Biogeosciences Discuss., 6, 7175–7205, 2009
www.biogeosciences-discuss.net/6/7175/2009/

Biogeosciences Discussions

Biogeosciences Discussions is the access reviewed discussion forum of *Biogeosciences*

Effects of an iron-light co-limitation on the elemental composition (Si, C, N) of the marine diatoms *Thalassiosira oceanica* and *Ditylum brightwellii*

E. Bucciarelli[1,2], **P. Pondaven**[1,2], **and G. Sarthou**[1,2]

[1]Université Européenne de Bretagne, France
[2]Université de Brest, CNRS, IRD, UMR 6539 LEMAR, IUEM; Technopôle Brest Iroise, Place Nicolas Corpernic, 29280 Plouzané, France

Received: 29 May 2009 – Accepted: 9 June 2009 – Published: 17 July 2009

Correspondence to: E. Bucciarelli (eva.bucciarelli@univ-brest.fr)

Published by Copernicus Publications on behalf of the European Geosciences Union.

Abstract

We examined the effect of iron (Fe) and Fe-light (Fe-L) co-limitation on cellular silica (BSi), carbon (C) and nitrogen (N) in two marine diatom species, *Thalassiosira oceanica* and *Ditylum brightwellii*. We showed that C and N per cell tend to decrease with increasing Fe and Fe-L co-limitation (i.e. decreasing growth rate). We observed an increase (*T. oceanica*, Fe-L co-limitation), no change (*T. oceanica*, Fe limitation) and a decrease (*D. brightwellii*, Fe and Fe-L limitations) in BSi per cell with increasing degree of limitation. When comparing our results to literature data, we noted that the trend in C and N per cell for other Fe limited diatoms was similar to ours. However there was no global trend in BSi, which suggests interspecific differences. The relative variations in C:N, Si:C and Si:N versus the relative variation in specific growth rate (i.e. μ:μ_{max}) followed the same patterns for both species under Fe and Fe-L co-limitation. The variations of C:N under Fe limitation reported in the literature for other diatoms are contrasted, which may thus be more related to growth conditions than to interspecific differences. Si:C and Si:N ratios increased by more than 2-fold between 100% and 40% of μ_{max}. Under more severe limitation (Fe or Fe-L), these ratios tend to decrease. To asses the field significance of our results, we compared them to those of artificial Fe fertilisation experiments. This comparison showed that Si:N increased between 100% and ~40% of μ_{max}, but decreased between 40% and 20% of μ_{max}, and increased again below 20% of μ_{max}. Between ~15% and 30% of μ_{max}, Si:N was even lower than under non limiting conditions. These results may have important biogeochemical implications on the understanding and the modeling of the oceanic biogeochemical cycles, e.g. carbon export.

1 Introduction

Warming of the climate system is now unequivocal and very likely due to the atmospheric increase of greenhouse gases such as carbon dioxide (CO_2) (IPCC, 2007).

BGD

6, 7175–7205, 2009

The rate of change in atmospheric CO_2, depends, however, not only on human activities but also on oceanic biogeochemical processes (Falkowski et al., 2000). Oceanic ecosystems indeed strongly affect the composition of the atmosphere, through CO_2 uptake by phytoplankton, and the export of that organic carbon from the surface to the ocean interior. In this regard, the phytoplanktonic group of the diatoms is thought to play a major role. These siliceous species contribute to up to 40% of the global oceanic primary production of carbon (Nelson et al., 1995) and the termination of their massive blooms export large quantities of organic carbon and biogenic silica from upper layers to the deep ocean (Smetacek, 1999). These export events may partly control the partitioning of carbon in the atmosphere-ocean-sediment system over geological timescales (Barber and Hiscock, 2006; Falkowski et al., 1998). Since the 1990's, it has been convincingly shown that the subnanomolar oceanic concentrations of iron (Fe) are low enough to limit primary production and in particular diatom growth in at least 40% of the ocean (de Baar et al., 2005). Iron limitation also induces a decoupling in the use of major macronutrients by phytoplankton, likely to influence the cycling of the major biogeochemical cycles (C, N, P, Si, S) over geological time scales (de Baar and La Roche, 2003).

Other abiotic parameters also control primary production and influence the elemental stoichiometry of phytoplankton (Geider and La Roche, 2002). For example, it has been shown that Fe-light co-limitation occurs in the subarctic Pacific Ocean (Maldonaldo et al., 1999), subantarctic waters (Boyd et al., 1999), central North Atlantic (Moore et al., 2006) and eastern North Pacific (Hopkinson and Barbeau, 2008). Differences in light intensity also seem to have played an important role during the Fe fertilisation experiments conducted to date and co-limitation by Fe and light may even best describe the HNLC regions than Fe alone (de Baar et al., 2005). Iron and light indeed interplay at the biochemical level, because phytoplanktonic cells need higher Fe:C for growth under low light (Sunda and Huntsman, 1997). However, despite the importance of this environmentally relevant co-limitation, very few studies explored its impact on the coupling of the major biogeochemical cycles. In the present study, we examined the effect of Fe-

BGD
6, 7175–7205, 2009

light co-limitation on cellular silica, carbon and nitrogen in two marine diatom species, *Thalassiosira oceanica* and *Ditylum brightwellii*, and explored the field-significance of our results.

2 Materials and methods

2.1 Culture conditions

Batch cultures of the centric diatoms *Thalassiosira oceanica* (axenic, oceanic species, CCMP 1005) and *Ditylum brightwellii* (xenic, coastal species, CCMP 358) were grown at 20°C in polycarbonate bottles. Cultures were grown under cool white fluorescent light at an irradiance of 75 (high light: HL) and 7.5 μmol photons m^{-2} s^{-1} (low light: LL) and a 14 h:10 h light:dark cycle. The culture media (see below) were sterilized by micro wave treatment (Keller et al., 1988). Cultures were grown as duplicates or triplicates at each Fe concentration. They were sampled during the exponential phase of growth for total cell concentration (CC), biogenic silica (BSi), and particulate (i.e. cellular) carbon (C) and nitrogen (N).

2.2 Culture media

The complete medium consisted of artificial AQUIL seawater enriched with 300 μmol L^{-1} nitrate, 10 μmol L^{-1} phosphate, 100 μmol L^{-1} silicate, 0.55 μg L^{-1} vitamin B_{12}, 0.5 μg L^{-1} biotin, 100 μg L^{-1} thiamin, 10 nmol L^{-1} selenite and 100 nmol L^{-1} molybdate (Price et al., 1988,1989). The medium also contained a trace metal ion buffer system consisting of 100 μmol L^{-1} ethylene diamine tetra acetic acid (EDTA), 19.6 nmol L^{-1} Cu, 50.3 nmol L^{-1} Co, 79.7 nmol L^{-1} Zn and 121 nmol L^{-1} Mn. The buffer system generated free ion concentrations of Cu, Co, Zn and Mn of $10^{-13.79}$, $10^{-10.88}$, $10^{-10.88}$ and $10^{-8.27}$ mol L^{-1}, respectively, at pH 8.1 (Price et al., 1988,1989). Added iron concentrations to the medium ranged from 0 (no addition) to 500 nmol L^{-1}.

BGD
6, 7175–7205, 2009

Background iron in the medium without EDTA (0.61 nmol L^{-1}) was measured by ICP-MS after preconcentration onto an 8-HQ resin. Total iron concentration was computed from the sum of added iron and the background iron concentration. In this medium, inorganic iron concentrations ([Fe']) can be estimated from total iron concentrations, and depend on the irradiance (Sunda and Huntsman, 1997). They varied between 0.85 and 699 pmol L^{-1} at HL and between 0.74 and 610 pmol L^{-1} at LL.

2.3 Specific growth rate

Cellular concentrations (CC, cells ml^{-1}_{medium}) were determined by microscopic counts. Specific growth rate (μ, d^{-1}) was determined by linear regression of the natural log CC versus time. The cells exhibited a constant daily specific growth rate over several days before the experiments.

2.4 Cellular nitrogen and carbon

All the glassware used for cell carbon and nitrogen determination (filter holders, filtration funnels, and vials) were washed with 10% HCl, rinsed with Milli-Q water and dried. They were then pre-combusted at 450°C for 4.5 h. Cells from culture samples were filtered as duplicates onto GF/F filters (pre-combusted as the glassware) and rinsed with artificial seawater containing no nutrient. Samples were stored frozen at –20°C and were dried before analysis. Samples were analyzed using a Carlo-Erba NA-1500 elemental analyzer.

2.5 Biogenic silica (BSi)

Culture samples were filtered as duplicates onto 0.6 μm polycarbonate membrane and rinsed with artificial seawater containing no nutrient. The filters were oven dried at 60°C for 24 h, digested for 7 days in 2.9 mol L^{-1} HF, and the resulting orthosilicic acid

was measured by spectrophotometry (Ragueneau and Tréguer, 1994).

3 Results

3.1 Specific growth rate

The specific growth rate decreased with the irradiance and the inorganic iron concentrations in the medium for both species (Fig. 1). The maximum specific growth rate (μ_{max}) and the half-saturation constant for growth with respect to iron ($K_{\mu Fe'}$) were determined using a Monod saturation function (Table 1). When the irradiance decreased by 10-fold, μ_{max} decreased by 1.8-fold and 1.6-fold for *T. oceanica* and *D. brightweilii*, respectively. In the same time, $K_{\mu Fe'}$ increased by 2.3-fold for *T. oceanica*, and did not seem to vary significantly for *D. brightwellii*, but this may be due to the large standard error of $K_{\mu Fe'}$ at LL.

3.2 Cellular nitrogen and carbon

The trends in variations in cellular carbon were similar for both diatoms under HL. Cellular C decreased with Fe limitation under HL from ~1 pmol cell^{-1} to ~0.5 pmol cell^{-1} for *T. oceanica* (Fig. 2a) and from ~50 pmol cell^{-1} to ~30 pmol cell^{-1} for *D. brightwellii* (Fig. 2b). At a given growth rate, the C content was higher under LL than under HL for both species (t-test, *T. oceanica*: t=3.06, p=0.007, n=18; *D. brightwellii*: t=3.96, p=0.002, n=14). Under LL, cellular C did not change with Fe limitation for *T. oceanica* (0.67±0.07 pmol cell^{-1}, mean±SD, n=16), while it decreased for *D. brightwellii* (from ~65 pmol cell^{-1} to ~37 pmol cell^{-1}).

For both species and light conditions, at μ higher than 0.1 d^{-1}, the nitrogen content decreased with Fe limitation (Fig. 2c, d). At a given specific growth rate, it was similar for *T. oceanica* under LL and HL (t-test, t=1.927, p=0.070, n=20) and decreased from ~0.1 pmol cell^{-1} at 1.2 d^{-1} to ~0.04 pmol cell^{-1} at 0.3 d^{-1}. At the most severe

BGD
6, 7175–7205, 2009

Fe-L co-limitation (μ=0.09 d^{-1}), the nitrogen content of *T. oceanica* increased up to 0.07 pmol $cell^{-1}$. For *D. brightwellii*, on the opposite, it was higher at LL than at HL (t-test, t=4.153, p=0.001, n=14), decreasing approximately from 11.5 to 5 pmol $cell^{-1}$ at 1 d^{-1} (LL and HL, respectively) and from 6 pmol $cell^{-1}$ to 4.5 pmol $cell^{-1}$ at 0.5 d^{-1} (LL and HL, respectively).

3.3 Biogenic silica (BSi)

The BSi content of *T. oceanica* was scattered under Fe limitation at HL and did not change significantly with the specific growth rate (r^2=0.01, p=0.64, n=17) (Fig. 2e). Under LL, however, BSi per cell was lower than at HL (t-test, t=4.89, p=0.0002, n=17) and increased with Fe limitation (r^2=0.60, p<0.05, n=13). The reverse trend was observed for *D. brightwellii*, with a decrease with Fe limitation (μ<0.6 d^{-1}) for both light conditions (Kruskall Wallis test, χ^2=12.55, p=0.0057, n=18) and similar values at a given specific growth rate under low and high light (Tukey test, p>0.05) (Fig. 2f).

3.4 Elemental ratios C:N, Si:C and Si:N

The molar ratio C:N was similar at LL and HL for *D. brightwellii* (t-test, t=3.24, p=0.071, n=14) and higher at LL than at HL for *T. oceanica* (t-test, t=3.63, p=0.002, n=20) (Fig. 3a, b). It increased for both diatoms when the specific growth rate decreased (~1.6-fold for *T. oceanica* and 1.4-fold for *D. brightwellii*), except at the most severe Fe-L co-limitation for *T. oceanica*, where it equalled the non limited value. Molar ratios Si:C (Fig. 3c, d) and Si:N (Fig. 3e, f) did not follow the same patterns for the two species. For *T. oceanica*, Si:C increased by ~1.8-fold and 1.4-fold under Fe limitation and Fe-L co-limitation, respectively, and Si:N increased by ~2-fold and 1.5-fold under Fe limitation and Fe-L co-limitation respectively, with the exception of the most severe Fe-L co-limitation where a decrease was observed. Si:C was lower under LL than under HL (t-test, t=6.13, p=0.00002, n=17), while Si:N was equivalent under HL and LL (t-test, t=0.55, p=0.59, n=17). For *D. brightwellii*, Si:C and Si:N increased respectively by

2-fold and 2.4-fold under Fe-limitation down to a specific growth rate of 0.6 d^{-1}. Below 0.6 d^{-1}, the ratios decreased to similar values as for non limited conditions. Under Fe-L co-limitation, the same pattern was observed, but it was harder to characterize due to the lower number of values. Besides, Si:C and Si:N were lower at LL than at HL for >0.6 d^{-1}, and similar for μ<0.6 d^{-1}.

To compare the effects of the limitations on the two diatoms, we will use in the discussion section the ratio R, defined as the relative variation of a given parameter between a limiting condition and the Fe-replete condition (e.g., at a specific growth rate μ, $R(Si:N)_\mu = (Si:N)_\mu : (Si:N)_{\mu\,max}$). The degree of limitation will be defined by its impact on the growth rate using the ratio $\mu:\mu_{max}$ (i.e. $R(\mu)$), with the value of maximum growth rate measured at the highest Fe concentration under HL for the Fe-limited experiment (e.g.: μ_{max} ~1.2 d^{-1} for *T. oceanica*), and under LL for the Fe-L co-limited experiment (e.g.: μ_{max}~0.7 d^{-1} for *T. oceanica*).

4 Discussion

4.1 Growth parameters and the decoupling of cellular C and N

The half-saturation constants for growth with respect to iron ($K_{\mu Fe'}$, Table 1) agree well with previous studies, showing a much lower value, i.e. a better adaptation to limitation (Fe and Fe-L) of a small diatom than of a large one (Sunda and Huntsman, 1995; Timmermans et al., 2001, 2004). Once the limitation relieved, however, the larger diatom would outgrow the smaller one due to its higher maximum specific growth rate. This is in agreement with in situ observations of large diatoms blooms following oceanic Fe enrichment both in situ and during incubation experiments (de Baar et al., 2005). The better adaptation of *T. oceanica* can be explained by a more favorable surface to volume ratio for a small species than for a bigger one (Hudson and Morel, 1990) and a general lower Fe requirement for growth in the oceanic species than in the coastal species (Sunda and Huntsman, 1995; Sunda et al., 1991). For example, oceanic

diatoms can synthesize flavodoxin instead of ferredoxin (La Roche et al., 1995). It has also recently been shown that *T. oceanica* uses the copper-containing plastocyanin instead of the functionally equivalent Fe-containing cytochrome c6 (Peers and Price, 2006), and has a different photosynthetic apparatus from a coastal species, i.e. lower cellular concentrations of Fe-rich cytochrome b6/f and PSI (Strzepek and Harrison, 2004). This could also explain the differences observed in the variations in cellular C between the two species (Fig. 2a, b). Cells acclimatize to low light by increasing their photosynthetic C-fixation rate (Sunda and Huntsman, 2004). It explains the increase in cellular C between HL and LL conditions for both species. However, cellular C decreased markedly with increasing Fe limitation under LL for *D. brightwellii*, while it remained constant for *T. oceanica* (Fig. 2a, b). Increasing the photosynthetic C-fixation rate indeed implies an increase in the photosynthetic capacity and thus cellular Fe (Sunda and Huntsman, 2004). Its photosynthetic apparatus allows *T. oceanica* to decrease its cellular iron requirements but not its photosynthetic rates (Strzepek and Harrison, 2004), and may thus help *T. oceanica* to better maintain its C content than *D. brightwellii* under LL and increasing Fe limitation.

The inefficiency of photosynthesis also reduces the efficiency of nitrite reduction by lowering the amount of reductants. This directly disrupts the metabolism of nitrogen, whose energetic needs are important (Timmermans et al., 1994; Muggli et al., 1996). Besides, Fe is the metal at the center of the nitrate and nitrite reductases. These combined effects of Fe limitation on N metabolism may explain why we observed a stronger effect of Fe on N than on C of Fe and Fe-L (co-)limited cells, except at the most severe degree of limitation for *T. oceanica* (i.e. at the highest degree of Fe-L co-limitation). If we exclude that singular point, N content indeed decreased by 60% and 50% for *T. oceanica* and *D. brightwellii*, respectively (same relative decrease at LL and HL, Fig. 2c, d) while C content decreased by ~40% for *T. oceanica* and *D. brightwellii* at HL and did not vary (*T. oceanica*) or decreased by 40% (*D. brightwellii*) at LL. As indicated above, however, at the highest degree of Fe-L (co)-limitation for *T. oceanica*, the N content doubled while C remained stable. This sharp increase might be explained

by the high level of Fe limitation, even more important under LL. It has been suggested that under severe Fe stress, *T. oceanica* may produce a Fe reductase that is also a plasmalemma bound form of nitrate reductase (Maldonado and Price, 2000). In that case, severely Fe-limited cells might increase their N quota while increasing Fe uptake. Our results give support to this hypothesis.

Many other studies focused on the intracellular C and/or N quota of Fe-limited diatoms. Their conclusions are rarely similar, even for the same species. To better compare all of these studies, we considered the relative variation in C and N per cell (i.e. R(C) and R(N)), versus the relative variation in the specific growth rate, i.e. R(μ) for 14 other Fe-limited species in six other studies (5 species of *Pseudonitzschia*: Marchetti and Harrison, 2007; 6 species of *Thalassiosira* including *T. oceanica*: Gallinari et al., 2009; Maldonado and Price, 1996; Timmermans et al., 2004; *Actinocyclus* sp.: Muggli et al., 1996; Timmermans et al., 2004; *Fragilariopsis kerguelensis* : Hoffmann et al., 2007; Timmermans et al., 2004; *Corethron pennatum* : Timmermans et al., 2004; and *Chaetoceros dichaeta*: Hoffmann et al., 2007). Results are reported on Fig. 4a and b. If we exclude our value of *T. oceanica* at the most severe limitation, R(C) and R(N) tend to decrease when Fe or Fe-L co-limitation increases (for R(C): r^2=0.58, p<0.00001, n=63, and R(N): r^2=0.54, p<0.00001, n=87). However, when considering the N or C quota per cell volume (when available: Gallinari et al., 2009; Maldonado and Price, 1996; Marchetti and Harrison, 2007; Muggli et al., 1996), there is no significant trend in R(C) or R(N), as also observed by Price (2005) for *T. weissflogii*. Given the importance of cell volume in comparing the different species and studies, we considered the relative variation in C:N, i.e. R(C:N), versus the relative variation in the specific growth rate, i.e. R(μ). In our study, and excluding our value of *T. oceanica* at the most severe limitation, we observed a similar increase with limitation for both species and both limitations (r^2=0.31, p=0.0001, n=47) (Fig. 5a). The relative variation in C:N of the other species cited above, however, does not show any dependency on R(μ) (r^2=0.003, p=0.8, n=24, data not shown). Growth conditions and species difference have been invoked to explain these contrasting results (Price, 2005). However, in the

same growth conditions, we did not observe a significant interspecific difference in our study. The contrasting results observed on the coupling or decoupling of C and N under Fe limitation may thus be more related to growth conditions (temperature, length of the daily cycle...) than to interspecific differences. On the whole, these results show that using a constant C:N ratio to infer Si:C from Si:N, as often done for modeling and in situ experiments, may lead to a bias.

4.2 Biogenic silica and ratios Si:C, Si:N

Most of the studies show an increase in biogenic silica under Fe limitation. We also observed a significant increase in the degree of silicification of Fe-L co-limited *T. oceanica*, but no clear trend under Fe limitation (Fig. 2e). The increase under Fe-L co-limitation may be due to light limitation only. Claquin et al. (2002) indeed showed that light limitation increases the amount of biogenic silica per cell of *Thalassiosira pseudonana*. Two recent studies also showed no change or a weak decrease in cellular biogenic silica of Fe-limited cells of *Chaetoceros dichaeta* (Hoffmann et al., 2007) and some clones of *Pseudonitzschia* (Marchetti and Harrison, 2007), respectively. These results are observed between two values ("low Fe" and "high Fe"), but the effect of Fe on silicification may depend on the degree of Fe limitation (this study, Timmermans et al., 2004). However, there is no significant trend in R(BSi) versus R(μ) when comparing different diatoms (Fig. 4c). This suggests interspecific differences in term of silicification in response to Fe or Fe-L limitation.

Marchetti and Harrison (2007) invoke different mechanisms likely to induce a decrease in biogenic silica under Fe limitation, like the changes in cell morphology and the existence of soluble pools. Although we did not study cell morphology or soluble pools, these hypotheses may be valid for *D. brightwellii*. Indeed, this species has spines, which may contain a large fraction of biogenic silica (e.g. *C. gracilis*, Rogerson et al., 1986). Timmermans et al. (2001) observed more/longer spines for the Fe-limited diatoms *C. calcitrans* and *C. brevis* when grown at LL. A decrease in their number or length in *D. brightweilii*, due to Fe and light limitations, may thus affect the BSi content.

Besides, Chisholm et al. (1978) showed that for *D. brightweilii*, the intracellular pool of Si may represent up to 50% of total cellular Si, and the size of internal soluble pool can be influenced by environmental variables (Martin-Jézéquel et al., 2000). However, although these mechanisms may explain why we observed a variation in the silicification of diatoms, the underlying processes are not explained. The causal link between iron and silicification has still to be discovered. A few hypotheses can be proposed, based on the silicification process and the possible role of the frustule as a defense mechanism. It is known that the energy for silicon metabolism is closely linked to respiration (Martin-Jézéquel et al., 2000). A recent paper showed that mitochondrial electron transport was down regulated in Fe limited cells of *Phaeodactylum tricornutum* (Allen et al., 2008). This may disrupt silicification. Another effect might be the control of Fe on the cell cycle via the cellular growth rate. Claquin et al. (2002) indeed showed for light and nutrient (N, P)-limited cells of *T. pseudonana* a relationship between the increased length of the G2 phase (during which Si is assimilated) and the higher degree of silicification under limitation. The increase in silicification of *T. oceanica* under the Fe-L co-limitation may indeed be due to an increase in the G2 phase duration (Claquin and Bucciarelli, 2009). If Fe limitation decreased the length of G2 phase for *D. brightwellii*, it might explain the decrease in BSi that we observed. This difference between the two species may also be related to their ability to escape grazing. Predation avoidance mechanisms include larger size and spines (Irigoien et al., 2005). The frustule is also an effective protection against zooplankton grazing (Hamm et al., 2003). A recent study showed a grazing-induced increase in cell wall silicification in the marine diatom *T. weissflogii* (Pondaven et al., 2007). Small, limited cells which are easier to graze may need stronger frustules than bigger cells. Under energy limitation (Fe and Fe-L), big cells that are not as sensitive as small ones to grazing may choose to reduce their silicification and save on respiratory energy.

Under mild Fe limitation (μ>40% μ_{max}), we observed an increase in Si:C and Si:N ratios (Fig. 3c–f), which has been noted previously by other studies (Hutchins and Bruland, 1998; Takeda, 1998; Timmermans et al., 2004). We also noted a decoupling

between Si, C and N under Fe-L co-limitation, which has been described recently for in situ studies (Hopkinson and Barbeau, 2008; Moore et al., 2007) but not for monospecific laboratory cultures yet. As changes in BSi were lower than in C or N under LL or HL at a given specific growth rate, the differences in Si:C and Si:N between the two irradiances depended mainly on the differences in the C and N contents. Under LL, the higher C content and the lower BSi value (for *T. oceanica*) and higher C and N content (for *D. brightwellii*), compared to HL conditions, induced a lower value of Si:C for *T. oceanica* at a given specific growth rate and a lower value of Si:C and Si:N for *D. brightwellii* at μ>0.6 d^{-1}. Besides, under severe limitation, we observed for the first time a decrease in these ratios. This pattern was especially clear for *D. brightwellii*. The decrease observed in this species was due to a larger decrease in biogenic silica under Fe limitation (by 60%) than in the cellular N and C content (by 50% and 40%, respectively).

4.3 Oceanographic relevance and use for modelling studies

We observed for both species and both limitations an increase in R(Si:C) and R(Si:N) from 100% to ~40% of μ_{max}, a decrease below ~40% of μ_{max}, and a possible increase in R(Si:C) below 20% of μ_{max} (Fig. 5b, c). To asses the field significance of our results, we estimated R(Si:N) for artificial Fe fertilisation experiments, when other nutrients were replete and when specific growth rate and Si:N ratios were reported or could be estimated (Fig. 6, Table 2). Those field data seem to follow a similar pattern as the one observed in our monospecific laboratory culture experiments. For values of μ:μ_{max} higher than 40%, a clear trend is observed with a decrease in Si:N ratio after fertilisation (i.e. when μ:μ_{max} increases):

$$R(Si:N) = 2.36(\pm 0.15) - 1.43(\pm 0.18) * R(\mu); r^2 = 0.60, p < 0.00001, n = 42$$

For values of μ:μ_{max} lower than 40%, the trend is less obvious. R(Si:N) tends to decrease between 20 and 40% of μ_{max} down to values below 1, i.e. below the value of

BGD
6, 7175–7205, 2009

Si:N at μ_{max}:

$$R(Si:N) = -1.34(\pm 0.60) + 8.64(\pm 1.84) * R(\mu); r^2 = 0.73, p = 0.0015, n = 10$$

It tends to increase again below 20% of μ_{max}:

$$R(Si:N) = 3.46(\pm 0.77) - 15.3(\pm 5.62) * R(\mu); r^2 = 0.60, p = 0.04, n = 7$$

Most of the artificial Fe fertilisation experiments exhibited decreasing Si:N values in the Fe fertlized patch compared to Fe or Fe-L limited conditions. However, an increase in Si:N during phase I of the SERIES bloom (Marchetti et al., 2006) and at the end of the EIFEX fertilisation (Hoffmann et al., 2006) was reported. If we consider the two different Fe infusions during EIFEX, it first showed a decrease in Si:N in the Fe fertilised patch compared to outside at the end of the first infusion (day 11–12, μ:μ_{max}<0.2), and then an increase compared to outside at the end of the second infusion (day 26–27, 0.2<μ:μ_{max}<0.4) (Hoffmann et al., 2007). The general trend was an increase in the Fe fertilised patch, which was attributed to a shift in the diatom community towards more silicified species (Hoffmann et al., 2006). A shift in dominant phytoplankton taxa towards diatoms was also invoked to explain the increase in Si:N during the phase I of SERIES (Marchetti et al., 2006). According to the present study, those increases in Si:N might also be due to an initial specific growth rate between ~15% and 30% of the maximum specific growth rate.

Previous studies indicating that diatoms increase their Si:N ratio under Fe limitation led to the assumptions (i) that more silicified, Fe limited diatoms would sink faster and that their frustule would be better preserved in sediments, with implications for the use of opal as a paleoproxy (Hutchins and Bruland, 1998; Takeda, 1998; Boyle, 1998) and (ii) that this decoupling may fuel the so-called silica pump in systems like the Southern Ocean or the Equatorial Pacific; systems which are known to be High Nitrate Low Chlorophyll Low Silicate environments (Dugdale et al., 1995). In biogeochemical models which consider the cycling of major nutrients such as C, N, P and Si, it is usually assumed that diatom Si content, Si:N and Si:C ratios increase under

limiting conditions, and that biogenic silica is efficiently exported below the mixed layer depth because of a lower remineralisation rate than organic C or N (Aumont et al., 2003; Moore et al., 2004). However, our results over a large range of Fe and Fe-L limitation and reduced specific growth rate show (i) that Fe and Fe-L limited diatoms may be less silicified than under conditions of optimal growth, and (ii) that the decoupling between Si, C and N in surface waters may be less straightforward than previously thought. The frustule of less silicified diatoms might dissolve more rapidly, which would decrease its preservation in the water column and sediments. High dissolution rates of BSi have actually been reported in Fe limited systems such as the Southern Ocean and the Equatorial Pacific (Brzezinski et al., 1998; Beucher et al., 2004). Besides, other mechanisms will influence the preservation of the frustule in the water column and its export from surface waters: grazing and formation of aggregates are the main sources of biogenic matter, including opal, towards the deep ocean (see review by Ragueneau et al., 2006). Grazing is recognized as one of the main factor that limits the development of phytoplanktonic blooms in HNLC waters, next to Fe and L limitation (de Baar and la Roche, 2003). It not only determines species succession but also has a major importance on the relative preservation of Si and C (presumably also N). Grazing indeed induces Si enrichment between food and fecal pellets, which can contribute to measured increase between production and export (Ragueneau et al., 2006). Export also depends on the incorporation of diatoms into aggregates (Moriceau et al., 2007). Formation of aggregates is promoted by the coagulation of sticky transparent exopolymer particles (TEP, formed from dissolved organic carbon released by phytoplankton) and other particles like cells (Passow et al., 2001). It has recently been shown that iron starved *Trichodesmium* produce more TEP and experience higher sedimentation rates (Berman Franck et al., 2007). According to our study, aggregation and sinking of Fe limited diatoms may export more nitrate (or carbon) than silicon between ~15% and ~30% of the maximum specific growth rate that can be achieved under non limiting conditions. These results may have important implications for the understanding and modeling of the biogeochemical cycles and estimates of C export.

5 Conclusions

General trends in the elemental composition of Fe limited and Fe-L co-limited diatoms could be determined by taking into account our results and literature data, and by considering the degree of limitation (i.e. reduction in growth rate).

We showed that C and N per cell tend to decrease with Fe and Fe-L co-limitation for all species, but an increase in C:N with increasing limitation was only significant for the species we studied. Contrasting results between literature data may be more related to growth conditions than to interspecific differences. There was no significant trend in silica content when comparing different Fe or Fe-L limited diatoms, which suggests interspecific differences. However, variations in Si:C or Si:N seem to be more constrained, even when comparing our results from monospecific laboratory cultures to in situ artificial Fe fertilisations. Under mild limitation ($\sim\mu$:μ_{max}>40%), a clear trend is observed with a decrease in Si:N ratio after fertilisation. Under more severe limitation, the trend is less obvious. Between ~15% and 30% of μ_{max}, Si:N is even lower than under non limiting conditions. More data are needed in that range of limitation, and this may have to be considered for parameterizing biogeochemical models to infer Si, C and N decoupling and C export.

Acknowledgements. This work was funded by the support from the European Commission's Marine Science and Technology Programme under Contract EVK2-1999-00227 (IRONAGES, "Iron Resources and Oceanic Nutrients – Advancements of Global Environment Simulations") and the BOA project (ANR-05-BLAN-0153). The iron concentration in the background medium was measured by Morgane Gallinari. Annick Masson performed carbon and nitrogen measurements. E. B. and G. S. would like to declare that they disagree with the scientific policy that the french government is presently pursuing, which will undoubtedly lead to a rapid decline in french fundamental research and which will deter students from pursuing research.

The publication of this article is financed by CNRS-INSU.

References

Allen, A. E., La Roche, J., Maheswari, U., Lommer, M., Schauer, N., Lopez, P. J., Finazzi, G., Fernie, A. R., and Bowler, C.: Whole-cell response of the pennate diatom *Phaeodactylum tricornutum* to iron starvation, P. Natl. Acad. Sci., 105, 10438–10443, 2008.

Aumont, O., Maier-Reimer, E., Blain, S., and Monfray, P.: An ecosystem model of the global ocean including Fe, Si, P co-limitations, Global Biogeochem. Cy., 17, 1060, doi:10.1029/2001GB001745, 2003.

Barber, R. T. and Hiscock, M. R.: A rising tide lifts all phytoplankton: growth response of other phytoplankton taxa in diatom-dominated blooms, Global Biogeochem. Cy., 20, GB4S03, doi:10.1029/2006GB002726, 2006.

Berman Franck, I., Rosenberg, G., Levitan, O., Haramaty, L., and Mari, X.: Coupling between autocatalytic cell detah and transparent expolymer particle production in the marine cyanobacterium *Trichodesmium*, Environ. Microbiol., 9, 1415–1422, 2007.

Beucher, C., Tréguer, P., Hapette, A.-M., Corvaisier, R., Metzl, N., and Pichon J.-J.: Intense summer Si-recycling in the surface Southern Ocean, Geophys. Res. Lett., 31, L09305, doi:10.1029/2003GL018998, 2004.

Boyd, P. W., LaRoche, J., Gall, M., Frew, R., and McKay, R. M. L.: Role of iron, light, and silicate in controlling algal biomass in subantarctic waters SE of New Zealand, J. Geophys. Res., 104, 13395–13408, 1999.

Boyd, P. W., Watson, A. J., Law, C. S., Abraham, E. R., Trull, T., Murdoch, R., Bakker, D. C. E., Bowie, A., Buesseler, K. O., Chang, H., Charette, M., Croot, P., Downing, K., Frew, R., Gall, M., Hadfield, M., Hall, J., Harvey, M., Jameson, G., LaRoche, J., Liddicoat, M., Ling, R., Maldonado, M. T., McKay, R. M., Nodder, S., Pickmere, S., Pridmore, R., Rintoul, S.,

Safi, K., Sutton, P., Strzepek, R., Tanneberger, K., Turner, S., Waite, A., and Zeldis, J.: A mesoscale phytoplankton bloom in the polar Southern Ocean stimulated by iron fertilisation, Nature, 407, 695–702, 2000.

Boyd, P. W., Law, C. S., Wong, C. S., Nojiri, Y., Tsuda, A., Levasseur, M., Takeda, S., Rivkin, R., Harrison, P. J., Strzepek, R., Gower, J., McKay, R. M., Abraham, E., Arychuk, M., Barwell-Clarke, J., Crawford, W., Crawford, D., Hale, M., Harada, K., Johnson, K., Kiyosawa, H., Kudo, I., Marchetti, A., Miller, W., Needoba, J., Nishioka, J., Ogawa, H., Page, J., Robert, M., Saito, H., Sastri, A., Sherry, N., Soutar, T., Sutherland, N., Taira, Y., Whitney, F., Wong, S.-K. E., and Yoshimura, T.: The decline and fate of an iron-induced subarctic phytoplankton bloom, Nature, 428, 549–553, 2004.

Boyle, E.: Pumping iron makes thinner diatoms, Nature, 393, 733–734, 1998.

Bozec, Y., Bakker, D. C. E., Hartmann, C., Thomas, H., Bellerby, R. G. J., Nightingale, P. D., Riebesell, U., Watson, A. J., and de Baar, H. J. W.: The CO_2 system in a Redfield context during an iron enrichment experiment in the Southern Ocean, Mar. Chem., 95, 89–105, 2005.

Brzezinski, M. A., Villareal, T. A., and Lipschultz, F.: Silica production and the contribution of diatoms to new and primary production in the Central North Pacific, Mar. Ecol. Prog. Ser., 167, 89–104, 1998.

Chisholm, S. W., Azam, F., and Eppley, R. W.: Silicic acid incorporation in marine diatoms on light:dark cycles: use as an essay for phased cell division, Limnol. Oceanogr., 23, 518–529, 1978.

Claquin, P., Martin-Jézéquel, V., Kromkamp, J. C., Veldhuis, M. J. W., and Kraay, G. W.: Uncoupling of silicon compared with carbon and nitrogen metabolisms and the role of the cell cycle in continuous cultures of *Thalassiosira pseudonana* (Bacillariophyceae) under light, nitrogen, and phosphorus control, J. Phycol., 38, 922–930, 2002.

Claquin, P. and Bucciarelli, E.: Cell cycle and silicification: Impact of an iron/light co-limitation on the marine diatom *Thalassiosira oceanica*, in preparation, 2009.

Coale, K. H., Johnson, K. S., Chavez, F. P., Buesseler, K. O., Barber, R. T., Brzezinski, M. A., Cochlan, W. P., Millero, F. J., Falkowski, P. G., Bauer, J. E., Wanninkhof, R. H., Kudela, R. M., Altabet, M. A., Hales, B. E., Takahashi, T., Landry, M. R., Bidigare, R. R., Wang, X., Chase, Z., Strutton, P. G., Friederich, G. E., Gorbunov, M. Y., Lance, V. P., Hilting, A. K., Hiscock, M. R., Demarest, M., Hiscock, W. T., Sullivan, K. F., Tanner, S. J., Gordon, R. M., Hunter, C. N., Elrod, V. A., Fitzwater, S. E., Jones, J. L., Tozzi, S., Koblizek, M., Roberts, A. E., Herndon,

J., Brewster, J., Ladizinsky, N., Smith, G., Cooper, D., Timothy, D., Brown, S. L., Selph, K. E., Sheridan, C. C., Twining, B. S., and Johnson, Z. L.: Southern Ocean iron enrichment experiment: carbon cycling in high- and low-Si waters, Science, 304, 408–414, 2004.

de Baar, H. J. W. and La Roche, J.: Trace metals in the oceans: evolution, biology and global change, in: Marine science frontiers for Europe, edited by: Wefer, G., Lamy, F., and Mantoura, F., Springer-Verlag, Berlin Heidelberg New York Tokyo, 79–105, 2003.

de Baar, H. J. W., Boyd, P., Coale, K. H., Landry, M. R., Tsuda, A., Assmy, P., Bakker, D. C. E., Bozec, Y., Barber, R. T., Brzezinski, M. A., Buesseler, K. O., Boyé, M., Croot, P. L., Gervais, F., Gorbunov, M. Y., Harrison, P. J., Hiscock, W. T., Laan, P., Lancelot, C., Law, C. S., Levasseur, M., Marchetti, A., Millero, F. J., Nishioka, J., Nojiri, Y., van Oijen, T., Riebesell, U., Rijkenberg, M. J. A., Saito, H., Takeda, S., Timmermans, K. R., Veldhuis, M. J. W., Waite, A. M., and Wong, C. S.: Synthesis of iron fertilisation experiments: from the Iron Age in the Age of Enlightenment, J. Geophys. Res., 110, C09S16, doi:10.1029/2004JC002601, 2005.

Dugdale, R. C., Wilkerson, F. P., and Minas, H. J.: The role of a silicate pump in driving new production, Deep Sea Res. I, 42, 697–719, 1995.

Falkowski, P. G., Barber, R. T., and Smetacek, V.: Biogeochemical controls and feedbacks on ocean primary production, Science, 281, 200–206, 1998.

Falkowski, P., Scholes, R. J., Boyle, E., Canadell, J., Canfield, D., Elser, J., Gruber, N., Hibbard, K., Högberg, P., Linder, S., Mackenzie, F. T., Moore III, B., Pedersen, T., Rosenthal, Y., Seitzinger, S., Smetacek, V., and Steffen, W.: The global carbon cycle: a test of our knowledge of Earth as a system, Science, 290, 291–296, 2000.

Frew, R., Bowie, A., Croot, P., and Pickmere, S.: Macronutrient and trace-metal geochemistry of an in situ iron-induced Southern Ocean bloom, Deep Sea Res. II, 48, 2467–2481, 2001.

Gallinari, M., Bucciarelli, E., Moriceau, B., and Ragueneau, O.: Dissolution properties of biogenic silica from diatoms grown under iron-replete and iron-limited conditions, in preparation, 2009.

Geider, R. J. and La Roche, J.: Redfield revisited: variability of C:N:P in marine microalgae and its biochemical basis, Eur. J. Phycol., 37, 1–17, 2002.

Gervais, F., Riebesell, U., and Gorbunov, M. Y.: Changes in primary productivity and chlorophyll *a* in response to iron fertilisation in the Southern Polar Frontal Zone, Limnol. Oceanogr., 47, 1324–1335, 2002.

Hamm, C. E., Merkel, R., Springer, O., Jurkojc, P., Maier, C., Prechtel, K., and Smetacek, V.: Architecture and material properties of diatom shells provide effective mechanical protection,

Nature, 421, 841–843, 2003.
Hoffmann, L. J., Peeken, I., Lochte, K., Assmy, P., and Veldhuis, M.: Different reactions of Southern Ocean phytoplankton size classes to iron fertilisation, Limnol. Oceanogr., 51, 1217–1229, 2006.
Hoffmann, L. J., Peeken, I., and Lochte, K.: Effects of iron on the elemental stoichiometry during EIFEX and in the diatoms *Fragilariopsis kerguelensis* and *Chaetoceros dichaeta*, Biogeosciences, 4, 569–579, 2007, http://www.biogeosciences.net/4/569/2007/.
Hopkinson, B. M. and Barbeau, K. A.: Interactive influences of iron and light limitation on phytoplankton at subsurface chlorophyll maxima in the eastern North Pacific, Limnol. Oceanogr., 53, 1303–1318, 2008.
Hudson, R. J. M. and Morel, F. M. M.: Iron transport in marine phytoplankton: kinetics of cellular and medium coordination reactions, Limnol. Oceanogr., 35, 1002–1020, 1990.
Hutchins, D. A. and Bruland, K. W.: Iron-limited diatom growth and Si:N uptake ratios in a coastal upwelling regime, Nature, 393, 561–564, 1998.
IPCC: IPCC fourth assessment report: Synthesis report, online available at: http://www.ipcc.ch/ipccreports/ar4-syr.htm, 2007.
Irigoien, X., Flynn, K. J., and Harris, R. P.: Phytoplankton blooms: a 'loophole' in microzooplankton grazing impact?, J. Plankton Res., 27, 313–321, 2005.
Keller, M. D., Bellows, W. K., and Guillard, R. R. L.: Microwave treatment for sterilization of phytoplankton culture media, J. Exp. Mar. Biol. Ecol., 117, 279–283, 1988.
Kudo, I., Noiri, Y., Imai, K., Nojiri, Y., Nishioka, J., and Tsuda, A.: Primary productivity and nitrogenous nutrient assimilation dynamics during the Subarctic Pacific Iron Experiment for Ecosystem Dynamics Study, Prog. Oceanogr., 64, 207–221, 2005.
La Roche, J., Murray, H., Orellana, M., and Newton, J.: Flavodoxin expression as an indicator of iron limitation in marine diatoms, J. Phycol., 31, 520–530, 1995.
Maldonado, M. T. and Price, N. M.: Influence of N substrate on Fe requirements of marine centric diatoms, Mar. Ecol. Prog. Ser., 141, 161–172, 1996.
Maldonado, M. T., Boyd, P. W., Harrison, P. J., and Price, N. M.: Co-limitation of phytoplankton growth by light and Fe during winter in the NE subarctic Pacific Ocean, Deep Sea Res. II, 46, 2475–2485, 1999.
Maldonado, M. T. and Price, N. M.: Nitrate regulation of Fe reduction and transport by Fe-limited *Thalassiosira oceanica*, Limnol. Oceanogr., 45, 814–826, 2000.

Marchetti, A., Juneau, P., Whitney, F. A., Wong, C. S., and Harrison, P. J.: Phytoplankton processes during a mesoscale iron enrichment in the NE subarctic Pacific: Part II - Nutrient utilization, Deep Sea Res. II, 53, 2114–2130, 2006.

Marchetti, A. and Harrison, P. J.: Coupled changes in the cell morphology and the elemental (C, N , and Si) composition of the pennate diatom *Pseudo-nitzschia* due to iron deficiency, Limnol. Oceanogr., 52, 2270–2284, 2007.

Martin-Jézéquel, V., Hildebrand, M., and Brzezinski, M. A.: Silicon metabolism in diatoms: implications for growth, J. Phycol., 36, 1–20, 2000.

Moore, C. M., Mills, M. M., Milne, A., Langlois, R., Achterberg, E. P., Lochte, K., Geider, R. J., and La Roche, J.: Iron limits primary productivity during spring bloom development in the central North Atlantic, Glob. Change Biol., 12, 626–634, 2006.

Moore, C. M., Hickman, A. E., Poulton, A. J., Seeyave, S., and Lucas, M. I.: Iron-light interactions during the CROZet natural iron bloom and EXport experiment (CROZEX): II – Taxonomic responses and elemental stoichiometry, Deep Sea Res. II, 54, 2066–2084, 2007.

Moore, J. K., Doney, S. C., and Lindsay, K.: Upper ocean ecosystem dynamics and iron cycling in a global three-dimensional model, Global Biogeochem. Cy., 18, GB4028, doi:10.1029/2004GB002220, 2004.

Moriceau, B., Soetaert, K., Gallinari, M., and Ragueneau, O.: Importance of particles formation to reconstruct water column biogenic silica fluxes, Global Biogeochem. Cy., 21, GB3012, doi:10.1029/2006GB002814, 2007.

Muggli, D. L., Lecourt, M., and Harrison, P. J.: Effects of iron and nitrogen source on the sinking rate, physiology and metal composition of an oceanic diatom from the subarctic Pacific, Mar. Ecol. Prog. Ser., 132, 215–227, 1996.

Nelson, D. M., Tréguer, P., Brzezinski, M. A., Leynaert, A., and Quéguiner, B.: Production and dissolution of biogenic silica in the ocean, Revised global estimates, comparison with regional data and relationship to biogenic sedimentation, Global Biogeochem. Cy., 9, 359–372, 1995.

Passow, U., Shipe, R. F., Murray, A., Pak, D. K., Brzezinski, M. A., and Alldredge, A. L.: Origin of transparent exopolymer particles (TEP) and their role in the sedimentation of particulate matter, Cont. Shelf Res., 21, 327–346, 2001.

Peers, G. and Price, N. M.: Copper-containing plastocyanin used for electron transport by an oceanic diatom, Nature, 441, 341–344, 2006.

Pondaven, P., Gallinari, M., Chollet, S., Bucciarelli, E., Sarthou, G., Schultes, S., and Jean, F.:

Grazing-induced changes in cell wall silicification in a marine diatom, Protist, 158, 21–28, 2007.
Price, N. M., Harrison, G. I., Hering, J. G., Hudson, R. J., Nirel, P. M. V., Palenik, B., and Morel, F. M. M.: Preparation and chemistry of the artificial algal culture medium Aquil, Biol. Oceanogr., 6, 443–461, 1988/1989.
Price, N. M.: The elemental stoichiometry and composition of an iron-limited diatom, Limnol. Oceanogr., 50, 1159–1171, 2005.
Ragueneau, O. and Tréguer, P.: Determination of biogenic silica in coastal waters : applicability and limits of the alkaline digestion method, Mar. Chem., 45, 43–51, 1994.
Ragueneau, O., Schultes, S., Bidle, K., Claquin, P., and Moriceau, B.: Si and C interactions in the world ocean: Importance of ecological processes and implications for the role of diatoms in the biological pump, Global Biogeochem. Cy., 20, GB4S02, doi:10.1029/2006GB002688, 2006.
Rogerson, A., DeFreitas, S. W., and McInnes, A. G.: Growth rates and ultrastructure of siliceous setae of *Chaetoceros gracilis* (Baccilariophyceae), J. Phycol., 22, 56–62, 1986.
Smetacek, V.: Diatoms and the Ocean Carbon Cycle, Protist, 150, 25–32, 1999.
Strzepek, R. F. and Harrison, P. J.: Photosynthetic architecture differs in coastal and oceanic diatoms, Nature, 431, 689–692, 2004.
Sunda, W. G., Swift, D. G., and Huntsman, S. A.: Low iron requirement for growth in oceanic phytoplankton, Nature, 351, 55–57, 1991.
Sunda, W. G. and Huntsman, S. A.: Iron uptake and growth limitation in oceanic and coastal phytoplankton, Mar. Chem., 50, 189–206, 1995.
Sunda, W. G. and Huntsman, S. A.: Interrelated influence of iron, light and cell size on marine phytoplankton growth, Nature, 390, 389–392, 1997.
Sunda, W. G. and Huntsman, S. A.: Relationships among photoperiod, carbon fixation, growth, chlorophyll *a*, and cellular iron and zinc in a costal diatom, Limnol. Oceanogr., 49, 1742–1753, 2004.
Takeda, S.: Influence of iron availability on nutrient consumption ratio of diatoms in oceanic waters, Nature, 393, 774–777, 1998.
Timmermans, K. R., Stolte, W., and de Baar, H. J. W.: Iron-mediated effects on nitrate reductase in marine phytoplankton, Mar. Biol., 121, 389–396, 1994.
Timmermans, K. R., Davey, M. S., van der Wagt, B., Snoek, J., Geider, R. J., Veldhuis, M. J. W., Gerringa, L. J. A., and de Baar, H. J. W.: Co-limitation by iron and light of *Chaetoceros*

brevis, *C. dichaeta* and *C. calcitrans* (Bacillariophyceae), Mar. Ecol. Prog. Ser., 217, 287–297, 2001.

Timmermans, K. R., van der Wagt, B., and de Baar, H. J. W.: Growth rates, half-saturation constants, and silicate, nitrate, and phosphate depletion in relation to iron availability of four large, open ocean diatoms from the Southern Ocean, Limnol. Oceanogr., 49, 2141–2151, 2004.

Tsuda, A., Kiyosawa, H., Kuwata, A., Mochizuki, M., Shiga, N., Saito, H., Chiba, S., Imai, K., Nishioka, J., and Ono, T.: Responses of diatoms to iron-enrichment (SEEDS) in the western subarctic Pacific, temporal and spatial comparisons, Prog. Oceanogr., 64, 189–205, 2005.

Table 1. Maximum specific growth rate μ (d^{-1}) and half-saturation constant for growth with respect to iron ($K_{\mu Fe'}$) for *T. oceanica* and *D. brightwellii* under Fe limitation (Fe lim) and Fe-L co-limitation (Fe-L co-lim). The squared correlation coefficient of the Monod saturation function versus inorganic Fe concentration (R^2) and the number of data used for the regression (n) are also given.

	Thalassiosira oceanica		*Ditylum brightwellii*	
	Fe lim	Fe-L co-lim	Fe lim	Fe-L co-lim
μ_{max} (d^{-1})	1.18±0.02	0.65±0.02	1.60±0.04	0.98±0.07
$K_{\mu Fe'}$ (pmol L^{-1})	1.29±0.17	3.01±0.43	25.4±1.99	36.5±9.79
n	20	24	19	10
R^2	0.92	0.93	0.97	0.88

Table 2. Data from six iron fertilisation experiments used for calculating the relative variation in specific growth rate (μ:μ_{max}) and the relative variation in molar ratio Si:N (R(Si:N)) presented in Fig. 6. For SOIREE, SOFeX South and EisenEx, IN and OUT patch data were available to calculate μ:μ_{max} and R(Si:N), with μ_{max} and (Si:N) at μ_{max} being IN patch values, and μ and Si:N being OUT patch values. For EIFEX, IN and OUT patch data were available for the first and second Fe infusions, which gave two values of R(Si:N) and μ:μ_{max}. For SERIES, the two phases of the bloom were considered, with comparison between IN patch and OUT patch data for phase I, and comparison of IN patch data between the end of phase I and the end of phase II. For SEEDS no OUT patch data were available, Fe replete conditions (exponential growth of the Fe induced bloom) were compared to Fe-L co-limited conditions (end of the experiment).

Fe fertilisation experiments	Specific growth rate (d^{-1})	Si:N (mol mol^{-1})
SEEDS (Subarctic Pacific) day 0–day 9: Fe enrichment day 9–day 13: Fe-L co-limitation	Calculated from IN patch chlorophyll data between d0 and d9 (μ_{max}) and d9–d13 (Fe-L co-limitation) after Tsuda et al. (2005) (Fig. 3)	Calculated from silicate and nitrate depletion (Fig. 5) and f-ratio (Fig. 12) in Kudo et al. (2005)
SERIES (Subarctic Pacific) day 0–day 10: Fe enrichment day 10–day 18: Fe limitation	Calculated from IN and OUT patch chlorophyll data after Boyd et al. (2004) (Fig. 1)	BSi: PON data in Marchetti et al. (2006) (Table 3)
EIFEX (Southern Ocean) day 0–day 11: 1st Fe infusion day 16–day 27: 2nd Fe infusion	Calculated from IN and OUT patch chlorophyll data after Hoffmann et al. (2006) (Fig. 1)	BSi: PON data in Hoffmann et al. (2007) (Fig. 2)
SOIREE (Southern Ocean) day 0–day 8: Fe enrichment	Calculated from IN and OUT patch chlorophyll data after Boyd et al. (2000) (Fig. 3)	Calculated from silicate and nitrate depletion (Table 1 and text) in Frew et al. (2001)
SOFeX South (Southern Ocean) day 0–day 20: Fe enrichment	OUT and IN patch community growth rates in Coale et al. (2004)	Calculated from silicate and nitrate uptake ratios and f-ratio in Coale et al. (2004)
EisenEx (Southern Ocean) day 1–day 21: Fe enrichment	Calculated from IN and OUT patch chlorophyll data after Gervais et al. (2002) (Fig. 2)	Calculated from silicate and nitrate depletion (Fig. 6) in Bozec et al. (2005)

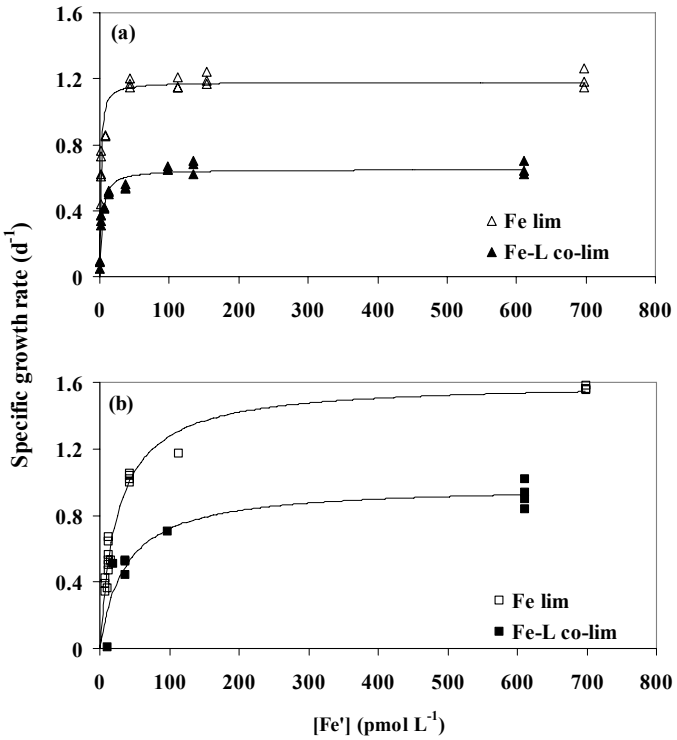

Fig. 1. Specific growth rate (μ, d^{-1}) of **(a)** *Thalassiosira oceanica* and **(b)** *Ditylum brightwellii* versus inorganic iron concentration in the medium ([Fe'], pmol L^{-1}) under high light ("Fe lim", open symbols) and low light ("Fe-L co-lim", closed symbols).

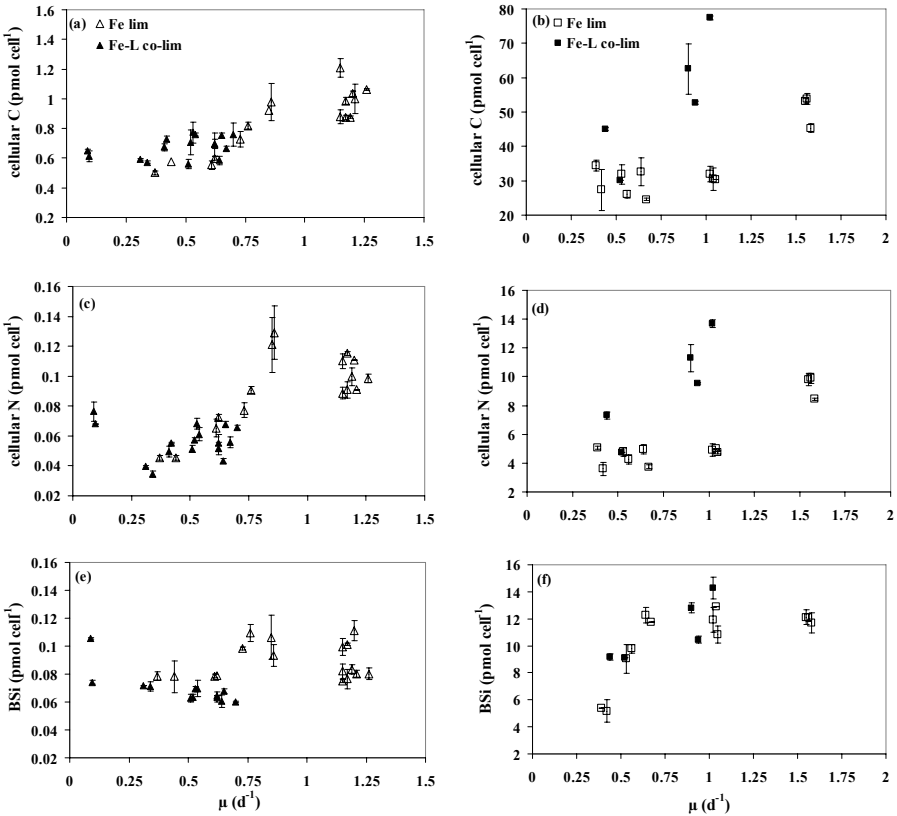

Fig. 2. Carbon **(a, b)**, nitrogen **(c, d)** and biogenic silica **(e, f)** per cell (in pmol cell^{-1}) versus specific growth rate (μ, d^{-1}) of *Thalassiosira oceanica* (left panels) and *Ditylum brightwellii* (right panels) under high light ("Fe lim", open symbols) and low light ("Fe-L co-lim", closed symbols).

BGD
6, 7175–7205, 2009

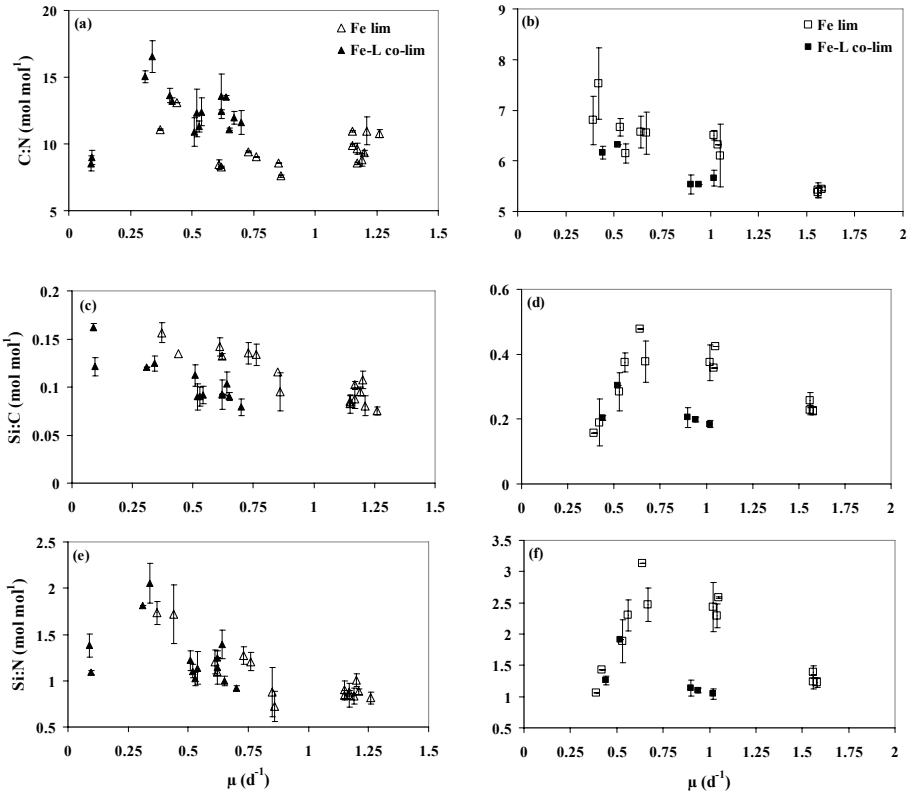

Fig. 3. Molar ratios of C:N **(a, b)**, Si:C **(c, d)** and Si:N **(e, f)** versus specific growth rate (μ, d^{-1}) of *Thalassiosira oceanica* (left panels) and *Ditylum brightwellii* (right panels) under high light ("Fe lim", open symbols) and low light ("Fe-L co-lim", closed symbols).

BGD
6, 7175–7205, 2009

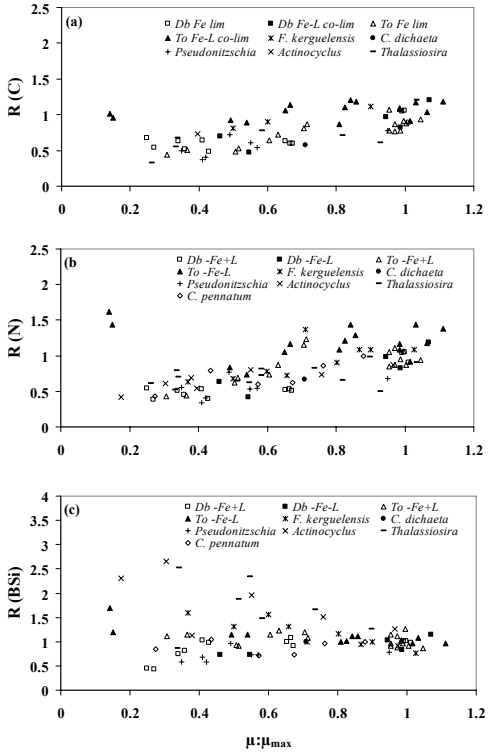

Fig. 4. Relative variation in **(a)** cellular C (R(C)), **(b)** cellular N (R(N)) and **(c)** cellular BSi (R(BSi)) versus relative variation in specific growth rate (μ:μ_{max}) for *Thalassiosira oceanica* (*To*, triangles), *Ditylum brightwellii* (*Db*, squares) under high light (open symbols) and low light (closed symbols), *Fragiloriopsis kerguelensis* (*F. kerguelensis*, *, Hoffmann et al., 2007; Timmermans et al., 2004), *Chaeotoceros dichaeta* (*C. dichaeta*, •, Hoffmann et al., 2007), *Pseudonitzshia heimii* type 1, *Pseudonitzshia*cf. *heimii* type 2, *Pseudonitzshia* cf. *turgidula*, *Pseudonitzshia multiseries*, *Pseudonitzshia* cf. *calliantha* (*Pseudonitzshia*, +, Marchetti and Harrison, 2007), *Actinocyclus* sp. (*Actinocyclus*, x, Muggli et al., 199; Timmermans et al., 2004), *Thalassiosira partheneia*, *Thalassiosira pseudonana*, *Thalassiosira weissflogii*, *Thalassiosira subtilis*, *Thalassiosira oceanica* 13-1, *Thalassiosira oceanica* 1003, *Thalassiosira* sp. (*Thalassiosira*, –, Gallinari et al., 2009; Maldonado and Price, 1996; Timmermans et al., 2004), *Corethron pennatum* (*C. pennatum*, ◇, Timmermans et al., 2004).

BGD
6, 7175–7205, 2009

Title Page
Abstract Introduction
Conclusions References
Tables Figures
◄◄ ►►
◄ ►
Back Close
Full Screen / Esc
Printer-friendly Version
Interactive Discussion

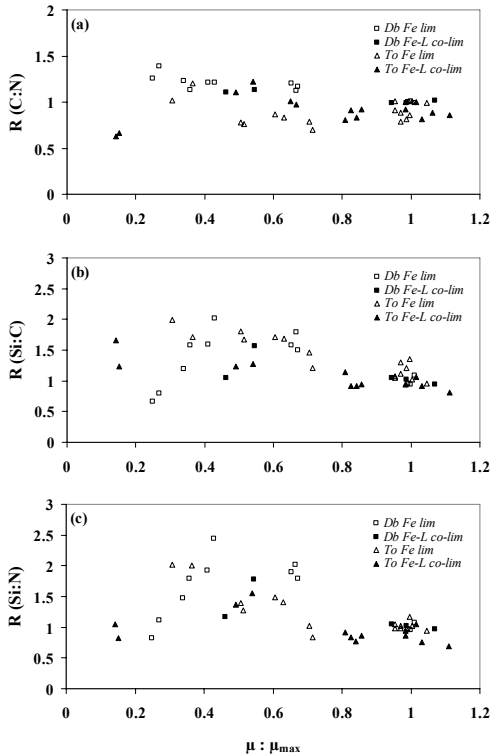

Fig. 5. Relative variation in molar ratio **(a)** C:N (R(C:N)), **(b)** Si:C (R(Si:C)), **(c)** Si:N (R(Si:N)) versus relative variation in specific growth rate (μ:μ_{max}) for *Thalassiosira oceanica* (*To*, triangles), *Ditylum brightwellii* (*Db*, squares) under high light (open symbols) and low light (closed symbols).

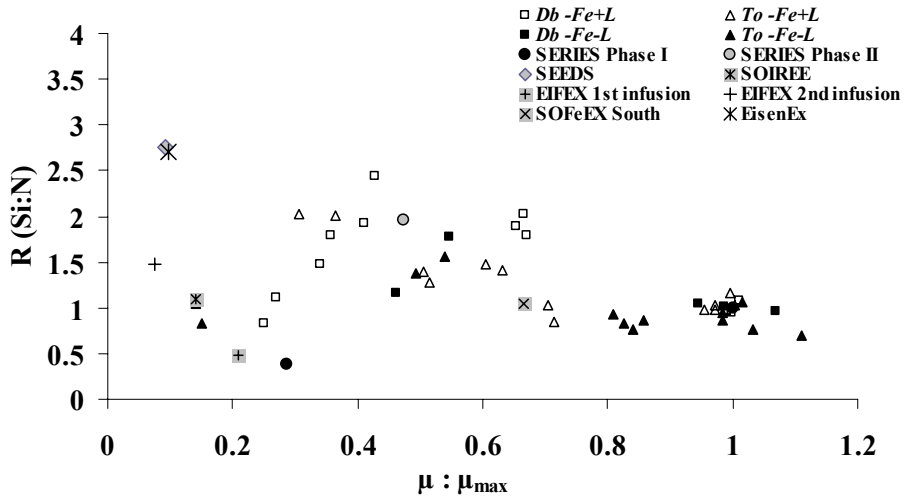

Fig. 6. Relative variation in molar ratio Si:N (R(Si:N)) versus relative variation in specific growth rate (μ:μ_{max}) for *Thalassiosira oceanica* (*To*, triangles), *Ditylum brightwellii* (*Db*, squares) under high light (open symbols) and low light (closed symbols) and six artificial Fe fertilisation experiments (SERIES phase I and II, SEEDS, SOIREE, EIFEX 1st and 2nd infusions, SOFEX South, EisenEx, see Table 2 for detail).

BGD
6, 7175–7205, 2009