

$\text{NH}_4^+$ ,  $\text{NO}_3^-$ , dissolved organic N (DON), and particulate organic N (PON) and considered the processes of ammonification, nitrification, and immobilization (Fig. 1, top). First-order kinetics were assumed for all processes except for lateral inputs. Concentrations were modeled as functions of distance. The least squares approach was used to fit the model to the main channel concentration data. The concentration data from the headwater sites were not used in the model. Constraints were placed on the model so that the initial concentrations, rate coefficients ( $k$ ), and uptake rates ( $r$ ) were greater than zero. Uptake lengths ( $1/k$ ) were calculated from the rate coefficients; an uptake length is an estimate of the average distance traveled by an element before it is removed from the water column.

Phosphate concentrations were very low at the headwater sites, averaging only  $0.3 \mu\text{M P}$  (data not shown). Further downstream in the main stem of the river,  $\text{PO}_4^{3-}$  was roughly the same as upstream ( $0.6 \mu\text{M}$ ). TDP concentrations showed no apparent downstream pattern and were only slightly higher than  $\text{PO}_4^{3-}$ , indicating very low DOP concentrations ( $0\text{--}1.5 \mu\text{M}$ ). DIN concentrations at the headwater sites were high, approaching  $60 \mu\text{M N}$  (Fig. 1, bottom). Further downstream in the main stem of the river, DIN concentrations were markedly lower (averaging  $17 \mu\text{M}$ ). TDN concentrations were much higher than DIN but, as with TDP, showed no apparent spatial pattern. Of the DIN fractions,  $\text{NO}_3^-$  exhibited a large drop in concentration with distance down the headwater stream. Although  $\text{NO}_3^-$  dropped,  $\text{NH}_4^+$  increased slightly, suggesting denitrification of the  $\text{NO}_3^-$  and ammonification without subsequent nitrification. In contrast, in the main channel,  $\text{NH}_4^+$  and  $\text{NO}_3^-$  concentrations were mirror images of each other but with  $\text{NH}_4^+$  dropping and  $\text{NO}_3^-$  increasing. This pattern suggests nitrification.

Our simple N model tracked observed data well (Fig. 1, bottom). Plots of predicted *versus* observed concentrations of  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ , and DON illustrated close agreement for all fractions. Slopes of regression lines were between 0.96 and 1.2 for the three components. Not only was there close agreement in a general sense, but the spatial patterns were also close to observed patterns for all components.

Rate coefficients determined with the model differed greatly between the various N fractions, ranging from  $0.0078 \text{ km}^{-1}$  for ammonification to  $10^7 \text{ km}^{-1}$  for plant uptake and microbial immobilization (Fig. 1, top). For all fractions, uptake, or transforma-

tion, lengths calculated from the rate coefficients were much longer than those usually reported for more pristine stream systems (2). The uptake lengths for  $\text{NH}_4^+$  and  $\text{NO}_3^-$  were 9 km and 13 km, respectively. The uptake length for  $\text{NO}_3^-$  was dominated by nitrification, as the rate of nitrification was  $10^6$  times that of plant uptake. The uptake length for DON exceeded the length of the river.  $\text{NH}_4^+$  uptake lengths reported in the literature for low-nitrogen, pristine stream systems are often between 30 and 400 m (2). Reported  $\text{NO}_3^-$  uptake lengths for similar systems typically range from 40 to 690 m (2). The long Ipswich River uptake lengths are probably due to the relatively high concentrations of inorganic and organic N in the Ipswich River. For a given rate of processing, calculated uptake rate coefficients vary inversely with concentration. The observed uptake lengths may also represent slow overall rates of N cycling in this system, which as evidenced by extremely high inorganic N:P ratios, is probably P limited.

Patterns of nutrient concentration and the results of the N model suggest that an important location for N retention or loss is in the headwater streams of the Ipswich River. Inorganic N concentrations decrease markedly in this region. The N-cycling model indicates very long uptake lengths in the mid and lower stretches of the river. It is possible that nutrient processing is greater in the headwaters because of greater relative contact with the riverbed. There may also be more active exchange between surface and hyporheic waters in the headwater streams. We would expect high rates of denitrification in anoxic hyporheic waters. Additional studies, such as tracer-nutrient releases and  $^{15}\text{N}$  additions could be profitably conducted in the upper reaches. Study of nutrient processing in the Ipswich River is increasingly important because N loading is rising in this rapidly urbanizing watershed. It is unclear how long the Ipswich River will be able to continue to process the high loads of inorganic N before the uptake capacity is reached.

This research was funded by NSF grants (LTER: OCE-9726921, DEB-9726862, and EAR-9807632) and a gift from the Jessie B. Cox Charitable Trust.

### Literature Cited

- Ingram, K. K., C. S. Hopkinson, K. Bowman, R. Garritt, and J. Vallino. 1994. *Biol. Bull.* 187: 277–278.
- Marti, E., and F. Sabater. 1996. *Ecology* 77: 854–869.

Reference: *Biol. Bull.* 197: 290–292. (October 1999)

### Increased Lability of Estuarine Dissolved Organic Nitrogen From Urbanized Watersheds

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Inputs of nitrogen from land can lead to eutrophication of estuaries (1–5, 6). Terrestrial N is transported as  $\text{NO}_3^-$ ,  $\text{NH}_4^+$ , PON (particulate organic nitrogen), and DON (dissolved organic

nitrogen), but most estimates of N loading are based on DIN ( $\text{NO}_3^- + \text{NH}_4^+$ ). DON had been thought to be mostly refractory to organisms, but recent studies show that some portion of the DON may be labile (6, 7). Land-derived DON may thus be mineralized within estuaries, and the  $\text{NH}_4^+$  released may be available to organisms (4). Most calculations of N inputs to estuaries

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are based on DIN; to the extent that DON is labile, nitrogen loads calculated on the basis of land-derived DIN inputs alone underestimate effective N loads (6, 7).

There is evidence that urbanization of watersheds alters the lability as well as the amount of DON loaded to receiving waters (6). We evaluated these issues by use of estuaries of Waquoit Bay, Massachusetts (Sage Lot Pond, Quashnet River, and Childs River), that receive different N inputs from their watersheds because of different degrees of urbanization (1, 4, 5). Sage Lot Pond (SLP) has a primarily forested watershed that provides a N load of 14 kg N ha<sup>-1</sup> y<sup>-1</sup>; Quashnet River (QR) has a watershed with an intermediate degree of urbanization and a N load of 350 kg N ha<sup>-1</sup> y<sup>-1</sup>; Childs River (CR) has a watershed with the greatest degree of urbanization, and a N load of 601 kg N ha<sup>-1</sup> y<sup>-1</sup> (4, 5).

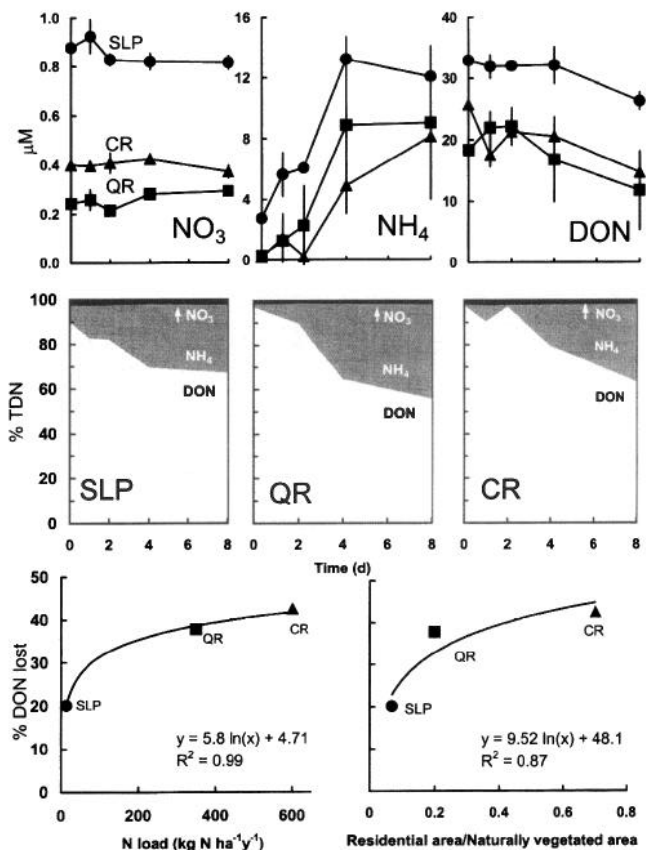
To gain a better understanding of how urbanization alters DON, we measured the lability of DON from the three different estuaries. In each estuary we collected surface water from a station with a salinity of 25‰. Samples were placed in acid-washed bottles and kept on ice. The water was filtered through precombusted 0.7- $\mu$ m glass fiber filters to remove larger consumers and particulates. Triplicate samples (1000-ml/flask) were incubated at 25°C in the dark with continuous shaking. Each flask was re-sampled at 12, 24, 36, 48, 96, and 192 h. The water samples were filtered (0.2- $\mu$ m Durapore membrane Millipore filter, pre-rinsed with deionized water); placed in acid-washed, high-density polyethylene bottles; and frozen for later analysis. Nutrient concentrations were determined by standard methods (Lachat QuikChem 8000 Automatic Ion Analyzer, 8).

DON concentrations decreased in all incubations (Fig. 1, top right) and as a percentage of the total N (Fig. 1, middle row). The loss of DON generated NH<sub>4</sub><sup>+</sup>, which increased in concentration during all incubations (Fig. 1, middle panel in top row, and all panels in middle row). NO<sub>3</sub><sup>-</sup> concentrations remained relatively constant during the incubations (Fig. 1, top left). The total dissolved nitrogen (TDN) did not change significantly (data not shown) within the measurement error (*F* values for regressions were 0.56 for SLP, 0.95 for QR, and 0.9 for CR), which implies that the transformation of TDN to particulate forms was not significant during the incubation; the concentrations of NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup>, and DON accounted for measured TDN throughout.

The degree of urbanization on the different watersheds did alter the lability of DON generated from the watersheds. Concentrations of DON were largest in SLP (Fig. 1, top right), the estuary with the smallest N load (Fig. 1, bottom left) and the largest ratio of forest to residential land on its watershed (Fig. 1, bottom right). Our results thus suggest that increased N loads during urbanization (Fig. 1, bottom right) are accompanied by proportionally more labile DON (Fig. 1, bottom right).

We conclude that since DON is quantitatively a large part of inputs from land (6), a labile fraction of 20%–40% (Fig. 1, bottom left) could indeed make a significant contribution of available N to estuarine organisms. Nutrient-loading protocols should therefore include assessments of the amounts and lability of DON entering estuaries. This is particularly important in cases of eutrophication of estuaries with watersheds exposed to greater degrees of urban land use.

The DON in the incubation was derived from a mix of terrestrial and marine sources. Internal sources (exudation from producers,



**Figure 1.** Top row: concentration (mean  $\pm$  SE) of nitrate, ammonium, and dissolved organic nitrogen (DON) during the incubation. Middle row: percentage composition of N measured in each of the three estuaries (Sage Lot Pond, Quashnet River, and Childs River) during the incubations. Bottom row: relationship of total percent DON lost during the incubations vs. land-derived nitrogen load (left panel) and vs. ratio of residential to vegetated land acreage on the watersheds (right panel).

regeneration from sediments) of DON are qualitatively important (our unpublished data). In addition, we collected samples from stations at which salinity was high and marine sources could have been important. Both of these features would diminish the possibility of our finding differences in lability due to land-derived DON. Nevertheless, we did find such differences, suggesting that despite the potentially confounding effects of internal estuarine and marine sources of DON, we can still detect measurable influences tied to land-use mosaics on watersheds. Clearly, further mass balance studies will be useful to clarify issues, but our present results point to a substantive coupling between land use on watersheds and the nature of the DON in estuarine waters. This coupling has considerable importance to the management of N loads to estuaries.

We thank Caroline Plugge, Kurt Hanselmann, Gabrielle Tomasky, and Jennifer Walters for analytical assistance. Also special thanks to Kenneth Foreman and The Ecosystems Center for use of the Lachat autoanalyzer and to Eric Davidson of the Woods Hole Research Center for suggestions. This research was supported by funds from the NSF Research Experience for Undergraduates program (OCE-9605099).

### Literature Cited

1. Valiela, I., J. Costa, K. Foreman, J. M. Teal, B. Howes, and D. Aubrey. 1990. *Biogeochemistry* 10: 177–197.
2. Valiela, I., et al. 1992. *Estuaries* 15: 443–457.
3. D'Avanzo, C., and J. Kremer. 1994. *Estuaries* 17: 131–139.
4. McClelland, J. W., and I. Valiela. 1998. *Limnol. Oceanogr.* 43: 577–585.
5. Valiela, I., G. Collins, J. Kremer, K. Lajtha, M. Geist, B. Seely, J. Brawley, and C. H. Sham. 1997. *Ecol. Appl.* 7: 358–380.
6. Seitzinger, S. P., and R. W. Sanders. 1997. *Mar. Ecol. Prog. Ser.* 159: 1–12.
7. Bronk, D. A., P. M. Gilbert, and B. B. Ward. 1994. *Science* 265: 1843–1846.
8. D'Elia, C. et al. 1977. *Limnol. Oceanogr.* 22: 760–764.

Reference: *Biol. Bull.* 197: 292–294. (October 1999)

## Effects of Increased Nitrogen Loading on the Abundance of Diatoms and Dinoflagellates in Estuarine Phytoplanktonic Communities

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Coastal and estuarine ecosystems are among the most anthropogenically affected ecosystems on earth (1). Increased urbanization, deforestation, and agricultural land uses are some of the main factors that cause increased nitrogen loading in the estuaries (2). The effects of this increased delivery of nitrogen on the receiving estuarine waters include increased abundance of benthic macroalgae and phytoplankton, reduced oxygen content of the water, and deterioration of shellfish and finfish populations (2, 3). However, knowledge of how and why estuaries subject to different rates of nitrogen loading differ in the composition of their phytoplanktonic communities is not as extensive, even though many laboratory experiments have addressed this question (4, 5, 6, 7). Changes in phytoplanktonic composition may have important effects on the receiving estuaries, such as changes in the food web structure (8) or in the sedimentation rates of organic matter (9).

In our study, we describe the abundance of diatoms and dinoflagellates in three estuaries of Waquoit Bay, Massachusetts, which have similar physical properties but differ greatly in their degree of urbanization and subsequent nitrogen loading rates (2). Childs River, Quashnet River, and Sage Lot Pond exhibit high ( $601 \text{ kg ha}^{-1} \text{ y}^{-1}$ ), medium ( $350 \text{ kg ha}^{-1} \text{ y}^{-1}$ ), and low ( $14 \text{ kg ha}^{-1} \text{ y}^{-1}$ ) nitrogen loading rates respectively (10). To describe the natural assemblages of diatoms and dinoflagellates in the three estuaries, we took water samples from one site at the mouth of each estuary, at  $\sim 2 \text{ m}$  depth on 12 November 1998. Temperature and salinity were very similar in the three sites examined. We took six samples from Childs River and Sage Lot Pond, and three samples from Quashnet River. Natural abundance was low during the sampling period; therefore, samples were concentrated 50 times by filtering 50 l of water through a  $10\text{-}\mu\text{m}$ -mesh filter to collect the phytoplankton. The phytoplankton was then placed in 1 l of water and fixed with Lugol's solution. The two phytoplanktonic groups were identified and cells were counted under compound microscopes.

In addition, we conducted a laboratory experiment to test whether the observed differences in composition of the phytoplanktonic groups examined were driven by increased nitrogen loading. Phytoplankton from Sage Lot Pond was collected using a  $10\text{-}\mu\text{m}$  mesh, and then placed in two 35-l tanks filled with Childs River water that had been previously filtered through  $1\text{-}\mu\text{m}$  filters.

The control tank contained phytoplankton from Sage Lot Pond placed in water from the same estuary using the procedure described above. Similar quantities of phytoplankton were placed in the three tanks. All tanks were oxygenated and kept with seasonal light and temperature conditions in an incubation chamber. In each tank, three replicates were taken at 0, 3, 6, and 9 days to measure phytoplankton abundance. We used the nitrogen content in the water column as a proxy for nitrogen loading. Nitrogen concentrations in Sage Lot Pond and Childs River water in November were 1 and  $5 \mu\text{M}$  respectively. Therefore, every 3 days we measured the nitrogen concentration in all tanks and added nitrogen as needed to maintain these natural levels.

Analysis of the natural abundance of diatoms and dinoflagellates showed that diatoms were the dominant group in all three estuaries (Fig. 1A), while dinoflagellates represented less than 10% of the phytoplanktonic community examined. Both diatom and dinoflagellate abundances increased from low- to high-nitrogen estuaries (Fig. 1A; ANOVA,  $P < 0.01$  for both groups). In addition, diatoms increased to a much greater extent than dinoflagellates did and, as a consequence, the ratio of diatoms to dinoflagellates increased more than one order of magnitude from low- to high-nitrogen estuaries (Fig. 1B; ANOVA,  $P < 0.01$ ). Dinoflagellates represented about 10% of the total community examined in Sage Lot Pond, but were less than 1% in Childs River (Fig. 1B). A further examination of the diatom community showed that both centric and pennate diatoms increased from low- to high-nitrogen estuaries (Fig. 1C; ANOVA,  $P < 0.01$  for both types). The two groups increased in similar proportions and, consequently, the ratio of centric to pennate did not change significantly with higher loading rates (ANOVA,  $P > 0.05$ ).

The results from the experiment showed that the Sage Lot diatom community in Childs River water responded differently than the control, the same community kept in its own water (Fig. 2). Diatom abundance in Childs River water increased over the course of the experiment ( $t$  test,  $P < 0.01$ ). Conversely, diatom abundance in Sage Lot water at the end of the experiment was not higher than the initial abundance ( $t$  test,  $P > 0.05$ ), in spite of the observed peak on the third day. At the end of the experiment, diatoms were more abundant in Childs River water than in Sage Lot water ( $t$  test,  $P < 0.01$ ). Dinoflagellate abundance increased