Nitrogen as the nutrient limiting the algal growth potential, for summer natural assemblages in the Gulf of Riga, eastern Baltic Sea

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Abstract: Nutrients potentially limiting the maximal biomass of natural assemblages were studied on the basis of samples collected in late July 1997, along a transect from the mouth of the river Daugava to the central part of the Gulf of Riga. The inorganic nutrient concentrations decreased strongly between the river mouth and the central part of the gulf from 17.3 to $<1\,\mu$M $NO_3^- + NO_2^- + NH_4^+$ (DIN), and from 3.5 to $0.03\,\mu$M PO_4^{3-}. DIN:PO_4^{3-} increased from 5 to 20. Similar trends occurred for SiO_3^{2-} whose concentration decreased from 72 to $1.6\,\mu$M, but DIN:SiO_3^{2-} only halved. Comparison of DIN:P-PO_4^{3-} ratios (atom:atom) with the Redfield's value indicated nitrogen to be the nutrient limiting the algal growth potential (AGP) in central waters, and phosphorus in near-shore waters; bioassays showed that nitrogen was the first limiting and phosphorus the second limiting nutrient in all samples tested, and the limitation index (growth in absence of one nutrient versus that in the unenriched control) indicated that in nearshore samples phosphorus was as limiting, or almost so, as nitrogen. Altogether, it is inferred that, in summer, there is a weak gradient in potential nutrient limitation from the estuary to the central part of the gulf. Nearshore, in the plume influenced by freshwater discharge, nitrogen and phosphorus are equally limiting, while in the central part of the gulf, AGP is clearly nitrogen-limited. Silicon and vitamins could become limiting if concentrations of nitrogen and phosphorus supplies should greatly increased. Iron appeared to be potentially limiting only in the central part of the gulf.

Key words: limiting nutrient, algal growth potential, natural assemblages, Gulf of Riga, Baltic Sea

Introduction

Human activities have significantly increased the input of nitrogenous and phosphorous nutrients to estuarine and coastal waters, while the silicon concentration has remained constant or has even decreased in riverine inflow (Schelscke & Stoermer 1972; Egge & Aksnes 1992). Hence, N:Si and P:Si nutrient ratios have increased (Rahm et al. 1996), and silicon limitation has become potentially more likely (Officer & Ryther 1980; Ragueneau et al. 1994). Accordingly, it has been assumed that a succession has occurred from diatoms to other phytoplankton species that do not depend on silicon, such as dinoflagellates and cyanobacteria (Kahru et al. 1994; Escavarage et al. 1996; Schöllhorn & Granéli 1996; Sanden & Hakanson 1996).

Cyanobacteria are the taxon most frequently cited as producing harmful blooms in the Baltic Sea in summer (Edler et al. 1985; Leppanen et al. 1995). These blooms seem to have increased in the past decades both in extent and duration (Kahru et al. 1994). Since the 1990s, blooms of the nitrogen-fixing species *Aphanizomenon flos-aquae* and *Nodularia spumigena* have also occurred in the Gulf of Riga, in summer (Balode & Purina 1996). Their appearance was related to a decrease in the DIN:DIP ratio (Yurkovskis et al. 1993). However, with samples collected at varied stations, bioassays carried out with two cultured strains showed that phosphorus was the nutrient most limiting the potential biomass in nitrogen-rich spring time waters, while in early summer the whole area was nitrogen-limited (Maestrini et al. 1997).

Here we report nutrients potentially limiting the maximal biomass of natural assemblages, in mid summer, along a

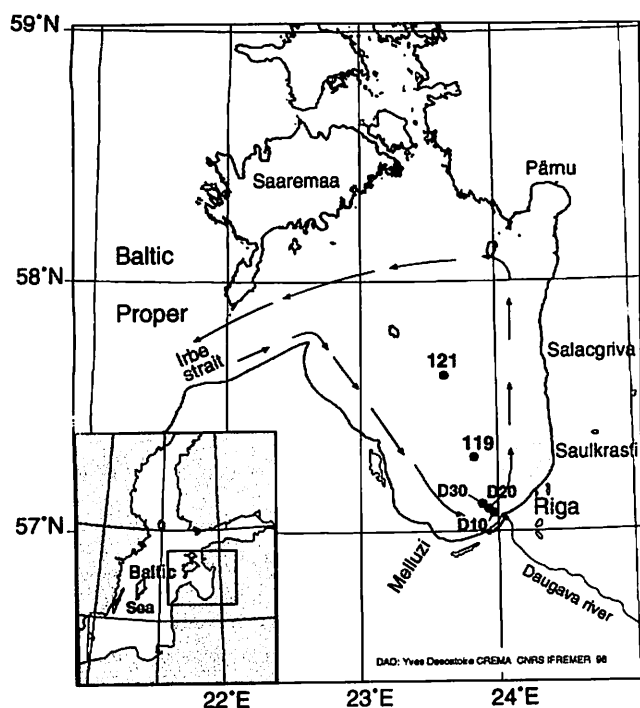Corresponding author: Serge Y. Maestrini; e-mail, smaestri@ifremer.fr

Fig. 1. Study area and positions of sampling stations. Arrows denote residual surface currents; dots indicate the river plume.

transect from the mouth of the main river to the central part of the gulf.

Materials and Methods

At six stations along a transect from the mouth of the river Daugava to the central part of the gulf (Fig. 1), a vertical profile of salinity and temperature was recorded with a CTD probe (AROP type, GMI, Denmark) on 25 and 29 July 1997. Water samples from the surface and within the thermocline were collected with an 8.2-liter Niskin bottle (Model 1010, General Oceanic, USA). Seawater was filtered through 150-μm mesh and brought to the laboratory within a few hours. Inorganic nutrient concentrations were immediately measured using manual protocols: nitrate, reduction to nitrite according to the protocol of Strickland & Parsons (1972); nitrite, method of Bendschneider & Robinson (1952); ammonium, method of Koroleff (1976); phosphate, method of Murphy & Riley (1962); silicate, method of Mullin & Riley (1965). Detection limits: $NO_3^--NO_2^-$, 0.01 μM; NH_4^+, 0.05 μM; PO_4^{3-}, 0.02 μM; SiO_3^{2-}, 0.1 μM (Aminot & Chaussepied 1983).

Bioassays were done to determine the nutrient(s) limiting the algal growth potential (AGP). Aliquots (25 ml) of 150-μm prefiltered seawater were added to 30-ml polycarbonate tubes in triplicates, and the 1-ml enrichment mixtures outlined in Table 1 were added. Initial nutrient concentrations in spike-enriched media were assumed to be low enough not to change the ecophysiological adaptations of the test algae and to be high enough to sustain an algal biomass sig-

Table 1. List and composition of spike enrichments used to bioassay the nutrient(s) limiting the growth potential of natural surface and thermocline assemblages collected on 25 July 1997. Concentrations are those of experimental cultures.

Reference number	Contents*	Reference number	Contents*
1	Nothing	7	All-P-Si
2	All	8	All-FeEDTA-Si
3	All-N	9	+N
4	All-P	10	+P
5	All-Si-Vitamins	11	N+P+Fe
6	All-N-Si	12	All+Metals

*All=N(KNO$_3$; 25 μM)+P(NaH$_2$PO$_4$, 2H$_2$O; 1.7 μM)+Si(Na$_2$SiO$_3$, 9H$_2$O; 30 μM)+Fe(FeCl$_3$; 100 nM)+EDTA(0.6 μM)+Vitamins
Vitamins=biotin(205 pM)+cyanocobalamin(67 pM)+thiamine (75 nM)
Metals=Co(CoSO$_4$, 4H$_2$O; 5 nM)+Mn(MnSO$_4$, H$_2$O; 50 nM)+ Mo (Na$_2$MoO$_4$, 2H$_2$O; 50 nM)

nificantly higher than that sustained by the unenriched controls; thus making clear which nutrients, if any, were present in situ at concentrations sufficiently high to sustain growth and which were not (for detailed discussion, see Maestrini et al. 1984). Incubation of the test cultures was carried out by placing the tubes in front of a north-facing window; the light period was roughly 16 h light and 8 h dark, at about 100 μmol m^{-2} s^{-1} at noon; temperature varied between 18 and 22°C with a circadian period. In vivo fluorescence was monitored daily for 6–10 d, 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. Cultures were stopped when two successive fluorescence values were similar after an obvious period of increase. At this time, the maximum biomass was estimated by analysis of the protein content (Petty et al. 1982). In plotting the biomass data, the value obtained after enrichment with all nutrients (abridged "All"; Table1) was defined as 100 %.

Data sets were first inspected by comparing the different enriched-culture bars with those of the unenriched control and the "All enriched" culture. For each set (one-water-sample-one test assemblage), this resulted in a number of limiting nutrients and a rank for each limiting nutrient. Then, in order to quantify the limiting role of each nutrient, the ratio of biomass in the "All minus one nutrient" enriched aliquots to the biomass in the unenriched control (abbreviated "All-X/control") was also calculated; hereafter, it is termed the "limitation index". In other words, for one nutrient missing in the spike and therefore present in the culture medium at its in situ concentration, this ratio is the increase in the coefficient of the natural AGP sustained by that nutrient. Hence, the lower the coefficient, the
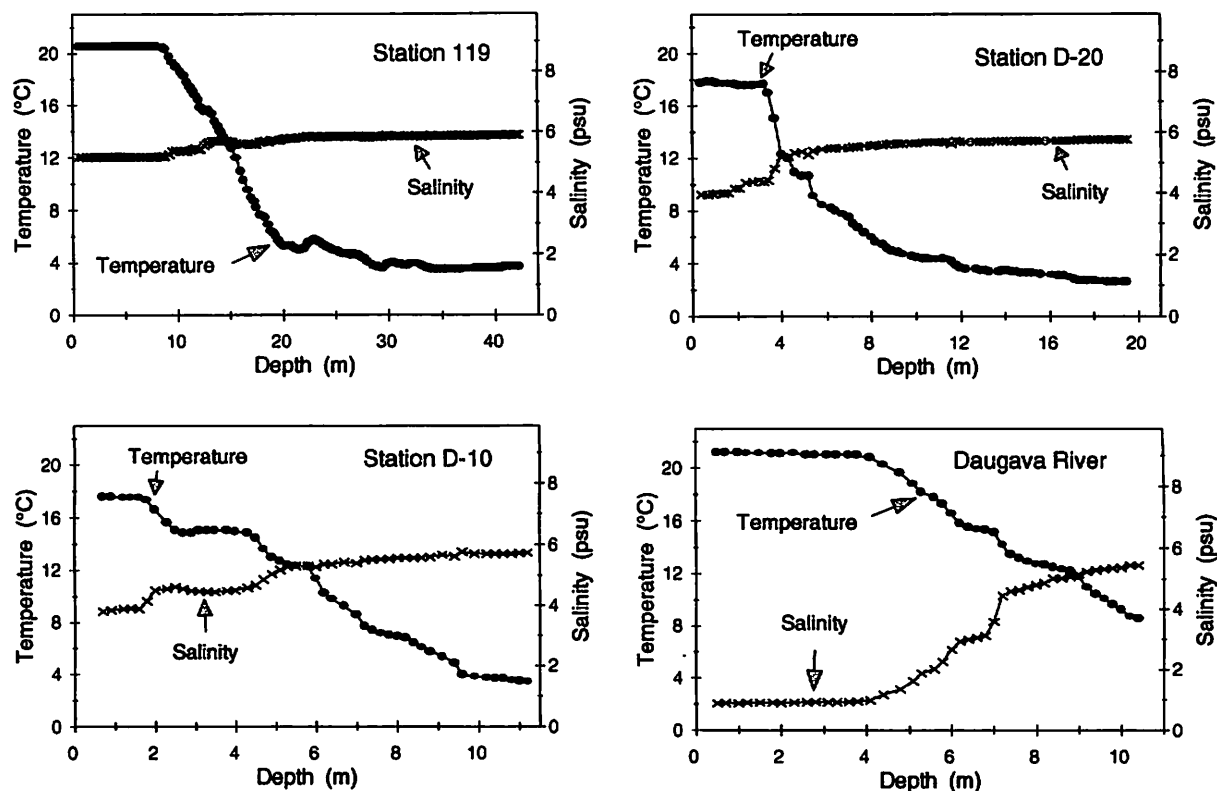
Fig. 2. Vertical distributions of temperature and salinity on 25 July 1997 in the Gulf of Riga; typical stations sampled are represented, along the transect from the Daugava River mouth to the central part of the gulf.

greater limiting effect, and vice versa. All included, 10 bioassay sets were done. All test cultures grew well, thus indicating the absence of adverse chemical conditions.

Results

Environmental conditions

The Gulf of Riga was thermally stratified at the time of sampling. At Stn 119, temperature (20.5°C) was uniform from the surface to a depth of 8.3 m; then it sharply decreased to 5.0°C at 21.5 m, with a decrease rate of 1.0°C m^{-1} (Fig. 2). The temperature further decreased gradually to 3.5°C at 42-m depth. In contrast, salinity varied little from the surface to the bottom, increasing only from 5.44 to 6.23 psu. At the other central station (Stn 121), the situation was similar, except that the temperature decreased more slowly, 0.9°C m^{-1}, in the thermocline layer, from 9.8 and 28.2-m depths. At Stns D-30 and D-20, although much closer to the coast, the thermocline was still sharp, 1.1°C m^{-1} from 2.4 to 18.8 m, and 1.8°C m^{-1} from 3.4 to 10.3 m, respectively. Salinity increased only slightly, from about 4 to 6 psu. The vertical structure of the water column changed markedly in the vicinity of the river mouth. At Stn D-10, there was a continuous decrease in temperature from about 17 to 4 °C between the surface and the bottom (12-m depth) (Fig. 2). The river water column was also stratified at the entrance of the estuary, though the thermocline was not

sharp. In the upper 3.8-m layer, temperature declined only from 21.2 to 21.0°C, and salinity increased from 0.89 to 0.95 psu. Then temperature continuously decreased to reach 8.6°C near the bottom, while salinity increased to 5.44 psu.

The inorganic nutrient concentration decreased strongly between the river mouth and the central part of the gulf (Stn 121; 37 nautical miles from the mouth, Table 2). In surface waters, the concentration of $NO_3^- + NO_2^- + NH_4^+$ (DIN) decreased 30 fold from 17.3 to 0.6 μM. At 9.3 km out (Stn D-30), DIN concentration was already <1 μM. A similar decrease occurred in the thermocline layer, but the difference was not as pronounced: DIN concentration decreased 20 fold from 10.3 to 0.5 μM. The overall decrease in PO_4^{3-} concentration was even more drastic; surface concentration at the river mouth was very high (3.5 μM), while it was near the lower limit of detectability in the central part of the gulf (0.03 μM), a 117 fold decrease. The decrease was slower than that of DIN, however; 14.8 km out, it was only 13 times lower. Relative variations in inorganic nitrogen and phosphorus concentrations led to an increase in the DIN: PO_4^{3-} ratio from 5 to 20 (Table 2). Similar trends occurred for dissolved inorganic silicate, whose concentration in surface water decreased 45 times: from 72.0 to 1.6 μM from the river mouth to the central part of the gulf. The ratio of silicon concentration to DIN concentration only halved over the distance from the river mouth to the central gulf.

Taken overall, the central part of the gulf (Stns 121 and

Table 2. Nutrient concentrations in samples collected on 25 July 1997, at the surface and in the thermocline, along a transect from the Daugava River mouth to the central part of the Gulf of Riga.

Sample		Inorganic nutrient (μM)							
Station	Depth (m)	NO_3^-	NO_2^-	NH_4^+	DIN	PO_4^{3-}	DIN : DIP	SiO_3^{2-}	SiO_3 : DIN
121	0	0	0	0.6	0.6	0.03	20	1.6	2.7
	15	0	0	0.5	0.5	0.07	7	1.6	3.2
119	0	0	0	0.6	0.6	0.05	14	1.4	2.3
	15	0	0	1.0	1.0	0.04	25	1.7	1.7
D-30	0	0.4	0	0.1	0.5	0.26	(2)	13.4	26.8
	8	0.1	0	0.7	0.8	0.01	80	2.1	2.6
D-20	0	4.3	0	0.4	4.7	0.26	18	25.2	5.4
	7	0.9	0.1	2.8	3.8	0.20	19	4.4	1.2
D-10	0	7.4	0.1	1.7	9.2	0.79	12	38.3	4.2
	7	1.3	0.2	2.3	3.8	0.21	18	4.9	1.3
Mouth	0	14.8	0.3	2.2	17.3	3.50	5	72.0	4.6
	7	7.0	0.3	3.0	10.3	1.00	10	30.3	2.9

119) and those coastal waters not enriched in nutrients through river discharge (Stn D-30) were clearly nitrogen- and phosphorus-poor, while waters closer to the river mouth (Stns D-20 and D-10) were clearly nitrogen- and phosphorus-rich. In the central gulf, DIN concentrations ranged between 0.5 and 1.0 μM, and PO_4^{3-} concentrations ranged between 0.03 and 0.07 μM, while in the plume the corresponding values were 3.8 and 9.2 μM DIN, and 0.20 and 0.79 μM PO_4^{3-}. Conversely, the whole study area was rather silicon-replete compared to nitrogen and phosphorus levels; in the central area, all concentrations were ≥ 1.4 μM, and the Si : DIN ratio was ≥ 1.7.

AGP bioassays

In unenriched controls, algal growth was low with respect to that in all-nutrient-enriched treatments. There were significant differences between nutrient-poor waters from the central part of the gulf and waters collected in the river-discharge plume (Stns D-10 and D-20, Fig. 1); the algal yield in unenriched surface and central waters within the thermocline (Stn 121) represented only 4.9% and 8.0% of that in all-nutrient-enriched cultures, while the corresponding values in water collected near the river mouth (Stn D-10) were 19.6% and 32.0%. For most samples, the nutrient-spiked cultures showed a significant increase over the initial in vivo fluorescence compared with the unspiked control cultures (Fig. 3); the overall averaged value of the limitation index (yield in spiked cultures versus yield in unenriched control culture) was 21.2 (SD=10.0, n=10, Table 4). Growth was only slightly enhanced, however, when either nitrogen or phosphorus was absent in the enrichment (Fig. 4). Omission of NO_3-N alone (All-N) usually gave a lower biomass than did omission of phosphorus alone (All-P). Omission of silicon and vitamins from the enriching mixtures decreased the algal yield slightly. In a few cases, the addition of Co, Mn and Mo slightly promoted growth.

Inspection of the bioassay sets showed that in all cases but one, the AGP was limited by at least two nutrients (Table 3); for surface water at Stn D-20, nitrogen was the single limiting nutrient. Over the 10 sets, one was limited by nitrogen and phosphorus equally. For all other waters, nitrogen was found to be the primary limiting nutrient and phosphorus to be the second any one. In central water (Stn 121), the third most limiting nutrient appeared to be chelated iron. With three samples, the absence of silicon and vitamins from spike enrichments led to a reduced yield; the decrease, however, was far less than that observed in the absence of nitrogen or phosphorus. Altogether, the nature of the nutrients most limiting the growth potential of the natural assemblages did not change from water near the
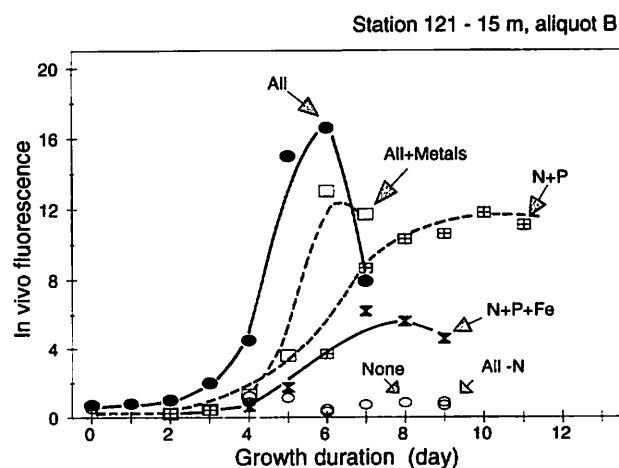

Fig. 3. In vivo fluorescence (arbitrary unit) versus time, in a differentially-enriched aliquot culture of the natural phytoplanktonic assemblage collected on 25 July in the Gulf of Riga (for the software to draw the figure, a few missing data points were added by calculating the means of preceding and following values). The example given is the sample collected in the thermocline (15-m depth), at Stn 121.
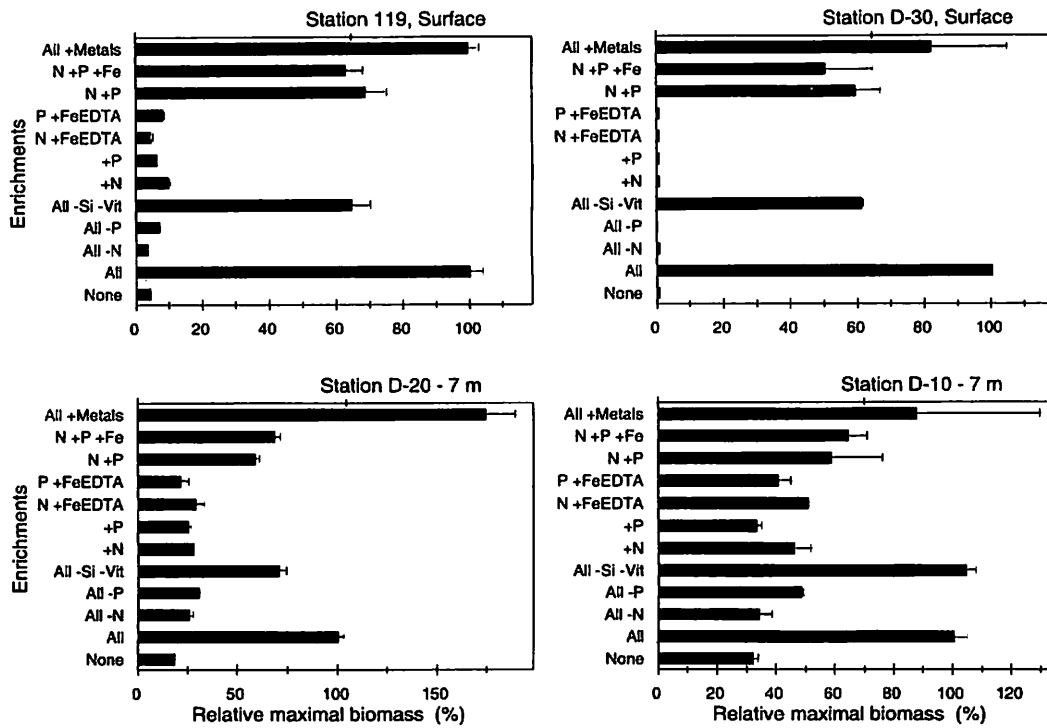
Fig. 4. Relative maximal algal biomass (%, measured as protein content) for different spike enrichments; typical sets of data are shown from the empirical analysis of nutrient limitation of AGP. Stn 119 (surface): N was the first limiting nutrient, P was the second limiting nutrient, silicon and vitamins could also become limiting; Stn D-30 (surface): N and P were equally limiting, silicon and vitamins could also become limiting; Stn D-20 (7 m): N was the first limiting and P the second limiting nutrient, silicon and vitamins could also become limiting, trace metals were stimulating; Stn D-10 (7 m): N was the first limiting and P the second limiting nutrient. Calculations were made using mean values from triplicates.

river mouth (Stn D-10) to waters in the central part of the gulf (Stns 119 and 121).

The mean values of the limitation index, reflecting the limiting effect of each nutrient, were 1.4 for nitrogen, 5.0 for phosphorus, and 13.3 for silicon and/or vitamins, while all nutrients added together gave a coefficient of 17.4 (Table 4). The addition of unchelated iron did not enhance the

yield beyond that sustained by the addition of both nitrogen and phosphorus. Moreover, the mean coefficient was greater for the N+P treatment (12.2), than for the N+P+Fe treatment (11.6). This was not valid for all samples, however; in the surface waters of Stns 121 and D-10, the addition of iron increased the algal yield. overall, the addition of trace metals slightly enhanced the growth potential, al-

Table 3. Bioassays. Nutrients limiting the potential biomass of phytoplankton in water sampled in the Gulf of Riga, on 25 July 1997, at five stations located along a transect from the Daugava River mouth to the central part of the gulf. *Numbers indicate the order in limitation; 1 is the most limiting, 2 is the next most limiting, 3 is the last limiting nutrient.

Station	Depth (m)	Limiting nutrient(s)			Additional comments
		1*	2	3	
121	0	N	P	EDTA	or Fe-EDTA limiting
121	15	N	P	Fe-E	Trace metals could be also limiting
119	0	N	P		Silicon and vitamins could become limiting
119	15	N	P		
D-30	0	N, P			Silicon and vitamins could become limiting
D-30	8	N	P		Trace metals were stimulating
D-20	0	N			
D-20	7	N	P		Silicon and vitamins could become limiting; trace metals were stimulating
D-10	0	N	P		
D-10	7	N	P		Iron could be also limiting

Table 4. Limitation index: growth of natural assemblages in the presence of different mixtures or in the absence of one nutrient relative to that in unenriched control water. For the "all samples" column, first number is the mean of 10 values (SD in parentheses). For stations 121 and D-10, the values shown are for surface water. Gowth was estimated as the increase of protein concentration.

	All samples ($n=10$)	Nutrient-poor waters (Stn 121)	Nutrient-rich waters (Stn D-10)
Presence of:			
All+metals	19.5 (13.9)	17.9	3.9
All nutrients	17.4 (12.2)	20.2	5.1
N+P+Fe	11.6 (7.5)	15.6	3.3
N+P	12.2 (8.0)	14.4	2.9
Absence of:			
N	1.4 (0.9)	0.5	1.2
P	5.0 (5.9)	1.0	2.6
Si and vitamins	13.3 (7.8)	24.6	4.7

though once again discrepancies occurred between water types. The absence of nitrogen and phosphorus limited AGP more markedly in water from the central part of the gulf than in water from near the river mouth; the respective growth coefficients were 0.5 and 1.0 in the offshore samples, and 1.2 and 2.6 in the nearshore ones. The absence of silicon and/or vitamins had no effect in water from the central part of the gulf; their limitation index coefficien (24.6) was 21% higher than that for the all-nutrient-added treatment.

Discussion

A few results conflicted in indicating which nutrients potentially most limited the phytoplankton growth potential. Outside the river plume, from 9 km from the river mouth (Stn D-30) to the central part of the gulf (Stn 121), both surface and thermocline-layer waters were very poor in DIN (Table 2). In three samples the DIN:DIP ratio (atom:atom) was lower than 16, thus indicating a potential nitrogen limitation (Redfield 1934), while in three other samples the ratio was higher than 16. As the phosphorus concentrations were near the detection limit, however, these ratios may be somewhat imprecise. In waters influenced by the Daugava River (Stn D-10 and D-20, 5.6 km from the mouth), concentrations of DIN equalled or exceeded 3.8 μM, and three over four DIN:DIP ratios exceeded 16, thus indicating potential phosphorus limitation. Conversely, in the water at the mouth of the river, the DIP concentration was high, and the DIN:DIP ratio was lower than 16.

Consideration of the bioassay results, however, suggests firstly that nitrogen was the nutrient potentially most limiting AGP (in all samples except one, limited equally by phosphorus and nitrogen; Table 3), and secondly that phosphorus was potentially the second-most limiting nutrient in most cases, and at a degree only slightly less than that of ni-

trogen (Table 4). In the same area, in mid spring 1996, phosphorus was clearly potentially the most limiting nutrient in nitrogen-rich waters greatly influenced by river discharge; in late spring, with a decrease in the DIN reserve, nitrogen and phosphorus then had an equal limiting role; and in early summer, when river discharge was lowest, the whole area was potentially nitrogen-limited (Maestrini et al. 1997). On the basis of these results, we believe that, in summer, there is a weak gradient in potential nutrient limitation from the estuary to the central part of the gulf. Nearshore, in the plume influenced by freshwater discharge, nitrogen and phosphorus are equally limiting, while in the central part of the gulf, AGP is clearly nitrogen-limited.

Similar features have been reported previously for the Baltic Sea. Lignell et al. (1992) observed that phytoplankton biomass was phosphorus-limited at the inlet of a fjord, whereas in the open sea both nitrogen and phosphorus were limiting. Pitkanen & Tamminen (1995) found that, in summer, from the river Neva to the open part of the Gulf of Finland, phosphorus alone potentially limited AGP in the river, phosphorus was the first and nitrogen was the next most limiting nutrient in the estuary, in the transition zone nitrogen was most limiting and phosphorus was the next most limiting nutrient, and in the open gulf nitrogen was the only limiting nutrient.

In our experiments, nutrients other than nitrogen and phosphorus were not the primary factors limiting AGP. All SiO_3^{2-}: DIN ratios ranged between 1.2 and 26.8, thus leading to the conclusion that silicon was not limiting in any sample. On occasion, however, silicon and vitamins appeared from the bioassays to be potentially limiting in several waters, although they clearly played a much weaker role (Table 4). Hence, they could fundamentally become limiting if concentrations of nitrogen and phosphorus supplies should ever increase greatly in the future. Notwithstanding, it is likely that the recently reported decrease in silicon loading in coastal waters and its subsequent enhanced importance in pushing the outcome of species competition towards a preponderance of summer-type harmful species (Smayda 1990; Sommer 1996) may not be critically important in the Gulf of Riga.

Iron appeared to be potentially limiting only in the central part of the gulf. This result is in agreement with the work of Forsgren et al. (1996) who showed that at least 40–90% of the iron transported by freshwater aggregated and sedimented when salinity reached 4–5 psu in the estuary of the Öve River, western Baltic. More research on the role of iron as a nutrient necessary for the growth of summer offshore phytoplankton is thus desirable.

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