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Dissolved Iron Cycling in the Subterranean Estuary of a Coastal Bay: Waquoit Bay, Massachusetts

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Iron oxides have a strong affinity for dissolved phosphates and certain trace metals (1). Charette and Sholkovitz (2) observed iron oxide-coated sands in sediment cores extracted from the intertidal zone of Waquoit Bay, Massachusetts, and determined that the iron oxides were intercepting phosphates entering the bay in groundwater. They subsequently hypothesized the formation of an "iron curtain," following the oxidation of dissolved ferrous iron to various iron oxides when iron-rich groundwater mixes with intruding saltwater in the subterranean estuary (3). Although the iron oxide-rich sands proved the formation of iron precipitates, more information was needed about the aqueous phase of iron and the conditions surrounding its oxidation. In this study, we have mapped the distribution and concentration of dissolved iron in the subterranean estuary of Waquoit Bay.

Water samples were collected from two transects on the north shore of Waquoit Bay. A map of Waquoit Bay is included in Charette *et al.* (4). One transect spanned 178 m and was orientated parallel to the beach; the second transect, which we examine in this paper, was placed perpendicular to the beach. This 17-m transect extended from the berm of the beach to the intertidal zone. Water samples were collected at 0.5-m intervals to depths of up to 8 m using retract-a-tip, well-point piezometers and a peristaltic pump. At each depth, water was pumped from the ground and immediately filtered to remove particulates, using 10% HCl-cleaned 0.22-μm filters. We measured dissolved ferrous iron, total dissolved iron, salinity, dissolved oxygen, phosphate, nitrate, ammonium, and silicate in each sample. Ferrous iron and total dissolved iron concentrations were determined using the ferrozine method (5). An automated Winkler titration system was used to determine dissolved oxygen concentrations, and a salinometer was used to measure salinity (6). Concentrations of phosphate, nitrate, ammonium, and silicate were measured colorimetrically, using a Lachat nutrient auto-analyzer (Zellweger Analytics, Quickchem© 8000 Series).

Using a 2D-cross-sectional view, we identified three distinct regions of high ferrous iron in groundwater and pore water profiles (Fig. 1). Region 1 was located in upland groundwater between 2.5 and 4.5 m below sea level, and from 15 to 6 m from the shoreline. Region 2 was found below 4.5 m in the high-salinity subterranean estuary, and region 3 was located between depths of 0.15 to 1 m in the bay sediments.

Region 1 contained the highest measured concentrations of ferrous iron (21.9 μM) and was located in completely freshwater (Fig. 1B). Dissolved ferrous iron concentrations in this plume decreased close to the shoreline (Fig. 1A). Dissolved oxygen did not exceed 1 mg/l in this region. We identify this region to be a plume of terrestrially derived ferrous iron, given the absence of saltwater and the fact that the pocket of high iron appeared to shoal as it neared the shoreline (Fig. 1A). Basic hydrology suggests that groundwater will discharge into coastal waters after travelling upward at the seepage face, in order to pass over heavier, saline waters that are encroaching into the aquifer. This path permits the mixing of ferrous iron-rich groundwater with shallower aquifer

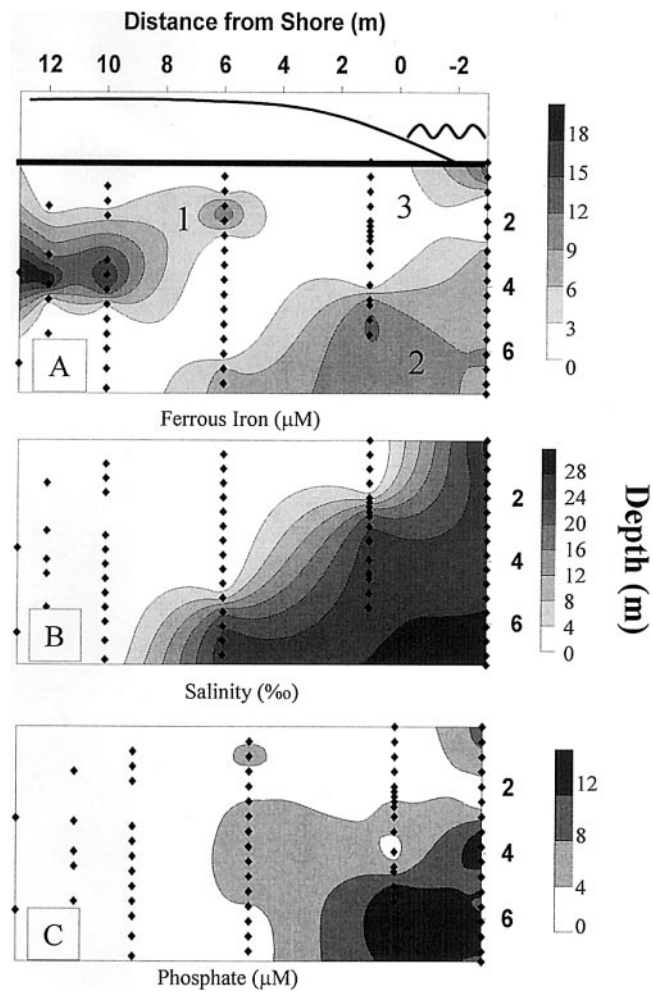

Figure 1. *Contour plots showing a 2D cross-sectional view of dissolved ferrous iron (A, contour line interval 3 μM), salinity (B, contour line interval 4‰), and dissolved phosphate (C, contour-line interval 4 μM) in water samples from the perpendicular transect. Diamonds (◆) represent where individual samples were extracted. Contours were generated using kriging in SURFER software (version 6.01), which statistically estimates the values of unknown data, based on actual data.*

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waters that contain higher concentrations of dissolved oxygen. In fact, we recorded a plume of oxygen-rich groundwater (~5 mg/l) at 1.5–2 m in all profiles in our transect parallel with the beach. Ferrous iron appears to oxidize when interacting with this plume. It should be noted that iron-oxidizing bacteria have been recognized to potentially occur in neutral pH groundwater at the oxic/anoxic boundary and could be contributing to iron oxidation (7).

Region 2 was associated with salinity values of 16 to 28‰ and also contained high concentrations of ferrous iron (Fig. 1A, B). This region is located where saltwater is encroaching into the aquifer. Given the hypoxic nature of the groundwater in this region (0.5–1 mg/l), iron-reducing bacteria may be active, utilizing iron oxides as an alternative electron acceptor to oxidize organic material (8). Hypoxia may prevail in the stagnant waters of this region, where fresh groundwater is passing over the saline waters. Encroaching saltwater may also deliver organic substrates from bay sediments that contribute to bacterial activity.

Region 3 contains high levels of ferrous iron in the first meter of sediment, where a black precipitate of iron sulfide exists just beneath the sediment surface. Organic matter deposition in this region results in the utilization of oxygen in decay processes that contribute to reducing conditions. In addition, peak temperatures in summer months increase respiration in benthic communities occupying these sediments, which reduce oxygen concentrations (9).

If we assume that a given concentration of iron oxides will scavenge a proportional amount of dissolved phosphate, then proportional concentrations of dissolved ferrous iron and dissolved phosphate should be released in regions where iron oxide deposits are dissolved. We did not, however, find a strong linear relationship between dissolved ferrous iron and dissolved phosphate in region 1 ($r^2 = 0.32$) (see Fig. 1A, C). This supports the idea that the ferrous iron is not released from previously oxidized iron, but is being transported to Waquoit Bay *via* groundwater. The correlation between ferrous iron and phosphate in region 2 is positive, but not strong ($r^2 = 0.24$). We did, however, find a strong linear correlation between ferrous iron and dissolved phosphate in region 3 ($r^2 = 0.89$), suggesting the reduction of iron oxides (see Fig. 1A, C) and the release of dissolved phosphate. In region 2, processes aside from iron oxide dissolution must be controlling the concentration of phosphate (see Fig. 1A, C), and these factors are probably present in region 3 as well. Likely processes are the mineralization of organic matter to release phosphate and the formation of iron-phosphorus minerals.

Charette and Sholkovitz (2) suggested that dissolved ferrous iron in the subterranean estuary was removed from solution *via* precipitation to iron oxides following mixing with oxygen-rich seawater. It appears the mechanisms responsible for groundwater iron oxidation are more complex. In fact, this study shows that the conversion of ferrous iron to various iron oxides occurred in almost completely salt-free water and that the oxidation and dissolution of iron is also a dynamic process.

We thank staff members of the Waquoit Bay National Estuarine Research Reserve for their support in our field efforts in June/July 2002 and for allowing us to utilize their facilities. This research was funded by a NSF-Research Experience for Undergraduates grant (OCE-0097498), a National Science Foundation grant (OCE-0095384) to M.A.C. and E.R.S., and a fellowship to M.A.C. from the Coastal Ocean Institute at WHOI.

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Nutrient and Freshwater Inputs From Sewage Effluent Discharge Alter Benthic Algal and Infaunal Communities in a Tidal Salt Marsh Creek

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Nutrient loading of coastal aquatic ecosystems is becoming a globally important issue. Elevated nitrogen, such as near sewage discharge pipes, has been found to be responsible for algal blooms in coastal areas (1). Raising nitrogen and phosphorus concentrations results in increased algal productivity and standing stock and has been shown to favor filamentous algae and diatom communities (2). Other studies demonstrated a decline in algal species diversity with nutrient inputs, although species richness was unaffected (3). Although a number of studies have linked nutrient loading with algal growth, the response of animal communities to nutrient inputs has been less well studied, especially in salt marsh estuaries (4).

Greenwood Creek, a tidal salt marsh creek in the Plum Island Sound estuarine system of northern Massachusetts, has been the site of sewage effluent input from the secondary wastewater treatment facility for the town of Ipswich, Massachusetts, for over 40

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years. Recent measurements of nitrogen inputs from the treatment plant indicate that the predominant form is nitrate ($NO_3 > 80\%$, dissolved organic nitrogen 10%–15%, NH_4 5%–10% [Deegan, unpubl. data]). Current inputs are 3500 m^3 of effluent per day and are oxygen (> 6.0 mg/l) regulated. We examined algal standing stock and the abundance of benthic invertebrates in Greenwood Creek and a nearby reference creek (Club Head Creek) to address the impact of sewage effluent (nutrients and freshwater) on benthic communities.

Two-kilometer transects along the stream were established, with sites spaced every 10 m for the first 30 m downstream of the point source of effluent (where we presumed the greatest sewage influence to be), and more widely spaced (about every 500 m) farther downstream. Sites in the reference creek were chosen to be similar in geomorphology to the sites in the sewage creek. All parameters were measured in late June or mid-July when the effects of sewage are expected to be fully developed. Nitrate (μM), temperature, and salinity were measured ($n = 1$) at high and low tide at each station ($n = 9$ stations). Mudflat algae samples were taken using a 2-cm-diameter syringe ($n = 3$ for each of 5 sites), and the uppermost 2 cm was analyzed for chlorophyll *a* (5). Infaunal mudflat invertebrates were collected using a 0.25 × 0.25 m quadrat ($n = 3$ for each site) dug to a depth of 0.05 m and sieved through a 500-μm sieve. Invertebrates were sorted, identified, and preserved in 70% ethanol. The snail *Ilyanassa obsoleta* was sampled by counting all snails in a 1-m-wide transect starting from the edge of the *Spartina patens* down into the creek channel. Two-way analyses of variance were used to test if ln-corrected concentration of chlorophyll *a*, abundance of the polychaete *Nereis*, or abundance of oligochaetes differed between creeks or among sites within a creek.

The sewage effluent input had elevated nitrate levels and lowered salinity. At low tide, nitrate was over 300 times that of the reference creek near the effluent source in the sewage creek, and declined downstream until it was 50 times that of the reference creek when it emptied into Plum Island Sound (Fig, 1A). Salinity was lowest (close to zero) near the effluent source and increased to 30 ppt downstream in the sewage creek. Salinity was high (32 ppt) and constant along the length of the reference creek (Fig. 1B). Temperature was similar between the two creeks (range of 19–21 °C). Chlorophyll *a* was lower at the upper sites of the sewage creek than in the reference creek, but farther downstream levels of chlorophyll *a* between the two streams were similar. The depressed chlorophyll levels in the sewage creek may be linked to the low salinity near the sewage input. Future studies could relate sediment NO_3^- levels to algal growth. As salinity increased, chlorophyll *a* concentration was similar to that of the reference creek (Fig. 1C).

Benthic invertebrate populations also differed between the two creeks. The infaunal samples were dominated by an oligochaete (f. *Enchytraeidae*), and a polychaete (*Nereis virens*). Oligochaetes were most abundant at the three sites closest to the sewage effluent input and declined in the downstream sites of the sewage creek to abundances similar to those in the reference creek (Fig. 1D). *Nereis* was only found in low numbers upstream in the sewage creek, and increased farther downstream as salinity increased. These polychaetes were less abundant in the reference creek and remained fairly constant along the transect (Fig. 1E). *Nereis* is typically found in more saline conditions (6), and, therefore, could be less able to survive upstream in the sewage creek, where salinity is lower and fluctuates more. *Ilyanassa* was very abundant in the downstream sites of the reference creek but was not present in the sewage creek (Fig. 1F). We do not yet understand why *Ilyanassa* was completely missing from the sewage creek.

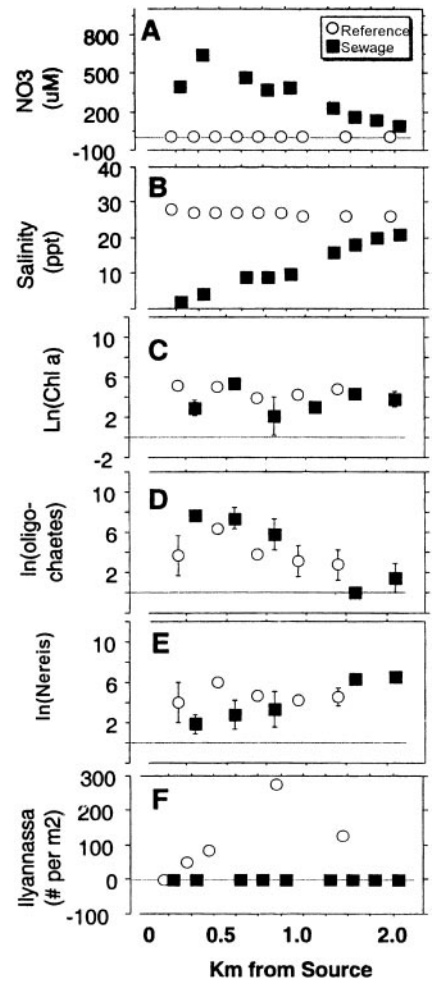

Figure 1. *Chemical and biological responses (mean ± 1 standard error) to sewage effluent input into a tidal salt marsh creek. The bottom was too rocky at station 1.1 in the sewage creek for the benthic invertebrate sampling technique to work. Two-way ANOVA* P*-values on ln-transformed values: Ln(chl a): Creek 0.02, Km 0.04, Creek*km 0.43; ln(Oligochaetes): Creek 0.21, Km 0.001, Creek*km 0.06; ln(Nereis): Creek 0.09, Km 0.33, Creek*km 0.0042. ANOVA was not run on* Ilyanassa, NO_3^-, *or salinity.*

We found differences in both the algal and benthic invertebrate communities in a tidal salt marsh creek influenced by sewage effluent compared to a reference creek with no sewage input. These differences are most apparent near the outfall and rapidly disappear downstream. They are probably linked to both freshwater input and nutrients, although the relative importance of these factors is not yet clear.

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Tidal Flushing of Ammonium From Intertidal Salt Marsh Sediments: The Relative Importance of Adsorbed Ammonium

Ketil Koop-Jakobsen (Roskilde University, Denmark) and Anne Giblin[1]

Intertidal sediments are areas of high nutrient processing. During low tides, air exposure of the sediment increases the rate of degradation of organic matter, causing the ammonium concentration in the sediment to increase between tidal inundations (1). At high tide, when the flooding water inundates the lower part of the salt marsh, ammonium is often released from the sediment to the water column by diffusion and convection (1, 2, 3). However, temporary seasonal import of ammonium from the water column to the sediment has also been observed (4).

In the sediment, a dynamic equilibrium exists between ammonium dissolved in the pore water and that adsorbed onto either inorganic particles or organic matter (5). The ratio between dissolved and adsorbed ammonium can be expressed by the unitless equilibrium constant K_D (6). Since ammonium adsorption to sediment particles and organic matter is a rapid and reversible process (5), not only does pore water ammonium have the potential of being flushed out of the sediment, but the ammonium adsorbed also has the potential of being released to the inundating water, due to a rapid shift in the equilibrium. This study investigates the importance of adsorbed ammonium during tidal flushing.

In the field, tidal flushing of ammonium was studied in late July 2002 in a salt marsh area in the Plum Island Sound, Massachusetts. The study area was dominated by *Spartina alterniflora* and was regularly flooded. Tidal flushing was studied during one tidal cycle in late July at temperatures of 20.9 °C, 22.2 °C, and 23.5 °C for sediment, inundating water, and air, respectively. The amount of ammonium flushed from the sediment was determined as the difference in sediment ammonium concentration before and after inundation. Sediment cores were collected in 6.5-cm diameter acrylic plastic cylinders 1 h before and 1 h after inundation. The cores were sliced in 1-cm sections immediately after sampling. From depths of 1, 2, 3, 5, 7, and 10 cm, subsamples of 3–8 g were collected and placed on ice until further analysis. Total pore water and exchangeable ammonium was extracted from the sediment using 2*M* KCl (5), and the ammonium concentration was determined by the phenol hypochlorite method (7).

In the laboratory, a K-value depth profile was measured under comparable conditions. Sediment cores were collected in the same sampling area during high tide when the sediment was inundated. The cores were placed in a lighted growth chamber at 25 °C and left inundated overnight to equilibrate core temperature. Subsequently, the water column was removed, and the sediment surface was left exposed to air for 12 h, after which the cores were sliced using the same method as in the field. Subsamples were collected for the determination of total ammonium concentration, pore water ammonium concentration, and sediment density. Pore waters were separated from sediments by centrifugation, and the concentration of adsorbed ammonium was determined as the difference between total- and pore-water ammonium. The K_D-value is determined as

$$K_D = \frac{[NH_4^+]_{adsorbed}(\mu mol/g.ww.sed.)}{[NH_4^+]_{porewater}(\mu mol/g.ww.sed.)}$$

and measured at the same depths as the ammonium concentration profiles.

The difference between the total ammonium profiles before and after inundation indicates that a substantial amount of ammonium is released from sediment at this location during inundation at high tides (Fig. 1A). The effect of flushing was greatest in the top 0–1-cm sediment layer, where 50% of the available ammonium was lost. The effect steadily decreased with depth, and at 10-cm depth only 10% of the available ammonium was released (Fig. 1B). In the upper 0–5 cm, more than 50% of the ammonium flushed from each sediment layer originated from the adsorbed pool; in the upper 0–1 cm, this value was as high as 95%. Hence the pool of adsorbed ammonium can account for a significant part of the ammonium released by tidal flushing.

Ammonium adsorbed onto sediment particles and organic matter is by far the largest ammonium pool in the sediment (Fig. 1C). In the upper 0–1-cm sediment layer, this pool can be up to 50 times larger than the pool of ammonium dissolved in the pore water. Farther down in the sediment, from the depths 2 cm to 10 cm, the pool of adsorbed ammonium is still dominant, but is here only about 10 times larger than the dissolved pool.

Since the proportion of ammonium released from the sediment exceeds the proportion of ammonium dissolved in the pore water, the flushing of ammonium from the sediment during inundation is not only a result of ammonium being released from the pore water by diffusive and convective forces, but much more a result of a change in the conditions determining the ammonium adsorption

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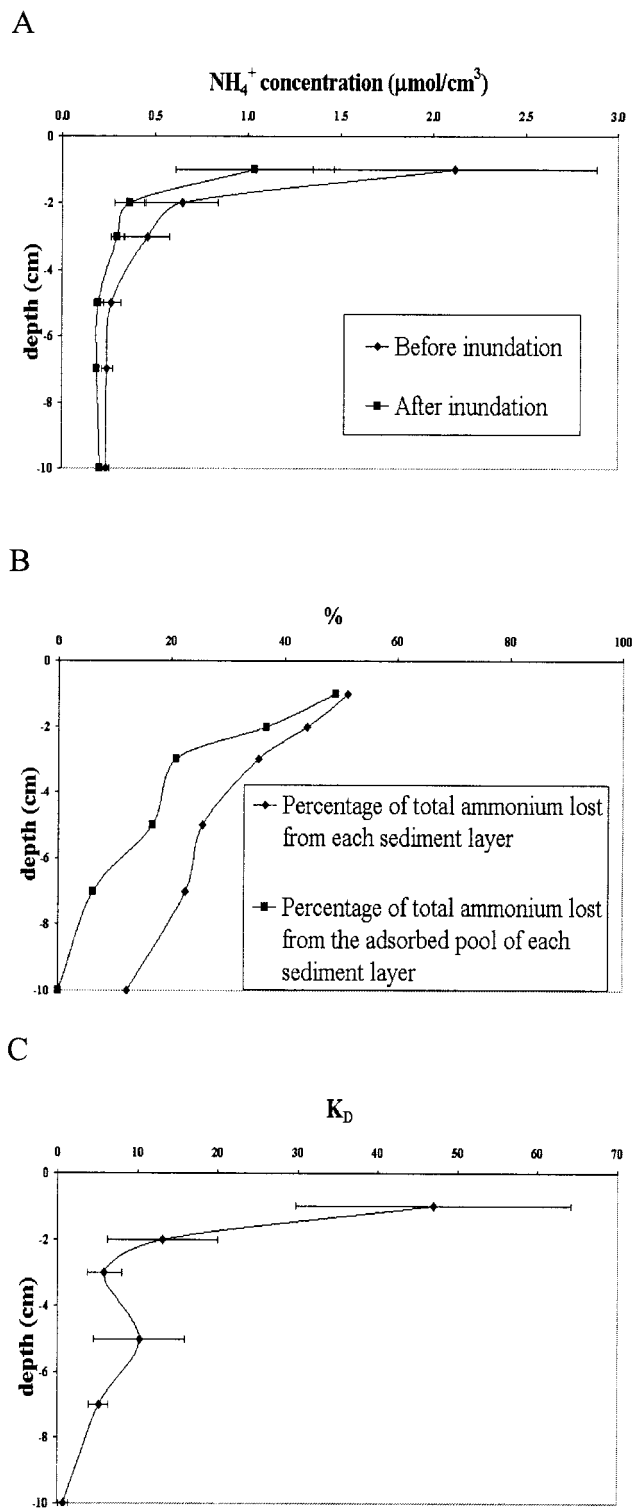

Figure 1. *(A) Ammonium concentration profiles before and after inundation. (B) Percentage of total ammonium removed from each sediment layer due to tidal flushing, and percentage of total ammonium lost from the adsorbed ammonium pool. (C) K_d-profile after 12 h of air exposure.*

equilibrium. Based on the difference in the ammonium concentration in the sediment before and after inundation, the release of ammonium from the sediment over a tidal cycle was calculated to be 18.6 mmol/m^2. That accounts for as much as 37% of the total ammonium in the upper 10-cm sediment layers, of which 80% originated from the adsorbed ammonium pool. These values are in accordance with other data for ammonium flushing (1, 2), and indicate that studies examining the loss of ammonium in sediments need to consider the adsorbed pools as well as the dissolved pools.

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Effects of Varying Salinity on Phytoplankton Growth in a Low-Salinity Coastal Pond Under Two Nutrient Conditions

Stacy Barron[1], *Carolyn Weber*[2], *Roxanne Marino*[3], *Eric Davidson*[4], *Gabrielle Tomasky*[5], *and Robert Howarth*[6] *(Ecosystems Center, Marine Biological Laboratory, Woods Hole, Massachusetts 02543)*

Coastal ponds are highly susceptible to negative effects from nutrient loading (1). The usual approach for managing such systems is to reduce nutrient input. Another possibility for some low-salinity systems may be to control salinity if salinity has a pronounced influence on phytoplankton growth. Freshwater species generally compose the phytoplankton of low-salinity systems. One might expect growth to slow as salinity increases until the assemblage switches from freshwater to marine. Similarly, phytoplankton native to systems with fairly constant salinity through space and time may not tolerate any change in salinity, as they may be adapted to that specific salinity (Valiela, Boston University, pers. comm.).

Oyster Pond (Falmouth, MA) is a brackish pond connected to Vineyard Sound through a lagoon. The pond is currently mesotrophic to eutrophic (based on chlorophyll levels; 1), perhaps due to nutrient loading from the expanding residential population surrounding the pond. Oyster Pond's salinity has decreased from 32‰ (open to the ocean) to less than 2‰ (road restricting Vineyard Sound inflow) (2). Currently, dredging and a weir maintain the salinity at a fairly constant 2.3‰. Oyster Pond managers have the option of manipulating salinity within the pond *via* the weir. While managers plan to manipulate salinity according to which fish populations they desire in the pond (Barry Norris, Oyster Pond Environmental Trust), we are interested in considering what effects salinity changes might have on resident phytoplankton populations. To determine if the general Oyster Pond phytoplankton population could adapt to changes in salinity, we added excess nutrients (nitrate and phosphate) under three salinity regimes. To determine if cyanobacteria could adapt to changes in salinity under N-depleted conditions, we added excess phosphate.

Water was collected from the northern end of Oyster Pond. Three salinity treatments (0.2‰, 2.3‰, and 5.0‰) under two nutrient conditions were created by mixing sieved Oyster Pond water (150-μm mesh to remove macrozooplankton), filtered Vineyard Sound water (GF/F), and deionized water in clear polycarbonate bottles. The 0.2‰ treatment contained 200 ml Oyster Pond water and 1800 ml deionized water. The 2.3‰ contained 200 ml Oyster Pond water, 129 ml Vineyard Sound water, and 1671 ml deionized water. The 5.0‰ treatment contained 200 ml Oyster Pond water, 298 ml Vineyard Sound water, and 1502 ml deionized water. Three replicate bottles in each salinity treatment were enriched with $NaNO_3$ and NaH_2PO_4 to final concentrations of 50 μM and 3 μM, respectively (N + P), while another three bottles at each salinity were enriched only with NaH_2PO_4 to a final concentration of 3 μM (P). Ambient nitrate and SRP (surface reactive phosphate) concentrations in the pond were 0.2 μM and less than 0.5 μM, respectively. Since Vineyard Sound water used to set up the 2.3‰ and 5.0‰ salinity treatments contained some nitrate and SRP (0.01 μM and less than 0.5 μM, respectively), nutrient additions were in excess to avoid a systematic bias. Two mM $NaHCO_3$ was added to each salinity treatment to buffer against CO_2 depletion and pH changes (3). Bottles were incubated from 24–29 °C with a 15:9 light:dark cycle. Light intensity ranged from ~280 to 350 $\mu E\ m^{-2}s^{-1}$.

For the N + P enrichments, 100 ml of water was taken from each bottle initially and daily over 8 days. Chlorophyll *a* concentration was measured fluorometrically after overnight extraction in acetone (4). P additions were sampled similarly over 10 days; phytoplankton samples were preserved with Lugol's solution initially and at 10 days. Cyanobacterial heterocysts were estimated using an inverted microscope and Sedgwick-Rafter counting chamber.

Phytoplankton grew well at all three salinities in the N + P enrichment over time (Fig. 1). These data suggest that, given ample nutrients, phytoplankton from north Oyster Pond tolerate salinities ranging from 0.2‰ to 5.0‰; they do not appear to be closely adapted to ambient salinity. The short-term physiological response observed in this experiment suggests that controlling pond salinity in the 0.2‰ to 5.0‰ range is not likely to result in large differences in overall phytoplankton growth when both N and P are available at high levels. We note that salinity manipulations can have effects on higher trophic levels, which may affect phytoplankton production and are not addressed by these experiments.

In the P treatment, phytoplankton growth over time was significantly slower, characteristic of a cyanobacteria response, and lower than in the N + P addition. With P addition alone, growth was significantly greater at ambient salinity (2.3‰) than at 5.0‰ (Fig. 1). The 0.2‰ treatment had intermediate rates of growth that were not significantly different from other treatments (Fig. 1). These data indicate that phytoplankton growth under P-enriched and N-depleted conditions may be differentially affected by salinity. Cyanobacterial heterocysts increased during the experiment at all salinities, indicating that nitrogen fixation was probably occurring. The largest increase in heterocyst numbers was in the 2.3‰ treatment (1307 ml^{-1} at 10 days *vs.* 6 ml^{-1} initially), indicating that N-fixing cyanobacteria present in Oyster Pond seem best adapted to ambient salinity. The 0.2‰ and 5.0‰ treatments increased from 6 ml^{-1} initially to 193 and 345 ml^{-1}, respectively. Note that only one sample was counted for each treatment at 10 days, so the difference in heterocyst numbers at 0.2‰ and 5.0‰ is

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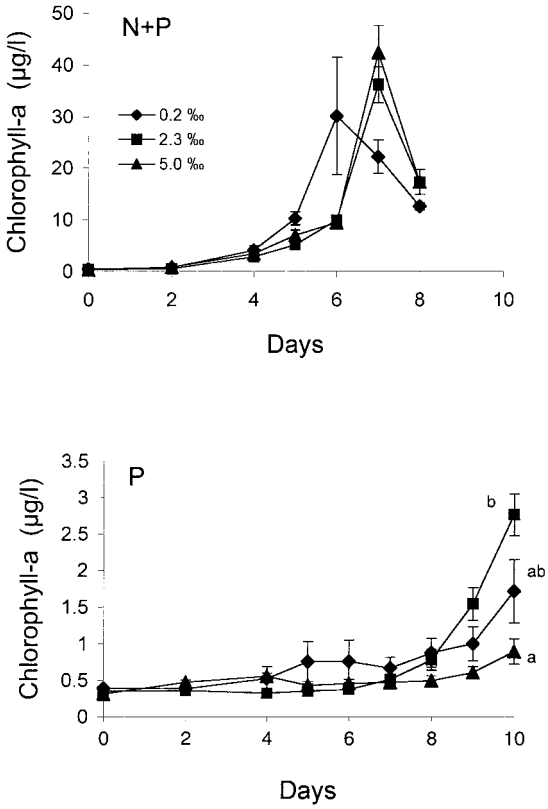

Figure 1. *Chlorophyll a (mean ± s.e.) through time in enrichment experiments done with N plus P additions and P additions only for three salinities (0.2‰, 2.3‰, and 5.0‰). Note scale differences on* x *and* y *axes. Different letters denote significant differences at the* $P < 0.05$ *level using one-way ANOVA and Tukey's honest significant difference test. Water was collected from the northern end of Oyster Pond and mixed with deionized and Vineyard Sound water to produce the salinities. The experiment was done on July 21, 2002.*

not statistically significant. The large increase in heterocysts in the 2.3‰ treatment may have influenced the final chlorophyll value by adding N to the water, allowing other species to grow.

The stimulation of phytoplankton growth in the P addition treatment contrasts with the finding of a companion study (5) which found that P additions to undiluted Oyster Pond water incubated under the same conditions did not significantly increase phytoplankton biomass. Two differences may explain this. The experiment described here ran for twice as long, allowing more time for the typically slow-growing cyanobacteria, present in the pond water at very low abundances, to respond. Further, our P addition treatment had much lower inorganic N (owing to the 10-fold dilution of Oyster Pond water), which also may have provided conditions more favorable for heterocyst development and N fixation, resulting in enough increase in N availability to increase phytoplankton biomass. This apparent difference between the two experiments bears further experimental investigation.

This short-term experiment should be interpreted with caution because over time cyanobacteria might adapt to a change in salinity. Cyanobacteria can grow and fix N up to 32‰ salinity, although they do so more slowly at higher salinities (3). Also, heterocyst abundance in Oyster Pond is low compared to lakes with high rates of N-fixation (6). Thus N-fixing cyanobacteria may not be present in great enough numbers in Oyster Pond at this time of year to alleviate N-limitation. Nonetheless, these experiments suggest that there may be a potential in Oyster Pond for eutrophication in response to both P enrichment alone as well as to N + P enrichment. Thus, managers should consider the sources of and possible controls on both N and P inputs to the pond. Further, it does not appear that manipulating salinity within the range tested here (0.2‰–5‰) will substantially affect phytoplankton growth directly.

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Nutrient Limitation of Phytoplankton Growth in Vineyard Sound and Oyster Pond, Falmouth, Massachusetts

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Phytoplankton growth requires nitrogen (N) and phosphorus (P) in an approximate molar ratio of 16:1 (the Redfield ratio; 1). N or P limitation in an aquatic system is considered to occur when the availability of N relative to P is well below or above this ratio, respectively (2, 3). Past studies have shown that marine systems of moderate to high productivity are typically N limited, while sim-

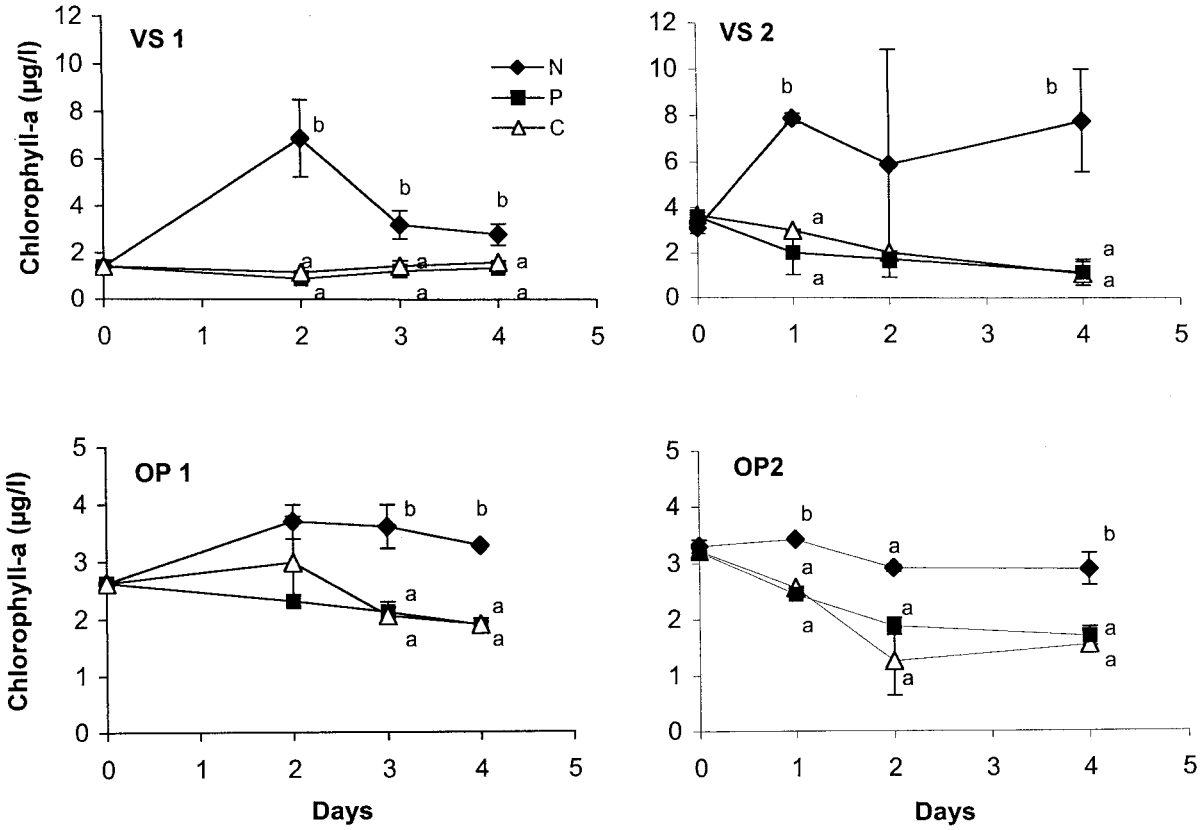

Figure 1. *Chlorophyll* a *concentrations (mean ± 1 s.e.) during experiment 1 and experiment 2 with water collected from Oyster Pond (OP, bottom panels) and Vineyard Sound (VS, top panels). Where standard errors cannot be seen they are smaller than the symbol. Statistical similarities and differences, as denoted by lowercase letters, were determined by a one-way ANOVA followed by Tukey's honest significant difference test (P < 0.05).*

ilarly productive freshwater systems are most often P limited (2, 3). However, relatively little is known about low-salinity estuaries. The Baltic Sea is perhaps the best-studied estuary of this type; there, productivity has been shown to be limited by P at salinities lower than 3 to 4‰ and by N at higher salinities (4).

Here, we report the results of a comparative set of nutrient limitation experiments in two coastal systems in Falmouth, Massachusetts, of very different salinities: Vineyard Sound and Oyster Pond (32‰ and 2.3‰, respectively). Previous studies have reported N limitation in Vineyard Sound (5, 6) as would be expected for a high-salinity coastal ecosystem (2, 3). In an October 1986 study, phytoplankton in Oyster Pond did not respond to N or P enrichments (5); Boston University Marine Program students obtained the same result from a similar experiment performed on Oyster Pond in October 2001. However, these experiments were not done during the peak growing season. Oyster Pond is currently considered to be mesotrophic to eutrophic (7), and with the watershed nearing buildout, effective management of nutrient inputs may be important in controlling eutrophication and algal blooms of concern.

We conducted two sets of bottle enrichment experiments, from June 30 to July 5, 2002, and from July 22 to July 26, 2002. For both experiments, we sieved water through a 150-μm mesh to remove large zooplankton. In the first experiment, 12 replicate, 2-l polycarbonate bottles from each system received enrichments of $NaNO_3$ or NaH_2PO_4 that increased ambient concentrations of nitrate by about 50 μM (N treatment) or phosphate by about 10 μM (P treatment); 12 control bottles from each system received no nutrient additions (C treatment). As a safeguard against short-term CO_2 depletion in the bottles, we added $NaHCO_3$ (2.0 m*M*) to the Oyster Pond samples. At the beginning of the experiment, nine bottles were sampled immediately (three each of controls and three each of the PO_4 and NO_3 additions) to determine initial chlorophyll *a* concentrations and confirm the effectiveness of the nutrient enrichments. The remaining bottles containing Oyster Pond or Vineyard Sound water were incubated 0.5 m to 1 m below the surface of Oyster Pond on a floating rack, at a light intensity of about 330–560 $\mu E\ m^{-2}s^{-1}$ (peak daylight hours). We collected three replicate bottles of each treatment on days 2, 3 and 4. Subsamples were filtered (GF/F) and chlorophyll *a* concentrations were determined fluorometrically (8).

We started our second set of experiments on July 22, 2002. The nutrient treatments were identical to the first experiment, except a treatment was added for Oyster Pond water in which both NO_3 and PO_4 were added to increase ambient concentrations to 50 μM and 3 μM, respectively, to parallel another concurrent set of experiments done in Oyster Pond (7). We repeatedly removed 100-ml samples from each of twelve 2-l bottles over time for chlorophyll analysis, rather than having replicate bottles for each time point.

We incubated the bottles in a growth chamber on a 15:9 h light: dark cycle at a light intensity of 280–350 μE m^{-2} s^{-1} and a temperature of 24 to 29 °C. All treatments were sampled initially, and on days 1, 2, and 4.

In the first experiment with Vineyard Sound water, chlorophyll *a* concentrations increased in the N-enriched treatment by day 2, and rapidly declined thereafter (Fig. 1); Concentrations were significantly higher than those of the controls and P-enriched treatment. In the second experiment, chlorophyll concentrations in the N-enriched bottles peaked on day 1 and were always significantly higher than the controls. In contrast, P-enriched treatments were never significantly different from the controls in either experiment (Fig. 1). Both experiments indicate that phytoplankton growth in Vineyard Sound was N limited, as previously reported (5, 6).

In the experiments with Oyster Pond water, chlorophyll *a* concentrations in the N-enriched treatment were significantly higher on two out of the three sampling dates for both experiments (Fig. 1). Chlorophyll *a* concentrations in P-enriched bottles did not differ significantly from controls at any time (Fig. 1). In the second experiment when both N and P were added, the response was far greater, with a final chlorophyll *a* concentration of 23.2 μg 1^{-1} on day 4 (data not shown). This suggests that P can quickly become limiting if enough N is supplied. The significant response in the N-enriched treatment in both our experiments differs from previous studies in Oyster Pond, which found no nutrient limitation (5), and from studies in low-salinity parts of the Baltic Sea (<3 to 4‰) which concluded that P was limiting (4).

Our results contribute to the large body of experimental evidence that finds N limitation in temperate coastal marine ecosystems of moderately high salinity, such as Vineyard Sound. For low-salinity estuaries, there are fewer studies on nutrient limitation, but our finding of N limitation is unusual. The difference between earlier studies in Oyster Pond and our study may reflect seasonal changes in nutrient limitation. Nitrogen may be limiting during the summer (our study) while neither N nor P is limiting in mid-fall (previous studies), either because there is less overall demand for nutrient late in the season or because N fixation over the summer and early fall has helped alleviate N limitation. Further research is needed to better understand nutrient limitation in low-salinity ecosystems, and to evaluate the relative importance of the many biogeochemical processes including N fixation that may regulate limitation in these systems. Nonetheless, our study suggests that N availability, rather than P, currently regulates phytoplankton growth in Oyster Pond during the summer.

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Response of Shrimp Populations to Land-Derived Nitrogen in Waquoit Bay, Massachusetts

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Land-derived nitrogen impacts are a major agent of change affecting estuarine populations. Some changes include macroalgae and phytoplankton blooms, which alter food webs and benthic habitats (1). N loads may influence the abundance, species composition, and growth rates of the shrimp species that are common in estuaries of Cape Cod, such as those in the genera *Palaemonetes* and *Crangon* (2). Estuaries of the Waquoit Bay estuarine system offer the opportunity to examine how shrimp of different species respond to different land-derived N loads, because different subestuaries are subject to different land-derived loads. For example, Sage Lot Pond, Quashnet River, and Childs River receive N loads of 15.9, 310.3, and 360 kg N ha^{-1} y^{-1}, respectively (3). The estuaries have similar water residence times, about 1–2 days, and range from 0–32 ppt (1).

In this study we assessed the effects of differences in land-derived N loads on shrimp abundance, shrimp species composition, growth rate, and reproduction in estuaries of Waquoit Bay, Massachusetts.

To estimate the abundance and size of shrimp of the different species, we walked a 5-m seine for 10 m in each of five arbitrary locations along the shore, beginning with the most fresh to the most saline of each estuary, during high tide. Shrimp were identified, counted, and measured from the tip of the rostrum to the end of the carapace. To estimate growth rates in *Palaemonetes pugio,* we first identified the modal carapace length of each cohort present, using the software program Mix 3.1.3, and calculated the increment in size per month. In addition, we recorded percent of ovigerous females in each estuary. We used ANOVA to compare species abundance and percent ovigerous females among the

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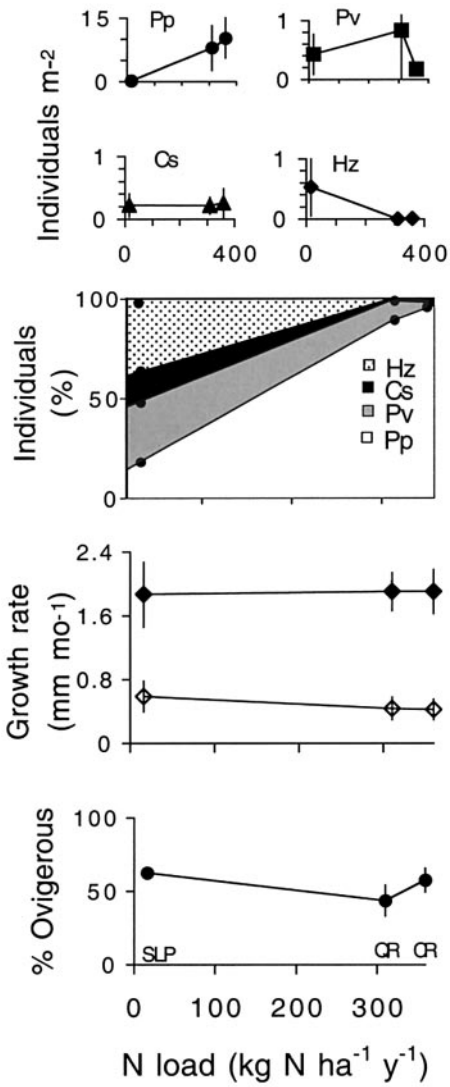

Figure 1. *Abundance ± SE of shrimp species (first panel), % species composition (second panel), growth rates of* Palaemonetes pugio *for subadults (◆) and adult cohorts (◇) (third panel), and percent ± SE of ovigerous females of* P. pugio *(fourth panel) in relation to land-derived N loads for the watersheds. Pp =* P. pugio*; Pv =* P. vulgaris*; Cs =* Crangon septemspinosa*; Hz =* Hippolyte zostericola*. CR = Childs River; QR = Quashnet River; SLP = Sage Lot Pond.*

estuaries. A $\log_{10}$ transformation was necessary to meet the ANOVA assumptions to compare abundances. An *a posteriori* Tukey test was used to test differences among estuaries.

We found four species of shrimp in the estuaries: *Palaemonetes pugio, P. vulgaris, Crangon septemspinosa,* and *Hippolyte zostericola* (Fig. 1, first panel). There was no detectable effect of salinity on the abundance of shrimp, so in this paper we pooled data from all stations. Abundance of *P. pugio* was significantly higher in Quashnet River and Childs River than in Sage Lot Pond ($F = 7.83$, df = 2, $P < 0.01$); abundance of *P. vulgaris* and *C. septemspinosa* did not change significantly ($F = 0.073$, df = 2, $P = 0.93$; $F = 0.016$, df = 2, $P = 0.9$). *H. zostericola* was found only in Sage Lot Pond, the estuary with the lowest N load (Fig. 1, first panel).

The differences in abundance of the four species created a clear shift in percent species composition as land-derived N load increased (Fig. 1, second panel). The dominance of *P. pugio* increased in relation to increasing N loading (Fig. 1, second panel); relative abundance of *P. vulgaris* and *C. septemspinosa* did not vary significantly; and *H. zostericola* was only present in the most pristine estuary. *P. pugio* and *H. zostericola* could be useful indicators of level of eutrophication.

We determined growth and reproductive effort for *P. pugio,* the most abundant shrimp species found (Fig. 1, second panel), as also reported in an earlier study (2). Size frequency histograms clearly revealed two cohorts of *P. pugio* in all estuaries. Individuals in cohort 1 had a carapace length of 8–13 mm, and individuals in cohort 2 had lengths of 14–18 mm. Growth rate of cohort 1 was higher than those of adults and was unaffected by N loads (Fig. 1, third panel). Growth rate of adults decreased slightly with increased N loads (Fig. 1, third panel). Egg-bearing females were only found in cohort 2 individuals. There was no effect of level of N loading on the percent of ovigerous females in cohort 2 ($F = 1.7$, df = 2, $P = 0.24$). This suggests that food supply in the estuaries was sufficient to support reproductive needs (4).

Higher N loads lead to higher populations of plants and algae, and the protein content of these are higher. *P. pugio* may be better at exploiting these resources then the other shrimp species (5, 6), although we lack an explanation for the mechanism underlying the different responses by these four shrimp species. We can say, however, that our results suggest that *P. pugio* may be a useful indicator species for increased N loads, and *H. zostericola* may indicate more pristine conditions.

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