymbiotic diatoms were higher in mesohaline waters (Fig. 7B).

Within the group of potentially symbiotic diatoms (Fig. 7C,D), the genus *Chaetoceros* dominated during both

investigations, with highest cell densities in transitional waters in April 2007 (up to 80,700 cells L<sup>-1</sup> at Sta. 19SP6) and in mesohaline waters in September 2008 (up to 58,000 cells L<sup>-1</sup> at Sta. 32). An exception was Sta. 23 (April 2007, transitional), where *Rhizosolenia* spp. dominated, and the mesohaline Sta. 10, 11, and 13 and Sta. 24 (salinity below 14) in September 2008, where *Guinardia* or *Hemiaulus* dominated. The second and third most abundant genera during both investigations were *Rhizosolenia* and *Bacteriastrium*. *Rhizosolenia* reached abundances of 6300 cells L<sup>-1</sup> (Sta. 10, oceanic) and 2850 cells L<sup>-1</sup> (Sta. 32, mesohaline) in April 2007 and September 2008, respectively. Cell densities of *Bacteriastrium* reached 5400 cells L<sup>-1</sup> (Sta. 19SP5, transitional) in April 2007 and 1800 cells L<sup>-1</sup> (Sta. 20, mesohaline) in September 2008. Overall, the abundances of potentially symbiotic diatoms seemed to increase along the flow path of the river plume (from the north toward the south) in April 2007 (Fig. 7C). In September 2008 only two stations showed high densities of symbiotic diatoms, Sta. 32 with 63,000 cells L<sup>-1</sup> and Sta. 34 with 44,000 cells L<sup>-1</sup>, both mesohaline waters north of the middle river arm.

Filamentous cyanobacteria were most abundant in transitional and oceanic waters in April 2007. We found highest trichome densities at transitional Sta. 9 and 16 (3300 and 5800 trichomes L<sup>-1</sup>, respectively; Fig. 7E). In September 2008 filamentous cyanobacteria of the genera *Anabaena* and *Spirulina* were encountered occasionally at low-salinity and mesohaline stations. *Trichodesmium* was present at three mesohaline stations. For all three genera, abundances were too low for a reliable count of cells or trichomes.

## Discussion

*Comparison of abiotic conditions during low and high discharge*—Differences in the freshwater supply of the Mekong River in the intermonsoon season in April and the SW monsoon season in September lead to a variable

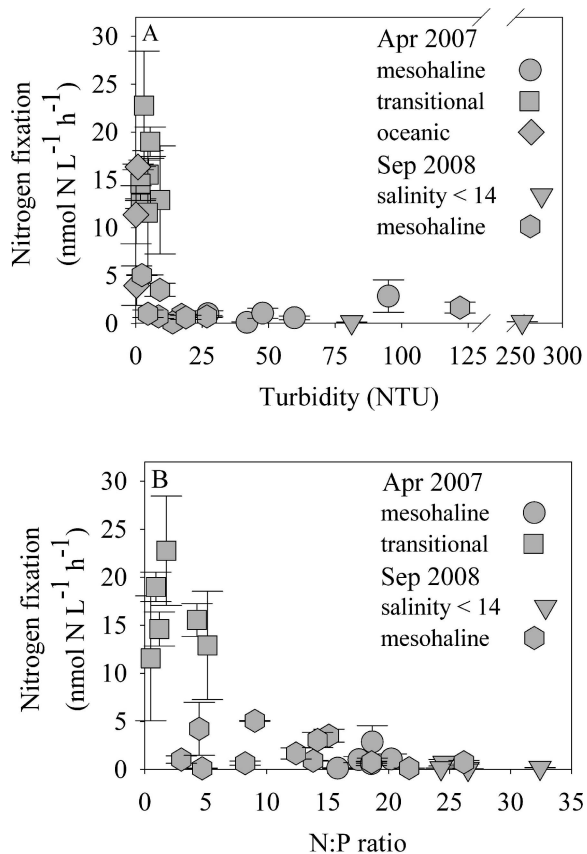


Fig. 6. (A) Relationship between turbidity and total  $N_2$  fixation rates. (B) Relationship between N:P ratio and total  $N_2$  fixation rates. Because of undetectable  $PO_4$  concentrations in oceanic waters, they are not included. Error bars indicate standard deviation of  $N_2$  fixation rates among triplicates.

extension of the river plume. In April the plume flows southward along the coast, and in September it reaches the oligotrophic waters in northeast direction (Voss et al. 2006). The region covered by our station grid extended into open sea waters in April 2007 but not in September 2008. We assume that the plume in September reached much farther offshore, although we collected waters only in the mesohaline parts of the plume. Thus, only mesohaline samples can directly be compared between the two cruises regarding all parameters. Mesohaline waters covered a larger area in September 2008, but the increase in salinity and the decrease in nutrient concentrations suggest that growth conditions were similar to April 2007. Concentrations of  $NO_3+NO_2 \leq 1.0 \mu\text{mol L}^{-1}$  can be considered to represent N limitation for phytoplankton (Goldman and Glibert 1983) but should not affect  $N_2$  fixation (Mulholland et al. 2001). N-limiting conditions were not found in the eutrophic mesohaline waters, but were found in transitional waters, which had detectable concentrations of  $PO_4$  and  $Si(OH)_4$ , and in oceanic waters, which were mostly depleted in both  $NO_3+NO_2$  and  $PO_4$  (Table 3).

The nutrient ratios can be used to further describe the differences in growth conditions for diazotrophs. The Mekong River water from the monitoring station MyTho (50 km upriver from Sta. 1) had a high N:P ratio of 42.8

and a low Si:N ratio of 4.2. These ratios clearly exceed those found at the river mouth stations in April 2007 (Sta. 1–3), where the N:P ratio was  $20.7 \pm 11.5$  (mean  $\pm$  SD;  $n = 3$ ) and the Si:N ratio was  $2.6 \pm 1.1$  ( $n = 3$ ). Similar N:P ratios of 30:1 were calculated from modeled TN and TP loads (Yoshimura and Takeuchi 2007). Data and model results suggest that the Mekong River water was actually P limited. The mechanisms by which the N:P ratios decreased toward higher salinities must remain speculative here but likely include denitrification in estuarine sediments as well as desorption of P from suspended particles or sediments as the river water mixed with ocean water (Seitzinger 1988; Jordan et al. 2008). The distribution patterns of N:P ratios show that during both low and high discharge, the mixing of Mekong and SCS ocean waters resulted in a mixed water body in which N:P ratios were lower than the Redfield ratio, indicating favorable conditions for diazotrophs (Table 3). As mentioned, nutrient concentrations alone would denote mesohaline waters as largely eutrophic and therefore as an unfavorable niche for diazotrophs. However, in September 2008, N:P ratios were below 16:1 at many mesohaline stations. This is interesting since in April 2007, N:P lower than 16:1 were almost exclusively found within the comparatively narrow salinity range of transitional stations (except for mesohaline Sta. 6, N:P = 11), while  $PO_4$  was undetectable at oceanic stations. Although we did not sample transitional and oceanic waters in September 2008, our findings imply that the part of the river plume in which conditions were favorable for  $N_2$  fixers covered a larger area in September 2008 than in April 2007. The  $Si(OH)_4$  concentrations in the freshwater from My Tho were about 4-fold higher than at coastal Sta. 1–3 (April 2007) but comparable to the  $Si(OH)_4$  concentrations in zero-salinity water in September 2008. High Si:N ratios should favor the growth of diatoms, as shown for a comparable environment with high freshwater inputs in the Gulf of Mexico (Turner et al. 2007). Si:N ratios tended to increase toward higher salinities in our investigation area, indicating  $Si(OH)_4$ -replete conditions coincident with N limitation. We assume that this selectively favored the growth of DDAs.

Another nutrient thought to be limiting for the growth of diazotrophs is iron (Mills et al. 2004); however, river plumes are generally not expected to be low in trace metals, and preliminary results from the Mekong plume in April showed total iron concentrations of 4–6 nmol L<sup>-1</sup> in surface waters (P. Croot unpubl.). Therefore, it seems unlikely that iron was limiting diazotroph growth during our investigation.

*Influence of the Mekong River plume on  $N_2$  fixation, primary production, and phytoplankton community composition*—As described previously, N:P ratios suggest that growth conditions were most favorable for diazotrophs in mesohaline waters in September 2008 and in transitional and oceanic waters in April 2007. And indeed, the highest rates of  $N_2$  fixation were found in these waters (Table 3; Fig. 4). While the exceptionally high  $N_2$  fixation rates coincided with oligotrophic conditions in transitional waters in April 2007, the highest rates found in September 2008 (between 1.66 and

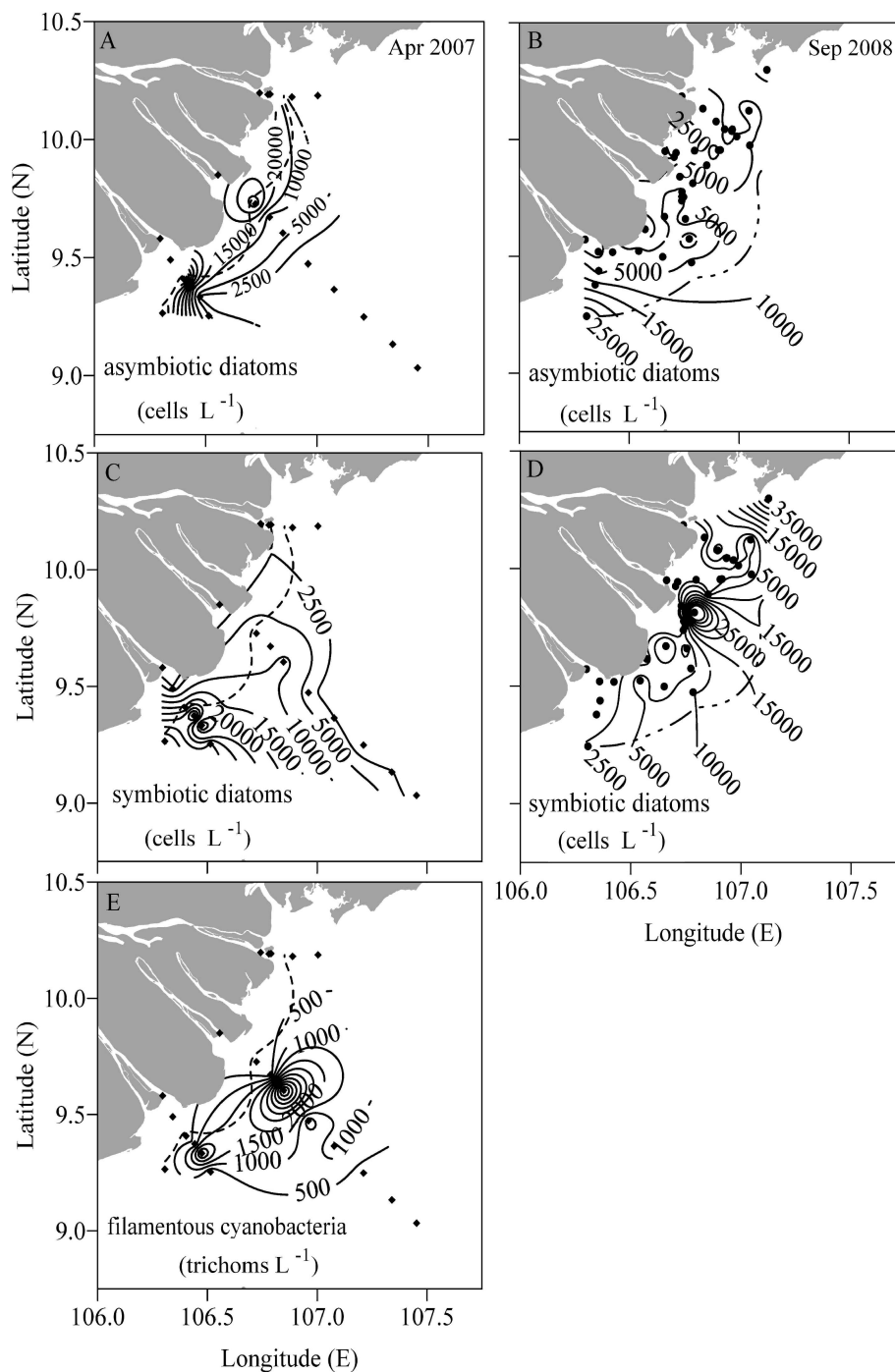


Fig. 7. Phytoplankton distribution in April 2007: (A) asymbiotic diatoms, (C) possible host species for *Richelia* and *Calothrix*, and (E) filamentous cyanobacteria. Phytoplankton distribution in September 2008: (B) asymbiotic diatoms and (D) possible host species for *Richelia* and *Calothrix*. Those species include *Chaetoceros* spp., *Rhizosolenia* spp., *Bacteriastrum* spp., *Hemiaulus* spp., and *Guinardia* spp. The dashed lines show the salinity boundary of 32.

5.05 nmol N L<sup>-1</sup> h<sup>-1</sup>) coincided with NO<sub>3</sub>+NO<sub>2</sub> concentrations between 1.3 and 4.3 μmol L<sup>-1</sup>, which can actually not be considered oligotrophic. However, the N:P ratios were always below 16:1 in these samples, suggesting that diazotrophs indeed benefited from a PO<sub>4</sub> surplus relative to phytoplankton nutrient requirements. The magnitude of the

rates found in these coastal waters is remarkable, considering that N<sub>2</sub> fixation is generally believed to be most important in areas having lowest concentrations of combined nitrogen (Capone and Carpenter 1982).

Asymbiotic diatoms were highly abundant in mesohaline waters, where the high concentrations of NO<sub>3</sub>+NO<sub>2</sub>, PO<sub>4</sub>,

and  $\text{Si}(\text{OH})_4$  presumably supported their growth. Abundances of over 20,000 cells  $\text{L}^{-1}$  were encountered close the coast in April 2007 and at southernmost stations in September 2008. At these stations, where no potential diazotrophs were found, we measured the lowest rates of  $\text{N}_2$  fixation ( $1.13 \pm 1.04$  nmol N  $\text{L}^{-1}$   $\text{h}^{-1}$  in April 2007). However, these rates are still similar to the highest  $\text{N}_2$  fixation rates of 1.2 nmol N  $\text{L}^{-1}$   $\text{h}^{-1}$  found by Voss et al. (2006) farther north in the SCS. Coinciding with highest rates of  $\text{N}_2$  fixation, abundances of potentially symbiotic diatoms were highest farther away from the coast in transitional (April 2007) and mesohaline waters (September 2008). These phytoplankton distributions and the high Si:N ratios in these waters further suggest that DDAs had a growth advantage. In contrast to the asymbiotic diatoms, abundances of DDAs seemed to increase along the flow path of the river plume (toward the south in April 2007 and toward the north in September 2008), possibly reflecting a phytoplankton community that underwent successive transformations as a response to changing nutrient conditions, including incipient N limitation and increasing Si:N ratios. Similar changes in the phytoplankton community were also seen along the Amazon River plume (Subramaniam et al. 2008) but on a much larger scale compared to the Mekong River outflow.

Unfortunately, we only speculate that the symbiotic diatoms indeed carried diazotrophic symbionts, but the picture is plausible for several reasons: (1) As mentioned, D. Bombar (unpubl.) showed that DDAs were present and actively fixed dinitrogen; (2) other studies in comparable environments found actively fixing DDAs (Foster et al. 2008); and (3) our high rates are in line with published ones from the Amazon plume. The highest  $\text{N}_2$  fixation rates encountered in the Mekong River plume were 14.47 nmol N  $\text{L}^{-1}$   $\text{h}^{-1}$  within the  $> 10\text{-}\mu\text{m}$  size fraction, and highest total rates were 22.77 nmol N  $\text{L}^{-1}$   $\text{h}^{-1}$  (April 2007). These values are very similar to rates found in a DDA bloom in the Amazon River plume ( $\sim 14$  nmol N  $\text{L}^{-1}$   $\text{h}^{-1}$ ; Carpenter et al. 1999) but are much higher than the mean values reported by Subramaniam et al. (2008) for the same area during different seasons ( $\sim 1$  nmol N  $\text{L}^{-1}$   $\text{h}^{-1}$ ). The high rates reported here seem rather unusual for unicellular species (Voss et al. 2004), although rates of the same magnitude were found, for example, in the pigment maximum of the Arafura Sea (Montoya et al. 2004). Blooms of *Trichodesmium* were not present in the mesohaline waters of the SCS in September 2008, further suggesting that DDAs were responsible for the high  $\text{N}_2$  fixation rates encountered. According to the *nifH* data by D. Bombar (unpubl.), *Calothrix* associated with *Chaetoceros* sp. was not abundant in the area, which is puzzling since *Chaetoceros* sp. was the most abundant diatom. It remains speculative if there were diazotroph symbionts associated with *Chaetoceros* and other diatoms in the SCS that were not detectable with the applied qPCR oligonucleotides. This has to be resolved in future studies.

High densities of *Trichodesmium* spp. tufts were exclusively observed in waters with salinities above 32 in April 2007. Thus, *Trichodesmium* seemed to be present mainly in full marine waters and occurred only sporadically in waters that

were influenced by the plume. This is in accordance with investigations in other river plumes (Carpenter et al. 1999; Foster et al. 2007, 2008). The absence of this typical marine diazotroph in mesohaline waters in September 2008 possibly shows that its growth was suppressed by the lower salinities and comparatively high concentrations of  $\text{NO}_3+\text{NO}_2$ .

Overall, it seems that a number of changes along the aging plume determined the species composition of the phytoplankton community in a similar way to the Amazon River plume (Subramaniam et al. 2008). Interestingly, the gradients in salinity and biogeochemical components seemed to be the same during both seasons, although the discharge volume was different. We had roughly 20 times higher discharge in September 2008, the plume was larger, and therefore the mesohaline, transitional, and open ocean water zones must have been larger as well. We suggest that the species composition found in April 2007 was also present in September 2008 but on larger spatial scales. This would include that DDAs became more abundant farther offshore from our easternmost station.

#### *Ecological importance of $\text{N}_2$ fixation in the Mekong plume—*

In the northern parts of the SCS,  $\text{N}_2$  fixation played a relatively minor role in satisfying the N demand of primary production (using a Redfield ratio of 6.6). Voss et al. (2006) reported values below 3% in the area between  $11^\circ\text{N}$  and  $13^\circ\text{N}$  when the Mekong River plume was not present, and Chen et al. (2004) published similar values for stations around  $20^\circ\text{N}$ . Even during the SW monsoon season, when the Mekong River plume was stimulating  $\text{N}_2$  fixation 200 km north of the river mouth,  $\text{N}_2$  fixation satisfied at most 8.2% of the N demand (Voss et al. 2006). The  $\text{N}_2$  fixation rates reported in this study supplied between 4.9% and 34.8% and between 1.0% and 21.7% of the total N demand of primary production in April 2007 and September 2008, respectively. These numbers are comparable to values calculated by Carpenter and others (1999) for the Amazon River plume and exceed values found in other parts of the Atlantic ( $\sim 12\%$  with  $\text{N}_2$  fixation rates of up to 2 nmol N  $\text{L}^{-1}$   $\text{h}^{-1}$ ; Voss et al. 2004) or in the Arabian Sea (13.5% with  $\text{N}_2$  fixation rates  $> 10$  nmol N  $\text{L}^{-1}$   $\text{h}^{-1}$ ; Capone et al. 1998).

As expected, the importance of  $\text{N}_2$  fixation as a nitrogen source for primary production was maximal in waters having N-limiting conditions, as shown by supplies of 13.3–34.8% of the N demand of primary producers in transitional waters (April 2007). It is noteworthy that this supply was still between 4.9% and 21.1% at mesohaline stations, where nutrient concentrations were high. In September 2008, N supplies by diazotrophs were in the lower range of those from April 2007 but increased with distance to the coast. We assume that in September 2008 the importance of  $\text{N}_2$  fixation in fueling primary production further increased in transitional waters, which we could not sample. All the aforementioned estimates of N supply are based on  $\text{N}_2$  fixation measured during the time of primary production, but we also report high  $\text{N}_2$  fixation rates from the night incubations. These rates were of the same magnitude as the rates measured during the day in the same area. DDAs are known to express *nifH* genes during the night (Church et al. 2005b), and we therefore propose that the rates measured during the night

have to be considered in the N supply estimates as well. This suggests that even 40–47% of the N demand of primary production may have been satisfied through this extra source of nitrogen. Our findings show that, overall, N<sub>2</sub> fixation is important in controlling the primary production in the Mekong River plume.

This study has presented a detailed analysis of the dynamics of N<sub>2</sub> fixation in the Vietnamese coastal waters affected by the Mekong River plume. A general picture seems to emerge for the Mekong River as well as other tropical rivers: They provide “new” nutrients, which are taken up quickly in the river mouth proximity and fuel phytoplankton growth, especially of diatoms. Additionally, farther offshore the excess in PO<sub>4</sub> and Si(OH)<sub>4</sub> seems to support N<sub>2</sub> fixation by a variety of diazotrophs. This may be a general phenomenon in tropical river plumes, which affect large areas of the ocean basins into which they discharge. The Amazon River plume covers thousands of square kilometers in the tropical North Atlantic but also in the SCS, and effects of the Mekong River discharge can be seen far offshore (Voss et al. 2006). In the SCS, there are pronounced differences in discharge of water and nutrients between different monsoon seasons, and therefore the importance of the Mekong River in influencing the oceanic N and C cycles will also differ between the seasons. We are only beginning to assess and understand these processes, but doing so seems critical for understanding the biogeochemistry of the SCS as well as for inferring hypotheses on how changes in land and water use will alter these complex dynamics.

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#### References

- BERGQUIST, B. A., AND E. A. BOYLE. 2006. Iron isotopes in the Amazon River system: Weathering and transport signatures. *Earth Planet. Sci. Lett.* **248**: 54–68, doi:10.1016/j.epsl.2006.05.004
- BONNET, S., I. C. BIEGALA, P. DUTRIEUX, L. O. SLEMONS, AND D. G. CAPONE. 2009. Nitrogen fixation in the western equatorial Pacific: Rates, diazotrophic cyanobacterial size class distribution, and biogeochemical significance. *Global Biogeochem. Cycles* **23**: GB3012, doi:10.1029/2008GB003439
- CAPONE, D. G., AND E. J. CARPENTER. 1982. Nitrogen fixation in the marine environment. *Science* **217**: 1140–1142, doi:10.1126/science.217.4565.1140
- , J. P. ZEHR, H. W. PAERL, B. BERGMAN, AND E. J. CARPENTER. 1997. *Trichodesmium*, a globally significant marine cyanobacterium. *Science* **276**: 1221–1229, doi:10.1126/science.276.5316.1221
- , AND OTHERS. 1998. An extensive bloom of the N<sub>2</sub>-fixing cyanobacteria *Trichodesmium erythraeum* in the central Arabian Sea. *Mar. Ecol. Prog. Ser.* **172**: 281–292, doi:10.3354/meps172281
- CARPENTER, E. J. 2002. Marine cyanobacterial symbioses. *Biol. Environ. Proc. R. Ir. Acad.* **102B**: 15–18, doi:10.3318/BIOE.2002.102.1.15
- , J. P. MONTOYA, J. BURNS, M. R. MULHOLLAND, A. SUBRAMANIAM, AND D. G. CAPONE. 1999. Extensive bloom of a N<sub>2</sub>-fixing diatom/cyanobacterial association in the tropical Atlantic Ocean. *Mar. Ecol. Prog. Ser.* **185**: 273–283, doi:10.3354/meps185273
- CHEN, Y.-L. L., H.-Y. CHEN, D. M. KARL, AND M. TAKAHASHI. 2004. Nitrogen modulates phytoplankton growth in spring in the South China Sea. *Cont. Shelf Res.* **24**: 527–541, doi:10.1016/j.csr.2003.12.006
- CHURCH, M. J., B. D. JENKINS, D. M. KARL, AND J. P. ZEHR. 2005a. Vertical distributions of nitrogen-fixing phylotypes at Stn ALOHA in the oligotrophic North Pacific Ocean. *Aquat. Microb. Ecol.* **38**: 3–14, doi:10.3354/ame038003
- , C. M. SHORT, B. D. JENKINS, D. M. KARL, AND J. P. ZEHR. 2005b. Temporal patterns of nitrogenase gene (*nifH*) expression in the oligotrophic North Pacific Ocean. *Appl. Environ. Microbiol.* **71**: 5362–5370, doi:10.1128/AEM.71.9.5362-5370.2005
- CLEAN WATER TEAM (CWT). 2004. Turbidity fact sheet, FS-3.1.5.0 (Turb). In *The clean water team guidance compendium for watershed monitoring and assessment, version 2.0*. Division of Water Quality, California State Water Resource Control Board. Available from [http://www.swrcb.ca.gov/water\\_issues/programs/swamp/docs/cwt/guidance/3150fs.pdf](http://www.swrcb.ca.gov/water_issues/programs/swamp/docs/cwt/guidance/3150fs.pdf)
- DEUTSCH, C., J. L. SARMIENTO, D. M. SIGMAN, N. GRUBER, AND J. P. DUNNE. 2007. Spatial coupling of nitrogen inputs and losses in the ocean. *Nature* **445**: 163–167, doi:10.1038/nature05392
- DIPPNER, J. W., K. V. NGUYEN, H. HEIN, T. OHDE, AND N. LOICK. 2007. Monsoon-induced upwelling off the Vietnamese coast. *Ocean Dyn.* **57**: 46–62, doi:10.1007/s10236-006-0091-0
- FANG, W., G. FANG, P. SHI, Q. HUANG, AND Q. XIE. 2002. Seasonal structure of upper layer circulation in the southern South China Sea from in-situ observations. *J. Geophys. Res.* **107**: 3202, doi:10.1029/2002JC001343
- FOSTER, R. A., AND G. D. O’MULLAN. 2008. Nitrogen-fixing and nitrifying symbioses in the marine environment, p. 1197–1218. In D. G. Capone, D. Bronk, M. Mulholland, and E. J. Carpenter [eds.], *Nitrogen in the marine environment*, 2nd ed. Elsevier Science.
- , A. SUBRAMANIAM, C. MAHAFFEY, E. J. CARPENTER, D. G. CAPONE, AND J. P. ZEHR. 2007. Influence of the Amazon River plume on distribution of free-living and symbiotic cyanobacteria in the western tropical North Atlantic Ocean. *Limnol. Oceanogr.* **52**: 517–532.
- , A. SUBRAMANIAM, AND J. P. ZEHR. 2008. Distribution and activity of diazotrophs in the Eastern Equatorial Atlantic. *Environ. Microbiol.* **11**: 741–750, doi:10.1111/j.1462-2920.2008.01796.x
- , AND J. P. ZEHR. 2006. Characterization of diatom-cyanobacteria symbioses on the basis of *nifH*, *hetR* and 16S rRNA sequences. *Environ. Microbiol.* **8**: 1913–1925, doi:10.1111/j.1462-2920.2006.01068.x
- GOLDMAN, J. C., AND P. M. GLIBERT. 1983. Kinetics of inorganic nitrogen uptake by phytoplankton, p. 233–274. In E. J. Carpenter and D. G. Capone [eds.], *Nitrogen in the marine environment*. Academic Press.
- GRASSHOFF, K. 1983. Determination of nutrients, p. 125–188. In K. Grasshoff, M. Ehrhardt, and K. Kremling [eds.], *Methods of seawater analysis*, 2nd ed. Verlag Chemie.
- HALLEGRAEFF, G. M. 1995. Harmful algal bloom: A global overview, p. 1–22. In G. M. Hallegraeff, D. M. Anderson, and A. D. Campbell [eds.], *Manual on harmful marine microalgae*. IOC Manual and Guides, No. 33. UNESCO.

- HELLERMAN, S., AND M. ROSENSTEIN. 1983. Normal monthly wind stress over the world ocean with error estimation. *J. Phys. Oceanogr.* **13**: 1093–1104, doi:10.1175/1520-0485(1983)013<1093:NMWSOT>2.0.CO;2
- HOA, L. T. V., N. H. NHAN, E. WOLANSKI, T. T. CONG, AND H. SHIGEKO. 2007. The combined impact on the flooding in Vietnam's Mekong River delta of local man-made structures, sea level rise, and dams upstream in the river catchment. *Estuar. Coast. Shelf Sci.* **71**: 110–116, doi:10.1016/j.ecss.2006.08.021
- JANSON, S., J. WOUTERS, B. BERGMAN, AND J. C. CARPENTER. 1999. Host specificity in the *Richelia*-diatom symbiosis revealed by *hetR* gene sequence analysis. *Environ. Microbiol.* **1**: 431–438, doi:10.1046/j.1462-2920.1999.00053.x
- JONES, G. B., C. BURDON-JONES, AND F. G. THOMAS. 1982. Influence of *Trichodesmium* red tides on trace metal cycling at a coastal station in the Great Barrier Reef Lagoon. *Oceanol. Acta* **4**: 319–326.
- JORDAN, T. E., J. C. CORNWELL, W. R. BOYNTON, AND J. T. ANDERSON. 2008. Changes in phosphorus biogeochemistry along an estuarine salinity gradient: The iron conveyor belt. *Limnol. Oceanogr.* **53**: 172–184.
- KORZUN, V. I. 1978. World water balance and water resources of the earth, p. 375–387. *In* V. I. Korzun [ed.], UNESCO series studies and reports in hydrology, No. 25. UNESCO.
- MILLS, M. M., C. RIDAME, M. DAVEY, J. LA ROCHE, AND R. J. GEIDER. 2004. Iron and phosphorus co-limit nitrogen fixation in the eastern tropical North Atlantic. *Nature* **429**: 292–294, doi:10.1038/nature02550
- MOISANDER, P. H., R. A. BEINART, M. VOSS, AND J. P. ZEHR. 2008. Diversity and abundance of diazotrophic microorganisms in the South China Sea during intermonsoon. *ISME J.* **2**: 954–967, doi:10.1038/ismej.2008.51
- MONTOYA, J. P., C. M. HOLL, J. P. ZEHR, A. HANSEN, T. A. VILLAREAL, AND D. G. CAPONE. 2004. High rates of N<sub>2</sub> fixation by unicellular diazotrophs in the oligotrophic Pacific Ocean. *Nature* **430**: 1027–1031, doi:10.1038/nature02824
- , M. VOSS, P. KÄHLER, AND D. G. CAPONE. 1996. A simple, high-precision, high-sensitive tracer assay for N<sub>2</sub> fixation. *Appl. Environ. Microbiol.* **62**: 986–993.
- MULHOLLAND, M. R. 2007. The fate of nitrogen fixed by diazotrophs in the ocean. *Biogeoscience* **4**: 37–51.
- , K. OHKI, AND D. G. CAPONE. 2001. Nutrient controls on nitrogen uptake and metabolism by natural populations and cultures of *Trichodesmium* (Cyanobacteria). *J. Phycol.* **37**: 1001–1009, doi:10.1046/j.1529-8817.2001.00080.x
- NGUYEN, A. D., H. H. G. SAVENJE, D. N. PHAM, AND D. T. TANG. 2008. Using salt intrusion measurements to determine the freshwater discharge distribution over the branches of a multi-channel estuary: The Mekong Delta case. *Estuar. Coast. Shelf Sci.* **77**: 433–445, doi:10.1016/j.ecss.2007.10.010
- NITTROUER, C. A., G. J. BRUNSKILL, AND A. G. FIGUEIREDO. 1995. Importance of tropical coastal environments. *Geo-Mar. Lett.* **15**: 121–126.
- REVELANTE, N., AND M. GILMARTIN. 1982. Dynamics of phytoplankton in the Great Barrier Reef lagoon. *J. Plankton Res.* **4**: 47–76, doi:10.1093/plankt/4.1.47
- SEITZINGER, S. P. 1988. Denitrification in freshwater and coastal marine ecosystems: Ecological and geochemical significance. *Limnol. Oceanogr.* **33**: 702–724, doi:10.4319/lo.1988.33.4\_part\_2.0702
- SUBRAMANIAM, A., AND OTHERS. 2008. Amazon River enhances diazotrophy and carbon sequestration in the tropical North Atlantic Ocean. *PNAS* **105**: 10460–10465, doi:10.1073/pnas.0710279105
- TURNER, R. E., N. N. RABALAIS, R. B. ALEXANDER, G. MCISAAC, AND R. W. HOWARTH. 2007. Characterization of nutrient, organic carbon, and sediment loads and concentrations from the Mississippi River into the Northern Gulf of Mexico. *Estuar. Coasts* **30**: 773–790.
- UTERMÖHL, V. H. 1958. Quantitative estimation of phytoplankton abundance and volume. *Mitt. Int. Ver. Theor. Angew. Limnol.* **9**: 1–38. [In German.]
- VILLAREAL, T. 1992. Marine nitrogen-fixing diatom-cyanobacteria symbioses, p. 163–175. *In* E. J. Carpenter, D. G. Capone, and J. G. Rueter [eds.], *Marine pelagic cyanobacteria: Trichodesmium and other diazotrophs*. Kluwer.
- VOSS, M., D. BOMBAR, N. LOICK, AND J. DIPPNER. 2006. Riverine influence on nitrogen fixation in the upwelling region off Vietnam, South China Sea. *Geophys. Res. Lett.* **33**: L07604, doi:10.1029/2005GL025569
- , P. CROOT, K. LOCHTE, M. MILLS, AND I. PEEKEN. 2004. Patterns of nitrogen fixation along 10°N in the tropical Atlantic. *Geophys. Res. Lett.* **31**: L23S09, doi:10.1029/2004GL020127
- WASMUND, N., I. TOPP, AND D. SCHORIES. 2006. Optimising the storage and extraction of chlorophyll samples. *Oceanologia* **48**: 125–144.
- YOSHIMURA, C., AND K. TAKEUCHI. 2007. Estimation of nutrient runoff processes in the Mekong River Basin using a distributed hydrological model. *J. Jpn. Soc. Hydrol. Water Resour.* **20**: 493–504, doi:10.3178/jjshwr.20.493

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