

# The influence of changes in nitrogen: silicon ratios on diatom growth dynamics

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## Abstract

Nitrate loading to coastal waters has increased over recent decades while silicon loading has remained relatively constant or decreased. As the N:Si ratio in coastal waters shifts due to these anthropogenic influences, silicate limitation of diatom biomass may become a feature of the biogeochemistry in coastal waters especially in regions of reduced exchange. Two sets of nutrient enrichment mesocosm experiments were conducted in successive years using a natural planktonic assemblage obtained from the Trondheimsfjord, Norway. The inorganic nutrient concentrations at the start of the experiments were manipulated to give a variety of N:Si concentrations at ratios representative of current and possible future values, should N loading continue. In June 1999 experiments were conducted with a gradient of inorganic N:Si ratios (1:2, 1:1, 2:1, 4:1) to investigate the influence of low and high N:Si ratio conditions and to determine the conditions that would generate Si limitation of diatom growth. In June 2000, based on 1999 data, highly replicated experiments were conducted at N:Si ratios of 1:1 and 4:1 which were expected to result in N and Si limitation of diatom growth, respectively; statistical differences in cellular composition were recorded. N limitation of diatom biomass increase was observed under the three lowest N:Si ratios: particulate carbon (C) accumulation continued to occur following N exhaustion resulting in an increase in the organic C:N ratio. Silicate limitation of diatom biomass increase only occurred at the highest N:Si ratio of 4:1. Silicate exhaustion was followed by continued nitrate uptake for several days, at a slower rate than previously. The resulting increase in organic N was accompanied by an increase in organic C such that the C:N ratio of the organic material at the highest N:Si ratio failed to increase to the extent observed under the N limited conditions. Statistically significant differences in chlorophyll-a yield per unit nitrate, C:chlorophyll-a ratios, C:N ratio and diatom cell yield per unit nitrate or Si were observed in Si compared to N limited conditions. All mesocosms became dominated numerically and in terms of biomass by the diatom *Skeletonema costatum*. The potential implications of changing N and Si regimes in coastal waters are discussed.

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**Keywords:** Nitrate:silicon ratios; N:Si; Diatoms; C:N; Nutrient limitation

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## 1. Introduction

Nitrogen (N) and silicon (Si) are fundamental inorganic nutrients required for diatom growth. The anthropogenic input of N to riverine systems, and

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hence to coastal waters, from agricultural and domestic sources has increased over recent decades possibly by a factor of two to three globally (Jickells, 1998 and references therein). Although concern over phosphate-based detergents has resulted in the development of some Si based alternatives, anthropogenic sources of Si have remained relatively constant over the same time period. Indeed, the damming of river systems for power generation, domestic consumption or irrigation can lead to a net decrease in the amount of silicate reaching the coast. Slowing river flow allows increased riverine primary production and nutrient uptake and the subsequent diatom sedimentation results in the loss of adsorbed silicate from the water column (Admiraal et al., 1990; Conley, 1997). In regions of restricted exchange such as the southern North Sea (Smayda, 1990), the Baltic (Rahm et al., 1996), the Kattegat (Smayda, 1990) and Chesapeake Bay, USA (Conley and Malone, 1992), these factors have resulted in an increase in the coastal inorganic N:Si ratio (Aure et al., 1998).

Although variation exists, many marine diatoms have a relatively balanced N:Si ratio within their biomass (Brzezinski, 1985). Hence, a consequence of increasing inorganic N:Si ratios may be Si limitation of diatom growth. A switch from N to Si limitation of diatom growth will potentially influence both the quantity (cell numbers and biomass) and quality (composition of this biomass) of a diatom population (Davidson and Gurney, 1999). Changes in the abundance of a particular diatom species or of the diatom assemblage as a whole, changes in cell metabolic state or composition will potentially have significant implications for microbial trophic transfer and for mesozooplankton fecundity (Miralto et al., 1999). Such changes are of more than academic interest since diatom species dominate the spring phytoplankton assemblage in temperate regions, are instrumental in carbon (C) export and other biogeochemical cycles, and are a major component of the marine food chain.

Moreover, although the conventional view is that a diatom-based food chain is benign (Officer and Ryther, 1980), changes in the relative availability of N and Si may influence the relative as well as absolute abundance of various diatom species. This may lead to the appearance of blooms of harmful diatom species such as *Pseudo-nitzschia* spp., in

which domoic acid toxin production may be related to silicate stress (Bates et al., 1998).

Considerable work has been conducted on diatom metabolism, mostly under N limitation (e.g. Collos, 1980; Geider et al., 1993; Lomas and Glibert, 2000), but also under Si limitation (Paasche, 1973a,b, 1980; Olsen and Paasche, 1986; Martin-Jézéquel et al., 2000). The influence of inorganic nutrient ratios rather than absolute amounts of a single limiting nutrient has also been studied in both laboratory culture and outdoor mesocosms. Much of this work centres on changes in the inorganic N:P ratio (Egge and Heimdahl, 1994; Jacobsen et al., 1995), however, N:Si ratios and their effects on diatom abundance have been studied by Sommer (1994a,b) and Egge and Aksnes (1992). In general, this work has been conducted at either high or low N:Si ratios where limitation by one nutrient is assured.

To complement these studies, this work therefore sought to investigate the influence of differing inorganic N:Si ratios on a diatom dominated natural phytoplankton assemblage at N and Si concentrations representative of present or future spring conditions in coastal water at temperate latitudes. Experiments were conducted in late spring/early summer in a relatively enclosed fjordic environment experiencing characteristically high freshwater runoff and reduced surface salinity. The transient changes in the diatom population that dominated the microbial assemblage, chlorophyll concentrations, and physiological (C:N) state were followed in each condition, and the potential impact on trophic transfer within the food chain assessed.

## 2. Materials and methods

Experiments were conducted in 1.5 m<sup>3</sup> mesocosm bags suspended in an outdoor seawater basin within the Trondheim Marine Systems Large Scale Facility during June 1999 and 2000. The bags were made of heat-sealed white snakefilm to achieve the desired dimensions of approximately 1 m diameter and 1.9 m depth. Transparent coverings of the same material were used to prevent aeolian input. The mesocosms were filled with a combination of 300 L surface and 1200 L deep water pumped from 3–5 m and 120 m, respectively, in Trondheimsfjord, in

order to generate suitable nutrient concentrations and relatively low inoculum cell densities. The salinity of the surface water collected was typical of enclosed fjords in late spring/early summer with a mean salinity of 27 psu. Water was first pumped into a large holding tank above the mesocosms and then allowed to flow into the individual bags. This process ensured a homogeneous nutrient concentration, salinity, and phytoplankton population was added to all mesocosm bags. Microscopic observations of samples taken before and after pumping indicated that the process had not damaged phytoplankton cells. Both surface and deep water were pre-filtered through a 200  $\mu\text{m}$  mesh to exclude mesozooplankton: a procedure routinely conducted in mesocosm studies (Escaravage et al., 1995; Sommer, 1998).

Nitrate (sodium nitrate,  $\text{NaNO}_3$ ) and silicate (sodium silicate,  $\text{Na}_2\text{SiO}_3 \cdot 5\text{H}_2\text{O}$ ) were added to mesocosms at time zero to obtain a number of

different N:Si ratios within the bags (Table 1). Phosphate (potassium dihydrogen phosphate,  $\text{KH}_2\text{PO}_4$ ) was added to excess (5  $\mu\text{M}$ ) and the use of fjord water eliminated potential micro-nutrient limitation.

Three linked experiments were conducted over two years using a range of six nutrient regimes to achieve five different inorganic N:Si ratios (Table 1). In the first year (1999), two sequential experiments were conducted; Experiment A of thirteen days (bags A1-1, A2-1, A3-1, A4-1) and Experiment B of ten days (bags B2-2, B4-2, B5-1). The three character identifier representing each bag is comprised of experiment (A–C), nutrient ratio (1–5) and replicate number (1–4), and is explained in detail in Table 1.

Year 1 experiments were exploratory in nature, and hence only semi replicated. A complete factorial design was not possible with the manpower available, as is commonly the case with such large-scale mesocosms (Egge and Aksnes, 1992). The

Table 1  
Initial N, Si & P concentrations ( $\mu\text{M}$ ) and resulting N:Si ratios in the mesocosm bags<sup>a</sup>

Experiment			Phosphate	Nitrate	Silicate	Approximate N:Si	
A	B	C					
A1-1			5	8	14	1:2	
A2-1			5	8	7	1:1	
A3-1			5	14	14		1:1
A4-1			5	28	14		2:1
	B2-2		5	8	7	1:1	
	B4-2		5	28	14		2:1
	B5-1		5	28	7		4:1
		C3-2	5	12	12		1:1
		C3-3	5	12	12		1:1
		C3-4	5	12	12		1:1
		C3-5	5	12	12		1:1
		C5-2	5	28	7		4:1
		C5-3	5	28	7		4:1
		C5-4	5	28	7		4:1
		C5-5	5	28	7		4:1

The three character identifier XX-X describes:

- Experiment (A,B or C)
- N:Si nutrient ratio where 1 is  $\sim 7:14$ , 2 is  $\sim 8:8$ , 3 is  $\sim 12:12$  or  $14:14$ , 4 is  $\sim 28:14$ , and 5 is  $\sim 28:7$
- Replicate number over the three combined experiments.

e.g. C5-5 represents: Experiment C, nutrient ratio 5 ( $28:7=4:1$ ) and replicate 5 (the 5th bag with this set of nutrient conditions in the 3 combined experiments).

<sup>a</sup> Experiments A and B were conducted in 1999 and together provide a gradient of N:Si ratios from 1:2 to 4:1. Experiment C was conducted in 2000 with replication at two nutrient ratios.

N:Si ratios in bags A2-1 (N:Si of 1:1) and A4-1 (N:Si of 2:1) were replicated in bags B2-2 and B4-2, respectively. Experiments A and B determined the two most appropriate nutrient regimes (N:Si ratios of 1:1 and 4:1) for highly replicated experiment C, in 2000. Experiment C, was therefore designed to include only these two nutrient ratios but with four replicate bags for each ratio, bags C3-2 to C3-5 at N:Si of 1:1 and bags C5-2 to C5-5 at N:Si of 4:1. These bags replicated the nutrient concentrations and ratios of bags A3-1 and B5-1 from experiments A and B, respectively, permitting comparison between years.

In 1999, mesocosms were run as batch cultures. In 2000, the mesocosms were run in batch mode until day 6, to allow the populations to become established. Subsequently, 20% of the volume was replaced every two days with new fjord water in the same 1 surface:4 deep proportions. Nutrients were added to ensure the concentrations in this new water were the same as those at time zero in each mesocosm. Experiment C therefore ran in semi-continuous mode prolonging the duration of active cell growth (Williams and Egge, 1998).

The contents of each mesocosm were mixed manually twice per day to maintain cells in suspension and prevent the development of stratification. Samples were collected at the same time daily in 1999, and every 2 days in 2000, immediately following mixing. A 1 L Ruttner water sampler was used at a depth of approximately 0.75 m. Sample bottles were placed in the dark and rapidly returned to the laboratory adjacent to the mesocosm facility. Temperature ( $^{\circ}\text{C}$ ) and irradiance ( $\text{Q s}^{-1} \text{cm}^{-2}$ ) in each bag were noted daily using a HUGRUN, Seamon mini temperature recorder and QSL100 Biospherical Instruments light meter, respectively. Light levels 10 cm subsurface ranged from approximately 0.2 to  $2 \times 10^{16} \text{Q s}^{-1} \text{cm}^{-2}$  on cloudy and sunny days, respectively, and were consistent between bags. Temperatures in the experimental bags ranged from 10.3–11.9  $^{\circ}\text{C}$  concurrent with ambient conditions. Variation between bags was within 0.2  $^{\circ}\text{C}$ .

Duplicate samples were filtered from each mesocosm bag for each of the analyses. Sub-samples for the determination of total elemental C and N were filtered onto precombusted 25 mm Gelman AE filters and frozen ( $-20^{\circ}\text{C}$ ) in individual petri

dishes until analysis. Size-fractionated chlorophyll-a samples were collected by sequentially filtering through 20  $\mu\text{m}$  and 0.2  $\mu\text{m}$  25 mm polycarbonate membranes which were placed directly into 5 ml cold 90% acetone and kept in the dark at 4  $^{\circ}\text{C}$ . Total chlorophyll-a calculated by summing the size fractions is presented in this paper. In experiment B only, sub-samples for glucan analysis were filtered through pre-combusted 25 mm GFC filters and frozen ( $-20^{\circ}\text{C}$ ) in individual eppendorf tubes until analysis. The filtrate from the chlorophyll and glucan methods was collected for inorganic nutrient analysis; 40 ml was stored in a plastic bottle and frozen for silicate determination and 50 ml was acidified with 0.5 ml  $\text{H}_2\text{SO}_4$  and stored in a glass bottle at room temperature until analysis for nitrate and phosphate concentration using a Technicon Autoanalyser. In 2000, filtrate from the chlorophyll procedure was stored at 4  $^{\circ}\text{C}$  and analysed for nitrate and silicate concentration within 2 d using standard manual methods (Strickland and Parsons, 1968). As is common in seawater, ammonium concentrations in the fjord water were low ( $<1 \mu\text{M}$ ) and pre-mixing of water ensured these to be identical in each bag. The contribution of ammonium to the pool of dissolved available inorganic nitrogen (DAIN) in each bag was deemed to be insignificant (nitrate 8–28  $\mu\text{M}$ ) and consequently, the term N:Si used throughout this paper quotes nitrate-N but can be assumed to dominate DAIN. Ammonium concentrations were not routinely measured during the experiments as rapid cycling would prevent the build-up of measurable concentrations.

Chlorophyll-a samples were allowed to extract for 24 h and the concentration was then calculated according to the method of Parsons et al. (1984) using a Turner Designs Fluorometer. In preparation for elemental analysis of particulate C and N, using a Carlo Erba NSC analyser NA1500, filters were oven dried for 6 h at 60 $^{\circ}\text{C}$ . Duplicate discs of 10 mm diameter were then bored out of the filters and carefully folded inside tin capsules which had previously been washed in acetone to remove any trace organic material. Glucan analysis was carried out according to Myklestad et al. (1982).

Diatom cell counts were conducted on duplicate 50 ml samples fixed with either 1% Lugol's Iodine or 4%

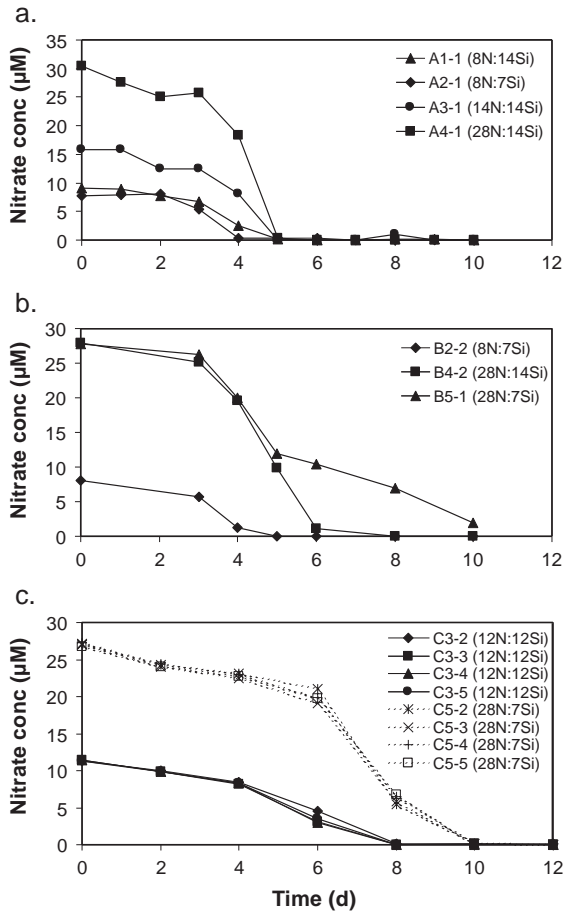


Fig. 1. The concentration (µM) of nitrate in (a) Experiment A, (b) Experiment B and (c) Experiment C.

glutaraldehyde and stored in the dark at 4 °C prior to analysis. Fixed sub-samples were settled and observed (Utermöhl, 1931) using a Leica DM IRB inverted microscope at 200 × for small diatoms and 100 × for larger cells.

In experiments A and B, diatom species were enumerated. In experiment C a more complete characterisation of the whole microbial assemblage was conducted (diatoms, bacteria, PNAN, HNAN, dinoflagellates and ciliates). This paper concentrates on the influence of new nutrients on the diatom population. The results pertaining to non-diatom species are reported in detail elsewhere (Roberts et al., 2003) but summarised here where necessary to aid discussion.

### 3. Results

#### 3.1. Nutrient concentrations

In experiments A and B, only a small decrease in the concentration of both nitrate (NO<sub>3</sub>) and silicate (Si) was noted during the first 3 d of the experiment, followed by a more marked decrease over a 3–4 d period (Figs. 1a, b and 2a, b). All mesocosms with N:Si ratios ≤ 2:1 (A1-1, A2-1, B2-2, A3-1, A4-1, B4-2) exhibited nitrate exhaustion together with the cessation of Si uptake. In contrast, bag B5-1 that had an N:Si of 4:1 exhibited Si exhaustion prior to NO<sub>3</sub>. These results were confirmed by those of experiment C (Figs.

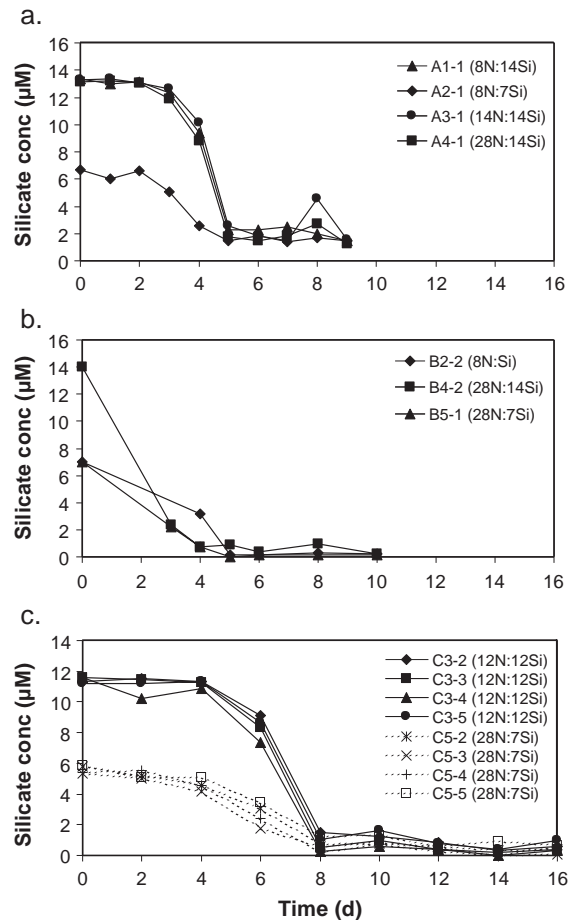


Fig. 2. The concentration (µM) of silicate in (a) Experiment A, (b) Experiment B and (c) Experiment C.

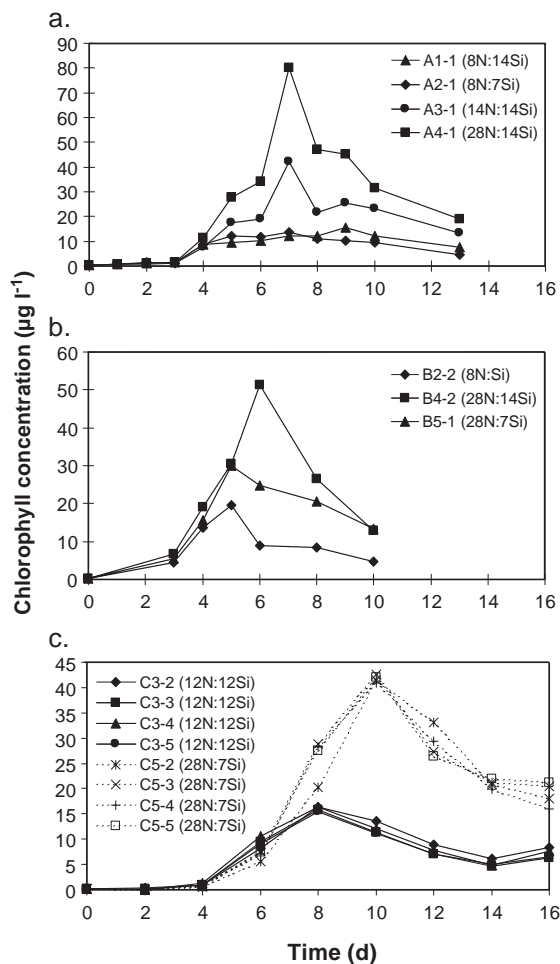


Fig. 3. Total chlorophyll-a concentration ( $\mu\text{g l}^{-1}$ ) in (a) Experiment A, (b) Experiment B and (c) Experiment C.

1c, 2c): in bags C5-2 to C5-5 silicate exhaustion was observed several days prior to nitrate depletion, potentially indicative of silicate limitation. The nutrient dynamics give an initial indication of which nutrient became limiting in each bag; further parameters providing supporting evidence will be discussed below. Hence, although nutrient (nitrate or silicate) depletion does not in itself guarantee limitation by a particular nutrient, for convenience we shall use the terms N limited for mesocosms with N:Si ratios  $\leq 2:1$  and Si limited for mesocosms with N:Si ratios of  $>2:1$  (i.e. B5-1 and C5-2 to C5-5).

### 3.2. Chlorophyll-a concentrations

Following a 3–4 d lag period, the total chlorophyll-a concentration increased in all mesocosms concurrent with nutrient uptake (Fig. 3). Peak chlorophyll-a concentrations occurred around the time of nutrient exhaustion. There was no sustained stationary phase, but rather, chlorophyll-a concentrations decreased, initially rapidly, indicating a marked collapse of the phytoplankton bloom post nutrient depletion. In all bags this rate of decrease slowed with time and in the N limited bags of experiment C an increase in chlorophyll-a concentration was evident after day 14 (bags C3-2 to C3-5) concurrent with an increase in the autotrophic flagellate population in these mesocosms (confirmed by microscopy, Roberts et al., 2003).

### 3.3. Chlorophyll-a/nitrate relationship

Visual inspection of the raw data suggested that the chlorophyll-a concentration may have been related to the quantity of nitrate supplied to the phytoplankton. For the data of experiments A and B a significant linear, and potentially predictive, relationship was apparent between the cumulative nitrate uptake (NU) and the peak chlorophyll-a yield (PC).  $PC = (2.47 \cdot NU) - 3.27$ ,  $r^2 = 0.92$ ,  $p = 0.002$ , (heavy solid line Fig. 4). However, this relationship was only

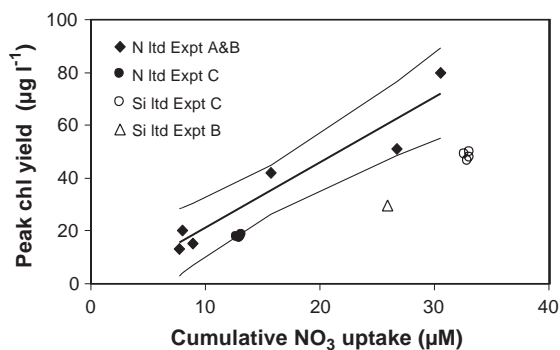


Fig. 4. Peak chlorophyll-a yield ( $\mu\text{g l}^{-1}$ ) as a function of cumulative nitrate uptake ( $\mu\text{M}$ ) during the three experiments. Heavy solid line is the regression line through the N limited data, equation defined in the text. Fine solid lines are the 95% confidence intervals of this regression line.

significant for those bags with N:Si ratios of  $\leq 2:1$  (termed N limited). In bag B5-1 with N:Si 4:1, the data fell outside the 95% confidence interval (CI) of the linear relationship. This was confirmed by experiment C, (included in Fig. 4) where data for N limited bags can be seen to fall on or within the 95% CI while Si limited bags fall well outside the 95% CI of the relationship. Hence under Si limited conditions the PC yield could not be explained on the basis of the calculated regression line derived for cumulative nitrate uptake under N limited conditions.

It was possible to fit a linear relationship through the complete data set for experiments A and B together with the mean values for low and high N:Si ratios in experiment C (mean values were used to prevent undue weighting of the regression by these highly replicated conditions). However, the gradient of this regression line was significantly different to that obtained in N limited conditions alone and data from the Si limited bags still fell outside the 95% CI of the line. Furthermore, calculation of mean PC per unit NU for the 1:1 and 4:1 N:Si regimes in experiment C produced significantly different values of 1.37 and 1.47  $\mu\text{g Chl-a } (\mu\text{molNO}_3)^{-1}$  respectively (2 sample t test,  $p=0.022$ ).

### 3.4. Phytoplankton abundance and taxonomy

In all mesocosms, diatoms numerically dominated the phytoplankton (in excess of 95% of numbers and biomass at the peak of the bloom, Roberts et al., 2003). In nutrient-replete conditions, diatom numbers increased approximately exponentially. There was little stationary phase; rather following nutrient depletion, diatom numbers decreased in all three experiments (Fig. 5).

The relationship between diatom cell yield and both cumulative nitrate and silicate uptake (SU) was assessed in the same manner as the Chl-a/ $\text{NO}_3$  relationship above. Although there was a general trend of increasing cell yield with increasing nutrient concentration for either nutrient, no significant linear relationship between cell number yield and the quantity of either  $\text{NO}_3$  or Si taken up or the N:Si ratio was found. Calculation of mean cell yield per unit NU for the 1:1 and 4:1 N:Si regimes in experiment C showed a significant difference (2 sample t test,  $p=0.000$ ) with mean values of  $4.3 \times 10^3$  and  $0.8 \times 10^3$  cells

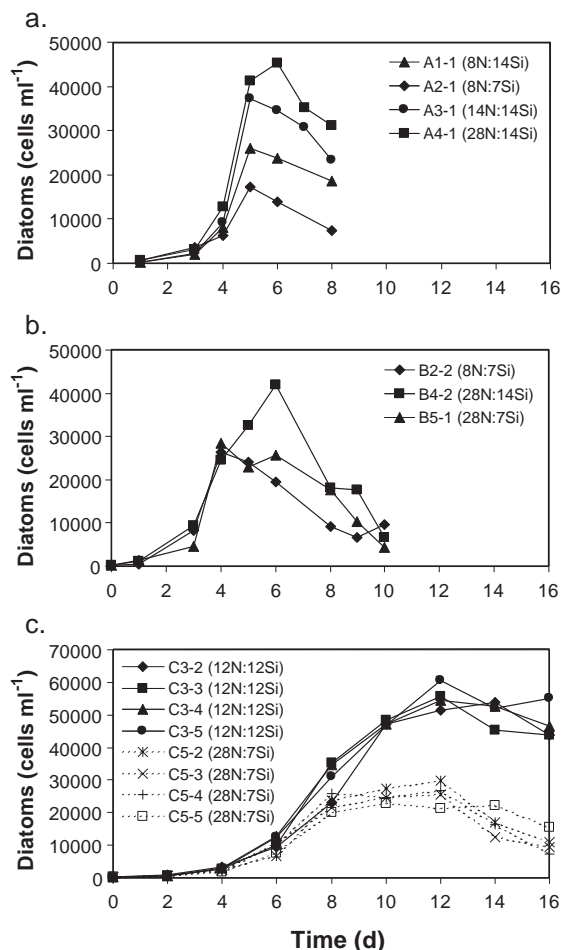


Fig. 5. Total diatom abundance (cells  $\text{ml}^{-1}$ ) in (a) Experiment A, (b) Experiment B and (c) Experiment C.

$(\mu\text{molNO}_3)^{-1}$  respectively. Similarly, mean cell yield per unit SU for the 1:1 and 4:1 N:Si regimes in experiment C showed a significant difference (2 sample t test,  $p=0.04$ ) with mean values of  $4.3 \times 10^3$  and  $3.8 \times 10^3$  cells  $(\mu\text{mol Si})^{-1}$  respectively.

The diatom assemblage was initially composed of fourteen species (Roberts et al., 2003). Of the diatoms present, only *Skeletonema costatum*, *Leptocylindricus minimus*, *Pseudo-nitzschia pseudodelicatissima* and a composite class of small *Chaetoceros* species were abundant at any time (here defined as greater than 1% of numbers or biomass). At inoculum, *Pseudo-nitzschia pseudodelicatissima* was the most abundant species. In all mesocosms the bloom rapidly became

dominated by *Skeletonema costatum*. After the establishment of the bloom, little change of species composition was noted during the experiments. Fig. 6 shows the mean percentage abundance of the four most abundant groups on day 0, (inoculum), day 8 (end of exponential phase) and day 16 (end of experiment) for both N and Si limited conditions in experiment C. Two sample t tests indicated no statistical difference with time or with limiting nutrient between the abundance of these different groups in either N or Si limiting conditions.

### 3.5. Particulate carbon and nitrogen

The particulate C and N content of the phytoplankton assemblage also reflected the time course of nutrient uptake, with a lag period followed by a phase of accumulation. An influence of inorganic N:Si ratio on the molar C:N ratio of particulate matter was noted. The C:N ratio remained relatively constant during the first four days of experiments A and B (Fig. 7a, b) and the first 6 days in experiment C (Fig. 7c) for all N:Si conditions. During this period all inorganic nutrients

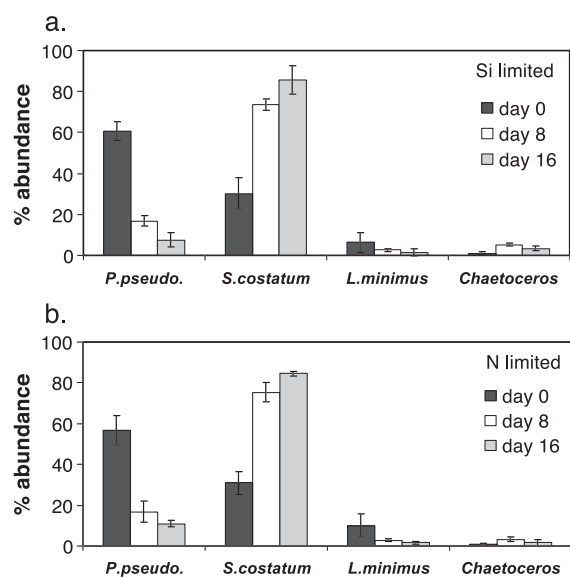


Fig. 6. The mean percentage abundance in terms of cell number in Experiment C for the four most abundant species/groups in the diatom assemblage on day 0 (dark columns), day 8 (open columns) and day 16 (light columns) in (a) Si limited and (b) N limited conditions.

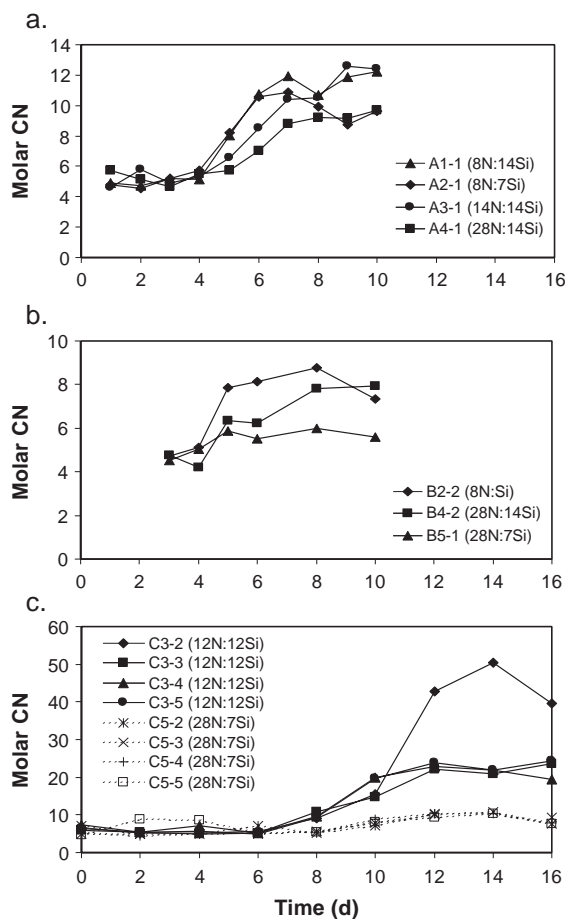


Fig. 7. The C:N molar ratio in (a) Experiment A, (b) Experiment B and (c) Experiment C.

were available and balanced growth was evident. Following exhaustion of the yield limiting nutrient the results for the high and low N:Si conditions diverged. A rapid increase in C:N ratio was observed in all N limited bags ( $N:Si \leq 2:1$ ) as a result of continued net C accumulation subsequent to  $NO_3$  exhaustion. The eventual cessation of net C fixation was reflected in a subsequent reduction in the rate of increase of the C:N ratio. In contrast, when Si limitation was evident (bags B5-1 and C5-2 to C5-5) the C:N ratio remained almost unchanged subsequent to both Si and  $NO_3$  exhaustion, indicating a balanced uptake of N and C for the duration of the experiments. Comparison of the mean maximum C:N ratio for N and Si limited conditions in experiment C, using a 2

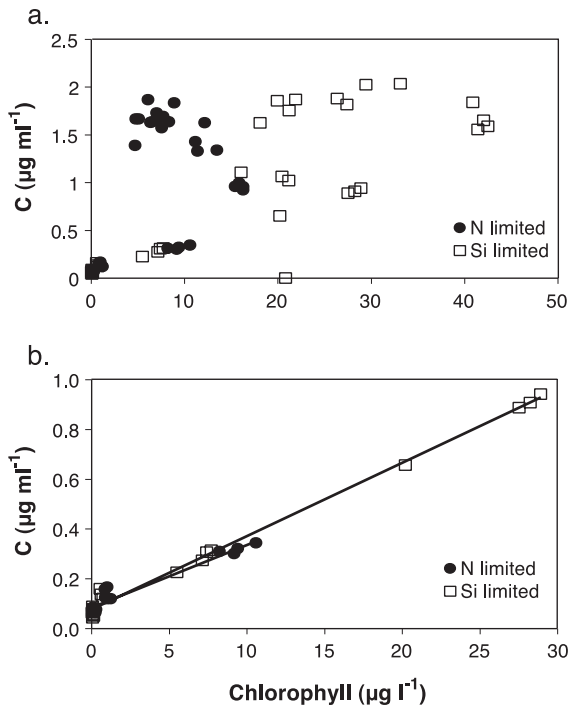


Fig. 8. The relationship between particulate C ( $\mu\text{g ml}^{-1}$ ) and chlorophyll-a ( $\mu\text{g l}^{-1}$ ) in Experiment C. Open squares: Si limited, high N:Si conditions. Closed circles: N limited, low N:Si conditions. (a) Complete data set. (b) Exponential growth phase only: High N:Si,  $C = 0.029 \cdot \text{chl-a} + 0.087$  ( $r^2 = 0.995$ ,  $p = 0.000$ ) Low N:Si,  $C = 0.024 \cdot \text{chl-a} + 0.093$  ( $r^2 = 0.94$ ,  $p = 0.000$ ).

sample *t* test, indicated a significant difference ( $p = 0.001$ ). The unrealistically high C:N of bag C3-2 that resulted from an underestimate of particulate N during the latter stages of the time course for this bag was first removed from this analysis. Should this data point be included, the non-parametric test that must then be conducted confirmed a significant difference in C:N between treatments.

### 3.6. Carbon:chlorophyll

Chlorophyll-a is commonly used as an index of C phytoplankton biomass by assuming a constant relationship between these parameters. Time course data indicated that chlorophyll-a peaked at approximately the time of exhaustion of the limiting nutrient, which was 2 and 4 d prior to the cessation of particulate C biomass accumulation for Si and N limited conditions, respectively. Indeed, for N limited conditions the

mean C biomass almost doubled between day 8 when net chlorophyll-a synthesis ceased and day 12 when net C biomass synthesis ceased. Subsequent to the peak in both parameters, the decrease in chlorophyll-a concentration was more rapid than that of C biomass.

The relationship between chlorophyll-a and C was therefore strongly non-linear in both nutrient regimes (Fig. 8a) and failed to meet the required assumptions for either correlation or regression analysis in both N and Si limited mesocosms. Further analysis of the data indicated that the relationship between C and chlorophyll-a was linear only during exponential growth (post lag phase and pre nutrient exhaustion, Fig. 8b). However, even under these restrictions the gradients

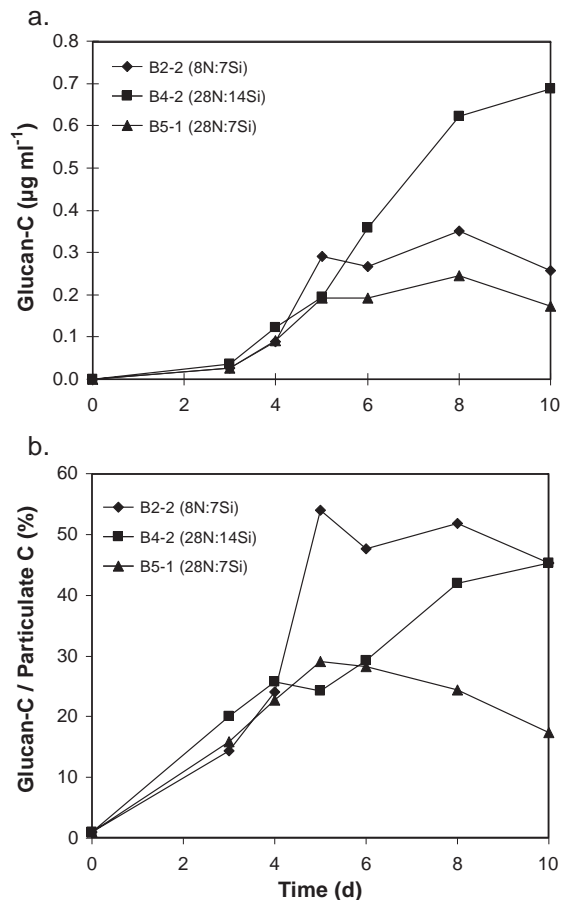


Fig. 9. (a) The concentration of glucan-carbon ( $\mu\text{g ml}^{-1}$ ) in Experiment B and (b) glucan-C expressed as a percentage of total particulate C.

of the relationships in high and low N:Si conditions were significantly different (General Linear Model,  $p=0.02$ ).

### 3.7. Glucan content

The concentration of the cellular metabolite  $\beta$ -glucan was determined during Experiment B. An initial increase in the glucan concentration with time was noted prior to nutrient exhaustion in all bags. In the N limited bags, concentrations of 0.3 and 0.7  $\mu\text{g ml}^{-1}$  were obtained (Fig. 9a). This was equivalent to  $\sim 50\%$  of the total particulate C in each bag (Fig. 9b). In contrast, the maximum amount of glucan recorded in the high N:Si bag (B5-1) was only 0.24  $\mu\text{g ml}^{-1}$ , representing less than 30% of the particulate C produced in the bag. The proportion of glucan-C was also observed to decrease following Si exhaustion on day 5 in this mesocosm.

## 4. Discussion

The coastal fjordic phytoplankton community examined was characterised by diatom dominance and exhibited a classical spring bloom increase until the exhaustion of one, or other, or both of the inorganic nutrients nitrate and silicate. The assayed parameters indicated that the N:Si ratio of this available inorganic nutrient influenced the behaviour and metabolism of the diatom community present.

### 4.1. Nutrient dynamics and limitation

The nutrient time courses demonstrated a response difference between those bags with elevated N:Si of 4:1 and all other N:Si conditions used. Those bags with N:Si of 4:1 were characterised by substantial Si exhaustion prior to that of nitrate. This alone would suggest, but not confirm, that diatom yield was limited by Si rather than nitrate in these high N:Si conditions. To confirm this hypothesis we followed Escaravage et al. (1999) in assigning nutrient limitation to particular mesocosms on the basis of the co-occurrence of a low molar ratio relative to the standard ratio of P:N:Si of 1:16:16 (Redfield, 1963; Officer and Ryther, 1980; Egge and Aksnes, 1992; Flynn and Martin-Jézéquel, 2000), and a concentration lower than the standard  $K_s$

value, assumed to be approximately 1  $\mu\text{M}$  for nitrate and Si for a *S. costatum* dominated assemblage (Conway and Harrison, 1977; Martin-Jézéquel et al., 2000). P was always in excess and applying these criteria indicated that N was limiting in all bags with initial N:Si of  $\leq 2:1$  and Si limited when N:Si was 4:1. The differences in chlorophyll-a/ $\text{NO}_3$ , C/chlorophyll-a, particulate C:N ratio and accumulation of cellular glucan (all discussed in more detail below) between the low and high N:Si regimes provided further evidence for a fundamentally different mode of nutrient limitation and resultant diatom physiology in the two sets of conditions.

As noted in the Methods section, ammonium was not routinely measured in these experiments. Ammonium concentrations in the fjord are low in summer ( $<1 \mu\text{M}$ ) and hence the nitrate concentration in our bags at inoculum and in renewal water exceeded ammonium by at least an order of magnitude. Ammonium regenerated by heterotrophs may be important in microbial assemblages, but this process was unlikely to have significantly influenced diatom dynamics in our experiments. The results of Roberts et al. (2003) show heterotrophic biomass formed  $<3\%$  of total until subsequent to the diatom peak. Hence, even if N regeneration efficiencies from heterotrophs were high, the amount of  $\text{NH}_4^+$  regenerated would be very low in comparison to nitrate available. In addition, rapid assimilation of recycled ammonium maintains low concentrations and yields no insight into the rate of heterotrophic activity.

N:Si ratios  $<1$  have been proposed to result in N limitation of diatom biomass accumulation and ratios  $>1$  to result in Si limitation (Levasseur and Theriault, 1987; Dortch and Whitley, 1992). The assumption of an approximate 1:1 N:Si ratio in diatom biomass (Officer and Ryther, 1980; Egge and Aksnes, 1992; Flynn and Martin-Jézéquel, 2000) is broadly confirmed by the results of Brzezinski (1985), who studied the N:Si ratios of 27 different diatom species. However, considerable variability exists; the atomic N:Si ratio for *S. costatum* was shown to reach 1.5 in continuous light (Brzezinski, 1985). Consistent with this, our results illustrated that Si limitation of a natural *S. costatum* dominated ecosystem did not develop until inorganic N:Si ratios exceeded 2:1. A degree of caution

should therefore be exercised in assigning a limiting nutrient unless the composition of the planktonic assemblage and the nutrient requirements of the constituent phytoplankters are known together with the nutrient conditions.

As *S. costatum* is the dominant diatom in many coastal planktonic ecosystems in differing locations e.g. the Adriatic (Miralto et al., 1999), and both Norwegian (Sakshaug and Mykkestad, 1973) and Scottish fjords (Tett, 1992), extrapolation of the current results to other *S. costatum* dominated ecosystems would imply the requirement of substantial N loading to achieve conditions suitable for the development of Si limitation of diatom biomass. However, large increases in N loading such as those observed by Aure et al. (1998) in coastal waters of the Skagerrak indicate that such substantial changes are indeed possible.

These results confirm recent suggestions that in order to develop realistic mathematical models of microbial ecosystems, such models should contain a multiple nutrient currency (for diatoms at least N and Si) to allow accurate simulation (Davidson and Gurney, 1999; Tett and Wilson, 2000). Moreover, models incorporating different phytoplankton functional groups (Taylor et al., 1993) and a 'mechanistic' approach to modelling (Flynn, 2001) may be necessary.

#### 4.2. Chlorophyll-a as a tracer of biomass

Chlorophyll-a is routinely used as an index of phytoplankton biomass as it can be assessed in the field relatively easily and in close to real time, while other methods such as microscopic estimation of volume or direct measurement of particulate C cannot. For this reason chlorophyll-a concentration is commonly used to assess water quality. By assuming a value,  $q$ , defined as the yield of chlorophyll-a per unit nutrient (usually dissolved available inorganic N), it is possible to predict the potential eutrophic influence of nutrient discharge into a water body in terms of chlorophyll increase. This principle was used by the UK's 'Comprehensive Studies Task Team' (CSTT), which defined a water quality standard of  $10 \text{ mg m}^{-3}$  chlorophyll, above which eutrophic conditions (in UK waters) are said to be present (CSTT, 1994, 1997).

The value assigned to the parameter  $q$  is therefore critical in determining the predicted chlorophyll and hence biomass. The calculation of  $q$  was originally based on a study of the yield of phytoplankton biomass from nutrient using a large data set from Scottish lochs (Gowen et al., 1992); values ranged from 0.25 to 4.4 with a median of  $1.05 \text{ mg chl (mmol N)}^{-1}$ . Subsequent laboratory experiments have studied the influence of season and the dominant form of N on the value of  $q$ , demonstrating marked variation (Edwards, 2001). Moreover, (at least) two assumptions underpin the use of  $q$  in screening models: that N is the limiting nutrient and that chlorophyll is a true index of phytoplankton biomass.

The results of the current work demonstrate that the significant linear relationship between peak chlorophyll-a and nitrate taken up broke down when Si limitation developed. As the calculated  $q$  value derived in the current study was significantly lower for the Si limited, high N:Si, conditions it would appear that in assessing the potential for eutrophication in marine waters it is important not only to monitor N inputs but also Si. Indicators of eutrophication as defined in terms of chlorophyll-a concentration will be suppressed if the diatom assemblage is Si rather than N limited.

Our results also indicated that the relationship between chlorophyll-a and C was variable and influenced by the inorganic nutrient ratio. Although differences between C: Chl-a for the two nutrient regimes were apparent, they occurred mainly after the peak of the bloom (in terms of chlorophyll). In both scenarios, but most markedly under N limitation, there was a large increase in C biomass subsequent to the cessation of net chlorophyll-a synthesis, which confirms previous suggestions that the use of chlorophyll to estimate phytoplankton C-biomass should be used with particular caution (Davidson et al., 1991; Cunningham, 1996) unless nutrient-replete conditions and exponential growth can be assured.

#### 4.3. Composition of the diatom assemblage

The lowest initial Si concentration used in our experiments was  $7 \mu\text{M}$ , representative of conditions found prior to the spring phytoplankton bloom in this region (Sakshaug and Mykkestad, 1973), and exceeding the  $2 \mu\text{M}$  threshold proposed by Egge and Aksnes

(1992) for diatom dominance. A diatom- rather than a flagellate-dominated assemblage was produced.

Smayda (1990) discussed the effect of change in N:Si ratios on planktonic assemblages, in terms of a possible shift from diatom to flagellate dominance and also with respect to changes in the species composition of the diatom assemblage itself. Such a shift has been noted previously in mesocosms of North Sea water in which *Phaeocystis* species often become dominant (Escaravage et al., 1995). Schøllhorn and Granéli (1996) studied the influence of different N:Si ratios on a phytoplankton population from Gullmar Fjord (Skagerrak) and found flagellate dominance at N:Si ratios of 4:1. However, their experiments used a summer phytoplankton community in which diatoms constituted only 11% of the total inoculum. The flagellate component of the spring assemblage used in the current study began to increase in the last 2 d of the experiments but still contributed less than 5% of the total biomass (Roberts et al., 2003). The phytoplankton in these mesocosms was therefore diatom dominated throughout the experiment.

Changes in the species composition of the diatom assemblage could have significant implications for the transfer of energy through the food chain as a result of differences in the efficiency of ingestion of species of varying morphology, differences in cellular lipid, protein and amino acid content, and differences in the elemental content of the cell as a whole. In the present study, changes in the N:Si ratio had little influence on the relative abundance of the most prevalent diatom species (*Skeletonema costatum*, *Pseudonitzschia pseudo-delicatissima*, *Leptocylindricus minimus* and small *Chaetoceros* spp.) with *S. costatum* dominating the bloom in both nutrient conditions. The switch from N to Si limitation did not appear to convey a competitive advantage on any single diatom species over the duration of the experiments or to produce a situation in which the relative availability of prey species to micro- and meso-zooplankton consumers would change. The structure of the diatom community in this typical fjordic location was therefore robust. Trondheimsfjord, in common with other fjords, experiences reduced surface salinity in late spring/summer through increased freshwater run off. Potentially, this reduced salinity may adversely

affect the vitality of some marine organisms, reducing their competitive ability which may act to buffer the system from any change in diatom community composition resulting from varying nutrient ratios.

Although no particular species benefited under different nutrient regimes, we were unable to derive linear relationships between diatom cell yield and utilisation of either N or Si or the N:Si ratio. This was a result of variation in the mean N and Si content of the cells between and within the different conditions and further highlights the difficulty of predicting the biological implications of changing inorganic nutrient concentrations.

#### 4.4. Diatom cellular composition

Although the relative species composition of the diatom assemblage remained approximately constant there was a change in cellular composition. It has long been established that nutrient stress influences the chemical composition of phytoplankton (Droop, 1968; Caperon and Meyer, 1972; Sakshaug et al., 1983). In Fig. 7, a significant impact on cell physiology in terms of cellular C and N content was recorded. In N limited mesocosms, net C accumulation continued for a number of days following N exhaustion resulting in an increase in C:N. In contrast, in Si limited conditions, both particulate C and N accumulation were found to continue following Si depletion, resulting in the C:N ratio remaining approximately constant.

Another index of metabolic state is glucan; the general term used for polymers of glucose. Myklestad (1974) and Myklestad et al. (1982) demonstrated that glucans form common storage products in diatoms. However, glucan only accumulates in quantity within diatoms when growth is suboptimal. Myklestad (1974) demonstrated this under conditions of N stress, where glucan-C constituted up to 81% of dry organic matter in a stationary phase culture of *S. costatum*. The greater concentration of glucan-C in low N:Si mesocosms in the present study is a further indication that N stress was experienced in mesocosms with N:Si ratios  $\leq 2:1$  but not at 4:1. In Si stressed cells, N uptake continues to fuel normal metabolic pathways, whereas protein synthesis is inhibited in N stressed cells where excess organic carbon is channelled, at least partly, into glucan synthesis.

Such differences in diatom C:N ratio and cellular composition under varying nutrient limitation may influence the energetic value of a cell to a predator and hence perturb energy fluxes through the marine food web. The ingestion of diatoms by meso-zooplankton grazers, such as copepods, is a major energy pathway. Recently there has been some discussion regarding the efficiency of this diatom-copepod pathway with the proposal that a diatom diet reduces the fecundity of grazing copepods (Miralto et al., 1999). Furthermore, based on data sets collected over 40 years, Turner et al. (1998) have proposed that changes in the N:Si ratio of the Mississippi delta, potentially shifting the diatom population from a state of N to Si limitation, has markedly influenced the species composition of the meso-zooplankton assemblage.

As discussed by Anderson (1994), prey C:N ratio and biochemical composition is an important indicator of food quality for mesozooplankton. Trophic transfer might be expected to be greatest when the C:N ratio of a prey item is approximately 7:1 (Anderson, 1994; Davidson and John, 2001). Our results indicate that the composition of a diatom cell in terms of C and N, and therefore its nutritional value as a food particle to a meso-zooplankton grazer, will vary according to the nutrient conditions in the environment; the C:N ratio in N limited ( $N:Si \leq 2:1$ ) cells increased compared to Si limited cells where the C:N ratio was not perturbed. Copepod recruitment predominantly occurs subsequent to the peak of the diatom spring bloom. Therefore, these results suggest that the extent of diatom-copepod trophic coupling may be directly influenced by the nature of the limiting nutrient during spring bloom decline.

## 5. Summary

Increased N loading (as nitrate) in comparison to Si resulted in a number of indicators suggesting that Si limitation of this coastal planktonic assemblage occurred at N:Si 4:1, the highest ratio studied. This switch from the N limitation observed at lower N:Si ratios did not change the species composition of the diatom assemblage but did influence diatom metabolism with implications for biomass estimation and the potential to alter pelagic food web dynamics.

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