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Variability in Inorganic and Organic Nitrogen Uptake Associated with Riverine Nutrient Input in the Gulf of Riga, Baltic Sea

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ABSTRACT: Concentrations and rates of uptake of dissolved organic nitrogen (DON, free amino acids, and urea) and inorganic nitrogen (DIN, nitrate, and ammonium) were measured along two transects in the Gulf of Riga, a sub-basin of the Baltic Sea, during May and July 1996. Concentrations of total dissolved nitrogen (TDN) were 23 ± 3 μg-at N l^{-1} in the northern region (mouth) and 41 ± 5 μg-at N l^{-1} in the southern region (head) of the Gulf. Rates of nitrogen uptake, determined with ^{15}N-labeled substrates, reflected differences in TDN concentration between the regions. In May, uptake of DIN+DON measured 0.17 and 0.43 μg-at N l^{-1} h^{-1} in the northern and southern parts of the Gulf, respectively. In July, DIN+DON uptake measured 0.38 and 0.68 μg-at N l^{-1} h^{-1} in the north and south, respectively. Most of the variability in total nitrogen flux between the northern and southern regions was due to heterogeneity of DON utilization. Uptake of urea and dissolved free amino acid were up to 6 and 3 times greater in the south compared to the north. As evidenced by size-fractionation, plankton size structure appeared to play a role in the uptake of DON. The community in the southern part was largely composed of cells < 5 μm, while up to 67% of the community in the northern part was composed of cells > 5 μm. Our results indicate that DON was a major source of nitrogen to phytoplankton, particularly in the southern part of the Gulf.

Introduction

Dissolved organic nitrogen (DON) comprises a substantial portion of the total nitrogen in marine and estuarine environments (Sharp 1983; Jackson and Williams 1985), and it is recognized as a dynamic pool, with many sources and sinks (Kieber et al. 1989; Bronk et al. 1994; Aluwihare et al. 1997; McCarthy et al. 1998). The relatively high rates of cycling of some high molecular weight compounds has contributed to the notion that DON is more labile than previously thought (Amon and Benner 1994, 1996; Cottrell and Kirchman 2000).

In recent years anthropogenic nutrient loading has proven to be a significant, and at times dominant, source of nitrogen to coastal systems (Peierls et al. 1991; Vitousek et al. 1997). External sources of nitrogen such as atmospheric deposition, land runoff, and river flow may play an important role in regulation of estuarine primary production and biomass accumulation (Malone et al. 1988; Paerl et al. 1990; Nixon 1995). As the transfer of dissolved inorganic nitrogen (DIN) through rivers and atmospheric deposition to marine systems has increased, so has the loading of DON (Correll and Ford 1982; Cornell et al. 1995). It has been hypothesized that when DIN becomes exhausted, DON may contribute to phytoplankton succession by fueling blooms of those species able to utilize it (Butler et al. 1979; LaRoche et al. 1997; Berman and Chava 1999). However, few studies have quantified the proportion of organic nitrogen to total nitrogen uptake by phytoplankton in situ, or how this relationship varies over the growing season.

The Gulf of Riga, Baltic Sea, has been a site of intensive study with regard to eutrophication (Wassman and Tamminen 1999). This region has witnessed decreased DIN concentrations in the surface water over the last decade (Yurkovskis et al. 1999), while the abundance of both nitrogen fixing and non-nitrogen fixing cyanobacteria has increased (Kahru et al. 1994; Balode and Purina 1996). The majority of the nitrogen input to the Gulf occurs via riverine transport, of which 70% is in the form of DON (Laznik et al. 1999; Stålnacke et al. 1999). Given the potential importance of DON in the Gulf of Riga, the present study was designed to examine the relationship between riverine nitrogen input, spatial and temporal variability in DIN and DON uptake, and community

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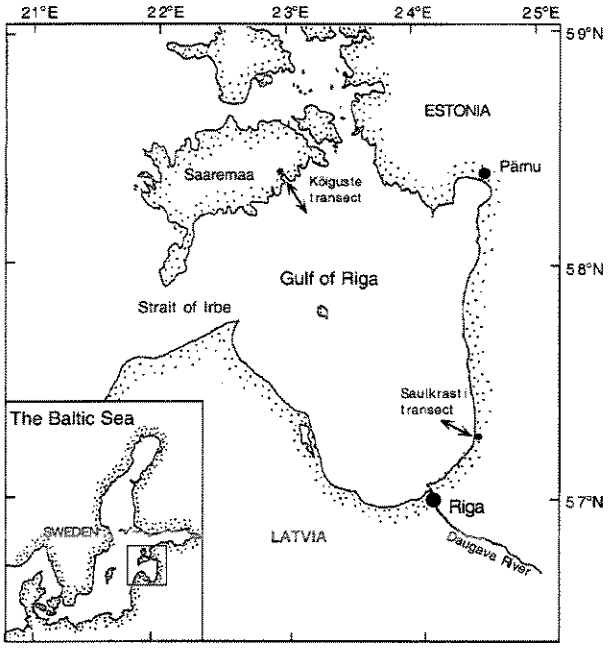

Fig. 1. Baltic proper. Inset: Gulf of Riga. Double headed arrows represent placement of the sampling transects. The Kõiguste transect represents the northern transect in Estonian territorial waters, the Saulkrasti transect represents the southern transect in Latvian territorial waters.

composition. Two contrasting sites in the southern and northern regions of the Gulf were chosen to examine the flux of nitrogen substrates in areas that were relatively influenced (south) and uninfluenced (north) by riverine nutrient inputs. Variation in concentrations of nitrogen, nitrogen uptake, and phytoplankton taxon-specific preferences were explored.

Materials and Methods

Study Location

The Gulf of Riga is a sub-basin of the Baltic Sea connected to the Baltic proper by two straits (Fig. 1). The average depth of the Gulf is 26 m, with a maximum depth of 62 m in the central part. Salinity ranges between 2–6 psu (this study: 2.2–5.8 psu) and is influenced by freshwater flow from several rivers in the southern and eastern parts of the Gulf, of which the Daugava River is the largest (Andrushaitis et al. 1995; Laznik et al. 1999). Two transects oriented perpendicular to land in the northern (Kõiguste, Estonia) and southern (Saulkrasti, Latvia) parts of the Gulf of Riga (Fig. 1) were sampled May 8–16 and July 24–31, 1996. Samples for phytoplankton enumeration were collected on May 4 and July 31. Stations were located at water depths of 2.5 (station 1), 5.0 (station 2; only Saulkrasti transect), 10 (station 3), and 20 m (station 4). Stations along the Kõiguste transect (Fig. 1, hereafter the northern transect) were 0.05, 4.8, and 13.2 km from shore. Stations along the Saulkrasti transect (Fig. 1, hereafter the southern transect) were 0.05, 0.25, 1.5, and 5.53 km from shore. Concentrations and rate determinations were measured on pooled water samples, representing the upper mixed layer. The same stations were sampled in May and July.

Analysis of Dissolved and Particulate Nitrogen

During May and July, concentrations of NH_4^+, $NO_2^-+NO_3^-$, urea, and total dissolved nitrogen (TDN) were determined 2–4 times along both transects. Samples for dissolved inorganic nitrogen concentrations (NH_4^+ and $NO_2^-+NO_3^-$) were filtered through precombusted GF/F filters and stored frozen until analysis on a Danish Sea Technology autoanalyzer at the nutrient analysis laboratory of the Danish Environmental Research Institute, Roskilde, Denmark using standard methods (Grasshoff et al. 1983). Samples for urea and TDN were filtered through 0.2 μm pore size Gelman acrodisks and stored frozen until analysis. Urea was analyzed according to Parsons et al. (1984). TDN was measured using an Antek 7000 high temperature combustion nitrogen analyzer. Samples were analyzed in triplicate with a mean coefficient of variation of 4.6% (n = 50). Concentrations of DON were calculated by subtracting inorganic nitrogen concentrations from TDN concentrations. Concentrations of dissolved free amino acids (DFAA) were determined in 0.2 μm Millipore HA filtered water samples. Individual DFAA were measured as fluorescent OPA-derivatives by HPLC according to Lindroth and Mopper (1979) and Jørgensen et al. (1993). Samples for particulate nitrogen (PN) were taken concomitant with measurements of nitrogen uptake. Unfiltered and < 5 μm size-fractionated water samples were filtered onto pre-combusted GF/F filters and dried immediately for later analysis of PN in the laboratory on a Control Equipment CHN Analyzer. Chlorophyll *a* (chl *a*) was analyzed fluorometrically after filtration (GF/F) and overnight extraction in 96% ethanol. Concentrations were corrected for phaeophytin content. On May 4 and July 31, samples for phytoplankton enumeration were collected along the southern transect. From each station, cell counts from discrete depths were averaged to give mean cell abundance and mean biomass for the entire water column. Samples were preserved in Lugol's acid solution and settled using the Utermohl method (1958) before being counted on an inverted microscope. The carbon biomass of the phytoplankton was estimated from their biovolu-

TABLE 1. Percent bacteria retained by the GF/F filter (RET), bacterial particulate nitrogen retained by the GF/F filter ($PN_{(bact)}$), cyanobacterial particulate nitrogen ($PN_{(cyano)}$), total particulate nitrogen (TPN), total chl *a* (CHL), and percent chl *a* in the <5 μm fraction (CHL < 5) in samples from the northern (N) transect (May 8 and 9, July 24, 1996) and the southern (S) transect (May 13 and 16, July 31, 1996). nm = not measured.

Date	Station	RET (%)	$PN_{(bact)}$ (μM)	$PN_{(cyano)}$ (μM)	TPN (μM)	CHL (μg l^{-1})	CHL < 5 (%)
8 May	1	40	1.6	nm	10	13.1	nm
8 May	4	40	1.4	nm	8.1	14.0	nm
9 May	1	40	1.6	nm	9.1	16.8	nm
9 May	3	40	1.6	nm	8.3	15.9	nm
9 May	4	40	1.4	nm	8.8	23.2	nm
13 May	1	50	4.6	0.1	11.6	23.0	nm
13 May	2	50	4.3	0.6	11.5	21.0	nm
13 May	3	50	4.3	0.1	12.3	27.0	nm
13 May	4	50	4.0	0.2	15.1	20.0	nm
16 May	1	50	5.7	nm	15.5	24.0	nm
16 May	2	50	4.7	nm	16.1	35.0	nm
16 May	3	50	4.4	nm	13.5	60.0	nm
16 May	4	50	4.3	nm	14	32.0	nm
24 Jul	1	45	4.3	nm	4.4	3.0	100
24 Jul	3	48	5.3	nm	4.2	3.8	76
24 Jul	4	32	4.8	nm	7.5	8.8	33
31 Jul	1	42	6.7	12.2	13.4	15.8	97
31 Jul	2	50	6.8	16.5	16.6	17.3	nm
31 Jul	3	58	8.4	7.6	16.1	24.0	nm
31 Jul	4	69	7.2	3.8	11.2	13.0	100

mes according to Balode et al. (1998). The fraction of PN due to cyanobacteria, PN_{cyano}, was calculated by converting from carbon to nitrogen using a molar C:N ratio of 7 (Table 1). Heterotrophic bacteria were enumerated from both the northern and southern transects during May and July after preservation in formaldehyde (4% final concentrations). Five to ten ml samples were filtered onto blackened 0.2-μm polycarbonate filters, stained with acridine orange (Hobbie et al. 1977) and enumerated using a Zeiss Universal epifluorescence microscope as described in Zweifel (1999). The fraction of PN due to heterotrophic bacteria, $PN_{(bact)}$, was obtained by converting the abundance of bacteria on the GF/F filter to PN according to Lee and Fuhrman (1987). The abundance of bacteria on the GF/F filter was calculated by subtracting the abundance of bacteria enumerated in the filtrate (generated by filtering the contents of the incubation bottle) from total bacterial abundance (Table 1).

Rate Measurements

Direct rates of nitrogen uptake were measured using ^{15}N labeled NH_4^+, urea, NO_3^-, and commercially available algal extract (Cambridge Isotope Laboratories, Inc. NLM-2161) consisting of a mixture of 12 amino acids (Table 2). Prior to use, the algal extract was passed through ion retardation resin (Biorad AG 11 A8 resin) to remove inorganic ions (Bronk and Glibert 1991). The composition of amino acids in the algal extract relative to the ambient composition is given in Table 2.

TABLE 2. Percent DFAA composition in the ^{15}N-labeled algal extract and in the water column during May and July in the southern part of the Gulf of Riga.

Amino acid	% ^{15}N-DFAA	% DFAA-May	% DFAA-July
Alanine	19.0	9.0	6.0
Valine	15.0	4.0	2.0
Leucine	14.0	3.0	2.0
Isoleucine	10.0	2.0	1.0
Threonine	9.0	3.0	0.3
Glycine	8.0	13.0	19.0
Serine	6.0	21.0	9.0
Aspartic acid	4.0	6.0	4.5
Glutamic acid	3.0	13.0	15.5
Glutamine	3.0	6.0	10.0
Histidine	3.0	4.0	14.5
Lysine	2.0	3.0	11.0

Trace amounts of > 98% enriched $^{15}NH_4^+$ (0.075–0.10 μg-at N l^{-1}), $^{15}NO_3^-$ (0.075–0.30 μg-at N l^{-1}), ^{15}N-urea (0.075 μg-at N l^{-1}), and ^{15}N-algal extract (0.015–0.030 μg-at N l^{-1}) were added separately to 500 ml acid-rinsed polycarbonate incubation flasks. Uptake rates were assayed on both unfiltered and < 5 μm (Nuclepore filter) size-fractionated water. The percent uptake in the < 5 μm fraction was calculated as:

$$\text{uptake}(\mu\text{g-at N } l^{-1} h^{-1})_{<5\mu m} \div \text{uptake}(\mu\text{g-at N } l^{-1} h^{-1})_{whole} \times 100$$

while the percent uptake in the > 5 μm fraction was calculated as:

$$100 - [\text{uptake}(\mu\text{g-at N } l^{-1} h^{-1})_{<5\mu m} \div \text{uptake}(\mu\text{g-at N } l^{-1} h^{-1})_{whole} \times 100].$$

Bottles were incubated in flowing, ambient seawater (4°C in May and 17°C in July) under neutral density screening simulating 45% incident natural irradiance. Incubations were terminated after 1 h in May and 0.5 h in July by filtration through precombusted GF/F filters (500°C, 1 h) at low pressure (< 100 mm Hg). Filters were dried overnight at 50°C and prepared for mass spectrometry according to Glibert et al. (1991). Samples were analyzed on a Nuclide 3″60° sector analyzer with dual mass collection. The ^{15}N atom percent of each sample was read in triplicate with a standard error ranging between 0.01% and 0.09%. Standard deviation in sample atom percent of samples filtered in duplicate ranged between 1.7–5.6%. Uptake rates were calculated according to Glibert and Capone (1993). The nitrogen specific rate, V (h^{-1}), was used when calculating the relationships between percent nitrogen uptake and percent biomass abundance of individual phytoplankton

groups along the southern transect. The absolute rate of uptake, ρ (μg-at N l^{-1} h^{-1}), was calculated as the product of the nitrogen-specific rate and PN concentration (Dugdale and Goering 1967). To date, picoplanktonic phytoplankton, including cyanobacteria, have been the groups mainly associated with uptake DFAA in field studies (cf., Paerl 1991; Mulholland et al. 1998). Therefore, we assumed that cyanobacteria were the primary consumers of DFAA in addition to heterotrophic bacteria and ρ_{DFAA} was calculated according to:

$$\rho_{DFAA}\ (\mu\text{g-at N } l^{-1}\ h^{-1}) = V\ (h^{-1}) \times PN_{(bact+cyano)}$$

Where $PN_{(bact+cyano)}$ includes the sum of heterotrophic-particulate and cyanobacterial-particulate nitrogen. In spring, cyanobacterial biomass was negligible and the equation reduced to:

$$\rho_{DFAA}\ (\mu\text{g-at N } l^{-1}\ h^{-1}) = V\ (h^{-1}) \times PN_{(bact)}$$

In July, both $PN_{(bact)}$ and $PN_{(cyano)}$ appeared overestimated at times relative to total PN. In these cases, total PN was used to calculate ρ_{DFAA}.

Results

Chlorophyll Concentrations and Phytoplankton Community Composition

Spring

Chlorophyll *a* concentrations along the southern transect, varying from 23 ± 1.8 μg l^{-1} to 42 ± 7.2 μg l^{-1}, averaged 2 times that of the northern transect, 16 ± 1.8 μg l^{-1} (Table 1). At this time, diatoms (*Bacillariophyceae*) comprised 90% or more of phytoplankton community biomass (Fig. 2a). *Thalassiosira baltica* was the most abundant diatom species followed by *Achnanthes taeniata* and *Diatoma elongatum*. The next abundant phytoplankton group was the dinoflagellates (*Dinophyceae*), consisting mainly of *Peridiniella catenata* (Fig. 2a). Identification and enumeration of phytoplankton along the northern transect was not conducted during spring.

Summer

Similar to spring, chl *a* concentration in the southern region, 18 ± 2.3 μg l^{-1}, averaged significantly more than the concentration in the northern region, 5 ± 1.5 μg l^{-1} (Table 1). These concentrations were less than half the respective spring values. Along the southern transect, most of the chl *a* was in the < 5 μm size fraction while along the northern transect, chl *a* in the < 5 μm fraction varied from > 90% at station 1 to 33% at station 4 (Table 1).

As in the spring, phytoplankton enumeration was only conducted along the southern transect. In contrast to spring, substantial variability in phytoplankton species composition was encountered and the phytoplankton consisted mainly of small-sized species. The cyanobacteria (*Cyanophyceae*) dominated phytoplankton abundance inshore, decreasing in abundance with distance from shore (Fig. 2b). This group was represented by 5 different species including the non-nitrogen fixing *Snowella lacustris, Microcystis* sp., and *Aphanothece* sp., and the nitrogen fixers *Aphanizomenon flos-aquae* and *Anabaena* sp. In addition to cyanobacteria, the biomass of prasinophytes (*Prasinophyceae*), largely *Pyramimonas* sp., decreased with distance from shore. The biomass of diatoms increased with distance from shore and dominated the offshore stations (Fig. 2b). This group was largely represented by *T. baltica*. The dinoflagellates *Dinophysis acuminata, Protoperidinium bipes,* and *Gymnodinium* spp. also increased in biomass offshore (Fig. 2b). Green algae (*Chlorophyceae*) and cryptophytes (*Cryptophyceae*),

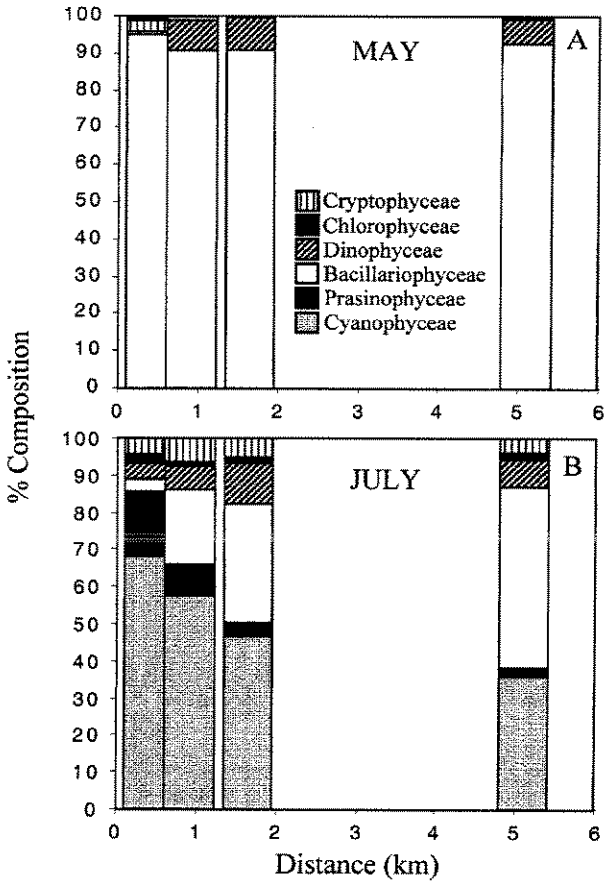

Fig. 2. A) Percent phytoplankton community composition ([taxon-specific biomass/total biomass] × 100) along the southern transect in May. B) Percent phytoplankton community composition along the southern transect in July. The horizontal axis represents the distance from shore of each station along the transects.

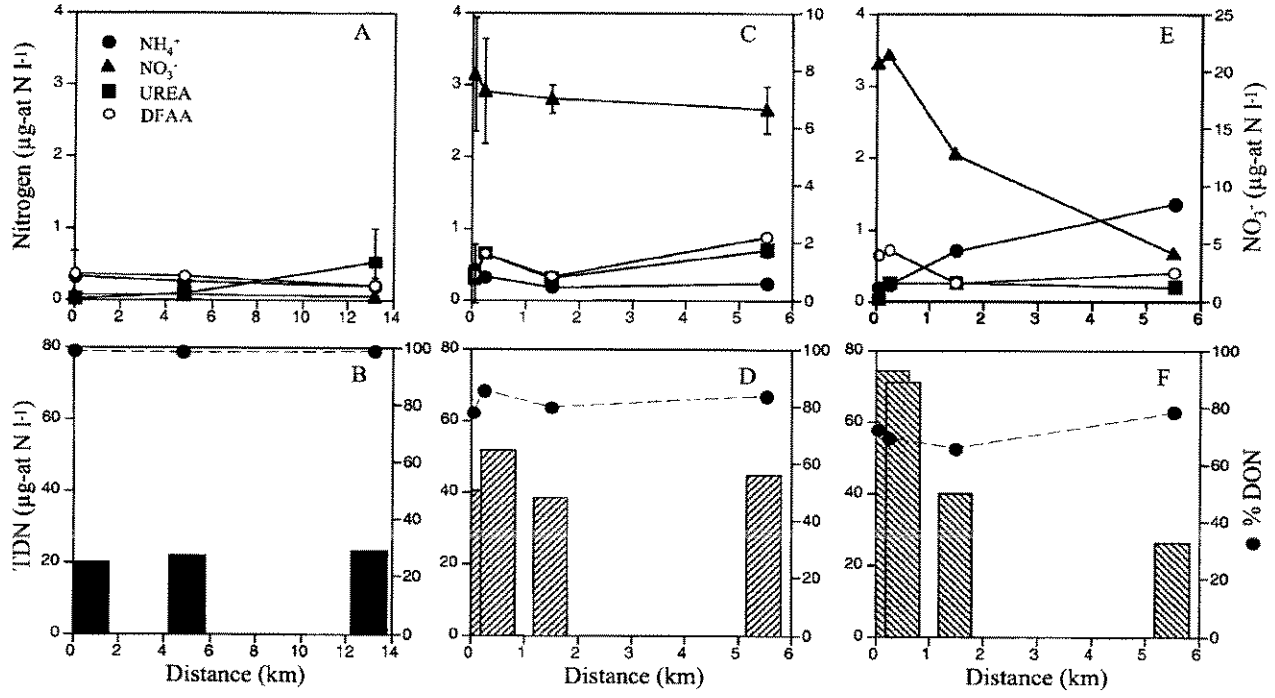

Fig. 3. Concentrations of NH_4^+, NO_3^-, urea, and DFAA (A), and DON concentration as a percent of TDN concentration (B) along the northern transect (A–B), along the southern transect pre-plume (C–D), and along the southern transect post-plume (E–F). The scale on the right in C and E represents concentrations of NO_3^-.

represented by *Monoraphidium contortum, Oocystis* spp., *Scenedesmus* spp., *Hemiselmis virescens, Plagioselmis prolonga,* and *Teleaulax* spp., were also present in lower abundance (Fig. 2b).

Nitrogen Concentrations

Spring

Along the northern transect, concentrations of DIN, urea, and DFAA were below 0.5 µg-at N l^{-1} (Fig. 3a). Concentrations of TDN averaged 18.6 ± 0.6 µg-at N l^{-1} and the DON pool represented close to 100% of the TDN pool (Fig. 3b).

In the southern part of the Gulf, the DIN pool was approximately 30 times greater than in the northern part due to elevated NO_3^- concentration (Fig. 3c). During the 4 d the southern transect was sampled, there was an intrusion of nutrient rich plume water from the Daugava River. The two distinct sampling regimes are identified as pre-plume (May 13; Fig. 3c,d) and post-plume (May 16; Fig. 3e,f), respectively. Pre-plume TDN concentrations varied between 38–51 µg-at N l^{-1} and DON represented 77–85% of the TDN pool (Fig. 3d). Following the nutrient plume intrusion NO_3^- increased 3-fold and a gradient in TDN was established ranging from 74 µg-at N l^{-1} inshore to 26 µg-at N l^{-1} offshore. Concentrations of DON represented 65–80% of the TDN pool post-plume (Fig. 3f). Using the average concentration of the pre-plume and post-plume periods, TDN concentration in the southern region was 2.3-fold higher than in the northern region during spring.

Summer

Concentrations of NH_4^+, urea, DFAA, and NO_3^- ranged from 0.1–0.7 µg-at N l^{-1} along the northern transect (Fig. 4a). As during spring, DON comprised the largest dissolved nitrogen pool, varying between 90–98% of TDN (Fig. 4b). Concentrations of NH_4^+, urea, DFAA, and NO_3^- in the southern part were similar to those in the northern part and were consistently < 0.7 µg-at N l^{-1} (Fig. 4c). In both parts of the Gulf, concentrations of DON decreased by about half from inshore to offshore from an initial value of ~40 µg-at N l^{-1} (Fig. 4b,d).

Nitrogen Uptake

Spring

In May, DIN contributed most to total nitrogen uptake (NO_3^- uptake was not measured along the northern transect in May). Following the plume intrusion, rates of NH_4^+ and NO_3^- uptake increased significantly compared to pre-plume rates along the southern transect (Fig. 5a). Rates of NH_4^+, urea, and DFAA uptake did not differ significantly between the northern and southern tran-

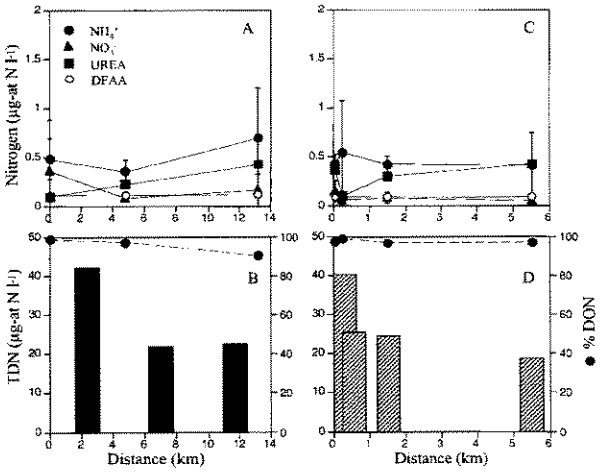

Fig. 4. Dissolved inorganic and organic nitrogen concentrations and DON as a percent of TDN along the northern transect, July 24 (A–B) and along the southern transect, July 31 (C–D).

sects prior to the nutrient plume intrusion (Fig. 5a). Rates of urea uptake ranged from 0.027–0.042 µg-at N l^{-1} h^{-1}, DFAA ranged from 0.045–0.067 µg-at N l^{-1} h^{-1}, NH_4^+ ranged from 0.063–0.097 µg-at N l^{-1} h^{-1}, and NO_3^- uptake ranged from 0.1–0.16 µg-at N l^{-1} h^{-1}.

Summer

In July, uptake of NH_4^+ (0.3–0.35 µg-at N l^{-1} h^{-1}) contributed most to total nitrogen uptake along both transects (Fig. 5b). With the exception of NH_4^+, uptake of all measured nitrogen substrates were greater in the southern compared to the northern part of the Gulf at this time (Fig. 5b). Urea uptake differed most between sites, with rates averaging < 0.05 µg-at N l^{-1} h^{-1} for the northern region and > 0.20 µg-at N l^{-1} h^{-1} for the southern region. In the northern region, the uptake of NH_4^+ was 3 times higher in July than in May, while uptake of urea was similar in May and July (Fig. 5a,b). In the southern region, the rate of NH_4^+ uptake in July was 4 times higher than the pre-plume rate measured in May, while it was similar to the uptake rate measured post-plume. The uptake of urea in July increased about 5-fold above the rate measured in May (Fig. 5a,b). Rates of NO_3^- and DFAA uptake were lower in July relative to May, representing only 6–8% of total nitrogen uptake along both transects (Fig. 5b).

During July, the pattern of nitrogen utilization differed between the northern and southern regions of the Gulf. Along the northern transect, NH_4^+ uptake consistently represented 80% of total nitrogen uptake while the remaining 20% of uptake was divided equally between the other nitrogen substrates (Fig. 6a). Along the southern transect, uptake of urea increased in importance going offshore, contributing equally with NH_4^+ uptake to total nitrogen uptake at the offshore station. Uptake of DFAA exhibited the opposite trend, decreasing in importance going offshore (Fig. 6b). The uptake of NO_3^- as a percent of total uptake remained constant along both transects.

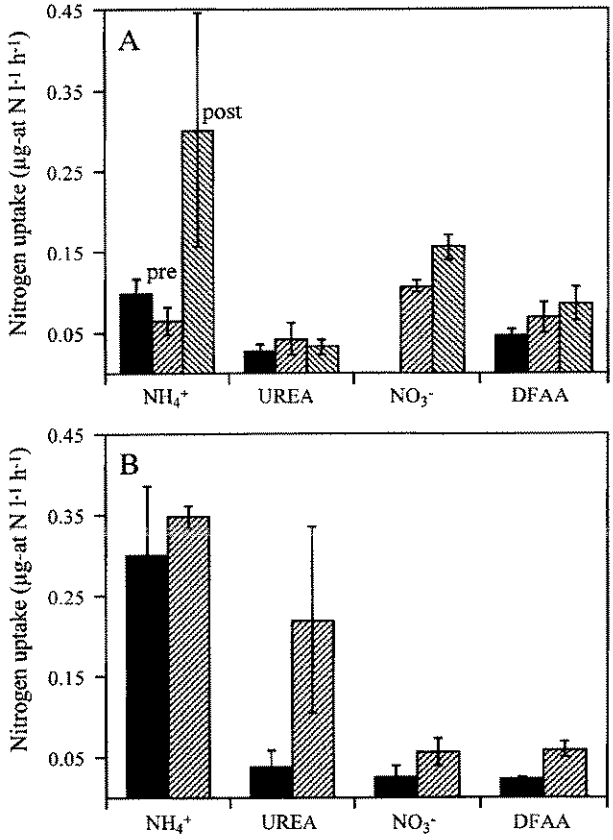

Fig. 5. Rates of uptake of NH_4^-, urea, NO_3^-, and DFAA in (A) May and (B) July. Black bars represent the northern transect and the stippled bars represent the southern transect pre-plume (pre) and post-plume (post), respectively. Error bars represent the standard deviation of 4 stations along the transect.

Size-fractionated uptake of nitrogen in July demonstrated that most of the DFAA, urea, and NH_4^+ was incorporated by organisms < 5 µm (Fig. 6c,d), except at the offshore station along the northern transect where half of the reduced nitrogen was taken up by > 5 µm phytoplankton. In the case of NO_3^- uptake, 80% was attributable to plankton > 5 µm at the offshore station along the northern transect, while 55% of NO_3^- uptake was by plankton > 5 µm at the inshore station (Fig. 6c). By contrast, along the southern transect 50% of NO_3^- uptake was due to plankton > 5 µm at the offshore

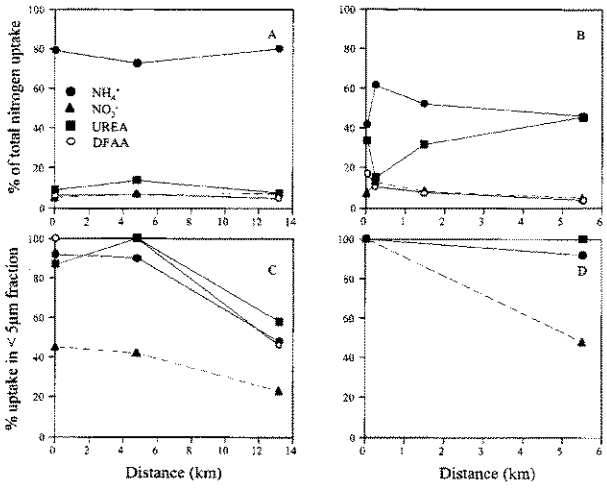

Fig. 6. Uptake of each nitrogen substrate as a percent of total nitrogen ($\rho_{NH4+} + \rho_{urea} + \rho_{NO3-} + \rho_{DFAA}$) uptake in July along the (A) northern transect and (B) southern transect. Percentage of NH_4^+, urea, DFAA, and NO_3^- uptake due to phytoplankton in the < 5 µm size fraction in July along the (C) northern transect and (D) southern transect.

station and close to 100% of NO_3^- uptake was by plankton < 5 µm at the inshore station (Fig. 6d).

Discussion

Eutrophication in the Gulf of Riga

The Gulf of Riga is considered a relatively eutrophic system, fueling annual primary productivity of 350 g C m^{-2} (Andrushaitis et al. 1992). During the summer months, most of the primary productivity in this region is attributable to cyanobacteria (Balode and Purina 1996). Recent increases in abundance of both nitrogen and non-nitrogen fixing cyanobacteria have been noted in the Gulf of Riga (Kahru et al. 1994; Baloda and Purina 1996), concurrent with decreases of 44–85% in surface water NO_3^- concentrations (Yurkovskis et al. 1996, 1999). Decreased concentrations of NO_3^- may have occurred as a consequence of reduced applications of commercial fertilizer and decreased water discharge after 1988 (Laznik et al. 1999; Vagstad et al. 2000). It has been hypothesized that changes in land-use practices and in the hydrography of the region have resulted in shifts in the quality of dissolved nitrogen and in the changes in community structure (Maestrini et al. 1997).

Terrestrial runoff and riverine input of both inorganic and organic nutrients to coastal regions may be considerable (Meybeck 1982). The Daugava River has been shown to be a major source of DON to the Gulf of Riga (Maestrini et al. 1999; Tamminen and Seppälä 1999), which in turn has a significant impact on the microbial community of the southern region (Jørgensen et al. 1999). In the Baltic proper, DON constitutes about 50% of the total nitrogen input (Stålnacke 1996), and our results demonstrate that surface water DON concentrations in the Gulf comprises greater than 85% of total nitrogen in spring and summer. The extent to which this pool of nitrogen may contribute to eutrophication is of considerable interest.

Comparison of the Northern and Southern Regions

The difference in average TDN between the northern, 23 ± 3 µM, and the southern, 41 ± 5 µM, regions agrees with previous studies indicating that the Daugava River is a significant source of nitrogen to the Gulf of Riga. During this study, differences in TDN concentrations were reflected in a 2-fold higher phytoplankton biomass in the south compared to the north. Likewise, total nitrogen uptake was 2-fold higher in the southern region compared to the northern region in both spring (assuming NO_3^- uptake to be 38% of total uptake along both transects) and summer.

In the summer, significantly higher rates of DFAA and urea uptake in the southern region coincided with accumulation of biomass in the < 5 µm fraction. Cyanobacteria and small diatoms comprised most of the chl *a* in this fraction. Although filamentous nitrogen-fixing cyanobacteria were also present, colonial cyanobacteria comprised the larger proportion of total cyanobacteria, particularly at the innermost stations. As with most size-fractionation experiments, there could have been some filtration artifacts pushing through filaments and breaking apart loose colonies of cyanobacteria such as *Snowella* and *Microcystis*. This could explain why virtually all of the measured chl *a* was in the < 5 µm fraction. We suggest that DFAA and urea, comprising over 40% of nitrogen uptake, may have contributed substantially to the chlorophyll standing stock at this time.

Uptake of DON

Dissolved organic nitrogen may support phytoplankton growth by several mechanisms. Qualitative evidence for direct uptake of radiolabeled DFAA by phytoplankton in the field exists (Wheeler et al. 1974; Paerl 1991). More generally, increases in phytoplankton cell numbers during a bloom can be mass-balanced by a decline in the DON pool (Berman 1997; LaRoche et al. 1997). Culture studies indicate that phytoplankton utilize DFAA mainly by direct uptake (Neilson and Larsson 1980; Flynn and Butler 1986; Antia et al. 1991; Palenik and Henderson 1997) and cell-surface deamination (Palenik and Morel 1990a,b). Finally, heterotrophic mineralization of DON releasing in-

TABLE 3. Percent nitrogen-specific uptake as a function of percent biomass of individual phytoplankton taxa (n = 4), southern transect July 1996. CYANO = *Cyanophyceae*, PRASINO = *Prasinophyceae*, DIATOM = *Bacillariophyceae*, DINO = *dinophyceae*, CRYPTO = *Cryptophyceae*, DFAA = dissolved free amino acids.

DFAA = 0.36(CYANO) − 7.2	$r^2 = 0.97$	$p < 0.01$
DFAA = 0.70(PRASINO) + 6.0	$r^2 = 0.94$	$p < 0.05$
DFAA = −0.26(DIATOM) + 18.8	$r^2 = 0.99$	$p < 0.01$
DFAA = −3.0(DINO) + 26.8	$r^2 = 0.93$	$p < 0.05$
UREA = −12.5(CRYPTO) + 95	$r^2 = 0.99$	$p < 0.001$

organic nitrogen indirectly supports phytoplankton growth.

It is difficult to attribute the utilization of a specific nitrogen substrate to a particular phytoplankton group in bulk incubation experiments. Even using size fractionation, the differentiation of phytoplankton groups as well as the differentiation between phytoplankton and bacteria is less than precise (cf., Wheeler and Kirchman 1986; Pantoja and Lee 1994; Hoch and Kirchman 1995). With the availability of phytoplankton compositional data, we conducted regression analysis to determine the relationships between phytoplankton groups and nitrogen-specific uptake rates. This analysis was conducted for the summer period, along the southern transect where the variability in phytoplankton composition was observed. Cyanobacteria and prasinophytes were positively correlated with the uptake of DFAA, while diatoms and dinoflagellates were negatively correlated with the uptake of DFAA (Table 3). Diatoms and dinoflagellates correlated with uptake of urea between stations 2 and 4 ($r^2 = 0.91$), but when station 1 was included, the relationship was not significant. The high rate of urea uptake at the innermost station suggests that cyanobacteria and prasinophytes also utilized urea. Cryptophytes were negatively correlated with uptake of urea suggesting they did not significantly contribute to the uptake of this nitrogen form along the transect relative to other groups (Table 3). Together with chlorophytes, the cryptophytes comprised a minor portion of the total phytoplankton community and did not significantly influence nitrogen uptake. Uptake of NH_4^+ did not vary specifically with any phytoplankton group probably because it was utilized by all groups.

Our results indicated that the northern part of the Gulf differed substantially from the southern part in terms of phytoplankton size structure and nitrogen assimilation pattern. In the northern part, up to 67% of chl *a* was in the > 5 μm size fraction (station 4) corresponding with the fraction that represented the majority of NO_3^- uptake. This agrees with a number of studies showing the abundance of larger plankton such as diatoms to be related to uptake of NO_3^- (Glibert et al. 1982; Price et al. 1991; Collos et al. 1992, 1997; Landry et al. 1997). However, of the reduced nitrogen (NH_4^+, urea, and DFAA), 50% of uptake occurred by phytoplankton > 5 μm. Larger phytoplankton such as diatoms, dinoflagellates (cf., Wheeler et al. 1974), and filamentous cyanobacteria possibly mediated uptake of DFAA in this fraction, occurring at slower rates relative to the southern part of the Gulf.

Utilization of organic nitrogen by cyanobacteria and other phytoplankton may be particularly important when DIN is depleted. In the summer, the DON pool can exceed DIN by orders of magnitude (Mopper and Lindroth 1982; Jackson and Williams 1985; LaRoche et al. 1997). Under these conditions, low molecular weight organic compounds continually regenerated from the bulk DON pool by heterotrophic microorganisms (Cho et al. 1996; Berman et al. 1999; Cottrell and Kirchman 2000) may benefit closely associated autotrophs (Antia et al. 1980). In the case of the Gulf of Riga, Maestrini et al. (1999) demonstrated the importance of DON for the growth of the cyanobacterium *Microcystis* sp. These authors were able to stimulate growth by adding ultra-filtered, riverine dissolved organic matter to natural phytoplankton from the southern part of the Gulf (Maestrini et al. 1999). DON may also be utilized by cyanobacteria which have the potential to fix nitrogen. Although the potential to fix nitrogen exists in several species of cyanobacteria (Mitsui et al. 1986), active fixation is an energy expensive process (Raven 1988) subject to control by the availability of reduced nitrogen (Luque et al. 1994; Bradley and Reddy 1997). Therefore, the proportion of nitrogen fixed to that taken up directly is a small part of total nitrogen assimilated by species such as *Aphanizomenon* sp. (Sörensson and Sahlsten 1987). In line with this reasoning, a study by Berman (1997) observed that *Aphanizomenon* sp. may significantly deplete the DON pool during blooms.

Equally important, cyanobacteria and other small-sized phytoplankton may also have benefited from nitrogen reminerlization by heterotrophic bacteria, as evidenced by differences in incorporation of amino acids using ^{15}N-labeled DFAA (this study) and ^{14}C-labeled DFAA (Jørgensen et al. 1999). Using net rates of bacterial productivity measured along the southern transect in 1996 (Zweifel 1999) and simultaneous net uptake of ^{14}C-DFAA (Jørgensen et al. 1999), DFAA were calculated to meet on average 130% (spring) and 80% (summer) of the bacterial nitrogen demand, assuming C:N ratios of 3.2 and 5 of amino acids and bacteria, respectively. However, the net ^{14}C-DFAA

uptake did not agree with the ^{15}N-DFAA uptake, as the former was found to on average 6 times (summer) and 4 times (spring) below the present net rates of ^{15}N-DFAA uptake. This discrepancy may be explained by a low bacterial growth efficiency. In support of this, the DOC growth efficiency was low in the southern part of the Gulf, ranging from 6.5% in summer to about 25% in spring (Zweifel 1999). Assuming correspondingly low growth efficiencies of DFAA, the bacterial gross nitrogen uptake would have been 5-fold to 12-fold higher than the bacterial nitrogen demand. If so, a significant release of nitrogen by the bacteria must have occurred, supplying other planktonic organisms such as cyanobacteria with nitrogen. Thus, when relating uptake of ^{15}N-DFAA to cyanobacterial production, the pathway of nitrogen may both have been direct and indirect (via heterotrophic bacteria).

Urea was also an important nitrogen source to the phytoplankton, but uptake varied substantially on a temporal and seasonal basis. Rates were higher in summer than in spring, and higher in the southern part compared to the northern part of the Gulf. Even in the southern region, rates measured in spring represented < 15% of uptake during summer. It can be assumed that variability in uptake of urea was closely associated with the spatial and temporal variability in production regulated by factors such as temperature and material flux to sediments. Urea is typically rapidly mineralized from dissolved and particulate organic matter, both in the water column and in sediments, either as an end product in itself or as an intermediate preceding further hydrolysis to NH_4^+ (Lomstein et al. 1989; Pedersen et al. 1993a,b; Cho and Azam 1995; Cho et al. 1996; Berman et al. 1999). Mineralization is coupled with phytoplankton assimilation, occurring at high rates in eutrophic, estuarine regions (McCarthy 1972; Kristiansen 1983; Sörensson and Sahlsten 1987; Glibert et al. 1991; Tamminen and Irmisch 1996; Berg et al. 1997). Therefore, greater nutrient inputs at the southern end of the Gulf may have been a contributing factor to the heterogeneity in urea uptake across the Gulf.

Summary

Phytoplankton nutrition and biomass in the Gulf of Riga were significantly influenced by the Daugava River. In spring, pulsed inputs of inorganic nitrogen resulted in rapid increases in rates of nitrogen uptake. In summer, inorganic nitrogen inputs were substantially lower and DON uptake was proportionally more important than in spring. Uptake of DON was correlated with small-sized phytoplankton, particularly cyanobacteria. These findings add to our growing recognition that riverine dissolved organic matter stimulates heterotrophic production (e.g., Seitzinger and Sanders 1997; Stepanauskas et al. 1999; Wikner and Hagström 1999), contributing to the production of certain phytoplankton bloom species (e.g., Berg et al. 1997; Berman 1997; Maestrini et al. 1999).

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