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# Phytoplankton phosphorus limitation in a North Atlantic coastal ecosystem not predicted by nutrient load

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Phytoplankton nutrient limitation patterns were investigated in an anthropogenically influenced coastal ecosystem with a high nutrient load. Weekly nutrient limitation bioassays, and water chemistry and stoichiometry measurements were performed in the Bay of Brest, France, from February to July 2011. Each limitation bioassay included phosphorus, nitrogen, silicate and all possible combined nutrient additions, and lasted over 72 h. Results showed that the phytoplankton community experienced a general P limitation from March to July. N limitation alone was observed only during 1 week in early March. Subsequently, all limitation bioassays revealed primary P limitation, indicated by significantly increased growth rates in all samples containing P additions. Besides P, Si was the second limiting nutrient for phytoplankton during 2 weeks at end of May to mid-June. Seston C:P ratios ranged between 198:1 and 749:1, and N:P ratios between 28:1 and 104:1. The highest effects on phytoplankton growth were reached with NPSi addition, suggesting synergistic macronutrient effects under single nutrient limitation. Our results demonstrate that dissolved nutrient ratios are not reliable in predicting actual phytoplankton nutrient limitation, since other processes and organisms strongly influence nutrient availability and resupply. The dominant P limitation emphasizes its importance for consideration in coastal water management.

**KEYWORDS:** phytoplankton; stoichiometry; bioassays; nutrient limitation; coastal ecosystem

## INTRODUCTION

Global changes in phytoplankton primary productivity throughout the world's oceans have become more evident in recent years. Although in the open ocean, phytoplankton biomass seems to be declining (Boyce *et al.*, 2010) and areas of low productivity expanding (Polovina *et al.*, 2008), eutrophication through human nutrient supply is the prevailing situation in coastal areas (Nixon, 1995; Beman *et al.*, 2005). Human influence through nutrient loading will increase in future with continued human population growth in coastal areas coupled with growing economic activity (Tilman *et al.*, 2001; Millennium Ecosystem Assessment, 2005). Coastal ecosystems suffer from nitrogen (N) and/or phosphorus (P) enrichment (Howarth, 1988; Nixon, 1995) and the resulting consequences of phytoplankton nutrient limitation (Ryther and Dunstan, 1971; Smith, 1984; Smith, 2006). Therefore, coastal zones are a focus to improve their water management by reducing nutrient enrichment caused by waste water and agricultural runoff. It is known that nutrient enrichment may lead to altered biogeochemical functioning and biological community structure of coastal waters (Cloern, 2001), such as increases in absolute phytoplankton biomass (Ryther and Dunstan, 1971; Beman *et al.*, 2005), oxygen depletion (Rabalais *et al.*, 2002), changes in community composition (harmful algal blooms) (Paerl, 1988; Heisler *et al.*, 2008) or changes in biomass stoichiometry (Downing, 1997; Klausmeier *et al.*, 2004a). In this context, one key topic of coastal studies is to assess system-specific responses of coastal ecosystems to nutrient enrichment and limitation of primary production. However, actual phytoplankton nutrient limitation patterns in nutrient enriched coastal environments have seldom been analysed in detail by comparing *in situ* nutrient limitation patterns with biogeochemical components (Granéli *et al.*, 1990; Tamminen and Andersen, 2007).

Nutrient limitations are often observed in phytoplankton communities, and can eventually lead to growth constraints (Hessen *et al.*, 2004), and stoichiometric changes in phytoplankton biomass (Hecky and Kilham, 1988; Klausmeier *et al.*, 2004a). Nutrients that can potentially limit primary ocean productivity include N, P, silicate (Si) and iron (Fe) (Hecky and Kilham, 1988; Boyd *et al.*, 2000; Leblanc *et al.*, 2005). Frequently, it is not just one nutrient that limits phytoplankton growth (Hecky *et al.*, 1993; Leynaert *et al.*, 2004; Harpole *et al.*, 2011), and nutrient limitation patterns change between consecutive seasons (D'Elia *et al.*, 1986; Fisher *et al.*, 1999) or even years (Granéli *et al.*, 1990). Typically, it is thought that freshwater systems are P limited, whereas estuarine and marine systems tend to be N limited (Hecky and Kilham,

1988; Elser *et al.*, 2007). Nutrient limitations in freshwater commonly become apparent in phytoplankton stoichiometry (Elser *et al.*, 2000), but phytoplankton in the marine environment appear to be more conservative and elemental stoichiometry seems less variable compared with freshwater seston (Hecky *et al.*, 1993; Sterner *et al.*, 2008; Trommer *et al.*, 2012). On average, the C:N:P stoichiometric ratio in marine seston lies between 106:16:1 and 166:20:1 (Redfield, 1958; Sterner *et al.*, 2008), with C:Si and N:Si ratios of 7.69 and 0.95, respectively, in planktonic diatoms (Brzezinski, 1985).

Phytoplankton physiological nutrient requirements are in general species specific (Quigg *et al.*, 2003; Lagus *et al.*, 2004). However, phytoplankton communities exhibit nutrient limitations that are primarily dependent on the temporal (Tyrrell, 1999) and spatial distribution of dissolved nutrients (Weber and Deutsch, 2010). Shallow coastal zones are particularly affected by river run off and turn over events, which underlie high seasonal fluctuations of dissolved nutrient availability (D'Elia *et al.*, 1986; Rudek *et al.*, 1991; Fisher *et al.*, 1992). In this process, N and P are of particular interest for phytoplankton nutrient limitation in estuaries and have been proposed for dual management strategies to reduce eutrophication (Howarth and Marino, 2006; Smith, 2006; Paerl, 2009). Additionally, attention should be placed on Si as the potential limiting nutrient for diatoms, since human activities have mainly influenced N and P loads in coastal waters, but not Si (Cloern, 2001). Fluctuations in nutrient availability can be manifested in the elemental composition of the phytoplankton community (Karl *et al.*, 2001; Klausmeier *et al.*, 2004a). However, the incorporating elemental ratio in phytoplankton biomass is also dependent on light intensity (Sterner *et al.*, 1997; Klausmeier *et al.*, 2004a) and dissolved nutrient concentrations (Klausmeier *et al.*, 2004b), and can be altered by growth rates (Klausmeier *et al.*, 2004a) and community species composition (Arrigo *et al.*, 1999; Rousseau *et al.*, 2002).

It has been shown that dissolved nutrient ratios independent of the Redfield ratio indicate phytoplankton nutrient limitation in some ecosystems (Ryther and Dunstan, 1971; Granéli *et al.*, 1990), but this does not apply for all regions (Tamminen and Andersen, 2007), probably since phytoplankton communities adapt their requirements according to the nutrient load (Schindler, 1977; Glibert and Burkholder, 2011). Although high nutrient load can result in increased algal biomass (Howarth, 1988; Beman *et al.*, 2005), the relationship between nutrient load, phytoplankton stoichiometry and nutrient limitation is complex. Studies to assess the role of nutrients in coastal areas are crucial; watersheds are in critical need of appropriate management actions, and research must be conducted to

evaluate required nutrient inputs. Data from a comprehensive nutrient limitation study in the Baltic Sea show that phytoplankton nutrient limitations cannot be inferred from dissolved nutrient ratios alone (Tamminen and Andersen, 2007), and N:P indicators, including particulate and dissolved nutrient components, have the highest explanatory power for *in situ* nutrient limitations (Ptacnik *et al.*, 2010). These studies should be given the highest priority for coastal water management; however, comparable studies in marine ecosystems with high temporal resolution remain lacking.

The well-investigated Bay of Brest represents a showcase of the eastern North Atlantic coastal ecosystems. The semi-enclosed bay is an agriculturally influenced area, which is characterized by river run off with high nitrogen load ( $\sim 200 \mu\text{M}$ ) (Ragueneau *et al.*, 1994). Several studies have been performed describing the seasonal variations in nutrient load in the bay with maximum nutrient concentrations during winter of  $\sim 20 \mu\text{M}$  N and  $\sim 0.5 \mu\text{M}$  P (Queguiner and Treguer, 1984; Ragueneau *et al.*, 1994). Phytoplankton succession indicates a diatom-dominated community with several biomass peaks during spring. Typically, the tidal range prevents the onset of bay stratification, and through most of the year the water column is well mixed (Ragueneau *et al.*, 1994; LePape *et al.*, 1996). From the 1970s, a continued increase in nitrogen loading was observed, leading to nutrient input imbalance. This nutrient discrepancy suggested potential Si limitation (an increase in the N:Si molar ratio), and a shift in the phytoplankton population with a greater likelihood of harmful algal blooms. Si deficiencies have been observed (Del Amo *et al.*, 1997; Ragueneau *et al.*, 2002), but diatoms still dominate the bay phytoplankton until mid-summer (Del Amo *et al.*, 1997; Beucher *et al.*, 2004). Until 1993, the bay has not yet suffered severe eutrophication (LePape *et al.*, 1996), indicated by strong increase in phytoplankton biomass likely to cause oxygen depletion. It is hypothesized that macrotidal dynamics in the bay itself, and short water residence time are responsible for preventing eutrophication in the bay (LePape *et al.*, 1996). Ragueneau *et al.* (Ragueneau *et al.*, 2002, 2005) emphasized the role of an invasive filter feeder, the slipper limpet *Crepidula fornicata*, in the silica cycle of the bay. In this context, the scientific attention in the Bay of Brest has focused mainly on the biogeochemical silica cycle coupled with phytoplankton dynamics.

In the present study, nutrient limitation patterns were evaluated on natural phytoplankton communities in an anthropogenic influenced coastal ecosystem in an exceptional three level approach from winter to summer 2011. Until now, similar investigations were conducted on the West Atlantic coast with bioassays with temporally limited resolution and focusing in most cases on N and P

(e.g. Fisher *et al.*, 1992; Rabalais *et al.*, 2002; Heil *et al.*, 2007). We performed sea water analyses in the Bay of Brest over a 20-week period and evaluated phytoplankton nutrient limitation patterns by comparison of (i) weekly *in situ* nutrient limitation bioassays in a full factorial design containing N, P and Si, (ii) dissolved nutrient ratios, and additional (iii) seston stoichiometric nutrient ratios. The data set provides high-resolution insights into phytoplankton nutrient limitation patterns during the main growth period in a coastal ecosystem exposed to anthropogenic nutrient loading and changing nutrient availability.

## METHOD

### Sampling procedure

Sampling was conducted within the framework of the CHIVAS program (“CHImie des VALves de la coquille Saint-Jacques européennes, archive multiproxies de la dynamique phytoplanktonique”) at a study site in the southern Bay of Brest on the French Atlantic coast ( $48^{\circ}17.750'N$ ,  $4^{\circ}27.50'W$ ; mean water depth 11 m; see map in Foullaron *et al.*, 2007). The bay is  $180 \text{ km}^2$  in size and is characterized by a coastal macrotidal system, exhibiting maximum tidal amplitudes of over 8 m during spring (Chauvaud *et al.*, 2000). The adjoining Iroise Sea is connected via a narrow strait (1.8 km width, 50 m depth) and allows rapid mixing with Atlantic Ocean water. Main freshwater input is provided by the Aulne and Elorn rivers. Water sampling was performed from the French R.V. “Albert Lucas”, weekly at mid-tide from 15th February 2011 until 4th July 2011, covering the main growth period and thus potential shifts in nutrient limitations. The sampling frequency was increased to two times per week during the most productive period (from March through May). Temperature and salinity data were acquired using a CTD (Sea-Bird SBE-911). Nutrients, chlorophyll *a* and particulate matter were measured from surface water collected with a 12 L Niskin bottle.

In the laboratory, 500 mL of surface water was filtered onto glass-fibre filters (GF/F Whatman) for chlorophyll *a* analysis. Chlorophyll *a* concentration was determined after extraction in 90% acetone, and fluorescence measurements in a Turner Design fluorometer. Two hundred fifty millilitres of surface water was fixed with Lugol's solution, and phytoplankton counts were carried out using an inverted microscope, and subsequently analysed with the Utermöhl method (Utermöhl, 1958). Relative abundances of algal groups by biovolume were determined after cell volume determination of the most abundant species (30–50 specimens) (Hillebrand *et al.*, 1999) and in

the case of less abundant species based on volumes determined in previous years. Dissolved  $\text{NO}_3$ ,  $\text{NO}_2$  and  $\text{Si}(\text{OH})_4$  concentrations were measured on a Technicon Auto Analyser II (Tréguer and Le Corre, 1975);  $\text{NH}_4$  on a Technicon Auto Analyser III (Aminot and Kérouel, 2007). Soluble phosphate was determined by filtering 100 mL of surface water through glass-fibre filters (GF/F Whatman). The filtrate was immediately frozen, and later analysed by the colorimetric method via a molybdate reaction following sulphuric acid digestion (Murphy and Riley, 1962).

Seston particulate organic carbon (POC) and particulate organic nitrogen (PON) were determined following filtration of 150 mL of surface water through pre-combusted glass-fibre filters (GF/F Whatman) using a CHN elemental analyser (EC Thermo Finnigan Flash 1112). Seston particulate phosphorus (PP) was determined by filtering 100 mL of surface water through pre-combusted acid washed glass-fibre filters (GF/C Whatman), and measured spectrophotometrically via a molybdate reaction following sulphuric acid digestion (Murphy and Riley, 1962). Biogenic silica (BSi) was determined from particulate matter collected by filtration of 1 L of surface water through 0.6  $\mu\text{m}$  polycarbonate membrane filters. Analyses were performed using the double alkaline leaching technique (Ragueneau and Treguer, 1994), and the Si/Al ratio was used to correct for mineral interference.

### Nutrient limitation bioassays

Immediately after return to the laboratory, *in situ* nutrient limitation bioassays were performed including eight nutrient treatments (two replicates per week). Due to logistical reasons, bioassays were performed once a week over the entire observation period. Twenty millilitres of 100  $\mu\text{m}$  pre-filtered surface water was filled in each of 16 vials. Two vials served as a control (i.e. they did not receive any nutrient treatments). In 14 vials, N, P and Si were added as nutrient pulses [three times the average natural winter concentration: 60  $\mu\text{M}$  N (1:1 molar solution of  $\text{KNO}_3$  and  $(\text{NH}_4)_2\text{SO}_4$ ), 1.5  $\mu\text{M}$  P (as  $\text{KH}_2\text{PO}_4$ ), 30  $\mu\text{M}$  Si (as  $\text{Na}_2\text{SiO}_3$ )] in the following combinations: N, P, Si, NP, NSi, SiP and NPSi (two replicates of each). Raw fluorescence (as a proxy for chlorophyll *a* content) was measured with an Aqua Pen-C AP-C 100 (Photon Systems Instruments, Brno, Czech Republic) fluorometer at 455 nm excitation wavelength. The “raw fluorescence” value of the Aqua Pen device is the integrated area of the chlorophyll *a* fluorescence transient (OJIP) curve, from which background fluorescence has automatically been subtracted. The conversion to chlorophyll *a* was calculated according to the company’s internal calibration. An

intercalibration of chlorophyll *a* from acetone extracted filters and Aqua Pen fluorescence data of cultures and natural phytoplankton samples showed a high degree of correlation  $r^2 > 0.85$  (Pondaven, unpublished data). Prior to the experiments, nutrient limitation bioassays were tested on different sample volumes (20 mL, 100 mL, 1000 mL; Stibor, unpublished results) with the Aqua Pen fluorometer, indicating no obvious bottle effects based on volumetric differences within 72 h. These results agree with studies, which show that incubation growth effects are not dependent on bottle volume (Hammes *et al.*, 2010) and phytoplankton nutrient limitation patterns can be reliably predicted by using small sample volumes over short time spans (50 mL, 48 h) (Yin *et al.*, 2001). To avoid misleading nutrient limitation results from endpoint sampling, we measured the raw fluorescence immediately following the nutrient pulse additions at 0 h, and subsequently after 24, 48 and 72 h ( $\pm 1$  h). In between measurements, all 16 vials were incubated at 16°C and 80  $\mu\text{M}$  quanta  $\text{m}^{-2} \text{s}^{-1}$  (12 h light/dark cycle). These climate chamber conditions are known from earlier experiments at the institute, to support good growth conditions for phytoplankton of the Bay of Brest.

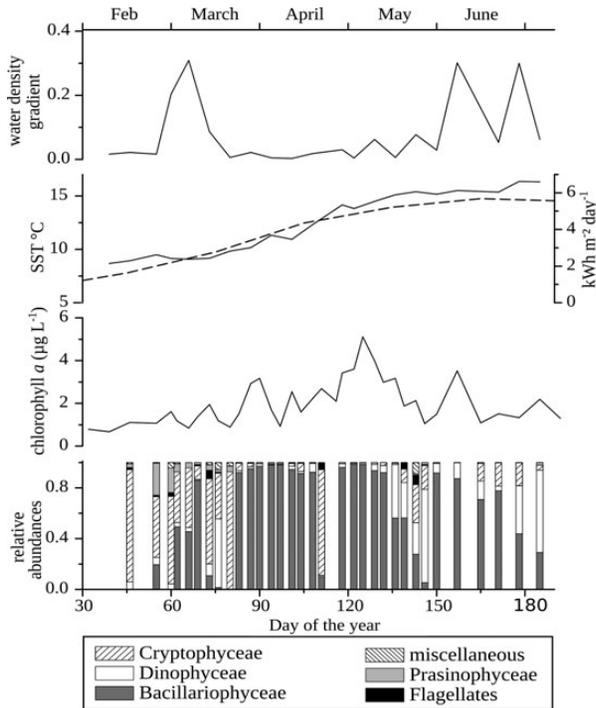
### Data analysis

The vertical water density gradient was used to calculate a stability index as the difference between surface and bottom water density ( $\delta\sigma$ ) divided by water depth (h) (Cloern, 1991). Linear regression slopes of nutrient limitation bioassays were calculated from ln-transformed raw fluorescence data over 72 h and used as an indicator of phytoplankton growth response. The nutrient limitation bioassays were statistically analysed with the STATISTICA software package by factorial analysis of variance with N, P and Si as categorical predictors. As response variable linear regression slopes of bioassays over 72 h were used. Significant differences between treatments were calculated with Fisher-LSD *post hoc* test and homogeneity of variance was tested with Levene’s test. As an additional parameter for nutrient limitation evaluation, the relative effects of nutrient addition compared with controls after 72 h were calculated [“effect size” =  $\text{LN}(\text{chlorophyll } a \text{ nutrient addition}/\text{chlorophyll } a \text{ control})$ ]. The highest size effect indicates the most limiting nutrient combination at the end of the bioassay.

## RESULTS

During the observation period, the mean vertical water density gradient was weak ( $< 0.31 \text{ kg m}^{-4}$ ), indicating a

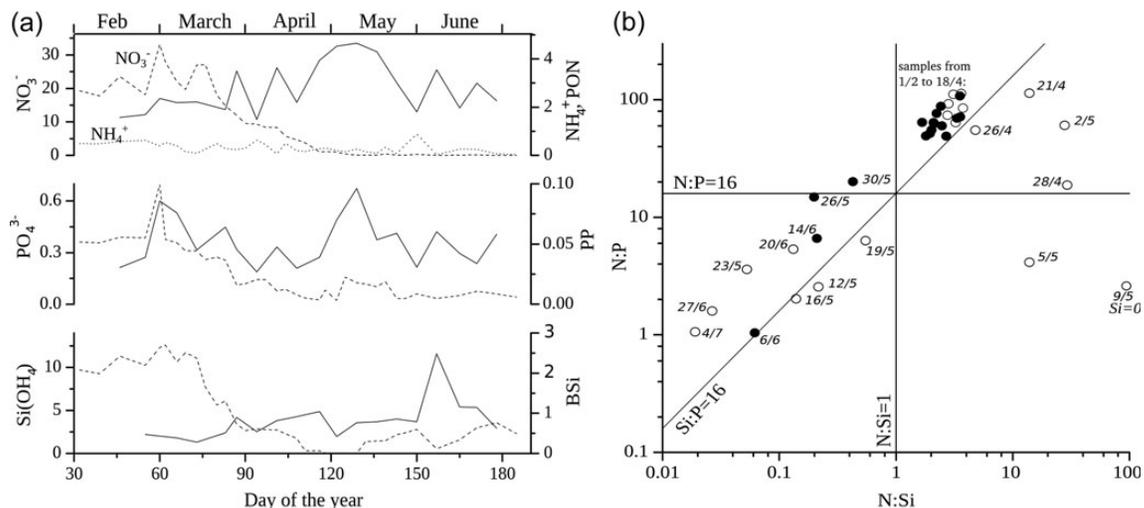
well mixed water column (Fig. 1). Small stratification peaks were measured at the beginning of March and in June (Fig. 1). Coincident with an increase in solar radiation, sea surface temperatures showed a steady increase,



**Fig. 1.** Temporal development of water density gradient ( $\text{kg m}^{-4}$ ), sea surface temperature, solar insolation (average 2001–2010; PVGIS Solar Radiation Database, <http://re.jrc.ec.europa.eu/pvgis/solres/solrespvgis.htm>), chlorophyll *a* and relative abundances of phytoplankton groups derived from biovolume during the observation period 2011.

and the chlorophyll *a* gradient exhibited the highest peak at the beginning of May with maximum values of  $5.1 \mu\text{g L}^{-1}$  and lower peaks in late-March and early-June (Fig. 1). Dominant phytoplankton groups included Bacillariophyceae, Cryptophyceae and Dinophyceae. At the beginning of the observation period, Cryptophyceae were the most dominant phytoplankton group, which were subsequently outcompeted by Bacillariophyceae starting in March. Diatoms clearly dominated the three visible chlorophyll *a* maxima at end of March, early-May and early-June (Fig. 1), which consisted of *Cerataulina* sp., *Chaetoceros* sp., *Dactyliosolen* sp., *Guinardia* sp., *Leptocylindrus* sp., *Pseudonitzschia* sp. and *Rhizosolenia* sp. as the main species. During the observation period, some harmful phytoplankton species were present (*Pseudonitzschia* sp., *Dinophysis acuminata*, *Cochlodinium* sp., *Gyrodinium* sp.), but made on average <1% of the total biomass. Only on 9 May 2011, *Dinophysis acuminata* accounted for ~8% of the total biomass. Towards the end of the observation period, Dinophyceae gained relatively more importance in total biomass (Fig. 1).

At the beginning of the study,  $\text{NO}_3^-$  concentrations were generally over  $20 \mu\text{M}$  and declined after the middle of March (Fig. 2a).  $\text{NH}_4^+$  concentrations remained steady throughout the study period ( $\pm 0.5 \mu\text{M}$ ). Initial  $\text{PO}_4^{3-}$  concentration was between  $0.35$  and  $0.4 \mu\text{M}$ , which subsequently declined after the beginning of March to almost a zero value by the end of the study period (Fig. 2a).  $\text{Si}(\text{OH})_4$  values were  $>10 \mu\text{M}$  until the middle of March, when a rapid decline was observed. By early-May  $\text{Si}(\text{OH})_4$  was below detection limit (Fig. 2a), but fluctuated around  $2.5 \mu\text{M}$  from mid-May until



**Fig. 2.** (a) Dissolved (dashed, dotted lines) and particulate (solid lines) nutrients (N, P and Si) in  $\mu\text{M}$  versus time. (b) Dissolved nutrient ratio relationships with dates. Circles indicate dates of primary phosphorus limitation as revealed from nutrient limitation bioassays (Table I). Solid lines indicate Redfield ratios.

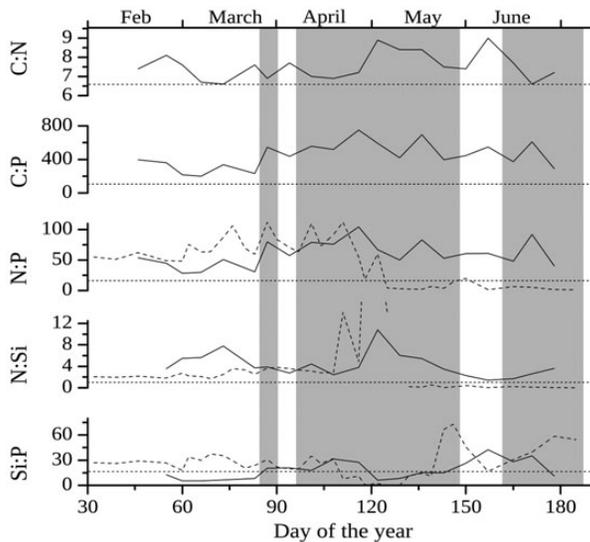
early-July. Total dissolved N:P ratios ranged from 50:1 and 130:1 until the beginning of May, and declined to values below 16:1 (Fig. 2b) between May and July (except 30 May 2011). A similar trend was observed in N:Si ratios, which exceeded 1:1 until the beginning of May, then declined to values below 1:1.

PP measurements were approximately 0.05  $\mu\text{M}$  over the observation period (the sample of 4 July 2011 was lost), indicating a homogeneous P content in phytoplankton despite fluctuations in the dissolved nutrient concentrations (Fig. 2a). PON measurements fluctuated between 1.5 and 4.7  $\mu\text{M}$ , with an average of 2.8  $\mu\text{M}$ , and a maximum in the second week of May (9 May 2011) (Fig. 2a). POC ranged between 11 and 40  $\mu\text{M}$  with a maximum in early-May (data not shown), which was consistent with the chlorophyll *a* peak. BSi fluctuated between 0.28 and 1.15  $\mu\text{M}$  with a maximum value at 2.48  $\mu\text{M}$  at the beginning of June (Fig. 2a). The resulting stoichiometry of seston C:P (C:N) ratios ranged between 198:1 (6.6:1) and 749:1 (9:1), with an average of 445:1 (7.5:1), indicating higher values than Redfield ratios (Fig. 3). Excess N conditions in the Bay of Brest were clearly evident in seston N:P and N:Si ratios, which fluctuated around 59:1 (between 28:1 and 104:1) and 4.3:1 (between 1.4:1 and 11:1) (Fig. 3). Seston Si:P fluctuated between 5:1 and 42:1, showing two increases in mid-April (31:1) and early June (42:1) (Fig. 3).

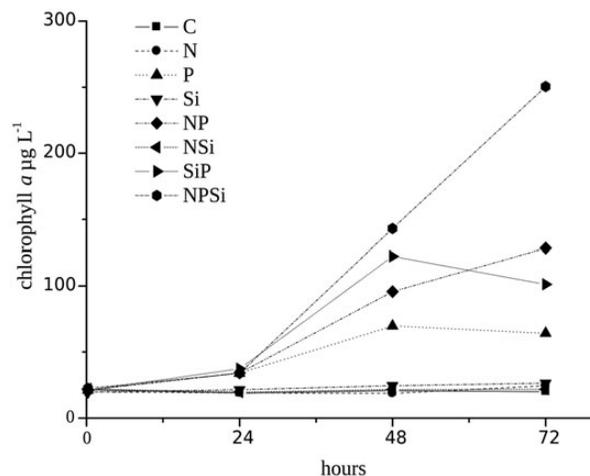
Concerning the nutrient limitation bioassays, variance homogeneity of linear regression slopes was fulfilled for the data set, using ln-transformed raw fluorescence data over 72 h ( $n = 320$ ,  $P = 0.797$ ). Factorial ANOVA of

bioassays revealed a dominant P limitation in the phytoplankton community from mid-March to July (ANOVA results of entire data set:  $P = 0.00$ ,  $F = 26.85$ ,  $r^2 = 0.38$ ; Fisher-LSD *post hoc* test  $P < 0.001$  for P, NP, SiP and NPSi addition), whereas N and Si played a minor role as limiting nutrients. In general, growth responses were highest in bioassays with P addition from week 5 to week 20 (Table I). Until the second week in March, no significant nutrient limitation could be observed. N limitation was only observed in week 4, whereas in week 5, P limitation was taking over. From then on, significant enhanced growth responses in all samples with P addition (see example in Fig. 4) in weeks 7, 9–15 and 18–20 suggest primary P limitation under natural conditions (Fig. 5). Only in late-May and at beginning of June did Si seem to be a secondary limiting nutrient as indicated in some significantly higher regression slopes (Table I). In most bioassays, samples with addition of all three nutrients (NPSi) resulted in the highest biomass after 72 h (Table I).

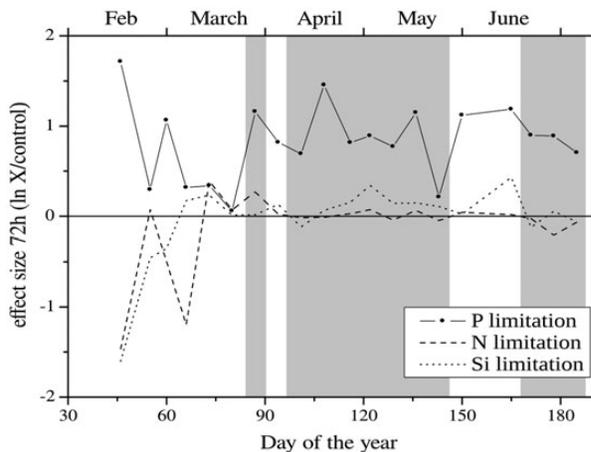
Based on our results summarized in Table I, we can sub-divide different phases of P limitation. During the first 6 weeks (mid-February until late-March), bioassays showed enhanced growth rates in P addition bioassays, but no significant dominance of P limitation was observed (only weeks 1, 5 and 6 show some significance) (Table I). However, highest effects on phytoplankton growth after 72 h included P addition in all six cases (single P addition in weeks 1 and 3, P combinations in weeks 2 and 4–6) (Table I). Seston stoichiometric C:P and N:P ratios in this period revealed persistent P deficiencies (Fig. 3), as well as dissolved N:P ratios (Fig. 2b). In this late winter to early spring period with relatively high available nutrient concentrations, P limitations were predominantly observed in



**Fig. 3.** Seston stoichiometry (solid lines) and dissolved nutrient ratios (dashed lines) versus time. Horizontal lines indicate Redfield ratios. Grey boxes indicate periods of primary phosphorus limitation as revealed from nutrient limitation bioassays (Table I).



**Fig. 4.** Typical bioassay example (5 May 2011) with all P addition bioassays increasing and the NPSi addition assay with highest effect at 72 h.



**Fig. 5.** Effect sizes of single P, N and Si nutrient additions on phytoplankton biomass after 72 h compared with control. Horizontal line at zero indicates no effect relative to control. Grey boxes indicate periods of primary phosphorus limitation as revealed from nutrient limitation bioassays (Table I).

stoichiometric ratios, and weakly in nutrient limitation bioassays. The second phase is dominated by primary P limitation during 9 weeks from the end of March until end of May (weeks 7–15), when P limitations significantly dominated the nutrient limitation bioassays (Table I, Fig. 5). Furthermore, high seston C:P and N:P ratios indicated severe P limitation (Fig. 3). The dissolved N:P ratio did not show a severe P limitation during this period, and even pointed towards N deficiency from 5 May 2011 until the end of the observation period (Figs 2b and 3). Dissolved Si:P ratios exhibited a similar pattern at the beginning of May, and dropped below 2:1, suggesting a Si limitation (Fig. 3). The second phase includes the chlorophyll *a* maximum and shows a decline in diatom abundance (Fig. 1) with declining Si:P ratios (Fig. 3). During the third phase (late-May through July), the phytoplankton nutrient limitation pattern remained dominated by primary P limitation, as indicated again by bioassays and seston stoichiometric ratios (Fig. 3). Si limitation appeared to play a role in the meantime, since Si and NSi addition increased the phytoplankton growth during weeks 16 and 17, respectively (Table I). Diatom abundances were low during this time. Dissolved nutrient ratios indicated a prevalent N limitation due to N:P ratios lower than 16:1 (Figs 2b and 3).

## DISCUSSION

The evaluation of phytoplankton nutrient limitation patterns is a precondition to regulate coastal ecosystem nutrient balance. Until now, coastal ecosystem experiments on nutrient limitation spanning several months in high

temporal resolution have rarely been conducted, although highly requested to prevent eutrophication (Millennium Ecosystem Assessment, 2005; Carpenter, 2008). Our study emphasizes the role of phosphorus in a North Atlantic coastal ecosystem. Indications for a dominant coastal spring P limitation were already found and described from similar anthropogenically influenced coastal ecosystems as for example the Mississippi River outflow (e.g. Rabalais *et al.*, 2002) and the Chesapeake Bay (e.g. Fisher *et al.*, 1992; Fisher *et al.*, 1999). Our results provide robust high-resolution data over one season, which support the increasing attention given to phosphorus in coastal marine ecosystems. We observed a continuous dominant phytoplankton P limitation from spring to summer, and no change in nutrient limitation patterns after the decrease of dissolved nutrient concentrations. During the significant P limitation period starting in late-March, seston N:P ratios were particularly high, exceeding critical N:P limitation ratios of 45:1 (Klausmeier *et al.*, 2004a) (Fig. 3). Biases in the interpretation of nutrient limitation arising from simple nutrient analyses or discrete bioassay sampling should be largely diminished, since the phytoplankton nutrient limitation analyses were simultaneously carried out on stoichiometry and bioassays under controlled laboratory conditions and at high temporal resolution.

Absolute dissolved nutrient concentrations during the observed period agree with previous studies (Queguiner and Treguer, 1984; Ragueneau *et al.*, 1994) and suggest no increase in total nutrient load from riverine input during the last ~30 years. The dissolved nutrient ratios clearly showed N excess until the beginning of May (Fig. 2b). The dissolved Si:P ratios indicated Si scarcity and P excess at the end of April and beginning of May, and a tendency towards Si excess during June and July (Fig. 2b). However, our results show that dissolved nutrient ratios poorly represent actual phytoplankton P limitation, which was derived by nutrient limitation bioassays and confirmed by seston stoichiometry. The combined analysis of dissolved nutrient ratios, seston stoichiometry and bioassays confirms a certain reservation of dissolved nutrient ratios as indicative of nutrient limitation (Smith, 1984). Our work supports a Baltic Sea study (Tamminen and Andersen, 2007), which showed that dissolved nutrient ratios often do not represent the actual nutrient limitations that affect natural phytoplankton populations. Our results demonstrated that during the spring bloom period, P remained the significantly limiting nutrient when chlorophyll *a* values were highest, although Si scarcity (Si:P < 1, N:Si > 1) was indicated during early May (Fig. 2). In the Bay of Brest, dissolved N:Si ratios of > 1 lead some authors (Hafsaoui *et al.*, 1985; Del Amo *et al.*, 1997) to the conclusion that silicate serves an

important nutrient, which is limiting for phytoplankton in this diatom-dominated environment (Ragueneau *et al.*, 2002). Del Amo (Del Amo, 1996) demonstrated Si limitation from kinetic uptake experiments using the <sup>32</sup>Si radioactive isotope, but its influence compared with other nutrients on total phytoplankton biomass production was not investigated. During our observation period, we found similar dissolved N:Si ratios, but seston stoichiometric measurements and nutrient limitation bioassays clearly revealed P as a limiting factor for biomass growth from winter to summer 2011. That dissolved nutrient ratios alone are not indicative of the actual phytoplankton, nutrient limitation in our study also becomes clear when considering the phytoplankton community composition. As a consequence of high diatom abundance (Fig. 1), Si was diminished, starting at the end of March (Fig. 2a), leading to dissolved nutrient ratios of Si:P < 1 and N:Si > 1, thus indicating Si limitation by end of April and early May (Fig. 2b and 3). However, nutrient limitation bioassays show that *in situ* phytoplankton actually experienced P limitation (Table I), which remains constant even after Si removal. Interestingly, diatom abundance started to decline in early May in accordance with negligible low Si concentrations (Fig. 2a), low biomass Si:P (Fig. 3) and low dissolved Si:P ratios (Fig. 2b). During the diatom decline, dissolved N:Si ratios switched from > 1 to < 1 (Fig. 2b), indicating favourable conditions for diatom growth. However, diatoms continue to decline and *in situ* nutrient limitation (Table I) as

well as biomass C:P and N:P ratios (Fig. 3) indicate constant P limitation. At the end of May and beginning of June, Si and NSi seem to play additional roles as second limiting factors besides P when diatoms were attaining higher abundances again (see BSi peak in early June in Fig. 2a). During this time, dissolved nutrient ratios resulted in Si:P > 1 and N:Si < 1, suggesting sufficient Si for diatoms to grow, but bioassays indicate nevertheless a stimulating effect of Si besides P during 2 weeks. These results reveal that bioassays include important information about *in situ* phytoplankton nutrient limitation, which cannot be derived from nutrient ratios and taxon abundances alone. Subsequently during June and July, the diatom decline seemed evidently independent from Si availability (Si:P > 1 and N:Si < 1, Fig. 2) and Cryptophyceae and Dinophyceae comprised a relatively greater proportion of total biomass during the later phase of P limitation (Fig. 1). This summer succession, besides a common year round diatom dominance, was observed earlier and explained by P competition under nutrient co-limitation (Beucher *et al.*, 2004). Secondary to Si limitation, N and P were classified as important limiting nutrients for phytoplankton in the Bay of Brest (Hafsaoui *et al.*, 1985; Del Amo *et al.*, 1997; Beucher *et al.*, 2004). On the contrary, our limitation results emphasize a primary P limitation in the Bay of Brest over the observed period (Fig. 5), and subordinate N and Si limitation in March and June (Table I). Over the study, no changes in nutrient load of the ecosystem were observed, since the absolute values

Table I: Statistical results of nutrient limitation bioassays: factorial ANOVAs (on regression slopes of ln-transformed raw fluorescence data over 72 h), post hoc comparisons (Fisher LSD) of all nutrient additions, and respective nutrient treatment with highest effect on biomass after 72 h

Date	Week	Day of the year	ANOVA	N	P	Si	NP	NSi	SiP	NPSi	Highest effect after 72 h
15 February 2011	1	46	***		***						P
24 February 2011	2	55									SiP
01 March 2011	3	60									P
07 March 2011	4	66	*	*				+			NPSi
14 March 2011	5	73	**	**	*		***		*	***	NPSi
21 March 2011	6	80	**				**		*	**	NP
28 March 2011	7	87	**		**		*		**	***	NPSi
04 April 2011	8	94	**		+				*	**	NPSi
11 April 2011	9	101	***		***		***		***	***	NPSi
18 April 2011	10	108	***		***		***		***	***	NPSi
26 April 2011	11	116	**		*		**		*	***	NPSi
02 May 2011	12	122	***		***		***		***	***	NPSi
09 May 2011	13	129	***		***		***		***	***	NPSi
16 May 2011	14	136	***		***		***		***	***	NPSi
23 May 2011	15	143	***		**		***		**	***	NP
30 May 2011	16	150	***		***		***	***	+	***	NPSi
14 June 2011	17	165	***		***	**	***		***	***	NPSi (after 96 h)
20 June 2011	18	171	***		**		***		***	***	NPSi
27 June 2011	19	178	***		***		***		***	***	NPSi
04 July 2011	20	185	***		***		***		***	***	NPSi (after 48 h)

Bold: primary phosphorus limitation, including exclusively all phosphorus addition combinations (P, NP, PSi, NPSi). Asterisks indicate significance levels: +0.05 < P < 0.1, \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001.

of nutrient concentrations remained within the same magnitude. That the actual nutrient limitations seem in most of our cases not analogous to the implied nutrient limitations derived from dissolved nutrient ratios suggests that phytoplankton nutrient limitation analysis should include seston particulate stoichiometry and bioassays to define the actual nutrient limitation as accurately as possible. To fully explain the observed nutrient limitation patterns, the influence of other factors such as food web composition and interactions, and nutrient recycling, have to be taken into account to explain the discrepancies in limitation results of older studies.

One hypothesis for silica recycling in the Bay of Brest was associated with the gastropod *Crepidula fornicata* (Chauvaud *et al.*, 2000; Ragueneau *et al.*, 2002), which serves as a suspension feeder and active silicate pump. Since silicate was primarily classified as a limiting nutrient in the past, Si recycling seemed to guarantee continuous diatom presence, and even dominance (Ragueneau *et al.*, 2002). In the present study, our results suggest a remaining dominant P limitation after Si draw down, which probably points towards a strong Si recycling through *Crepidula fornicata*, and even a complete compensation of the former Si limitation for phytoplankton. Ragueneau *et al.* (Ragueneau *et al.*, 2002) reported high Si recycling from late-July, but high recycling might be occurring from as early as the end of May. However, biogenic BSi dissolution might also have contributed to a diminished Si limitation, since it occurs soon after the first diatom maximum in the Bay of Brest and may resupply dissolved Si as early as May (Beucher *et al.*, 2004). Another explanation would be high *Crepidula fornicata* abundances, which were shown to increase the Si benthic flux (Ragueneau *et al.*, 2005). In Bay of Brest mesocosm experiments (Fouillaron *et al.*, 2007), it was shown that gastropod abundances altered the magnitude of the phytoplankton bloom but not the long-term persistence of diatom dominance, implying that sufficient total Si was present, and strong nutrient recycling fertilized phytoplankton. Kinetic uptake experiments clearly indicated phytoplankton Si limitation during the mesocosm experiments (Fouillaron *et al.*, 2007). The fact that, in our study, P limitation was the dominant nutrient limitation may on the one hand be due to stronger Si recycling in 2011 than that which was observed before. On the other hand, since the kinetic uptake experiments were not performed with other nutrients than Si (Fouillaron *et al.*, 2007), their results may not necessarily reflect a comprehensive evaluation of total phytoplankton nutrient limitation. However, in contrast to the use of Si mainly by diatoms, it is important to emphasize that nutrients such as N and P are required by all species. When nutrient recycling from gastropods is involved and N is available from river discharge, P is likely to become limiting in the first place.

Herbivorous zooplankton are also a strong sink for N and P, which provides nutrients to phytoplankton due to nutrient recycling and so alters the dissolved nutrient ratios. Copepods, for example, have relatively high N demands (Hessen and Lyche, 1991; Gismervik, 1997) compared with appendicularians (Sommer *et al.*, 2004) or cladocerans with higher P demands (Gismervik, 1997). In general, phytoplankton with high N:P stoichiometry is considered to have low food quality (Sterner and Elser, 2002; Andersen *et al.*, 2004), and should therefore affect organisms with high P demand in particular. It has been suggested that the seston C:P ratio of higher than 330, as in our study, has a strong potential for the occurrence of P-based food quality constraints on growth of zooplankton (Elser and Hassett, 1994). In theory, copepods with high N demands should have an advantage in the Bay of Brest due to high N input, because the stoichiometric mismatch between copepods (with high body N:P ratio) and phytoplankton (with high biomass N:P ratios) is low. In 2004, copepods (*Acartia* sp., *Paracalanus* sp., among others) represented a large zooplankton group in the bay (up to 40%), but appendicularians (*Oikopleura* sp.) and *Noctiluca* sp. reached peak abundances of a respective 55 and 80% of the zooplankton in early summer (June) (Fouillaron *et al.*, 2007; Pondaven, unpublished data). Sommer *et al.* (Sommer *et al.*, 2004) found that copepods increased phytoplankton N limitation via a trophic cascade effect. However, N limitation would probably not be maintained in this N excess environment like the Bay of Brest with riverine input. Recently, Trommer *et al.* (Trommer *et al.*, 2012) reported that natural copepod as well as rotifer concentrations are able to shift phytoplankton nutrient limitation patterns towards co-limitation by nutrient recycling, particularly during non-Redfield conditions. In our study, results indicated that in 18 out of 20 cases primary phytoplankton P limitation was co-limitation (Table I, highest effect), and consumer-driven nutrient recycling might therefore have played a significant role. In addition, higher trophic levels such as fish, which exhibit considerable P demands (Hjerne and Hansson, 2002), can theoretically also be affected by a low trophic transfer of P in the food web based on P limited, high N:P phytoplankton. The combined nutrient cycling interactions in the food web contribute to nutrient limitation patterns observed in phytoplankton, and thus should be considered before drawing conclusions based on dissolved nutrient load alone.

The identification of P as limiting factor for phytoplankton growth in this coastal environment has implications for local water management (Fisher *et al.*, 1992). To date, the main focus in attempts to avoid eutrophication has been on nitrogen supply, since due to the utilization of agricultural fertilizers, reactive nitrogen is easily distributed

by rivers or via precipitation into ecosystems, which enhances primary productivity (Millennium Ecosystem Assessment, 2005; Schlesinger, 2009). In contrast, phosphorus is mainly accessible through weathering processes of rocks, but can also be anthropogenically mobilized, and transported into coastal ecosystems. Although nitrogen is regarded as the limiting nutrient in most marine ecosystems (Howarth, 1988; Howarth and Marino, 2006; Elser *et al.*, 2007), the role of phosphorus as a limiting nutrient in coastal environments seems underestimated (Carpenter, 2008). For example, the proposed resistance to eutrophication in the Bay of Brest, “despite” high N concentrations (LePape *et al.*, 1996), is, among other factors, likely due to low P nutrient loading. In coastal ecosystems known to contain high P concentrations such as the Gulf of California, agricultural N supply through river run-off does indeed lead to large phytoplankton blooms (Beman *et al.*, 2005). Our results indicate that in coastal ecosystems with high N nutrient loading, like the Bay of Brest, eutrophication seems rather to be dependent on the P supply, similar to the classical limnological understanding (Lampert and Sommer, 2007). The high N supply in the Bay of Brest through winter and spring leads to a high stoichiometric imbalance of dissolved nutrients with N:P ratios of higher than 20:1 (Hafsaoui *et al.*, 1985; Ragueneau *et al.*, 1994; LePape *et al.*, 1996; this study). Although our study shows that the dissolved nutrient ratios do not necessarily point towards actual phytoplankton limitation, it indicates the logical cause and effect case of high N load followed by primary P limitation during this time of the year. Stoichiometric imbalance with high N:P ratios in an anthropogenic influenced estuary and the resulting phytoplankton P limitation has already been observed in the Paerl River estuary (Yin *et al.*, 2001). During the summer months, June to August, the dissolved N:P ratio in the Bay of Brest seems to point towards N limitation (Ragueneau *et al.*, 1994; LePape *et al.*, 1996), which is however not reflected in our bioassay and biomass stoichiometry results. Since the primary P limitation is characterized by nutrient co-limitation (Table I), nutrient recycling of other trophic levels might account for our observed pattern during June. More importantly, nutrient recycling of zooplankton and zoobenthos would then act as a buffer to the predicted nutrient limitation by dissolved nutrients in this coastal environment. In the case of the Bay of Brest, P supply should be given greater attention in order to maintain the present ecological balance, which is already showing the first signs of eutrophication at the macrobenthos level (Chauvaud *et al.*, 2000).

In any case, in order to mitigate phytoplankton blooms, coastal water management should always address both N

and P supply after including *in situ* phytoplankton nutrient limitation measurements. It has already been strongly emphasized by several authors that the exclusive focus on the reduction of one nutrient load is inadequate to prevent eutrophication (e.g. Smith, 2006; Schindler *et al.*, 2008; Paerl, 2009). Synergistic effects of nutrients may result in altered ecosystem processes (Howarth and Marino, 2006; Elser *et al.*, 2007) and therefore P management should be maintained to guarantee long-term phytoplankton production sustainability (Schindler *et al.*, 2008). This is similarly important in relation to harmful algal blooms, since not just the total quantity but also the nutrient pool composition influences such blooms (Heisler *et al.*, 2008). Evidently, shifts towards lower Si:P ratios may favour harmful algae blooms (Smayda, 1990), as well as shifts towards lower N:P ratios (Hodgkiss and Ho, 1997; Schindler *et al.*, 2008). In either case, if P limitation is the dominant nutrient condition, phytoplankton species with a higher P affinity will dominate the community. Our study demonstrates that dissolved nutrient ratios alone are insufficient indicators for the actual nutrient limitation affecting phytoplankton and additional approaches for its determination should be considered. This was similarly shown for the brackish environment of the Baltic Sea (Tamminen and Andersen, 2007). Our results provide data to extend this understanding of phytoplankton limitation to rather full marine coastal ecosystems. However, changes in available nutrient ratios through water management activities are by all means able to alter the phytoplankton community composition (Lie *et al.*, 2011), and influence the food web (Elser *et al.*, 2000). Therefore, in order to manage potential future eutrophication, and related effects in coastal ecosystems, additional investigations of nutrient loading combined with phytoplankton nutrient limitation patterns are critical to uncover links of cause and effects to assist water management activities. Coastal water management should not only focus on nitrogen nutrient load, but also identify and monitor *in situ* phytoplankton nutrient limitation patterns. The controlled, long-term nutrient regulation of combined N and P is the only means to a sustainable coastal water management (Granéli *et al.*, 1990; Carpenter, 2008).

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