

*Limnol. Oceanogr.*, 46(5), 2001, 1237–1242  
© 2001, by the American Society of Limnology and Oceanography, Inc.

## Variation in cellular nutrient status within a population of *Dinophysis norvegica* (Dinophyceae) growing in situ: Single-cell elemental analysis by use of a nuclear microprobe

**Abstract**—Elemental analysis (C, N, and P) of single cells of *Dinophysis norvegica* collected from the Baltic Sea was performed by use of a nuclear microprobe (NMP). For each cell, elemental maps were created and the cellular C, N, and P content was obtained. Concentrates of *D. norvegica* cells were collected simultaneously for measurement of particulate C, N, and P content by traditional methods for comparison. The traditional measurements of C, N, and P yielded 2.2–2.5 times higher values per cell than the ones obtained by the NMP method. Nevertheless, the averages for the elemental ratios were very similar for the traditional measurements and NMP. All the analyzed cells were deficient in both N and P, compared with the Redfield ratio. The data show that cellular nutrient status, especially cell quotas of N, can vary considerably within a population of one algal species growing under the same environmental conditions in situ.

Cellular composition of macro-/micronutrients is often used to infer nutrient limitation of phytoplankton growth by one or several nutrients (e.g., Goldman et al. 1979; Goldman 1980). Intracellular nutrient ratios in phytoplankton have often been shown to respond to nutrient deficiency in the growth medium and to be related to growth rate in unialgal cultures (Droop 1974). In field studies, algal nutrient ratios are commonly measured on whole or size-fractionated plankton communities—i.e., the material that is retained on a glass-fiber filter. This material includes, depending on pre-fractionation of the sample, numerous phytoplankton species, heterotrophic protists, metazooplankton, parts of the bacterial biomass, and dead particulate organic material. Very little is known about the nutrient status of individual phytoplankton species growing in situ. Data on the variation in nutrient status between individual cells in a population of one phytoplankton species (in situ or in culture) are nonexistent.

With the development of single-cell techniques, like flow cytometry and quantitative image analysis, more interest is being paid to inter- and intraspecific heterogeneity in and among microbial populations. It is therefore of great interest to find a suitable technique to measure nutrient composition of single phytoplankton cells. Some attempts have been made in recent years to analyze the chemical composition of single cells. X-ray microanalysis (XRMA) with use of scanning electron microscopy (SEM) has been applied for this purpose, mainly on bacteria cells (Norland et al. 1995; Fagerbakke et al. 1996), but also on algae and even crustacean zooplankton (Sigeo and Holland 1997; Vrede 1998). A pioneering work on the use of such a technique is the study by Biscaye and Olsen (1976). These authors used a combination of SEM and energy-dispersive X-ray fluorescence to measure the trace metal content of suspended marine particles. The use of electrons to induce X-ray emission is mainly suited for the anal-

ysis of heavy elements and for light element analysis on very small cells, such as bacteria. For light elements like carbon and nitrogen, the background of the X-ray energy spectrum is high when electrons are used, because of “bremsstrahlung”—i.e., radiation in the X-ray spectral line due to the slowing down of incident electrons in the sample (Llabador and Moretto 1998). Moreover, the measurement of C and N with use of electron-induced X-ray emission is severely impaired by X-ray absorption within the sample, which results in a limited penetration depth. Because it cannot be assumed that elements are homogeneously distributed within larger cells, it would be preferable to measure the whole volume of the cell to obtain total elemental contents rather than to extrapolate from a scan on the surface of the cell. This can be accomplished by use of a high-energy proton beam instead of electrons. This technique, analogous to XRMA with use of electrons, is generally referred to as “nuclear microanalysis.” This technique allows absolute quantification of elements in single cells and elemental mapping to visualize elemental distribution in microscopic specimens (Fig. 1).

Nuclear microanalysis includes several different techniques with regard to detection, but they have in common that the target atoms are excited by a high-energy ion beam, in this case a proton beam. The detection techniques used in this study include particle-induced X-ray emission (PIXE) for the quantification of phosphorus, proton backscattering (PBS) for the measurement of carbon and nitrogen, and scanning transmission ion microscopy (STIM) for cell thickness (areal mass density). The principles of detection are, in short,

(1) PIXE: The atoms in the sample are excited by the incident protons—i.e., when electrons from inner shells are ejected, vacancies are created. When these vacancies in the inner shells are refilled by electrons from outer shells, the excess energy is released as photons with wavelengths in the X-ray band. The energy of these photons is related to electron transitions in atoms of different elements that are allowable according to quantum theory, and the X-ray emission from different elements can thus be identified from the X-ray energy spectrum. The amount of different elements in the sample can be quantified from this spectrum, by comparison with standards containing known amounts of the elements of interest. This technique is best suited for quantifying elements heavier than aluminium.

(2) PBS: The energy of protons that are backscattered from elastic collisions with nuclei of target atoms in the sample will be related to the mass of the target nuclei. Thus, the energy spectrum of backscattered protons can be used to separate different elements, and the amount of each element can be quantified by use of standards of known elemental composition. This technique has proved to be a good choice for quantifying light elements such as carbon and nitrogen.

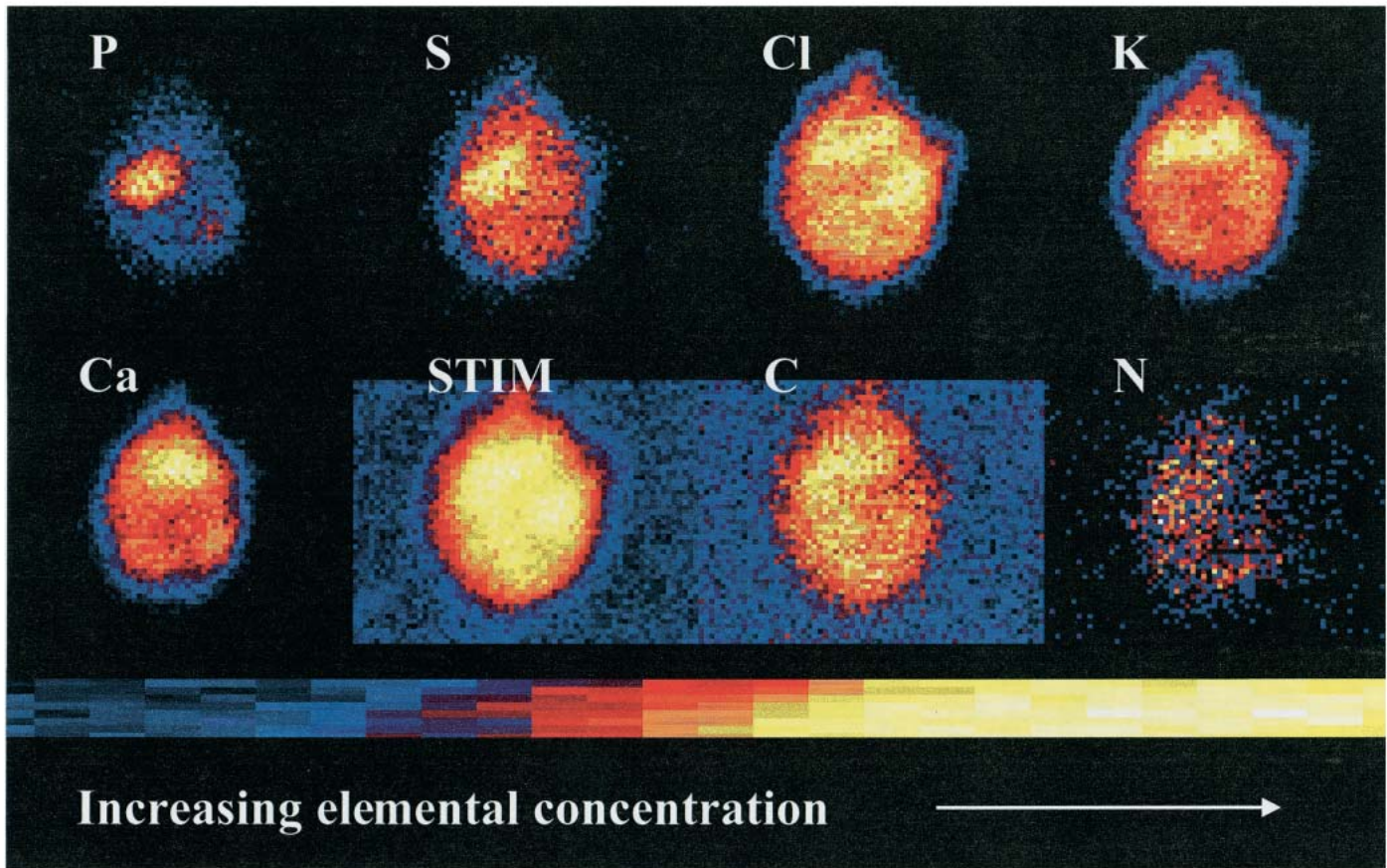


Fig. 1. Elemental maps of a *D. norvegica* cell. Maps are shown for PIXE analysis of P, S, Cl, K, and Ca. Proton backscattering was used for the C and N maps. The STIM map shows areal mass density. The resolution of the maps are 5  $\mu\text{m}$ . A resolution of 2  $\mu\text{m}$  can be obtained but with a substantial cost of scanning time for specimens of this size. The elemental maps are used for orientation only and are not corrected for background and spectral interferences between elements.

(3) STIM: The energy loss of protons passing through the sample is measured. This energy loss is proportional to the thickness of the sample or, more precisely, the areal mass density ( $\text{mg cm}^2$ ). The penetration depth of 2.55 Mev protons in the sample is 10  $\text{mg cm}^{-2}$ . STIM is used to produce an image of the sample and can also be used for X-ray absorption corrections if the sample thickness exceeds a given limit.

A short description of the principles of nuclear microanalysis can be found in Tapper and Malmqvist (1991) and a more comprehensive review in Llabador and Moretto (1998). Nuclear microanalysis has been applied with success to study elemental distributions in biomedical specimens (Pallon and Knox 1993; Pallon et al. 1996; Pinheiro et al. 1996). The method has also been tested on cultured phytoplankton cells (Pallon et al. 1999).

In this article, we present the first measurements of C, N, and P content of single phytoplankton cells growing in situ. The analyses were performed on the dinoflagellate species *Dinophysis norvegica* collected from the Baltic Sea. The primary objective is to describe the variation in elemental composition within a natural phytoplankton population and to discuss the potential of this single-cell technique for further studies relating growth conditions with elemental composition and growth rate of phytoplankton populations in situ.

*Materials and methods*—Samples were collected in the Gotland deep area in the Baltic Sea (57°18'N, 20°05'E) on 31 July and 2 August 1998 during a cruise on the R/V *Petr Kottsov*, organized by the Institute for Baltic Research in Warnemünde, Germany. Cells were collected by vertical hauls with a 25- $\mu\text{m}$  mesh plankton net from a depth of 25 m. The phytoplankton concentrates obtained this way were dominated by the filamentous cyanobacteria *Aphanizomenon* cf. “*baltica*” and *Nodularia spumigena* and the dinoflagellate *D. norvegica*. The cyanobacteria were removed by repeated filtrations through 200- and 100- $\mu\text{m}$  mesh nets. The filtrate was concentrated further onto a 20- $\mu\text{m}$  mesh net. The resulting concentrate, dominated to >95% (biovolume) by *D. norvegica* cells, was divided into aliquots for cell counts; particulate C, N, and P analysis with traditional methods; and nuclear microprobe (NMP) analyses.

The samples for NMP analysis were filtered onto 20- $\mu\text{m}$  mesh nets cut to 25-mm diameter circles and placed in a polycarbonate filtration funnel. Directly after filtration, each net was quickly rinsed in the funnel with a few ml ice-cold Milli-Q water under gentle vacuum, to remove salt. The cells on the net were transferred onto a sample target made of Kimfol® by turning the net upside down on the Kimfol and then removing the net again. The Kimfol sample targets were

immediately frozen to  $-20^{\circ}\text{C}$ . This procedure was carefully tested in advance with use of cultured dinoflagellates and was found to produce minimal cell breakage prior to freezing. The purpose of the filtration and rinsing process is to remove NaCl that would otherwise deposit as crystals on the cells during freeze-drying. Absorption in a salt layer would reduce the number of X-rays reaching the detectors. Moreover, a high Cl peak would increase the detection limit for P, because the P and Cl peaks are adjacent in the X-ray energy spectrum (Pallon et al. 1999). Test samples were also taken during the cruise, treated the same way, and checked under the microscope immediately after Milli-Q rinsing and transfer to Kimfol, to ensure that the *D. norvegica* cells did not break before freezing. The frozen cells on the Kimfol sample targets were freeze-dried within 2 weeks.

Depth profiles for cell counts (1 liter, from 1, 5, 10, 15, 20, and 25 m depth) were taken on 28, 29, and 31 July and on 3 August. Particulate C, N, and P (500 ml from 1, 10, and 15 m and 1,000 ml from 25 m) were filtered onto pre-combusted glass-fiber filters (Gelman A/E). The samples for cell counts were conserved with Lugol's Iodine and counted on an inverted microscope (Nikon Diaphot).

The *Dinophysis* cell concentrates for traditional particulate C, N, and P analysis were centrifuged at  $300 \times g$  for 5 min and were washed with filtered Baltic seawater (Gelman A/E) once, to ensure minimal contamination of bacteria and smaller phytoplankton in the samples. Finally, the water was aspirated and the cell pellets frozen immediately. The CN samples were freeze-dried 2 weeks later at the laboratory. All particulate CN samples (cell concentrates and filters) were analysed with a Fisons NA1500 CN analyzer. Particulate phosphorus was analysed according to the method described by Solórzano and Sharp (1980).

Cell volumes of *D. norvegica* were estimated by use of average linear dimensions measured on 10 cells in a Lugol-fixed sample and the formula for a rotational ellipsoid with ellipse-shaped cross-section  $[(L \times W \times T)\pi/6]$  ( $L$  = length,  $W$  = width, and  $T$  = thickness). All statistical analyses were performed with Statistica software for Windows (StatSoft, Inc. 1998).

**NMP analysis**—The samples for NMP analyses were measured by use of the Lund Nuclear Microprobe at the department for Nuclear Physics, University of Lund, Sweden. The NMP consists of a particle accelerator that produces a 2.55-MeV proton beam, a beam focusing and alignment system composed of magnetic quadrupole lenses in a triplet configuration, a sample holder where the sample can be viewed under a microscope, particle detectors, and a data acquisition system. Si(Li) detectors with a spectral resolution of 160 eV were used to collect X-rays.

Cells on the sample membrane (Kimfol) were located under the microscope and were chosen in a semirandom way (cells that appeared to be intact). A rectangular scan area was set to cover the cell and some area around it. A scan was started, and the emerging elemental maps were used to decrease the scan area to cover only the cell, if possible. Since the time necessary to scan a cell was  $\sim 0.5$ –1 h, the scan area had to be chosen with care. For element quantification, each cell was delineated manually to obtain data from

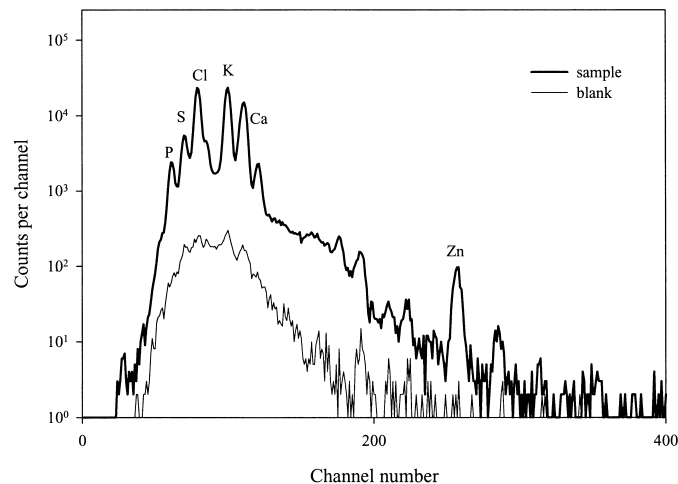


Fig. 2. Energy spectra for PIXE. The channel number on the  $x$ -axis reflects X-ray energy. The lower spectrum is from a blank support membrane (Kimfol).

the area covered by the cell only (CAMAC/Mac computer and KMAX Sparrow software). Energy spectra for X-rays (PIXE) (Fig. 2) and backscattered protons (PBS) were used for each sample, to separate and quantify the different elements by computer fitting. For each cell that was analyzed, an area of the Kimfol sample support film beside the cell was scanned, to obtain a blank spectrum. This blank spectrum was used to correct for background from the support film (including possible dried organic material on the samples). The Kimfol used in this work had a C value of  $136 \mu\text{g cm}^{-2}$  and an O value of  $34 \mu\text{g cm}^{-2}$ . The relative SDs for carbon and oxygen in the blanks measured for each sample were 6.37% and 9.48%, respectively ( $n = 36$ ). For details on the analytical setup (detection of X-rays and backscattered protons, data collection, and fitting of energy spectra), see Pallon et al. (1999). PIXE data are quantified by relating the number of detected X-rays to the number of protons that have passed the sample. In principle, this is an absolute quantitative technique, in that, once it has been calibrated, reliable measurements can be done without the use of standards. However, to guarantee analytical quality, pure elemental standards were analyzed in connection with the samples. In this study, the main PIXE standard was GaP to calibrate P. SiO, KCl, and CaF were also used to check for neighboring elements. For PBS, C and N were checked by use of a thin standard plastic foil containing known amounts of C and N (AP1, Moxtek®). The relative SDs for carbon and nitrogen measured on this standard film was 2.85% and 6.63%, respectively ( $n = 5$ ).

**Results**—No significant differences were found in the NMP measurements of C, N, or P between the analyzed *D. norvegica* cells sampled on 31 July and 2 August (15 cells analyzed from each date, Mann-Whitney  $U$ -test). Consequently, the samples from the two sampling occasions were pooled. The average C content per cell measured by combustion was  $891 \text{ pmol cell}^{-1}$ , whereas NMP yielded values ranging from 267 to  $583 \text{ pmol cell}^{-1}$  (mean  $\pm$  SD,  $400 \pm 90$ ). The combustion measurements of N gave an average of

Table 1. C, N, and P contents and ratios (mean  $\pm$  SD) of *D. norvegica* obtained in this study. Number of measurements are in parentheses.

	pmol C cell <sup>-1</sup>	pmol N cell <sup>-1</sup>	pmol P cell <sup>-1</sup>	C:N	C:P	N:P
All cells	400 $\pm$ 90.3 (n = 30)	18.5 $\pm$ 9.02 (n = 30)	1.11 $\pm$ 0.35 (n = 30)	26.3 $\pm$ 12.2 21.6*	383 $\pm$ 114 359*	17.0 $\pm$ 7.81 16.6*
Range, all cells	267–583	5.95–38.0	0.56–2.12	11.8–53.7	242–695	6.26–36.3
N:P > 16	429 $\pm$ 106 (n = 16)	24.3 $\pm$ 7.96 (n = 16)	1.11 $\pm$ 0.40 (n = 16)	19.0 $\pm$ 6.72 17.6*	414 $\pm$ 124 386*	22.7 $\pm$ 6.21 21.9*
N:P < 16	366 $\pm$ 54.3 (n = 14)	11.7 $\pm$ 4.14 (n = 14)	1.12 $\pm$ 0.30 (n = 14)	34.7 $\pm$ 11.7 31.2*	348 $\pm$ 92.6 328*	10.5 $\pm$ 2.40 10.5*
Thecae	97.2 $\pm$ 52.6 (n = 6)	2.59 $\pm$ 1.12 (n = 6)	0.08 $\pm$ 0.06 (n = 6)	38.4 $\pm$ 11.6 37.5*	1465 $\pm$ 571 1,279*	42.5 $\pm$ 24.1 34.1*
Traditional CNP on cell concentrate	891 $\pm$ 20.6 (n = 6)	44.6 $\pm$ 2.39 (n = 6)	2.78 $\pm$ 0.35 (n = 5)	20.0	320	16.0

\*Signifies ratios calculated as ratios of mean elemental contents per cell in the group.

44.6 pmol cell<sup>-1</sup>, and oxidation and hydrolysis of P gave 2.83 pmol P cell<sup>-1</sup>. The corresponding NMP analysis ranged from 5.95 to 38.0 pmol N cell<sup>-1</sup> (mean  $\pm$  SD, 18.46  $\pm$  9.02) and from 0.56 to 2.12 pmol P cell<sup>-1</sup> (mean  $\pm$  SD, 1.11  $\pm$  0.35). Average C, N, and P content of the thecae were (mean  $\pm$  SD) 97.2  $\pm$  52.6 pmol C, 2.59  $\pm$  1.12 pmol N, and 0.08  $\pm$  0.06 pmol P, respectively (Table 1). The mean biovolume of the Lugol-preserved *D. norvegica* cells was calculated to 4.8  $10^4 \mu\text{m}^3$ .

The ratios of C:N, C:P, and N:P agreed well between the NMP analyses and traditional particulate analyses on cell concentrates (Table 1). For this comparison, ratios of the mean C, N, and P contents measured by NMP were used, rather than the means of the ratios, which were substantially different in some cases (Table 1).

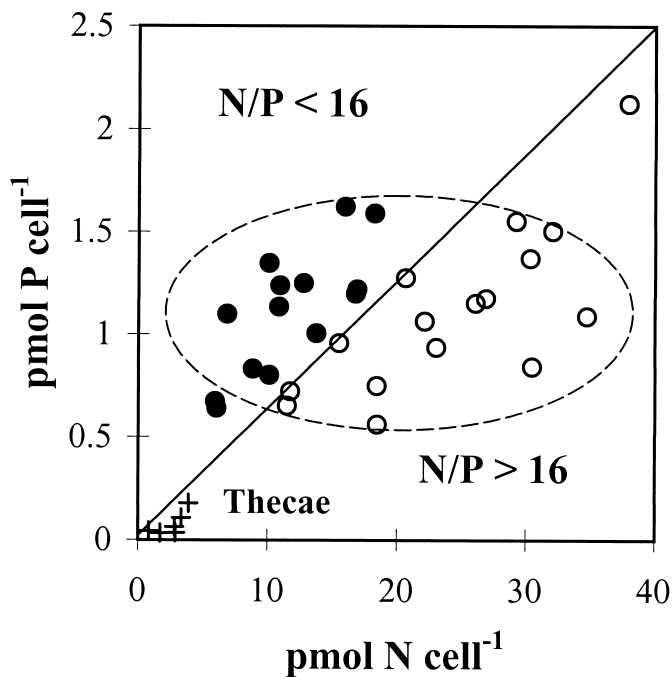


Fig. 3. P vs. N content of all *D. norvegica* cells and thecae measured. The line represents a atomic N:P ratio of 16 (Redfield). The variability in the N:P ratio is more strongly coupled to the N content of the cells than to the P content.

All of the analyzed cells were both N- and P-deficient in comparison with the Redfield atomic C:N ratio of 6.6 and C:P ratio of 106 (Redfield 1958). To investigate the relation between N and P deficiency within the studied micropopulation, the cells were divided into two groups with regard to the Redfield atomic N:P ratio: cells with N:P < 16 and cells with N:P > 16 (Fig. 3). Differences between the groups were tested by use of the Mann-Whitney *U*-test (Statistica software). The two groups were significantly different in average N content per cell ( $U = 16$ ,  $P < 0.001$ ), whereas average C and P content per cell were not significantly different. The average C:N ratios (average of ratios) also differed between the two subsets ( $U = 23$ ,  $P < 0.001$ ), whereas the C:P ratios did not (Fig. 4).

The particulate C collected on filters on 31 July was 25–26  $\mu\text{M}$  from 1 to 15 m depth and 13  $\mu\text{M}$  at 25 m. Total particulate N was 2.7–3  $\mu\text{M}$  from 1 to 15 m and 1.4  $\mu\text{M}$  at 25m, and particulate P was 0.20–0.22  $\mu\text{M}$  from 1 to 15 m

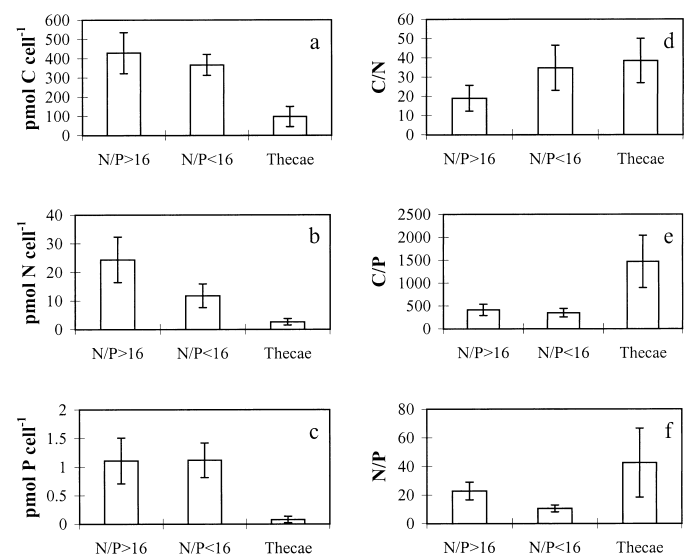


Fig. 4. Average C, N, and P contents (a–c) and ratios (d–f) in cells with N:P > 16 and N:P < 16, respectively. The two groups differed significantly in N content but not in C and P content. The C:N ratios also differed significantly between the groups, whereas the C:P ratios did not (Mann-Whitney *U*-test).

and  $0.13 \mu\text{M}$  at 25 m. The C:N ratio of the total particulate fraction was  $\sim 10$  throughout the water column, and the N:P ratio was between 10 and 14.

*D. norvegica* cell concentrations ranged from  $1,000 \text{ L}^{-1}$  in the surface water to  $4,000 \text{ L}^{-1}$  at 20–25 m depth. The highest cell concentrations are usually found at the thermocline at  $\sim 20$  m depth during summer, and *D. norvegica* are normally almost absent from the surface water. The quite high concentrations at the surface during this study were due to strong wind-induced mixing of the water column the days before sampling.

Nitrate concentrations ranged between  $0.12$  and  $0.19 \mu\text{M}$  from the surface to 40 m depth and increased to  $1.2 \mu\text{M}$  at 50 m depth. Ammonium varied between  $0.11$  and  $0.35 \mu\text{M}$ , with the highest concentration measured at 15 m. Phosphate concentrations were below  $0.02 \mu\text{M}$  down to 20 m and then gradually increased to  $0.48 \mu\text{M}$  at 50 m. There were no major differences in nutrient concentrations between the two sampling dates. The dissolved inorganic N:dissolved inorganic P ratio was  $>20$  down to 20 m depth and was  $<4$  from 25 to 50 m (not shown). The surface salinity was 7‰, and surface temperature was  $15^\circ\text{C}$ .

**Discussion**—The average C:N and C:P ratios of the *D. norvegica* population, as measured both by NMP and traditional methods, would suggest that the population was severely deficient in both N and P. The lowest cellular C:N ratio in this data set was 11.8, and the cell with the highest N content ( $38.0 \text{ pmol}$ ) had a C:N ratio of 14.6. Dinoflagellate C:N ratios in exponentially growing cultures under balanced nutrient conditions have often been shown to exceed the Redfield ratio of 6.6. Parsons et al. (1961) obtained C:N ratios of 9.0 for *Amphidinium carteri* and 8.9 for *Exuviella* sp., whereas Sakshaug et al. (1984) found a C:N ratio of 11.4 in exponentially growing cultures of *Ceratium tripos* under balanced nutrient conditions. Sakshaug et al. (1984) suggested that the high C:N ratio of *Ceratium* was due to the thick cellulose theca. This does not seem to be an explanation regarding the *D. norvegica* in our data set. If the average C and N contents of the measured thecae are subtracted from the average C and N contents of the intact cells, we still obtain a “cytoplasmic” C:N ratio of 19.0 for the whole data set or 15.2 for the N:P  $> 16$  subset. The cell with the lowest C:N ratio (11.78) would have a cytoplasmic C:N ratio of 9.03, and the one with the highest N content would have a C:N ratio of 12.9 in the cytoplasm. From this, we conclude that all the analyzed cells were N-deficient.

The N:P ratios had a population average of 16–17 measured with the two methods applied. However, it ranged between 7.5 and 36 when measured with NMP on single cells, which reveals a large intraspecific variation in nutritional status within the population. The variation in N:P ratios were mainly dependent on cellular N content. N content per cell varied 6.4 times within the population, whereas P  $\text{cell}^{-1}$  varied 3.4 times and C  $\text{cell}^{-1}$  2.2 times. When the data are divided into subsets with N:P ratios above and below 16, it is also clear that the subsets differ in N content and C:N ratios but not in P content or C:P ratios. This makes conclusions regarding P deficiency uncertain, but it seems likely that the “moderately” N-deficient cells were more P-deficient

than N-deficient (N:P  $> 16$ ), whereas the most N-deficient cells had a N:P ratio below 16 and should thus be characterised as dominantly N-deficient.

The observation that the cell quota of N varies so much within the population, clearly more so than C or P, would imply that the N available to *D. norvegica* is heterogeneously distributed in the microscale. Some *Dinophysis* species are heterotrophic, and photosynthetic *Dinophysis* species have been shown to also be able to ingest cells (Jacobson and Andersen 1994). If *D. norvegica* ingests cells primarily to sequester N, this could explain the large variation in N content between individual cells, since cellular N is more heterogeneously distributed than dissolved N. It cannot be excluded that the differences in cellular N content reflects the depth from which the cells were sampled, since the samples contain cells from 25 m depth to the surface. This will be subject to further investigation.

There is no specific figure of reproducibility for biological materials analyzed by NMP; the only way to establish this is by repeated tests on materials that are known to have equal compositions. PIXE, when analyzing thin samples, can be shown to have an in-precision better than 10%, typically 5%. For PBS, a measurement of the reproducibility could be found from the measured contents of carbon in the plastic backing foil (Kimfol), since for each algal cell a corresponding blank value was measured. The relative SD for carbon in those blanks was 6.37%, which includes variation in dried dissolved material that might be present on the samples. SDs for C and N in the Moxtek film used as a standard were 2.85% and 6.63%, respectively ( $n = 5$ ). The variation in C and N content between cells is thus an order of magnitude larger than these estimates of analytical precision.

The reason for the traditional C, N, and P measurements being more than twice as high calculated per cell as the NMP analyses is not entirely clear. The cell concentrates that were used for the bulk measurements appeared to be  $>95\%$  *D. norvegica* on microscopic examination and biovolume estimation (minor amounts of *D. rotundata* and *D. norvegica* thecae and cyanobacteria fragments were present but were  $<5\%$  by biovolume estimation). One possible source of error lies in the “mapping” of the cells. The size of the proton beam used in this setup was  $5 \mu\text{m}$ , which means that the sample is scanned by squares of  $5 \mu\text{m}$ . These squares cannot be divided, but the whole square is measured for the elemental concentrations. Squares that cover the edge of the cell could also cover some of the surrounding support film. The area measured is still  $25 \mu\text{m}^2$ , but some of the area is outside the cell, yielding a lower concentration to area ratio. This could be a source of underestimation of elemental contents when analyzing smaller cells but should not be of major significance in this case, since the *D. norvegica* cells had an area of  $1,200$ – $2,200 \mu\text{m}^2$  in freeze-dried form.

A critical step in sample preparation is the drying of the cells. Both NMP and combustion analysis of C and N require dry samples. Even if no cell breakage could be detected when the NMP samples were prepared, it cannot be excluded that leaking of cell material took place. This could also be a problem when preparing samples for traditional CN analysis but probably not to the same extent, since elemental contents are averaged for hundreds of thousand cells, where-

as NMP analysis usually includes a few tens of cells. There is no reason to believe that leaking should be element specific, so elemental ratios should not be affected by leakage of cell material. For each cell measured by NMP, an area beside the cell is measured as a blank. If dissolved cell material has dried on the support film, this could affect the blank values that are subtracted from the cell values and thus lead to underestimation of cellular element levels. This would, however, be discovered when blank values are compared with a clean Kimfol film.

The use of the calculated mean biovolume of  $4.8 \cdot 10^4 \mu\text{m}^3$  and a carbon-to-volume conversion factor of  $0.16 \text{ pg C } \mu\text{m}^{-3}$  (Verity et al. 1992) gives a C content of  $639 \text{ pmol cell}^{-1}$  in *D. norvegica* in our samples. We, however, did not include cells larger than  $10^3 \mu\text{m}^3$  in our study but concluded that both C and N density decreased with increasing cell size. Application of a conversion factor of  $0.13 \text{ pg C cell}^{-1}$  (Smetacek 1975) would yield a C content of  $520 \text{ pmol cell}^{-1}$ . Both of these conversion factors yield C contents closer to the NMP measurements in this study than to the bulk analyses on the cell concentrates, but the values are, of course, subject to unknown errors in both the biovolume estimation and the conversion factors.

The NMP is a promising tool for obtaining species-specific nutrient status of phytoplankton. More work is needed to calibrate and verify absolute elemental contents of single cells, but elemental ratios seem to agree well when the different methods of measurement are compared.

Lars-Åke Gisselson<sup>1</sup> and Edna Granéli

Department of Marine Sciences  
University of Kalmar  
SE-391 82, Kalmar, Sweden

Jan Pallon

Department of Nuclear Physics  
University of Lund  
Box 118  
SE-22100 Lund, Sweden

## References

- BISCAYE, P. E., AND C. R. OLSEN. 1976. Suspended particle concentrations and compositions in the New York Bight. *Limnol. Oceanogr. Spec. Symp.* **2**: 124–137.
- DROOP, M. R. 1974. The nutrient status of algal cells in continuous culture. *J. Mar. Biol. Assoc. U. K.* **54**: 825–855.
- FAGERBAKKE, K. M., M. HELDAL, AND S. NORLAND. 1996. Content of carbon, nitrogen, oxygen, sulfur and phosphorus in native aquatic and cultured bacteria. *Aquat. Microb. Ecol.* **10**: 15–27.
- GOLDMAN, J. C. 1980. Physiological processes, nutrient availability, and the concept of relative growth rate in marine phytoplankton ecology, p. 179–192. *In* P. G. Falkowski [ed.], *Primary productivity in the sea*. Plenum.
- , J. J. MCCARTHY, AND D. G. PEAVEY. 1979. Growth rate influence on the chemical composition of phytoplankton in oceanic waters. *Nature* **279**: 210–215.
- JACOBSON, D. M., AND R. A. ANDERSEN. 1994. The discovery of mixotrophy in photosynthetic species of *Dinophysis* (Dinophyceae): Light and electron microscopical observations of food vacuoles in *Dinophysis acuminata*, *D. norvegica* and two heterotrophic dinophysoid dinoflagellates. *Phycologia* **33**: 97–110.
- LLABADOR, Y., AND P. MORETTO. 1998. Nuclear microprobes in the life sciences. World Scientific.
- NORLAND, S., K. M. FAGERBAKKE, AND M. HELDAL. 1995. Light element analysis of individual bacteria using X-ray microanalysis. *Appl. Environ. Microbiol.* **61**: 1357–1362.
- PALLON, J., M. ELFMAN, P. KRISTIANSSON, K. MALMQVIST, E. GRANÉLI, A. SELLBORN, AND C. KARLSSON. 1999. Elemental analysis of single phytoplankton cells using the Lund nuclear microprobe. *Nucl. Instrum. Methods Phys. Res. B* **158**: 312–316.
- , AND J. KNOX. 1993. Quantitative elemental mapping of biomedical specimens using the nuclear microprobe. *Scanning Microsc.* **7**: 1207–1214.
- , K. G. MALMQVIST, Y. WERNER-LINDE, B. FORSLIND. 1996. PIXE analysis of pathological skin with special reference to psoriasis and atopic dry skin. *Cell. Mol. Biol.* **42**: 111–118.
- PARSONS, T. R., K. STEPHENS, AND J. D. H. STRICKLAND. 1961. On the chemical composition of eleven species of marine phytoplankters. *J. Fish Res. Board Can.* **18**: 1001–1016.
- PINHEIRO, T., J. PALLON, R. FERNANDES, M. J. HALPERN, P. HOMMAN, AND K. MALMQVIST. 1996. Nuclear microprobe applied to the study of coronary artery walls—a distinct look at atherosclerosis. *Cell. Mol. Biol.* **42**: 89–102.
- REDFIELD, A. C. 1958. The biological control of chemical factors in the environment. *Am. Sci.* **46**: 205–222.
- SAKSHAUG, E., E. GRANÉLI, M. ELBRÄCHTER, AND H. KAYSER. 1984. Chemical composition and alkaline phosphatase activity of nutrient-saturated and P-deficient cells of four marine dinoflagellates. *J. Exp. Mar. Biol. Ecol.* **77**: 241–254.
- SIGEE, D. C., AND R. HOLLAND. 1997. Elemental composition, correlations and ratios within a population of *Staurastrum planctonicum* (zygnematales): An X-ray microanalytical study. *J. Phycol.* **33**: 182–190.
- SMETACEK, V. 1975. Die sukzession des phytoplankton in der westlichen Kieler Bucht. Ph.D. thesis, Institut für Meereskunde, Kiel.
- SOLÓRZANO, L., AND J. H. SHARP. 1980. Determination of total dissolved phosphorus and particulate phosphorus in natural waters. *Limnol. Oceanogr.* **25**: 758–760.
- TAPPER, U. A. S., AND K. G. MALMQVIST. 1991. Analysis, imaging and modification of microscopic specimens with accelerator beams. *Anal. Chem.* **63**: 715–725.
- VERITY, P. G., C. Y. ROBERTSON, C. R. TRONZO, M. G. ANDREWS, J. R. NELSON, AND M. E. SIERACKI. 1992. Relationships between cell volume and the carbon and nitrogen content of marine photosynthetic nanoplankton. *Limnol. Oceanogr.* **37**: 1434–1446.
- VREDE, T. 1998. Elemental composition (C:N:P) and growth rates of bacteria and *Rhodomonas* grazed by *Daphnia*. *J. Plankton Res.* **20**: 455–470.

<sup>1</sup> Corresponding author (lars-ake.gisselson@hik.se)

## Acknowledgments

Financial support for this study was provided by the Swedish Natural Science Research Council. We acknowledge the support from the European Community's Marine Science and Technology program under contract MAS3-CT97-0103.

Received: 5 July 2000  
Accepted: 20 February 2001  
Amended: 12 March 2001