Growth physiology and fate of diatoms in the ocean: a review

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Abstract

Diatoms are a major component of phytoplankton community. They tend to dominate under natural high-nutrient concentrations, as well as during artificial Fe fertilisation experiments. They are main players in the biogeochemical cycle of carbon (C), as they can account for 40% of the total primary production in the Ocean and dominate export production, as well as in the biogeochemical cycles of the other macro-nutrients, nitrogen (N), phosphorus (P), and silicon (Si). Another important nutrient is Fe, which was shown to have a direct or indirect effect on nearly all the biogeochemical parameters of diatoms. In the present paper, an inventory is made of the growth, physiology and fate of many diatom species, including maximum growth rate, photosynthetic parameters (maximum specific rate of photosynthesis, photosynthetic efficiency and light adaptation parameter), nutrient limitation (half-saturation constant for growth/uptake), cellular elemental ratios, and loss terms (sinking rates, autolysis rates and grazing rates). This is a first step for improvement of the parameterisation of physiologically based phytoplankton growth and global 3D carbon models. This review is a synthesis of a large number of published laboratory experiments using monospecific cultures as well as field data. Our compilation confirms that size is an important factor explaining variations of biogeochemical parameters of diatoms (e.g. maximum growth rate, photosynthesis parameters, half-saturation constants, sinking rate, and grazing). Some variations of elemental ratios can be explained by adaptation of intracellular requirements or storage of Fe, and P, for instance. The important loss processes of diatoms pointed out by this synthesis are (i) sinking, as single cells as well as through aggregation which generally greatly increases sinking rate, (ii) cell autolysis, which can significantly reduce net growth rates, especially under nutrient limitation when gross growth rates are low, and (iii) grazing by both meso- and micro-zooplankton. This review also defines gaps concerning our knowledge on some important points. For example, we need to better know which iron species is available for phytoplankton, as well as the impact of Fe on the variation of the elemental ratios, especially in terms of assimilation and regeneration of C and N. A better quantification of prey selection by microzooplankton and mesozooplankton in natural environments is also needed, including preference for the various phytoplankton and zooplankton species as well as for aggregates and faecal pellets.

Keywords: Diatoms; Biogeochemistry; Growth physiology; Nutrient limitation; Iron; Elemental ratios; Losses

1. Introduction

Diatoms are one of the predominant contributors to global carbon fixation. They account for 40% of the total primary production in the Ocean (Nelson
et al., 1995; Tréguer et al., 1995; Mann, 1999; Smetacek, 1999; Tréguer and Pondaven, 2000). Evidence from sediment traps (Honjo, 1982; Smetacek, 1985; Takahashi, 1986) and in situ photographs of the sea floor (Billet et al., 1983) testifies that significant vertical export of primary production from the upper ocean to the deep sea occurs as episodic, seasonal events following phytoplankton blooms. Simultaneous aggregation of an entire phytoplankton population community and its subsequent sedimentation was shown to occur on a time-scale of 24 hours (Alldredge and Gotschalk, 1989). A high enrichment of micro-organisms in marine snow aggregates relative to the surrounding waters was reported by many investigators (cf. Riebesell, 1991); this rapidly sinking phytoplankton is mainly dominated by diatoms, which sink in the form of large, quickly settling flocs of marine snow, amorphous aggregates 0.5 mm or greater in diameter (Smetacek, 1985; Takahashi, 1986; Allredge and Silver, 1988; Krarck and Milligan, 1988; Riebesell, 1991). Diatoms are main players in the biogeochemical cycles of carbon (C), nitrogen (N), phosphorus (P), silicon (Si), and iron (Fe), and tend to dominate export production (Buessler, 1998). Nevertheless, their relatively low surface to volume ratios will need nutrient-rich conditions for growth in contrast to smaller phytoplankton, such as nano- or pico-plankton, with higher surface to volume ratios, which allow a more efficient exploitation of low nutrient concentrations (Chisholm, 1992). Therefore, diatoms will dominate phyto-

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<tr>
<td>Maximum specific rate of photosynthesis $P_{\text{chl}}^{\text{m}}$</td>
<td>$2.6 \pm 1.0$ (n = 10)</td>
<td>gC gChl a$^{-1}$ h$^{-1}$</td>
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<tr>
<td>Photosynthetic efficiency: $\alpha_{\text{chl}}$</td>
<td>$0.021 \pm 0.005$ (n = 10)</td>
<td>gC gChl a$^{-1}$ h$^{-1}$ m$^{-2}$ s$^{-1}$ μmol$^{-1}$</td>
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<td>Light adaptation parameter: $E_k = P_{\text{chl}}^{\text{m}}/\alpha_{\text{chl}}$</td>
<td>$95 \pm 40$ (n = 10)</td>
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<td>Maximum specific growth rate: $\mu$</td>
<td>$0.4 - 3.3$</td>
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<td>$V = \text{cell volume}$</td>
<td>$\mu = 3.4 V^{-0.13}$</td>
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<td>Nutrient limitation</td>
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<tr>
<td>$K_s(Si)$</td>
<td>$3.9 \pm 5.0$ (n = 25)</td>
<td>μM</td>
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<td>$K_s(N)$</td>
<td>$1.6 \pm 1.9$ (n = 35)</td>
<td>μM</td>
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<tr>
<td>$K_s(P)$</td>
<td>$0.24 \pm 0.29$ (n = 14)</td>
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<td>$K_\mu(Fe)$</td>
<td>$0.35 \pm 0.44$ (n = 12)</td>
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<td>Elemental composition</td>
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<tr>
<td>C:N</td>
<td>$7.3 \pm 1.2$ (n = 86)</td>
<td>mol/mol</td>
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<tr>
<td>N:P</td>
<td>$10 \pm 4$ (n = 27)</td>
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<tr>
<td>Si:C</td>
<td>$0.11 \pm 0.04$ (n = 47)</td>
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<td>Si:N</td>
<td>$0.8 \pm 0.3$ (n = 50)</td>
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<td>Si:P</td>
<td>$5.9 \pm 1.3$ (n = 5)</td>
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<tr>
<td>Fe:C</td>
<td>$35 \pm 25$ (n = 21)</td>
<td>mol/mol</td>
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<td>Loss terms</td>
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<td>Sinking rate (individual cells) Fe-replete</td>
<td>$-0.1 - 1.5$</td>
<td>m d$^{-1}$</td>
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<td>Sinking rate (aggregates/flocs)</td>
<td>$-184 - 175$</td>
<td>m d$^{-1}$</td>
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<tr>
<td>Grazing rate</td>
<td>$0.2 - 1.8$</td>
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<tr>
<td>Cell lysis</td>
<td>$0.005 - 0.24$</td>
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plankton communities under high-nutrient concentrations. The dominance of (large) diatoms is also observed in man-made perturbations (iron additions) in so-called High-Nutrient, Low-Chlorophyll (HNLC) regions. In-situ iron fertilisations, in the Equatorial Pacific, the Southern Ocean, the Subarctic Pacific, as well as natural fertilisations, all induced a shift in the natural phytoplankton assemblage towards a predominance of (larger) diatom species, when Si does not become limiting (Martin et al., 1994; De Baar et al., 1995; Coale et al., 1996b, 2004; Boyd et al., 2000; Landry et al., 2000b; Blain et al., 2001; Bucciarelli et al., 2001; Gall et al., 2001; Smetacek, 2001; Takeda et al., 2002; Boyd et al., 2004). In spite of so many studies on diatoms, the real contribution of diatoms to carbon export, as well as the impact on Fe on this important phytoplankton group and on the global carbon cycle still needs to be assessed.

One tool to answer these questions is the use of physiologically based phytoplankton growth models (e.g. SWANCO model, Lancelot et al., 2000) and global 3D carbon models (e.g. PISCES model, Aumont et al., 2003). These models may also help to better understand why diatom species dominate in some environments. In recent years, planktonic ecological models have become increasingly complex and the parameter estimation becomes more and more difficult. The aim of this review is then to compile and synthesise the existing data on the growth physiology and fate of diatom in the ocean and the effect of Fe on these parameters (Table 1). We define the gaps in our knowledge concerning these parameters and give some recommendations for additional laboratory experiments in order to improve/extend our knowledge on this important group of autotrophs.

2. Growth and photosynthesis

Maximum specific growth rates ($\mu_{\text{max}}$), obtained under conditions of saturating light and nutrient sufficiency, range from 0.2 d$^{-1}$ to 3.3 d$^{-1}$ with an average value of 1.5 ± 0.8 d$^{-1}$ (n = 67, see Fig. 1). Values vary by more than one order of magnitude, but they can be related to cell size, which has long been recognised as an important cause of interspecific variability of $\mu_{\text{max}}$, the smallest cells having the highest growth rates (see e.g. Geider et al., 1986; Raven, 1986; Langdon, 1988; Tang, 1995; Raven and Kübler, 2002). When plotting $\mu_{\text{max}}$ (in d$^{-1}$) vs. cell volume (in $\mu$m$^3$) of 67 diatom species obtained from literature (Fig. 1), the size dependence of $\mu_{\text{max}}$ was described by the following allometric relation:

$$\mu_{\text{max}} = 3.4 \cdot V^{-0.13}$$

(1)

The magnitude of the exponent has been the subject of some discussions (Chan, 1978; Banse,
Although the correlation coefficient remains low ($r^2 = 0.48$) when considering a range of variation from 13 $\mu$m$^3$ to 7 $10^5$ $\mu$m$^3$, it appears that size dependence of growth rate becomes statistically more significant when considering this large range of sizes (Geider et al., 1986).

The reduction of $\mu_{\text{max}}$ with increasing cell size implies that small cells have a distinct catalytic advantage over large ones and should out-compete them (Raven, 1986). The increase in cell size induces a decrease in the capacity of solute influx or efflux on a volume basis, due to a thicker diffusion boundary layer and a smaller area of membrane lipid and number of transporters allowing solute fluxes (Raven and Kübler, 2002). Moreover, the increase in cell size decreases the effectiveness of increased pigmentation in harvesting light, the so-called ‘package effect’ (Kirk, 1994; Finkel and Irwin, 2000). The energy used to synthesise pigment-protein complexes takes longer to repay in terms of absorbed energy in a given external radiation field on larger cells (Raven, 1984). In other terms, assuming a constant concentration of chlorophyll (Chl) per cell volume and a constant spherical geometry, the ‘package effect’ implies that each individual Chl molecule has a lower probability of absorbing photons in a given radiation field in a large cell than in a small one, the Chl-a specific absorption cross-section coefficient increasing with cell size (Geider et al., 1986; Raven and Kübler, 2002). On the other hand, intracellular concentration Chl-a of diatoms tends to decrease with cell size, resulting in a moderation in the increase in the package effect with cell size (Finkel, 2001). Clearly, the quantification of the influence of the ‘package effect’ in algae of different sizes and shapes, as well as for cells exposed to low irradiance, is of great importance in mechanistic interpretations of rates of growth and photosynthesis in natural conditions (Raven and Kübler, 2002).

The rate of photosynthesis is controlled by the efficiency of light utilisation to drive the overall photosynthetic reactions to carbon fixation (Falkowski and Raven, 1997). Photosynthesis vs. light can be represented by a P-E curve (Webb et al., 1974), which is now widely accepted as a useful relationship for examining the photosynthesis physiology of microalgae, despite the uncertainties introduced by differences in methodology (Henley, 1993). Indeed, the shape and the magnitude of the P-E curve reflect the underlying biophysical, biochemical, and metabolic processes that regulate photosynthesis (Falkowski and Raven, 1997). Typically the P-E curve can be divided into light-limited, light-saturated and photoinhibition regions. In nature pronounced photoinhibition in P-E curve is commonly observed only in samples collected below the surface-mixed layer (Platt et al., 1980). For a recent review on photoacclimation of photosynthesis, see MacIntyre et al. (2002).

We compiled values for the maximum specific rate of photosynthesis ($P_m$), the photosynthetic efficiency ($\alpha$), which represents the initial slope of the P-E curve, and the light adaptation parameter ($E_k$) defined as the ratio between $P_m$ and $\alpha$. Values (normalised to Chl-a content for $P_m^{\text{chla}}$ and $\alpha^{\text{chla}}$) are reported in Appendix 1 (www.nioz.nl/projects/ironages). $P_m^{\text{chla}}$ ranges from 1.2 to 11.4 gC gChl$^{-1}$ h$^{-1}$, with an average value of 2.6 $\pm$ 1.0 gC gChl$^{-1}$ h$^{-1}$ ($n=10$). $\alpha^{\text{chla}}$ ranged from 0.013 to 0.087 gC gChl$^{-1}$ h$^{-1}$ $\mu$mol$^{-1}$ m$^{-2}$ s$^{-1}$, with an average value of 0.021 $\pm$ 0.005 gC gChl$^{-1}$ h$^{-1}$ $\mu$mol$^{-1}$ m$^{-2}$ s$^{-1}$ ($n=10$). Finally, $E_k$ varied between 46 and 498 $\mu$mol photons m$^{-2}$ s$^{-1}$, with an average value of 95 $\pm$ 120 $\mu$mol photons m$^{-2}$ s$^{-1}$ ($n=10$). The three extremely large values as shown in Appendix 1 (www.nioz.nl/projects/ironages) were excluded for the calculation of the average values.

Variability in the P-E curve, together with variability in the Chl$\alpha$:C ratio is used to assess photoacclimation, which involves changes in the macromolecular composition and ultrastructure of the photosynthetic apparatus (Durfor and Falkowski, 1997; Falkowski and Raven, 1997). There is much work on the change in Chl$\alpha$ quota as a function of irradiance (e.g. Geider, 1987), with general agreement that this can be from 2 to 10 times. Thus the ‘down-regulation’ of the photosynthetic apparatus mostly occurs in response to irradiance. Next to the photon flux density, varying the cellular chlorophyll$\alpha$ quota up to one order of magnitude (Geider, 1987; Sosik et al., 1989; Veldhuis and Kraay, 2004), iron also induces major changes in the photosynthetic characteristics (Greene et al., 1991; Sosik and Olson, 2002). Indeed, iron is essential for many major biogeochemical processes, including photosynthesis (Geider and La Roche, 1994). Raven
(1990) estimated that 80% of the iron required by phytoplankton is in the photosynthetic electron transfer. The high Fe requirement of the photosynthetic apparatus makes photosynthesis a principal target of Fe deficiency. In the marine diatom *Phaeodactylum tricornutum*, Fe limitation induces a 2-fold reduction of maximum photosynthesis rate and a 1.3-fold increase of the photosynthesis efficiency with Fe limitation (Greene et al., 1991). The decrease in the maximum photosynthesis rate was explained by the decrease in the minimal turnover time required for an electron to pass from water to CO₂ (Greene et al., 1991). The increase in the photosynthetic efficiency was explained by a decrease in light-harvesting pigment content, reducing the package effect related to the optical properties of a heterogeneous suspension of particles (Berner et al., 1989; Greene et al., 1991). There is universal agreement that a reduction in photosynthetic pigment content (chlorosis) accompanies Fe limitation (Greene et al., 1991; Geider and La Roche, 1994). Fe limitation may either directly induce chlorosis by influencing the formation of protochlorophyllide (Spiller et al., 1982), or indirectly, simply by reduction of the cellular abundance or activity of enzymes that require Fe and that are involved in the Chl biosynthesis pathway (Davey and Geider, 2001).

### 3. Nutrient limitation: half-saturation constants for growth/uptake

For a given nutrient (Nut), the specific uptake rate ($V_{\text{Nut}}$) and the specific growth rate ($\mu$) follow Michaelis-Menten (Eq. 3) and Monod (1942) (Eq. 4) saturation functions, respectively:

$$V_{\text{Nut}} = \frac{V_{\text{max}}[\text{Nut}]}{([\text{Nut}] + K_{S})}$$  \hspace{1cm} (3)

$$\mu = \frac{\mu_{\text{max}}[\text{Nut}]}{([\text{Nut}] + K_{\mu})}$$  \hspace{1cm} (4)

where $V_{\text{max}}$ and $\mu_{\text{max}}$ represent the maximum rates of uptake and growth, respectively, at infinite substrate concentration, $K_s$ the concentration of the nutrient that limits $V_{\text{Nut}}$ to 0.5$V_{\text{max}}$ and $K_\mu$ the concentration of the nutrient that limits $\mu$ to 0.5$\mu_{\text{max}}$. $[\text{Nut}]$ is the concentration of the added nutrient.

At steady state, the specific growth rate and the specific uptake rate are in balance and thus equivalent. The large difference which exists between the half-saturation constants for growth $K_\mu$ and short-term uptake $K_s$ is due to acclimation capabilities of the organisms. Over the acclimation range, $K_\mu$ to $K_s$, the algae can maintain maximum growth rates by modulating both their internal nutrient quotas and their maximum short-term nutrient uptake rates in response to variations in external nutrients concentrations (Morel, 1987).

Half-saturation constants for nitrate ($K_s(\text{N})$), phosphate ($K_s(\text{P})$), orthosilicic acid ($K_s(\text{Si})$) and iron ($K_\mu(\text{Fe})$) have been reported in Appendix 2 (www.nioz.nl/projects/ironages). For iron, $K_\mu(\text{Fe})$ is generally expressed using inorganic Fe concentrations ($Fe^\text{in}$) when adding an artificial chelator, ethylenediaminetetraacetate (EDTA) as a metal buffer (Morel et al., 1979; Sunda et al., 1991; Sunda and Huntsman, 1995), but total concentrations of dissolved Fe ($Fe_{\text{diss}}$) are chosen as master variable, when no EDTA is added (Coale et al., 1996a; Timmermans et al., 2001b). The form of Fe that is available for phytoplankton growth is not yet known. It was primarily thought that only $Fe^\text{in}$ was bioavailable (cf. Maranger et al., 1998). However, more than 99% of dissolved Fe is organically complexed in seawater (Gledhill and Van den Berg, 1994, 1995; Rue and Bruland, 1995). The corresponding $Fe^\text{in}$ is then far too low for sustaining growth of even the small phytoplankton species (Timmermans et al., 2001b), and some Fe organic complexes may be a source of available Fe for phytoplankton growth (Soria-Dengg and Horstmann, 1995; Hutchins et al., 1999; Maldonado and Price, 1999). When concentrations of natural organic ligands in the diatom cultures and their conditional stability constants with Fe are known, or considering a theoretical percentage of organically complexed Fe in seawater (Timmermans et al., 2001b), $K_\mu(Fediss)$ can be calculated from $K_\mu(Fe^\text{in})$ (Gledhill and Van den Berg, 1994; Rue and Bruland, 1995). However, it is then hard to judge whether the differences of $K_\mu(Fe)$ are caused by the physiological differences between the species, or whether calculation of the concentration of $Fe^\text{in}$ plays a role. In this review, $K_\mu(Fe)$ refers to inorganic Fe concentrations when EDTA is added to culture medium and to dissolved Fe concentrations when incubations are performed in natural seawater. This assumes that
Fe' and dissolved Fe are the fractions that are bioavailable in the former and latter cases, respectively. This hypothesis clearly shows that more studies are needed to better know which iron species is available for phytoplankton.

$K_s(N)$ values ranged from 0.02 µM to 10.2 µM, with an average value of 1.6 ± 1.9 µM (n = 35). $K_s(P)$ ranged from 0.01 µM to 8.9 µM, with an average value of 1.2 ± 2.5 µM (n = 18). $K_s(Si)$ values range from 0.2 to 22 µM, with an average value of 3.9 ± 5.0 µM (n = 25). Values of $K_m(Fe)$ varied between 0.59 pM to 1.12 nM, with an average value of 0.35 ± 0.44 nM (n = 12).

The range of variations of half-saturation constants is very wide and the absolute values of the average half saturation values indicate that generally these are well above the annual sea surface concentrations (Levitus et al., 1993; Johnson et al., 1997). Therefore, diatoms may dominate phytoplankton community only under conditions of high-nutrient concentrations. Such conditions are found in spring and early summer blooms in open ocean waters (Ragueneau et al., 2000). This implies that at the beginning of the growth season the diatoms should not experience nutrient limitation. This is what is seen in the spring development of the phytoplankton, where the diatoms belong to the groups with a rapid development. Only after the depletion of nutrients (mainly silicate), do other phytoplankton species take over.

On the other hand, diatoms can adapt to low nutrient concentrations by reducing their size. Small cells are more proficient (have a smaller half-saturation constant) than large cells (Eppley et al., 1969). They have greater surface to volume (S/V) ratio, which increases the exchanges of gases and solutes across the cell surface, then decreasing the diffusion limitation of nutrient uptake (Morel et al., 1991a). Comparing half-saturation constants of macro- and micro-algae, Hein et al. (1995) determined a power law relationship between $K_s(N)$ values and surface to volume (S/V) ratios:

$$K_s(N) = 0.61(S/V)^{-0.58}$$

with $K_s(N)$ in µM and S/V in µm⁻¹, $r^2 = 0.4$, n = 32.

Similarly, very recently, Leynaert et al. (2004) observed a strong relationship between S/V ratios and $K_s(Si)$ for one diatom species, whose size was reduced by Fe limitation:

$$K_s(Si) = 0.64(S/V)^{-3.66}$$

with $K_s(Si)$ in µM and S/V in µm⁻¹, $r^2 = 0.9$, n = 5.

For $K_m(Fe)$, Smith and Kalff (1982) showed little evidence of significant variations of $K_m(P)$ among a group of eight co-occurring phytoplankton species (diatom and non-diatom species). On the other hand, Donald et al. (1997) showed that Thalassiosira weiss-flogii was able to use two phosphate uptake systems depending on P concentrations, with each phase possessing very different $K_s(P)$ values. The phase I uptake could occur in the oligotrophic ocean where phosphate concentrations are low. The organisms would acquire phosphate at very low concentration without being able to acquire it quickly into the cell (Donald et al., 1997). The phase II uptake could occur in microscale phosphate-enriched patches and allows for rapid uptake (Rivkin and Swift, 1982; Donald et al., 1997). The range of variation found in the literature may then be related to the existence of these two phase uptake systems. However, when not considering the extreme values, $K_m(P)$ values average 0.24 ± 0.29 µM (n = 14).

For $K_m(Fe)$, using data reported in Appendix 2 (www.nioz.nl/projects/ironages), we also established a power law relationship between $K_m(Fe)$ and S/V:

$$K_m(Fe) = 12 10^{-3}(S/V)^{-1.77}$$

with $K_m(Fe)$ in nM and S/V in µm⁻¹, $r^2 = 0.7$, n = 9.

The very low value of $K_m(Fe)$ for the small oceanic Antarctic species Chaetoceros brevis indicates that this species has adapted to very low Fe concentrations and would never experience Fe limitation in the Southern Ocean, whereas the large chain-forming diatom species Chaetoceros dichaeta will only bloom following an Fe input (Timmermans et al., 2001b).

### 4. Elemental ratios

Elemental ratios for 47 different diatom species at nutrient-replete conditions are reported in Appendix 3 (www.nioz.nl/projects/ironages).
C:N ratios have an averaged value of $8.0 \pm 3.5$ mol/mol ($n = 101$), ranging from 2.7 to 29.7 mol/mol (Fig. 2). This range of variation is very wide but we observed that more than 85% of diatom species have C:N varying from 5.0 to 9.7 mol/mol (Fig. 2). Three diatom species have very high C:N ratios (>19.4, see Appendix 3 (www.nioz.nl/projects/ironages)). When not considering these three very high values, which may be the result of some peculiarity of the culture conditions (Brzezinski, 1985), C:N ratios have an averaged value of $7.3 \pm 1.2$ mol/mol ($n = 86$), which is close to the 6.6 C:N ratio reported by Redfield et al. (1963). N:P ratios have an average value of $10 \pm 4$ ($n = 27$) and are widely distributed from 5 to 18 mol/mol (Fig. 2). More than 90% of the values are lower than the Redfield ratio of 16. Revisiting the C:N:P ratios for several phytoplankton groups, Geider and La Roche (2002) also observed in nutrient-replete cultures that the C:N ratio, although variable, has a typical value close to that of the Redfield ratio and that the N:P ratio fall below the Redfield ratio. Using mass balance calculations based on the physiologically achievable range of the dominant macromolecules in the cell, they could only explain the low N:P ratios by an intracellular storage of P.

Si:C ratios have an averaged value of $0.14 \pm 0.13$ mol/mol ($n = 54$), ranging from 0.04 to 0.95 mol/mol. More than 88% of diatom species have Si:C ratios between 0.05 and 0.19 mol/mol (Fig. 2), which means a factor 4 of variation. One diatom species, the giant diatom *Ethmodiscus* (Villareal et al., 1999a), has a very high Si:C ratio (0.95 mol/mol), due to a low C content per cell volume (Villareal et al., 1999a). When not considering the extreme values, Si:C ratios have an averaged value of $0.11 \pm 0.04$ mol/mol ($n = 47$). Si:N ratios have an average value of $1.0 \pm 1.0$ mol/mol ($n = 57$), ranging from 0.17 to 7.24. The very high value (7.24) is also observed for the giant diatom *Ethmodiscus*, and is also due to a low N content per cell volume (Villareal et al., 1999a). 88% of diatoms species have values ranging from 0.4 to 1.55 mol/mol, which means a factor 4 of variation, as for Si:C ratios. When not considering the extreme values, Si:N ratios have an average value of $0.8 \pm 0.3$ mol/mol ($n = 50$).

Fe:C ratios had an average value of $65 \pm 91$ mol/mol ($n = 25$), ranging over more than 2 orders of magnitude, from 2.3 to 370 µmol/mol. As seen in Fig. 2, Fe:C ratios are widely distributed. 84% of diatom species had Fe:C ratios between 2.3 and 89 µmol/mol, corresponding to an average value of $35 \pm 25$ µmol/mol ($n = 21$). The Fe:C ratios of diatom
species are highly dependent on iron concentration in the medium (Sunda et al., 1991; Sunda and Huntsman, 1995; Maldonado and Price, 1996). Bruland et al. (2001) showed that the variation of Fe:C ratio with Fe availability has to be taken into account for the prediction of iron depletion or nitrate depletion in upwelling systems. Oceanic species, which are growing in seawater with very low Fe concentrations (100–1000 times lower than in the coastal waters) have lower Fe:C ratios than related estuarine species (Sunda and Huntsman, 1995).

Reduction of size cell is not the only strategy for adaptation to low-iron oceanic conditions (see above). Another strategy for the adaptation to low Fe concentrations is reducing their Fe requirement for growth (thus reducing their Fe:C ratios; Sunda et al., 1991; Sunda and Huntsman, 1995; Maldonado and Price, 1996; Muggli et al., 1996; Muggli and Harrison, 1997; Schmidt et al., 1999). The mechanism is still unknown. It could be a more efficient exchange of iron within intracellular pools during Fe starvation (Sunda et al., 1991), a minimisation of metabolic pathways that requires large amount of Fe (Stefels and Van Leeuwe, 1998), or a replacement of Fe-containing proteins by non-Fe-containing proteins possessing other metal catalysts (La Roche et al., 1996). Under low Fe conditions, flavodoxin replaces the Fe-containing electron transfer catalyst ferrodoxin as a means of decreasing the cellular Fe requirement (Entsch et al., 1983; Raven, 1988; La Roche et al., 1993, 1996, 1999; McKay et al., 1999). On the other hand, during periods of high Fe availability, some coastal diatoms are able to accumulate excess Fe, having Fe:C ratios 20–30 times higher than those needed for maximal growth (Sunda and Huntsman, 1995). Diatoms could then out-compete other species if Fe became limiting (Bruland et al., 2001).

Recent seawater and culture experiments showed an increase in Si:C and Si:N ratios of diatoms under Fe limitation (Martin and Fitzwater, 1988; Hutchins and Bruland, 1998; Hutchins et al., 1998; Takeda, 1998; De la Rocha et al., 2000; Franck et al., 2000; Bucciarelli et al., ms in review; Brzezinski et al., 2003). Iron-stressed diatoms should therefore deplete surface waters of orthosilicic acid before nitrate, driving the system towards Si limitation of the phytoplankton community. The effect of Fe on the uncoupling between Si, C, and N uptake and assimilation is currently not well understood. It may be due to the different ways Fe is involved in intracellular mechanisms (Stefels and Van Leeuwe, 1998; Bucciarelli et al., ms in review) and to the differences between silicon, carbon and nitrogen cyclings (Martin-Jézéquel et al., 2000; Claquin et al., 2002). Fe is essential to many metabolic processes that require electron transfer reactions, such as photosynthesis, respiration and nitrogen assimilation inducing that C and N assimilations are directly affected by Fe limitation. On the other hand, Si is mainly linked to the growth rate and the duration of the cell wall synthesis phase (Martin-Jézéquel et al., 2000; Claquin et al., 2002). Si assimilation may be indirectly affected by Fe limitation through a reduction of growth rate (Bucciarelli et al., ms in review). By examining gross and net utilisation of carbon, nitrogen and silicon in the Southern Ocean, Brzezinski et al. (2003) hypothesised that the main effect of Fe limitation on nutrient uptake is the Antarctic Circumpolar Current is to diminish the relative use of new nitrogen, i.e. to increase reliance on regenerated nitrogen by inhibiting nitrate uptake, rather than to alter the stoichiometry of gross nutrient uptake by phytoplankton. Clearly more experimental work is needed to better understand and quantify the different processes involved in this positive feedback of Fe limitation on the ‘silicate pump’ (Dugdale et al., 1995).

5. Losses

5.1. Sinking rates of diatom cells

Sinking rate of a diatom cell is not only a function of its size and shape but is also strongly dependent on the physiological state of the cell (Brzezinski and Nelson, 1988). In healthy, non-aggregated cells, sinking and ascent rates are generally described by Stokes’ equation (Hutchinson, 1967). Increasing senescence of a population is accompanied by an increasing sinking rate. The means of this control was in part found to be through the selective exchange of heavier ions for lighter ions in the vacuole, the so-called ionic pump, which is an energy-requiring process (Anderson and Sweeney, 1978). Nutrient (N, P, Si, and Fe) and energy limitations increase cell
sinking rates several fold (Smayda, 1970; Bienfang et al., 1982; Bienfang and Harrison, 1984; Harrison et al., 1986; Waite et al., 1992; Muggli et al., 1996). Muggli et al. (1996) observed a 5-time increase from Fe-replete to Fe-stress conditions in the sinking rate of the oceanic diatom *Actinocyclus* sp., from 0.17 m d$^{-1}$ to 0.93 m d$^{-1}$. Fe limitation may affect phytoplankton sinking rates by stressing the energy-producing pathways needed by the cell to maintain their buoyancy. Furthermore, the higher silicification of diatoms under Fe-stress (Martin and Fitzwater, 1988; Hutchins and Bruland, 1998; Hutchins et al., 1998; Takeda, 1998; De la Rocha et al., 2000; Franck et al., 2000; Bucciarelli et al., ms in review) may increase the sinking rate via thicker siliceous frustules. Recently, during the Southern Ocean RElease Experiment (SOIREE, in Feb. 1999), Watson et al. (2000) observed a two-fold decrease of Si:C ratio inside the patch as opposed to outside the patch and Waite and Nodder (2001) noted a four-fold decrease in the sinking rate inside the patch as opposed to outside the patch, on day 8. This decrease may be in agreement with the hypothesis of a lower sinking rate due to thinner frustules when Fe was added. However, the sinking rate response was very rapid, with almost a three-fold increase in 24 h, on days 11 and 12 when algae were again Fe stressed due to Fe complexation by organic ligands (Maldonado et al., 2001). This fast response is then more consistent with ionic changes in cell protoplast (Waite and Nodder, 2001).

Some large-sized diatoms adopted a strategy of buoyancy-mediated vertical migration to access nutrient-enriched waters located below the nutricline (Villareal, 1992; Villareal et al., 1993, 1999b; Moore and Villareal, 1996; McKay et al., 2000). These species are able to grow under low-light conditions (Goldman and Mc Gillicuddy, 2003) and, although there are virtually no data on nutrient uptake by large diatoms for extremely low light environments, large diatoms may play a major role in primary and export production (Kemp et al., 2000; Goldman and Mc Gillicuddy, 2003).

5.2. Aggregation

Aggregation processes generally increase sinking rates (Alldredge and Silver, 1988). In theory, the combined effect of cell sinking rates increases and aggregation should have the largest effect on the magnitude of diatom export flux (Jackson and Lochmann, 1992). Diatom aggregates are the primary source of marine snow aggregates, which were shown to sink at a mean rate of ~ 50–100 m d$^{-1}$ (Billet et al., 1983; Alldredge and Silver, 1988; Mann, 1999). For a detailed review on diatom aggregation in the sea, see Thornton (2002). Both biological and physical processes are responsible for the formation of large, rapidly sinking aggregates (McCave, 1984). The repackaging of particulate material into faecal pellets through the feeding activities of animals is the biologically mediated aggregation and dominates when primary productivity is directly consumed by grazers (McCave, 1984; Kiorboe et al., 1996; Wassmann et al., 1999). On the other hand, physical mechanisms dominate during intense phytoplankton blooms, when an uncoupling between primary and secondary productivity occurs (Jackson, 1990; Riebesell and Wolf-Gladrow, 1992). The rate of physical formation of algal flocs depends on biomass accumulation and concentration, cell size and shape, including setae, the abundance of chain-forming species, stickiness, and whether the cells are solitary or chain-forming, and the mechanisms responsible for particle collision, such as Brownian motion, differential settlement and shear (Jackson, 1990; Jackson and Lochmann, 1992; Riebesell and Wolf-Gladrow, 1992; Kiorboe et al., 1998). Another important agent of diatom aggregation is the presence of transparent exopolymer particles (TEP) (Riebesell, 1991; Alldredge et al., 1993; Passow et al., 1994, 2001; Engel, 2004) For a recent review on TEP, see Passow (2002). TEP are probably generated abiotically from dissolved extracellular polysaccharides (Passow et al., 1994), which are released by phytoplankton, especially diatoms, and bacteria (Hama and Handa, 1987; Decho, 1990; Williams, 1990). The major importance of TEP for aggregation of diatom blooms appears to be the much higher sticking coefficients of TEP compared to diatom cells (Kiorboe et al., 1990; Kiorboe and Hansen, 1993; Passow et al., 1994; Logan et al., 1995).

Not all diatom flocs settle immediately from surface waters. Large marine snow aggregates were observed to be retained in the surface layer for several days, including the floating mats of *Rhizosolenia* spp. (Villareal, 1988; Kemp et al., 2000) and marine snow
aggregates with neutral buoyancy or even slow ascending rates, related to gas bubble formation within the flocs (Stachanowitsch et al., 1990; Riebesell, 1992). Turbulence may help retain some large aggregates in the mixed layer for many days where they age and dissociate (Alldredge and Cohen, 1987). Aggregate feeders may capture aggregates and prevent them from sinking to the sea-floor, resulting in increased remineralisation rates in the euphotic zone (Jackson, 1993; Green and Dagg, 1997).

5.3. Cell lysis

Lysis of phytoplankton cells is increasingly recognised as a potentially important factor in phytoplankton population dynamics (Brussaard et al., 1995; Augusti et al., 1998, 2001; Kirchman, 1999; Augusti and Duarte, 2000). One type of cell lysis is the viral lysis, but diatoms appear to be relatively well protected against viruses. In Ross Sea habitats, of over 29 700 diatom cells examined none was infected by large viruses (Gowing, 2003). Another type of cell lysis is death due to nutrient stress. Brussaard et al. (1997) first studied the autolysis kinetics of a marine diatom under nutrient stress. Although the autolysis rates in their study do not seem very high (mostly lower than 0.13 d\(^{-1}\)), these loss rates can significantly reduce net growth rates, especially under nutrient limitation when gross growth rates are low. Veldhuis et al. (2001) published detailed results of viability and cell death of, amongst others, Chaetoceros calcitrans. They showed that, even in apparently uniform populations, distinct differences in viability could exist. The exact consequences of loss of membrane integrity used in the Veldhuis et al. (2001) study as the indicator of viability are yet unknown. Similarly, data on the Antarctic diatom Chaetoceros brevis seemed to show that increased mortality could occur after relief from iron limitation (Timmermans et al., 2001b).

5.4. Grazing

In the last decade there has been considerable debate about whether grazing pressure or Fe supply control algal stock in HNLC regions (Cullen, 1991; Frost, 1991; Morel et al., 1991b; Banse, 1992; Price et al., 1994; Landry et al., 1997). Current opinion suggests that both are important and that they form the basis for the ecumenical Fe hypothesis (Cullen et al., 1992). In the NE subarctic Pacific, the findings of Boyd et al. (1996), from in-vitro Fe enrichments in which representative number of mesozooplankton were present, indicate that Fe supply rather than grazing pressure exerts the ultimate control over the magnitude of diatom stocks in this region. In-vitro experiments at Ocean Station Papa, where mesozooplankton stock was increased in order to increase grazing pressure, showed that more than five times ambient grazing pressure is required to prevent the accumulation of algal biomass following an episodic Fe supply (Boyd et al., 1999). On the contrary, during monsoons, in the Arabian Sea, which is a typical HNLC area where Fe concentrations are not likely to be limiting (Measures and Vink, 1999), grow-out experiments showed that diatoms were the only group capable of blooming in the absence of mesozooplankton, i.e. their abundance in situ was more likely to be controlled by mesozooplankton grazing than by Fe availability.

The most common grazers of diatoms are large organisms such as copepods (Smetacek, 1999). Some studies question the role of diatoms as a key food and for reproductive success in copepods (Klepple et al., 1991; Klepple, 1993; Poulet et al., 1994, 1995; Uye, 1996; Ban et al., 1997; Ianora et al., 1999; Miralto et al., 1999; Tang and Dam, 2001). Even if diatoms do not always show a negative effect on egg production and hatching (Starr et al., 1999; Irigoien et al., 2000; Shin et al., 2003), some inhibitory compounds that block female copepod embryogenesis (Poulet et al., 1995; Uye, 1996) may represent a defence mechanism by diatoms against grazing by copepods, thereby prolonging diatom blooms (Ban et al., 1997). Grazing impacts of copepods vary between values lower than 1% and values around 45% of primary productivity (e.g. Dam et al., 1995; Verity et al., 1996b; Dubischar and Bathmann, 1997; Roman et al., 2000; Urban-Rich et al., 2001; Zeldis, 2001; Cabal et al., 2002). Some studies contradict the paradigm that microzooplankton are constrained to diets of nanoplankton and strongly suggest that they feed on phytoplankton larger than themselves (e.g. Hansen and Calado,
1999, and references herein). Heterotrophic dinoflagellates, for example, achieve that either by engulfing prey in a membrane extruded from the dinoflagellate cell (Jacobson and Anderson, 1986) or by drawing in chains and compressing them in food vacuoles (Buck and Newton, 1995). In coastal, as well as open ocean environments, 45–110% of the standing stock of diatoms can be removed each day by microzooplankton grazing (Verity et al., 1996a; Nejstgaard et al., 1997; Landry et al., 2000a; Calbet, 2001; Strom et al., 2001; Olson and Strom, 2002; Suzuki et al., 2002). A predictive understanding of diatoms grazing losses must then take into account microzooplankton as important and sometimes dominant consumers of bloom-forming diatoms. However, a better quantification of prey selection by microzooplankton and mesozooplankton in natural environments is needed, including preference for the various phytoplankton and zooplankton species as well as for aggregates and faecal pellets.

6. Conclusions and recommendations

Diatoms are the most important phytoplankton group in marine environments and are the key players in the ocean carbon cycle. In spite of so much research on diatoms, the real contribution of diatoms to carbon export, as well as the impact on Fe on this important phytoplankton group and on the global carbon cycle still needs to be assessed. The biochemical parameters vary among the species and size can account for much of interspecific variability of maximum growth rate, optical absorption cross-section, half-saturation constants for nitrate, orthosilicic acid, and iron, and sinking rate of individual cells. However, so far very few studies have investigated the variations of these parameters, as well as the impact of Fe limitation on the very large diatom species, which significantly contribute to annual new production (Goldman et al., 1992; Goldman, 1993; Pudsey and King, 1997; Gersonde and Barcena, 1998; Timmermans et al., 2001b; Goldman and McGillicuddy, 2003; Timmermans et al., in press). There is then an obvious need for more studies on the biogeochemical parameters of isolated, ecologically relevant, open ocean, bloom forming large diatoms, in particular for a better constrain of the allometric relationships. However, these species might be not easy to grow and maintain for longer periods in the laboratory.

The elemental ratios, the C:N, Si:C, Si:N ratios, although variable, have typical values close to Redfield/Brzezinski ratios. The largest difference is observed for N:P and Si:P, and could be explained by an intracellular storage of P (Geider and La Roche, 2002). Fe:C ratio could also be explained by a reduction of Fe requirement, through a more efficient use of intracellular pools (Sunda et al., 1991), a minimisation of metabolic pathways (Stefels and Van Leeuwe, 1998), and/or a replacement of Fe-containing proteins by non Fe-containing proteins (La Roche et al., 1996). A luxury uptake of Fe can also happen when Fe availability is high (Sunda and Huntsman, 1995) and may explain part of the variability in Fe:C ratios if Fe concentrations in culture media are different from one experiment to another one. Recent studies showed the strong impact on Fe limitation on the elemental ratios Si:N and Si:C (Marin and Fitzwater, 1988; Hutchins and Bruland, 1998; Hutchins et al., 1998; Takeda, 1998; De La Rocha et al., 2000; Franck et al., 2000; Bucciarelli et al., ms in review; Brzezinski et al., 2003). However, the mechanisms underlying this impact are still not clear and need more studies including the effect on the cell cycle, and on the assimilation and regeneration of C and N.

Concerning the losses, diatoms can sink quite rapidly because of their large size and because they form aggregates which generally increase sinking rates. Diatoms are also subject to cell lysis by autolysis (Brussaard et al., 1997; Veldhuis et al., 2001) as they seem to be relatively well protected against viruses (Gowing, 2003). Diatom cells are commonly grazed by mesozooplankton species such as copepods; however, one cannot ignore that microzooplankton species are sometimes dominant consumers of bloom-forming diatoms. However, a better quantification of prey selection by microzooplankton and mesozooplankton in natural environments is needed.

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