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Phosphorus and nitrogen limitation of primary production in a simulated estuarine gradient

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ABSTRACT: The transition between phosphorus limitation of primary production in freshwater and nitrogen limitation in seawater was examined along an estuarine gradient simulated in 4 large 13 m^3 enclosures connected in a series and containing pelagic and benthic subsystems. Nominal salinities of 0, 5, 10 and 25 ppt were maintained by exchanging appropriate volumes of water between enclosures. River water, which served as a freshwater endmember, was naturally high in N relative to P, while the oceanic endmember (water from Narragansett Bay, RI, USA) was low in N relative to P. Production in the water column was supported by external inputs and recycled nutrients. Bioassays, inorganic nutrient concentrations and N:P ratios of the seston and inorganic nutrients indicated that phosphorus was limiting at 0, 5 and 10 ppt, while nitrogen was limiting at 25 ppt. Coincident with this shift in limiting nutrient was a shift in the N:P ratio of nutrient supply from greater than the Redfield ratio of 16 to less than 16. External inputs established relative rates of supply in each enclosure. The relative proportion of N and P in external inputs was largely a function of the hydrodynamic mixing of fresh (high N, low P) and salt water (low N, high P) endmembers. At the scale of the estuarine segment or enclosure, neither recycled inputs from the benthos and water column, nitrogen fixation nor internal losses of N and P to sedimentation and/or denitrification materially altered relative supply rates, despite a hydrodynamic residence time of 27 d.

KEY WORDS: Estuaries · Primary production · Nutrient limitation · Nitrogen · Phosphorus

INTRODUCTION

When primary production is nutrient limited, it is most often the supply of phosphorus which regulates production in freshwater (Schindler 1974) whereas it is usually nitrogen which is critical in saline waters (Ryther & Dunstan 1971, Oviatt et al. 1995). While this difference in limiting nutrient may hold in general, exceptions exist (Howarth 1988, Krom et al. 1991). Inherent in the concept of limitation is the supply of one resource or nutrient relative to another. Thus, the difference in limiting nutrient between freshwater and marine systems may be caused by changes in the nitrogen supply, the phosphorus supply, or both.

Although nitrogen fixation can maintain phosphorus limitation in lakes (Schindler 1977), this does not appear to be true in marine systems (Howarth et al. 1988a). These apparent differences in nitrogen fixation potential, coupled with observations that rates of denitrification both achieve higher values (Seitzinger 1988) and can contribute to nitrogen limitation in coastal marine systems (Nixon et al. 1980), have focused attention on processes which alter the nitrogen supply.

While the focus on nitrogen is relatively recent, the biogeochemistry of phosphorus in fresh and marine waters has been a lively area of inquiry for some years (Carritt & Goodgal 1954, Hutchinson 1957, Jitts 1959, Pomeroy et al. 1965, Fox et al. 1986, Levine et al. 1986, Froelich 1988). The interactions of phosphorus with particles, either suspended or in the sediments, differ in fresh and salt water. The geochemical release of phosphorus from terrigenous sediments upon contact with seawater (Froelich 1988, Jordan et al. 1991), and

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the relatively high fluxes of phosphorus from marine sediments (Pomeroy et al. 1965), have led to the hypothesis that changes in the phosphorus cycle may account for the difference in limiting nutrient (Pomeroy et al. 1965, Caraco et al. 1989, 1990).

Conceptually, estuaries may be well suited for examining nutrient limitation. Estuaries are the transition zone between fresh and ocean water, and shifts in limiting nutrient can occur in these systems (D'Elia et al. 1986, Caraco 1988, Fisher et al. 1992). However, the temporal and spatial variability of and difficulty in quantifying input to these systems often make them experimentally unmanageable. As a controllable alternative we have used large, outdoor, experimental enclosures (vol 13.1 m^3), connected in a series, to simulate the horizontal salinity gradient of a shallow (5 m) well-mixed estuary. The experimental gradient was also configured to simulate the transition from freshwater to marine sediments that occurs in an estuary. The enclosures, or mesocosms, are located at the Marine Ecosystem Research Laboratory (MERL) in Rhode Island, USA. In essence, this physical configuration of the enclosures divides a conceptual estuary into segments of constant salinity in the same manner as mathematical box models do (Officer 1980). The purpose of the present investigation was to (1) evaluate sources and supply rates of nitrogen and phosphorus with respect to the demand required to support production in each enclosure or segment in the gradient and (2) identify key processes and factors which alter rates of nutrient supply and hence determine nitrogen or phosphorus limitation in estuarine systems. Specifically, we address 3 major processes or factors thought to determine the limiting nutrient in most aquatic systems and a fourth which is peculiar to estuaries. These are the composition of external inputs, preferential loss of phosphorus to the sediments or nitrogen to sedimentation and denitrification, and nitrogen fixation (Howarth 1988). For estuaries in particular, the hydrodynamic mixing of endmembers may also be important (Smith 1991).

METHODS

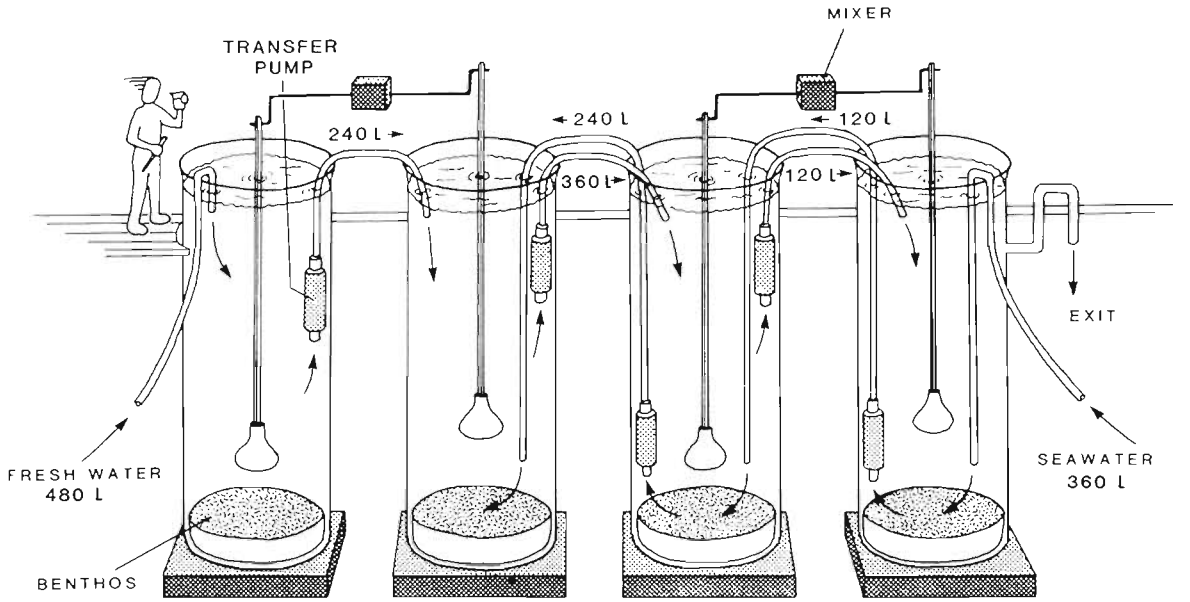
Estuarine gradient. The design and operation of the experimental estuarine gradient (Fig. 1) has been described by Klos (1988). The gradient consisted of 4 mesocosms (vol 13.1 m^3; depth 5.0 m) ranging in nominal salinity from 0 to 25 ppt (Fig. 1). Mesocosms were interconnected by air driven pumps. The total volume (480 l d^{-1}) pumped to each tank in 4 pulses d^{-1} (every 6 h) was derived from adjacent tanks in appropriate proportion to produce the desired salinities. The constraints imposed by this mixing regime necessitated the inclusion of an overflow or drain for each mesocosm (Fig. 1). While each mesocosm received 480 l d^{-1} from adjacent enclosures as inflow, somewhat less than this was pumped out to produce salinities in those enclosures immediately up- or downstream. The excess water drained by gravity through a standpipe (shown for the 25 ppt mesocosm in Fig. 1). The inclusion of an overflow for each mesocosm allowed a constant and equal hydrodynamic residence time (27 d) at each salinity along the gradient, a feature rarely found in a natural estuary but experimentally desirable because salinity and residence time of water do not covary. The actual volume transferred by the pumps was measured weekly. Mixers (Fig. 1) rotating (5 rpm) in a vertical elliptical orbit for 2 of every 6 h maintained a well-mixed water column. Heat exchangers (not shown in Fig. 1) held water temperatures to within 2°C of those in nearby Narragansett Bay.

Sediments (2.52 m^2, depth 30 to 40 cm) for the mesocosms were collected (30 May to 2 June 1989) from 2 sites in the Pawcatuck River Estuary in southern Rhode Island (RI). Sediments for the 0, 5 and 10 ppt treatments came from a low salinity station (annual range 3 to 22 ppt) while those for the 25 ppt treatment were collected further down the estuary (annual range 20 to 30 ppt). Methods of collection are summarized in Hunt & Smith (1983).

The Pawcatuck River also served as a source of freshwater for the gradient. Drawn at Branford, RI, water was transported by tanker truck, held in a mesocosm without sediment and used as needed. Water was resupplied every 2 to 3 wk. Saltwater was supplied to the gradient via diaphragm pump from Narragansett Bay. The experiment commenced on June 13, 1989, and lasted 123 d.

Water column measurements. Parameters and their frequency of measurement are shown in Table 1. The water column of each mesocosm was sampled by siphoning a composite sample (depth 0.1, 2.5, 4.5 m) during a mixing cycle. Salinity was measured on an Autosal Model 8400 inductive salinometer. Particulate carbon, nitrogen (PN) and phosphorus (PP) samples were passed manually (60 ml plastic syringe) through Whatman GF/F glass fiber filters. Carbon and nitrogen were analyzed by elemental analysis using a Carlo Erba Model 1106 Elemental (CHN) Analyzer. PP was analyzed colorometrically on a Technicon II Autoanalyzer after ashing at 500°C and extraction in HCl (Froelich et al. 1982). Total nitrogen and phosphorus were analyzed by persulphate digestion after Valderrama (1981).

Phytoplankton biomass was assessed both by analysis of chlorophyll *a* (chl *a*) (Yentsch & Menzel 1963, Lorenzen 1966) and *in vivo* fluorescence with and without the addition of DCMU [3-(3,4-dichlorophenyl)-1,1-dimethylurea] (Keller & Rice 1989). The



TREATMENT	0 ‰	5 ‰	10 ‰	25 ‰
OVERFLOW VOLUME (l/day)	240	120	120	360
OBSERVED SALINITY (‰)	—	5.35 ± 0.39	10.87 ± 0.39	27.14 ± 1.58
PREDICTED SALINITY (‰)	—	5.72 ± 0.30	10.78 ± 0.40	26.62 ± 1.92

Fig. 1 Experimental estuarine salinity gradient. Freshwater (0‰) and seawater were introduced at either end. To maintain desired salinity, water was transferred between tanks every 6 h via pumps with each enclosure receiving a total of 480 l d^{-1}. Of this 480 l, only a fraction was required to meet volume and salinity demands of adjacent enclosures. Excess water was drained by gravity via an overflow (shown only for the 25 ppt treatment). Nominal overflow volumes for each treatment are given at the bottom of the figure. Observed salinities are mean ± SD of measurements taken 2 times per week throughout the experiment. Salinities ± SD predicted from measured pump volumes are also shown

Table 1. Parameters and their frequency of measurement during the experiment

Parameter	Frequency
Water column	
Salinity	Twice weekly
Chlorophyll *a*	Weekly
Particulate carbon and nitrogen	Weekly
Particulate phosphate	Weekly
Total nitrogen and phosphorus	Fortnightly
System metabolism (O_2)	Weekly at dawn, dusk, dawn
Dissolved inorganic nutrients (NH_4^+, NO_3^{2-}, NO_2^-, PO_4^{3-})	Weekly at dawn, dusk, dawn
Nutrient limitation bioassays	Fortnightly
Nitrogen fixation	September 20
Benthos	
Benthic flux (NH_4^+, NO_3^{2-}, NO_2^-, PO_4^{-3}, CO_2, O_2)	Fortnightly
Denitrification	July 24
Rain	
Dissolved inorganic nutrients	Per event

resultant values of the latter measurement, in relative units, were normalized to a coproporphyrin standard of known concentration. This procedure corrects for drift in the fluorometer (Turner Designs Model 10) and yields values which are comparable over time (Donaghay & Klos 1985). Chl *a* (µg l^{-1}) and DCMU-enhanced fluorescence (relative units) were significantly correlated (linear) in each of the 4 mesocosms in the gradient ($r = 0.802$ for 0 ppt, 0.693 for 5 ppt, 0.889 for 10 ppt, 0.580 for 25 ppt, $n = 15$ and $p < 0.05$ in each case). The fluorometric determination of chl *a* suffers from interference by chl *b*, leading to an underestimation of chl *a* and an overestimation of phaeopigments (Lorenzen 1981, Welschmeyer 1994). If selective underestimation occurred along the gradient, then PN to chl *a* ratios should differ among the treatments. PN:chl *a* ratios (± 95% CI), calculated from the slopes of linear regressions, for the 0, 5, 10 and 25 ppt treatments were, respectively, 10.1 ± 3.0, 15.6 ± 6.1, 11.0 ± 7.9, and 7.4 ± 4.2. Statistically, all these slopes are significantly different from zero ($p < 0.05$). However, their confidence intervals overlap, showing that they are statistically equivalent to each other. These considerations

argue against significant interference by chl *b* and indicate that chl *a* suitably reflected phytoplankton biomass.

Dissolved inorganic nutrient samples were manually filtered through 47 mm diameter, 0.4 µm pore size membrane filters (Nuclepore) into 60 ml polypropylene jars. These were frozen until analysis on a Technicon II Autoanalyzer (Lambert & Oviatt 1986). Samples were taken weekly at dawn, dusk and the following dawn. Weekly concentrations reported here represent the average of these 3 measurements. Concentrations of samples from all treatments were calculated from standard curves employing artificial seawater (30 ppt) solutions. Following Froelich & Pilson (1978), raw sample concentrations were adjusted by a correction factor which accounted for differences between the refractive index of artificial seawater and that of the sample water due to differences in salinity. For samples with a salinity below 15 ppt, Collos et al. (1992) recommend buffering (pH = 8.5) the ammonium chloride reagent used in the analysis of nitrate to prevent reduction beyond nitrite. We did not use buffered ammonium chloride. A subsequent comparison of samples analyzed with pure and buffered ammonium chloride on our autoanalyzer showed the pure reagent to yield results, at most, 5% lower than the buffered. Thus, estimates of dissolved inorganic nitrogen may be a maximum of 5% too low in the 0, 5 and 10 ppt treatments. The concentrations of dissolved organic nitrogen and phosphorus (DON, DOP) were calculated by difference between the total and sum of particulate and dissolved inorganic.

Nitrogen fixation potential was assessed by traditional acetylene reduction methods using 50 ml serum bottles incubated in the light (200 ft-candles) at ambient temperatures (Stewart et al. 1967). A 1.0 m poropak-N (80/100) column at 60°C was used with an injection temperature of 110°C. Samples were taken once during the experiment (Table 1).

System metabolism. System production and respiration were estimated weekly by measuring oxygen concentrations at dawn, dusk and the following dawn in the enclosure water columns. Dissolved oxygen was measured by the Winkler titration (Lambert & Oviatt 1984). Dawn minus dusk concentrations were corrected for diffusion to yield an estimate of system production (Oviatt et al. 1987). The difference between dusk concentration and the following dawn concentration provided a measurement of system respiration (Oviatt et al. 1987).

Total system production (TSP) and total system respiration (TSR) were calculated by integrating weekly measurements over the duration of the experiment. These oxygen metabolism measurements include respiration of both the water column and the heterotrophic benthos. Both system level measurements of production and respiration were adjusted for benthic oxygen consumption (see 'Benthos', below) to furnish estimates of net daytime water column production and nighttime respiration. Since benthic respiration is a 24 h process and system production and respiration occur only during the day or night, respectively, each is adjusted by 1/2 the benthic respiration following Doering (1989) (Table 2). Rates integrated over the entire experiment were used in these calculations.

The nutrient supply of nitrogen and phosphorus required to support production can be calculated following Doering (1989) (Table 2) from integrated water column production of oxygen and the average elemental composition of the seston. Since water column production is in units of oxygen and seston C:N and C:P ratios are used, a production quotient (PQ) of 1 mol O_2 mol^{-1} CO_2 is assumed. Both calculations assume constant stoichiometry and no differential cycling of O, C, N, or P.

Similarly, the regeneration of nutrients in the water column at night can be calculated from integrated water column respiration of oxygen and the C:N or C:P

Table 2. Equations used for calculation of (1) water column production (WCP) and water column respiration (WCR) from total system production (TSP), total system respiration (TSR) and benthic oxygen consumption (BR); (2) nitrogen and phosphorus demand of production; PQ: production quotient; (3) recycling of nutrients in the water at night; RQ: respiratory quotient; (4) denitrification from benthic fluxes of dissolved inorganic carbon (BFDIC) and dissolved inorganic nitrogen (BFDIN); and (5) nitrogen fixation from the net utilization of both (a) Total P and (b) DIP. 'In' refers to external input and 'out' to output via pumps and overflow; BFDIP: benthic input of DIP. See 'System metabolism' in 'Methods'

Water column metabolism

WCP = TSP + ½BR
WCR = TSR – ½BR

Nutrient requirement of production (assumes PQ = 1.0)

Nitrogen required = WCP/(C:N of seston)
Phosphorus required = WCP/(C:P of seston)

Nutrients recycled in the water column (assumes RQ = 1.0)

Nitrogen recycled = WCR/(C:N of seston)
Phosphorus recycled = WCR/(C:P of seston)

Denitrification

Denitrification = [(BFDIC)/(C:N seston)] – (BFDIN)

Nitrogen fixation

(a) $[(\text{Total P}_{in} + \text{BFDIP} - \text{Total P}_{out}) \times (\text{N:P seston})] - (\text{Total N}_{in} + \text{BFDIN} - \text{Total N}_{out})$

(b) $[(\text{DIP}_{in} + \text{BFDIP} - \text{DIP}_{out}) \times (\text{N:P seston})] - (\text{DIN}_{in} + \text{BFDIN} - \text{DIN}_{out})$

ratio of seston following Doering (1989). Since water column respiration is in units of O_2 and a C:N or C:P ratio is used, the method assumes a respiratory quotient (RQ) of 1 mol CO_2 mol^{-1} O_2. Both calculations employ constant PQs and RQs. Although these vary in the short term, Oviatt et al. (1986) found that use of such traditional corrections of oxygen to carbon for system measures of metabolism for long-term integrated data sets was justified. Both calculations also employ long-term average nutrient ratios. Given the considerable short-term variability of nutrient uptake measurements, the ratio approach may be more appropriate for calculating longer-term average rates, as done here (Boynton & Kemp 1985).

Benthos. Exchanges of dissolved inorganic nutrients, dissolved oxygen and dissolved inorganic carbon (DIC) across the sediment water interface were measured by following concentration changes over time within a clear plastic chamber (vol ~320 l) covering the entire benthos (Doering et al. 1987). Control bottles were simultaneously incubated on top of the chamber to account for changes occurring within the water in the chamber. Fluxes were calculated using a 3 point time series and linear regression of concentration on time. Dissolved inorganic nutrients and dissolved oxygen were analyzed as above. DIC concentrations were measured on a O.I. Corp. Model 700 Total Carbon Analyzer.

The loss of fixed nitrogen as N_2 gas through denitrification was estimated from intact sediment cores in N_2-free, gas-tight chambers (Nowicki 1994, Seitzinger et al. 1984). Observed N_2 gas production rates were corrected for N_2 gas produced during de-gassing of sediment pore waters and from atmospheric contamination through use of anoxic control cores (Nowicki 1994). Cores were incubated at 18°C. At the time of sampling (Table 1) tank temperatures were about 20°C.

Rain. Rainfall samples were collected in acid-cleaned, 500 ml poly-bottles fitted with a glass funnel (10 cm diameter) inserted through a stopper in each bottle's neck (Nowicki & Oviatt 1990). Replicate samplers remained outside for the duration of a storm and were then retrieved and the collected precipitation analyzed for dissolved inorganic nutrients. To calculate input to the enclosures, volumes of rain collected were prorated to the surface area of the tanks. Dryfall input was not measured.

Nutrient limitation bioassays. Water from each of the 4 mesocosms was distributed directly, or filtered through a 144 µm mesh net if zooplankton were prevalent, into 4 sets of four 250 ml polycarbonate bottles. One set remained unamended while nitrogen (NH_4Cl), phosphorus (KH_2PO_4) and both nutrients were added to one each of the remaining 3 sets. Nutrients were added to produce nominal concentrations of 2 µmol l^{-1} P and 32 µmol l^{-1} N above ambient. All 64 bottles were incubated outdoors in a running seawater bath covered with a screen which reduced ambient light levels by 58%. Each day 1 bottle from each set of additions to each salinity treatment was harvested. Phytoplankton biomass was measured by fluorescence, enhanced by addition of DCMU, on a Turner Designs Model 10 Field Fluorometer. Chl *a* and fluorescence were significantly correlated in the mesocosms (see 'Water column measurements', above) showing that fluorescence adequately measured biomass in our case.

Bioassay results were expressed as an N to P response ratio (Elser et al. 1988). Calculation of the response ratio involved 2 steps and employed data only from the control, N addition and P addition. First, the maximum DCMU fluorescences obtained in N and P additions during a bioassay were each divided by the maximum observed in unamended controls, yielding a normalized N response and a normalized P response. The ratio of the N response to the P response is the N to P response ratio. A value greater than 1.0 indicates that the response to N addition was greater than that to P addition and suggests potential N limitation. A value less than 1.0 indicates that the response to P addition was greater than the response to N addition, suggesting potential limitation by phosphorus.

Water fluxes between enclosures. An important part of the nutrient budget (see 'Nutrient inputs and outputs', below) was the nutrients transferred between enclosures by the pumps. The volume of water pumped was a critical term in this calculation. One way to check the accuracy of the pumps was to use the measured flow rates to predict salinity in the gradient over the course of the experiment.

Salinity was estimated for each treatment on each day of the experiment using the computer program Stella (Richmond et al. 1987). The model employed was a mathematical representation of Fig. 1 in which freshwater entered at one end of the gradient while saltwater entered at the other. Water flowed between enclosures at the measured pump rates. These data were interpolated (linear) to give daily estimates. Thus, the mass of salt in a tank on any day was estimated by the following equation:

$$S_t \times V = S_{t-1} \times V + (Q_{in} \times S_{in} - Q_{out} \times S_{out})$$

where S_t was the tank salinity on day t; S_{t-1} the tank salinity on the previous day; Q_{in} the inflow of water and S_{in} the salinity of that water over the period $t-1$ to t; and Q_{out} the outflow of water and S_{out} the salinity of that water over the period $t-1$ to t. S_{in} was the salinity in adjacent tanks and S_{out} was the salinity in the tank of interest. V equals the volume of a mesocosm. The input and output of water from the enclosures was

adjusted for rain. The program ran with a daily time step, performing 2 calculations during each time step using a second order Runge-Kutta method (Richmond et al. 1987). On average the daily salinity values calculated from the model differed from those measured by 1 to 7% ($p < 0.05$; Fig. 1). This comparison demonstrates that the measured pump flows reasonably reflected the actual transfer of water between mesocosms.

Nutrient inputs and outputs. The external nutrient inputs to the water column of each mesocosm included pumped transfer from adjacent enclosures and rain. Outputs included pumped transfer to adjacent mesocosms and overflows. Net changes in the standing stocks of nutrients in the water column from beginning to end of the experiment (initial concentration minus final concentration) were considered as external input if the change was positive or output if the change was negative.

The pumped transfer of nutrients between mesocosms was calculated as the product of concentration and water flux. These were interpolated (linear) to give daily estimates, which in turn were summed over the 123 d experiment to yield integrated values. Benthic flux measurements were expressed as daily rates and integrated as above. Nutrient inputs via rain were calculated for each rain event and added to provide a total for the experiment. Recycling of nutrients in the water column at night was calculated using integrated (123 d) water column respiration estimates and average elemental ratios of the seston.

Nitrogen fixation, measured in the water column, was an additional source of nitrogen. Denitrification by sediments was an additional output. Although not necessarily representing a direct loss from the water column, this process may be viewed as reducing the sedimentary input of inorganic nitrogen to the water column. Denitrification and nitrogen fixation were measured only once during the experiment. Measured rates were assumed to have been constant and applied to the entire 123 d experimental period.

The extrapolation of single measurements of nitrogen fixation and denitrification over the entire experimental period is perhaps too ambitious. Both, however, may be calculated from our data. Potential nitrogen fixation was calculated using nutrient inputs to the water column and nutrient outputs from the water column after Smith (1984) (Table 2). The first term in either equation presented in Table 2 is the net utilization of phosphorus by the water column. This is multiplied by the N:P ratio of the seston to estimate the net utilization of nitrogen required to form seston of that N:P. The last term in either equation is the observed net utilization of nitrogen by the water column. An estimated nitrogen utilization (first term) greater than the observed (second term) implies a nitrogen deficit made up by nitrogen fixation.

The indirect calculation of nitrogen fixation carries with it certain provisos. As Smith (1991) has noted, the quantity yielded by the calculation represents the net result of the opposing processes: nitrogen fixation and denitrification. Using total N and P in the calculation will overestimate available N relative to P because organic P is more available than organic N (Howarth 1988), and the calculated N deficit will be underestimated. Using DIN (dissolved organic nitrogen) and DIP (dissolved inorganic phosphorus) will underestimate available N relative to available P because some organic P is included in the measurement of DIP (Howarth & Marino 1990), and the calculated N deficit will be overestimated. As originally formulated by Smith (1984) the calculation does not include recycling and may yield inflated estimates (Howarth et al. 1988a). Therefore, we have included the benthic input of nitrogen and phosphorus in the calculation. In order to bracket potential N fixation we have used both total and dissolved inorganic N and P.

Denitrification by sediments may be indirectly calculated from measurements of oxygen and DIN flux (Nixon et al. 1976). Instead of oxygen flux which measures only aerobic respiration we have substituted DIC production which more closely approximates total community respiration. The first term in the equation (Table 2) is the measured production of dissolved inorganic carbon. Dividing by the C:N ratio of the seston estimates the expected DIN produced by benthic respiration of material with a ratio equivalent to the seston. The last term in the equation is the observed DIN production by sediments. A deficit between expected and observed implies denitrification. The indirect calculation of N fixation and denitrification assumes constant stoichiometry and no differential cycling of C, N, or P.

RESULTS

Nutrient and chlorophyll concentration

Phytoplankton biomass, as measured by the concentration of chl *a*, exhibited at least 1 major peak (>10 µg chl *a* l^{-1}) in each treatment during the experiment (Fig. 2). Chl *a* and the concentration of DIN fluctuated inversely over time in the 0, 5 and 10 ppt treatments (linear correlation coefficient $r = -0.868, -0.762, -0.540$ respectively, $p < 0.05$ in all cases). Although this same pattern was evident for the 25 ppt treatment, the correlation was not statistically significant ($r = -0.255$, $p > 0.05$). This behavior indicates that fluctuations in phytoplankton biomass were caused by *in situ* growth and

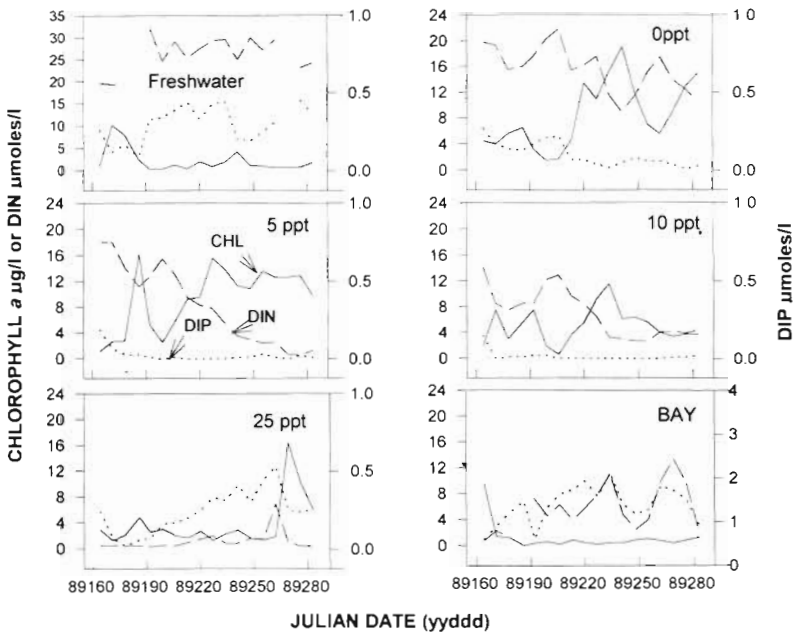


Fig. 2. Chlorophyll *a*, dissolved inorganic nitrogen (DIN), and dissolved inorganic phosphorus (DIP) in the water column of each salinity treatment, and the fresh and salt (Bay) water endmembers over the course of the experiment

mortality rather than advection from adjacent enclosures. This result is further supported by the lack of concordance between endmember chlorophyll concentrations and adjacent endmembers of the gradient (freshwater vs 0 ppt, $r = -0.018$; Bay vs 25 ppt, $r = -0.075$; $p > 0.05$ in both cases).

Nutrient concentrations exhibited different patterns of temporal fluctuation and potentials for limiting phytoplankton growth in the treatments. Although providing only a first approximation, comparison of nutrient concentrations with literature values for the half-saturation constant of nutrient uptake furnishes a measure of nutrient limitation potential (Fisher et al. 1988). Half-saturation constants range between 1 and 2 µmol l^{-1} for DIN and 0.1 and 0.5 µmol l^{-1} for DIP. Concentrations below these ranges indicate a potential for nutrient limitation (Fisher et al. 1988).

DIN concentrations, averaged over the experimental period, declined with increasing salinity (Table 3). DIN concentrations also declined over time in the 0, 5, and 10 ppt treatments (Fig. 2). Nevertheless concentrations remained high and fell below the lower limit of the half-saturation constant (1 µmol l^{-1}) only twice in the 5 ppt treatment towards the end of the experiment. By contrast DIN concentrations were relatively low in the 25 ppt treatment remaining below 1 µmol l^{-1} for 13 of the 18 weekly measurements.

Over the experimental period, average DIP concentrations were low in the 0, 5, and 10 ppt treatments and on the order of 2 to 10 times higher in the 25 ppt

Table 3. Mean concentrations (± SD) of nutrients during the 123 d experiment. Parenthetical numbers are the number of measurements. FW: freshwater source; SW: saltwater source pumped from Narragansett Bay. The dissolved organic fraction was calculated as the difference between total N or P and the sum of dissolved inorganic and particulate. Concentrations are in µmol l^{-1}, except for chlorophyll *a*, which is µg l^{-1}

	Salinity treatment					
	FW	0 ppt	5 ppt	10 ppt	25 ppt	SW
Nitrogen						
Dissolved inorganic (16 to 18)	25.94 ± 4.11	15.59 ± 3.60	8.18 ± 6.06	6.92 ± 3.68	1.14 ± 1.52	5.73 ± 3.70
Particulate (18)	3.89 ± 2.12	8.74 ± 4.25	12.45 ± 6.16	8.08 ± 3.65	5.35 ± 2.98	3.52 ± 1.69
Dissolved organic (10)	15.5 ± 8.7	21.0 ± 4.2	13.4 ± 5.3	15.0 ± 4.3	14.0 ± 2.3	10.0 ± 6.6
Phosphorus						
Dissolved inorganic (17 to 18)	0.30 ± 0.12	0.10 ± 0.08	0.02 ± 0.05	0.01 ± 0.03	0.23 ± 0.14	1.38 ± 0.43
Particulate (18)	0.26 ± 0.18	0.46 ± 0.18	0.42 ± 0.14	0.31 ± 0.07	0.35 ± 0.13	0.25 ± 0.13
Dissolved organic (10)	0.16 ± 0.16	0.30 ± 0.32	0.11 ± 0.13	0.10 ± 0.16	0.16 ± 0.18	0.04 ± 0.10
Chlorophyll *a* (18)	2.14 ± 2.72	8.40 ± 5.17	9.31 ± 4.83	5.05 ± 2.75	3.69 ± 3.83	1.19 ± 2.14

Table 4 Mean molar ratios of carbon (C), nitrogen (N), and phosphorus (P) in the seston. Errors are 95% confidence intervals (n = 18). Also given are the molar ratios of N and P in external inputs to each treatment over the entire 123 d experiment and DIN:DIP (dissolved inorganic nitrogen and phosphorus) ratio of the water column pool calculated from average concentrations

	Salinity treatment			
	0 ppt	5 ppt	10 ppt	25 ppt
Seston				
N:P	20.4 ± 4.3	28.9 ± 4.7	25.9 ± 5.1	15.6 ± 3.6
C:N	11.0 ± 1.9	9.2 ± 0.9	8.9 ± 1.5	6.3 ± 1.4
C:P	221.6 ± 47.9	267.2 ± 46.9	225.9 ± 44.0	95.8 ± 29.7
External inputs				
Total N:P	54	59	55	15.1
DIN:DIP	78	162	88	6.3
Water column concentrations				
DIN:DIP	156	409	692	5.0

treatment (Table 3). DIP declined over the first half of the experiment in the 0 ppt treatment and, although never exhausted, remained below the lower limit of the half-saturation constant (0.1 µmol l^{-1}) for the last 73 d of the experiment (Fig. 2). In the 5 and 10 ppt treatments DIP often reached nondetectable levels and remained below 0.1 µmol l^{-1} for almost the entire experiment (17 of 18 measurements). DIP was always detectable in the 25 ppt treatment, and, after an initial decline, accumulated until a phytoplankton bloom occurred towards the end of the experiment. Concentrations fell below 0.1 µmol l^{-1} on only 4 of 18 occasions.

Inorganic nutrient concentrations, when compared to half-saturation uptake constants, indicate that the potential for P limitation was higher in the 0, 5, and 10 ppt treatments than at 25 ppt. The potential for N limitation was higher at 25 ppt than in the lower salinity treatments.

On average within the estuarine gradient, total nitrogen occurred predominantly in the dissolved organic and particulate forms (Table 3). However, the percentage of the total comprised by DIN varied, being 20 to 35% in the 0, 5, and 10 ppt treatments and only 6% at 25 ppt. Total phosphorus also occurred primarily as dissolved organic and particulate. In contrast to nitrogen, DIP comprised a small percentage of the total (2 to 12%) in the 0, 5 and 10 ppt treatments but a larger one (30%) at 25 ppt.

Average ratios of C, N, and P in suspended particulate matter (Table 4) were all greater than Redfield ratios (Redfield et al. 1963) in the 0, 5 and 10 ppt treatments. As judged by the width of the 95% confidence intervals, organic matter was formed nearly in Redfield proportions in the 25 ppt treatment. Average DIN:DIP concentration ratios were greater than ratios in the seston at low salinities (0, 5 and 10 ppt) but less than the seston ratio at 25 ppt (Table 4).

Oxygen metabolism

Integrated daily system production of oxygen in the 0 and 5 ppt treatments was about half that at 10 and 25 ppt (Table 5). Integrated nighttime system respiration varied by a factor of about 3, increasing steadily as salinity treatment increased. Although benthic oxygen consumption also increased with increasing salinity, it varied by a factor of only 1.8 across the gradient (Table 5). Calculated water column production showed the same pattern as system production. Water column respiration increased with increasing salinity treatment and varied by a factor of 3.5 from lowest to highest value (Table 5).

Table 5. Time integrated total system oxygen metabolism, benthic fluxes, calculated water column production and respiration of oxygen, calculated nutrient demand of production, and calculated recycling of inorganic nutrients in the water column at night. Units for oxygen are mol O_2 enclosure^{-1} 123 d^{-1}; for nutrients, mmol N or P enclosure^{-1} 123 d^{-1} See 'Results'

	Salinity treatment			
	0 ppt	5 ppt	10 ppt	25 ppt
System oxygen metabolism				
Production	6.22	5.23	10.08	12.90
Respiration	5.34	7.57	10.39	16.25
Benthic fluxes				
Oxygen consumption	3.06	3.98	4.07	5.62
DIN production	80.1	200.4	292.8	750.6
DIP production	7.6	0.24	2.3	30.9
Water column oxygen metabolism				
Production	7.75	7.22	12.12	15.71
Respiration	3.81	5.58	8.36	13.44
Water column nutrient cycling				
Demand of production				
Nitrogen	704	788	1363	2505
Phosphorus	35.0	27.0	53.7	164.0
Recycling at night				
Nitrogen	346	609	640	2143
Phosphorus	17	21	37	140

Net system production of oxygen (Production – Respiration for whole system) was slightly positive at 0 ppt, indicating autotrophy, and negative elsewhere, indicating heterotrophy. Net production for the water column was positive everywhere, indicating autotrophy. Thus, during this summer experiment, benthic oxygen demand drove net system metabolism toward heterotrophy.

While the calculated nitrogen demand of production increased along the 0 to 25 ppt gradient, the phosphorus demand did not. The DIP demand at 5 ppt was lower than at 0 or 10 ppt, not because of lower production but because of the comparatively high C:P ratio of particulate matter formed in this treatment (Table 4).

External nutrient inputs and outputs

External inputs for P (Table 6) and N (Table 7) include pumped transfers, rain, and net changes in the water column standing stock. The 'net' term (Total in – Total out of the water column) represents net retention (+) or export (–) of nutrients. While P is presumably retained in the sediment, for N this term quantifies the minimum amount of N available for retention in the sediments or denitrification. N fixation would increase the magnitude of this term.

Phosphorus

The total external input of P declined from the 0 ppt treatment to the to 10 ppt treatment and increased to its highest level at 25 ppt (Table 6). While the total input was mainly composed of DOP+PP at 0 ppt (61%), 5 ppt (87%) and 10 ppt (84%), DIP dominated at 25 ppt (71%). As a result of these differences in composition and magnitude, the external input of DIP was 3 to 12 times higher in the 25 ppt treatment than at lower salinities. Except for a small contribution from rain, almost all the input of total P and DIP to the 25 ppt treatment was via inflow water. At lower salinities, inflow water also dominated the external input of total P and DIP, but changes in standing stock were an additional, significant source of DIP.

Phosphorus was primarily exported as DOP+PP in all treatments, with outflow water being the major component. Increases in standing stock in the water column accounted for 10 to 20% of the total measured output along the gradient (Table 6). Export of DIP steadily decreased in both absolute magnitude and proportion of the total in the low salinity treatments (0 ppt, 9%; 5 ppt, 3%; 10 ppt, 2%). At 25 ppt DIP export was both an order of magnitude higher and formed a greater proportion of the total (27%) than at lower salinities.

Nitrogen

The external input of total N declined steadily across the 0 to 25 ppt gradient. Inflow water was by far the major source of total N, with changes in standing stock and rain being less significant (Table 7). While DIN comprised about half the total N at 0 ppt, this fraction fell to between a quarter and a third at higher salinities. The external input of DIN also fell steadily from 0 to 25 ppt, being 4 times higher at 0 ppt than at 25 ppt.

The export of total nitrogen declined across the 0 ppt to 25 ppt gradient (Table 7). In all treatments nitrogen was exported primarily in outflow water as DON+PN. The export of DIN in outflow water declined progressively from the 0 ppt to the 25 ppt treatment.

Table 6. External inputs and outputs of phosphorus for each treatment along the estuarine gradient. DIP: dissolved inorganic phosphorus; DOP: dissolved organic phosphorus; PP: particulate phosphorus. Positive phosphorus terms indicate input and negative terms output. Total input: sum of all positive terms; total output: sum of all negative terms; total net: sum of total input and output

Salinity treatment	Source	DIP	DOP	PP	Totals	
0 ppt	Inflow	16.6	13.9	12.8	Input	52.1
	Rain	0.07			Output	–56.9
	Δ Standing stock	3.2	–5.6	5.5	Net	–4.8
	Outflow	–5.2	–22.5	–23.6		
5 ppt	Inflow	2.8	14.6	20.4	Input	40.3
	Rain	0.07			Output	–39.0
	Δ Standing stock	2.4	–2.9	–4.2	Net	1.3
	Outflow	–1.0	–7.1	–23.8		
10 ppt	Inflow	4.1	8.0	22.7	Input	36.4
	Rain	0.07			Output	–32.6
	Δ Standing stock	1.5	–5.8	–1.1	Net	3.7
	Outflow	–0.6	–7.5	–17.6		
25 ppt	Inflow	60.9	4.1	14.5	Input	85.5
	Rain	0.07			Output	–52.2
	Δ Standing stock	–0.13	–5.5	5.9	Net	33.3
	Outflow	–14.2	–11.8	–20.6		

Table 7 External inputs and outputs of nitrogen for each treatment along the estuarine gradient. DIN: dissolved inorganic nitrogen; DON: dissolved organic nitrogen; PN: particulate nitrogen. Positive terms indicate input and negative terms output. Total input: sum of all positive terms; total output: sum of all negative terms; total net: sum of total input and output.

Salinity treatment	Source	DIN	DON	PN	Totals	
0 ppt	Inflow	1395.4	987.9	205.5	Input	2820.3
	Rain	34.7			Output	-2615.00
	Δ Standing stock	120.0	76.8	-163.8	Net	205.3
	Outflow	-840.2	-1122.7	-488.3		
5 ppt	Inflow	600.4	890.0	478.2	Input	2367.4
	Rain	34.7			Output	-2095.0
	Δ Standing stock	219.6	144.5	-239.5	Net	272.4
	Outflow	-429.7	-698.5	-727.3		
10 ppt	Inflow	337.4	710.6	618.4	Input	1974.6
	Rain	34.7			Output	-1768.2
	Δ Standing stock	126.9	146.6	-128.4	Net	206.4
	Outflow	-378.3	-785.4	-476.1		
25 ppt	Inflow	348.6	611.8	276.4	Input	1293.5
	Rain	34.7			Output	-1258.8
	Δ Standing stock	1.3	20.7	-70.4	Net	34.7
	Outflow	-70.2	-794.8	-323.4		

Endmembers

The fresh- and saltwater endmember inputs via inflow water from the freshwater reservoir and Narragansett Bay differed in both composition and relative magnitudes of N and P. The total N input was 3 times and that of DIN 5 times higher from freshwater than from saltwater. By contrast the total P load from saltwater was 1.7 times higher and the DIP load 3.7 times higher than from freshwater. Examination of Fig. 1 and Table 3 indicates that these differences were primarily due to differences in absolute and relative concentrations rather than water transport. While DIN accounted for 50 and 30% of the total nutrient load in fresh- and saltwater respectively, DIP accounted for 40% of the total in freshwater but over 80% in saltwater. Thus, there was less N and more phosphorus in seawater as compared to freshwater and the P in seawater had a distinctly higher proportion of DIP.

N:P ratios

At 0, 5, and 10 ppt, the ratios of N to P in both total and inorganic external loads exceeded the Redfield ratio and the N:P of the seston (Table 4), indicating phosphorus limitation. At 25 ppt, N to P loading ratios were near (total) or well below the N:P of the seston, suggesting nitrogen limitation. The N:P ratios of the freshwater endmember (DIN:DIP = 84; TN:TP = 60) were consistent with phosphorus limitation while those for the saltwater endmember (DIN:DIP = 4.2; TN:TP = 11.3) indicated nitrogen limitation.

Net retention

All treatments retained N, which was available for storage in the sediments or denitrification (Table 7). At low salinity (0, 5, 10 ppt), retention was 7 to 11% of the total input compared with only 3% at 25 ppt. The net (total in - total out; Table 6) term for P ranged from export to retention. At 0 ppt 9% of the external input of P was exported in outflow water indicating a net loss of P from this treatment. At 5 and 10 ppt, net retention was low (3 to 10% of the external input), while at 25 ppt 40% of the external input was retained. N:P ratios were: -43 at 0 ppt, 209 at 5 ppt, 41 at 10 ppt and 1 at 25 ppt.

Recycled inputs

Benthic input of DIN increased steadily up the gradient until at 25 ppt it exceeded input from inflow water (Table 5). The benthic input of DIP exhibited no consistent pattern across the gradient, except that DIP flux at 25 ppt was 3 to 100 times greater than at lower salinities (Table 5). DIN:DIP ratios were 10.5, 835, 127 and 24.3 at 0, 5, 10 and 25 ppt respectively.

As expected, recycling of nutrients in the water column followed the same pattern as oxygen respiration (Table 5). Estimated rates of water column recycling exceeded rates of benthic recycling in all cases. For the 10 and 25 ppt treatments, rates were high and comparable to the external input.

Nitrogen fixation

N fixation, as measured by acetylene reduction, was detectable nowhere along the experimental gradient (Table 8). Calculations for the 0, 5 and 10 ppt treatments were all negative indicating that N supplied by other sources more than accounted for the utilization of

total P or DIP (Table 8). Thus for these treatments, both measurement and calculation agree that N fixation did not occur.

The results for the 25 ppt treatment were not as demonstrative. The calculated range is based on the 95% surrounding the mean N:P ratio of seston (Table 4). In so far as this range overlaps zero, both measurement and calculation agree. Midpoints of the 2 ranges are 144 and 216 mmol N enclosure^{-1} 123 d^{-1}. Prorating to a 200 d growing season for comparison, these are 1.3 and 2.0 g N m^{-2} yr^{-1} and rather high when judged against estuarine rates summarized by Howarth et al. (1988a).

Table 8. Nitrogen fixation and denitrification. Nitrogen fixation measured by the acetylene reduction technique and calculated indirectly using the net utilization of total P and N, and dissolved inorganic N (DIN) and P (DIP). Range in calculated nitrogen fixation is based on the 95% CI around the mean N:P of the seston. Denitrification measured and calculated from observed benthic fluxes of dissolved inorganic carbon (DIC) and DIN. Units are mmol enclosure^{-1} 123 d^{-1}

	Salinity treatment			
	0 ppt	5 ppt	10 ppt	25 ppt
Nitrogen fixation				
Measured	0	0	0	0
Total N, P	−240 to −216	−435 to −421	−372 to −310	−15 to +447
DIN, DIP	−431 to −240	−516 to −474	−260 to −185	−135 to +424
Denitrification				
Measured	15	45	0	15
Calculated	225	314	126	447
Benthic DIC flux	3360	4720	3720	7510

Denitrification

Calculated rates of benthic denitrification were an order of magnitude higher than measured rates (Table 8). This discrepancy clearly illustrates the problems inherent in extrapolating from a single measurement to a 123 d budget period. Measurements of denitrification made in salinity gradients in subsequent years ranged from 3 to 37 µmol N_2 m^{-2} h^{-1} or 45 to 550 mmol N 123 d^{-1} (Nowicki 1994), with highest rates observed in the enclosures with highest salinities. Field studies of estuarine denitrification rates have shown considerable variation in rates from a single site (B. Nowicki, J. Kelly, E. Requintina & D. Van Kueren unpubl.). It is not uncommon to observe nondetectable rates (even at warmer temperature) at stations which have also exhibited relatively high rates of denitrification. Calculated rates ranged from 126 mmol N mesocosm^{-1} 123 d^{-1} at 10 ppt to a high of 447 mmol N mesocosm^{-1} 123 d^{-1} at 25 ppt. These rates imply a range of 8.4 µmol N_2 m^{-2} h^{-1} to 30.0 µmol N_2 m^{-2} h^{-1} which compares favorably with estuarine and lacustrine rates reported by Seitzinger (1988). In agreement with Seitzinger (1988) and Seitzinger et al. (1991), calculated rates comprised a high percentage of the total benthic N flux (DIN + denitrification) at 0 ppt (74%) and 5 ppt (61%) and a relatively lower fraction at 10 ppt (29%) and 25 ppt (37%).

Bioassays

N to P response ratios calculated from bioassay data indicated that phosphorus (N to P response ratio < 1.0) was potentially limiting in the 0, 5 and 10 ppt treatments. Nitrogen (N to P response ratio > 1.0) was potentially limiting in the 25 ppt treatment (Fig. 3). This pattern was invariant over time in all treatments.

DISCUSSION

The purpose of the experiment was to identify sources and supply rates of nutrients that support production along an estuarine gradient. A second goal was to identify processes that changed relative supply rates and hence the limiting nutrient. These objectives were predicated first on the logic of Officer & Ryther (1980) that the relative supply rates of nutrients determine which becomes limiting. Secondly, an estuarine environment appeared a likely place to observe changes in relative supply rates because fresh- and saltwater endmembers typically have different limiting nutrients, and estuaries represent the mixing/transition zone between them. Although several attributes of estuaries could have been varied (hydrodynamic residence time, degree of stratification) we chose salinity because the concentrations and reactivity of many substances vary with salinity (Burton & Liss 1976, Sholkovitz 1976, Froelich 1988). We also varied the sediments because these are an important site in nutrient cycles of both lakes (Schindler 1974, Levine et al. 1986, Levine & Shindler 1992) and estuaries (Nixon et al. 1976, 1980, Doering 1989).

Uncertainties in estimates of nutrient inputs to estuaries frustrates attempts to understand nutrient limitation in these systems (Howarth 1988). The issue of which nutrient input, total or dissolved inorganic, is appropriate adds further complication (Howarth & Marino 1990). One of the critical terms in calculating nutrient inputs was the transfer of nutrients between enclosures by the pumps. That the concentration of a

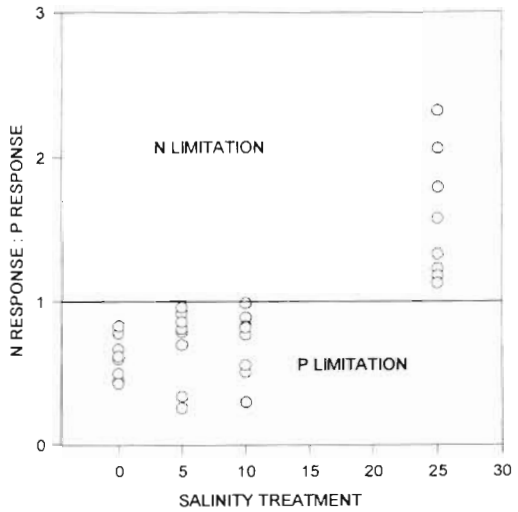


Fig. 3. Phytoplankton bioassay response to nutrient addition versus average salinity of each treatment. N and P responses were quantified by DCMU enhanced fluorescence and normalized to controls. A response ratio greater than 1.0 (horizontal line) indicates N limitation. A ratio less than 1.0 indicates P limitation

conservative property (salinity) could be estimated within 1 to 7% using measured pump flows demonstrates that transfers of nutrients by pumps were well constrained. Errors associated with nutrient input by rain are on the order of 5% (Nowicki & Oviatt 1990). The errors associated with the integrated estimates of benthic flux varied between treatments. For the 0, 5, 10 and 25 ppt treatments respectively, the standard error (Ramette 1981) as a percentage of the mean for DIN fluxes was 27, 11, 21 and 4% respectively. For DIP fluxes these were 13, 17, 128 and 47% respectively. We also calculated estimates of denitrification and nitrogen fixation which, according to Smith (1991), may have an error of 50%.

Nutrient supply and water column production

In this section, we compare the nutrient demand required to support water column production with external inputs of dissolved inorganic nutrients (inflow + rain + changes in standing stock) and recycled inputs from the benthos and water column. N fixation, an additional external input, is discussed separately below.

While both the recycled inputs of DIN from the benthos (measured) and the water column (calculated) increased across the 0 to 25 ppt gradient, the external supply decreased so that the total supply became progressively dominated by recycling as salinity increased (Fig. 4). At 0 ppt total supply of DIP was about evenly divided between external and recycled inputs. From 5 to 25 ppt both external and recycled inputs increased, with recycled inputs comprising the major portion of the total (Fig. 4).

In the 0 and 5 ppt treatments, external inputs supplied enough DIN to support water column production (Fig. 4). Although available, recycled nutrients from the benthos and water column were not necessary to fulfill the demand. By contrast, external inputs were not large enough to fulfill the demand of production at 10 and 25 ppt. Additional N was required, and recycling by the benthos and in the water column at night sufficiently accounted for the remaining deficit (Fig. 4).

In contrast to N, external inputs of DIP did not supply enough phosphorus to fuel production in any treatment (Table 4). Total supply rates (external + recycled inputs; Fig. 4) agreed reasonably with the phosphorus demand of production. Thus the demand of production for both N and P could be accounted for by external and recycled inputs in all treatments. The DIN:DIP ratios for the total supply rates of nutrients (external + recycled inputs) were for 0 ppt, 44; 5 ppt, 63; 10 ppt, 39; and 25 ppt, 14.

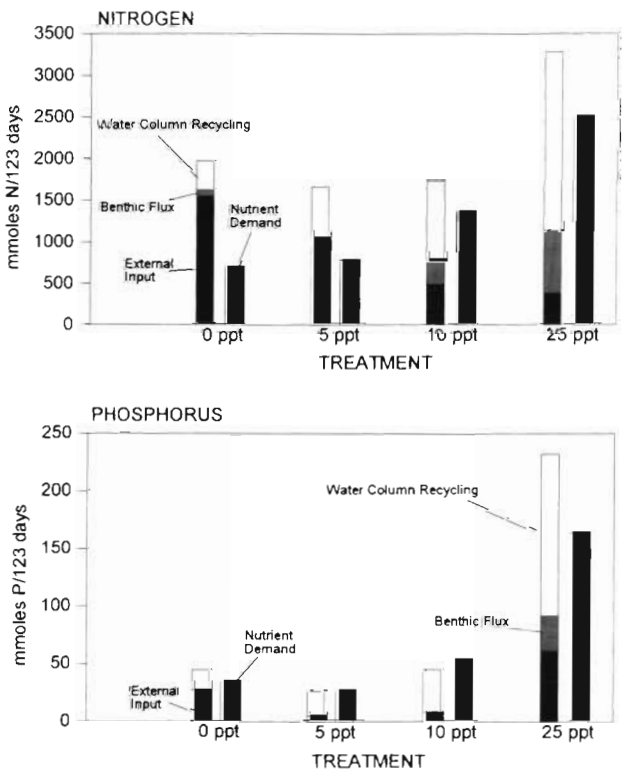


Fig. 4. Nutrient demand of production compared with external nutrient supplies (rain, inflow water, and water column concentration changes) and recycled nutrient supplies from the benthos and water column

Nutrient limitation along the gradient

The nutrient limitation bioassays indicated that phosphorus was potentially limiting in the 0, 5 and 10 ppt treatments, while nitrogen was potentially limiting at 25 ppt. This pattern was invariant over time. The bioassays themselves identify which nutrient (N or P) would become limiting were the water column cut off from all external supplies (Hecky & Kilham 1988). This information does not unequivocally establish which nutrient was in shortest supply. However, since the bioassay results were consistent over time, they do show that the balance between supply and demand maintained standing stocks in which P (0, 5 and 10 ppt) or N (25 ppt) would always be exhausted first.

However flawed (Hecky & Kilham 1988), other commonly employed indicators of nutrient limitation such as dissolved inorganic nutrient concentrations (Nixon & Pilson 1983), external DIN:DIP loading ratios (Magnien et al. 1992), and elemental composition of particulate matter (Goldman et al. 1979) are consistent with bioassay results. All suggest P limitation at 0, 5 and 10 ppt and N limitation at 25 ppt.

At the center of the concept of nutrient limitation is the axiom that the limiting nutrient will be in shorter supply than others. The relative amounts of N and P that are needed to support production, after accounting for external inputs, furnishes a measure of 'shortness of supply' Any additional nutrient requirement over and above that supplied by external sources (inflow + rain + standing stock) must come from recycling or, in the case of N, from N fixation as well.

The data presented here support this concept. In the 0 and 5 ppt treatments, the DIN demand of production was less than external input (Fig. 4) and DIN was present in excess (Fig. 2). In contrast, the P demand of production exceeded P input and DIP was present at trace levels during most of the experiment. Recycled P was necessary to support the observed level of production and DIP was in shorter supply than DIN in these enclosures. In both the 10 and 25 ppt treatments both N and P recycling were required to supply water column production. Comparing the ratio of additional N to additional P required from recycling with the N:P ratio of the seston identifies which of the 2 nutrients was in shorter supply. At 10 ppt, N and P were incorporated into seston at a ratio of 26 N for every P (Table 4). In addition to the nutrients already supplied by external input, 864 mmol of N and 48 mmol of P were needed to support water column production (Fig. 4). Only (864/48) 18 additional N were required for every P incorporated into seston forming at 26 N:1 P. Thus relatively less N than P was required, and P was in shortest supply. At 25 ppt, seston formed at a ratio of 16 N:1 P (Table 4). After accounting for external input, 2120 mmol of N and 103 mmol of P were needed to fulfill the demand of production (Fig. 4). An additional (2120/103) 21 N were required for each P incorporated into seston forming at 16 N:1 P. Relatively more N than P was required and N was in shortest supply.

Along the experimental gradient, we observed a transition from potential P limitation at 0, 5 and 10 ppt to potential N limitation at 25 ppt. Accompanying this transition was a change in the relative supply rates of nutrients such that P was in relatively short supply at 0, 5 and 10 ppt and N at 25 ppt.

Changes in rates of supply

Howarth (1988) identified 3 major factors which control whether N or P is more likely to be limiting in aquatic systems: (1) the preferential loss of N or P from the photic zone due to denitrification, preferential sedimentation of N in zooplankton fecal pellets, or adsorption of P; (2) the extent to which any relative deficit in N availability is made up through N fixation; and (3) the ratio of N to P in external inputs. For estuaries in particular, endmember inputs and their subsequent hydrodynamic mixing may also be important (Smith 1991). Below, we assess the importance of these 4 factors in our experiment.

Preferential loss

The 'net' term in the N and P budgets (Tables 6 & 7) represents nutrients available for export (negative sign) or storage in the sediments (positive sign) and, in the case of N, denitrification. At 0 ppt, P was exported while N was stored in the sediments or denitrified. For each mole of P exported, 43 moles of N were lost to sedimentation or denitrification. In other treatments, losses were all to the sediments or denitrification. N:P ratios of net losses at low salinities (0, 5 and 10 ppt) were all above Redfield and those of the seston, showing preferential loss of N relative to P. At 25 ppt the N:P ratio of net loss was 1.0 and well below either the Redfield ratio or that of the seston, suggesting preferential retention of P relative to N.

While preferential loss of nutrients appears to have occurred in the experimental gradient, its effect within any one treatment condition appears to have been minor. A preferential loss of P in a situation where P was already in short supply would have made a scarce nutrient scarcer. Where P was in short supply, N was preferentially lost and where N was scarce, P was preferentially lost. In absolute magnitude the net loss of N

was higher at 0, 5, and 10 ppt, where N was not limiting, than at 25 ppt where it was limiting. The loss of P was highest at 25 ppt, where P was not limiting.

Because measured and calculated rates of denitrification in the experimental gradient do not agree, the loss of N cannot be apportioned between denitrification and storage in the sediment. If the higher calculated rates are accepted, these are comparable in magnitude to the 'net' term in the N budgets for the 0, 5 and 10 ppt treatments (Table 7). This agreement indicates that, in these treatments, enough N was supplied to support both production and some net storage or denitrification. At 25 ppt the discrepancy between the calculated rate of denitrification and the budgetary 'net' term is large. A discrepancy would arise if the denitrified nitrogen came from nitrogen deposited in the sediments before the budgeting period began. Although plausible, we have no evidence in support of this explanation.

Nitrogen fixation

N fixation may have occurred only in the 25 ppt treatment. At 0, 5 and 10 ppt both measurement and calculation indicate a lack of N fixation. On the basis of the high total N:total P loading ratios (Table 4) and the high average concentration of DIN in these treatments, N fixation would not be expected (Howarth et al. 1988b). In the 25 ppt treatment, the acetylene reduction technique detected no N fixation. Although results of the indirect calculation (Smith 1984, 1991) were equivocal, potential rates were high relative to those reported for other systems (Howarth et al. 1988a).

Calculating the potential contribution of N fixation to the N demand of production in the 25 ppt treatment places this process in perspective. After accounting for other external inputs (inflow, rain, changes in standing stock), 2120 mmol of N were still required from other sources, including recycled inputs from the benthos and the water column and potential N fixation (Fig. 4). If we accept the 2 midpoint estimates of 144 and 216 mmol N enclosure^{-1} 123 d^{-1}, then N and P would be required in ratios of 18 to 19 instead of the 21 calculated assuming no N fixation. Even if N fixation occurred at a very high rate, the formation of organic matter with an N:P of 16:1 would still have required relatively more N than P. Despite N fixation, N would have remained in shorter supply than P.

N:P ratio in external inputs

N:P loading ratios, whether expressed as total N:total P or DIN:DIP, are surrogate measures of the relative availability of N and P (Howarth & Marino 1990). Inorganic nutrients, readily taken up by phytoplankton, may more closely approximate nutrient availability than total nutrients, some fraction of which must be remineralized before becoming available. Both ratios are imperfect predictors of nutrient availability, and hence limiting nutrient, because biogeochemical processes in an aquatic system may alter relative availabilities of N and P compared to loading inputs (Howarth & Marino 1990).

The nutrient (N or P) found to be in shortest supply and potentially limiting in each enclosure by bioassays, minimum nutrient concentrations, concentration ratios and elemental composition of seston, was already in shortest supply in external inputs (Table 4). DIN:DIP ratios for the calculated total supply rates (external + recycled inputs; Fig. 4) showed the same qualitative pattern as external inputs alone: greater than 16 at 0, 5 and 10 ppt and less than 16 at 25 ppt. External inputs determined which nutrient could become limiting in each treatment.

Hydrodynamic mixing

Biogeochemical processes within an estuary function against a background of hydrodynamic mixing (Officer 1980, Smith 1991). The variation in the relative external supplies of N and P may be a function of endmember composition and hydrodynamic mixing. This hypothesis can be examined using a simplified version of the Stella model already described for salinity. This conservative model includes only 2 processes: nutrient input from fresh- and saltwater endmembers and hydrodynamic mixing throughout the experimental gradient.

The pumped transfers between enclosures and overflows were set at the nominal values shown in Fig. 1 DIN and DIP inputs via freshwater inflow to the 0 ppt treatment and via seawater inflow to the 25 ppt treatment were set at the daily measured average computed from Tables 6 & 7. Nutrient inputs were not adjusted for rain or changes in standing stock in this simplified version because external inputs to the enclosures were, in general, dominated by inflow water. Initial nutrient concentrations in each treatment were set at 0.0 µmol l^{-1} and the model was run until steady state was achieved. As before, the program ran with a daily time step calculating daily nutrient concentrations and mass transfers between treatments.

The steady state inputs via inflow water of DIN and DIP to each treatment are shown in Fig. 5. The modelled relationship between DIN and DIP inputs along the gradient is the same as that observed for external inputs. DIN:DIP ratios are well above 16:1 at 0, 5 and 10 ppt and well below Redfield at 25 ppt. Thus, changes in the N:P of external inputs, which ultimately determined relative rates of nutrient supply, can be

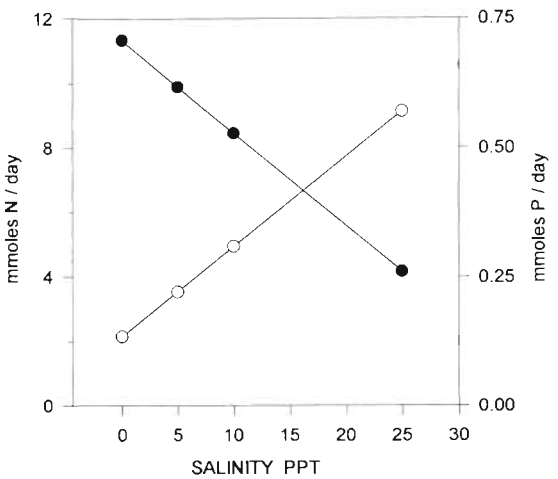


Fig. 5. Predicted nutrient input to each enclosure via inflow water given average composition of fresh- and saltwater endmembers and water transport as in Fig 1. Ratio of the 2 axes is 16 N:1 P. (O) Phosphorus; (●) nitrogen

explained largely by the hydrodynamic mixing of endmembers with contrasting magnitudes and proportionalities of DIN and DIP.

Transition from P to N limitation

The transition from P to N limitation along the experimental estuarine gradient was examined at the scale of each component enclosure or segment. At this scale, the ratio of DIN:DIP in the external supply established the relative amounts of DIN and DIP available to support productivity in the water column. The ratio of DIN:DIP in the external nutrient supply was largely a function of the hydrodynamic mixing of endmembers. Even with a relatively long hydrodynamic residence time in each enclosure (27 d), the effects of recycling, nitrogen fixation and relative net loss of N and P to sedimentation/denitrification were not large enough to change the nutrient already in short supply in external inputs. Rather, the net effect of internal biogeochemical processes was to allow the formation of seston with N:P ratios closer to Redfield than dictated by external inputs.

Our results emphasize the importance of external nutrient supplies in determining the limiting nutrient in an estuary. This experimental evidence supports the implications of recently described historical trends in the Chesapeake Bay. These suggest that an increase in the N:P ratio of external inputs has resulted in a greater potential for phosphorus limitation of phytoplankton productivity (Magnien et al. 1992). The roles of hydrodynamic mixing and endmember composition in establishing the N:P ratio of external supplies demonstrates that processes and events occurring in adjacent ecosystems (rivers, ocean) can directly influence nutrient limitation in estuaries (e.g. Vitousek & Howarth 1991). Further, our results provide experimental evidence that the spatial and perhaps seasonal 'switches' from P to N limitation often observed in estuaries (D'Elia et al. 1986, Caraco 1988, Webb 1988, Fisher et al. 1992) can be caused proximately by changes in external supplies. Ultimately, such changes can be a function of endmember composition and their pattern of mixing within the estuary.

Lastly, our results led us to address the question of appropriate spatial scales of processes as they influence nutrient limitation. Estuaries are unique hydrodynamic systems. Unlike lakes and rivers, which are flow-through systems whose discharge does not affect input, estuaries discharge into and mix with coastal waters. In the well-mixed case studied here, these coastal waters, in turn, serve as an input. The discharge of an estuary can influence one of its inputs: the oceanic endmember.

At the spatial scale of the single enclosure or estuarine segment, internal biogeochemical processes did not determine which nutrient would become limiting. Our summer experiment lasted 123 d or about ⅓ of the year. As expected from the work of Nixon (1986), the immediate nutrient requirements of phytoplankton production were fulfilled by external inputs and recycling. In agreement with Howarth (1988), N fixation, if it occurred, did not relieve N limitation. Preferential loss of both N and P occurred in the gradient. Yet, losses of N to sedimentation/denitrification, at least from budgetary considerations, were highest where N was not limiting. P losses were highest where P was not limiting. Along the gradient, there was a spatial displacement between the limiting nutrient and the preferential loss which might promote its shortness of supply. To influence relative supply rates of N and P such preferential losses must act over larger spatial scales. Summing the net losses of N and P across the entire experimental gradient (Tables 6 & 7) reveals that N was lost preferentially to P (N:P = 22:1). This preferential loss at the scale of the whole gradient would contribute to the transition between freshwater (high N, low P) and saltwater (low N, high P) endmembers that we observed. Although not as dramatic, our results are similar to those of Nixon et al. (1986), who showed that, in Narragansett Bay as a whole, N was lost preferentially to P at a rate of 140 N:1 P m^{-2} yr^{-1}. Alteration of nutrient supplies by estuarine biogeochemical processes can be important at the scale of the whole estuary, with their cumulative effect exerted upon the composition of the oceanic endmember. This endmember, as our results show, contributes to the spatial pattern of nutrient limitation in the estuary itself.

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