



Biogeochemical Transformations of Inorganic Nutrients in the Mixing Zone between the Danube River and the North-western Black Sea

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Received November 1998 and accepted in revised form November 1999

Biogeochemical transformations of inorganic nutrients were studied in the mixing zone between the Danube and the north-western Black Sea in July 1995 and in the winter–spring transition period of 1997. Inorganic nutrients, phytoplankton carbon-biomass and biogenic silica were analysed at 10 different locations across the entire salinity gradient in combination with parallel measurements of phytoplankton carbon and inorganic nutrient uptake rates, making use of different tracers (¹⁴C, ¹⁵N, ³²Si and ³²P). The quantitative and qualitative nutrient signature of the Danube outflow into the Black Sea varied seasonally, depending on upwards processes of consumption, remineralization, transformation and elimination. Danube DSi (dissolved silicon) and DIN concentrations decreased from winter to summer, although PO₄ remained constant for the whole season. The winter distribution of inorganic nutrients along the salinity gradient was conservative, and phytoplankton biomasses and activities were very low due to strong light limitation. However, significant phytoplankton carbon and inorganic nutrient uptake rates were measured in early spring and during summer at salinities depending on the hydrodynamics of the Danube and Black Sea mixing. Between a salinity of 0 and 10 (using the Practical Salinity Scale), phytoplankton (diatom) growth and mortality processes have been evidenced, which at first strongly lower the inputs of inorganic nutrients to the coastal zone, but also affect the balance among which nutrients are spread over the shelf. These modifications have important consequences for phytoplankton dynamics and species dominance at salinities above 10; diatom growth being apparently P-limited during spring and N-limited during summer. Surprisingly, no DSi limitation of diatom growth has been shown during this study, and a background DSi level of 2–3 μM was measured in the entire shelf area. Causes for such an observation appear to be (1) an efficient biotic and abiotic removal of PO₄ during mixing, (2) an important denitrification in the upper estuary and (3) an important recycling of Si, both in the water column and at the sediment–water interface. The negative long-term effect of the quantitative and qualitative (deficiency in DSi) changes of the Danube inputs on the functioning of the Black Sea pelagic and benthic food-webs have been clearly reported in the literature and cannot be questioned by the present data set. Rather, this study simply highlights the complexity of the mechanisms by which propagation of the Danube eutrophication beyond the river plume possibly occurs.

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Keywords: Danube; north-western Black Sea; nutrients; phytoplankton

Introduction

The north-western shelf of the Black Sea, a shallow semi-enclosed basin that receives the discharge of the rivers Dniepr, Dniestr and Danube, is one of the areas where human-induced eutrophication has been

reported as causing extensive damage to the pelagic (Bodeanu, 1992) and benthic communities (Gomoiu, 1992). Since the 1960s and until the late 1980s, riverine nitrogen (DIN=5NO₃⁻+NH₄⁺) and phosphorus (PO₄) inputs to the north-western Black Sea have increased by a factor 5 and 3, respectively (Cociasu *et al.*, 1996). This is the result of the

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development of economic activities (industrial and agricultural) and urbanization in the watershed of the main tributaries and along the shoreline (Tolmazin, 1985; Mee, 1992). Over the same period, dissolved silicon (DSi) carried by the rivers significantly decreased as a result of numerous hydraulic management programmes (Tolmazin, 1985; Humborg *et al.*, 1997). Hence, the nutrient environment of the north-western Black Sea phytoplankton was dramatically modified, both in quantity and quality. This is particularly well documented in the coastal zone under the influence of the Danube River, discharging about 70% of the total freshwater load to the Black Sea (Tolmazin, 1985). The deficiency of DSi with respect to DIN and PO₄ has been hypothesized to be at the basis of the replacement of diatoms by non-siliceous, often less desirable, phytoplankton (Officer & Ryther, 1980; review in Smayda, 1990; see Humborg *et al.*, 1997, for the Black Sea). Accompanying these changes in inorganic nutrients, a qualitative shift occurred among the oxidation degree of nitrogen compounds being delivered to the coastal areas due to eutrophication in the freshwater reservoirs (Sapozhnikov, 1992). Taken together, these processes have stimulated phytoplankton blooms of altered species composition (Bodeanu, 1992). This change in the phytoplankton community structure towards non-siliceous mixotrophic species (Bodeanu, 1984; Bologna *et al.*, 1995; Bouvier *et al.*, 1998) and its cascading effect on the pelagic food chain structure appear to be at the basis of the Black Sea ecosystem destabilization (e.g. Mee, 1992; Van Eeckhout & Lancelot, 1997).

However, the link between nutrient changes in the Danube River and the phytoplankton dynamics of the Black Sea is not direct, due to biogeochemical transformations of nutrients in the Danube estuary. The importance of these processes depends on the residence time of Danube waters in the estuary, and varies seasonally. Yet inorganic nutrient removal in the Danube estuary has been shown to occur in spring and summer and was ascribed to phytoplankton growth in the well-illuminated shallow Danube plume (Humborg *et al.*, 1997). However, no direct evidence of phytoplankton nutrient uptake rates in the Danube estuary have been given to date, and the factors controlling these uptakes remain unknown.

Such data are presented in this paper, which discusses the seasonal modulation of biogeochemical transformations of inorganic nutrients in the Danube–Black Sea mixing zone. Results are based on the analysis of inorganic nutrients, phytoplankton carbon-biomass and biogenic silica distributions along salinity gradients in combination with parallel measurements of phytoplankton carbon and inorganic nutrient

uptake, making use of different tracers (¹⁴C, ¹⁵N, ³²Si and ³²P). These measurements were conducted in summer 1995 and in the winter–spring transition period of April–May 1997. A shipboard artificial salinity gradient was additionally run in April 1997 to assess the fate of freshwater phytoplankton when mixed with salt waters. Results are discussed in terms of changes in the nutrient balance along the estuary and their consequences for the dynamics of coastal phytoplankton and the spreading of inorganic nutrients over the north-western shelf.

Materials and methods

Study area

With a mean annual discharge of 210 km³, the Danube outflows directly into the Black Sea through three branches (Chilia, Sulina and St. Gheorghe) of its delta (Figure 1) and mixes with Black Sea waters on the shelf (Serpoianu *et al.*, 1982). The spatial and temporal scales of the mixing processes rely on the river discharge and the prevailing wind conditions (direction, speed and frequency), which both display large seasonal fluctuations. In winter, the mixing is generally rapid and the river plume is not well formed. By spring time, a 0.5–3-m deep river plume flows on top of the shelf water (Humborg, 1997), which is itself strongly stratified from spring to autumn. This river plume usually moves southward along the Romanian coast, down to the Bulgarian coast and the Bosphorus strait, owing to the Coriolis force and the prevailing northern winds blowing in this region (Cociasu *et al.*, 1996).

Sampling

Samples were collected during Leg 1 of the EROS 2000 (1 July–2 August 1995) and EROS 21 (9 April–5 May 1997) cruises of RV *Professor Vodyanitsky* (Sevastopol, Ukraine) in the Ukrainian, Romanian and Bulgarian exclusive economic zones of the north-western Black Sea. Four salinity gradients of high resolution (10 samples in the 0–18 salinity range) were performed in parallel with the sampling of the shelf, in surface waters off the St. Gheorghe branch (July 1995), the Sulina branch (18 April 1997 and 5 May 1997) and the Chilia branch (15 April 1997) of the delta (Figure 1). Sampling carried out in 1997 was conducted aboard the Romanian *Filioara* and Ukrainian *Sprut* coastal boats. A rubber boat was used in 1995. Surface water samples were collected with clean plastic carboys or glass bottles and brought back

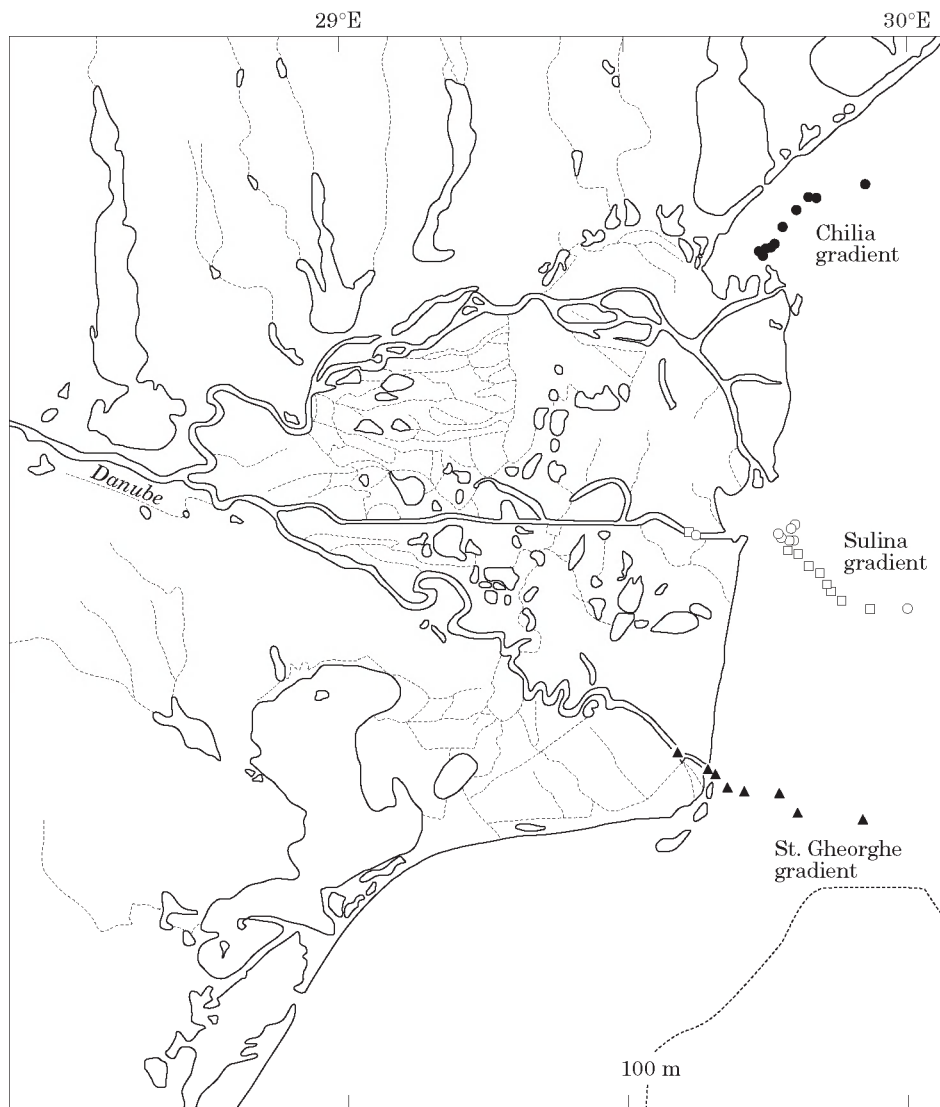


FIGURE 1. Map of the research area showing the four salinity gradients sampled off the St. Gheorghe branch (summer 1995, black triangles), the Chilia branch (winter 1997, black circles) and the Sulina branch (winter 1997, open circles; spring 1997, open squares).

to RV *Professor Vodyanitsky*, where they were immediately processed. The marine end-member was sampled, from surface to bottom with RV *Professor Vodyanitsky*, making use of a CTD-Mark III equipped with a Go FLO rosette. Sampling was completed in less than 10 h, which allows the observation of quasi-stationary physical and chemical properties given to the hydrodynamics of the mixing between freshwaters and marine waters (Bol'shakov, 1970).

The resistance of freshwater phytoplankton to salinity increase was investigated by running a ship-board artificial gradient. A phytoplankton community sampled at a salinity of 0.3 was mixed with different volumes of 0.2- μm filtered seawater of salinity 18

(marine reference) and amended with inorganic nutrients to correct the dilution effect. A total of 10 salinities were reconstructed, and samples were processed for the measurement of chemical stocks and biological activity as described below. Note that this experiment does not mimic *in situ* mixing processes, as freshwater phytoplankton was submitted instantaneously to various salinities. Rather, this experiment provides an indication of the extent to which the whole freshwater assemblage, or part of it, is affected by salinity, and which salinity is lethal for some species. Both sources of information prove to be very helpful for the interpretation of carbon and inorganic nutrient uptake rate measurements.

Optical measurements

Incident photosynthetically available radiation (PAR) was continuously recorded making use of a cosine Li-COR sensor set up on the upper deck of the ship. The vertical light attenuation coefficient K_{PAR} was determined from the vertical distribution of PAR obtained by deploying the PNF profiler from RV *Professor Vodyanitsky*. The Secchi disk was used during the salinity gradient sampling. K_{PAR} was then calculated using the following empirical relationship based on parallel deployments of the PNF and Secchi disk in the investigated area:

$$K_{\text{PAR}} = 1.55 [(\text{Secchi})^{1.2}]^{-1}.$$

Chemical methods

Inorganic nutrients were determined directly after sampling, following classical spectrophotometric methods, i.e. Strickland and Parsons (1972) for NO_3 , Grasshoff *et al.* (1983) for DSi and PO_4 and Korolef (1969) for NH_4 .

Samples collected for particulate organic carbon (POC), particulate organic nitrogen (PON), chlorophyll *a* (Chl *a*) and biogenic silica (BSi) were filtered immediately after sampling. Filters for Chl *a* determination (Whatman GF/F filters, $\sim 0.7 \mu\text{m}$ pore size) were stored in liquid nitrogen until analysis. Filters for POC and PON (pre-combusted Whatman GF/F filters $\sim 0.7 \mu\text{m}$ pore size) and filters for BSi (Nucleopore, $0.6 \mu\text{m}$ pore size to allow comparison with Chl *a*) were dried at 60°C overnight and stored at an ambient temperature until analysis.

Chl *a* and phaeo-pigment *a* concentrations were determined by spectrophotometric analysis using the Lorenzen (1967) method. POC and PON were analysed (Cauwet, 1994) with a Solid Sample Module (SSM-5000) associated with a Shimadzu TOC-5000 total carbon analyser and a chemiluminescent nitrogen detector (SIEVERS NO 270B).

The protocol of Brzezinski and Nelson (1989) was adapted to estimate biogenic silica from the total particulate silica in coastal waters. The original method consisted of two successive alkaline digestions, the first one (NaOH 0.2 M at 100°C for 40 min) dissolved all the BSi and the second one (HF 2.9 M at ambient temperature for 48 h) dissolved the remaining particulate silica, the lithogenic fraction (LSi). In coastal areas, however, a significant fraction (c. 10%) of LSi dissolves during NaOH digestion (Ragueneau & Tréguer, 1994), which may lead to overestimates of BSi. To correct this interference, an additional NaOH 0.2 M digestion was added before

the use of HF for the determination of the Si/Al ratio of the dissolving silicate minerals. Applied to each sample, this method yielded Si/Al molar ratios of 2.0 (± 0.5), which are consistent with those determined in Tokyo Bay by Kamatani and Takano (1984). This ratio was then used to estimate, from concomitant measurements of Al concentration, the LSi extracted during the first alkaline digestion of the samples and then correct BSi measurements from the mineral interference. As an example, Danube waters contained 54 and $180 \mu\text{M}$ of LSi in winter 1997 and summer 1995, respectively. Using the correction described above yielded a difference between the apparent and corrected BSi concentrations that corresponded to a dissolution of 15 and 7% of the LSi during the NaOH digestion, respectively. This range is in close agreement to the one provided for pure silicate minerals by Ragueneau and Tréguer (1994) and for other coastal environments (Ragueneau *et al.*, in prep). The absence of correction would generate overestimates of the BSi by almost 100% for the highest values of the LSi/BSi ratio.

Phytoplankton chemical composition

POC, PON and BSi may all contain a significant detrital component that needs to be estimated. The living fraction of the BSi as well as the phytoplankton carbon- and nitrogen-biomass were estimated from Chl *a*, using the significant linear relationships, between POC and Chl *a* ($R^2=0.52$, $N=14$), between PON and Chl *a* ($R^2=0.71$; $N=14$) and between BSi and Chl *a* ($R^2=0.88$; $N=18$), obtained when combining all data recorded across the salinity gradient of the Danube estuary during the vegetative period (Figure 2). This statistical analysis gives a POC/Chl *a* ratio ($\mu\text{g}:\mu\text{g}$) of 41, which is in reasonable agreement with ratios typical of healthy cells growing with excess nutrients (Banse, 1977; Montagnes *et al.*, 1994). It provides a PON/Chl *a* ratio ($\mu\text{g}:\mu\text{g}$) of 6.05, i.e. a C/N ratio of 6.77, which is very close to the Redfield ratio (Redfield *et al.*, 1963). The excellent relationship between BSi and Chl *a* concentrations, and the very low BSi level at zero Chl *a*, strongly suggest that little detrital BSi is present in the mixing area during the productive period and that diatoms are a major constituent of the phytoplankton community. Accordingly, the combination of POC/Chl *a* and BSi/Chl *a* slopes gives a Si/C molar ratio of 0.21 for the estuarine phytoplankton assemblage. As would be expected for estuarine species, this value is higher than the Si/C stoichiometry of 0.13 given for coastal diatoms growing under nutrient-repleted conditions (Brzezinski, 1985) and lower than Si/C of 0.4 reported for

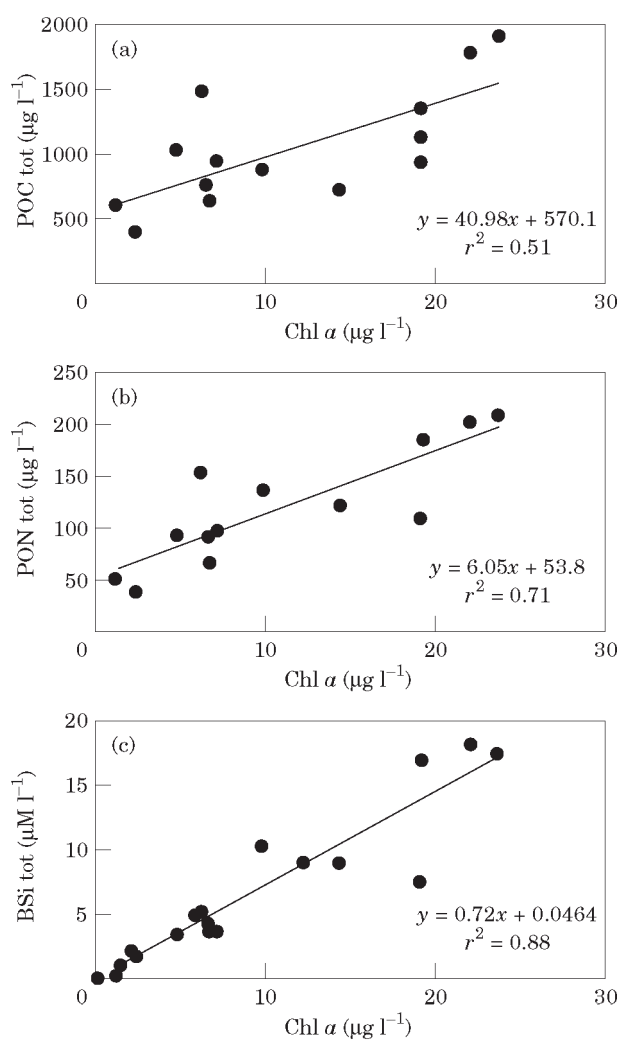


FIGURE 2. Linear relationships between (a) POC and Chl *a*, (b) PON and Chl *a* and (c) BSi and Chl *a* for the productive period.

freshwater diatoms (Conley *et al.*, 1989). No relationship could be shown between POC, PON and BSi, on the one hand, and the Chl *a*, on the other, for the winter period due to the low phytoplankton activity and the significant contribution of detritus to the bulk of organic matter. Therefore, the ratios determined for the productive period were used to calculate the contribution of phytoplankton to the winter bulk of particulate organic matter.

Phytoplankton photosynthetic characteristics

Photosynthetic characteristics of phytoplankton were determined from short-term (2–3 h) experiments made of ¹⁴C uptake at different light intensities (P/E curve). ¹⁴C-bicarbonate (Amersham) was added to 100 ml of seawater at a rate of 0.1 µCi ml⁻¹. After

incubation, phytoplankton cells were collected by filtration through GF/F Whatman filters. Radioactivity incorporated by phytoplankton was determined by liquid scintillation according to the protocol described in Mathot *et al.* (1992). Photosynthetic parameters—the maximal photosynthesis rate and the light adaptation parameter I_k —were determined by mathematical adjustment of P/E data making use of the equation by Platt and Gallegos (1980).

Carbon and inorganic nutrient uptake rates

Parallel measurements of carbon and nutrient uptake rates by surface layer phytoplankton were conducted under incident-surface light conditions making use of tracer techniques (¹⁴C, ¹⁵N, ³²Si and ³²P). Twenty-four-hour incubations of phytoplankton communities spiked with C, N and Si tracers were conducted in on-deck incubators, under *in situ*-simulated conditions. These measurements will be referred to as optimal rates, as incubations were performed at 100% of the incident radiation. P tracer incubations were achieved under artificial light in a temperature-controlled cabinet. Light intensity was comparable to the average light received by the on-deck incubators. Due to the high turnover rate of PO₄, the incubation duration was followed kinetically over 2 h. These results can be extrapolated to 24 h as phytoplankton PO₄ uptake has been shown to proceed linearly over a daily cycle (Veldhuis *et al.*, 1986).

NO₃ and NH₄ uptake rate experiments were performed with a standard ¹⁵N methodology. Experiments were launched after tracer addition (99% ¹⁵NO₃ or ¹⁵NH₄) to the seawater sample, confined in 2.7-l polycarbonate bottles. The particulate matter in the sample was collected after incubation by filtration on pre-ashed Whatman GF/F filters. The filters were stored in polystyrene petri dishes and dried at 50 °C. ¹⁵N accumulation in the particulate nitrogen (PN) was measured by conversion of the PN to N₂ using a modified Dumas combustion technique. Isotope detection was achieved by optical emission spectrometry making use of a Jasco NIA-1 Analyser; ¹⁵N abundances were calibrated against certified standards (Goeyens *et al.*, 1995). Uptake rates were calculated according to the formulae of Dugdale and Wilkerson (1986) and Collos (1987).

PO₄ uptake rates were measured by amending natural samples with ³²P-orthophosphoric acid (Amersham). Parallel radiotracer incubations with HgCl₂-poisoned samples (70 ppm, final concentration) were run to determine the passive adsorption of PO₄ on particles. After incubation, size fractionation (10-, 2-, 1.2-, 0.45- and 0.2-µm Sartorius

membranes) was performed in order to separate the bacterial and phytoplanktonic contributions to the PO_4 uptake. Phytoplankton PO_4 uptake was obtained by cumulating the radioactivity incorporated in the fractions >10 and $2\text{--}10\ \mu\text{m}$, corrected from the passive adsorption on the same fractions, which can be very important in the low salinity region. Additional incubations were occasionally run with natural communities enriched with different concentrations of inorganic PO_4 , up to 10 times the ambient level, in order to determine the kinetic properties of PO_4 uptake for different Black Sea phytoplankton populations.

DSi uptake rates were measured using the radioactive ^{32}Si technique (Tréguer *et al.*, 1991). Forty μl of the stock $^{32}\text{Si}(\text{OH})_4$ solution ($0.6\ \mu\text{Ci ml}^{-1}$, Los Alamos, U.S.A.) were added to 250-ml incubation flasks. After incubation, radioactive cells were collected by filtration on $0.6\text{-}\mu\text{m}$ Nucleopore membranes. ^{32}Si incorporation was estimated using the detection of the Cerenkov radiation of the daughter, ^{32}P , after secular equilibrium was reached, i.e. about 4 months after termination of the cruise. As for P tracer experiments, parallel incubations were run with HgCl_2 -poisoned samples in order to determine passive adsorption of DSi on particles. No adsorption could be detected, in agreement with previous observations of DSi behaviour in estuaries (Mayer & Gloss, 1980).

Results

Meteorological and hydrological conditions

The feature of the Danube River plume strongly depends on the freshwater discharge and the meteorological conditions (Tolmazin, 1985). In April–May 1997, the monthly river discharge was $20.58\ \text{km}^3\ \text{month}^{-1}$, close to the monthly average calculated for the post-Iron Gate dam construction (1975–1995; Cociasu *et al.*, 1997). Up to 25 April 1997, however, typical winter meteorological conditions prevailed, and the $7.8\ ^\circ\text{C}$ Danube waters outflowed into the well-mixed $5.8\ ^\circ\text{C}$ Black Sea (Guieu & Martin, 2002). No real river plume was formed and the Danube–Black Sea mixing was rapid, extending to less than 10 miles offshore. This sampling period will be referred as the ‘winter situation’. Stable spring meteorological conditions prevailed after 25 April 1997, enhancing the thermal stratification of the Black Sea, whose surface layer reached $9\ ^\circ\text{C}$ in early May 1997. The Danube temperature was above $12\ ^\circ\text{C}$ at this time and a stable well-formed 2-m-deep river plume outflowed over the shelf Black Sea waters, up to a

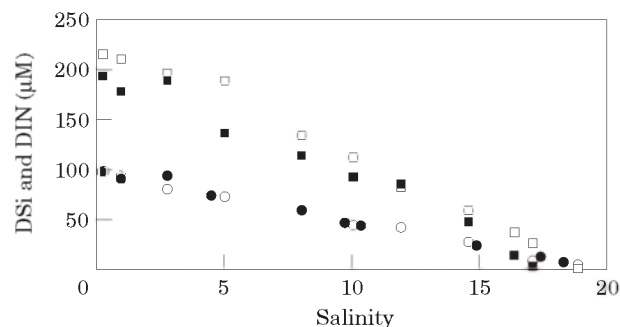


FIGURE 3. DSi (circles) and DIN (squares) winter mixing diagrams for the Chilia (open symbols) and Sulina (filled symbols) branches of the Danube delta.

distance of 40 miles offshore. In July 1995, the Danube–Black Sea mixing zone was between the winter and spring features recorded in 1997. The monthly river discharge was $21.7\ \text{km}^3$, significantly higher than the July average calculated for the post-Iron Gate dam construction ($17.5\ \text{km}^3\ \text{month}^{-1}$, Cociasu *et al.*, 1997). The temperature of the Black Sea surface waters was $24\ ^\circ\text{C}$, and a 20 m-deep, strong thermocline isolated the surface from the $8\ ^\circ\text{C}$ deep saline waters. Due to changing wind conditions, hydrodynamic instability prevailed in the vicinity of the Danube delta mouth where transient wind mixing events destroyed the river plume. The latter, however, was reformed very rapidly, depending on the duration, strength and direction of wind events.

Nutrient transformations in the Danube river plume

Similarity of mixing processes at two Danube delta branch outflows. Figure 3 compares the mixing diagrams of DSi and DIN concentrations recorded across the salinity gradient in front of the Chilia and Sulina branches of the Danube delta during winter. The two salinity profiles appear homogeneous and, according to trace metal distributions, this similarity can be extended to the St. Gheorghe branch (Guieu & Martin, 2002). This allows the comparison of the three seasonal snapshots of nutrient transformations in the Danube River plume, although based on data recorded in front of two different branches of the delta, namely the Sulina (winter–spring transition period) and the St. Gheorghe (summer).

Winter: conservative mixing. By early April 1997, all nutrients behaved in a so-called conservative way in relation to theoretical dilution, suggesting that the mixing of the Danube with Black Sea waters was alone responsible for the observed distribution of nutrients [Figure 4 (a,d,g,i)]. Extrapolation of the regression

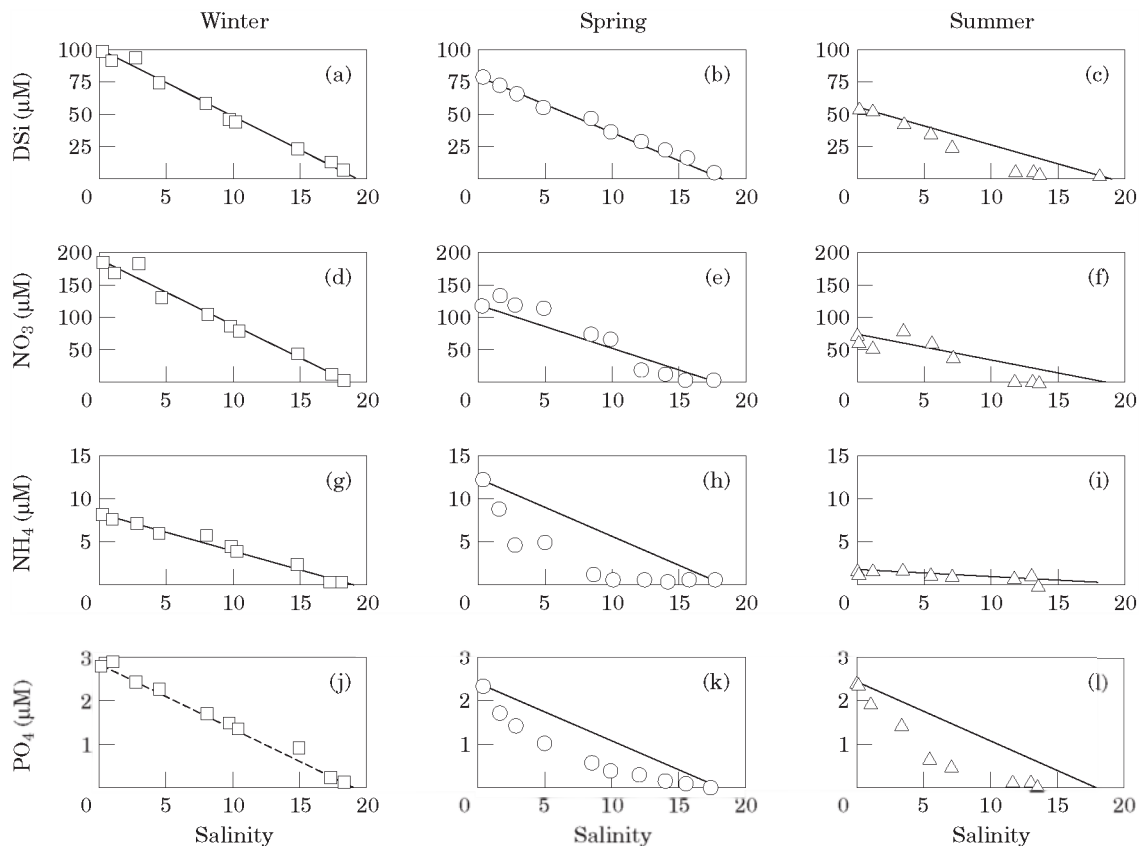


FIGURE 4. Nutrient distributions in the Sulina and St. Gheorghe branches of the Danube delta during summer 1995 (triangles, right panels), winter 1997 (squares, left panels) and spring 1997 (circles, middle panels). (a, b, c) DSi, (d, e, f) NO_3^- , (g, h, i) ammonia and (j, k, l) PO_4 . Conservative mixing is shown by the linear dilution curves between the river and marine end-members.

lines to zero-salinity estimated 100, 200, 8 and 3 μM for the respective concentrations of DSi, NO_3^- , NH_4 and PO_4 at the Danube outflow (Figure 4), in close agreement with observations over recent years (Cociasu *et al.*, 1997). Compared to the Si:N:P of 16:16:1, summarizing the nutrient needs of coastal phytoplankton and diatoms (Redfield *et al.*, 1963; Brzezinski, 1985), the outflowing Danube waters were 'NO₃-excess' with respect to DSi and PO_4 and reasonably balanced between PO_4 and DSi (Figure 5). In contrast, winter concentrations of the marine end-member (salinity 18.2) were largely DSi-excess (DSi=6.9 μM) with respect to NO_3^- , NH_4 and PO_4 , whose concentrations were typically oligotrophic, i.e. 1.8, 0.4 and 0.1 μM , respectively (Figure 4). Accordingly, phytoplankton activity was light-limited across the entire salinity gradient (Table 1), and low absolute [Figure 7 (a,c,f)] and specific [Figure 8 (a,c)] optimal rates of nutrient uptake were measured during that winter period.

Beyond the conservative properties shown in winter for inorganic nutrients, an important feature of river

characteristics in this season is the importance of the freshwater-derived detrital carbon and BSi, which represent more than 50% of the total organic matter. Detrital POC and BSi were not linearly distributed along the salinity gradient [Figure 6 (d,g)], being partly removed in 5–8 salinity section. This sink is particularly important for BSi, suggesting a massive sedimentation of freshwater diatom-derived material [Figure 6(h)].

Spring: evidence for complex nutrient transformations in the river plume. By early spring, DSi, NO_3^- and PO_4 decreased in the river end-member by 20–30% [Figure 4(b,e,k)]. In contrast, NH_4 increased by 50% [Figure 4(h)]. Interestingly, the nutrients displayed a completely different behaviour during the mixing of freshwater with the saline waters of the Black Sea (Figure 4). DSi exhibited the most apparent conservative behaviour [Figure 4(b)]. An apparent source of NO_3^- was observed at 3–5 salinity, while removal was apparent at salinities above 10 [Figure 4(e)]. NH_4 was exponentially removed at low salinity, decreasing from

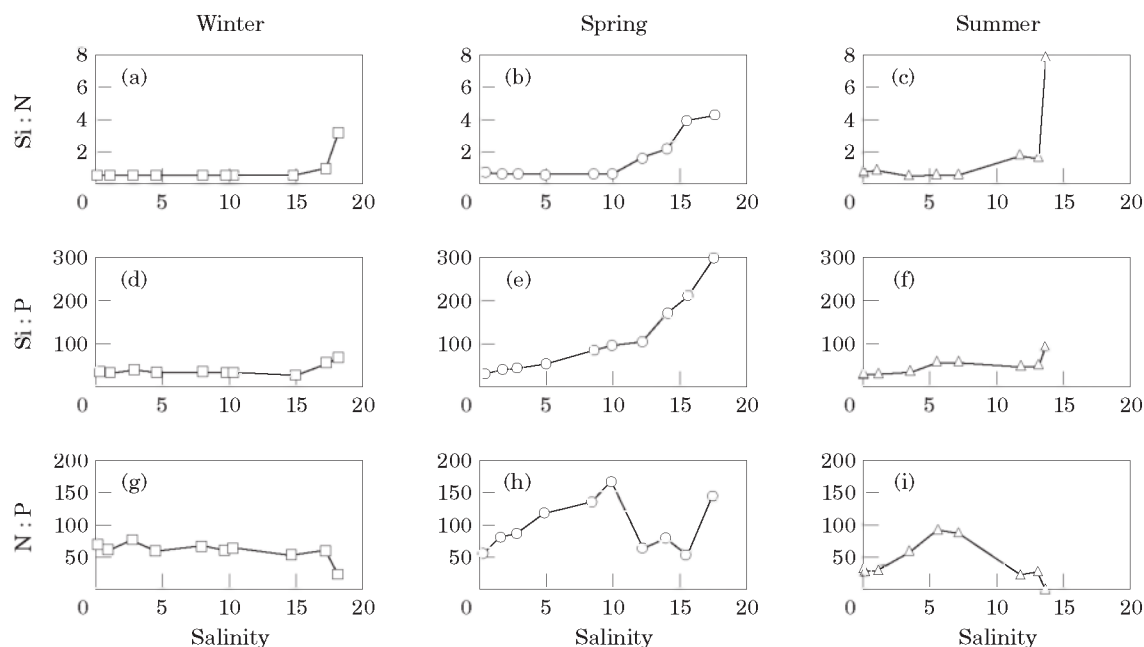


FIGURE 5. Horizontal modification of the nutrient ratios in the Sulina and St. Gheorghe branches of the Danube delta during summer 1995 (triangles, right panels), winter 1997 (squares, left panels) and spring 1997 (circles, middle panels). (a, b, c) Si:N, (d, e, f) Si:P and (g, h, i) N:P.

TABLE 1. Ambient PAR and light adaptation of phytoplankton

Season	Area	I_0 ($\mu\text{M m}^{-2} \text{s}^{-1}$)	I_a ($\mu\text{M m}^{-2} \text{s}^{-1}$)	I_k ($\mu\text{M m}^{-2} \text{s}^{-1}$)	I_a/I_k
winter	freshwater	313 (115–460)	14	83	0.17
	mixing	313 (115–460)	58 (46–82)	119 (55–185)	0.48 (0.37–0.63)
	marine	313 (115–460)	79 (53–104)	50 (40–60)	1.70 (0.90–2.50)
spring	freshwater	470			0.45 (0.10–0.80)
	mixing		276 (64–420)	142 (72–217)	2.00 (1.00–2.50)
	marine		167	147	1.10
summer	mixing	560 (405–623)	194 (86–424)	232 (119–520)	0.88 (0.30–2.00)
	marine	624	249	119	2.10

I_0 =incident surface PAR, I_a is the average PAR in the upper surface layer and I_k is the light adaptation parameter (Platt *et al.*, 1980). When I_a/I_k is higher than 1, light does not limit phytoplankton growth.

12 μM at the Danube mouth to less than 1 μM at a salinity of 10 [Figure 4(h)]. Finally, strong removal of PO_4 can be inferred from the strongly negative deviation of the PO_4 mixing curve from the theoretical dilution curve [Figure 4(k)].

This differential behaviour among nutrients induces modifications in the ratios at which nutrients are spread within the coastal zone [Figure 5(b,e,h)]. The Si/N ratio shifts from Si-deficiency to Si-excess above a salinity of 10 [Figure 5(b)]. Incidentally, this shift coincides with a very low PO_4 concentration, below 0.5 μM [Figure 4(k)]. The Si/P [Figure 5(e)] and N/P [Figure 5(g)] ratios regularly increase for salinity

ranging from 0 to 10, due to the exponential removal of PO_4 [Figure 4(k)]. In particular, the N/P ratio reaches very high values (*c.* 150) at a salinity of 10, compared to 50 in the river end-member. Interestingly, the Si/P ratio keeps increasing above a salinity of 10, while the N/P ratio displays a strong decrease for a salinity between 10 and 15 [Figure 5(e,h)].

In agreement with the apparent nutrient removal, phytoplankton biomass increased along the salinity gradient, substantially at a salinity of between 3 and 5 and moderately at an intermediate salinity around 10–13 [Figure 6(b,e,h)]. Clearly, these biomass increases resulted from phytoplankton photosynthetic

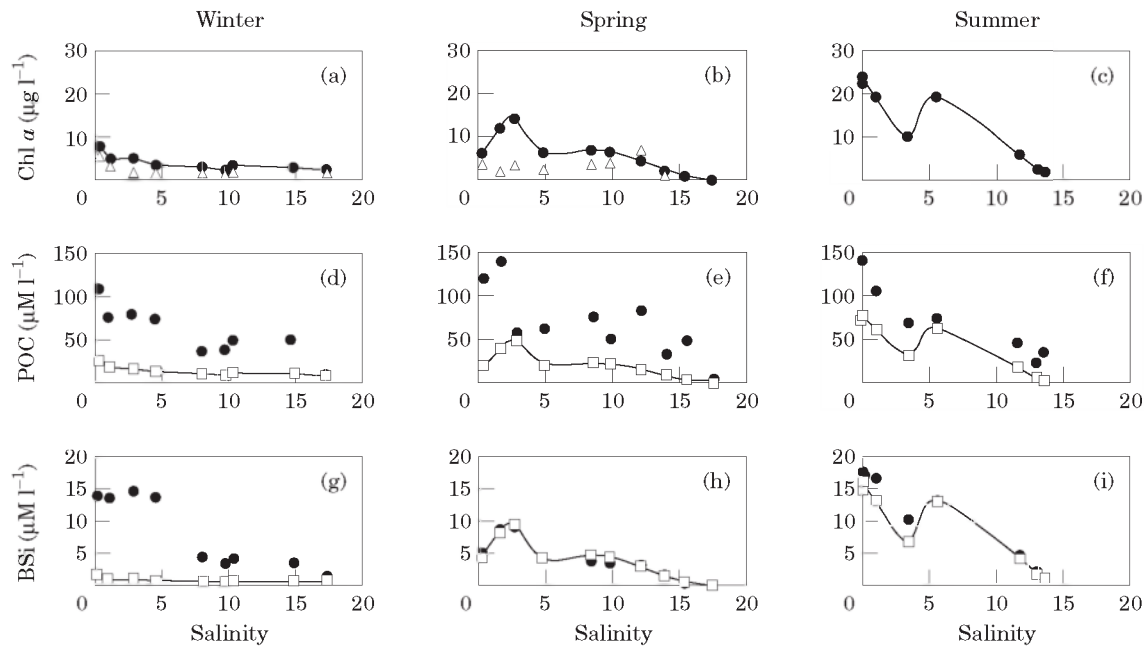


FIGURE 6. Distribution of (a, b, c) Chlorophyll (filled circles) and phaeophytin (open triangles), (d, e, f) POC and (g, h, i) BSi in the Sulina and St. Gheorghe branches of the Danube delta during summer 1995 (right panels), winter 1997 (left panels) and spring 1997 (middle panels). For POC and BSi, the total (filled circles) and living (open squares) fractions are indicated.

activity and nutrient uptake processes occurring all along the river plume, but mainly at a salinity of between 2 and 5 (Figures 7, 8). Below salinity 2, phytoplankton activity was typically light-limited due to the high load of suspended matter (Figures 7, 8; Table 1). Above this salinity, sufficient light was available for phytoplankton (Table 1) and Si, N and P uptakes proceeded at a high rate (Figure 7), reaching their maximum specific uptake at salinity 5 (Figure 8). The high ambient light and nutrient concentrations at low salinity allowed phytoplankton to grow at a high daily specific rate of about 1 (Figure 8). Regarding the N-uptake regime of phytoplankton, it is interesting to observe an NH_4 -based nitrogen uptake in the freshwater section of the Danube estuary for both periods [Figure 7(f,g)]. This most probably reflects inhibition of NO_3 uptake by the high concentrations of NH_4 (review in Dortch, 1990). At the intermediate salinities, the N-uptake shifted from an NH_4 -based uptake regime in winter to a NO_3 -based one in spring [Figure 7(g)]. Above a salinity of 5, specific rates of inorganic nutrient uptake were decreased dramatically, reaching values comparable to the winter level at a salinity of between 8 and 13 [Figure 8(b,d)]. Photosynthesis exhibited a distinct behaviour, as a high specific rate was maintained up to a salinity of 10, above which levels comparable to winter were reached [Figure 8(b,d)]. This discrepancy between C and

Si/P/N assimilation (Figure 7) suggests the occurrence, above a salinity of 5, of severe nutrient limitation, or of species-related mortality processes. The mortality/inactivation of freshwater phytoplankton when mixed with salt water was shown by the ‘artificial gradient’ experiment, run under saturated light and nutrient conditions [Figure 9(a)]. As much as 80% of the initial Si uptake was lost at a salinity of 8 [Figure 9(b)], suggesting that freshwater diatoms were poorly resistant to the salt increase. Interestingly enough, phytoplankton C-assimilation losses did not parallel diatom mortality, remaining below 50% up to a salinity of 13 [Figure 9(b)].

Summer: upstream nitrification and phytoplankton growth at intermediate salinity. During summer, nutrient concentrations in the river end-member were further reduced for DSi , NO_3 and NH_4 , but interestingly, not for PO_4 (Figure 4). DSi concentrations deviated from the dilution curve at salinities above 5 [Figure 4(c)]. The same patterns as those described for the early spring period can be observed for both NO_3 and PO_4 . Compared to spring, nitrification was more pronounced and shifted at a higher salinity [Figure 4(f)]. NH_4 concentration at the river outflow was very low and simply diluted with seawater [Figure 4(i)]. This suggests that upstream nitrification proceeded without transient accumulation of NH_4 , and that NO_3 was the

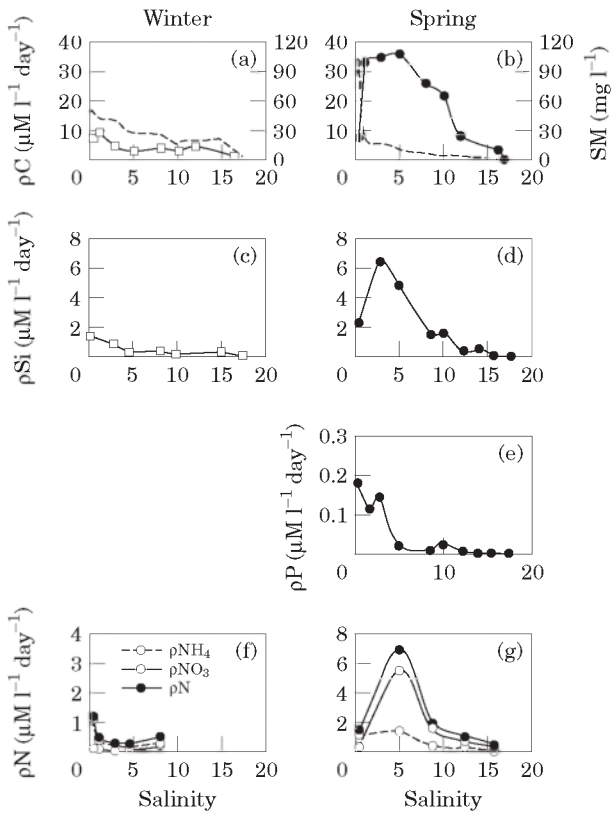


FIGURE 7. Nutrient uptake rates along the salinity gradient of the Sulina branch during winter (left panels) and spring (right panels). (a, b) ρC , (c, d) ρSi , (e) ρP and (f, g) ρN . In (a, b), the dotted line shows the suspended matter (SM) concentration. P uptake rate has been measured along salinity gradients only during spring (see e). In (f, g), the open circles, solid line refer to ρNO_3 , while the open circles, dotted line refer to ρNH_4 .

nitrogen form of phytoplankton N-uptake within the Danube–Black Sea mixing zone. The Si/N shift from Si to N deficiency occurred at a salinity of between 8 and 12 [Figure 5(c)]. Interestingly enough, N/P ratios shifted towards N deficiency in the same salinity range [Figure 5(i)]. Freshwater phytoplankton rapidly decreased from a Chl *a* maximum concentration of $24 \mu g l^{-1}$ at the Danube outflow to $10 \mu g Chl a l^{-1}$ at a salinity of 4 [Figure 6(c)], most probably due to salt-induced mortality (Figure 9) followed by mass sedimentation or intense degradation. Unfortunately, no measurements of phytoplankton activity were performed during the summer of 1995 below a salinity of 10.

Discussion

Biogeochemical signature of the Danube end-member

The concentrations of inorganic nutrients and particulate biogenic matter at the Danube outflow (limit

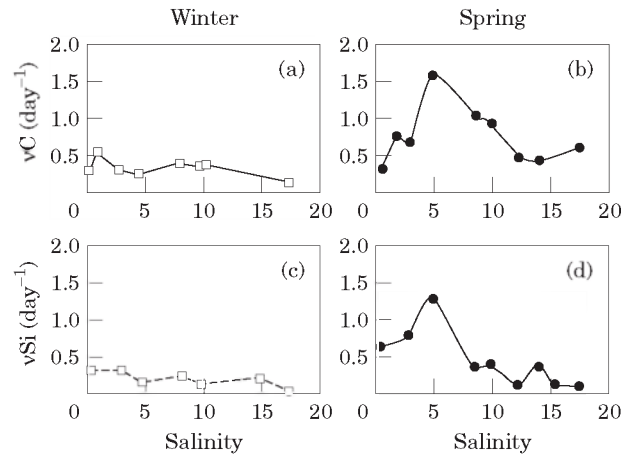


FIGURE 8. (a, b) Carbon and (c and d) silicon specific uptake rates along the salinity gradients sampled in the Sulina branch of the delta, under winter (left panels) and spring (right panels) conditions.

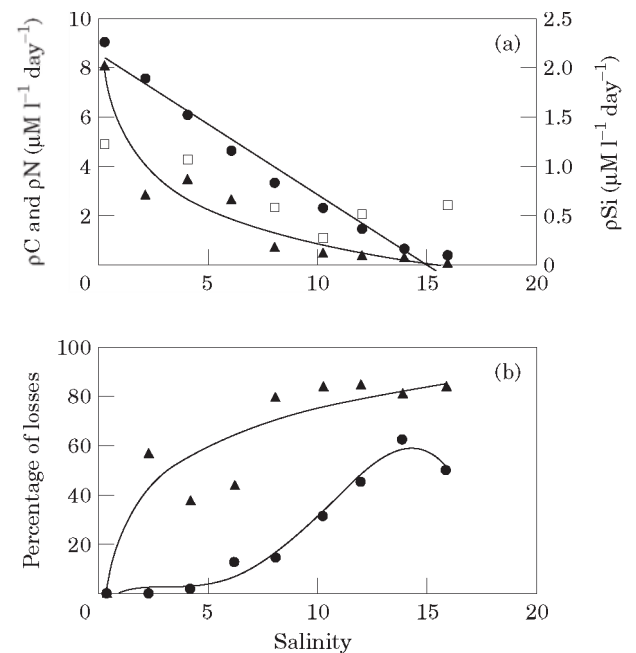


FIGURE 9. Artificial mixing experiment. (a) Salinity change of C (filled circles), N (open squares) and Si (filled triangles) uptake rates. (b) Salinity change of the calculated specific losses of C (filled circles) and Si (filled triangles) uptake rates (see text).

of salt intrusion) are determined by the input of nutrients from the catchment basin and by biogeochemical processes that occur in the river system (see Garnier *et al.*, 2002) and vary seasonally. Between winter and summer, the decreasing nutrient concentrations (Figure 4) and the concomitant increase of Chl *a*, POC and BSi concentrations (Figure 6)

TABLE 2. Spring–summer biogeochemical signature at the Danube outflow in the Black Sea

Element	1992–1993 (Humborg, 1997)	1995–1997 (this study)
DIN (μM)	120–220	54–120
PO ₄ (μM)	1.8–4.5	2.2
DSi (μM)	10–58	50–75
Chl <i>a</i> ($\mu\text{g l}^{-1}$)	1–8	5–22
BSi ($\mu\text{M l}^{-1}$)	0.8–6 ^a	5–18

^a Calculated from Chl *a* concentration using BSi/Chl *a* ratio determined in this study.

indicate active phytoplankton production in the river in general, and diatom growth in particular. Furthermore, the strong reduction of DIN, combined with the lack of apparent seasonal change of PO₄ (Figure 4), especially between spring and summer, can be seen as the result of upstream anoxic events that lead to both denitrification and desorption of particle-bound phosphorus (Conley *et al.*, 1995). Accumulations of the released PO₄ may indeed have been masked by direct phytoplankton uptake, as suggested by the observed 30% decrease of DSi and DIN at the same location (Figure 4). As a direct result of river primary production in spring–summer, the Danube also carries with it large amounts of biogenic particles, as indicated by the high levels of Chl *a*, a freshwater-derived detrital particulate carbon and biogenic silica recorded at the river outflow in the Black Sea (Figure 6). In particular, up to 24% of the total Si delivered by the Danube is in the form of BSi during the summer, which is in reasonable agreement with other estuaries (14–37% for global river end-members, Conley, 1997). This confirms the importance of riverine particulates in the total nutrient load to coastal ecosystems, as reported by Conley (1997) and Mayer *et al.* (1998) for Si and N, respectively. These inputs provide an important potential source of dissolved organic and inorganic nutrients, which is available for phytoplankton and bacteria after phytoplankton lysis (Figure 9) and nutrient remineralization.

Beside this strong seasonality of river biological processes, there also exists an important interannual variability in the system, as evidenced by the comparison to similar studies conducted in the spring–summer period of 1992–1993 by Humborg (1997). Of particular interest are the significantly higher DSi, but lower DIN and PO₄, concentrations at the Danube outflow, recorded in recent years compared to 1992–1993 (Table 2). This could result from changes in the timing and magnitude of river

biological processes and/or land use. In their analysis of the long-term evolution of nutrient concentrations in the Danube and the north-western shelf, Cociasu and Popa (2002) indeed indicate that a significant decrease of Danube DIN and PO₄ loads has been observed since 1995. At the same time a slight increase of DSi inputs was recorded. On the other hand, the extremely low Danube DSi concentration in the summers of 1992–1993 could well be the result of an intense diatom production that may have occurred just upstream of the limit of salt intrusion, as observed in the Chesapeake Bay estuaries by Anderson (1986). The accompanying low Chl *a* and calculated diatom-BSi (Table 2) suggests that most of the BSi is delivered as diatom-derived detrital matter. However, in the absence of such data, sedimentation of freshwater diatoms in the upper part of the river and delta cannot be excluded.

Transformations of inorganic nutrients during mixing

Once mixed with marine waters, inorganic nutrients and phytoplankton of a river origin are progressively transformed along the salinity gradient. Complex biogeochemical processes occur in the low salinity (0–10) part of the mixing zone during spring and summer (Figures 4–9), which are attributable to freshwater phytoplankton growth and mortality processes, as well as to bacterial organic matter degradation (Becquevort *et al.*, 2002). Both the intensity of these processes and the salinity at which they reach their maximal rates are strongly determined by the hydrodynamics of the Danube–Black Sea mixing zone (see DeMaster *et al.*, 1983; Lohrenz *et al.*, 1990; Turner *et al.*, 1990, for interesting comparisons in the Amazon, Mississippi and Yellow rivers, respectively). Depending on wind events—direction, intensity, duration (Tolmazin, 1985)—a very stable 2-m deep river plume spreading over the surface layer of the Black Sea is formed. Such favourable wind conditions were met by early May 1997, and a typically ‘estuarine’ succession of hetero- and autotrophic events (e.g. Fisher *et al.*, 1988) were recorded across the salinity gradient in the range of 0–10. These events led to an accumulation of NO₃ in the lower part of the salinity gradient, apparent conservative behaviour for DSi and strong removal of PO₄ (Figure 4).

During the spring of 1997, a well-defined succession of bacterial (figure 2 of Becquevort *et al.*, 2002) and phytoplankton (Figure 7) productions was indeed observed below a salinity of 10 (Figure 11). Although total phytoplankton production culminates between a salinity of 2 and 10 (Figure 7), one can calculate that it corresponds with the intense development of a

freshwater diatom community at a salinity of 2–5, followed by a more modest growth at a salinity of 8–10 of a non-siliceous assemblage (Figure 11). This succession was reconstructed based on data of total primary production and the conversion of Si uptake rates in diatom C-fixation, making use of a Si/C ratio of 0.21. The latter was in turn deduced from the consistency between Si/C uptake (Figure 7) and biomass (Figure 6) ratios. Note that our value ranges between the silicification level reported for marine (0.13, Brzezinski, 1985) and freshwater (~ 0.4 , Conley *et al.*, 1989) diatoms. Together, this indicates that phytoplankton production at a salinity of 2–5 is only due to freshwater diatoms. Bacterial production, on the other hand, peaks at a salinity of below 2 and between 5 and 8, i.e. just between the two phytoplankton developments (Figure 11). The occurrence of maximal bacterial growth at the decline of freshwater diatoms strongly suggests that bacterial activity was stimulated by the supply of labile organic matter released after the lysis of freshwater diatoms. The latter process was most probably caused by the salinity increase, as suggested by the results of the artificial mixing experiment (Figure 9).

The nutrient distributions reflect these processes, but with contradictory net effects (Figure 4). Both auto- and heterotrophic production utilize PO_4 as phosphorus (see Becquevort *et al.*, 2002, for the relative contribution of phytoplankton and bacteria in the uptake of PO_4). Therefore, both mechanisms add to the contribution of the significant PO_4 removal observed during spring and summer (Figure 4). Additionally, passive adsorption of PO_4 onto newly formed biogenic particles, as enhanced by the pH increase (see Guieu & Martin, 2002), accelerates PO_4 removal due to active photosynthesis in the lower part of the salinity gradient (Figures 4 and 7). Consequently, depleted levels of PO_4 are already recorded at a salinity of 10, which will have important implications for phytoplankton dynamics at higher salinities.

In contrast, the primary production, which is light-limited at a salinity of below 2 (Figure 7; Table 1), and the concomitant active bacterial production (Figure 11) have, as a net effect, the accumulation of NO_3 in the low salinity section of the gradient (< salinity 5). Because NO_3 accumulation occurs despite the significant NO_3 uptake by phytoplankton between a salinity of 2 and 5 (Figure 7), this phenomenon can be attributed to the nitrification of NH_4 released by bacterial organic matter. Such upstream nitrification processes are also suggested to occur in the Amazon estuary (De Master & Pope, 1996). Their importance in the low salinity section of

the Danube estuary relies on the presence of biodegradable organic matter in the Danube and on the possible accumulation of NH_4 at the river outflow (Figure 4).

Finally, and contrary to NO_3 , NH_4 and PO_4 , DSi mixes in an apparent conservative mode in the low salinity region during all seasons (Figure 4). Apparently, this is in contrast with the intense spring diatom bloom at a salinity of between 2 and 5 (Figure 7), and suggests the occurrence of significant BSi dissolution to compensate the removal of DSi by actively growing freshwater diatoms. BSi dissolution may occur in the water column and at the sediment–water interface. The latter process has been demonstrated in several estuaries (D’Elia *et al.*, 1983; Anderson, 1986; Ragueneau *et al.*, 1994), especially in spring and summer when temperature increases the dissolution rate of diatom frustules (Lawson *et al.*, 1978). Our data set on winter BSi [Figure 6(g)] and DSi [Figure 4(a)] distribution suggests massive sedimentation of detrital BSi at a salinity of between 5 and 8. Indeed, the strong decrease of detrital BSi [Figure 6(g)] is not compensated by DSi production [Figure 4(a)], which would have been expected from BSi remineralization in the absence of significant diatom production [Figure 7(c)]. Overall, this suggests that as in other estuaries, benthic dissolution of BSi takes place in the spring when the temperature increases. Significant water column BSi dissolution, on the other hand, is expected in the lower part of the salinity gradient during the spring–summer period, following the lysis of freshwater diatoms. Two mechanisms possibly contribute to the acceleration of the dissolution process: the increasing electrolyte concentration (Hurd, 1983) and the bacterial activity (Figure 11; Becquevort *et al.*, 2002), which removes the protective layer of organic matter coating the opal surfaces (Lewin, 1961; Bidle & Azam, 1999).

Seasonal dynamics of nutrient limitation in the Danube River plume

Two important consequences arise from the combination of estuarine processes of nutrient uptake, mineralization and the transformation described above. Firstly, nutrient concentrations decrease extremely rapidly across the salinity gradient, especially PO_4 , which reaches depletion levels at a salinity of 10 in spring and summer [Figure 4(k,l)]. Secondly, as a result of distinct behaviours of nutrients as they get mixed with Black Sea waters and involved in biological processes, the nutrient balance is modified along the estuary (Figure 5). Both the level of nutrients and their ratios are important when

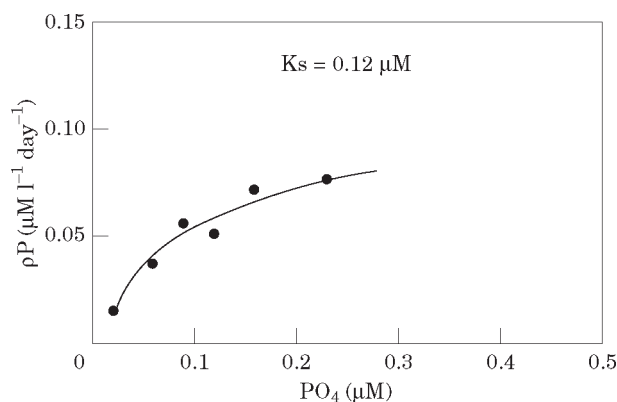


FIGURE 10. Kinetic experiment performed by adding ^{32}P (up to 10 times the ambient PO_4 concentration of $0.02 \mu\text{M}$) to a diatom-dominated community sampled at station 30 ($44^\circ 58' \text{N}$, $29^\circ 45' \text{E}$).

considering nutrient limitation of phytoplankton growth. Ambient nutrient ratios are usually compared to idealized phytoplankton stoichiometry (Redfield *et al.*, 1963; Brzezinski, 1985). Such a comparison, however, is only indicative of the potential limitation of phytoplankton growth by a given nutrient (Dortch & Whitley, 1992). It needs to be confirmed by careful examination of ambient nutrient concentrations and, if possible, compared to experimental values of half-saturation Michaelis–Menten constants describing nutrient uptake. Both Si/P and Si/N ratios significantly increased across the salinity gradient, rapidly relieving the DSi limitation, which existed potentially when analysing the nutrient concentrations of the river end-member (Si/N ratio lower than 1; Figure 5). The combination of PO_4 removal processes and of organic matter degradation and nitrification of the regenerated NH_4 contribute to increase the N/P ratio from *c.* 50 in the river end-member (not necessarily indicative of a potential PO_4 limitation. Wynne & Rhee, 1986) to about 150 at a salinity of 10 (Figure 5). Together, the elevated Si/P and N/P ratios at a salinity of greater than 3 induce a potential limitation of brackish diatom activity by ambient PO_4 . Results of a kinetic experiment of P uptake conducted on a diatom-dominated community sampled at a salinity of 16 suggest a half-saturation constant (K_s) value of $0.12 \mu\text{M}$ (Figure 10). This value is compatible with values reported for other river plumes (e.g. 0.2 M in the Mississippi River plume; Dortch & Whiteledge, 1992) and coastal areas (Taft *et al.*, 1975). More generally, it fits perfectly with the $0.1\text{--}0.5 \mu\text{M}$ range of half saturation constants for PO_4 uptake by phytoplankton, as summarized by Nalewajko and Lean (1980). Ambient PO_4 concentration approached this value of $0.12 \mu\text{M}$ at a salinity

of as low as 10 and was clearly below this value for a salinity of above 14. Thus, the combination of high Si/P and N/P ratios with low ambient PO_4 concentration strongly suggests that the growth of phytoplankton in general, and of diatom in particular, was limited by PO_4 availability during the spring period at a salinity of as low as 10.

Between spring and summer, the system lost inorganic nitrogen with respect to phosphate, possibly through denitrification as observed in many estuarine systems (Seitzinger, 1988; Kamp-Nielsen, 1992; Friederich *et al.*, 2002). NO_3 is almost depleted at a salinity of as low as 12 (Figure 4), and the phytoplankton N-uptake regime was based on ammonium uptake in the entire Danube–Black Sea mixing zone, except in the salinity region lower than 9 where nitrate assimilation was significant (Vervilmeren, unpubl. data). Nitrogen limitation of phytoplankton growth can thus be suggested for this period of the year, although co-limitation with P availability might well occur. The extent of N limitation, is however, difficult to establish (Hecky & Kilham, 1988) and depends upon the efficiency of N regeneration. Note that Friederich *et al.* (2002) demonstrate a differential benthic recycling between these two elements, with a five-fold decrease in the N/P ratio in the benthic flux compared to the ratio in the Danube, thereby suggesting that N, rather than P, may limit phytoplankton growth during the summer.

In contrast to spring and summer depletion levels of NO_3 and PO_4 at a relatively low salinity, DSi never reaches concentrations lower than $3\text{--}5 \mu\text{M}$ (Figure 4). Such ‘background’ DSi is typical of the Black Sea marine waters as well (Cociasu & Popa, 2002). This suggests that despite the potentially Si-limiting signature of the Danube waters (Figure 5), DSi does not limit diatom growth in the Danube estuary and north-western Black Sea. Rather, PO_4 depletion in spring 1997 and NO_3 and PO_4 depletion in summer 1995 could be responsible for the negligible diatom growth in the lower Danube estuary and Black Sea waters (Figure 11; Bouvier 1998). Thus, the presence or the absence of diatoms at a salinity of above 10 does not appear to be affected by DSi concentrations, which remained well above the threshold of $2 \mu\text{M}$ defined by Egge and Aksnes (1992) for diatom competitive growth. This result demonstrates that the eutrophication mechanism cannot be described in terms of only one type of nutrient input and that estuaries play a significant role in changing quantitatively and qualitatively the riverine nutrients spread over the coastal zone. It also shows that if the absence of DSi affects diatom growth and phytoplankton dynamics (Officer & Ryther, 1980; Conley & Malone, 1992; Ragueneau

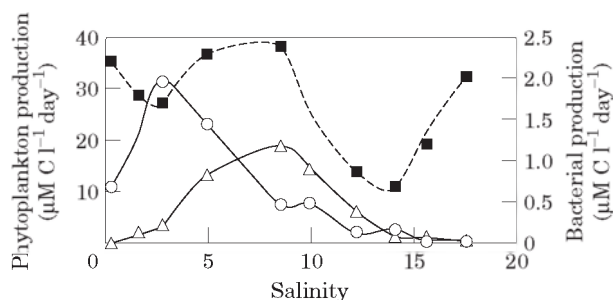


FIGURE 11. Distribution of diatoms (open circles), non-diatoms (open triangles) and bacterial (filled squares) production in the Sulina branch of the delta, during spring 1997.

et al., 1994; Del Amo *et al.*, 1997), its presence does not necessarily imply that diatoms will be dominant. This is a direct application of the enclosure results obtained by Egge (1998), suggesting that diatoms are poor competitors at low PO_4 concentrations, even if DSi is present in excess, as was the case in the Danube estuary during this study.

Danube River plume processes and eutrophication of the Black Sea

There is an apparent contradiction between DIN and PO_4 removal in the Danube estuary as observed in this study and also in 1992 and 1993 (Humborg, 1997), and the well-established negative effects of land use and river management on the functioning of the coastal ecosystem over the entire north-western shelf (Bodeanu, 1992; Gomoiu, 1992; Cociasu *et al.*, 1996) and in the hydrochemistry of the deep basin as well (Murray *et al.*, 1989; Tugrul *et al.*, 1992; Humborg *et al.*, 1997). Thus, mechanisms must exist that allow the propagation of Danube nutrient loads to the entire Black Sea. Our data strongly suggest that these mechanisms are complex and vary seasonally.

During winter, nutrients are simply diluted during mixing with marine waters and transported in their dissolved form along the Romanian shelf. Cross-frontal exchanges between the river plume and the adjacent waters of the Black Sea can occur along the entire path of the plume to the Bosphorus, allowing some of these nutrients to be spread over the entire shelf and to the deep basin of the Black Sea (Kononov *et al.*, 1999). During spring and summer, it has been suggested that most of the propagation of Danube eutrophication takes place in the form of biogenic matter, which can be exported with water masses, but which also flows to higher trophic levels or to the sediment. The relative importance of these pathways determines when and where nutrient re-

mineralization processes take place. Thus, it is possible that the Danube's properties are propagated to the entire shelf via the production, sinking and remineralization of organic matter and biogenic silica carried by the river and/or produced within the plume. Aubrey *et al.* (1996) describe a mechanism involving plume dynamics, mixing and downwelling over the shelf break and slope, and coastal upwelling as potential hydrological processes susceptible to determine the fate of anthropogenic nutrients on the north-western shelf of the Black Sea. Significant sedimentation of biogenic material derived from lysed freshwater diatoms at a salinity of above 5, as suggested in Figure 6, might be one of the possible mechanisms by which biogenic matter produced in the plume is temporarily retained within the estuarine system. Differential recycling rates will, therefore, play a major role, along with hydrodynamics, in replenishing the surface waters with nutrients of a modified balance. Friederich *et al.* (2002) show that PO_4 is recycled at a much faster rate than DSi and DIN. Clearly, budgets need to be constructed for each nutrient, which will describe the complex pathway of nutrients through the pelagic and benthic food-webs along the entire land-ocean continuum, before one can fully understand the mechanisms by which eutrophication can affect the entire Black Sea basin and, possibly, the adjacent Mediterranean waters.

Acknowledgements

We are very grateful to the crew of the RV *Professor Vodyanitsky* and to our Romanian colleagues for their hospitality and help during the EROS cruises. Thanks are also due to Professors J.-M. Martin, V. Egorov and N. Panin for the general organization of the cruises. Thanks also to J.-Y. Parent, who carried out the Chl *a* measurements, and to J. Cotten, who provided assistance in the A1 measurement by means of ICP-AES at the University of Brest. This work is publication No. 176 of the EU-ELOISE initiative of the Environment and MAST Programmes in the framework of the EROS 2000/EROS 21 Black Sea projects funded by the Environment and Climate Programme of the European Commission (contract N° EV5V-CT94-0501 and ENV4-CT96-0286).

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