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*Published in:*  
Ecological Monographs

*DOI:*  
[10.1890/0012-9615\(2002\)072\[0579:SIORNT\]2.0.CO;2](https://doi.org/10.1890/0012-9615(2002)072[0579:SIORNT]2.0.CO;2)

2002

[Link to publication](#)

*Citation for published version (APA):*  
Stepanauskas, R., Jorgensen, N. O. G., Eigaard, O. R., Zvikas, A., Tranvik, L. J., & Leonardson, L. (2002). Summer inputs of riverine nutrients to the Baltic Sea: Bioavailability and eutrophication relevance. *Ecological Monographs*, 72(4), 579-597. [https://doi.org/10.1890/0012-9615\(2002\)072\[0579:SIORNT\]2.0.CO;2](https://doi.org/10.1890/0012-9615(2002)072[0579:SIORNT]2.0.CO;2)

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## SUMMER INPUTS OF RIVERINE NUTRIENTS TO THE BALTIC SEA: BIOAVAILABILITY AND EUTROPHICATION RELEVANCE

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**Abstract.** Most nitrogen and phosphorus transported by world rivers to the oceans is associated with dissolved organic matter. However, organic matter as a potential source of N and P has hitherto been largely neglected in studies of coastal microbial food webs. We examined 50 rivers, draining a major part of the Baltic Sea watershed, with respect to summer concentrations, chemical composition, and biological availability of N and P. The broad spectrum of rivers studied enabled us to assess whether the input of terrigenous organic matter can be an important nutrient source, at various levels of anthropogenic loading of inorganic N and P.

Concentrations of total N and P ranged from 9 to 220  $\mu\text{mol/L}$  and from 0.14 to 5.56  $\mu\text{mol/L}$ , respectively, with the highest concentrations in the southern part of the Baltic Sea drainage area and in several rivers on the Finnish western coast. Urea and dissolved combined amino acids (DCAA) each constituted 4–20% of dissolved organic nitrogen (DON), while dissolved free amino acids (DFAA) made up <3% of DON. The contribution of urea and amino acids to the DON pool was inversely correlated with DON concentration. Bacterial regrowth bioassays in selected rivers demonstrated that ~30% of DON and ~75% of dissolved organic phosphorus (DOP) was potentially available to the indigenous bacterial assemblage of the Baltic Sea, and hence susceptible to mineralization within the pelagic food web. Our study is among the first to demonstrate that bacterioplankton are able to utilize a major part of DON and DOP from a broad spectrum of natural waters.

The C:N ratio, absorbance spectra, and fluorescence properties of the organic matter suggest that the observed high bioavailability of DON and DOP was due to a large contribution of organic matter from riverine primary production compared to the humic matter derived from terrestrial vascular plants. In addition, algal and bacterial cells dominated the transport of particulate organic material, further enhancing productivity of coastal waters. No correlations were found between DON bioavailability and the fraction of DON bound in urea and amino acids, indicating a utilization of other N compounds (e.g., amides) by the bacteria.

We estimate that the input of summer riverine N to the Baltic Sea consists of 48% dissolved inorganic N, 41% DON, and 11% particulate N. Corresponding values for phosphorus are 46%, 18%, and 36% of dissolved inorganic P, DOP, and particulate P, respectively. During the thermal summer stratification, when freshwater inputs are trapped in the surface layer, rivers contribute ~30% of N and ~5% of P needed to support the export production (plankton sedimenting out of the photic layer) in the Baltic Sea. The high availability to bacteria suggests that DOP is a major stimulator of pelagic productivity in the P-limited northern part of the Baltic Sea. Based on reported concentrations in other areas, we suggest that the global contribution of riverine organic N and P to the primary production of coastal waters is comparable to the contribution of inorganic nutrients.

**Key words:** Baltic Sea; bioassay; bioavailability; biogeochemistry; dissolved organic nitrogen, DON; dissolved organic phosphorus, DOP; eutrophication; nitrogen; nutrients; riverine; contribution to coastal waters; phosphorus; riverine transport.

Manuscript received 26 April 2000; revised 8 May 2001; accepted 21 June 2001; final version received 18 October 2001.

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### INTRODUCTION

Dissolved organic matter (DOM) is a widely recognized source of energy for aquatic biota. Bacteria in the water column and their predators benefit from or-

ganic matter derived from the indigenous primary producers (Pomeroy 1974, Azam et al. 1983). In addition, bacteria often form a link between terrestrial and aquatic food webs, as they utilize organic matter originating from terrestrial plants in the watershed. The role of bacteria in this transfer of energy between different ecosystems has received considerable interest (Carpenter and Pace 1997, Hessen and Tranvik 1998). In addition to being an energy source, the DOM is also an important potential vector of organically bound nutrients between terrestrial and aquatic ecosystems (Bushaw et al. 1996, Seitzinger and Sanders 1997, Carlsson et al. 1999, Stepanauskas et al. 1999a, b, 2000b). Accordingly, about 70% of all nitrogen transported by rivers into coastal marine areas is associated with the DOM (Meybeck 1982). Although this pathway potentially has a highly significant impact on the nutrition of aquatic biota and on connections between major ecosystems, it has hitherto been largely neglected in studies of coastal marine microbial food webs.

It is well known that bacteria and some species of algae are able to utilize simple organic nitrogen compounds, e.g., urea (Eppley et al. 1971) and amino acids (Palenik and Morel 1990). The direct utilization of organic phosphorus compounds by algae is well documented (Boström et al. 1988, Berman et al. 1991) and extracellular enzymes for the acquisition of phosphorus from organic sources (phosphatases) are ubiquitous in the aquatic environment (Münster and De Haan 1998). Still, very little is known about the microbial availability of bulk dissolved organic nitrogen and phosphorus (DON, DOP).

Recent reports show that 2–70% of riverine DON is potentially bioavailable, as determined by bacterial regrowth bioassays (Seitzinger and Sanders 1997, Carlsson et al. 1999, Stepanauskas et al. 1999b, 2000b). Nitrogen in terrigenous dissolved humic substances may become available for algal growth after microbial assimilation and mineralization into inorganic N species (Carlsson et al. 1999). Thus, after mineralization within the aquatic food web, DON may contribute significantly to the supply of inorganic N to algae. Possibly, riverine DOP contributes in an analogous way to algal nutrition, although there is a lack of corresponding studies of bulk DOP bioavailability. If this is the case, riverine DOM contributes to the occurrence of marine coastal algal blooms as a source of both N and P, considered to be the major limiting nutrients for phytoplankton growth (Ryther and Dunstan 1971, Granéli et al. 1990).

In this paper we employ a new bioassay method to quantify the bioavailability of DON and DOP in a large number of rivers discharging to the Baltic Sea. During the last century, the Baltic Sea has been subjected to a fourfold increase in the loading of N and an eightfold increase in the loading of P, caused by anthropogenic inputs (Larsson et al. 1985). In addition, the Baltic Sea receives high amounts of terrigenous DOM from the

northern boreal forest region (Stålnacke et al. 1999). The choice of study area enabled us to assess whether a considerable input of terrigenous organic matter can still be an important nutrient source, despite high anthropogenic loading of inorganic N and P. We examined the potential bioavailability of riverine organic N and P loaded to the Baltic Sea, and evaluated their role in stimulating coastal primary production. Our results suggest that organic N and P from relatively pristine areas may play a significant role in the development of coastal blooms in the Baltic Sea, and probably also in other coastal regions.

## MATERIALS AND METHODS

### *Study area*

The Baltic (surface area =  $0.37 \times 10^6$  km<sup>2</sup>, excluding Kattegat and Skagerrak) is a semi-enclosed, brackish sea bordering northern Europe. It is exceptionally susceptible to riverine nutrient inputs due to its large drainage area ( $1.63 \times 10^6$  km<sup>2</sup>), small mean depth (54 m), and limited water exchange with the Atlantic Ocean (Wulff et al. 1990, Bergström and Carlsson 1994). The drainage basin of the Baltic Sea (Fig. 1) includes 14 countries and has ~85 million inhabitants (Sweitzer et al. 1996). High population density and intensive agriculture characterize the southern part of the basin, while the northern part is almost entirely covered by boreal forest. Riverine discharge is responsible for 60% and 70% of the annual total N and P inputs to the Baltic Sea, respectively (Stålnacke et al. [1999] and references therein). Other sources of nutrients include atmospheric deposition, N<sub>2</sub> fixation, and coastal point sources.

Typically from May through October a thermocline is formed at 10–20 m in the Baltic Sea (Eilola and Stigebrandt 1998). In this paper we refer to these six months as “summer.” More than half of the annual net primary production occurs during this period (Elmgren 1989), and nuisance algal blooms during late summer are a major environmental problem (Granéli et al. 1990). Since the Secchi-disk depth is only ~6 m deep during summer (Cederwall and Elmgren 1990), photosynthesis occurs solely in the well-mixed surface layer.

### *Sampling strategy*

Our sampling approach was to collect a single water sample from a maximal number of rivers that account for a major part of the Baltic Sea drainage area and represent diverse types of environmental conditions. The measured concentrations of inorganic and total nutrients were compared to and calibrated with the long-term averages for the entire summer period (see *Use of historic nutrient-transport data*, below). Water was collected during an expedition lasting 23 June–26 July 1999, when all rivers were near their base flow due to the virtual absence of precipitation in the region during May–July 1999.

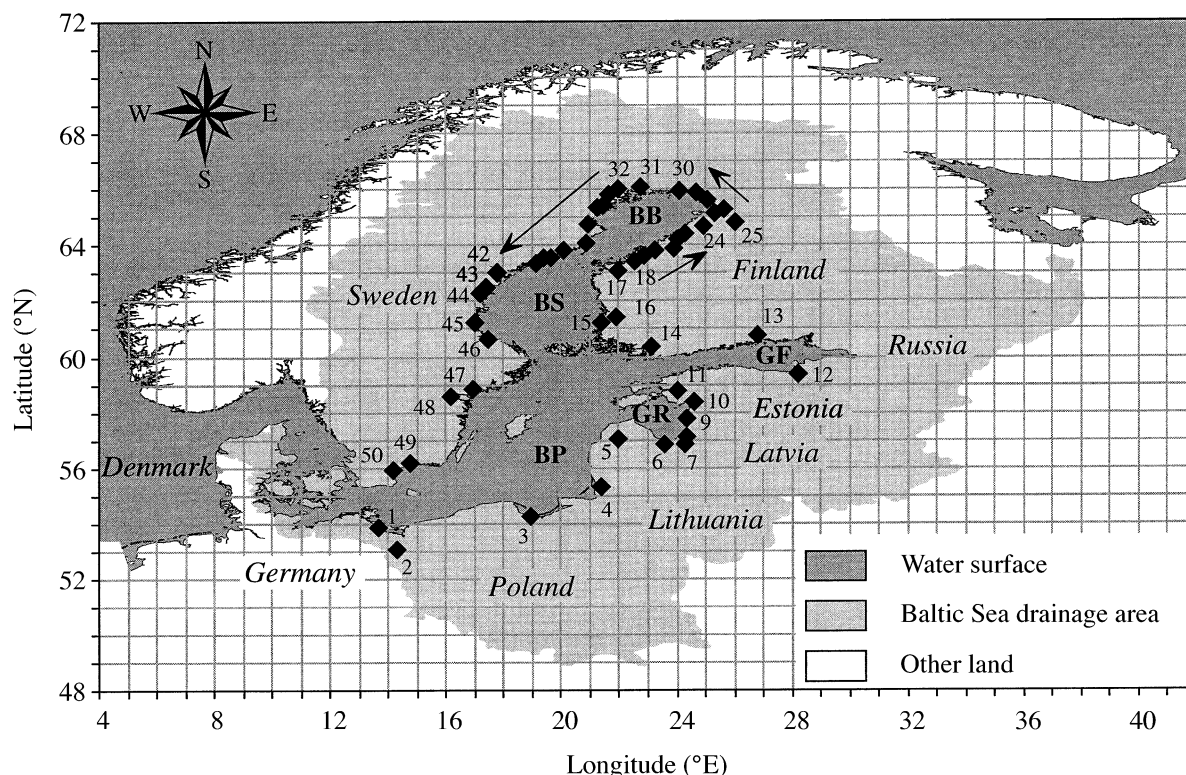


FIG. 1. Map of the 50 river sampling points (black diamonds) from the rivers that account for a major part of the Baltic Sea drainage. For river names, see Appendix A. Subbasins of the Baltic Sea: BB = Bothnian Bay, BS = Bothnian Sea, GF = Gulf of Finland, GR = Gulf of Riga, BP = Baltic Sea proper. One longitude degree corresponds to ~56 km, and one latitude degree corresponds to ~111 km in the center of the map.

The study was restricted to the rivers discharging to the Baltic Sea proper, the Gulf of Riga, the Gulf of Finland, the Bothnian Sea, and the Bothnian Bay (Fig. 1, Appendix A). Thus, rivers discharging to the Danish Straits, the Kattegat, and the Skagerrak were omitted, since the latter areas are geographically, hydrologically, and biologically distinct from the main Baltic Sea body.

First, 35 rivers representing the major annual discharge to the Baltic Sea were selected (Appendix B). Rivers Neva and Pregol were omitted due to administrative obstacles to collect samples in Russia. Second, 15 smaller rivers were included, representing widely different parts of the drainage area and covering a broad range of river size. We selected only rivers where we had access to historical data on discharge and nutrient concentrations. River Peene was included despite the lack of available historical data, since it is the largest river discharging from Germany to the Baltic Sea. Selected rivers, excluding Peene, accounted for 64% of total N, 69% of nitrate, 51% of total P, and 46% of phosphate summer loads from the entire watershed of the Baltic Sea, respectively (Baltic drainage database [BDDDB, Stålnake 1996]; see *Use of historic nutrient-transport data*, below).

Dissolved organic nitrogen (DON) and dissolved or-

ganic phosphorus (DOP) bioavailability assays were run for 15 of the selected rivers, in which dissolved inorganic N (DIN) was  $<15 \mu\text{mol/L}$  and  $\text{DON:DIN} > 2$ . Higher concentrations of inorganic nutrients impair the accuracy of such bioassays (Stepanaukas et al. 1999b). Information on the DIN and DON pools were provided either from historical data or from actual measurements of N concentrations in the rivers prior to the bioassays.

#### Collection of riverine water

A single sample was collected from each of the selected rivers. Sampling sites were chosen as close to the river mouths as possible, but upstream from the estuarine mixing zone (based on conductivity measurements in the field) and upstream from towns situated off the seashore (Fig. 1, Appendix A). Water was collected in a polyethylene bottle from bridges in the middle of the main stream, 1 m above the bottom. In small and shallow rivers, surface water samples were taken from the shore.

Immediately after collection, water was filtered through GF/F glass-fiber filters (Whatman International, Kelt, UK) followed by  $0.2\text{-}\mu\text{m}$  pore size Supor filters (Gelman Sciences, Ann Arbor, Michigan, USA). GF/F filters were stored frozen for the analyses of particulate N and P, and chlorophyll *a*. For absorbance and fluo-



rescence analysis, filtered water was stored refrigerated in acid-washed polyethylene vials. For dissolved organic carbon (DOC) and total dissolved nitrogen analysis, filtered water was stored frozen in pre-combusted glass vials with Teflon lids. For bacterial counts, unfiltered water was fixed with 2% (final concentration) borax-buffered particle-free formaldehyde and stored refrigerated. For the remaining analyses, filtered water was stored frozen in acid-washed polyethylene vials.

#### *DON and DOP bioavailability assays*

We determined the potential bioavailability of DON employing N-limited bacterial regrowth cultures (Stepanauskas et al. 1999a, b, 2000b). An analogous protocol employing P-limited cultures was introduced for DOP bioavailability determination.

Water for bacterial inoculum was collected from the shore of the Baltic Sea at Stenshuvud (55°39' N, 14°16' E) one month prior to the expedition, and was stored at 20°C in the dark. The long pre-incubation of the inoculum aimed to minimize fluctuations in bacterial abundance and composition during the expedition. The inoculum was filtered through GF/F glass fiber filter to remove bacterivores immediately before adding it to the cultures.

To prepare bacterial cultures, we added sea salts (Sigma-Aldrich [Saint Louis, Missouri, USA]; final salinity 6.5‰), glucose (to avoid carbon limitation; final concentration 140 µmol/L), and inoculum (5% by volume) to the filtered (as above) riverine water. For each river, 16 aliquots of 125 mL of the cultures were distributed into polystyrene flasks. To ensure N limitation in eight flasks and P limitation in another eight flasks, phosphate (NaH<sub>2</sub>PO<sub>4</sub>, final concentration 30 µmol/L) or nitrate (NaNO<sub>3</sub>, final concentration 175 µmol/L) was added, respectively. Four of the N-limited cultures received nitrate spikes (7 µmol/L, final concentration), and four of the P-limited cultures received phosphate spikes (2.5 µmol/L, final concentration).

Blank treatments were prepared to evaluate N and P contamination from the inoculum, salts, and handling. Thus, four replicate N-limited blanks were prepared by mixing sea salts, glucose, phosphate, and inoculum (as above) with deionized water instead of riverine water. Correspondingly, four replicate P-limited blanks were prepared by mixing sea salts, glucose, nitrate, and inoculum (as above) with deionized water. One full set of blank cultures was initiated at the beginning of the expedition, and another full set was initiated at the end of the expedition. No significant differences in bacterial numbers were found in the two series of blanks, and the mean values were used in the calculations of DON and DOP bioavailability.

All cultures were incubated at 18°–25°C in the dark for 14 d, to ensure that the bacteria reached stationary growth phase. The cultures had pH 8.1 ± 0.2 (means ± 1 SD) in the beginning of incubations and pH 7.9 ± 0.3 at the end of incubations. After a gentle shaking

of the cultures, samples for the determination of cell abundance were taken daily, fixed with borax-buffered, particle-free formaldehyde (final concentration 2%) and stored refrigerated. Cell counts were performed within a month after the sampling.

From each culture, only the highest bacterial abundance reached was used for bioavailability calculations. First, nitrogen content of an average cell in N-limited cultures ( $N_{\text{cell}}$ ) was calculated:  $N_{\text{cell}} = s / (D_{\text{spike}} - D_{\text{river}})$ , where  $s$  is the concentration of the nitrate spike,  $D_{\text{spike}}$  is the average cell abundance in cultures with riverine N and nitrate spikes, and  $D_{\text{river}}$  is the average cell abundance in cultures with solely riverine N. Concentration of potentially bioavailable DON ( $\text{DON}_{\text{PB}}$ ) in the river water was estimated as:  $\text{DON}_{\text{PB}} = N_{\text{cell}} \times D_{\text{river}} - N_{\text{cell}} \times D_{\text{blank}} - N_{\text{i}}$ , where  $D_{\text{blank}}$  is the average cell abundance in N-limited blank cultures, and  $N_{\text{i}}$  is the concentration of inorganic N in the water. In an analogous way, P content of an average cell and the concentration of bioavailable DOP was estimated using cell abundances in P-limited cultures. The estimated average N content per cell in N-limited cultures was 2.45 fmol and was similar to the values (1.89 and 2.28 fmol) from similar studies by Stepanauskas et al. (1999b, 2000b). The estimated average P content per cell in P-limited cultures was 0.189 fmol. Due to insufficient analytical sensitivity of DON and DOP measurements, we did not perform a direct monitoring of the decline of DON and DOP concentrations in the cultures.

To avoid long water storage before the initiation of bioassays, bacterial cultures were set up immediately after water collection, and the incubations were performed in a camper-based mobile laboratory. Exceptions were rivers Nemunas, Venta, Daugava, Gauja, Salaca, Kasari, and Narva. Filtered (as above) water from the latter rivers was stored refrigerated until the end of the expedition (less than one month). Then the water was filtered once more through a 0.2-µm pore size Supor filter and the bioassays were initiated. The second 0.2-µm filtration removed 1–5% of the total N and P.

Bacterial bioassays for DON and DOP bioavailability were set up for a total of 15 rivers. Due to bacterivore (as determined by microscopic observations) or viral infections, some cultures did not exhibit a regular sigmoid growth and did not reach a distinct stationary phase. These cultures were excluded from bioavailability estimates. As a result, DON and DOP bioavailability was determined for 13 and 11 rivers, respectively.

#### *Measurements of bacterial biomass*

Bacterial abundance in the field and bioassay samples was measured using a flow-cytometric method (del Giorgio et al. 1996). We added SYTO-13 stain (50 µmol/L, Molecular Probes [Eugene, Oregon, USA]) and Fluoresbrite Carboxy YG microspheres (1.58 µm diameter, ~3 × 10<sup>5</sup>/mL, Polysciences [Warrington,

Pennsylvania, USA) to 1-mL subsamples and analyzed them with a Becton Dickinson FacSort flow cytometer (Becton Dickinson, Franklin Lakes, New Jersey, USA) at a low sample flow rate ( $\sim 12 \mu\text{L}/\text{min}$ ). The cytometer was controlled with the CellQuest 1.2 software (Becton Dickinson, Franklin Lakes, New Jersey, USA). Bacterial cells and microspheres were separated in a log-log scattergram of green fluorescence intensity (FL1) and side scatter (SSC). Voltages for these parameters were set to 560V and 400V, respectively. Samples were run for 1 min or until 10 000 cells were counted. Bacterial abundance in the samples was calculated using microspheres as an internal standard. The abundance of the microspheres in a stock solution was analyzed by epifluorescence microscopy on a weekly basis.

The flow-cytometric cell abundance measurements of field samples were compared with manual microscopic counts. Microscope slides were prepared by staining 2-mL subsamples with 3 g/L acridine orange on 0.2- $\mu\text{m}$  pore size black polycarbonate membranes (Osmonics, Kent, Washington, USA). A Nikon Labophot-2 epifluorescence microscope equipped with a Nikon Plan Fluor100 $\times$ /1.30 oil immersion lens was used. From each slide, at least 250 cells and 10 fields of view were counted. There was a good agreement between the two methods ( $r^2 = 0.89$ ,  $n = 50$  measurements), although on average flow-cytometric counts were 5% higher than microscopic counts. Similarly, bacterial counts employing SYTO-13 stain and desktop flow cytometers compared well with direct microscopic counts for field samples from diverse lakes (del Giorgio et al. 1996) and for samples from bacterial cultures (Bertilsson et al. 1999, Stepanauskas et al. 1999b), proving it a rigid and convenient method. Bacterial abundance values, measured flow cytometrically, were further used in this study.

The mean bacterial cell volume was determined for field samples. From each microscope slide, prepared as above, five randomly selected 10-bit images were acquired with a Nikon Labophot-2 epifluorescence microscope equipped with a Nikon Plan Fluor100 $\times$ /1.30 oil immersion lens, connected to a Hamamatsu C4742-95 CCD digital camera ( $1280 \times 1024$  active pixels,  $8.58 \times 6.86$  mm sensing area [Hamamatsu Phototronics K.K., Hamamatsu City, Japan] via a Nikon TV-lens (C-0.6 $\times$ ). This gave a final pixel size of 0.09  $\mu\text{m}$ . The camera was controlled by the QED Imaging software (QED Imaging, Pittsburgh, Pennsylvania, USA). Further processing of the pictures was done using IPLab Spectrum 3.1a software (Signal Analytics, Vienna, Virginia, USA). Cell edge detection was performed by applying a Marr-Hildreth operator and thresholding the image to zero, according to Ramsing et al. (1996). Overlapping and out-of-focus cells were excluded manually.

Cell volume ( $V$ ) was calculated as a cylinder with hemispherical ends:  $V = (\pi/4) \times w^2 \times (l - w/3)$ , where  $l$  is the major axis of the best fitting ellipsoid, and  $w$

is the effective diameter, calculated according to the formula:  $w = 2/(\pi - 4) \times [(l^2 + (\pi - 4) \times A)^{0.5} - l]$ , where  $A$  is the area of the bacterial image. The total bacterial biovolume was estimated by multiplying cell abundance (determined flow cytometrically) by the average cell volume (determined by image analysis). Biovolumes were converted to bacterial carbon biomass assuming that C comprises 50% of the bacterial dry mass and according to the biomass-to-biovolume function:  $M = 435 \times B^{0.86}$ , where  $M$  is the dry cell biomass (femtograms per cell) and  $B$  is the bacterial volume in cubic micrometers (Loferer-Kröbächer et al. 1998). The N and P content of bacterial biomass was estimated assuming the cell C:N:P atom ratio to be 50:5:1 (Fagerbakke et al. 1996).

### Chemical analyses

Standard methods were applied to measure nitrate plus nitrite (Wood et al. 1967) and ammonium (Chaney and Marbach 1962), and their sum was defined as dissolved inorganic nitrogen (DIN). Total dissolved nitrogen (TDN) was measured with an ANTEK 9000 high temperature combustion total nitrogen analyzer (ANTEK Instruments, Houston, Texas, USA). Dissolved organic nitrogen (DON) concentrations were calculated by subtracting DIN from TDN. Particulate nitrogen (PN) was analyzed after conversion of material, collected on GF/F filters, to dissolved inorganic phosphorus (DIP) and nitrate by persulfate oxidation (Koroleff 1983). Total nitrogen (TN) concentrations were obtained by summing PN and TDN.

DIP was determined as molybdate-reactive P, while total dissolved phosphorus (TDP) and particulate phosphorus (PP) were analyzed after conversion of dissolved and particulate fractions to DIP by persulfate oxidation (Koroleff 1983). Dissolved organic phosphorus (DOP) was calculated by subtracting DIP from TDP. Total phosphorus (TP) concentration was obtained by summing PP and TDP.

Dissolved organic carbon (DOC) was analyzed by the platinum-catalyzed high-temperature combustion method using a Shimadzu TOC-5000 total carbon analyzer (Shimadzu Scientific Instruments, Columbia, Maryland, USA) after acidification of the samples and purging of inorganic C. Absorbance spectra of dissolved organic matter (DOM) were measured spectrophotometrically (bandpass 1 nm) in a 1-cm cell. The slope of ln-transformed spectra at 280–400 nm ( $A_{280-400}$ ) was determined according to Davies-Colley and Vant (1987). DOM fluorescence upon excitation at 370 nm was measured at 450 and 500 nm (excitation and emission bandwidth 5 nm) in a 1-cm cell with a spectrofluorometer.

Urea was measured according to Price and Harrison (1987). Dissolved free and combined amino acids (DFAA and DCAA) were quantified as fluorescent o-phthalaldehyde derivatives by high-pressure liquid chromatography according to Lindroth and Mopper (1979) and Jørgensen et al. (1993). The following in-

dividual amino acids were detected: asp, glu, asn, ser, gln, his gly, thr, arg, ala,  $\gamma$ -aminobutyric acid (GABA), tyr, met, val, phe, ile, leu, orn, and lys. DCAA were hydrolyzed to individual DFAA by a microwave technique (Jørgensen and Jensen 1997). To convert molar concentrations of amino acids to moles of N, an average of 1.1 N atom per amino acid was assumed. The sum of N bound into urea, DCAA, and DFAA was defined as identified dissolved organic nitrogen (IDON).

Chlorophyll *a* concentration was determined by spectrophotometer after ethanol extraction (Jespersen and Christoffersen (1987). Algal biomass was estimated by using a carbon:chlorophyll *a* ratio 62:1 (Valiela 1995) and the Redfield C:N:P atom ratio, 106:16:1.

#### *Use of historical nutrient-transport data*

A database compiled by Stålnacke (1996) was used in nutrient load calculations; we refer to this database as "BDDDB" (Baltic drainage database). BDDDB contains monthly records of runoff and transport of TN, nitrate, TP, and DIP from more than 100 large rivers discharging to the Baltic Sea for the period 1970–1990. Monitored rivers account for 81% of runoff, 74% of TN, 77% of nitrate, 61% of TP, and 54% of DIP total riverine transport to the Baltic Sea. We calculated mean monthly transport by individual rivers during the 1970–1990 period and used it as a basis for further estimates.

Average summer (May–October) loads of different N and P fractions from the entire Baltic Sea watershed were calculated in the following way:

1) TN, DIN, TP, and DIP loads were obtained by taking the sum of summer loads of BDDDB rivers and dividing it by a corresponding BDDDB coverage coefficient (0.74, 0.77, 0.61, and 0.54, respectively).

2) The loads of DON, PN, urea, DCAA, DFAA, DOP, PP, and bioavailable DON and DOP were calculated for the rivers covered by the expedition, using measured concentrations and BDDDB-based average summer runoff values. The fractions of loads of different organic N and P forms were assumed to have the same distribution in the entire watershed of the Baltic Sea as in the rivers covered by the expedition.

#### *Statistical analyses*

Statview 5.0 software (SAS Institute Inc.) was used for correlation analysis.

Partial least-squares regression analysis (PLS; Eriksson et al. 1995) was performed using The Unscrambler 6.11 software (Camo ASA, Oslo, Norway) in an attempt to explain the variance in the two response parameters, the bioavailability of dissolved organic N and P, by background water chemical parameters. Unlike multiple linear regression, PLS is not sensitive to covariance among the predictor variables and does not require the analytical error in predictor variables to be negligible.

All parameters were autoscaled before use in the

model: the geometric means were centered to zero and all data were normalized for standard deviation. Full cross validation was used in the modeling procedure, one sample being excluded at a time. The response parameter for each excluded sample was then predicted from a PLS model created from the remaining samples. The predictive strength of the model was assessed by linear regressions of measured values in the cross-validation procedure. For this regression  $Q^2$  is the coefficient of determination, i.e., the proportion of variance in the response parameter being explained by the model; RMSEP is the root mean square error of prediction, i.e., the average difference between the measured and predicted values of the response variable.

The bioavailability of DON and DOP were modeled in separate PLS analyses. Initially, all available water chemistry and biological parameters were included as predictor variables in the PLS (concentrations of TN, TP, DIN, DIP, DON, DOP, DOC, PN, PP, urea, DCAA, DFAA, IDON, and chlorophyll *a*; bacterial abundance and biovolume; the proportion of urea, DCAA, DFAA, and IDON in DON; DOC:DON,  $A_{430}$ :DOC,  $A_{280-400}$ , and  $F_{280-400}$ ) (Appendices B, C, and D). Then predictor variables with the least impact on the model (e.g., lowest loading weights) were sequentially excluded until the highest  $Q^2$  was achieved.

#### RESULTS

Concentrations of TN (total N) and TP (total P) in the sampled rivers were 9–220  $\mu\text{mol/L}$  and 0.14–5.56  $\mu\text{mol/L}$ , respectively (Appendix B, Fig. 2). The concentrations were highest in the southern part of the Baltic Sea drainage area, and in several rivers on the Finnish western coast. The lowest concentrations were found in the northern and northwestern rivers, numbers 29–46. Similar patterns emerged for DIN (dissolved inorganic N) and DIP (dissolved inorganic P) concentrations, which constituted 1–69% (mean = 23%) of TN and 1–56% (mean = 18%) of TP (Appendix B). Concentrations of DON (dissolved organic N) and DOP (dissolved organic P) showed less variation among the rivers, and they constituted 24–88% (mean = 63%) of TN and 11–70% (mean = 44%) of TP. As for TN and TP, the concentrations of DON and DOP were lowest in the northern and northwestern rivers, numbers 29–46. The distribution of particulate nitrogen and phosphorus (PN and PP) was more complex, but the highest concentrations occurred in the southern and eastern parts of the Baltic Sea drainage area. PN and PP constituted 3–41% (mean = 14%) of TN and 14–87% (mean = 37%) of TP.

Concentrations of PN (particulate N) and PP (particulate P) correlated strongly with the concentration of chlorophyll *a* (Fig. 3) and with each other (log-transformed values,  $R = 0.84$ ,  $P < 0.001$ ). PN and PP correlations with bacterial biomass were less pronounced (log-transformed values,  $R = 0.53$  and  $0.54$ , respectively,  $P < 0.001$ ). Similarly, the correlation be-



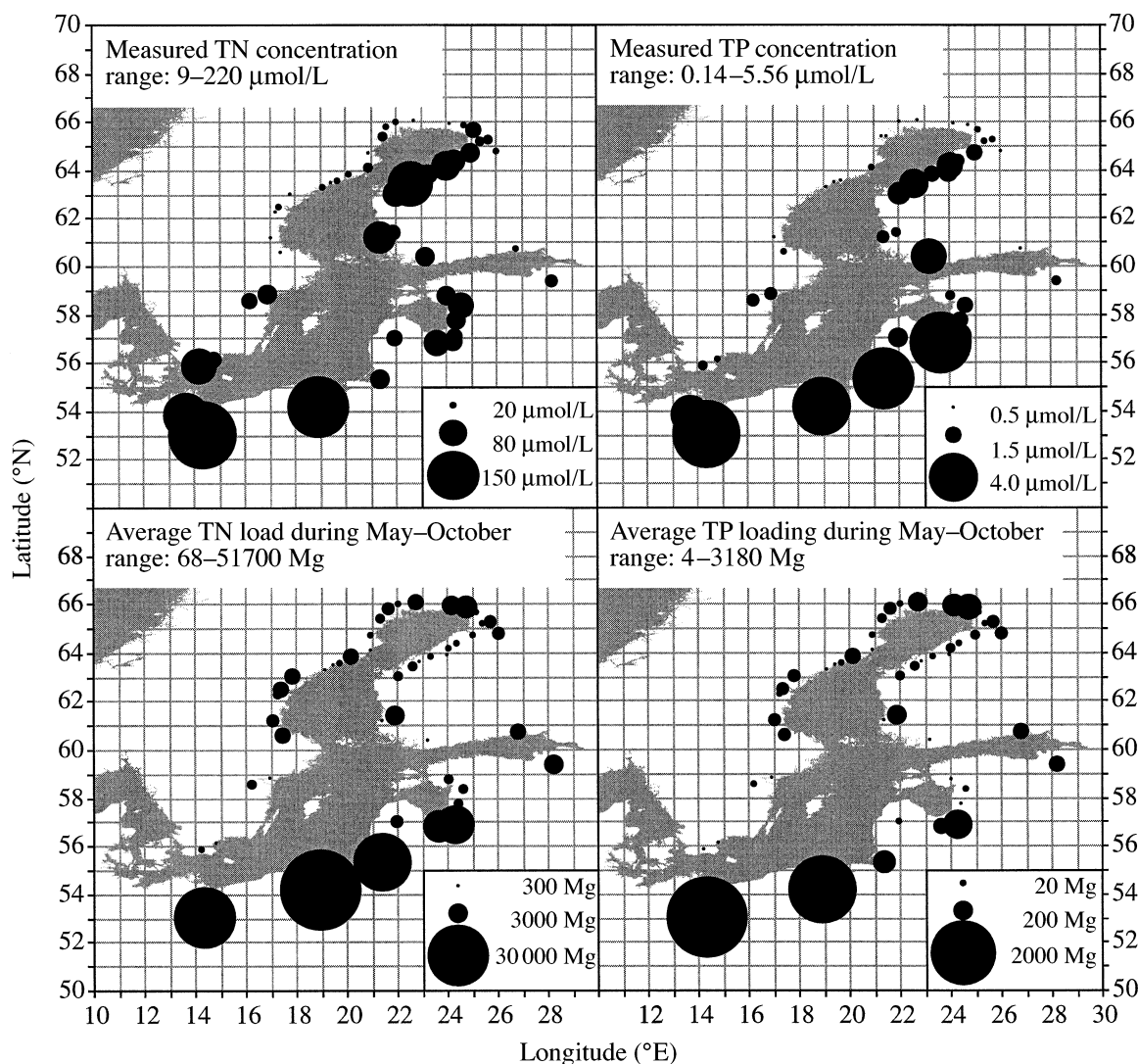


FIG. 2. Concentrations and loads of total nitrogen (TN) and total phosphorus (TP), from rivers discharging to the Baltic Sea. Upper panels: Measured concentrations of TN and TP in the collected river water. Lower panels: Average loads of TN and TP carried by the studied rivers during May–October, extracted from the Baltic drainage database (Stålnacke 1996; see *Materials and methods: Use of historical nutrient-transport data*).

tween the chlorophyll *a* and bacterial biomass was relatively weak ( $R = 0.52$ ,  $P < 0.001$ ). Nitrogen and phosphorus, estimated to be bound into algal and bacterial biomass, accounted for 72–372% of PN and 74–794% of PP (Appendix C). On average, the estimated algal N and P was higher than bacterial N and P by factors 7.4 and 4.5, respectively.

Urea, dissolved combined amino acids (DCAA), and dissolved free amino acids (DFAA) constituted 4–23%, 4–19%, and 0.2–3% of DON in various rivers (Appendix B). Thus, 8–45% of DON was identified (IDON). Higher proportions of urea tended to occur in the northern part of the Baltic Sea watershed. The proportions of DCAA and DFAA did not have clear geographical tendencies. The ratio IDON:DON correlated negatively with the concentration of DON (Table 1).

Concentrations of urea, DCAA, and DFAA were intercorrelated (Table 1).

The bulk DOC (dissolved organic C):DON ratio and spectral properties of dissolved organic matter varied substantially among the studied rivers (Fig. 4, Appendix D). The atom DOC:DON ratio ranged from 9 to 59, the DOC-specific absorbance ( $A_{430}$ :DOC) varied from 11 to 52 ( $\text{mol/L}^{-1}\cdot\text{cm}^{-1}$ ), the slope of the absorbance spectrum in the ultraviolet (UV) region ( $A_{280-400}$ ) varied from 0.0142 to 0.0199  $\text{nm}^{-1}$ , and the 450:500 nm fluorescence ratio ( $F_{450:500}$ ) varied from 1.29 to 1.45. The highest  $A_{430}$ :DOC and DOC:DON, and the lowest  $A_{280-400}$  and  $F_{450:500}$  occurred mostly in the northern rivers. All four parameters correlated with each other (Table 2).

The bioavailability of DON (determined for 13 rivers) varied 8–72% (mean = 31%), and the bioavail-

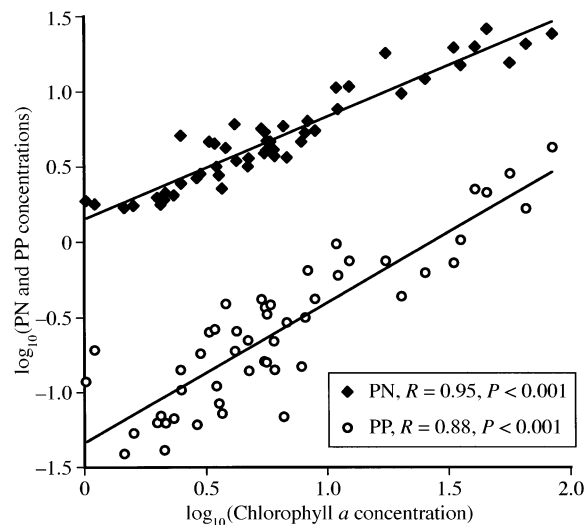


FIG. 3. Correlations between the concentrations of chlorophyll *a* (in micrograms per liter) and concentrations of particulate nitrogen (PN) and phosphorus (PP) (both in micromoles per liter) in the collected river water.

ability of DOP (determined for 11 rivers) varied 4–131% (mean = 75%) (Appendix D). DON bioavailability did not correlate significantly with DON concentration, or with the proportion of IDON, urea, DCAA, and DFAA in DON (Table 1). Only the proportion of DFAA in DON tended to correlate positively with DON bioavailability. In several rivers, the bioavailable proportion of DON exceeded the proportion of DON that was identified as DFAA, DCAA, or urea (IDON) (Appendix B). All rivers, where the proportion of bioavailable DON was lower than the proportion of IDON, were situated in the northeastern part of the Bothnian Bay drainage area.

DON bioavailability correlated positively with the slope of the UV absorbance spectrum of DOM ( $A_{280-400}$ ) and the 450:500 nm fluorescence ratio ( $F_{450:500}$ ), while it correlated negatively with DOC:DON and the DOC-specific absorbance ( $A_{430}$ :DOC; Table 2). Similarly, DOP bioavailability tended to correlate negatively with  $A_{430}$ :DOC and positively with  $A_{280-400}$  and  $F_{450:500}$ .

Partial least-squares regression models (PLS<sub>1</sub>) were

developed to predict DON and DOP bioavailability from background water chemistry data. The best prediction (highest coefficient of determination,  $Q^2$ ) of DON bioavailability was obtained from a model with three predictor variables:  $A_{430}$ :DOC,  $A_{280-400}$ , and DIN concentration (Fig. 5). Almost all of the explained variance in DON bioavailability was due to the first latent variable ( $LV_1$ ), where  $A_{430}$ :DOC had the greatest impact. However, the precision of the model was low (slope = 0.67,  $Q^2$  = 0.27, RMSEP (root mean square error of prediction) = 20), and a model with  $A_{430}$ :DOC as the only predictor variable had only slightly lower precision (slope = 0.63,  $Q^2$  = 0.22, RMSEP = 20). The best prediction of DOP bioavailability was obtained from a model with DOP concentration as the only predictor variable (slope = 3.13,  $Q^2$  = 0.70, RMSEP = 50).

According to BDDDB (Baltic drainage database [Stålnacke 1996]), rivers in the Baltic Sea drainage area carry 53% of water, 40% of TN, 32% of DIN, 49% of TP, and 45% DIP during summer months (May–October). Assuming our measured concentrations to be constant throughout the summer, and taking mean monthly discharge from BDDDB, our studied rivers carried about 125 000 t of TN and 6820 t TP during summer (Fig. 2, Table 3: column A). Of the N load, DIN, DON, and PN constituted 44%, 44%, and 12%, respectively. Accordingly, DIP, DOP, and PP constituted 24%, 25%, and 51% of TP load, respectively. The BDDDB-based average transport from the studied rivers during the same months is 179 000 t TN and 9250 t TP, and inorganic forms comprise 50% of TN and 43% of TP loads (Table 3: column B). We estimate that DIN, DON, and PN constitute 48%, 41%, and 11%, respectively, of the riverine nitrogen loads from the entire Baltic drainage area (Table 3: column C). The summer riverine phosphorus load to the Baltic Sea was estimated to be 46% DIP, 18% DOP, and 36% PP. Urea, DCAA, and DFAA together comprised 16% of the total load of DON. About 13% of TN load and ~14% of TP load was constituted of bioavailable dissolved organic N and P, assuming 31% of DON and 75% of DOP to be bioavailable (Appendix D).

TABLE 1. Coefficients of correlation (*R*) between various nitrogen parameters.

| Variable          | DON<br>bioavail-<br>ability | DON<br>concentration | IDON<br>(% of DON) | Urea<br>(% of DON) | DFAA<br>(% of DON) |
|-------------------|-----------------------------|----------------------|--------------------|--------------------|--------------------|
| DON concentration | 0.28                        |                      |                    |                    |                    |
| IDON (% of DON)   | 0.09                        | −0.56***             |                    |                    |                    |
| Urea (% of DON)   | 0.07                        | −0.50***             | 0.94***            |                    |                    |
| DFAA (% of DON)   | 0.42                        | −0.37*               | 0.74***            | 0.58***            |                    |
| DCAA (% of DON)   | 0.02                        | −0.56***             | 0.90***            | 0.70***            | 0.74***            |

Notes: DON = dissolved organic N, IDON = identified DON, DFAA = dissolved free amino acids, and DCAA = dissolved combined amino acids. *N* = 13 measurements for DON bioavailability; *N* = 50 measurements for the remaining parameters.

\*  $P < 0.05$ ; \*\*\*  $P < 0.001$ .

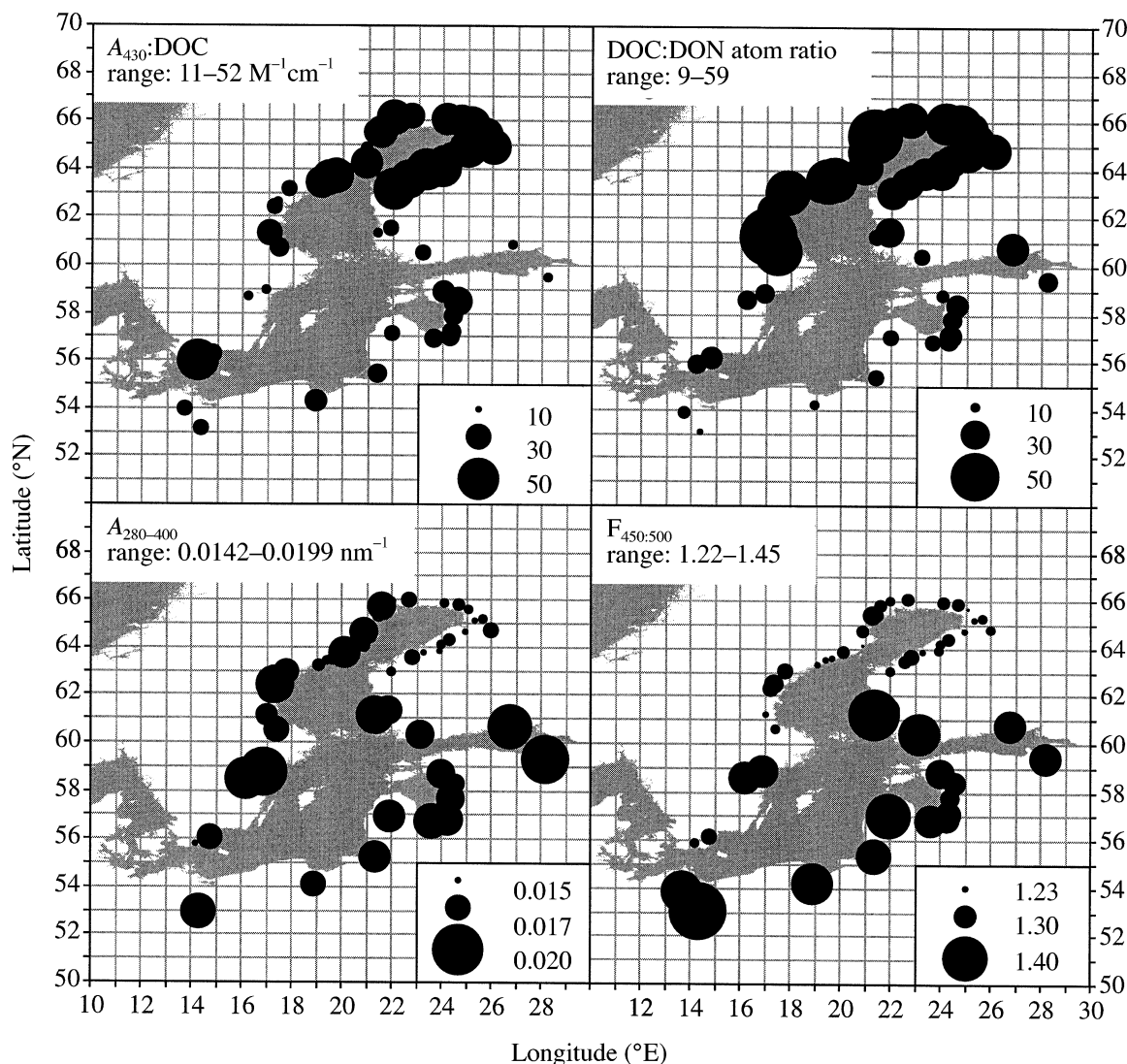


FIG. 4. Chemical characteristics of the dissolved organic matter in the collected river water. Presented are dissolved organic C (DOC)-specific light absorption at 430 nm ( $A_{430}:\text{DOC}$ ), DOC:DON (dissolved organic N), slope of ln-transformed light absorption at 280–400 nm ( $A_{280-400}$ ), and the ratio of fluorescence at 450 and 500 nm with excitation at 370 nm ( $F_{450:500}$ ).

## DISCUSSION

### *Concentrations of riverine N and P*

The highest concentrations of total and inorganic N and P occurred in rivers draining the southern and eastern part of the Baltic Proper watershed (Fig. 2, Appendix B). Most probably this reflects the more dense human population and the more intensive agriculture in the southeastern part of the drainage area (Sweitzer et al. 1996). Globally, human activity is reported to be the dominant controlling factor for riverine inorganic nutrient export (Caraco and Cole 1999). Accordingly, Stålnacke et al. (1996) estimate that ~50% of all riverine inputs of TN (total N) and TP (total P) to the Baltic Sea originate from the six largest rivers in the southern and eastern parts of the drainage area (Oder, Vistula, Nemunas, Daugava, Narva, and Neva).

On average, the measured concentrations of DIN (dissolved inorganic N), DIP (dissolved inorganic P), TN, and TP were below previously reported long-term mean summer concentrations (Appendix B). As a consequence, loads of TN and TP derived from our measurements were ~40% lower than estimates based on long-term average concentrations (Table 3: column B). Our measured share of DIP in the TP load was also lower than the long-term average (Fig. 6, Table 3: columns A and B). This disparity could be caused by methodological differences, since inorganic N and P given in the Baltic drainage database (BDDb) apparently were measured on unfiltered river water, and therefore include both dissolved and particulate reactive inorganic forms. Our DIN and DIP measurements were performed on particle-free water. Another explanation for the measured lower nutrient concentrations



TABLE 2. Correlation coefficients ( $R$ ) for the potential bioavailability of dissolved organic matter (DOM) and DOM chemical parameters.

| Variable       | DON bioav. | DOP bioav. | DOC: DON | $A_{430}$ : DOC | $A_{280-400}$ |
|----------------|------------|------------|----------|-----------------|---------------|
| DOP bioav.     | 0.51       |            |          |                 |               |
| DOC:DON        | -0.55*     | -0.03      |          |                 |               |
| $A_{430}$ :DOC | -0.66*     | -0.34      | 0.45***  |                 |               |
| $A_{280-400}$  | 0.65*      | 0.25       | -0.51*** | -0.96***        |               |
| $F_{450-500}$  | 0.61*      | 0.40       | -0.68*** | -0.71***        | 0.71***       |

Notes: DON bioav. = dissolved organic N bioavailability, DOP bioav. = dissolved organic P bioavailability, DOC = dissolved organic C,  $A_{430}$  = absorbance at 430 nm,  $A_{280-400}$  = the reverse slope of the log-transformed absorbance in the range of 280–400 nm,  $F_{450-500}$  = the ratio of fluorescence at 450 and 500 nm with excitation at 370 nm.  $N = 13$  measurements for DON bioavailability,  $N = 11$  measurements for DOP bioavailability,  $N = 47$  measurements for  $A_{280-400}$  slope, and  $N = 50$  measurements for the remaining parameters.

\*  $P < 0.05$ ; \*\*\*  $P < 0.001$ .

could be that our samples were taken during an unusually dry summer, 1999. Since only a single sample was taken in each river in the present study, temporal variation could not be assessed. Therefore, the extrapolation of nutrient loads to the entire Baltic Sea drainage area was adjusted to the long-term average values (see *Material and methods: Use of historical nutrient-transport data*, above and Table 3).

Standard river surveys do not distinguish between dissolved and particulate organic nutrients (e.g., Stålnacke et al. 1999). Our study showed that 20% of organic N and as much as 70% of organic P loaded by rivers to the Baltic Sea during summer was particle bound, assuming that all particulate N (PN) and particulate P (PP) was organic (Table 3). PN and PP ap-

peared to be dominated by algal and bacterial cells (Appendix C) and probably had a direct effect on the productivity of coastal waters in the Baltic. It is possible that high riverine loads of cell-bound nutrients are typical for many temperate coastal waters during summer.

#### DON bioavailability

The potential bioavailability of dissolved organic N (DON) varied considerably among the studied rivers, from 8% to 72% (Fig. 5, Appendix D). The lowest DON bioavailability, 8–14%, was found in rivers situated in the northeastern part of the Bothnian Bay drainage area (river numbers 24–30; Fig. 1 and 5B). Probably the low bioavailability in these rivers was due to a large proportion of their DON originating from peat bogs, which are abundant in this area (Sweitzer et al. 1996). This is consistent with the bioavailability of DON being negatively correlated with the dissolved organic C (DOC)-specific absorbance (Table 2, Fig. 5A), since humic matter from peat bogs exhibits high absorbance (McKnight and Aiken 1998). Similar DON bioavailability (8–16%) is reported for DON from a wetland-draining stream in southern Sweden (Stepanauskas et al. 1999b). In the remaining Baltic drainage rivers, DON bioavailability varied from 29% to 72% (Appendix D), and was similar to the range (40–72%) reported by Seitzinger and Sanders (1997) from two large North American rivers. This may indicate that substantial amounts of DON in these latter Baltic rivers were autochthonous, produced by phytoplankton and macrophytes. Phytoplankton, which was abundant in many rivers (Appendix C), is reported to excrete 30–40% of gross DIN uptake in the form of DON (Bronk et al.

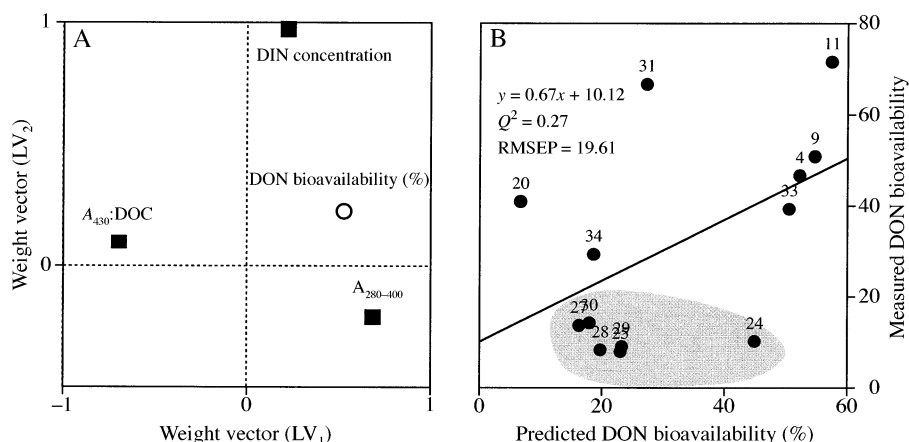


FIG. 5. (A) Partial least-squares (PLS) model weight-vector plot for predictor variables (solid squares) and the response variable (open circle) for the first ( $LV_1$ ) and second ( $LV_2$ ) latent variable.  $A_{430}$ :DOC is dissolved organic C (DOC)-specific light absorption at 430 nm, and  $A_{280-400}$  is the slope of ln-transformed light absorption at 280–400 nm; DON = dissolved organic N, and DIN = dissolved inorganic N. (B) PLS-modeling correlation plot of predicted vs. measured DON bioavailability. The line indicates linear regression ( $N = 13$ ).  $Q^2$  is the coefficient of determination, and RMSEP is the root mean square error of prediction. Numbers by the data points indicate river number (see Fig. 1 and Appendix A). The gray polygon groups rivers characterized by low DON bioavailability and located in a geographically confined area in the northwestern part of the Bothnian Bay drainage area.



TABLE 3. Estimated riverine transport of nitrogen and phosphorus to the Baltic Sea during May–October.

| Parameters†                     | Transport by the 48 studied rivers‡ |         | Transport from the entire drainage area, C§ |
|---------------------------------|-------------------------------------|---------|---------------------------------------------|
|                                 | A                                   | B       |                                             |
| Total N, TN (Mg)                | 125 000                             | 179 000 | 288 000                                     |
| Inorganic N (% of TN)           | 44                                  | 50¶     | 48                                          |
| DON (% of TN)                   | 44                                  | ...     | 41                                          |
| Particulate organic N (% of TN) | 12                                  | ...     | 11                                          |
| Bioavailable DON (% of TN)#     | 14                                  | ...     | 13                                          |
| Urea (Mg N)                     | 4583                                | ...     | 8834                                        |
| DCAA (Mg N)                     | 3794                                | ...     | 7313                                        |
| DFAA (Mg N)                     | 478                                 | ...     | 921                                         |
| Total P, TP (Mg)                | 6820                                | 9250    | 18 000                                      |
| Inorganic P (% of TP)           | 24                                  | 43¶     | 46                                          |
| DOP (% of TP)                   | 25                                  | ...     | 18                                          |
| Particulate organic P (% of TP) | 51                                  | ...     | 36                                          |
| Bioavailable DOP (% of TP)#     | 21                                  | ...     | 14                                          |

† DON = dissolved organic N, DOP = dissolved organic P; DCAA = dissolved combined amino acids, DFAA = dissolved free amino acids.

‡ Rivers Peene and Eurajoki are excluded due to missing data in the Baltic drainage database (BDDB; Stalnacke 1996). Column A estimates are based on concentrations measured in the field and on the long-term average runoff extracted from BDDB. Column B estimates are based on the long-term average concentrations and runoff extracted from BDDB.

§ The estimate is normalized for year-to-year variation. See *Materials and Methods: Use of historical nutrient-transport data* for the method of estimation.

|| Only the dissolved fraction.

¶ Both dissolved and particulate fractions.

# Mean values of DON and DOP bioavailability (31% and 75%; Appendix D) were used for rivers where the actual bioavailability was not measured.

1994), and therefore could be an important source of DON.

Most of the larger rivers in the Baltic Sea drainage area flow through lake systems and artificial reservoirs. In northern Sweden, up to 70% of the annual discharge, dominated by spring flood water, is stored in reservoirs, and is gradually released during summer (Bergström and Carlsson 1994). Concentration and bioavailability of DON in boreal streams are high during spring flood (Stepanaukas et al. 2000b). Thus, storage of spring flood water in lakes and reservoirs may contribute to the observed high DON bioavailability at the river mouths during summer.

According to our estimate, summer riverine input of bioavailable DON to the Baltic Sea was 4.4-fold lower than the input of DIN (Table 3: column C). The high proportion of DIN was mainly due to a few major rivers discharging to the southern Baltic Sea proper (Oder, Vistula, Nemunas, and Daugava; Appendix B, Stalnacke et al. 1999). The Bothnian Bay and the Bothnian Sea receive a substantially higher proportion of DON than the Baltic Sea proper. However, P limitation prevails in the Bothnian Bay and often occurs in the Bothnian Sea (Granéli et al. 1990). Thus, riverine loads of DON probably do not enhance productivity of these waters. Nonetheless, riverine DON may significantly enhance primary production in certain N-limited pristine coastal waters in the Baltic as well as in other marine areas, like the Arctic Ocean and most tropical regions. Accordingly, Opsahl et al. (1999) report that up to 33% of dissolved organic matter (DOM) with

molecular mass >1000 kDa is terrigenous in the surface Arctic. Assuming that the average riverine DON bioavailability is 30% (Appendix D) and that DON constitutes 70% of the total N in world rivers (Meybeck 1982), around 40% of the readily bioavailable N loading globally to coastal areas is DON.

#### DOP bioavailability

The potential bioavailability of dissolved organic P (DOP), determined in P-limited bacterial cultures, was extremely variable among the studied rivers, between 4% and 131% (Appendix D). Overestimation of DOP bioavailability (values >100%) probably was caused by underestimates of the DOP concentration. In support of this hypothesis, partial least squares (PLS) regression analysis indicated a strong negative relationship between the apparent concentration and bioavailability of DOP. We calculated the concentration of DOP by subtracting DIP (measured as molybdate-reactive P) from total dissolved P (TDP). To determine TDP, we used persulfate oxidation with a subsequent measurement of DIP. Persulfate oxidation is a standard method, but recent studies show that it often underestimates the concentration of TP (Monaghan and Ruttenberg 1999) and, consequently, DOP. Moreover, it is suspected that molybdate-reactive P may include some organic P, further underscoring the concentration of DOP (Rigler 1968, Boström et al. 1988). Finally, riverine TDP and DIP concentrations were often very low, close to the detection limit of the methods. This might cause further

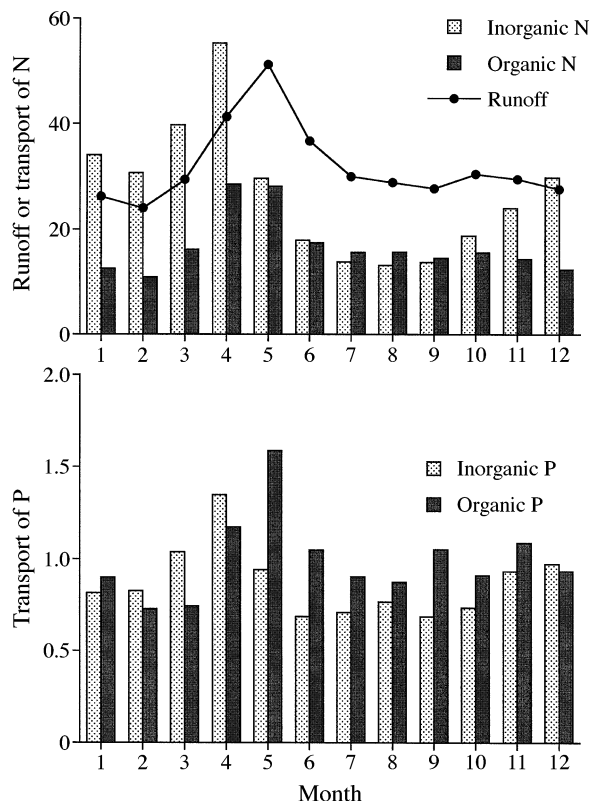


FIG. 6. Monthly runoff and the transport of nitrogen and phosphorus to the Baltic Sea by the rivers covered by the Baltic drainage database (Stålnacke 1996; see *Materials and Methods: Use of historical nutrient-transport data*); average values for the period 1970–1990. For transport of N and transport of P the y-axis scale numbers are thousands of metric tons per month (1 metric ton =  $10^3$  kg = 1 Mg); for runoff the scale numbers are billions ( $10^9$ ) of cubic meters per month. Monitored rivers account for 81% of runoff, 74% of N transport, and 61% of P transport from the entire Baltic Sea drainage area. Chemical analyses were performed on unfiltered water and therefore include dissolved as well as particulate forms.

discrepancies in the calculated DOP concentration and bioavailability in oligotrophic rivers.

Due to the uncertainties in DOP concentration measurements, the exact DOP bioavailability values should be viewed with caution. Nevertheless, our results demonstrate that heterotrophic bacteria were able to utilize a larger fraction of dissolved P than the molybdate-reactive P in all of the studied rivers (Appendix D). In many cases, the concentration of potentially bioavailable dissolved P was close to the TDP concentration. The apparent DOP bioavailability tended to correlate positively with indicators of plankton-derived organic material and with the bioavailability of DON (Table 2).

We are not aware of any published studies on the potential bioavailability of bulk DOP to bacterioplankton, although extracellular enzymes for the acquisition of P from organic sources (phosphatases) are ubiqui-

tous in aquatic environments (Münster and De Haan 1998). Most investigations dealing with potential DOP bioavailability employ axenic algal cultures and test specific artificial DOP substrates (Boström et al. 1988, Berman et al. 1991). Hence, these results do not reflect P availability in natural ecosystems with complex algal and bacterial assemblages and with diverse DOP compounds. The role of heterotrophic bacteria in DOP utilization seems to be neglected, although several studies show higher DOP uptake rates by bacterioplankton than by phytoplankton (Currie and Kalff 1984, Berman 1988, Boon 1993, Panosso and Esteves 1999). Our study is among the first to demonstrate that bacterioplankton is able to utilize a major part of DOP from a broad spectrum of natural waters.

On the entire Baltic Sea scale, summer riverine load of bioavailable DOP was 3.3-fold lower than the load of DIP (Table 3: column C). However, the share of DOP in the load of TP to the Bothnian Bay and the Bothnian Sea is as high as 65% (Stålnacke et al. 1999). Since DOP appears to be highly bioavailable (Appendix D), we propose that riverine organic P is a major stimulator of algal production in the Bothnian Bay and the Bothnian Sea, where P limitation prevails (Granéli et al. 1990). Similarly, DOP inputs may be an important productivity regulator in other phosphorus-deficient coastal marine regions and lakes.

#### *DON and DOP bioavailability assays*

Natural dissolved organic matter (DOM) is an extremely diverse collection of compounds, the exact chemical composition of which is poorly understood (McKnight and Aiken 1998). Similarly, little is known about the capabilities of individual bacterioplankton strains to degrade specific substrates. To account for the existing chemical and metabolic diversity, bacterial regrowth bioassays (also called “batch” or “dilution cultures”) with natural inocula are widely used to examine the bioavailability of dissolved organic carbon (DOC; for reviews, see Søndergaard and Middelboe [1995] and Tranvik [1998]).

Similar assays have been adopted to determine the potential bioavailability of natural DON (Bushaw et al. 1996, Seitzinger and Sanders 1997, Carlsson et al. 1999, Stepanauskas et al. 1999a, b, 2000b, this study) and DOP (this study). First, water is filtered to reduce bacterial abundance by several orders of magnitude and to remove bacterivores. Then the cultures are manipulated to be N limited (for DON bioavailability assays) or P limited (for DOP bioavailability assays) and incubated until they reach the carrying-capacity biomass, which usually takes up to two weeks. The amount of bioavailable DON and DOP is estimated from the measurements of bacterial biomass, with subsequent conversion into units of consumed N and P.

It is assumed that all bioavailable DOM is consumed after bacteria have reached the stationary growth phase. However, slow growth could still continue undetected

during the apparent stationary phase, causing bioavailability underestimates. On a longer time scale, various environmental factors may alter DOM bioavailability in natural waters. Examples of such factors are solar radiation (Bushaw et al. 1996) and changes in water ionic strength (Stepanauskas et al. 1999a, b, Wikner et al. 1999). In addition, bacterial assemblages in bioassays most likely are different from natural assemblages, due to the lack of grazing pressure and light, altered temperature and substrate composition, and nutrient additions. Therefore, the bioavailability values should be viewed as potential, operationally defined bioavailability.

In bioavailability estimates we did not use literature-based values of nutrient content per bacterial cells and the biovolume-to-biomass ratio, because the reported numbers vary several-fold between different systems (Fry 1988, Fagerbakke et al. 1996). Instead, we calibrated bacterial growth response to the concentration of bioavailable nutrients by applying inorganic nutrient spikes (see *Materials and Methods: DON and DOP bioavailability assays*, above). The main assumption is that different potentially bioavailable N and P compounds yield identical cell abundance. In support of this, Stepanauskas et al. (1999b) found no difference in cell yield after additions of nitrate or ammonium in assays identical to those used in the present study. Furthermore, there was no difference in the average cell size of bacteria cultivated on DON substrates alone and with nitrate spike added during the stationary growth phase. This is not surprising, because inorganic spikes are depleted during the first few days. After that time, media in cultures with and without spikes become identical, probably resulting in similar microbial assemblages.

Bacteria might use part of N and P from DOM and spikes for the production of extracellular material (e.g., extracellular mucilage and enzymes). This would decrease the spike-induced biomass yield. Consequently, N and P content of an average cell ( $N_{\text{cell}}$  and  $P_{\text{cell}}$ ) would be overestimated. A similar bias could also be caused by the growth of bacteria on the walls of the cultivation flasks. However, excretion and growth on the walls is unlikely to affect bioavailability estimates, since  $N_{\text{cell}}$  and  $P_{\text{cell}}$  would be overestimated to the same degree as ( $D_{\text{river}}$  [the average cell abundance in cultures with solely riverine N] –  $D_{\text{blank}}$  [the average cell abundance in N-limited blank cultures]) would be underestimated. It must be pointed out that bioavailability estimated using DIN and DIP spikes includes nutrients used by bacteria not only for the cell build up, but also for the production of extracellular material. Thus, the estimated  $N_{\text{cell}}$  and  $P_{\text{cell}}$  should only be viewed as operational coefficients. However, growth on the walls and excretion does not seem to account for a large portion of nutrient net flow in such bioassays (Stepanauskas et al. 1999b).

A similar, inorganic nutrient spike-based approach has been used in algal regrowth assays when the bio-

availability of DOP was investigated (Boström et al. 1988). However, the concept is not applicable to DOC bioavailability studies, because spikes of labile DOC (e.g., glucose) would not give growth efficiency representative of natural DOC. Growth efficiency is not involved in our DON and DOP bioavailability calculations, because N and P are not likely to be respired under oxic incubations.

#### *Chemical composition of DON*

Between 8% and 45% of DON was identified as urea, dissolved combined amino acids (DCAA), and dissolved free amino acids (DFAA) in the collected river water (Appendix B). Similarly, urea and amino acids have been previously found to comprise 5–18% of DON in two streams from the Bothnian Bay drainage area (Stepanauskas et al. 2000b). These numbers are low compared to lakes and marine environments, where DCAA alone are reported to comprise 10–30% of DON (Jørgensen and Jensen 1997, Jørgensen et al. 1999). Urea and DCAA were estimated to constitute 7% and 6% of the summer riverine DON load to the Baltic Sea, while the share of DFAA was <1% (Table 3).

The proportion of identified N compounds did not correlate with DON bioavailability (Table 1, PLS data). In many of the rivers, the concentrations of potentially bioavailable DON ( $\text{DON}_{\text{PB}}$ ) exceeded DON bound in urea, DFAA, and DCAA (Appendix B). Similarly,  $\text{DON}_{\text{PB}}$  exceeded identified DON (IDON) by a factor of 2–9 in boreal streams during a spring flood (Stepanauskas et al. 2000b). This indicates that aquatic bacteria readily utilize other N-containing organic compounds besides those identified in this study (e.g., non-DCAA amides), which are suggested to dominate DON in the ocean (McCarthy et al. 1997) and in soils (Schulten and Schnitzer 1998). On the other hand, several rivers from the northeastern part of the Bothnian Bay drainage area had  $\text{DOD}_{\text{PB}}$  concentrations lower than the concentrations of IDON. This may possibly be explained by a substantial part of DCAA being inaccessible for bacterial uptake (Keil and Kirchman 1994, Tranvik and Jørgensen 1995, McCarthy et al. 1997).

Our study indicates that concentrations of urea, DFAA, and DCAA do not reflect the amount of DON readily available to bacterioplankton in aquatic environments. Thus, a major fraction of bioavailable DON appears to be chemically unidentified. On the other hand, a substantial part of identified DON is not readily accessible for microbial uptake in some waters, possibly due to steric hindrance by and/or chemical bonds to the macromolecular matrix. Further research is needed to clarify the relationships between the chemical composition and the biological availability of aquatic DON.

#### *Indicators of DON bioavailability*

Spectroscopic properties of dissolved organic matter and the bulk DOC:DON ratio were better predictors of

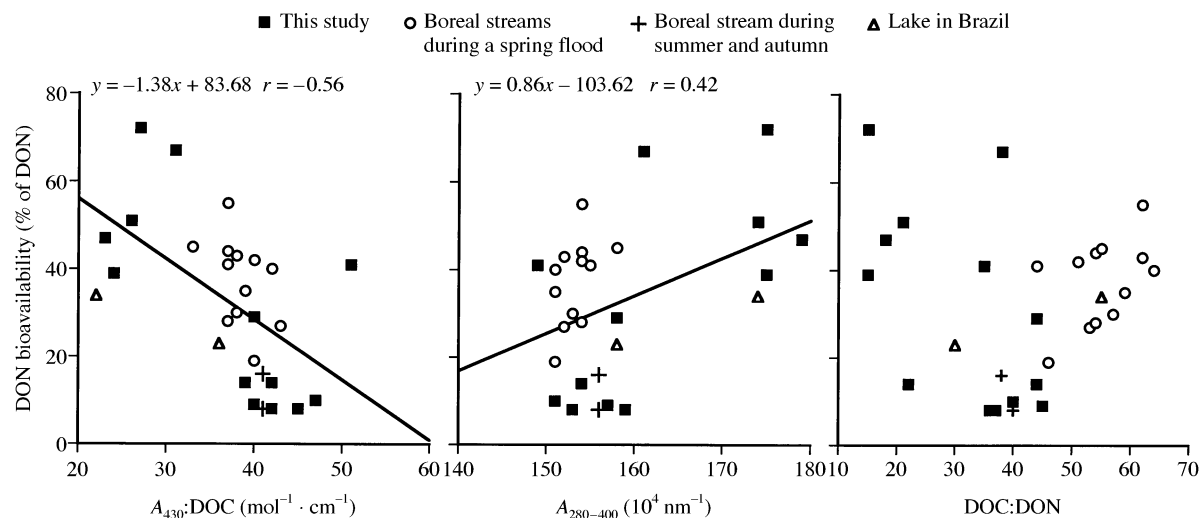


FIG. 7. Correlations between dissolved organic N (DON) bioavailability and various characteristics of dissolved organic matter. Presented are dissolved organic C (DOC)-specific light absorption at 430 nm ( $A_{430}:\text{DOC}$ ), the slope of ln-transformed light absorption at 280–400 nm ( $A_{280-400}$ ), and the atom ratio of DOC and DON. The cited values of  $A_{280-400}$  were recalculated from the ratio of light absorbance at 250 and 365 nm ( $y = [x + 5.5]/656.4$ ;  $R^2 = 0.96$ ,  $N = 47$  measurements; data for the model (solid squares) are taken from this study). The potential DON bioavailability was determined employing N-limited bacterial regrowth cultures with indigenous inocula. The data for boreal streams during a spring flood (open circles) are from Stepanauskas et al. (2000b), for boreal stream during summer and autumn (plus symbols [+]) from Stepanauskas et al. (1999b), and for lake in Brazil (open triangles) from Stepanauskas et al. (2000a).

DON bioavailability than the proportion of urea and amino acids in DON (Fig. 5, Tables 1 and 2). Low DOC:DON, low DOC-specific absorbance ( $A_{430}:\text{DOC}$ ), a steep slope of the ultraviolet (UV) absorbance spectrum (high  $A_{280-400}$ ), and a high ratio of fluorescence at 450 and 500 nm ( $F_{450:500}$ ) were associated with high DON bioavailability, in agreement with results of Stepanauskas et al. (1999a, 2000b). Among these variables, the  $A_{430}:\text{DOC}$  ratio appeared to predict DON bioavailability best, both in this study (Fig. 5) and when data from diverse locations were compiled (Fig. 7).

High DOC:DON and DOC-specific absorbance are typical for aromatic, high mean molecular mass DOM from terrestrial sources (McKnight and Aiken 1998). Several of the studied rivers, mostly in the southern part of the Baltic Sea drainage area, had a DOC:DON atom ratio similar to the Redfield ratio (6.6), indicating a freshly produced and yet undegraded material (Fig. 4, Appendix D). In contrast, the DOC:DON ratio in most rivers discharging into the Bothnian Bay and the Bothnian Sea was between 30 and 60, pointing out that a substantial part of their DOM originated from terrestrial sources (e.g., forests and peat bogs) prevailing in this part of the Baltic Sea drainage area (Sweitzer et al. 1996). Our results suggest that nitrogen bound into high-molecular-mass, allochthonous DOM, rich in aromatic compounds, is more resistant to microbial degradation than algal-derived DON. Similar conclusions for the bioavailability of DOC of diverse origin were drawn by Søndergaard and Middelboe (1995) and Sun et al. (1997).

Increasing the ratio of fluorescence at 450 and 500

nm,  $F_{450:500}$ , in a range of values from 1.4 to 1.9, has been proposed to indicate a decreasing share of terrestrially derived organic material (McKnight et al. 2001). Our study indirectly supports this concept, since  $F_{450:500}$  was negatively correlated with DOC:DON and  $A_{430}:\text{DOC}$  (Table 2). However, our observed  $F_{450:500}$  values ranged from 1.22 to 1.45 (Appendix D) and were mostly below the values 1.4–1.9 given by McKnight et al. (2001). Thus, if the model proposed by McKnight et al. (2001) would be applied to our data, DOM of all our studied rivers should be considered extremely allochthonous and, in most cases, out of range. This does not seem to be reasonable, as suggested by other indicators of DOM quality. A possible explanation for the disagreement between the two data sets might be different vegetation and soils prevailing in the study areas of McKnight et al. (2001) and in the Baltic Sea drainage area. More research is needed before using  $F_{450:500}$  as a universal indicator of DOM quality.

Steep slopes of absorbance spectra in the UV range (high  $A_{280-400}$  values) indicate low average molecular mass of DOM (de Haan 1972, Pages and Gadel 1990). The  $A_{280-400}$  values in our studied rivers (0.0142–0.0199  $\text{nm}^{-1}$ ; Appendix D) ranged from similar to peat bogs (0.008–0.013  $\text{nm}^{-1}$ ; Pages and Gadel 1990) to typical for eutrophic lakes (0.0173–0.0205  $\text{nm}^{-1}$ ; Davies-Colley and Vant 1987). Positive correlations between  $A_{280-400}$  and DON bioavailability (Fig. 5, Table 2) indicate that low molecular mass DON was more bioavailable than high molecular mass DON, in agreement with work based on ultrafiltration by Stepanauskas et al. (1999a, b). Furthermore, a positive correlation between  $A_{280-400}$  and  $F_{450:500}$  and neg-



ative correlations between  $A_{280-400}$  and DOC:DON and  $A_{430}$ :DOC (Table 2), imply that allochthonous DOM was dominated by larger molecules than autochthonous DOM, in support of the general view on DOM cycling (McKnight and Aiken 1998).

#### Composition of PN and PP

Strong correlations of the chlorophyll *a* concentration with the concentrations of particulate N and P (Fig. 3) suggest that algal biomass constituted a major part of the particulate organic matter (POM). This is consistent with the high concentrations of PN and PP estimated to be bound into algal and bacterial biomass (Appendix C).

Bacterial and algal cells were estimated to account for more particulate P (175%) and N (265%) than the actually measured concentrations of PN and PP. This could be caused by several factors. First, the use of the persulfate oxidation method could underestimate PP and PN concentrations (Monaghan and Ruttenberg 1999). Second, concentrations of PN and PP could be underestimated due to bacterial cells passing through the GF/F filters, on which particulate matter was collected. Most bacterial cells were smaller (Appendix C) than the pores of these filters ( $\sim 0.7 \mu\text{m}$ ), and could pass the filter undetected as PN and PP. Finally, estimates of N and P bound into biomass were based on highly variable conversion factors. Thus, the ratio of chlorophyll *a* to phytoplankton carbon may range between 25 and 154, mostly depending on algal species and growth conditions (Valiela 1995). Similarly, variation is large in the bacterial biovolume : biomass ratio and C:N:P ratios of phytoplankton and bacterioplankton (Valiela 1995, Fagerbakke et al. 1996). We used mean values in order to get a rough estimate of planktonic N and P (see *Materials and methods: Chemical analyses*, above).

Despite the uncertainties in estimates, the combined results strongly suggest that algal and bacterial cells dominated particulate organic material in most of the studied rivers. Similarly, chemical biomarker analyses by Canuel et al. (1995) and Canuel and Zimmerman (1999) indicate that phytoplankton dominate POM in a number of American rivers. This contradicts the prevailing belief that riverine POM is mostly composed of allochthonous and refractory detritus (Vannote et al. 1980, Meybeck 1982). Allochthonous particles may dominate in certain fluvial systems, as demonstrated for the Amazon River (Hedges et al. 1994), which has an extensive input of organic material from the floodplain. However, our results suggest that phytoplankton and bacterioplankton dominate POM in most rivers in temperate and boreal regions during the summer period.

This finding has important implications regarding riverine impacts on coastal waters. Riverine plankton can be easily consumed by marine phagotrophs and in this way contribute to coastal secondary production.

Furthermore, part of the algal and bacterial cells discharged by rivers probably retain their photosynthetic activity in brackish estuaries and contribute to coastal primary production. Hence, freshwater phytoplankton (e.g., *Aphanizomenon flos-aquae*, *Anabaena*, *Cyano-dictyon*, *Aphanocapsa*, *Aphanothece*, *Lemmermanniella* spp.) prevail in the coastal waters of the Baltic Sea, while typical brackish-water phytoplankton (e.g., *No-dularia spumigena*, *Heterocapsa triquetra*, and *Aphanizomenon* sp.) dominate in the open Baltic Sea (Gertrud Cronberg [Lund University], *personal communication*). Assuming that half of the riverine PN and PP load (Table 3: column C) consists of algal cells, the riverine algal input would correspond to  $\sim 2\%$  of the export production of the entire sea. Thus, river-born algae can constitute a major part of phytoplankton in brackish coastal waters in the Baltic Sea as well as in other geographical areas.

#### Nutrient cycling within rivers

Chlorophyll *a* concentrations averaged  $16 \mu\text{g/L}$  and bacterial abundance averaged  $4 \times 10^9$  individuals/L (Appendix C) in the studied rivers, corresponding to levels found in eutrophic lakes (Wetzel 1983). Phytoplankton and bacterioplankton were abundant even in northern rivers draining boreal forests. To support this high biomass, considerable quantities of nutrients must be cycled by the riverine plankton. Organically bound N and P comprised about half of the summer riverine nutrient loads to the Baltic Sea (Table 3, Fig. 6), and significant DIN and DIP concentrations occurred only in a few southern rivers with densely populated drainage areas (Appendix B). As discussed above, most of the particulate N and P appeared to be bound into microbial biomass, and a large part of DON and DOP probably was autochthonous.

We suggest that within-river conversion of inorganic nutrients into dissolved and particulate organic matter deserves increased attention. Apparently, inorganic nutrients collected from the drainage area are intensively cycled by planktonic and benthic biota in rivers before discharging into the sea. This might severely alter nutrient retention mechanisms. For example, incorporation of nitrate into PN and DON may prevent denitrification and in this way decrease N retention. Caraco and Cole (1999) estimate that, as a worldwide average,  $\sim 15\%$  of N loaded to watersheds with fertilizers and atmospheric deposition reach river mouths as nitrate. We propose that a substantial additional part of anthropogenic N may be converted to DON and PN, and reach the sea in a largely bioavailable organic form.

The river continuum concept implies that the bioavailability of bulk organic matter should decrease downstream due to continuous microbial degradation of bioavailable fractions (Vannote et al. 1980, Wetzel 1992). Experimental evidence for a downstream decrease in DOM availability was provided from a black-water river by Leff and Meyer (1991). However, high

DON and DOP bioavailability in the lower parts of large rivers (Appendix D, Seitzinger and Sanders 1997) contradicts the river continuum concept. Consistent with this, Hedges et al. (1994) and Benner et al. (1995) did not detect qualitative changes in DOM along the Amazon River system. Furthermore, stable-isotope analyses demonstrate the importance of autochthonous primary production in the riverine food web (Thorp et al. 1998). We propose that in most large rivers the bioavailability of bulk DOM does not decrease downstream as predicted by the river continuum concept, due to the continuous internal production of fresh autochthonous material and due to the external inputs of new allochthonous DOM from groundwater, runoff, point sources, and tributaries. Furthermore, bioavailability of riverine DOM may be enhanced at river mouths, in response to mixing with saline water (Stepanauskas et al. 1999a, b, Wikner et al. 1999).

*The potential contribution of summer riverine loads of N and P to primary production in the Baltic Sea*

Riverine water discharging into the Baltic Sea during summer is trapped in the surface layer by the seasonal thermal stratification, and is called "juvenile freshwater" (Eilola and Stigebrandt 1998). The usual mixing depth during summer is 15 m (Eilola and Stigebrandt 1999), corresponding to a volume of  $\sim 5500 \text{ km}^3$  above the seasonal thermocline. Assuming complete mixing of the above-thermocline water volume, summer riverine input (Table 3: column C) can increase TN and TP concentrations in the surface water of the Baltic Sea by an average of 3.7 and 0.1  $\mu\text{mol/L}$ , respectively. The corresponding increase in dissolved bioavailable forms excluding particulate material (DIN + bioavailable DON and DIP + bioavailable DOP) is 2.2  $\mu\text{mol/L}$  N and 60 nmol/L P. Assuming that algae take up nutrients according to the Redfield ratio, summer riverine inputs of dissolved N and P can support new primary production equal to 6.5 g C/m<sup>2</sup> and 1.2 g C/m<sup>2</sup>, respectively. Since N limitation prevails in most parts of the Baltic Sea (Granéli et al. 1990), the N-based calculation is the most relevant.

The annual export production (the part of primary production sedimented out of the photic zone) is  $\sim 49 \text{ g C/m}^2$  in the Baltic Proper,  $\sim 32 \text{ g C/m}^2$  in the Bothnian Sea, and  $\sim 7 \text{ g C/m}^2$  in the Bothnian Bay (Stigebrandt 1991). Assuming that production in the Gulf of Finland and in the Gulf of Riga is the same as in the Baltic Sea proper, and that 55% of the annual production takes place during summer (Elmgren 1989), the summer export production in the entire Baltic Sea is 23 g C/m<sup>2</sup>. Thus, juvenile freshwater provides  $\sim 28\%$  of N and  $\sim 5\%$  of P needed to support the export production in the Baltic during summer.

About 130 000 t N/yr is fixed by cyanobacteria in the Baltic Sea (Brattberg 1980). This is somewhat less than the estimated summer riverine inputs of DIN and bioavailable DON (180 000 Mg; Table 3). In addition,

$\sim 400\,000 \text{ Mg N/yr}$  and  $18\,500 \text{ Mg P/yr}$  are supplied to the Baltic Sea from coastal point sources and through atmospheric deposition (Stålnacke 1996). Unfortunately, no published data are available on seasonal variation and bioavailability of these loads. For a rough estimate, we assumed that 50% of the annual loads from point sources and atmospheric deposition occurs during the summer period, and that this N and P is 100% bioavailable. A calculation as above reveals that summer inputs from rivers, atmosphere, point sources, and N<sub>2</sub> fixation can supply 70% of N and 12% of P required for the export production during the seasonal stratification.

Despite continuous external input, summer concentrations of DIN are very low in the open Baltic Sea ( $<0.5 \mu\text{mol/L}$ ; BED database, Sokolov and Wulff 1999). Exceptions are the Bothnian Bay, which is P limited (Granéli et al. 1990), and the plumes of large rivers. The study by Jørgensen et al. (1999) in Riga Bay demonstrates that riverine DIN and DON are quickly transformed into plankton cells within a close distance from the Daugava river mouth. Thus, productivity of coastal waters is directly dependent on riverine nutrient inputs.

About 30–40% of DIN utilized by phytoplankton is excreted into the water column as labile organic compounds (Bronk et al. 1994). Another portion of the incorporated N is remineralized in the phagotrophic food web. Only  $\sim 30\%$  of the gross primary production is transported below the thermocline in the Baltic Sea on an annual basis (Stigebrandt 1991). Since horizontal transport of juvenile freshwater is fast,  $\sim 4.3 \text{ km/d}$ , mixing of surface water is effective throughout the entire Baltic Sea during one stratified period (Eilola and Stigebrandt 1998). Therefore, we suggest that recycled nutrients from the juvenile freshwater can be an important factor contributing to eutrophication throughout the Baltic Sea.

In addition to their role as sources of N and P to the production of biomass, river-borne organic substances could have a more subtle effect on the community structure and productivity of phytoplankton in the Baltic Sea. Certain primary producers are favored against others in the presence of DOM. Among them are cyanobacteria (Paerl et al. 1987) and dinoflagellates (Granéli and Moreira 1990), both of which dominate summer blooms in the Baltic Sea.

### Conclusions

Inputs of riverine N and P are to a large extent responsible for the worldwide eutrophication of coastal marine waters. Our study demonstrates that contribution of river-borne organic nutrients can be of similar importance as inorganic forms. Chemical characterization and microbial bioassays revealed that an autochthonous, labile component dominated loads of particulate and dissolved organic material from the Baltic Sea watershed during summer. This indicates that riv-

erine plankton converts substantial amounts of nutrients into biomass and DOM, which reach the sea in a largely bioavailable form. Our study is among the first to demonstrate that bacterioplankton is able to utilize a major part of DON and DOP from a broad spectrum of natural waters.

Spectroscopic analyses suggest that high-molecular-mass, allochthonous DOM is more resistant to microbial degradation than algal-derived DOM. The DOC-specific light absorbance ( $A_{430}$ :DOC) appeared to be a relatively good DON bioavailability predictor for a broad spectrum of samples. Interestingly, the concentration of potentially bioavailable DON considerably exceeded concentrations of urea and amino acids in most studied rivers. Thus, a major fraction of bioavailable DON appears to be chemically unidentified. Further research is needed to clarify the relationships between the chemical composition and the biological availability of aquatic organic material.

Our present investigation demonstrates that inorganic, organic, and particulate pools of nutrients, as well as their biological bioavailability, must be considered when establishing a nutrient mass balance of a diverse and complex ecosystem like the Baltic Sea.

#### ACKNOWLEDGMENTS

We are grateful to Regitze E. Jensen for skillful analysis of amino acids, Jan Johansson for optical DOM measurements, and Hanna Eriksson and Elisabeth Fröberg (ITM) for basic water chemistry analyses. Karin Larsson is acknowledged for the help with GIS, and Per Stålnacke, Miguel R. Medina, and Fredrik Wulff for providing access to the BDDb and BED databases. We thank Andrzej Tonderski for lending the water collection device and, together with Per Stålnacke, for useful practical advice arranging the expedition. We thank Eva Waldemarson and Ragnhild Ohlin for lending instruments for the field work, and Ričardas Paškauskas for help with our expedition in Lithuania. Thanks are extended to Fredrik Wulff, Per Stålnacke, Wilhelm Granéli, and Gertrud Cronberg for valuable comments on the paper and Stefan Bertilsson for assistance with multivariate statistics. Financial support for the study was provided by MISTRA, the foundation Oscar och Lili Lamms Minne, the EU TMR research network "Wetland Ecology and Technology" (WET, contract ERBFMRX-CT960051), the Swedish Institute, and the Danish Natural Science Research Council.

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## APPENDIX A

Basic data on the studied rivers [subbasins of the Baltic Sea where rivers discharge, average water flow during May–October 1970–1990 (extracted from the Baltic drainage database; BDDb), and location and date of the water collection] are available in ESA's Electronic Data Archive: *Ecological Archives* M072-008-A1.

## APPENDIX B

Concentrations of total P and total N, nutrient distribution among particulate, dissolved organic, and dissolved inorganic forms, and the proportion of dissolved organic N (DON) bound in urea, dissolved combined amino acids (DCAA), and dissolved free amino acids (DFAA) are available in ESA's Electronic Data Archive: *Ecological Archives* M072-008-A2.

## APPENDIX C

Concentrations of chlorophyll *a*, bacterial cell abundance, bacterial cell mean volume, the estimated concentrations of N and P bound into algal and bacterial biomass, and the percentage of particulate nitrogen explained by the sum of algal and bacterial biomass are available in ESA's Electronic Data Archive: *Ecological Archives* M072-008-A3.

## APPENDIX D

The concentration, chemical characteristics, and potential bioavailability of dissolved organic matter (DOM) in the collected riverine water are available in ESA's Electronic Data Archive: *Ecological Archives* M072-008-A4.