Nitrogen loading from land is a principal cause of eutrophication of shallow estuaries (1, 2, 3). In regions such as Cape Cod, Massachusetts, which are underlain by unconsolidated sands, the major mechanism that transports nitrogen to estuaries is groundwater flow, and the major nitrogen source (primarily in the form of nitrate, NO\textsubscript{3}) is often wastewater from septic systems (1, 2, 3). Wastewater nitrate concentrations decrease during travel in groundwater due to dilution with clean groundwater and to loss by denitrification (4). The loss of nitrogen during flow between a septic tank and receiving estuary can be calculated by determining the reduction in concentration of dissolved inorganic nitrogen relative to the change in concentration of a passive tracer that accounts for dilution.

We investigated losses of nitrate for a domestic septic system in the watershed of Quashnet River, Cape Cod. Effluent from septic systems moves downstream within plumes containing high concentrations of nitrate. In addition, the study area has plumes derived from fertilized turf or fields. To sort out the different plumes, we measured boron (B, a passive tracer derived from laundry detergents and associated with wastewater sources [5, 6, 7]) and potassium (K, associated with both wastewater and fertilizer sources [8, 9]) in the samples of groundwater.

To calculate loss of nitrate along the plumes, we collected samples from nine wells downstream from the septic system. Each well was furnished with 14 ports that allowed us to sample groundwater at intervals of 1–2 m. We collected 300 ml of water from 129 ports during June and July 2000 and measured concentrations of nitrate (NO\textsubscript{3} + NO\textsubscript{2}) and ammonium (NH\textsubscript{4}) using colorimetric and fluorometric techniques, respectively. We selected samples with nitrate concentrations above 8 μM and conductivities less than 4,000 μS/cm for measurements of B and K. These samples were analyzed by Ward Laboratories (Kearney, NE).

Examination of vertical and horizontal profiles of nitrate and ammonium suggested that there were three distinct plumes within our well field (Fig. 1). The upper plume moved along near the surface of the water table and contained the highest nitrate concentration of the three plumes; at nearly 3000 μM, it was similar to literature values (8) for septic effluent that has just left the leaching field. The nitrate, B, and K concentrations in this plume differed considerably from those of the other plumes (Fig. 2, A and B).

In contrast, the lower plume showed no increase in nitrate relative to increase in B (Fig. 2, A). It did, however, show a positive relationship to K, and at a given K concentration had a much higher nitrate concentration than did the upper plume (Fig. 2, B). This evidence suggests that the lower plume might be due to fertilizer use upgradient of our septic system.

The middle plume had no significant relationships between nitrate and B or K, perhaps because of the small number of samples and the low concentrations. The concentrations of nitrate, B, and K from the middle plume do, however, fit on the lower portions of the curves for the upper plume (Fig. 2, A and B). These circumstances lead us to think that the middle plume was probably the leading edge of a plume from a septic system located farther upgradient from our septic system. We therefore used data for the upper and middle plumes in our examination of the fate of septic system nitrogen in this watershed.

Concentrations of nitrate and B diminished as water parcels aged (age, Fig. 2, C and D, calculated from Vogel equations [10] that predict time since recharge as a function of depth in aquifer). To allow for dilution, we normalized the data by expressing concentrations as NO\textsubscript{3}/B (Fig. 2, E). We estimated the NO\textsubscript{3}/B in the effluent that had just left the septic system (age 0) by using a literature value (8) (Fig. 2, E, upper dashed line). The NO\textsubscript{3}/B values we used came from a Cape Cod site near our study area, and the data dated from 1992, only a 7–8 year difference from our date of collection. We presume that differences in B were therefore a reasonable proxy for those in our study system. We calculated losses of NO\textsubscript{3} as the difference between the age 0 nitrate concentration, allowing for dilution, and the measured nitrate concentration.

Losses of nitrate in excess of dilution were quite rapid, with rates reaching 50% loss at 0.2 years (Fig. 2, F). The loss rates diminished with time, which suggests that, if these data are representative of losses elsewhere, N losses by denitrification and retention take place primarily near the septic system source. Extrapolating the curve of Figure 2 (F), we find that near-complete losses may be reached at 4.8 years, which is equivalent to 480–730 m from the septic system, assuming a travel rate of 100–150 m per year (11).

As a minimum estimate of loss, we also calculated loss relative to our highest measured NO\textsubscript{3}/B ratio (Fig. 2, E, lower dashed line). If our initial NO\textsubscript{3}/B ratio were closer to this measured value, our estimate of time to 50% NO\textsubscript{3} loss would increase to 0.6 years; but the estimate of time to 100% loss was not affected. The extrapolation to 100% loss assumes that the relationship between percent loss NO\textsubscript{3} and age continues to hold beyond our oldest sample. This would not be the case if the availability of labile organic carbon were to limit NO\textsubscript{3} loss before 100% loss is achieved.
Figure 1. Vertical cross section from the soil surface, water table, and aquifer through our field of multiple sampling wells (elevation relative to mean low water [MLW]). The numbers are concentrations of NO₃ (µM) for water samples collected from each of the 14 ports in each of the 9 wells. Although the wells were not all in one plane, for simplicity they are shown as if they were. Contour lines are drawn to indicate NO₃ concentrations of 32, 128, 512, and 2048 µM. Position of salty water determined from salinity of water samples.

Figure 2. A: NO₃ concentration versus B concentration for samples collected from upper (●), middle (■), and lower (△) plumes. B: NO₃ concentration versus K concentration for all three plumes. C: NO₃ concentration versus age for upper and middle plumes. D: B concentration versus age for upper and middle plumes. E: NO₃ to B ratio versus age for upper and middle plumes. F: Percent loss of NO₃ versus age for upper and middle plumes.
If coastal zone managers wish to regulate septic nitrogen loads, they could concentrate on management of septic systems that lie within 480–730 m of the shore, since these appeared to be the major contributors of nitrate to receiving estuaries. Septic sources farther upgradient probably contribute less significantly. 

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Effects of Nitrogen Load and Irradiance on Photosynthetic Pigment Concentrations in *Cladophora vagabunda* and *Gracilaria tikvahiae* in Estuaries of Waquoit Bay

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Two major controls of activity and standing crop in macroalgae are nitrogen supply and irradiance (1). Increased nitrogen loads increase production (2) and biomass of macroalgae such as *Cladophora vagabunda* and *Gracilaria tikvahiae* (E. Stieve, unpub. data). Lower light availability lowers growth rates of macroalgae, although this effect varies among species (3). Because of exponential attenuation and self shading within algal mats, the irradiance available for benthic algae depends on water depth (1). Supply as well as storage of nitrogen and photons affect the concentration of photosynthetic components in macroalgae (1, 4). Photosynthetic pigments such as phycocyanin also act as nitrogen pools, and macroalgae acclimate to different irradiance regimes by changing pigment concentrations (5).

To examine the effects of different nutrient supplies on photosynthetic pigment concentrations in a green and a red alga, we collected samples of *Cladophora vagabunda* (L.) van den Hoek and *Gracilaria tikvahiae* McLachlan from five estuaries within Waquoit Bay (Childs River, Eel River, Quashnet River, Sage Lot Pond, and Timms Pond) that are exposed to different nitrogen loads (6). To study the effect of irradiance on pigment concentration we collected samples at a range of depths (80 to 210 cm). Irradiance at each sampling depth was measured using a spherical underwater sensor attached to a Li-Cor DataLogger LI-1000. Samples were collected during one day in early June.

At each site, six samples of benthic macroalgal material were collected and sorted to isolate fronds of *C. vagabunda* and *G. tikvahiae*. The samples were sorted by species. Chlorophylls a, b, and carotenoids were extracted as described by Figueroa et al. (7); phycochlorophylls were extracted as described by Beer and Eshel (8). Pigments were extracted within 36 h of collecting and were kept at 5°C until extraction to avoid pigment degradation. Concentrations of pigments were determined by use of a Perkin Elmer UV/VIS spectrophotometer (8, 9, 10). Chlorophyll a and carotenoid concentrations were measured in both *C. vagabunda* and *G. tikvahiae*. Chlorophyll b concentrations were measured in *C. vagabunda*. Phycoerythrin concentrations were measured in *G. tikvahiae*. To further ascertain the internal storage of nitrogen and carbon under different nitrogen and irradiance regimes, we dried macroalgal samples and measured percent nitrogen and percent carbon in a Perkin Elmer elemental analyzer according to the manufacturer’s instructions.

Concentrations of chlorophyll a (Fig. 1A) and carotenoids (Fig 1B) in both species of macroalgae increased as nitrogen load to the estuaries increased. There was no consistent difference between upstream and downstream sites within the estuaries, and there was no apparent effect of different salinities at the sites of collection (range of 10‰ to 32‰) on pigment concentrations (data not shown), so data were pooled within each estuary. Concentrations of chlorophyll a and carotenoids in *C. vagabunda* and *G. tikvahiae* are within the range found in other published literature (5); more importantly, the nitrogen loads increase pigment concentrations from values characteristic of nitrogen-poor waters to those of nitrogen-rich estuaries (Fig. 1A, B) (5). The response of *C. vagabunda* to nitrogen supply was more pronounced than that of *G. tikvahiae* (Fig. 1A, B).

These results suggest that nitrogen supply has important effects on pigment concentrations and nitrogen content of fronds, and that the response depends on the species. Surprisingly, concentrations of phycoerythrin in *G. tikvahiae* did not increase as nitrogen load

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