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# Nitrogenous organic substances as potential nitrogen sources, for summer phytoplankton in the Gulf of Riga, eastern Baltic Sea

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**Abstract:** To investigate whether uptake of dissolved organic compounds might favour the growth potential of toxic cyanobacteria and dinoflagellates, surface water was collected in late July 1997 in the Gulf of Riga, and enriched with different nitrogen sources. (1) Organic substances were added (25 $\mu$M N) under P-, Si- and Fe-replete conditions. Urea sustained a biomass increase (protein content) 20% greater than that with $NH_4^+$. Glycine, hypoxanthine and putrescine gave only 50% of the biomass obtained with $NH_4^+$. Glutamic acid and spermine supported growth in only one case each, and guanine in none. (2) The >1000 dalton fraction of dissolved organic matter (DOM) taken from the Daugava River was provided at concentrations of 5, 15 and 49 $\mu$M dissolved organic nitrogen (DON); P, Si and Fe were not added, except in the control. DON sustained algal growth as much as ammonium and $NO_3^-$, but data were somewhat inconsistent. (3) The cyanobacterium *Microcystis aeruginosa* was cultured in filtered (0.45 $\mu$m) water supplemented with organic substances (25 $\mu$M N) and DOM (15 $\mu$M DON), under P-, Si-, and Fe-replete conditions. Growth with urea, glycine, hypoxanthine, putrescine or spermine ranged between 145 and 175% of that obtained with $NO_3^-$. That 15 $\mu$M DON sustained a biomass increase representing only 70% that gained with $NO_3^-$ might be explained by the different relative concentrations of DON and $NO_3^-$. Altogether, it is inferred that urea is an important potential nitrogen source for summer phytoplankton as a whole, while dissolved free amino acids (DFAA) and DON of terrestrial origin may partly sustain growth in some individual species.

**Key words:** phytoplankton, terrigenous DON, nitrogenous nutrients, Baltic Sea

## Introduction

In recent decades, toxic algal events have emerged as a major environmental problem in many coastal waters (Hallegraeff 1995). The suggestion has been made that organic compounds from land drainage, such as humic acids, have favoured dinoflagellate growth versus that of diatoms (Carlsson & Granéli 1993; Carlsson et al. 1995; Lara et al. 1993; Moran & Hodson 1994). This suggestion has been supported by the fact that several eukaryotic algae grow in culture with organic compounds as the sole source of nitrogen (Flynn & Butler 1986; Berman et al. 1991). Dissolved free amino-acids (DFAA) have also been reported to sustain a significant part of algal growth in the Chesapeake Bay (Glibert et al. 1991). Moreover, Palenik et al. (1988–1989) showed that some phytoplankters can use various forms of dissolved organic nitrogen (DON) without initial transport into the cell, by using cell-surface enzymes to degrade organic forms of nitrogen to $NH_4^+$.

In contrast, less attention has been paid to the range of nitrogenous organic substances utilizable by cyanobacteria. All cultured strains tested by Kratz & Myers (1955), Van Baalen (1962), Wyatt et al. (1971) and Kapp et al. (1975) showed good growth with urea and purines as nitrogen sources. Several amino acids sustained growth of only some strains, and other amino acids were not utilized at all. Berman (1997) showed that components of the DON pool were the major source of nitrogen for a natural freshwater assemblage dominated by the cyanobacterium *Aphanizomenon ovalisporum*, a monoculture of which grew well in mineral media supplemented with urea, hypoxanthine, lysine, guanine and glucosamine.

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**Table 1.** List and composition of spike enrichments used to test the effect of dissolved organic matter (fraction >1000 Da) taken from the Daugava River on the growth of natural assemblages collected on 25 July 1997. Concentrations are that of experimental cultures.

| Reference number | Nitrogen source and concentration | Other nutrients added |
|---|---|---|
| 01 | None | |
| 02 | | EDTA (N: 25 $\mu$M) |
| 03 | | P (1.7 $\mu$M)+Si (30 $\mu$M)+FeEDTA |
| 04 | $NO_3^-$ (25 $\mu$M) | P+Si+FeEDTA (100 nM, 0.6 $\mu$M) |
| 05 | $NO_3^-$ (25 $\mu$M) | |
| 06 | $NH_4^+$ (25 $\mu$M) | |
| 07 | Urea (N: 25 $\mu$M) | |
| 08 | Putrescine (N: 25 $\mu$M) | |
| 09 | DOM (DON: 5 $\mu$M; DIN: 0.5 $\mu$M) | |
| 10 | DOM (DON: 15 $\mu$M; DIN:1.3 $\mu$M) | |
| 11 | DOM (DON: 49 $\mu$M; DIN: 4.5 $\mu$M) | |
| 12 | DOM (DON: 15 $\mu$M; DIN:1.3 $\mu$M) | P+Si+Fe |
| 13 | DOM (DON: 15 $\mu$M; DIN:1.3 $\mu$M) | P+Si+FeEDTA |
| 14 | DOM (DON: 15 $\mu$M; DIN:1.3 $\mu$M) | Fe (100 nM) |

Cyanobacteria are the taxon most frequently cited as producing harmful blooms in the Baltic Sea in summer (Edler et al. 1985; Leppänen et al. 1995). Toxin-producing dinoflagellates that develop in waters poor in inorganic nutrients have also been recently reported in the same area (Willén et al. 1990; Carpenter et al. 1995; Balode & Purina 1996).

On this basis, we have endeavoured to investigate whether uptake of dissolved organic compounds might favour the growth potential of toxic cyanobacteria and dinoflagellates. Here we report the first published results concerning the role of dissolved organic substances and dissolved organic matter (DOM) of terrestrial origin as a potential source of nitrogen, in summer, for natural assemblages and one cyanobacterial cultured strain.

## Materials and Methods

Surface water samples were collected on 25 and 29 July 1997, at six stations along a transect from the mouth of the river Daugava to the central part of the gulf (see Maestrini et al. 1999). Seawater was filtered through 150-$\mu$m mesh and brought to the laboratory in a few hours.

DOM was extracted from water of the Daugava River, in the estuary area, by using a tangential flow ultrafilter (Guo et al. 1995; Benner et al. 1997). First, water was filtered through 1.2-$\mu$m and 0.2-$\mu$m Opticap filter units (Millipore); then, DOM was concentrated with a tangential device, the Prep/scale™ TFF 6 ft$^2$ cartridge (Millipore). Two fractions ($M=10^3-10^6$ daltons [Da] [abridged >1000], and $M<10^3$ Da [<1000]) were extracted. Total nitrogen was obtained after U.V. oxidation (Collos & Mornet 1993), and then treated as for soluble reactive nitrogen; DON was calculated as the difference between total nitrogen and dissolved inorganic nitrogen (DIN) concentrations.

Analysis of total DON contained in extracted DOM could not be carried out immediately; consequently, samples for experiments (see below) were spiked with tentatively calculated amounts of nutrients. Calculations turned out to be fairly accurate for the fraction >1000 Da, but they were too low by one order of magnitude for the fraction <1000 Da. Measured concentrations of the two fractions were: (1) fraction <1000 Da, $\Sigma N=90\,\mu$M consisted of 30 $\mu$M of DIN and 60 $\mu$M of DON including 7 $\mu$M urea; (2) fraction >1000 Da, $\Sigma N=803\,\mu$M consisted of 67 $\mu$M of DIN and 736 $\mu$M of DON including 56 $\mu$M urea. DIN and urea were measured using manual protocols: nitrate, reduction to nitrite according to protocol of Strickland & Parsons (1972); nitrite method of Bendschneider & Robinson (1952); ammonium, method of Koroleff (1976a); urea, method of Koroleff (1976b). Since the initial DON concentration for the cultures treated with the fraction <1000 Da was in fact 1.5 $\mu$M instead of 15–25 $\mu$M as in the other treatments, the data obtained have been discarded.

Three sets of experiments were done:

(1) Enrichments with nitrogenous organic compounds under P-, Si- and Fe-replete conditions. Surface water was enriched with $PO_4^{3-}$, $SiO_3^{2-}$ and Fe-EDTA at balanced concentrations (16N:1P:1.1Si, atom/atom, after Redfield 1934) with respect to that of nitrogen, and 150 ml was added to 300-ml polycarbonate Erlenmeyer flasks. Ammonium, glutamic acid, glycine, guanine, hypoxanthine, putrescine, spermine and urea were then added singly (25 $\mu$M). Treatments were done in duplicate. Culture conditions were as for algal growth potential (AGP) bioassays carried out in parallel (Maestrini et al. 1999). In vivo fluorescence was monitored daily, with a 10 AU Turner Fluorometer (Brand et al. 1981), until maximum growth had been obtained in each case; for each culture, the time-course data were corrected by subtracting the respective initial fluorescence value.

(2) Enrichments with DOM taken from the Daugava River. Surface water from the central part of the gulf (Stn 121) was added (150 ml) to 300-ml polycarbonate Erlenmeyer flasks, and then differentially enriched (Table 1).

Treatments were done in duplicate. Culture conditions were as for the AGP bioassays. In vivo fluorescence was measured daily. At the termination of growth, cell concentration was measured, and the maximum biomass was estimated by analysis of the protein content (Petty et al. 1982).

(3) Growth of the potentially toxic cyanobacteria *Microcystis aeruginosa* in the presence of DOM extracted from the Daugava River. Surface water collected in the central part of the gulf (Stn 119) was filtered through a 0.45 $\mu$m membrane filter, enriched with $PO_4^{3-}$ (1.7 $\mu$M) and Fe-EDTA (100 nM, 0.6 $\mu$M), and added (25 ml) to 30-ml polycarbonate tubes. Then the following nitrogen sources (25 $\mu$M) were added individually to 1-ml volumes of: $NO_3^-$, $NH_4^+$, glycine, hypoxanthine, putrescine, spermine, urea; 3-ml DOM>1000 Da was added giving an initial concentration of 15-$\mu$M DON. All treatments were done in triplicate. The initial concentration of *M. aeruginosa* was $60\times 10^6$ cells $l^{-1}$. Culture conditions were as for the AGP bioassays. In vivo fluorescence was monotored daily. At the termination of growth, cell concentration was measured, and the protein concentration was determined.

As for any experiment with natural assemblages or non axenic cultured strains, the final protein contents may include those of heterotrophic bacteria. Nevertheless, indications from protein content and in vivo fluorescence were similar. We thus assumed bacterial uptake not to have been important. Also, since seawater was prefiltered at 150 $\mu$m and in vivo fluorescence and protein content showed a continous increase over the time course of the experiment, we assume that grazing was not significant.

## Results

### Environmental conditions

Structure of the water column and nutrient concentrations along the gradient from the river mouth to the central part of the gulf have been already reported (Maestrini et al. 1999).

### Algal biomass and dominant species in initial assemblages

In surface waters most influenced by the river discharge, there was an increase in algal biomass from the river mouth towards the open gulf; the concentration of Chlorphyll *a* (Chl *a*) increased from 10.8 $\mu$g $l^{-1}$ at Stn D-10, to 19.5 $\mu$g $l^{-1}$ at Stn D-20, and to 32.5 $\mu$g $l^{-1}$ at Stn D-30. Notwithstanding, in all waters within the thermocline, Chl-*a* concentrations were similar and much lower than at the surface: respective recorded values were 3.6, 4.5 and 5.9 $\mu$g $l^{-1}$. Altogether, surface communities were dominated by cryptomonads (*Hemiselmis virescens*, *Teleaulax acuta*, *T. amphioxeia*, *Plagioselmis prolonga*), and prasinophytes (*Pyramimonas* spp.); diatoms (*Skeletonema costatum*, *Thalassiosira baltica*) and green algae (*Oocystis borgei*, *Moraphidium contortum*) were also present. The cyanobacteria *Aphanizomenon flos-aquae*, *Merismopedia elegans* and *Snowella lacustris*, and the dinoflagellate *Dinophysis acuminata* were recorded in nearshore waters (Stns D-30 and D-20) not immediately influenced by river discharge. In waters within the thermocline, the relative importance of cryptomonads and prasinophytes greatly decreased, whilst that of *D. acuminata* and *Thalassiosira baltica* increased.

In offshore surface waters, the algal biomass was much lower than near the river outlet and decreased towards the open sea; Chl-*a* concentration was 5.2 $\mu$g $l^{-1}$ at Stn 119 and 4.1 $\mu$g $l^{-1}$ at Stn 121. As for nearshore waters, within the thermocline were low in biomass compared to surface waters; respective Chl-*a* concentrations were 1.4 and 0.9 $\mu$g $l^{-1}$. Both in surface waters and those within the thermocline, species diversity was lower than in nearshore waters. Cryptomonads and prasinophytes were still present, but other species represented a greater fraction of the total assemblage. *A. flos-aquae*, *D. acuminata* and *Thalassiosira baltica* at Stn 119; the diatoms *Thalassiosira baltica* and *Scostatum*, and the green algae *O. borgei* and *O. submarina* at Stn 121 were dominant.

Community structure and changes during growth in the presence of various organic compounds as the sole nitrogen source will be reported in detail in a later manuscript.

### Growth of natural assemblages in the presence of nitrogenous organic compounds

The growth curve of phytoplankton is outlined in Fig. 1. Biomass peaked by Day 3 and Day 4 in central waters (Stn 121), while maximum biomass occurred as soon as Day 2 in nearshore waters (Stn D-10). Growth in central water increased markedly when most of the organic subtances were added; final biomasses usually represented from 58 to 118% those obtained with ammonium, while the value for the nitrogen-unenriched control was 17%. Guanine, however, sustained negligible growth (Stn 121, Fig. 2). In contrast, growth in water from near the river mouth increased only slightly when nitrogenous organic compounds were added; the final biomass in the nitrogen-unenriched control represented 70% of that gained with the addition of ammonium, and respective values for the organic substances ranged from 82 to 119% (Stn D-10, Fig. 2). In water of intermediate nature (Stn D-30), all control replicates and those incubations enriched with guanine and spermine showed low to negligible growth. Altogether, maximum biomass in the most effective treatment was twice as much in water from the river plume (Stn D-10) as it was in central water (Stn 121).

Urea appeared to be the best organic nitrogen source. For all the waters studied, growth in the presence of additional urea-N was roughly 20% greater than that in the presence of additional $NH_4^+$-N (Fig. 2). However, in the water from the river plume (Stn D-10), the presence of an important reservoir of natural DIN (9.2 $\mu$M) somewhat biased the comparison. Glycine, hypoxanthine and putrescine were the other three nitrogen sources used by all three phytoplankton

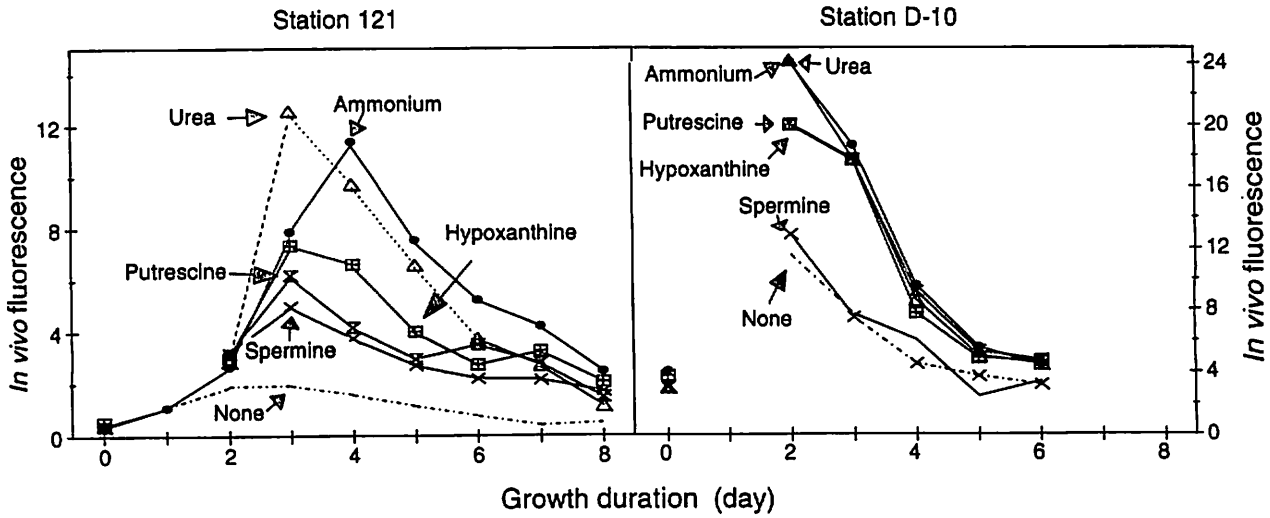


**Fig. 1.** In vivo fluorescence (arbitrary units) versus time for natural phytoplanktonic assemblages collected in offshore (Stn 121) and nearshore (Stn D-10) surface waters, and cultured in the presence of added inorganic and organic nitrogenous substances, under P-, Si-, Fe- and vitamin-replete conditions.

assemblages, with growth reaching at least 50% of that gained with ammonium. Growth in the presence of other nitrogenous substances varied; glutamic acid and spermine sustained high growth in water from near the river (Stn D-10), but low to negligible in water from Stn D-30. Guanine was the only compound that did not sustain growth in any of the water samples tested.

## Growth of natural assemblages in the presence of DOM taken from the Daugava River

The addition of $NO_3^-$+P+Si+Fe (All) to water led to a rapid increase in algal biomass; on Day 4, the in vivo fluoresoecnce representing a 60-fold increase in the initial biomass (Fig. 3), for a growth rate of 1.5 division $d^{-1}$. The absence of nitrate from this mixture (All -N) prevented phytoplankton from growing more than in the unenriched control, thus reflecting strong nitrogen limitation in the water sample; growth represented about 18% of that in the all-inorganic-nutrient treatment (Fig. 5). When added alone, inorganic ($NO_3^-$, $NH_4^+$) or organic (urea, putrescine) nitrogenous compounds promoted a biomass increase representing between 35 and 51% that gained in the all-inorganic-nutrient treatment—urea being the best nutrient substrate. The single addition of EDTA only slightly promoted algal growth.

Addition of DOM extracted from the Daugava River led to surprising results. Overall, DON provided by DOM sustained algal growth at the same level as ammonium and nitrate (Fig. 5). However, additions of DON at increasing concentrations of 5, 15 and 49 $\mu$M sustained maximum biomasses representing 53, 32 and 41% that gained with the addition of all-inorganic-nutrients, respectively. Addition of phosphorus, silicon and iron with 15 $\mu$M DON increased the final biomass, to 45% that gained with all-inorganic-nutrients, with the corresponding value for 15 $\mu$M DON added alone being only 32%.

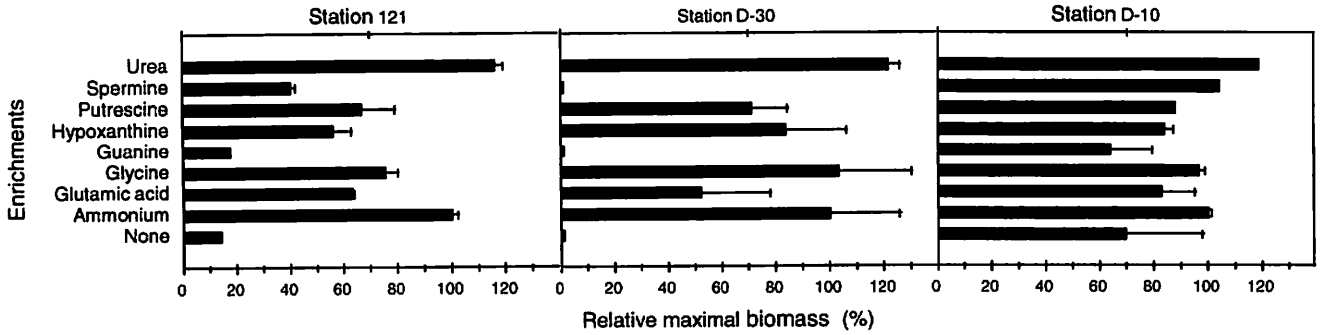


**Fig. 2.** Relative (%) maximal in vivo fluorescence in phytoplanktonic assemblages from offshore (Stns 121 and D-30) and nearshore (Stn D-10) waters grown in the presence of different organic nitrogenous substances. Growth took place under P- Si-, Fe- and vitamin-replete conditions. Calculations have been made using mean values of duplicates.

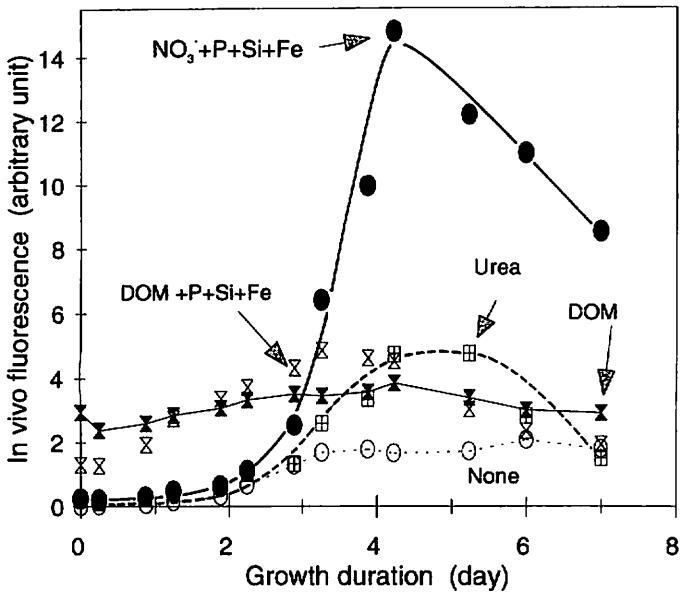


**Fig. 3.** In vivo fluorescence (arbitrary unit) versus time of surface phytoplankton from offshore waters (Stn 121), cultured in the presence of nitrogenous compounds, and DOM (fraction >1000 Da, 15 μM DON) taken from the Daugava River. Examples given are for the best-growing aliquots.

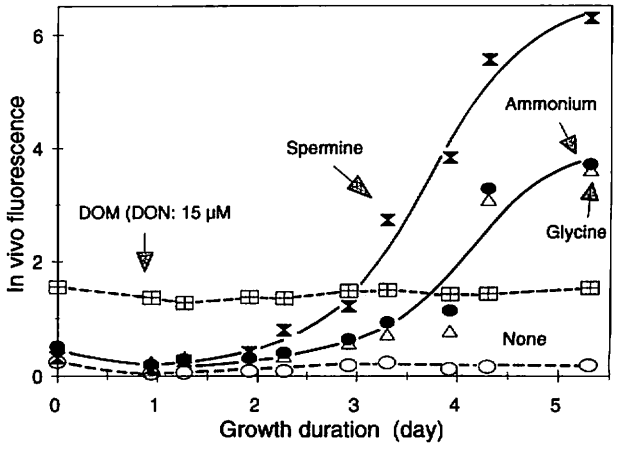


**Fig. 5.** In vivo fluorescence (arbitrary unit) versus time for *Microcystis aeruginosa* cultured from offshore surface water (Stn 119) in the presence of various inorganic and organic nitrogenous compounds, and DOM (fraction >1000 Da) taken from the Daugava River, under P- and Fe-replete conditions. Examples given are the best-growing aliquots.

Growth of *Microcystis aeruginosa* in the presence of river DOM and nitrogenous organic compounds

Growth started after a lag of approximately one day. The addition of spermine and ammonium to water led to large increases in in vivo fluorescence; in the fastest growing replicates after 5 d of culturing, the initial value had increased 48-fold (Fig. 5), thus reflecting a growth rate of 1.1 division $d^{-1}$. Growth as reflected by fluorescence activity was slower in the presence of nitrate. In cultures where DOM extracted from the Daugava River was added, the initial value of the in vivo fluorescence was much higher than in the other treatments; no increase was recorded at the end of culturing. No increase of the in vivo fluorescence was observed in the unenriched control, while a slight increase occurred in water spiked only with P+FeEDTA.

Final biomasses, calculated as protein yield, showed different patterns to those seen with in vivo fluorescence; furthermore, differences appeared reduced. The best nitrogen

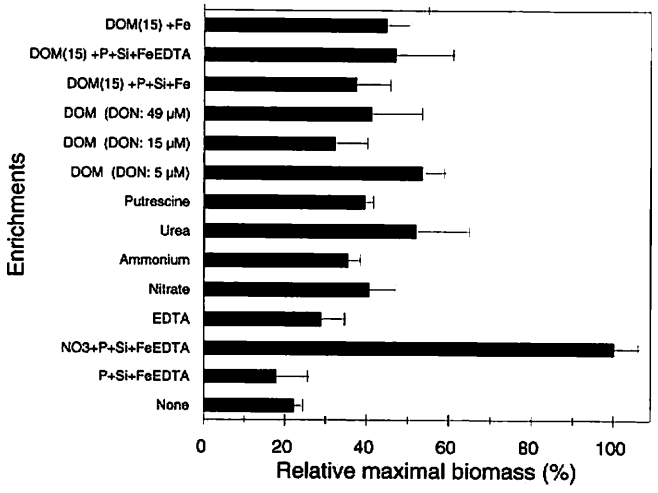


**Fig. 4.** Relative maximumal biomass (%; measured as protein content), versus maximumal biomass in the presence of $NO_3^- + PO_4^{3-} + SiO_3^{2-}$ + FeEDTA (All), of surface phytoplankton from offshore waters (Stn 121), cultured in the presence of various combinations of the following: DIN, urea, putrescine and DOM (fraction >1000 Da) taken from the Daugava River. Calculations have been made using mean values of duplicates.

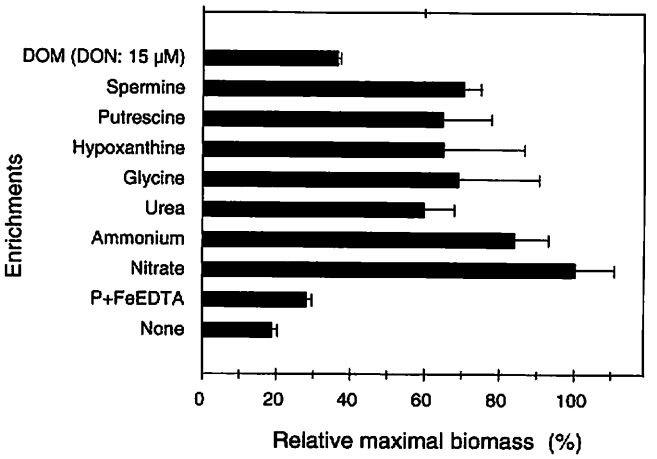


**Fig. 6.** Relative maximumal biomass (%; measured as protein content) versus maximumal biomass in the presence of $NO_3^-$ of *Microcystis aeruginosa* cultured from surface offshore water (Stn 119) in the presence of various inorganic and organic nitrogenous compounds, and DOM (fraction >1000 Da) taken from the Daugava River, under P-, and Fe-replete conditions (except "None" which did not receive any additions). Calculations have been made using mean values of triplicates.

source was nitrate. Respective growth with urea, glycine, hypoxanthine, putrescine and spermine as sole sources of nitrogen ranged between 62 and 72% that gained with nitrate (Fig. 6). Organic matter from the Daugava River sustained growth at a level of 38% of that gained with nitrate, even though the concentration of DON was only 15 $\mu$M, while that of nitrate was 25 $\mu$M. Addition of phosphorus and chelated iron with no addition of nitrogen led to a biomass increase representing 28% that gained with nitrate, while in the unenriched control the corresponding value was 19%.

## Discussion

### Potential uptake of urea and DFAA in nitrogen-limited waters

Results from the addition of nitrogenous organic substances in P-, Si- and Fe-replete conditions, support the idea that nitrogen plays the primary role in potentially limiting AGP. This is true both for natural phytoplankton (Fig. 2) and for the cyanobacterium *Microcystis aeruginosa* (Fig. 6) growing in central water: the yield in the nitrogen-unenriched controls was negligible compared to yields when nitrogenous compounds were added. Such conditions are expected to stimulate photoheterotrophy (Flynn & Butler 1986; Glibert et al. 1991) and other mixotrophic ways of obtaining nutrients in organic form (Havskum & Riemann 1996; Stoecker et al. 1997). From all the experiments, urea appears to be the best organic source of N for natural communities (Figs 2, 4). Glycine, hypoxanthine and putrescine were the next best nitrogen sources for all natural assemblages tested, while glutamic acid and spermine supported growth only in one case each (Figs 2, 6). Guanine was not significantly used by any of the three assemblages tested.

It has long been known that algae can grow in the laboratory with organic nitrogen compounds as their sole nitrogen source (Antia et al. 1991; Hillebrand & Sommer 1996). A critical question follows: does this also occur under natural conditions? The aim of most laboratory studies has been to characterise biochemically some strains, in order to differentiate them by their growth patterns, for taxonomic purposes. Most investigators choose experimental conditions where the concentration of nitrogen source is 100 or even 1000 times higher than the values characteristic in the sea. They may thus be activating one or more different uptake mechanisms not active at natural concentrations.

Urea, a terminal product of nitrogen catabolism, is excreted by zooplankton and other metazoans (Corner & Newel 1967; Båmstedt 1985; Wright et al. 1995). Thus in situ urea-N concentration can reach values as high as 8.9 $\mu$M in estuaries (Remsem et al. 1972). In the open part of the Gulf of Riga, urea-N concentrations ranging from 0.5 to 1.5 $\mu$M were recorded in August 1988 (A. Yurkowskis, pers. comm.), while all values but one recorded from May to July 1996 at various stations ranged between 1.3 and 5.6 $\mu$M (Maestrini et al. 1997). Such values are consistent with the $K$ values for the two enzymes acting in the initial step of urea metabolism (McCarthy 1972; Lomas et al. 1996). It is therefore not surprising that field research has shown that urea is an important potential nitrogen source for natural assemblages in several coastal areas (Turley 1986; Glibert et al. 1991; Fernandez et al. 1996). In the western Baltic Sea, the uptake rate for urea can be five times higher than that for nitrate (Sörensson et al. 1989), and urea appears to be an important nitrogen source during the regenerative phase of plankton succession in the Gulf of Finland (Tamminen & Irmisch 1996). The concentration we used in our enrichment experiments (25 $\mu$M) was 5 times higher than in situ concentrations. Notwithstanding, it was 20 times lower than that in most laboratory experiments, and 1000 times lower than concentrations required by some species for adaptation to growth with urea as the sole nitrogen source (Antia et al. 1977). Thus we can infer that urea is an important potential nitrogen source for summer phytoplankton in the Gulf of Riga.

Amino acids are also metabolites naturally released by both phytoplankton and zooplankton (Hammer et al. 1981; Poulet et al. 1991). In coastal waters, the huge biomass of fixed organisms can also contribute to an increase in DFAA concentrations (Tupas & Koike 1990). Altogether, total DFAA concentrations in various coastal waters have been reported to vary in the $\mu$M range (Daumas 1976; Dawson & Gocke 1978; Jorgensen 1982), thus representing a significant fraction both of DON and of the total nitrogen nutrient pool, particularly in summer when the DIN pool is exhausted or near exhaustion. For other waters, Lee & Bada (1977) and Fuhrman & Ferguson (1986) reported concentrations only in the nanomolar range.

Several species have been reported to take up DFAA at concentrations close to those in situ (Wheeler et al. 1974). Clark et al. (1972) moreover observed that many littoral phytoplankters have transport systems that allow them to accumulate and assimilate amino acids at concentrations characteristic of natural waters. In nature, however, phytoplankton uptake of DFAA is dependent on the presence of other nitrogenous forms. In addition, amino acids having the same charge reciprocally inhibit transport activities (Antia et al 1991). Accordingly, one can assume that DFAA are not taken up under conditions of DIN repletion, as they usually are in the Gulf of Riga during the spring-bloom period (Yurkovskis et al. 1993), while they may well become a significant potential nitrogen source in DIN-poor summer waters.

Only a few reports have shown growth in natural phytoplankton assemblages to be partly sustained by DFAA. Schell (1974) showed utilization of glycine and glutamic acid by natural assemblages at low but detectable rates compared with those of nitrate and ammonia. Rivkin & Putt (1987) reported that diatoms isolated from Antarctic communities assimilated amino acids at ambient concentrations (4–25 nM) in both light and dark conditions. In contrast,

Glibert et al. (1991) concluded that DFAA contributed to only a very small fraction of the total N utilized by phytoplankton in the Chesapeake Bay. Moreover, bacteria can be the primary consumers of DFAA in coastal and estuarine waters (Azam & Hodson 1977; Sepers 1977).

We can thus conclude that DFAA are not a key potential nitrogen source for bulk phytoplankton in the Gulf of Riga. Notwithstanding, on occasion they might be a significant nitrogen source for some species, especially in summer. Experiments to separate bacterial and algal DFAA uptake should be the next step in this research.

### Potential uptake of DON of terrestrial origin by central water assemblages

The single addition of DOM, taken from the Daugava River, to the central water assemblage led to a biomass increase similar to that sustained by the addition of nitrate alone (Fig. 4). However, in one assay, DOM was provided at a concentration of 49 $\mu$M DON plus 4.4 $\mu$M DIN, twice that of nitrate (25 $\mu$M) in the control ($NO_3$+P+Si+FeEDATA), with similar growth. In another, DOM was provided along with P and Si whose concentrations in the experimental treatments were 0.24 $\mu$M and 13.6 $\mu$M, respectively. This treatment, containing 15 $\mu$M DON in the presence of $PO_4^{3-}$, $SiO_3^{2-}$ and Fe-EDTA, should have led to a biomass representing around 60% that of the control "All". However, the biomass that was recorded represented only 48% that of the control when EDTA was present with iron, and only 38% when EDTA was omitted. A similar discrepancy was observed by Gedziorowska & Plinski (1986) who supplied natural phytoplankton assemblages isolated from the Bay of Gdansk, Baltic Sea, with DOM extracted from the same water.

Since algae are thought not to be able to compete with bacteria in the uptake of labile dissolved organic substrates (Hass & Webb 1979), most research on DOM as a nutrient source has focused on bacteria (Cherrier et al. 1996; Gardner et al. 1996). Notwithstanding, Granéli et al. (1985, 1989), Carlsson & Granéli (1993) and Carlsson et al. (1993) reported that dinoflagellate biomass increased several fold when humic acid compounds extracted from rivers were available with nitrate; there was evidence of both bacterial degradation of humic compounds and algal growth sustained by humic-bound nitrogen. In contrast, further research by Carlsson et al. (1995) showed that humic acids extracted from river water only slightly enhanced total phytoplankton biomass and primary production when added to natural assemblages grown in mesocosms; the growth of small flagellates was significantly stimulated, however. On the other hand, Palenik et al. (1988–1989) showed that some phytoplankters can use various forms of DON without initial transport into the cell, by using cell-surface enzymes to degrade these forms of nitrogen to $NH_4^+$, and Berman et al. (forthcoming) have shown that a significant fraction of DON can be degraded to $NH_4^+$ or urea by free dissolved enzymes, as well as by indigenous bacteria.

Coastal waters close to estuaries receive large quantities of DOM from land sources. In the Greenland Sea, total DON concentration has been reported to be as high as 5 $\mu$M (Lara et al. 1993). Hence, DON of terrestrial origin could directly or indirectly provide amounts of nitrogen significant for phytoplankton growth. We infer that DON with a terrestrial origin likely plays a role as a nitrogen source for the natural summer assemblages in the Gulf of Riga, but at present whether this role is critical or not remains an open question.

### Potential uptake of DON by the cyanobacterium *Microcystis aeruginosa*

Respective growth with urea, glycine, hypoxanthine, putrescine and spermine as sole sources of nitrogen ranged between 62 and 72% that gained with nitrate (Fig. 6). Organic matter from the Daugava River sustained growth representing 38% that gained with nitrate, even though the concentration of DON was only 15 $\mu$M, while that of nitrate was 25 $\mu$M. Addition of phosphorus and chelated iron with no addition of nitrogen led to a biomass representing 28% that gained with nitrate, while in the unenriched control the corresponding value was 19%.

The five nitrogenous compounds tested as sole sources of nitrogen gave yields representing between 62 and 72% that gained with nitrate (Fig. 6), while the corresponding value for DON extracted from the Daugava River was only 38%. The concentration of DON was only 15 $\mu$M, while nitrate was present at 25 $\mu$M. Hence, one can speculate that the yield in the presence of DON would have been 63% if the initial DON concentration were 25 $\mu$M. If this is true, DON of terrigenous origin would be a potential nitrogen source for *M. aeruginosa* as good as urea, glycine and the purine derivatives we tested. Therefore, although at present no clear conclusion can be derived from the data we obtained, it can be tentatively hypothesised that DON from the Daugava River is a possible nitrogen source for *M. aeruginosa*. Further research with improved extraction method, and DON added at concentrations equal to those under natural conditions is clearly required.

### In situ versus in vitro role of organic nitrogen sources

In situ, algae and cyanobacteria can potentially meet their nitrogen needs from the uptake of several inorganic and organic chemical nitrogenous forms, while bacteria are mostly competitors for organic compounds and ammonia. Heterotrophic bacteria have been shown to account for $<10\%$ of the total nitrate uptake (Kirchmann 1994).

Presence of ammonium at significant concentrations inhibits the uptake of nitrate and urea (Eppley et al. 1969; McCarthy et al. 1977; Tamminen 1995). However, Dortch (1990) argued that the presence of ammonium does not reduce the uptake of other nitrogenous forms to the degree that is generally believed; concomitant uptake of several

chemical forms is well documented. Moreover, in the picoflagellate *Micromonas pusilla*, the presence of urea resulted in a reduction in nitrate uptake and both N substrata were taken up simultaneously (Cochlan & Harrison 1991). Furthermore, amino acid uptake by the non-diazotrophic cyanobacterium *Oscillatoria rubescens* was not inhibited by the presence of ammonium (Feuillade & Krupka 1986). Hence, the contribution of organic compounds to the nitrogenous nutrition of natural assemblages is assumed to be potentially significant, especially insummer. Further research with the help of labelled compounds is suggested.

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