



# Ecophysiological linkage of nitrogen enrichment to heavily silicified diatoms in winter

Dongyan Liu<sup>1,\*</sup>, Patricia M. Glibert<sup>2</sup>

<sup>1</sup>State Key Laboratory of Estuarine and Coastal Research, East China Normal University, Shanghai 200062, PR China

<sup>2</sup>University of Maryland Center for Environmental Science, Horn Point Laboratory, Cambridge, MD 21613, USA

**ABSTRACT:** Over recent decades, increased anthropogenic nitrogen and reduced land-based loading of silica in many coastal waters have asymmetrically changed the nitrogen:silica ratios. These changes have contributed to shifts in phytoplankton assemblages from diatoms to non-diatoms, as well as increases in the frequency and magnitude of non-diatom harmful algal blooms. Here we show a subtle and counterintuitive change in diatom assemblage, i.e. heavily silicified diatoms significantly increased in winter after nitrogen enrichment, based on paleoecological and contemporary seasonal water column data from 2 eutrophic bays in the Yellow Sea. The heavily silicified diatom *Paralia sulcata* showed an increasing trend over time in parallel with nitrogen enrichment, which was associated with low temperature, and low dissolved inorganic nitrogen:phosphorus, high dissolved nitrogen:silica, and low ammonium:nitrate ratios on a seasonal basis. Applying recent insights regarding diatom nitrogen metabolism and its putative urea cycle, a physiological mechanism linking nitrogen, carbon, and silica metabolism is suggested to explain the phenomenon of increased silicification under winter conditions at the cellular level. Winter sequestration of silica in *P. sulcata* valves also has biogeochemical consequences, including a weakening of the silica pump and a slowing of biogenic silica dissolution, thereby reducing the availability of silica for further diatom growth in subsequent seasons and increasing the window of opportunity for summer growth of non-diatom harmful algal bloom species.

**KEY WORDS:** *Paralia sulcata* · Nitrification · Diatom metabolism · Photosynthesis · Phytoplankton shift · Si cycling

## INTRODUCTION

The composition and abundance of phytoplankton assemblages influence the structure and function of marine food webs and intricately control the biogeochemical cycling of nutrient elements. Nutrient enrichment (eutrophication) and the resulting alteration in the balance and stoichiometry of different nutrients is one of the most important and recognized mechanisms for explaining shifts in phytoplankton assemblages in coastal ecosystems (e.g. Smayda 1990, Conley et al. 1993, Anderson et al. 2002, Glibert & Burford 2017). In contrast to increases in N and P, there is evidence for decreasing dissolved silica (DSi) concentrations in coastal waters, largely due to

sediment trapping and elemental transformations following construction of dams (e.g. Vörösmarty et al. 2003, Beusen et al. 2009). Such asymmetric changes between N, P, and Si have resulted in a gradual move from N to Si limitation in many coastal systems (Conley & Malone 1992, Ragueneau et al. 1994), and long-term increases in N:Si and N:P have been associated with compositional shifts in phytoplankton assemblages, away from diatoms to proportionately more flagellates and cyanobacteria, many of which are harmful algal bloom (HAB) species (e.g. Anderson et al. 2002, Heisler et al. 2008, Glibert & Burford 2017).

Some systems have also experienced a shift in diatom assemblage: small and heavily silicified species increased, especially in winter and early spring.

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\*Corresponding author: dyliau@sklec.ecnu.edu.cn

Time series from the subarctic North Pacific show that the abundance of the diatom *Neodenticula semi-nae* increases and cells become smaller in size and more silicified in autumn and winter after the period of vertical mixing and nutrient injection (Shimada et al. 2009). In the Baltic Sea, *Thalassiosira baltica*, a small and heavily silicified centric diatom, has become the principal species of the early spring bloom (Wasmund et al. 1998) since the period of accelerated N loading (Olli et al. 2008). Similarly, the abundance and seasonality of *Paralia sulcata*, a tythropelagic diatom species with robust chain-forming valves, has been increasing in Helgoland Roads, North Sea (Gebühr et al. 2009), and in the Yellow Sea, China (D. Liu et al. 2008, 2013), in recent decades, associated with nutrient enrichment. To date there has been no ecophysiological explanation as to why heavily silicified diatoms increase under the condition of N pollution and cold temperatures. Rather, given the trend toward increasing Si limitation in the water column, cells that accumulate proportionately more Si would not be expected (Conley et al. 1993). Here, we confirm the shift that has occurred in diatom speciation in some systems and explore its physiological basis and biogeochemical consequences, based on paleoecological and contemporary water column data from 2 eutrophic embayments in the Yellow Sea, China.

The Yellow Sea experienced 2- to 5-fold increases in dissolved inorganic nitrogen (DIN) and phosphorus (DIP) between 1970 and 2000 and that trajectory of change is continuing (Liu et al. 2013, Stokal et al. 2014). The change in DIN loading and associated water column concentrations have been documented to result from China's agricultural and economic development as well as urbanization (D. Liu et al. 2008, 2013). With accelerating N loads, a further skewing of DIN:DSi ratios is expected in the Yellow Sea in years to come (Stokal et al. 2014), and the probability of increasing seasonal DSi limitation from spring to summer has increased from approx. 10% to 94% in the Shishli Bay (Zhao et al. 2000) in recent decades (Wang et al. 2012, Liu et al. 2013). As a consequence, the Yellow Sea now routinely experiences increasingly larger HAB occurrences, particularly in coastal bays. For example, since 2000, numerous summer HAB events in Sishili Bay, one of the embayments in the Yellow Sea, have reached an aerial extent of >60 km<sup>2</sup>, often involving the HAB dinoflagellate *Akashiwo sanguinea*, at cell densities ranging up to 4 × 10<sup>6</sup> cells l<sup>-1</sup> (Ye et al. 2006, Yu & Hao 2009, Hao et al. 2011, Liu et al. 2013, Chen et al. 2015). In addition, large blooms of *Noctiluca scintillans* and *Prorocentrum micans* as well as the raphido-

phyte *Chattonella marina* have occurred since the early 2000s (Ye et al. 2006, Jiang et al. 2011, Liu et al. 2013). In Jiaozhou Bay, *Mesodinium rubrum* and *N. scintillans* and several raphidophyte species are among the current, common HAB taxa in late summer (Yu et al. 2011, Yuan et al. 2017).

Coincident with the increasing proliferation of HAB species, a winter diatom shift has also been observed. *P. sulcata* with heavily silicified valves is now common, if not dominant, during the winter months (D. Liu et al. 2008, 2013). In Sishili Bay, the relationship between eutrophication and the increase in *P. sulcata* was previously established from examination of the biomass and species composition of diatom valves and dinoflagellate cysts and their sterol biomarkers in sediment cores as paleoecological indicators (Liu et al. 2013). Results were evaluated in the context of increased nutrient loading, changes in phytoplankton assemblages in the upper water body, regional climate change, and historic knowledge of Yantai city's development through time, i.e. agricultural reform in the early stages of Chinese economic reforms during 1975 to 1985 led to significant fertilizer use and consequently increased high nutrient flux from rivers to coastal waters and caused shifts in phytoplankton assemblages (Liu et al. 2013). These analyses clearly documented the shift away from larger diatoms such as *Coscinodiscus radiatus* (89–115 μm), toward smaller diatoms, including *P. sulcata*. These relationships were further supported by Di et al. (2013), who also reported that *P. sulcata* valves dominated in the surface sediment at most stations of the Yellow Sea. Moreover, some additional environmental correlates of *P. sulcata* have been well documented in other systems (e.g. Huang et al. 1996, McQuoid & Nordberg 2003, D. Liu et al. 2008, Olli et al. 2008). In coastal Sweden, *P. sulcata* cells have been shown to be associated with upwelled, nutrient-rich water (McQuoid & Nordberg 2003). Relationships with cold temperatures have also been established (Hobson & McQuoid 1997, Zong 1997). Gebühr et al. (2009, p. 195), on observing increases in this species in Helgoland Roads, North Sea, described *P. sulcata* as 'a typical winter alga adapted to low light conditions under colder water temperatures but high concentrations of nutrients'.

Our study builds on these previous findings and specifically addresses several questions with respect to increasing *P. sulcata* in winter and nitrogen enrichment in the Yellow Sea. Why might heavy silicification occur under conditions of high N availability and cold temperatures? Are these winter diatoms related

to DSi availability, to changes in N, and/or their proportion? Is there a physiological basis for increasing silicification based on observed changes in nutrient composition and known biochemical pathways of silicification? How might silicification by these winter diatoms alter biogeochemical cycling and the propensity for HABs? Paleocological data and contemporary water quality data from 2 inland bays of the Yellow Sea are used to address these questions and confirm both the long-term trends and seasonal patterns of this diatom in relation to environmental factors.

## MATERIALS AND METHODS

### Site descriptions

Jiaozhou Bay and Sishili Bay are both semi-enclosed water bodies within the Yellow Sea, sur-

rounded by the major cities of Qingdao and Yantai, respectively (Fig. 1). Jiaozhou Bay has a surface area of 390 km<sup>2</sup> and an average depth of 6–7 m (D. Liu et al. 2008). It has a number of seasonal streams, including the Yang, Dagu, Moshui, Baisha, and Licun rivers, that empty into the bay, but due to water use and damming within the watershed, river flow into the bay has decreased by an order of magnitude since the 1980s (Yu et al. 2011). Sishili Bay is much smaller, with an area of 130 km<sup>2</sup> and an average depth of 15 m (Liu et al. 2013). It receives freshwater flow from the Guangdang, Xinan, and Xiaoyuniao rivers. Two periods of economic development have significantly impacted the water quality in the 2 bays. First, in the 1970s–1980s, agriculture expanded throughout the watershed, with its associated use of nitrogenous fertilizers, and second, in the 1990s–2000s, sewage discharge and marine aquaculture activity increased substantially as the population rapidly expanded (Fig. 2).

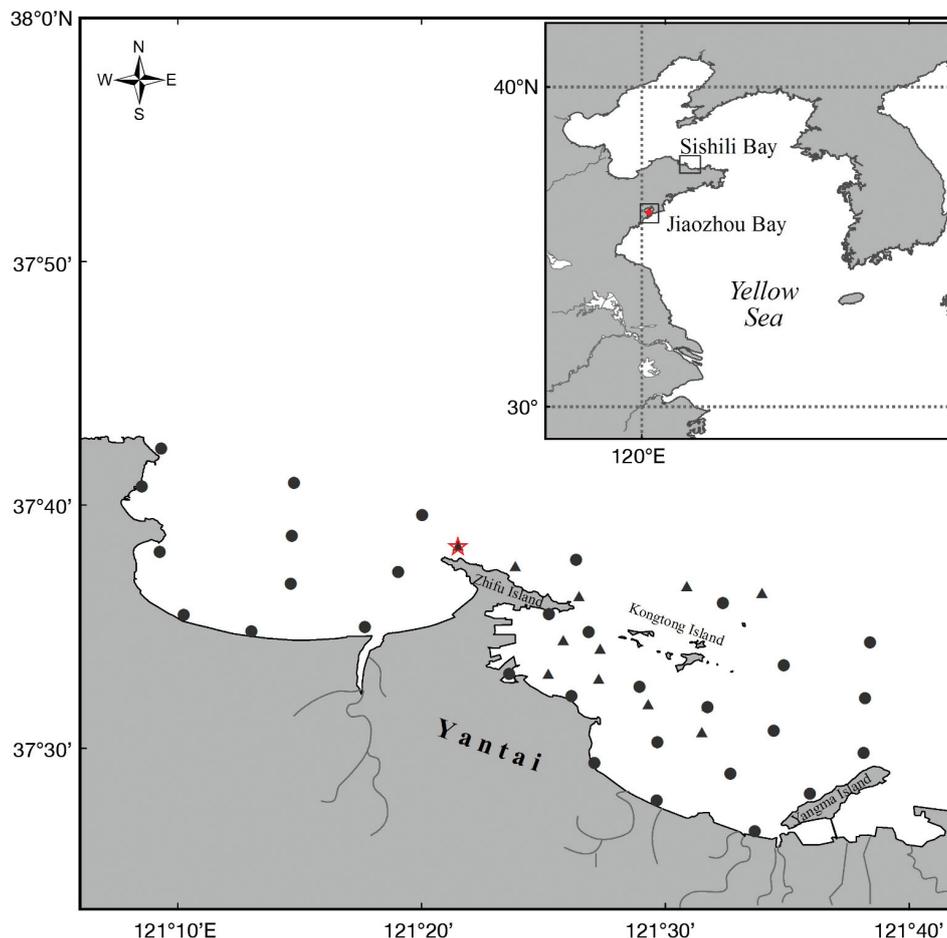


Fig. 1. Map showing the location of Jiaozhou Bay and Sishili Bay, Yellow Sea, and the sites of sediment sampling in both bays and of water column sampling in Sishili Bay. Star: site of core collection; triangles: sampling sites during December 2008 to March 2010; circles: sampling sites during April 2010 to March 2011

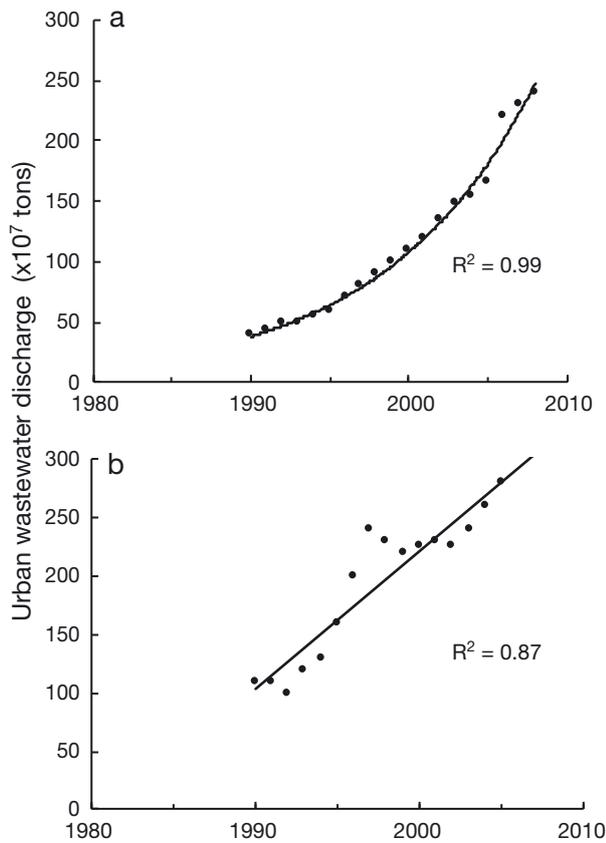


Fig. 2. Change in urban wastewater discharge for (a) Jiaozhou Bay and (b) Sishili Bay since 1990. Data for (a) are derived from Yu et al. (2011) and for (b) from Liu et al. (2013)

### Sampling and analysis

Sediment cores were collected from Jiaozhou Bay (36° 5' 23" N, 120° 14' 36" E; water depth of 20.6 m; June 2001) and Sishili Bay (37° 38' 20.37" N, 121° 21' 31.03" E; water depth ~12 m; December 2008; Fig. 1). Standard methods for the analysis of diatom fossils were conducted following Battarbee et al. (2001) and D. Liu et al. (2008, 2013). Briefly, sediment samples were dried at 105°C, and then treated with 10% HCl and 30% to remove carbonate and organic matter, respectively. After rinsing, zinc bromide (specific gravity 2.4) was added to the samples to suspend the siliceous valves, and then the samples were centrifuged at  $760 \times g$  (10 min). An aliquot of suspended valves was placed onto a cover slip and then dried. Permanent slides for enumeration were made by mounting the coverslips in Naphrax™. Valves were examined under an Olympus CX-31 light microscope. At least 300 valves were counted in each sample, and abundance was calculated as the number of valves per g of dry sediment weight (valves g<sup>-1</sup> DW).

Water quality and phytoplankton data were obtained from monthly to bimonthly sampling that took place at 12–30 stations in the water column of Sishili Bay, undertaken over a period of  $\geq 2$  yr (December 2008–March 2011). Near-surface seawater samples were collected at each station using a Go-Flo bottle (5 l). Nutrient samples were initially filtered through cellulose acetate membranes (Whatman, 0.45  $\mu$ m) and frozen for later analysis. All inorganic nutrients ( $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ,  $\text{NH}_4^+$ ,  $\text{PO}_4^{3-}$ , and DSi) were subsequently analyzed using Flow Injection Analysis (AA3, Bran & Luebbe). Chlorophyll a (chl a) was determined on return to the laboratory by filtering 1 l water samples using GF/F filters under low vacuum, and concentrations were detected using spectrophotometry (TU-1800, Persee) after samples were extracted with 15 ml of 90% acetone in the dark for 24 h in the refrigerator. Mixed phytoplankton samples taken from near-surface waters were fixed with Lugol's iodine for cell enumeration and species identification, using an inverted microscope at  $\times 100$ –400 magnification after sedimentation for 24 h in 25 ml glass chambers (Utermöhl 1958), as further described in Wang et al. (2012, 2013) and Liu et al. (2013).

### Data analysis

Monthly means of all station data were calculated to determine seasonal patterns. Trend lines were fitted using Excel functions. Median values for various parameters were compared using the Mann-Whitney test.

## RESULTS

### Paleoecological trends

Paleoecological evidence from both Jiaozhou and Sishili bays showed highly variable total diatom contributions with time, but consistent increases in the diatom *Paralia sulcata* since 1990 (Fig. 3). In Jiaozhou Bay, the overall diatom abundance was considerably higher than that in Sishili Bay, and there was a decline in total diatom abundance from the 1960s to about 1990, after which it consistently increased (Fig. 3a). The increase from 1990 to 2001 was defined linearly with  $R^2 = 0.51$  ( $n = 19$ ,  $p < 0.001$ ). Total diatoms in Sishili Bay, in contrast, were lower in the sediment prior to 1940 but increased consistently after that time (Fig. 3b). In both cores, there was a consistent, significant ( $p < 0.001$ ) increase in the proportion

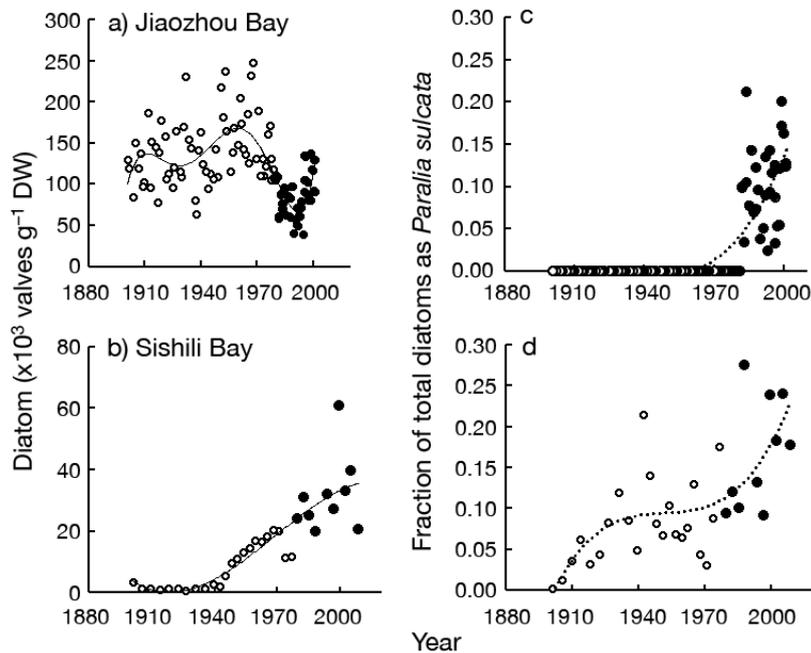


Fig. 3. Total diatoms in cores collected from (a) Jiaozhou Bay (June 2001) and (b) Sishili Bay (December 2008), Yellow Sea. Change in the fraction of the diatom *Paralia sulcata* in the preserved diatom community of (c) Jiaozhou Bay and (d) Sishili Bay over time. Cores were dated as described in D. Liu et al. (2008, 2013). Post-1980 data are shown with black symbols

of *P. sulcata* to total diatoms since the 1980s (Fig. 3c,d). In Jiaozhou Bay, this species was not found prior to 1980, while in the Sishili Bay, it has increased from ~10% to >25% of the preserved diatom assemblage since the 1980s.

### Contemporary water column trends

Data from water column sampling in Sishili Bay over a period of  $\geq 2$  yr provide a contemporary, seasonal perspective of water quality and phytoplankton community. Temperature ranged from a low of 1.02°C to a high of 24.1°C (Fig. 4a), and chl *a* reached its annual maximum during the warmest months (Fig. 4b). Diatoms dominated from January to April, declining in summer, and increasing again later in the year (Fig. 4c); of these diatoms, *P. sulcata* comprised >80% of the community early in the year, but a lesser proportion of total diatoms from June to October (Fig. 4d). Concentrations of  $\text{NO}_3^-$  reached levels of 12–15  $\mu\text{M-N}$  from winter to spring, but averaged <3  $\mu\text{M}$  during the summer months when non-diatoms predominated (Fig. 4e). Concentrations of  $\text{NH}_4^+$  were especially elevated in spring and fall when concentrations of 3–6  $\mu\text{M-N}$  were commonly seen, with some values equaling or exceeding

9  $\mu\text{M-N}$  (Fig. 4f). Concentrations of  $\text{PO}_4^{3-}$  remained <0.5  $\mu\text{M-P}$  throughout most of the year, but increased to >1.0  $\mu\text{M-P}$  in late fall (Fig. 4g). Concentrations of DSi varied widely during the 2 winters when samples were collected, and were <3  $\mu\text{M-Si}$  in late winter through spring, but were consistently >3  $\mu\text{M-Si}$  from late summer to fall (Fig. 4h).

Some of environmental parameters during the time period of *P. sulcata* dominance could be distinguished from those associated with the periods of either non-*P. sulcata* diatoms or non-diatom dominance. *P. sulcata* dominated ( $\geq 50\%$  of all diatoms) when water temperatures were <5°C, whereas non-*P. sulcata* diatoms were more abundant ( $\geq 50\%$  of all diatoms) over a broader temperature range (interquartile range 5.5–22.5°C), and non-diatoms ( $\geq 50\%$  of phytoplankton assemblage) were dominant at temperature >17°C; *P. sulcata* occurred at a significantly lower temperature

than the other taxa ( $p < 0.001$ ; Fig. 5a). Diatom (both non-*P. sulcata* and *P. sulcata*) dominance was associated with a median  $\text{NO}_3^-$  water column concentration significantly higher than that associated with the period of non-diatoms ( $p < 0.001$ ; Fig. 5b), but no significant difference was seen across dominant groups with respect to  $\text{NH}_4^+$  concentrations, although *P. sulcata* was found across the broadest range in  $\text{NH}_4^+$  (Fig. 5c). When *P. sulcata* was dominant, significantly higher median concentrations of  $\text{PO}_4^{3-}$  were observed ( $p < 0.001$ ), as well as the largest range in  $\text{PO}_4^{3-}$  concentrations (Fig. 5d). Finally, concentrations of DSi during *P. sulcata* dominance were lower than those during the period of growth of either non-diatoms or non-*P. sulcata* diatoms ( $p < 0.001$ ), but the period of non-*P. sulcata* had the widest range in concentration (Fig. 5e).

There was no significant difference in the dominance of non-diatoms and mixed assemblage diatoms with respect to the proportion of DIN:DIP, but *P. sulcata* diatoms occurred at a significantly lower DIN:DIP ratio ( $p < 0.001$ ; Fig. 5f). All phytoplankton groups were associated with a median DIN:DIP ratio within a factor of 2 of classically considered stoichiometric proportions, and there was a broad range in all cases (Fig. 5f). With respect to the proportion of DIN:DSi, *P. sulcata* dominated at a median ratio that

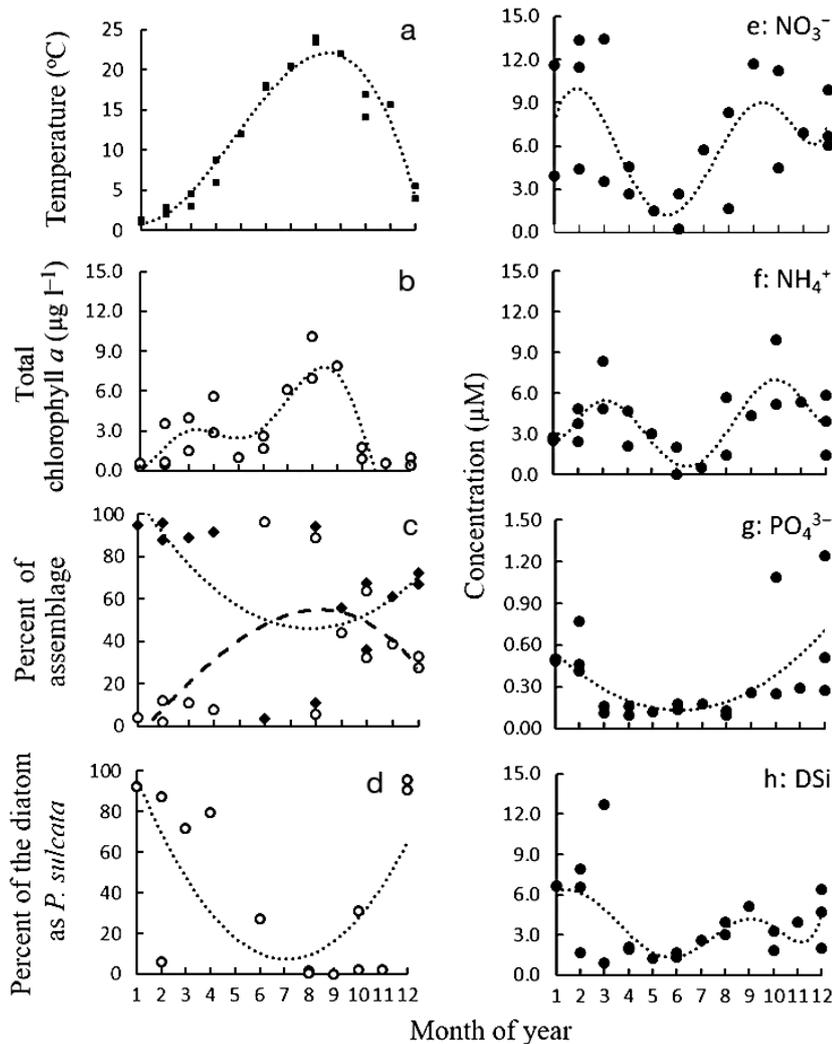


Fig. 4. Seasonal patterns in temperature, phytoplankton biomass, and composition in Sishili Bay, Yellow Sea, based on mean monthly values of 12–30 stations collected from late 2008–early 2011. (a) Temperature. (b) Total chlorophyll a. (c) Percent of total phytoplankton assemblage as diatoms (◆; dotted line showing trend) versus non-diatoms (○; dashed line showing trend). (d) Percent of total phytoplankton assemblage as the heavily silicified diatom species *Paralia sulcata*. (e–h) Water column concentrations of (e)  $\text{NO}_3^-$ , (f)  $\text{NH}_4^+$ , (g)  $\text{PO}_4^{3-}$ , and (h) DSi. Trend lines are drawn to aid visual comparisons

was >3-fold higher than that when non-diatoms were dominant and 30% higher than that when non-*P. sulcata* diatoms were most common (Fig. 5g). Each of the major taxonomic groups considered here occurred at significantly different DIN:DSi ratios ( $p < 0.001$ ), with those associated with *P. sulcata* being the highest.

With regard to forms of N, *P. sulcata* was most common when  $\text{NO}_3^-$  was the dominant inorganic N form (interquartile range of the  $\text{NH}_4^+:\text{NO}_3^-$  ratio 0.34–0.89), while non-*P. sulcata* diatoms spanned a broader range in this ratio (interquartile range 0.42–2.13), and non-diatoms occurred when  $\text{NH}_4^+$  was the dominant N form (interquartile range 1.98–4.52) and each

taxonomic group occurred at significantly different proportions of  $\text{NH}_4^+:\text{NO}_3^-$  ( $p < 0.001$ ; Fig. 5h). This latter relationship was explored in more detail (Fig. 6). From cell abundance data, it is clear that non-diatoms increased in absolute numbers and in proportion as the  $\text{NH}_4^+:\text{NO}_3^-$  ratio increased (Fig. 6a). For the non-*P. sulcata* diatoms, while they spanned a range of  $\text{NH}_4^+:\text{NO}_3^-$  proportion, their absolute numbers decreased as this proportion increased (Fig. 6b). *P. sulcata* was mostly found when  $\text{NH}_4^+:\text{NO}_3^-$  proportions were <1, and although there were instances when they occurred when  $\text{NH}_4^+:\text{NO}_3^-$  proportions exceeded 1, their absolute cell abundances were exceedingly low under these conditions (Fig. 6c).

## DISCUSSION

Phytoplankton composition in the Yellow Sea has changed significantly over the past several decades, with diatoms giving way to many more HAB taxa and more frequent blooms. Altered nutrient loads and proportions, including increasing N and declining DSi with increasing eutrophication and numerous anthropogenic changes have played key roles in these changes observed in phytoplankton assemblages. What is less appreciated is that diatom composition changed with eutrophication. As shown here, and as previously docu-

mented (Gebühr et al. 2009, D. Liu et al. 2008, 2013), the small and heavy silicified diatom *Paralia sulcata*, under the condition of disproportionately high DIN:DSi ratios, has taken the place of the previously dominant large diatoms in winter–spring since the latter decades of the 20th century.

The formation of new siliceous valves appears to occur just before cell division and accordingly has been associated with the G2 and M periods of cell division; an increase in the phase length of these periods leads to more Si uptake and deposition (Martin-Jézéquel et al. 2000, Claquin et al. 2002). Thus, the thickness of diatom valves with its associated Si

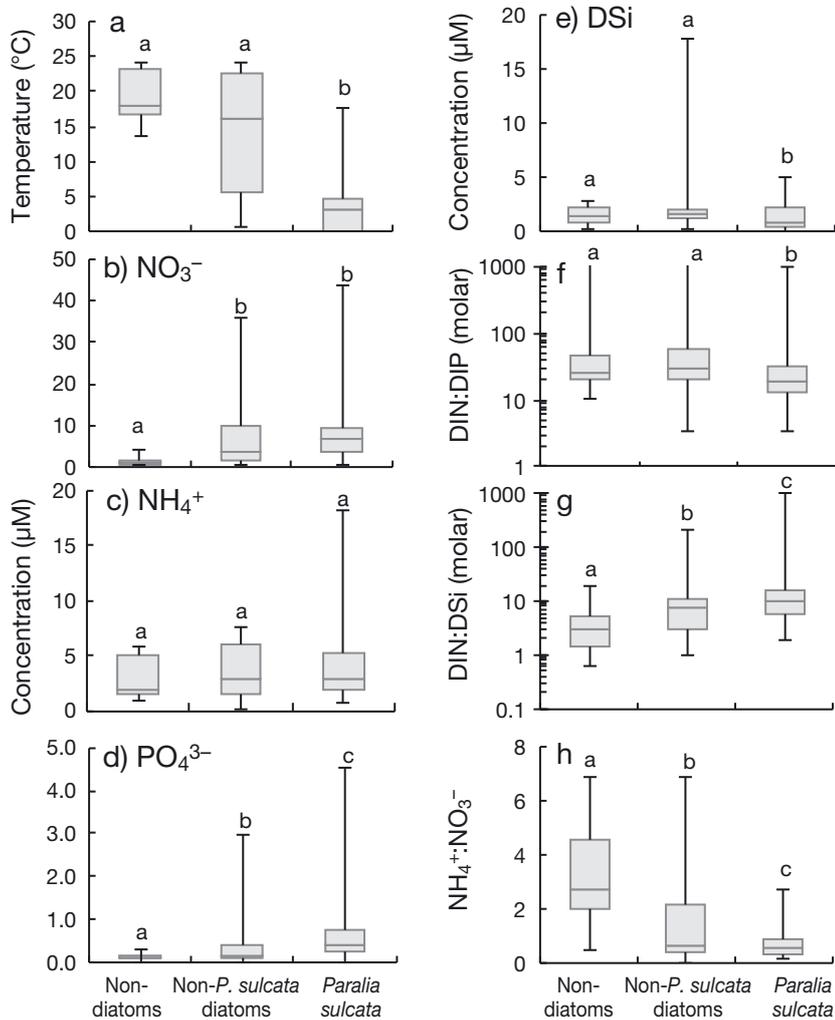


Fig. 5. Comparisons of environmental conditions in Sishili Bay during periods of dominance by different phytoplankton groups, as non-diatoms, non-*Paralia sulcata* diatoms, and *P. sulcata* (each respective group represented  $\geq 50\%$  of the phytoplankton cell abundance). (a) Temperature. (b–e) Water column concentrations of (b)  $\text{NO}_3^-$ , (c)  $\text{NH}_4^+$ , (d)  $\text{PO}_4^{3-}$  and (e) DSi. (f–h) Ratios of (f) DIN:DIP, (g) DIN:DSi, and (h)  $\text{NH}_4^+:\text{NO}_3^-$ . In the box and whisker plots, the median values for all observations (based on 12–30 stations over a period of  $\geq 2$  yr) are shown as horizontal lines. The boxes represent the interquartile (25–75% range of all observations), and the whiskers illustrate the full range of observed values. Significant differences ( $p < 0.01$ ) between phytoplankton groups are shown with different lower case letters. Note that only  $\text{NH}_4^+$  had no significant differences between phytoplankton groups

content has been previously associated with a decline in growth rate, as well as the amount of Si uptake, the latter a function of the availability of substrate (Claquin et al. 2002, 2006). In contrast to the classic notion of increasing silicification being a function of limitation of growth by nutrients, iron or light, factors that prolong the cell cycle (e.g. Hutchins & Bruland 1998, Martin-Jézéquel et al. 2000, Claquin et al. 2006), trends in diatom silicification in the Baltic

Sea, North Sea, and Yellow Sea are occurring coincident with nutrient enrichment, rather than limitation. Thus, this phenomenon is seemingly counterintuitive and has no physiological explanation. Here, applying recent insights regarding diatom nitrogen metabolism and its putative urea cycle, we suggest a physiological mechanism linking nitrogen, carbon, and silica metabolism to explain this cold-water phenomenon at the cellular level.

### Diatom physiology and stress responses

Growth in cold water, especially the  $< 5^\circ\text{C}$  temperatures when *P. sulcata* dominates, is physiologically stressful. Cold temperatures cause stress because the biophysical light reactions of photosynthesis are relatively temperature insensitive, but the biochemical reactions (e.g. Calvin cycle reactions) are temperature sensitive and the excess reductant produced can lead to over-reduction of the electron transport chain, in turn leading to photoinhibition, photodamage, and loss of growth potential (Lomas & Glibert 1999, Glibert et al. 2016). A further limitation of temperature is the effect on enzyme activities, including the N reduction enzymes,  $\text{NO}_3^-$  reductase (NR) and  $\text{NO}_2^-$  reductase (NiR). Heavily dependent on  $\text{NO}_3^-$  as the N substrate for growth during these winter conditions, the average temperature of growth may, in fact, be suboptimal for the assimilation of  $\text{NO}_3^-$ , as the activity of NR in many diatoms has a temperature optimum of 12–15°C and

that of NiR is often higher (e.g. Gao et al. 1993, Lomas & Glibert 2000). Clearly, *P. sulcata* must overcome these stresses to dominate in these conditions and has the physiological mechanisms to do so, as the optimal temperature for *P. sulcata* growth has been reported be  $\sim 7^\circ\text{C}$  (Hobson & McQuoid 1997, Zong 1997).

Diatoms have 2 significant pathways to balance cellular energy demands and the uncoupling of N

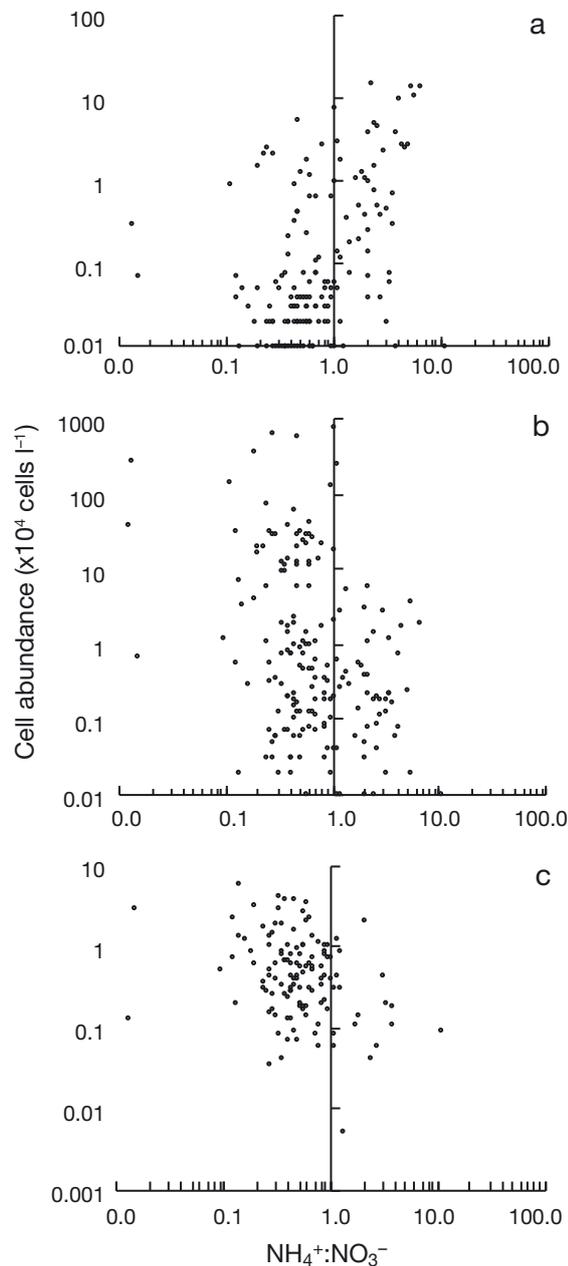


Fig. 6. Relationships between cell abundance of dominant phytoplankton groups of Sishili Bay, Yellow Sea, and the proportion of  $\text{NH}_4^+:\text{NO}_3^-$  in the water column based on data from 12–30 stations collected monthly from late 2008–early 2011. (a) Non-diatoms. (b) Non-*Paralia sulcata* diatoms. (c) *P. sulcata*. The y-axis is drawn at a  $\text{NH}_4^+:\text{NO}_3^-$  ratio = 1 to facilitate visual comparisons

and C that can occur under conditions of energy or redox imbalance imposed by temperature stress. First, diatoms are recognized to be  $\text{NO}_3^-$  specialists, often disproportionately favoring  $\text{NO}_3^-$  over  $\text{NH}_4^+$  as a N substrate, using the reduction of  $\text{NO}_3^-$  and  $\text{NO}_2^-$  via the enzymes in a non-assimilatory mode that

complements their function in the assimilation of N (Lomas & Glibert 1999, Parker & Armbrust 2005, Kamp et al. 2011, Glibert et al. 2016; Fig. 7a) and the proportionately lower ratios of  $\text{NH}_4^+:\text{NO}_3^-$  associated with the diatom communities compared with non-diatoms (Fig. 5h) is consistent with this notion. The dissimilatory reduction of  $\text{NO}_3^-$  to  $\text{NO}_2^-$ , and then of  $\text{NO}_2^-$  to  $\text{NH}_4^+$ , serves as a sink for reducing power, derived from the splitting of water that may develop when photochemistry exceeds (C) assimilatory capacity (Fig. 7a), making  $\text{NO}_3^-$  reduction a form of photoprotection. With water column concentrations of  $\text{NO}_3^-$  that averaged  $7 \mu\text{M-N}$  during the period of both non-*P. sulcata* diatom and *P. sulcata* dominance, there was seemingly sufficient availability of this preferred form of N for the cells to carry out both assimilatory and dissimilatory reduction as needed (Fig. 4e). Yet, even with sufficient substrate available, the low temperatures of the winter may suppress NR activity and therefore the effectiveness of this pathway in cellular energy balance.

A second important reaction in cellular redox homeostasis is photorespiration, initiated by oxygenation catalyzed by RUBISCO (D-ribulose-1,5-bisphosphate carboxylase/oxygenase), the enzyme responsible for the fixation of  $\text{CO}_2$  via the carboxylase reaction (Fig. 7b). Photorespiration exports reducing equivalents to the peroxisome and the mitochondria and, accordingly, increases under stress conditions, including under cold conditions, where it also serves as a protection against photoinhibition (Peterhansel et al. 2010, Rosenwasser et al. 2014).

An increase in photorespiration has significant effects on the production of silicified cell walls in diatoms. Photorespiration increases the flow of glycolate through the peroxisome and then to the mitochondria where its metabolism is normally linked to a putatively, fully functioning urea cycle (Armbrust et al. 2004, Allen et al. 2006, 2011; Fig. 7). The urea cycle plays important roles in the exchange of nutrients between organelles, and one of its key intermediates is polyamines, the suite of molecules attached to polypeptides of silifins, the Si-precipitating proteins (Kröger et al. 2000, Allen et al. 2006). Importantly, when polyamine synthesis is overexpressed, more cell wall precursors are made, ultimately leading to production of thickened silica frustules (Nunn et al. 2013; Fig. 7b).

Enhancements in photorespiration, glycolate production, and urea cycle activity are thus hypothesized to have led to higher polyamine production and therefore production of *P. sulcata* with thicker cell walls in the winter waters of the Yellow Sea. All of

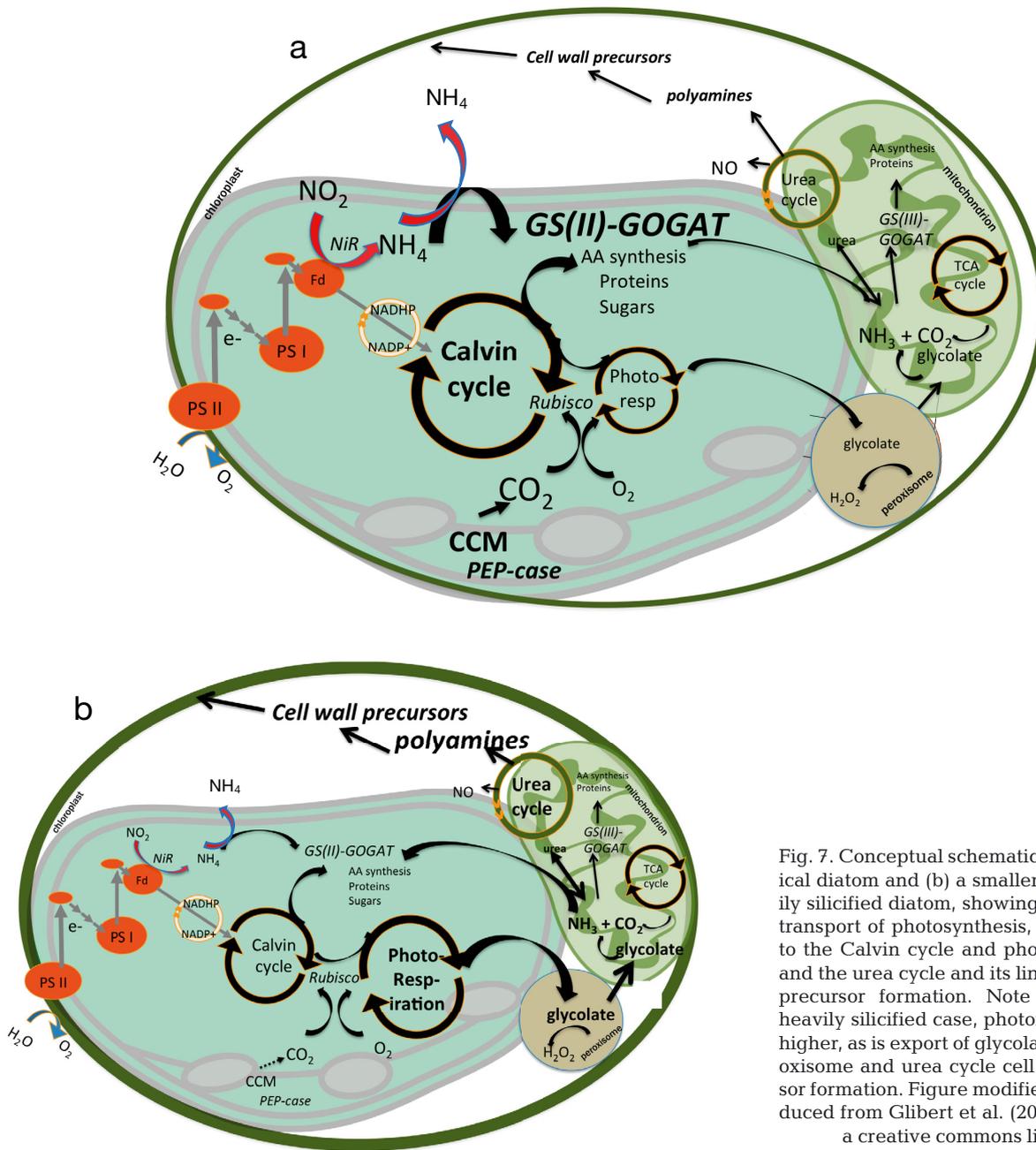


Fig. 7. Conceptual schematics of (a) a typical diatom and (b) a smaller, more heavily silicified diatom, showing the electron transport of photosynthesis, the coupling to the Calvin cycle and photorespiration and the urea cycle and its link to cell wall precursor formation. Note that in the heavily silicified case, photorespiration is higher, as is export of glycolate to the peroxisome and urea cycle cell wall precursor formation. Figure modified and reproduced from Glibert et al. (2016) based on a creative commons license

these pathways would be enhanced under conditions of elevated N and stressful cold temperatures. Why a diverse assemblage of diatoms did not display the same response in the cold-water conditions of the Yellow Sea is yet unresolved, but the diversity of N-reducing enzymes and polyamines even among diatoms may be significant in this regard (e.g. Kröger et al. 2000, Allen et al. 2005). As temperatures warmed seasonally, both the need to balance cellular energy from over-reduction and pathways by which diatoms likely balanced their cellular energy shifted.

As temperatures warmed and neared the optimal temperature for NR activity, *P. sulcata* was replaced by an assemblage of diatoms that could balance their cellular energy to a great extent through dissimilatory NO<sub>3</sub><sup>-</sup> reduction, leading to the decrease in the proportion of *P. sulcata* relative to non-*P. sulcata* diatoms.

Other factors that may have contributed to the success of *P. sulcata* at the expense of other taxa are possible. Limitation by Fe in the Yellow Sea during this period is possible but does not explain the unique

response by *P. sulcata* relative to other diatom species. Fe limitation limits NiR, thereby forcing the cell into a state of lack of reducing power, the same physiological stress imposed by excess  $\text{NH}_4^+$  and cold (Milligan & Harrison 2000). Temperature alone can be ruled out as a sole factor contributing to *P. sulcata* increases in the Yellow Sea, as it has for the North Sea (Gebühr et al. 2009), as both the paleoecological records and anthropogenic discharge trends show that increases in the heavily silicified species occurred coincident with, or shortly after the onset of eutrophication (Figs. 2 & 3), while winter temperatures have remained either constant or have increased only more recently than the onset of *P. sulcata*. The winter communities require sufficient N as well as  $\text{PO}_4^{3-}$  to drive these photoprotective mechanisms, and the concentrations in the Yellow Sea during this season confirm that these nutrients were available. The comparable increase of *Thalassiosira baltica* in the winter–spring blooms of the Baltic Sea and of *P. sulcata* in the North Sea may result from similar mechanisms, as these species also increased in proportion only after eutrophication became significant (Conley et al. 2008, Gebühr et al. 2009).

### Biogeochemical implications

There are important trophic and biogeochemical implications for diatom communities composed of more heavily silicified cells. Recent studies have shown that diatoms with thickened frustules are poorly grazed by copepods compared with less-silicified diatoms, and those copepods that do feed on heavily silicified diatoms may have reduced egg production and hatching success (Liu et al. 2016). Heavily silicified cells sink faster than lightly silicified cells, sequestering both carbon (C) and biogenic Si (BSi) in the sediment, the so-called Si pump (Dugdale et al. 1995). Moreover, fecal pellets from copepods grazing on heavily silicified cells are denser, and sink faster from the water column, increasing Si export from the photic zone as well as C export (Dugdale et al. 1995). The dissolution rate of BSi from diatoms that have been incorporated in fecal pellets appears to be reduced by factors of 2–3 relative to diatoms that are not aggregated (Claquin et al. 2006).

*T. baltica* blooms in the Baltic Sea have fundamentally altered DSi availability, from ca. 36  $\mu\text{M}$ -Si a century ago compared with ca. 13  $\mu\text{M}$ -Si observed today (Conley et al. 2008).

Although we do not know the contribution of *P. sulcata* to DSi availability in the Yellow Sea, a rough

contribution of *P. sulcata* to BSi in the sediments of Jiaozhou Bay can be estimated according to the assumption of 10 pM-Si cell<sup>-1</sup> and the total valves (Fig. 3b). Previous publications have shown that the range of BSi in the sediments of Jiaozhou Bay is approximately 0.9–1.9% and the range of Si is approximately 50–200  $\mu\text{mol g}^{-1}$  dry sediment (S. M. Liu et al. 2002, 2008). We summarize these data using mid-range values for Si (125  $\mu\text{mol g}^{-1}$ ) and BSi (1.35%), yielding a value of 18% contribution of *P. sulcata* in the surface sediment. The tychopelagic nature of *P. sulcata* and its documented preservation in sediments suggests that it could play a role in the cycling of BSi. Sequestration of Si in the frustules of these diatoms not only contributes to the long-term decline in Si concentrations but also may have a seasonal consequence. Here, we propose a simple model to show the difference in seasonal succession of phytoplankton assemblages under heavily silicified and non-heavily silicified diatom dominance in winter (Fig. 8). By locking up more BSi in sediments, heavily silicified diatoms, and fecal pellets, the biogeochemical remineralization of DSi back to the water will be slower for heavily silicified diatoms than for less-silicified diatoms. Prolonged BSi cycling and largely consumed DSi in winter and spring can accelerate DSi limitation in the water column before the rainy season comes, and consequently increase the window of opportunity for non-siliceous species, including HAB taxa, to proliferate with the coming summer and increasing temperature. With anthropogenic loads of N and P increasing in the water column, non-diatom communities can subsequently attain higher total biomass for prolonged periods of time, especially during the early summer (May–June) in temperate seas compared with pre-eutrophic conditions (Fig. 8). Clearly, the biogeochemical implications of altered diatom communities need to be further explored by comparing with other sea regions, e.g. Baltic Sea (Conley et al. 2008), North Sea (Gebühr et al. 2009), and even the Antarctic (Abelmann et al. 2006), where heavily silicified diatoms have increased over time or space.

### CONCLUSIONS

The results shown here build on previously established relationships between the increasing abundance of the diatom *Paralia sulcata* and eutrophication, and illustrate the potential interactions of temperature, availability of N forms, enzyme activities, photosynthesis, and silicification at the cellular

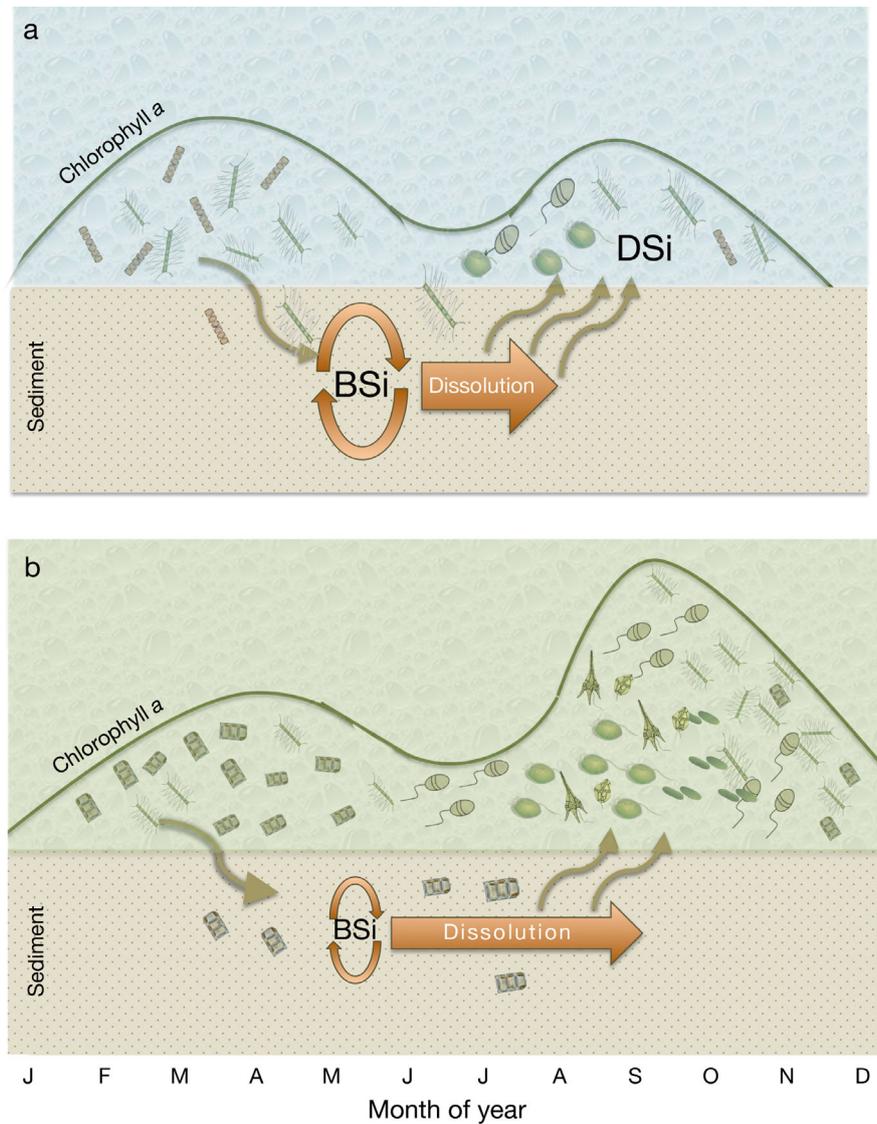


Fig. 8. Conceptual diagram of the seasonal succession of phytoplankton and corresponding rates of BSi dissolution under (a) pre-eutrophic conditions and (b) nutrient-enriched conditions. Note that in the pre-enrichment state, winter diatoms are more diverse, silica dissolution is more rapid, and the proportional accumulation of non-diatoms during the warmer months is relatively lower. In the eutrophic condition, more heavily silicified diatoms dominate in winter, prolonging the period of BSi dissolution and cycling, enhancing the period for non-diatoms to accumulate during the warmer months

level, which in turn regulate the community dynamics and ultimately affect trophic dynamics and biogeochemistry. What is proposed here, but which will require further physiological experimentation, is that a condition of highly silicified diatoms may be amplified by increasing proportions of the anthropogenic input of N in the cold winter conditions via changes in cellular energy balance. The biochemistry of diatom metabolism tightly regulates the biogeochemistry of BSi cycling, with increasing silicification augmenting the Si pump (Dugdale et al. 1995) that drives systems toward Si limitation and dependence on recycled nutrients, and increases the propensity for large-scale shifts at all levels of the food web. The intertwining of the cycles of the major nutrient elements is abundantly clear, as is the magnitude of

human perturbations of their respective cycles. The profound and complex effects of the changes in these cycles on ecosystem structure and function are just beginning to be unraveled at the species level. The need to reduce N loads, even in the relatively slow-growing winter periods, should be ever more apparent, as the magnitude of the resulting heavily silicified diatom blooms sets the trajectory for production and biogeochemistry for the subsequent seasons and beyond.

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