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ABSTRACT: The significance of silicon in the diatom bloom dynamics of eutrophicated Belgian coastal waters (Southern Bight of the North Sea) was assessed by investigating diatom species succession and silicification level (Si:C) in relation with environmental control during an annual cycle from early February to mid-December 1995. The Si:C of natural diatom communities was determined by 2 methods. The measurement of biogenic silica after alkaline digestion of particulate material and the estimate of diatom carbon biomass derived from microscope observations were performed on weekly samples from Stn 330 in Belgian coastal waters. Alternatively, parallel 24 h kinetics experiments of ^{32}Si uptake and ^{14}C incorporation into proteins, as an index of phytoplankton biomass production, into diatom-dominated natural communities were conducted in simulated natural conditions. The seasonal pattern of diatom growth was characterised by 3 diatom assemblages dominated by respectively small colonial species, *Chaetoceros* spp.-*Schroederella* sp. and *Rhizosolenia* spp. The colonial haptophyte *Phaeocystis* bloomed for 2 mo, co-occurring with the 2 latter diatom communities. The 3 diatom assemblages were characterised by distinct Si:C with values varying from 0.80 to 0.05 during the growing season. The Si:C of the blooming assemblages are discussed with respect to inter- and intra-specific variations based on changes in ambient controlling factors (nutrient, light and temperature) and compared with information available from the literature for individual diatom species. The results suggest that silicic acid availability controls the Si:C of the main diatom assemblages. This is supported by the positive relationship between the diatom Si:C and silicic acid concentrations. We conclude that silicic acid availability is an important factor in the selection of diatom species, shaping the diatom succession in temperate coastal waters.

KEY WORDS: North Sea · Diatoms · Silicification · Silicic acid

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INTRODUCTION

Diatoms have an absolute requirement for silicon and the availability of silicic acid can limit diatom growth. Depletion of silicic acid relative to inorganic nitrogen and phosphate has been observed to select for non-siliceous algae (Smayda 1990, Egge & Aksnes

1992). As conceptually discussed in Officer & Ryther (1980) and Billen et al. (1991), this shift is exacerbated in coastal waters receiving anthropogenic inputs of nitrogen and phosphorus and can end with the dominance of often hardly edible non-siliceous algae, which can result in harmful consequences. Such is the case of Belgian coastal waters (Southern Bight of the North Sea) largely influenced by freshwater sources with high concentrations of anthropogenic nutrients. The winter signature of these coastal waters (Lancelot et

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al. 1991, Lancelot 1995) shows nitrogen (mostly nitrate) excess over phosphate and silicic acid when compared to nitrogen and silicic acid requirements of diatoms (Brzezinski 1985) and to nitrogen and phosphorus needs of phytoplankton in general (Redfield et al. 1963). As a consequence, the spring phytoplankton bloom, while initiated by an early spring diatom bloom, is recurrently dominated by large *Phaeocystis* colonies (Lancelot et al. 1987, 1998, Cadée & Hegeman 1991, Lancelot 1995, Rousseau et al. 2000).

Nutrient changes during the spring bloom suggest that the growth of early spring diatoms is limited by silicic acid availability (Billen et al. 1991, Schoemann et al. 1998) while the magnitude of the *Phaeocystis* bloom is determined by nitrate left over after the decline of early diatoms (Lancelot 1995, Lancelot et al. 1998, Rousseau 2000). The bloom of *Phaeocystis* colonies in Belgian coastal waters occurs between April and May and persists for 20 to 40 d (Rousseau 2000). Conversely, diatoms are present throughout the year and contribute significantly to the phytoplankton community throughout the vegetative season; this includes periods of *Phaeocystis* occurrence when ambient silicic acid is very low (Lancelot et al. 1998, Rousseau et al. 2000). During these periods, 2 diatom assemblages composed of different species succeed consecutively to the early spring diatom community (Lancelot et al. 1998). This highlights the role of silicic acid availability in shaping diatom species succession in this ecosystem, compared to other limiting factors such as temperature, light and other nutrients (N, P).

The literature on diatom silica requirement is contradictory. Earlier microscope observations of phytoplankton in the English Channel (Cooper 1933) reported that a thin-walled species (*Cerataulina pelagica*) did indeed succeed to a thick-walled one (*Coscinodiscus granii*) when silicic acid concentration declined during the spring bloom. Unfortunately, these observations were not supported by quantitative measurements of the diatom silica content. An extensive laboratory study conducted on 27 marine diatom species reported an inter-specific variability of the diatom silicification level, expressed as the molar Si:C ratio, of about 1 order of magnitude (0.04 to 0.42; Brzezinski 1985). On the contrary, Paasche & Østergren (1980) linked the decrease of the silica content of one single blooming diatom to silicic acid limitation. Hence, the link between diatom succession and silicic acid availability in natural environments appears more complex due to a possible intra-specific variability which shows lower silica content of species under silicic acid limitation conditions (Paasche 1973a, Harrison et al. 1976, Tilman & Kilham 1976). Besides silicic acid availability, the silicification level (Si:C) of diatoms is also strongly affected by other factors regulating diatom growth rate

such as light intensity and photoperiod, temperature, nutrient (N, P) or trace metal ($Fe^{2+/3+}$, Zn^{2+}) concentrations (see review in Martin-Jézéquel et al. 2000).

Understanding the significance of silicon in eutrophicated coastal seas requires a better knowledge of factors regulating diatom succession patterns. The possible role of silicic acid availability in the diatom succession has been mostly tested on cultured diatoms (e.g. Paasche, 1973a, Tilman & Kilham 1976) but poorly investigated in the natural environment. This is due to the lack of adequate methods for measuring correctly the biogenic silica (BSi) associated with diatoms and their carbon biomass (C biomass). In this study, we measure and compare the Si:C of the main diatom communities blooming in Belgian coastal waters during an annual cycle from early February to mid-December 1995. The Si:C of the blooming assemblages are discussed with respect to inter- and intra-specific variations based on changes in ambient controlling factors (nutrient, light and temperature) and compared with information available from the literature.

To obtain the best estimates of the diatom Si:C, we combined different methods (chemical, microscopic and radioisotopic) in order to eliminate the interference from non-diatom carbon and detrital particulate silicon of various origins (lithogenic silica and detrital diatom frustules). In one method, the Si:C of field diatoms was determined on the basis of the estimate of diatom C biomass derived from microscope observations and from the measurement of BSi after alkaline digestion of particulate material (Paasche 1980, Krausse et al. 1983). The protocol of Ragueneau & Tréguer (1994) was used to remove the interference with lithogenic silica (LSi). As an alternative method, parallel time course measurements of ^{14}C and ^{32}Si incorporation were conducted with diatom-dominated field assemblages. Their Si:C was estimated as the ratio between the rate of silicic acid uptake and that of protein synthesis. This latter has been demonstrated to be the best index for phytoplankton biomass production (Cuhel et al. 1984, Lancelot et al. 1986). The ^{32}Si technique (Tréguer et al. 1991, Brzezinski & Phillips 1997) has been applied successfully in various marine environments to measure the BSi production associated with living diatoms (e.g. Tréguer et al. 1991, Nelson & Dortch 1996, Brzezinski et al. 1997, Ragueneau et al. 2002).

MATERIALS AND METHODS

Field sampling. Measurements were conducted on samples collected at Stn 330 (51° 26.00′ N, 002° 48.50′ E) of the Belgian monitoring network. Since 1988, this station has been used as the reference station for mon-

itoring seasonal and interannual changes in Belgian coastal waters (Southern Bight of the North Sea), owing to its average physico-chemical characteristics (depth, temperature, salinity and nutrient enrichment level). These continental coastal waters are composed of north-eastward flowing Atlantic water mass influenced by freshwater discharges of the rivers Seine, Somme (Lancelot et al. 1991), Scheldt (Yang 1998) and Rhine/Meuse (van Bennekom & Wetsteijn 1990). The strong tidal currents combined with a shallow water column ensure complete vertical mixing of the water column (Simpson 1994). The discharge of continental suspended matter together with a quasi-permanent resuspension of sandy and silty sediments due to the highly turbulent regime, supplies these coastal waters with large amounts of particulate matter.

Surface seawater samples were collected with a bucket aboard RV 'Belgica'. Sampling of Stn 330 was conducted weekly between February and December 1995. Sub-samples were taken for major nutrient (NH_4, NO_3, PO_4 and $Si(OH)_4$) and BSi concentration analyses and phytoplankton species determination. Radiotracer experiments were run with 4 diatom-dominated phytoplankton communities collected during the spring and fall. Three of them were collected at Stn 330 on 24 and 31 March and 27 October 1995. Due to extreme weather conditions and logistic problems, sampling of mid-February 1995 was conducted at co-ordinates 51° 08.00′ N, 002° 10.00′ E Microscope observations showed similar diatom species dominance as normally observed at Stn 330 during this period (Lancelot et al. 1998).

Temperature and light measurement. Seawater temperature was measured with a thermosalinometer (Beckman). The average photosynthetically active radiation PAR (µmol quanta m^{-2} s^{-1}) available to phytoplankton in the water column during the photoperiod was calculated by using the equation of Riley (1957) on the basis of the incident PAR, the vertical light attenuation coefficient and the depth of the station (17 m). The average PAR during the photoperiod was converted from global solar radiation recorded at Stn Ostende (Institut Royal de Météorologie of Belgium). It was calculated using the empirical relationship derived from the direct calibration of the instrument measuring global radiation GR (J cm^{-2} 30 min^{-1}) with a PAR sensor (Li-Cor):

$$GR = 3.43 \times 10^{-6} \times PAR^2 + 0.0805 \times PAR$$

The vertical light attenuation coefficient K_e was calculated from the suspended matter (SM) content according to the empirical relationship established for Belgian coastal waters (Rousseau unpubl. data):

$$K_e = 0.024 \times SM + 0.188 \ (n = 38; r = 0.89)$$

where K_e was derived from optical vertical profiles.

SM content was determined by weighting particulate matter collected by filtration on a GF/F (Whatman) pre-weighted filter and dried for 4 h at 105°C.

Analytical methods. Concentrations of major nutrients (NH_4, NO_3, PO_4 and $Si(OH)_4$) were determined according to the colorimetric methods described in Grasshoff et al. (1983), on filtered seawater samples (0.6 µm, Nuclepore), stored frozen until analysis. Diatoms as well as *Phaeocystis* colonies and free-living cells were enumerated with an inverted microscope (Leitz Fluovert) using the Utermöhl method (Hasle 1978). Samples were preserved with 1% (final concentration) Lugol-glutaraldehyde solution and stored at 4°C in the dark until analysis. Magnification for enumeration was chosen according to cell or colony size: 40 or 100× for *Phaeocystis* colonies; 100 or 200× for diatoms; and 320× for cells of size less than 50 µm. At least 400 cells were counted in each sample resulting in an error amounting to 10% at the 95% confidence level (Lund et al. 1958); 100 cells of the most abundant species were enumerated. Diatoms were enumerated and identified to the genus level unless a species was easily identifiable or dominant.

The C biomass of diatoms was calculated on the basis of cell density and biometry determined for each species. A specific average conversion factor was calculated from biovolumes measured on a cell population throughout the period of its development. Biovolumes were calculated from linear dimensions measured at 320 or 400× and using specific geometric models (Hillebrand et al. 1999) and were converted into C biomass using the size-dependent density relationship as recommended for diatoms by Menden-Deuer & Lessard (2000). The C biomass of *Phaeocystis* colonies (cellular and mucilaginous) and free-living cells was calculated according to Rousseau et al. (1990).

BSi was determined on particulate material collected on 47 mm diameter polycarbonate membrane filters (0.6 µm pore size; Nuclepore). Filters were dried immediately at 50°C for 12 h and stored at room temperature in sealed petri dishes until analysis. BSi concentrations were determined according to the sequential Na(OH)/HF digestion method (Ragueneau & Tréguer 1994), which allows the determination of a factor correcting the particulate silica extracted during the NaOH digestion for the leaching of LSi. A linear relationship holds between the particulate silica and LSi extracted after NaOH and HF digestion, respectively, for samples collected during period of low biological activity. The slope of the regression represents the proportion of LSi extracted with BSi. Application of this method on samples collected at Stn 330 during winter and during low diatom biomass periods in 1995, estimates that the contribution of LSi to BSi was 0.28 (n = 6; r = 0.86).

Tracer experiments. The Si:C of natural diatoms was alternatively estimated by running parallel 24 h time course experiments of ^{32}Si uptake and ^{14}C incorporation into proteins. Compared to biomass measurements, this method is specific for algae in active growth. However, it has to be selective for siliceous forms in order to avoid the contribution of non-diatoms to carbon production. For this reason, radiotracer experiments were conducted during periods of total diatom dominance. Although it has been shown under laboratory conditions that most of the diatom silicic acid uptake and silica deposition is confined to a short specific period of the cell cycle (Sullivan 1977), it is assumed that cell division of diatoms in the natural environment is not synchronous. Silicic acid uptake rate measurements at the time scale involved in these experiments can thus be considered as an index of diatom population growth (Ragueneau et al. 2000). On this basis, the diatom Si:C was estimated from the ratio between the rate of Si uptake and that of C protein synthesis, the latter converted to biomass production. A 72% protein contribution to the diatom C biomass was considered as typical for coastal North Sea communities (Lancelot et al. 1986).

Experiments of ^{14}C incorporation and ^{32}Si uptake were performed according to the protocols of Mathot et al. (1992) and Tréguer et al. (1991), respectively. These procedures were applied as follows. Large volumes of seawater were inoculated with solutions of ^{14}C-bicarbonate (45 mCi mmol^{-1}, Amersham) or ^{32}Si (OH)$_4$ (1.125 µCi µgSi^{-1} in NaOH 0.1 N; Los Alamos, USA) at a rate of 100 and 0.6 µCi l^{-1} of seawater, respectively. The large volumes were then distributed into 6 to 9 aliquots and incubated at *in situ* temperature under a light intensity of 200 µmol quanta m^{-2} s^{-1} and a 12:12 h light:dark cycle. At various times over 24 h, ^{14}C subsamples were filtered on GF/C filters (Whatman) and proteins were specifically isolated from other

cellular constituents after trichloroacetic acid (TCA) precipitation using the protocol of Lancelot & Mathot (1985). Subsamples for ^{32}Si incorporation were collected by filtration on 0.6 µm polycarbonate membrane filters (Nuclepore) and rinsed with filtered (0.2 µm) seawater. Radioactivity incorporated into proteins was measured by liquid scintillation (Packard Tri-Carb 1600CA) after dissolution of proteins in 10 ml Ready Safe (Beckman). The ^{32}Si incorporation by diatoms was estimated by detection of the Cerenkov radiation (Packard Tri-Carb 1600CA) of the daughter ^{32}P after secular equilibrium was reached (after about 4 mo).

RESULTS

Diatoms and *Phaeocystis* succession in Belgian coastal waters in 1995

Fig. 1 shows seasonal variations of diatoms and *Phaeocystis* at Stn 330 of Belgian coastal waters in 1995. Diatoms were present all through the productive season; by contrast, the occurrence of *Phaeocystis* colonies was limited to 2 mo in spring (Fig. 1). Diatoms initiated the phytoplankton succession in mid-February. At this time, their C biomass increased from a background level of less than 10 mg C m^{-3} up to a maximum of 223 mg C m^{-3} at the end of April. Diatoms maintained a level as high as 164 mg C m^{-3} during the *Phaeocystis* bloom. During June, diatoms nearly disappeared from the water column and their C biomass varied from 0.6 to 6.4 mg C m^{-3}. A summer diatom bloom (133 mg C m^{-3}) occurred between July and August and a late rather modest diatom growth occurred in October (26 mg C m^{-3}). In 1995, *Phaeocystis* colonies bloomed between late March and end of May (Fig. 1). The total *Phaeocystis* C biomass, including both free-living and colonies, was on average similar to diatom biomass except in mid-May when a *Phaeocystis* biomass of 540 mg C m^{-3} was suddenly recorded. The contribution of cells to *Phaeocystis* C biomass was considerably lower, reaching a maximum of 114 mg C m^{-3} in mid-May (Fig. 1).

Three seasonally distinct diatom assemblages were identified from the taxonomic analysis (Fig. 2). The early spring diatom community (Assemblage 1) present in mid-March was characterised by small colony forming species: *Asterionellopsis glacialis*, *A. kariana*, *Thalassiosira* spp., *Thalassionema nitzschioides*, *Skeletonema costatum*, *Melosira sulcata* and *Plagiogramma brockmanii* (Fig. 2a). The second diatom assemblage (Assemblage 2) composed of *Chaetoceros* spp. and *Schroederella* sp. bloomed from mid-March to early-April (Fig. 2b). This community partly co-occurred with *Phaeocystis* colonies (Figs. 1 & 2b) but was rapidly re-
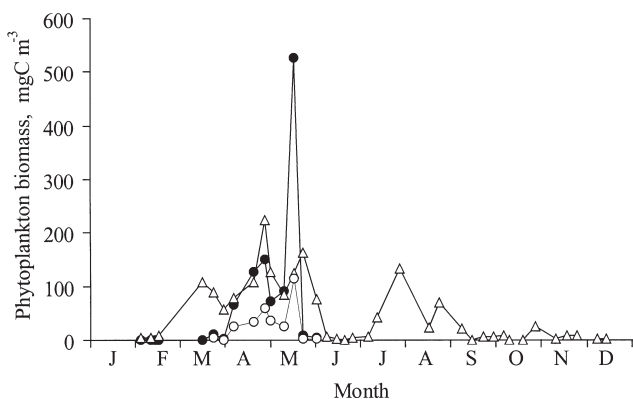

Fig. 1. Seasonal changes of diatoms (△), *Phaeocystis* colony (●) and cellular (○) C biomass measured at Stn 330 of Belgian coastal waters in 1995
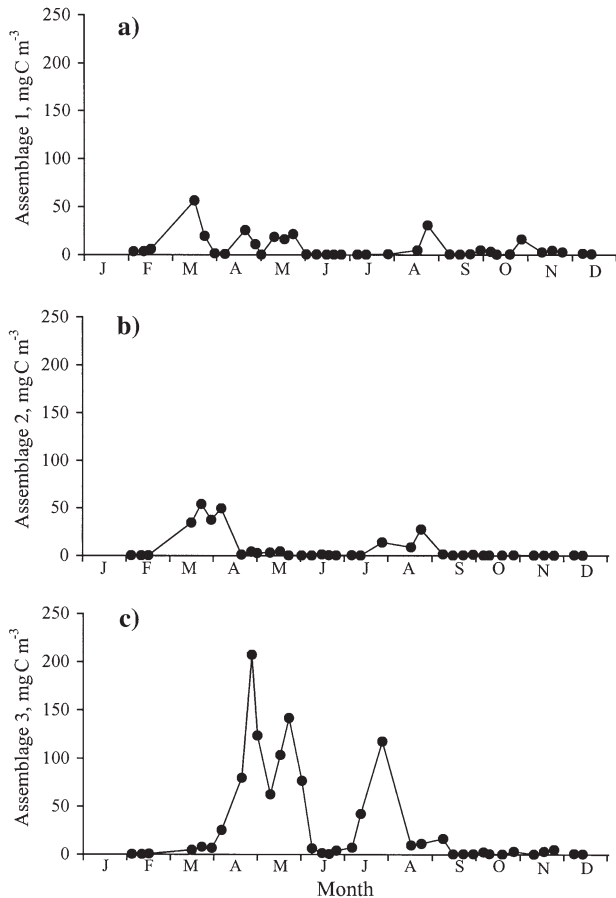
Fig. 2. Seasonal changes of: (a) Assemblage 1 (early spring and autumn diatoms); (b) Assemblage 2 (*Chaetoceros* spp.-*Schroederella* sp.); and (c) Assemblage 3 (*Rhizosolenia* spp.) blooming at Stn 330 of Belgian coastal waters in 1995

placed in mid-April by a diatom assemblage dominated by *Rhizosolenia* spp. (mainly *R. stolterfothii* and *R. delicatula*) in the presence of some *Cerataulina pelagica*, *Guinardia flaccida* and *Ditylum brightwellii*. This *Rhizosolenia*-dominated community (Assemblage 3) was present during the *Phaeocystis* bloom and accounted for the maximum diatom biomass recorded in the second half of April (223 mg C m^{-3}; Fig. 2c). During summer, the diatom community was once again dominated by the genus *Rhizosolenia* (mainly *R. delicatula* and *R. shrubsolei*) which composed the bulk diatom biomass at the end of July (Fig. 2c). The autumn diatom community was basically similar to Assemblage 1 with, however, occurrence of some large *Coscinodiscus* spp. in September and October (Fig. 2a).

Phytoplankton dominance and environmental conditions

The bloom of Assemblage 1 between February and March coincided with the decrease of Si(OH)$_4$ and PO$_4$

winter stocks, whose concentrations were 13 and 2.7 µM, respectively (Figs. 2a & 3b,c). At the end of March, 3.9 µM of Si(OH)$_4$ and 0.3 µM of PO$_4$ were left over when the diatom community shifted towards Assemblage 2. The latter community was maintained at low ambient Si(OH)$_4$ concentrations, i.e. 1.5 µM up to mid-April (Figs. 2b & 3b,c). At that time, a peak of 1.1 µM of PO$_4$ was recorded. In contrast, NO$_3$ concentrations varied from 36 to 22 µM during this period (Fig. 3a). This large excess of NO$_3$ compared to Si(OH)$_4$ and PO$_4$ was nearly depleted at the time of *Phaeocystis* colony bloom maximum (Figs. 1 & 3a). In the second half of May, the decline of the *Phaeocystis* and *Rhizosolenia* spp. bloom corresponded to the lowest DIN (3.3 µM), Si(OH)$_4$ (0.9 µM) and PO$_4$ (0.03 µM) concentrations recorded during spring (Figs. 2 & 3). From early June, DIN, Si(OH)$_4$ and PO$_4$ concentrations increased up to 18, 6.4 and 0.6 µM, respectively (Fig. 3) and persisted before being taken up by summer *Rhizosolenia* spp. (Figs. 2c & 3). From early September, nutrient concentrations progressively increased up to their winter levels while phytoplankton biomass decreased.
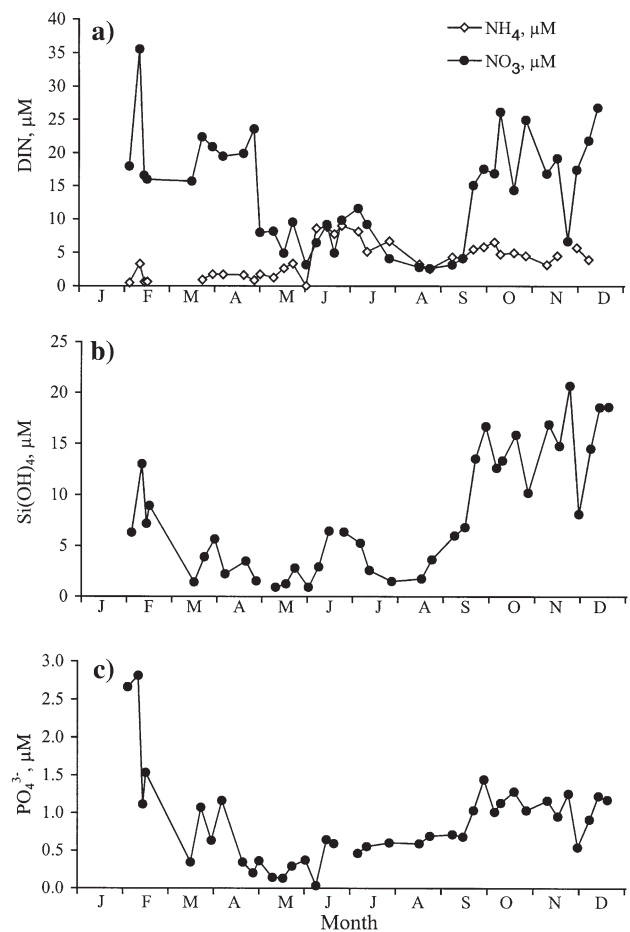

Fig. 3. Seasonal changes of major inorganic nutrient concentrations (µM): (a) Dissolved inorganic nitrogen, DIN (NO$_3$, NH$_4$); (b) Si(OH)$_4$; and (c) PO$_4$ recorded at Stn 330 in 1995

Table 1. Biomass-based silicification level (expressed as Si:C molar ratio) of the main diatom communities blooming in Belgian coastal waters in 1995. Seawater temperature and average light in the water column (WC light) are also reported

Diatom community	Season	Date (d/mo)	Temperature (°C)	WC light (µmol quanta m^{-2} s^{-1})	Si:C
Assemblage 1 (small diatoms-*Coscinodiscus* spp.)	Early spring and autumn	15/02	7.5	12	0.49
		17/03	8.1	26	0.42
		27/10	14.9	16	0.80
Assemblage 2 (*Chaetoceros* spp.-*Schroederella* sp.)	Spring	24/03	8.0	28	0.30
		31/03	8.0	32	0.27
		07/04	8.5	36	0.17
Assemblage 3 (*Rhizosolenia* spp.)	Spring and summer	21/04	9.0	35	0.05
		18/05	11.5	47	0.06
		28/07	18.0	57	0.10

Average light availability in the water column and temperature were different during the occurrence of the 3 diatom assemblages (Table 1). In early spring, Assemblage 1 was blooming when light intensity and temperature were at their lowest. During the fall, this diatom assemblage was present under similar light conditions (16 µmol quanta m^{-2} s^{-1}) but at quite higher temperature (14.9°C). During the blooms of Assemblages 2 and 3, both light intensity and temperature increased from 28 to 57 µmol quanta m^{-2} s^{-1} and 8 to 18°C, respectively.

Si:C of the main diatom assemblages

The seasonal evolution of BSi (Fig. 4) shows a clear bimodal pattern which contrasts with that of the diatom C biomass (Fig. 2). BSi concentrations as high as 3.7 µmol l^{-1} were associated with the modest bloom of Assemblage 1 (Figs. 2a & 4). A significant drop was observed in late March-early April and corresponded to the blooming of Assemblages 2 and 3 (Figs. 2c & 4).
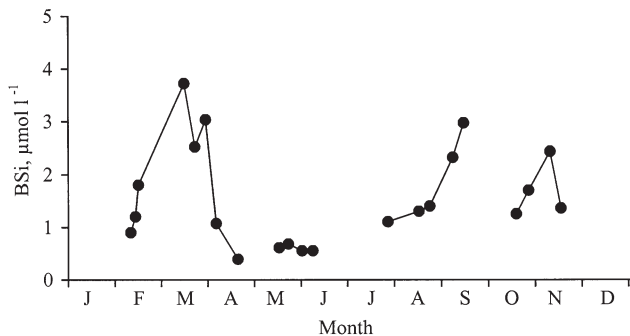

Fig. 4. Seasonal changes of biogenic silica (BSi) concentrations recorded at Stn 330 in 1995

Low BSi concentrations of 0.6 µmol l^{-1} were recorded during the spring and summer periods when *Rhizosolenia* spp. dominated the diatom community (Figs. 2c & 4). Once again, high BSi concentrations of about 2 µmol l^{-1} were measured in the autumn but these were not associated with diatom C biomass maxima (Figs. 2 & 4). Taken together, this suggests a large variability of the Si:C between the different diatom assemblages. However, a variable contribution to the BSi pool of dead diatoms and faecal pellets containing diatom frustules cannot be excluded. The alkaline digestion method does indeed measure bulk diatom silica, including empty or broken frustules in suspension or in faecal pellets. The Si:C of diatoms based on BSi measurement and diatom C biomass in the field can therefore be overestimated. To circumvent such bias, our calculations of Si:C were restricted only to samples corresponding to periods of maximum biomass of each of the 3 diatom assemblages (Fig. 2, Table 1). The absence of empty or broken frustules and faecal pellets was checked by microscopy. Results of these calculations are reported in Table 1: This shows distinct Si:C for the various diatom assemblages blooming throughout the growth season (Fig. 2). The early spring and autumn Assemblage 1, composed of the small colony forming diatoms with some *Coscinodiscus* sp. during the autumn, is the most highly silicified community with Si:C varying between 0.42 and 0.80. The late spring and summer *Rhizosolenia* spp. (Assemblage 3) are the least silicified (Si:C ≈ 0.05 to 0.10); while *Chaetoceros* spp. and *Schroederella* sp. (Assemblage 2) show intermediate Si:C (0.17 to 0.30).

Further Si:C estimations were made for 4 diatom communities of Assemblages 1 and 2 by performing parallel 24 h time course radiotracer experiments (Fig. 2). In all experiments, ^{32}Si uptake and protein synthesis rates were shown to proceed linearly during

the light and dark period (Table 2). This is illustrated in Fig. 5 which shows the tight coupling between both processes for the diatom community (Assemblage 1) sampled in mid-February. The diatom Si:C was estimated from the ratio between the rate of Si uptake and that of C biomass production. Process rates were determined as the slopes of the linear regression between radiotracer incorporation and incubation time (Fig. 5). Results of these calculations are reported in Table 2, which shows that the diatom Si:C estimated from process measurements ranged between 0.38 and 0.74 for Assemblage 1 and between 0.20 and 0.24 for Assemblage 2.

DISCUSSION

Determination of diatom Si:C in shallow coastal waters

Accurate determination of the Si:C of marine diatoms constitutes one prerequisite for assessing the role of silicic acid in the diatom bloom dynamics of coastal waters. No direct method exists to measure specifically the diatom Si:C in natural aquatic ecosystems due to several interferences. Despite that, comparing measurements of BSi to diatom C biomass, the latter being derived from microscopic observations, constitutes one of the best approaches. However, a major problem associated with the determination of BSi in coastal waters when applying classical alkaline digestion methods (Krausse et al. 1983), results from the contribution of detrital BSi and of some LSi. In this work, the contribution of LSi has been assessed by using the method of Ragueneau & Tréguer (1994), who showed that the interference of LSi is related to its concentration modulated by a correction factor specific for the investigated area. We thereby determined a correction factor specific for Belgian coastal waters.

More complicated is the estimate of the contribution of detrital BSi which includes both empty or broken frustules, diatom-derived aggregates and faecal pel-
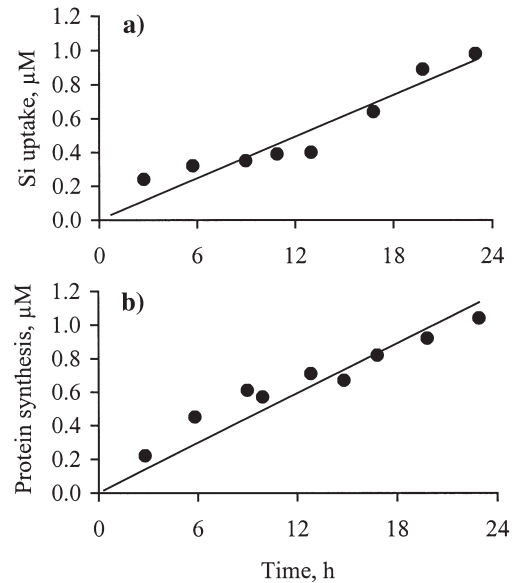

Fig. 5. Time course experiments of ^{32}Si uptake (a) and ^{14}C incorporation into proteins (b) by a diatom community (Assemblage 1) sampled in February 1995. C biomass was 8 mg C m^{-3} and silicic acid concentration was 19.6 µM

lets, all of which vary greatly throughout the season. This is particularly important during periods of diatom bloom decline and high mesozooplankton activity (from mid-May to September in Belgian coastal waters; Hecq 1980). The presence, as revealed by qualitative microscope observations, of empty frustules and/or copepod faecal pellets in the samples of the summer and autumn 1995, when BSi was at a maximum and the diatom C biomass was reduced (Figs. 2 & 4), argues for the significant contribution of detrital BSi to the pool of measured BSi. It may be concluded that the determination of diatom Si:C based on BSi measurements and diatom C biomass estimates are acceptable during periods of diatom growth; however, they are most probably overestimated outside these periods. On the other hand, the determination of the diatom C bio-

Table 2. Process-based Si:C of 4 diatom assemblages sampled in Belgian coastal waters in 1995. Si:C are calculated as the ratio between the rates of ^{32}Si uptake and ^{14}C incorporation into proteins determined from parallel 24 h experiments. Rates (µM h^{-1}) are expressed as the slopes of the linear regression between radiotracer incorporation and incubation time. Statistical coefficients of the linear regressions (n, r^2 at p < 0.05) are indicated

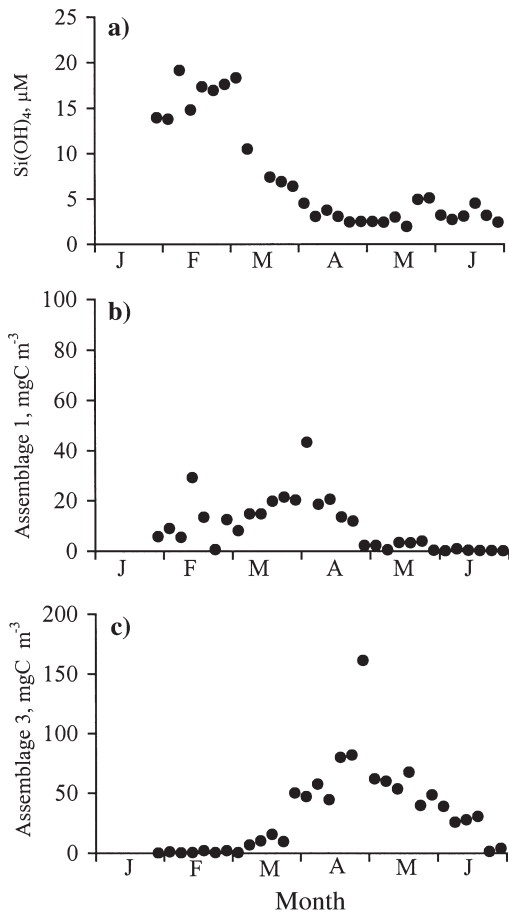| Diatom community	Date (d/mo)	^{32}Si uptake rates			^{14}C protein synthesis			Si:C
		Rate	n	r^2	Rate	n	r^2	
Assemblage 1	15/02	0.0381	8	0.91	0.0369	9	0.96	0.74
	27/10	0.070	6	0.81	0.134	6	0.99	0.38
Assemblage 2	24/03	0.0911	7	0.84	0.3355	7	0.99	0.20
	31/03	0.0828	6	0.94	0.2516	6	0.98	0.24

Fig. 6. Data recorded at Stn 330 averaged over 5 d periods from February to June for the years 1993 to 1999: (a) silicic acid concentration; (b) Assemblage 1 biomass; and (c) Assemblage 3 biomass

mass based on cell biometry adds to the uncertainty of the Si:C. Some bias in our estimation of the diatom C biomass can indeed result from the use of an average size for each diatom species throughout the blooming period; however, it could also result from the use of an empirical relationship between biovolume and C content, which has been shown to vary greatly with growth conditions (Montagnes et al. 1994, Menden-Deuer & Lessard 2000). A comparison of calculations of diatom C biomass using the conversion factors proposed by Edler (1979) and by Menden-Deuer & Lessard (2000) indicates, however, that the variation linked to empirical conversion factors is on average 8% (data not shown).

The second approach used was based on the calculation of the ratio between the silicic acid (^{32}Si) uptake and C protein synthesis (^{14}C) rates. The correlation observed between the kinetics of ^{32}Si uptake and ^{14}C incorporation into diatom proteins (Fig. 5, Table 1) indicates close coupling between silicic acid uptake,

silica deposition and protein synthesis over a 24 h period (Sullivan 1986). This contrasts with other observations (e.g. Brzezinski et al. 1990, Martin-Jézéquel et al. 2000) reporting that diatom silicic acid uptake is a discontinuous process, tightly geared to the formation of the cell wall (G_2 phase of the cell cycle). The linear ^{32}Si uptake observed for our 24 h experiments would reflect asynchronous growth of field diatoms rather than a continuous uptake of silicic acid by siliceous algae. Relating the uptake rate of silicic acid to diatom growth (here measured as protein synthesis) seems to be a valuable approach in estimating the Si:C of field diatoms, provided that they are the dominant carbon producers. If not, other algae will interfere with the protein synthesis measurement and the Si:C of diatoms will therefore be underestimated.

Diatom dynamics in Belgian coastal waters

The phytoplankton seasonal pattern recorded in Belgian coastal waters in 1995 was similar to that recorded every year at Stn 330 since 1988 (Lancelot et al. 1998, Rousseau 2000). The main features are the occurrence in spring of 3 consecutive diatom assemblages respectively dominated by small colonial species (Assemblage 1), *Chaetoceros* spp.-*Schroederella* sp. (Assemblage 2) and *Rhizosolenia* spp. (Assemblage 3). Superimposed on the 2 latter, the colonial haptophyte *Phaeocystis* blooms for about 2 mo. Similar patterns of diatom community succession have been reported in other regions of the Eastern Southern Bight of the North Sea, i.e. in French (H. Grossel pers. comm.) and Dutch (Gieskes & Kraay 1975, Cadée 1986, Peperzak et al. 1998, Philippart et al. 2000) coastal waters. This similarity suggests that this succession pattern is typical for the Southern Bight of the North Sea.

The 3 diatom assemblages identified in Belgian coastal waters in 1995 were submitted to different light, temperature and nutrient conditions (Figs. 2 & 3, Table 1). Assemblage 1 was present at the highest nutrient concentrations, the lowest light intensity but quite different temperature. Assemblage 3 was blooming within a wide range of light intensity and temperature but at rather low nutrient concentrations. Environmental conditions prevailing during the short-living bloom of Assemblage 2 were intermediate. Seasonal variations of phytoplankton and nutrient concentrations at Stn 330 could result from a change in water masses as well. A statistical analysis of time series data (1993 to 1999) of diatom biomass and nutrient concentrations recorded during spring at Stn 330 suggests, however, the existence of a recurrent seasonal pattern in spite of highly variable hydrodynamical conditions (unpubl. data). This is particularly illustrated by sea-

sonal changes of silicic acid concentrations and of C biomass of Assemblages 1 and 3 (Fig. 6). Assemblage 1 is indeed associated with the depletion of the winter stock of silicic acid while Assemblage 3 blooms at low silicic acid concentrations during the late spring period.

Inter- and intra-specific variability of Si:C

The 3 diatom assemblages observed in Belgian coastal waters in 1995 have been characterised by distinct silicification level with Si:C values varying from 0.80 to 0.05 (Table 1) through the season. These different Si:C reflect both intra- and inter-specific variabilities. Seasonal changes of Si:C in relation to diatom succession have been very poorly documented in the literature. Most of the field observations are related to intra-specific variability, i.e. the decrease of the silica content of the marine *Skeletonema costatum* (Paasche & Østergren 1980) and the freshwater diatom *Asterionella formosa* (Krivtsov et al. 2000) throughout the course of a spring bloom. The 2-fold variability of the diatom Si:C recorded within each assemblage of Belgian coastal waters (Tables 1 & 2) would suggest that intra-specific variability might be important as well. Unfortunately, the few data available for each blooming assemblage do not allow us to assess the control of the intra-specific variability of Si:C.

To better understand the reason of this variability, we compared our data with available information on the inter- and intra-specific variability of individual diatom species. Table 3 summarises the available information for cultured marine diatoms selected with respect to the 3 diatom assemblages identified in our study. The inter-specific variability of Si:C was assessed by comparing data of Table 3 for diatoms in exponential growth phase but no distinction was made between light and temperature conditions. Globally, the literature reports inter-specific variation of diatom Si:C between 0.04 and 0.52, which provides strong support to our field data (Tables 1 & 2). However, the variability of Si:C between the different assemblages (Table 3) is not as distinct as measured in Belgian coastal waters (Tables 1 & 2). On average, the range of the silicification level of diatoms of Assemblage 1 is significantly higher than those of Assemblages 2 and 3. Some diatoms of Assemblage 1 such as *Thalassiosira* spp., *Thalassionema nitzschioides* and *Coscinodiscus* sp. are well characterised by Si:C as high as those measured in Belgian coastal waters (0.38 to 0.80; Tables 1 & 2). However, Si:C of other species of Assemblage 1 such as *Skeletonema costatum* is significantly lower (0.07 to 0.29, Table 3). Additionally, and contrary to what has been measured for diatoms of Belgian coastal waters (Tables 1 & 2), no clear distinction can be made between cultured diatoms of Assemblages 2 and 3 based on their Si:C (Table 3). The inter-specific variability of Si:C within Assemblage 2 ranges between 0.04 and 0.16 while *Rhizosolenia* sp. and *Ditylum brightwellii* (Assemblage 3) show variations between 0.11 and 0.20 (Table 3).

Part of the variability reported in Table 3 is most probably due to intra-specific or clonal variability. This is particularly suggested when several Si:C values are available for the same species (Table 3). An intra-specific variability of about 2 to 4 is reported for some

Table 3. Literature review of the range of silicification levels (Si:C molar ratio) of cultured diatoms during exponential growth. No distinction has been made between studies using various light and temperature conditions

Diatom species	Si:C	Reference
Assemblage 1		
Asterionellopsis glacialis	0.11–0.12	Brzezinski (1985)
Skeletonema costatum	0.07–0.15	Brzezinski (1985)
	0.07–0.10	Paasche (1980)
	0.11	Harrison et al. (1977)
	0.20	Parsons et al. (1961)
	0.25	N. Daoud (unpubl. data)
	0.29	Llewellyn & Gibb (2000)
Thalassionema nitzschioides	0.42	Brzezinski (1985)
Thalassiosira spp.	0.52	Llewellyn & Gibb (2000)
Thalassiosira weissflogii	0.05–0.15	Brzezinski (1985)
	0.05–0.07	Brzezinski (1985)
	0.10	De La Rocha et al. (2000)
Thalassiosira rotula	0.13	Llewellyn & Gibb (2000)
	0.16	Harrison et al. (1977)
Thalassiosira pseudonana	0.09–0.11	Brzezinski (1985)
	0.13	Llewellyn & Gibb (2000)
	0.06–0.12	Brzezinski (1985)
Coscinodiscus granii	0.07	Paasche (1980)
	0.16	Harrison et al. (1977)
	0.22–0.36	Brzezinski (1985)
Assemblage 2		
Chaetoceros sp.	0.04–0.16	Brzezinski (1985)
	0.13–0.15	Llewellyn & Gibb (2000)
	0.17	Harrison et al. (1977)
Leptocylindrus danicus	0.08–0.11	Brzezinski (1985)
Assemblage 3		
Rhizosolenia sp.	0.14	Brzezinski (1985)
Rhizosolenia fragilissima	0.15	Paasche (1980)
Ditylum brightwellii	0.11–0.20	Brzezinski (1985)

species of Table 3. Intraspecific variability of the diatom
Si:C has been attributed to changes in light intensity,
photoperiod, temperature and nutrient availability
(Eppley et al. 1967, Harrison et al. 1976, Paasche 1980,
Brzezinski 1985, Takeda 1998, De La Rocha et al. 2000).
The effect of changing light and temperature on the
diatom Si:C was re-examined by Brzezinski (1985). No
obvious generic trend was observed when changing
the photoperiod and temperature although a 2-fold
variation of Si:C was observed. On the contrary, low
light (Furnas 1978) and limitation of nitrogen (Harrison
et al. 1976), iron (Takeda 1998, De La Rocha et al. 2000)
and zinc (De La Rocha et al. 2000) had a net effect of
increasing the diatom Si:C. Although alteration of the
kinetics of silica production by iron and zinc stress has
been reported (De La Rocha et al. 2000), the increase of
Si:C may be attributed to a slowdown of the growth
process by light and nutrients without affecting the
diatom silicic acid uptake and silica deposition (Martin-
Jézéquel et al. 2000). In accordance, a 2- to 3-fold in-
crease of the Si:C was reported for a natural *Chaeto-
ceros*-dominated diatom population when nitrate was
decreasing to depletion and silicic acid was abundant
(Kudo et al. 2000). Llewellyn & Gibb (2000) who com-
pared Si:C of 10 diatom species in the exponential and
stationary stages of their growth obtained inconclusive
results. An increase of Si:C was observed for weakly
silicified diatoms in the course of growth. The reverse
was observed for highly silicified diatoms such as *Tha-
lassionema* sp. and *Navicula* sp. (Llewellyn & Gibb
2000). These results suggest a more complex pattern of
nutrient interactions on the silicification level of dia-
toms, with opposing effects of depletion of silicic acid
and of nitrogen/iron. Indeed the ability of diatoms to
vary their cell wall thickness and consequently the Si:C
with silicic acid availability has been demonstrated by
Paasche (1973b, 1980) and Harrison et al. (1977). The
physiological adaptation to low silicic acid concentra-
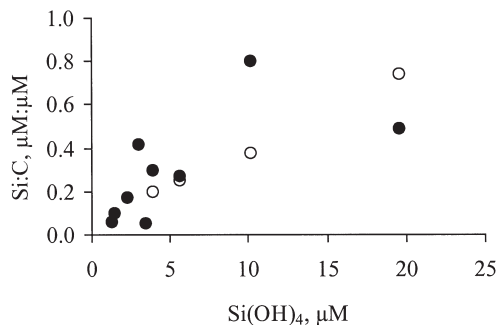tions by individual species was reported by Nelson &
Dortch (1996).

Fig. 7. Relationship between Si:C of diatoms estimated from
biomass (●) and process (○) based measurements and ambient
silicic acid concentrations

Diatom succession and silicic acid availability

Based on the previous information on the variability
of Si:C, it could be concluded that the distinct Si:C esti-
mated for the 3 diatom assemblages of Belgian coastal
waters reflect both specific properties and environ-
mental control. It seems plausible that the high ambi-
ent silicic acid of the autumn and late winter, in com-
bination with sufficient inorganic nitrogen and phos-
phate as well as low light and temperature conditions
lead to the early spring dominance of highly silicified
diatoms in Belgian coastal waters (Fig. 3, Table 1). The
pattern is more complex for the spring and summer
diatom communities (Assemblages 2 and 3) which
grow under higher light and temperature but low sili-
cic acid and phosphate conditions (Fig. 3, Table 1).
Indeed, high light and temperature conditions which
are associated to low silicic acid levels would de-
crease the diatom Si:C, while the opposite would result
from phosphate depletion. However, the occurrence of
Assemblages 1 and 3 under very different light and
temperature conditions (Table 1) would preclude these
environmental factors to control Si:C of these diatoms.
The decrease of ambient silicic acid from 19.6 μM to a
minimum of 1.3 μM (Fig. 3b) suggests that silicic acid
availability could control the diatom Si:C. The ob-
served seasonal pattern of diatoms might be due in
part to differences in their silica requirement. Accord-
ingly, a positive relationship was observed between
the diatom Si:C and ambient silicic acid concentrations
(Fig. 7). Our relationship (Fig. 7) fits well with the
general observation in the aquatic environment that
diatom Si:C is related to silicic acid concentrations.
Freshwater diatoms are much more heavily silicified
than the marine ones (Conley et al. 1989), the silicic
acid concentrations in freshwaters being much higher
than those of marine waters. Among marine diatoms,
those growing in the Southern Ocean, where silicic
acid concentrations as high as 80 μM have been re-
corded, are characterised by a Si:C as high as 0.65
(Nelson & Smith 1986).

Although the nutrient limitation status of the coastal
diatom communities was not assessed during this
study, the positive relationship that exists between
Si:C of the different diatom communities and silicic
acid availability suggests that the succeeding diatom
communities in Belgian coastal waters are well
adapted to their silicic acid environment. This has been
particularly well documented for the genus *Rhizosole-
nia* which was the dominant diatom during late spring
and summer (Fig. 2, Lancelot et al. 1998). *Rhizosolenia*
spp. is known to form huge blooms of 4×10^5 cells l^{-1}
between May and June in the English Channel (Grall
1972, Sournia et al. 1987) and in the Bay of Brest
(Ragueneau et al. 1994) when silicic acid concentra-

tions are low. In the Bay of Brest, the shift observed from the early spring diatom bloom dominated by *Thalassiosira* spp. to a *Rhizosolenia*-dominated community was also related to silicic acid availability (Del Amo et al. 1997b). Furthermore, Del Amo et al. (1997a,b) showed the ability of the *Rhizosolenia* to grow on remineralized silicic acid from sedimented early spring diatoms, suggesting that this diatom is particularly well adapted to take advantage of low silicic acid concentrations. Taken together, these results support the hypothesis that ambient silicic acid is an important factor for shaping the diatom succession in temperate coastal ecosystems. This conclusion reveals the complexity of the link between silicic acid availability, diatom blooms and coastal eutrophication. The latter is indeed usually described as a succession of diatom and non-siliceous algae in response to silicic acid limitation. There is consequently a need to re-explore the role of diatoms in eutrophicated environments on the basis of the specific physiology of the different blooming diatom communities.

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