

Determination of Denitrification in the Chesapeake Bay from Measurements of N_2 Accumulation in Bottom Water

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ABSTRACT: This study demonstrates the feasibility of using direct N_2 measurements in an estuary for determination of denitrification. High precision measurements of dinitrogen : argon ratios (N_2 : Ar) were made by membrane inlet mass spectrometry on water samples taken along the length of the Chesapeake Bay in July and October 2004. The N_2 : Ar ratio in low salinity surface water was elevated relative to air saturation by 0.3–0.5% with no systematic change along the length of the Bay. N_2 : Ar in high salinity bottom water exhibited a linear increase in the landward direction along a 144-km longitudinal section. In this section of the Bay covering 20% of the main stem, the bottom water salinity was statistically uniform and the increase in N_2 : Ar was in the direction of net residual current flow. The system was analyzed as a capped river with the assumption that N_2 entered the water from the underlying sediment where denitrification is known to take place. The rate of denitrification needed to support the measured increase in N_2 was calculated using an average residual current velocity and water column depth. The increase in N_2 with distance ($0.046 \mu\text{mol N l}^{-1} \text{ km}^{-1}$) equated to an average denitrification flux of $73 \mu\text{mol N m}^{-2} \text{ h}^{-1}$. N_2 fluxes determined on sediment cores taken from the source and terminus regions of the delineated water mass were 45 ± 23 and $83 \pm 39 \mu\text{mol N m}^{-2} \text{ hr}^{-1}$, respectively, which were not statistically different from the whole system estimate. The measured change in oxygen concentration within the bottom water was used to estimate nitrogen remineralization and the efficiency of denitrification. Denitrification efficiency (nitrogen denitrified/nitrogen remineralized) was estimated to be in the range of 22–28% for the bottom water sediment system and 30–37% considering the sediment zone alone.

Introduction

Understanding denitrification in estuaries has increased significantly in the last 10 years as a result of new mass spectrometric techniques for measuring dinitrogen (N_2) concentrations in environmental samples. Two methods, in particular, spurred interest in studying denitrification in estuaries: the isotope pairing technique (Nielsen 1992), which traces $^{15}\text{N-NO}_3^-$ to N_2 and the net N_2 flux method aided by high precision membrane inlet mass spectrometry (MIMS; Kana et al. 1994). These methods were well suited to the requirement for high precision measurements of N_2 in aquatic sediment systems. Although the two methods have different measurement capabilities (Cornwell et al. 1999), they share one important characteristic—they are incubation methods requiring containerized samples. Incubation techniques have some advantages. Most importantly they allow for the manipulation and control of experimental variables, which allow the investigation of single factors such as oxygen (Rysgaard et al. 1994), nitrate (NO_3^- ; Kana et al. 1998), and macrofauna (Pelegrí et al. 1994). The methods have also been used to measure system-level denitrification fluxes under simulated

natural conditions (e.g., Nielsen et al. 1995; Trimmer et al. 2000). For this later application, incubation techniques have some disadvantages, including limited spatial and temporal scales of measurement and difficulty measuring complex substrates within an ecosystem (e.g., vegetated sediments, rocky substrates, intertidal habitats; Cornwell et al. 1999). Measurements of ecosystem-scale processes using methods that inherently integrate those processes over length and time scales more commensurate with the dominant scale of an ecosystem are considered desirable and complementary to incubation techniques. Appropriate time and space scales for studying denitrification in estuaries would be on the order of water residency times and the physical dimension of the estuary.

There are complementary denitrification methods that depend on measurements that reflect the larger scale of whole ecosystems. These methods include mass balance determinations (Nixon et al. 1996; David and Gentry 2000; Pribyl et al. 2005), stoichiometric relationships to other measurable material fluxes (Richards 1965; Nixon 1981; Giblin et al. 1997; Codispoti et al. 2001), natural abundance of ^{15}N (Voss et al. 2001; Altabet et al. 2002), and N_2 excess determinations (Laursen and Seitzinger 2002; McCutchen et al. 2003). The advantages of these open system methods with respect to

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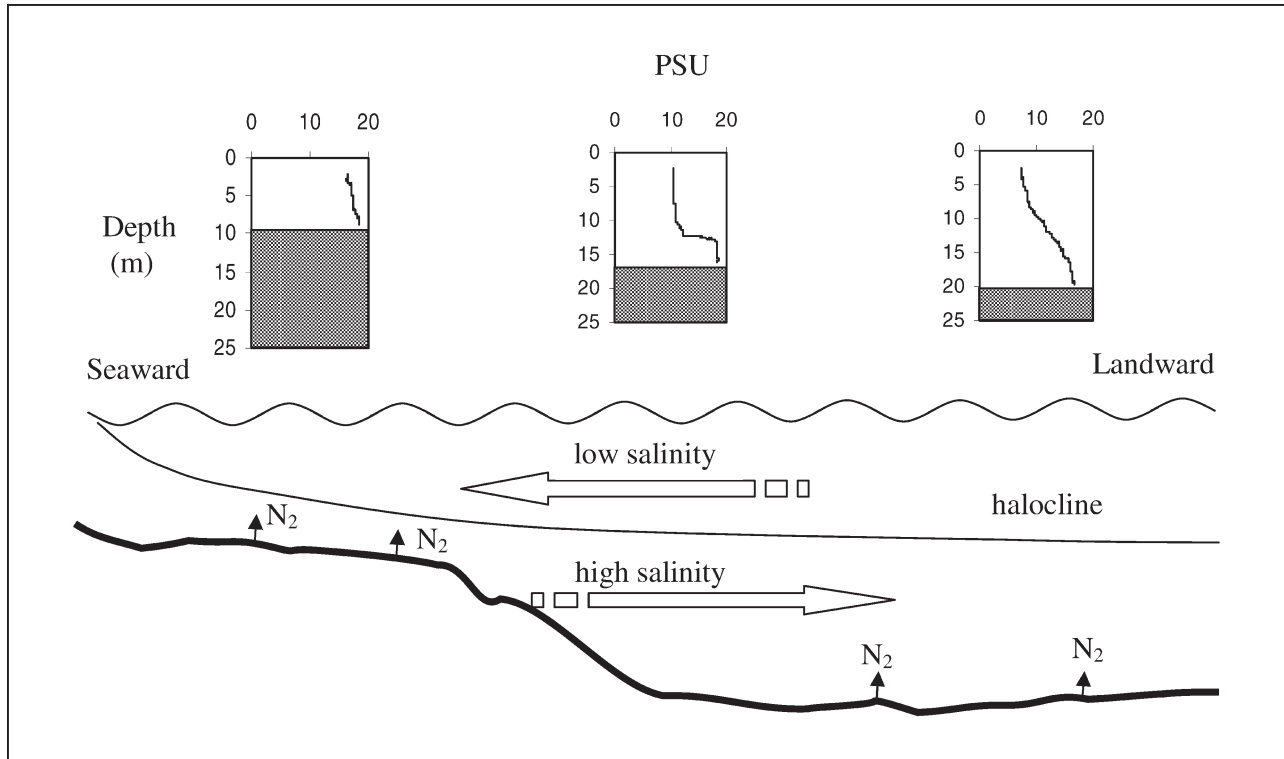


Fig. 1. Diagrammatic longitudinal cross section of the Chesapeake Bay two-layer flow with source of N₂ depicted. Plots are salinity profiles from three stations in October 2005. Left: km = 0, middle: km = 73, right: km = 144.

process scale are often counteracted by disadvantages, including for some studies the lack of a direct measurement of denitrification, the lack of a measurable time basis to determine rates, or the use of assumptions that are poorly constrained. It was in this context that we sought a method of measuring denitrification in the Chesapeake Bay from open system measurements of N₂. The results presented here demonstrate the feasibility of using measurements of in situ N₂ concentrations for determining denitrification in estuarine systems that exhibit two-layer flow characteristics.

The Chesapeake Bay is a large (6,500 km²) microtidal estuary with normally well-developed stratification and two-layer flow (Pritchard 1956; Schubel and Pritchard 1987; Boicourt 1992). In the lower (mouth end) and mid bay regions, high salinity water exhibits net flow in the landward direction underneath a seaward flowing surface layer. We surmised that denitrification activity in the sediments would enrich the bottom water with N₂, and that there would be a positive concentration gradient in the landward direction (Fig. 1). This is analogous to distance gradients of N₂ in rivers (Laursen and Seitzinger 2002) except that we anticipated greater measurement sensitivity in our system due to the capping effect of the low salinity

water. This report describes results from two cruises that demonstrate enrichment of N₂ in the Chesapeake Bay.

Materials and Methods

Sampling of the Chesapeake Bay was conducted in July and October 2004 aboard the R/V *Cape Henlopen*. Station locations were along the length of the Chesapeake Bay. Results from the July cruise indicated that additional stations were needed to identify the bottom water salt wedge and to increase the degrees of freedom for a regression analysis. The October cruise included additional stations spaced at c. 18-km intervals. Hydrographic data were determined using a Sea Bird conductivity temperature depth recorder (CTD).

DISSOLVED GAS MEASUREMENTS

Water for N₂ : argon (Ar) ratio measurements was collected using a CTD rosette sampler with 10-l Niskin bottles. Water for N₂ : Ar analysis was always sampled first from the Niskin bottles immediately upon retrieval deck side. Water from a Niskin bottle was gravity pressure fed from the spigot through a short tube with the tube's outlet placed at the bottom of a 20 ml test tube. The test tube was flooded for 5+ s while manipulating the dip tube to

assure no bubbles were trapped in the test tube during flooding. Estimated 5+ volumes of water flooded the test tube before it was sealed with a glass standard taper stopper. Eight test tube samples were collected from each depth (Niskin bottle) although typically only 4 tubes were analyzed. Sample tubes were kept in dim laboratory light while awaiting analysis. All N_2 : Ar measurements were completed within 2 h of collection using a shipboard MIMS (Kana et al. 1994; Kana and Weiss 2004).

The MIMS instrument included a Pfeiffer Vacuum model 422 quadrupole mass spectrometer (QMA 400 analyzer with cross-beam ion source) with a flow-through silicone capillary membrane inlet (Bay Instruments, Easton, Maryland). The inlet is a modification of Kana et al. (1994), consisting of a glass vacuum line and support for the membrane (Silastic tubing, 0.5 mm i.d.), capillary supply lines, and U-tube. Water was pumped through the stainless steel capillary tube using a Rainin RP-1 peristaltic pump at maximum speed (48 rpm). The water passed through a 75-cm length of tubing immersed in a thermostated water bath to assure constant sample water temperature when it flowed through the membrane tube. The U-tube in the vacuum line located between the membrane and mass spectrometer was immersed in liquid nitrogen in order to remove water vapor and CO_2 from the pervaporated gas mixture prior to it entering the mass spectrometer. Water used to calibrate the instrument signals was obtained from c. 600 ml of deionized water held within a 1-l spherical flask that was fully immersed (except ports) in the same thermostated bath that contained the membrane apparatus. The flask openings were fitted with wetted sponges to insure saturated water vapor pressure. Temperature of the bath was held constant ($\pm 0.01^\circ C$) at a temperature within a few degrees Celsius of the in situ sample water temperature. The standardization water was stirred without bubble entrainment using a paddle attached to an overhead stirrer. The time constant ($1/e$) for reaeration of water with this setup is 10–20 min depending on water volume and stir rate. Standard water was equilibrated overnight (>30 time constants) prior to use, which assured that it was at solubility equilibrium. Independent tests have shown that this standardization method with barometric pressure correction can yield reproducible O_2 concentration measurements to within 0.2% for analyses separated by several weeks time (Kana unpublished).

Sample water was pumped from the bottom of the sample test tube and through the membrane until the mass spectrometer signals stabilized, usually within 2 min. Avoidance of air contamination after opening the test tube was minimized

because of the long path length of the overlying sample water within the tube and short analysis time. N_2 : Ar ratios were determined in multiple ion detection mode. Masses 28, 32, and 40 were monitored in a continuous cycle with the cold cathode vacuum sensor deactivated to prevent it from causing instabilities in the signals. Ion detection was by Faraday collector. Evaluations of oxygen effects on N_2 determinations have been made for this instrument (Kana and Weiss 2004) and tested separately on these cruises by comparing N_2 : Ar ratios of selected baywater samples before and after the addition of powdered sodium dithionite, which removes O_2 . The stabilized signals were recorded in a spreadsheet along with the analysis time of day (hh:mm:ss). Instrument drift was measured by frequent analyses of standard water along with their respective measurement times. Pseudoreplicated standards were measured in triplicate by cycling the peristaltic pump off and on and recording the stabilized signals after each pump perturbation. This provided a statistical sampling to assess precision and drift during operation of the instrument. A typical analysis run would involve triplicate recordings of standard water followed by one or more quadruplicate sample series measurements, with the number of sample series depending on the degree of instrument drift. Drift records on the hour-scale indicate that linear interpolation of calibration factors according to measurement time was justified when standards were recorded on the order of quarter-hour to hourly intervals. Calibration factors for each standard were calculated for N_2 : Ar ratios based on solubility equations (Weiss 1970; Colt 1984).

Oxygen concentrations in the water masses were measured using a polarographic oxygen sensor attached to the CTD-rosette sampler. Instrument calibration was provided by ship operations of the University of Delaware.

CORE INCUBATION EXPERIMENTS

Sediment was collected by a box corer and subsampled using 7 cm diameter \times 30 cm tall acrylic cylinders. Approximately 15 cm of sediment was contained in each cylinder. Sediment cores were preincubated for 2+ h in a fully submersed condition within a donut-shaped bath with top caps off and with the bath water being gently bubbled. Bath water consisted of previously collected bottom water. Top caps were inserted at time 0. Stirring of the isolated headspace above each core was carried out by a suspended magnet that was rotated by a magnetic turntable in the center of the bath housing. Subsamples were collected at 1.5–2 h intervals for solute and dissolved gas measurements. Four time-points were collected during each in-

cubation. Make-up water was automatically dispensed into the headspace. Samples for gas analysis were collected in 7-ml ground glass stoppered tubes, forced by gravity flow from the ambient water. Mercuric chloride (0.1% v : v saturated solution) was added as a preservative and the sample tubes were stored underwater at ambient or subambient temperatures until measurement, usually within 24 h of collection. Nutrient samples were collected in 20-ml syringes, filtered (0.2 μm), and frozen for subsequent analysis. Concentrations of N₂ and O₂ were determined by MIMS as described above.

SOLUTE MEASUREMENTS

Salinity was determined from conductivity measurements from the CTD profiler. NO₃⁻ was analyzed colorimetrically (Parsons et al. 1984) on 0.45- μm filtered samples that had been stored frozen.

WATER MASS SPATIAL AND DEPTH CALCULATIONS

A Regional Ocean Modeling System (ROMS) was used to calculate the spatial coverage and average depth of the bottom water layer. The Chesapeake ROMS model (Li et al. 2005) was designed with an orthogonal curvilinear coordinate system to follow the general orientation of the deep channel and coastlines of the main stem of the Bay. The model had a grid size of c. 1 km in the horizontal direction and 20 levels in the vertical direction. The depth of the pycnocline at each station was input to the model and extended between stations by linear interpolation. This line was then extended laterally across the bathymetry to determine the horizontal coverage of the water mass. A spatially averaged depth was calculated for the area covered by the bottom water mass.

Results and Discussion

A vertical profile of the N₂ : Ar ratio for one of the deeper stations is shown in Fig. 2. In both the surface and deep water layers, the N₂ : Ar ratio was higher than the ratio expected under air-equilibrium conditions. It is notable that the observed ratios exhibited a constant offset within the surface layer and within the deep layer indicating that the gas profiles were well mixed within each uniform density layer. This observation simplifies the calculation of N₂ inventories in the bottom layer, allowing use of salinity as an indicator of the distribution of N₂ within a given density structure. The N₂ : Ar ratio in the surface layer was elevated at all stations during both July and October sampling periods, but there was no pattern in the data at either time of year (the slopes of the linear regressions with respect to distance were not significant). In July, surface ratios averaged 0.31%

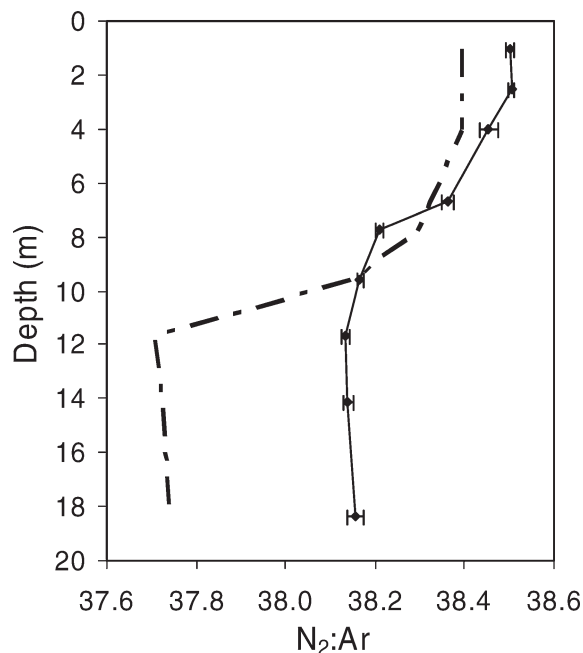


Fig. 2. Vertical distribution of the N₂ : Ar ratio (filled diamonds) at km 144 in July 2005. Error bars correspond to 1 SD. Broken line corresponds to the ratio in air-equilibrated water at the temperature and salinity recorded at each sample depth.

$\pm 0.09\%$ ($n = 7$) above the solubility ratio; in October the surface ratios averaged $0.50\% \pm 0.14\%$ ($n = 12$) above the solubility ratio. Normalized N₂ : Ar ratios in surface ocean water are typically near 1% excess due to the injection of more N₂ relative to Ar from gas exchange with bubbles mediated by wave action (Emerson et al. 1991). Our data are consistent with bubble injection albeit to a lower extent than in oceanic provinces. This would be expected for a confined water body.

The station locations during the October 2004 cruise are shown in Fig. 3 and the salinity values in the surface and deep water samples are shown in Fig. 4. Inspection of the salinity patterns indicates that there was complete mixing of the water column in the region closest to the mouth of the Chesapeake Bay (lowest kilometer stations), evidenced by the similar salinities at the surface and bottom. This region extended to $37^{\circ}16'N$, north of which was a departure in the salinities between surface and bottom water. We designated the station where the surface and deep salinities initially diverged as kilometer (km) 0 for this presentation. Between km 0 and km 144, bottom water salinity exhibited little dilution effect from mixing with surface water. The negative slope of salinity with respect to distance along the bottom water was $0.0097 \text{ psu km}^{-1}$, but it was statistically insignificant ($p = 0.26$).

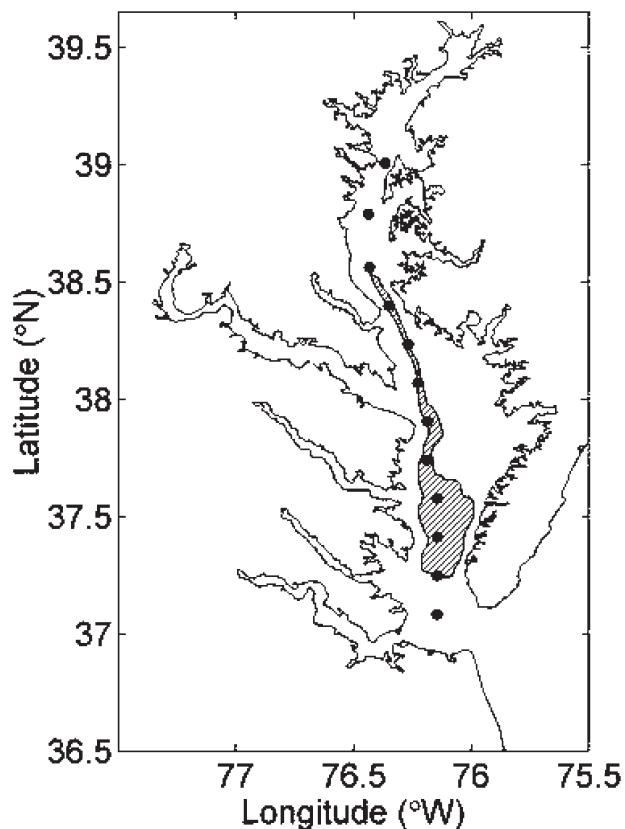


Fig. 3. Map of the Chesapeake Bay with station locations (filled circles). Horizontal distribution of bottom water in October 2005 is represented by the hashed area. Km 0 corresponds to the southern most station at the edge of the hashed area.

We concluded that the bottom layer was effectively isolated from the surface layer over this 144-km reach and we analyzed the changes in this water mass as a closed system with regard to any potential vertical exchange processes between the two density layers. The horizontal extent of the bottom water layer (Fig. 3) was determined to be c. 1,200 km² or 20% of the Bay area, not including tributaries, with an average depth below the pycnocline of 4.4 m. Our subsequent discussion is restricted to the km 0-144 region of uniform salinity bottom water. As points of reference, km 0 corresponds to the South Bay (SB) station and km 144 corresponds to the Mid Bay (MB) station referenced in other Chesapeake Bay studies.

Our conceptual model for this study is analogous to a river undergoing changes in chemical concentrations as it flows downstream. Time is referenced to distance through flow rate, and changes in chemistry (e.g., gas composition) reflect in situ processes and exchanges with the underlying sediment. We could ignore material exchange with

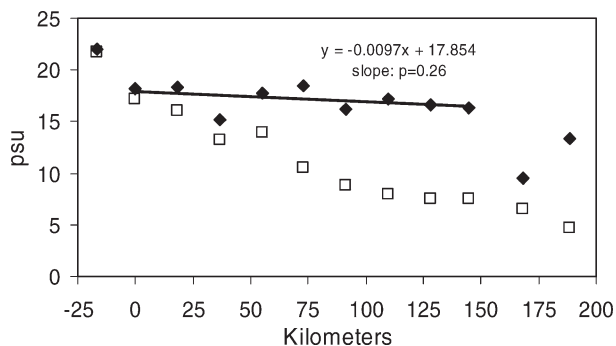


Fig. 4. Salinity in bottom water (filled diamonds) and surface water (open squares) as a function of distance along the transect. Line equation is for bottom water data points in the range of 0-144 km.

the surface layer given the lack of a horizontal salt gradient in the delineated bottom layer. Our system was modeled as a capped and flowing water mass with the dissolved gases in the water layer only being affected by processes within the high salinity water mass and exchange with the sediment layer. Measured N₂:Ar ratios from each station in October were normalized to the N₂:Ar ratio observed at km 0, where the water subducted a surface layer and initially became isolated from the atmosphere. (A separate analysis of N₂:Ar ratios normalized to the local solubility ratio resulted in an identical system gradient [slope] at the level of the two most significant digits.) The spatial gradient is exhibited in Fig. 5. Under the assumption that the Ar concentration did not change, we calculated the correlated change in nitrogen (as N₂) along this reach. The data fit a linear model with a slope of 0.046 mmol N m⁻³ km⁻¹ ($p < 0.001$; $r^2 = 0.82$). In July, fewer stations were sampled and data were obtained from only four locations that had similar salinity bottom water. The up-bay and down-bay limits of the high salinity bottom water coincided with the km 0 and 144 end member stations in the October data set. A positive slope was observed in July with a linear least squares estimate of 0.068 mmol N m⁻³ km⁻¹ ($r^2 = 0.81$), but the slope was not statistically significant ($p = 0.1$) and the confidence limits were large. We did not use those data for estimates of denitrification. The positive slope at a 10% probability does lend some support for the general notion that progressive N₂ enrichment in bottom water may be a normal characteristic of the Chesapeake Bay.

A linear spatial gradient in N₂ developed as the subsurface flow moved toward the head of the Bay. The rate of change in N₂ along this gradient is related to the net residual flow rate of the bottom water and N₂ production and influxes. Net residual flows have been measured in bottom water in the

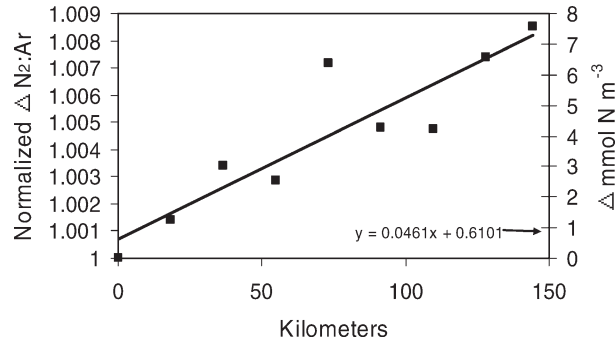


Fig. 5. N₂ : Ar ratios of bottom water normalized to the N₂ : Ar ratio observed at km 0 (left axis). Corresponding change in N as N₂ assuming no change in Ar concentration (right axis).

Chesapeake Bay in the region of interest (Boicourt 1992) and a long-term average flow rate of 10 cm s⁻¹ (= 0.36 km h⁻¹) was used in this study to convert the spatial gradient to a volumetric flux. We used this flow velocity to calculate the time rate of change of nitrogen as N₂ (0.046 mmol N m⁻³ km⁻¹ × 0.36 km h⁻¹) yielding a volumetric flux of 0.017 mmol N m⁻³ h⁻¹. The transit time over the 144-km reach was c. 17 d, indicating that this method integrated processes over a 2–3 wk time scale. The areal flux required to support the volumetric flux in a water column height of 4.4 m (average depth of the bottom water layer) was 73 μmol N m⁻² h⁻¹.

Areal flux rates were also determined using a sediment core incubation method with sediments collected from the end member stations along the 144-km transect (Table 1). The average denitrification rate at km 144 (83 ± 39 μmol N m⁻² h⁻¹) was nearly twice the rate of that at km 0 (45 ± 23 μmol N m⁻² h⁻¹), although the variance among triplicate subcores made the difference statistically insignificant (one-tail *t*-test: *p* = 0.11). If we assume areal flux over the 144-km reach can be estimated by the average of the fluxes measured at the end positions, we find that the estimate from the sediment core measurements (64 μmol N m⁻² h⁻¹) is close to the estimate from the open system measurements (73 μmol N m⁻² h⁻¹).

These independent estimates of denitrification are the first to be reported for the Chesapeake Bay main stem (excluding tributaries) that use direct N₂ methods. Published quantitative estimates of denitrification in the Chesapeake Bay main stem are limited. Kemp et al. (1990) measured denitrification three times a year at laterally separated stations in the vicinity of our km 144 station using both an acetylene block assay and an inferred relationship to NO₃⁻ flux. Rates determined using the acetylene block method, which is recognized to underesti-

TABLE 1. Rates of denitrification and oxygen removal in bottom water along the Chesapeake Bay transect in October 2005. Core fluxes (average ± SD) were made by measuring N₂ : Ar changes in replicated (*n* = 3) incubation chambers containing sediment cores (station designated as km location). Oxygen uptake for chamber measurements is for sediment oxygen demand (sediment chamber flux – blank water flux).

Measurement	Denitrification (μmol N m ⁻² h ⁻¹)	Oxygen Uptake (mmol O ₂ m ⁻² h ⁻¹)
Open (N ₂ : Ar)	73	-1.740
Core (km 0)	45 (23)	-0.937 (0.083)
Core (km 144)	83 (39)	-1.337 (0.004)

mate denitrification when nitrification is important (Seitzinger et al. 1993), were generally lower than rates determined by NO₃⁻ balance, and the authors concluded that the NO₃⁻ balance rates provided the best estimate of denitrification in their study. Seasonal estimates of denitrification (by NO₃⁻ balance) in the mid bay region ranged from 0 to 83 μmol N m⁻² h⁻¹ depending on season, with denitrification being lowest in summer when dissolved oxygen levels were lowest and highest in spring when NO₃⁻ flux was highest. The flux estimated for November, which included a 3-mo integrated NO₃⁻ balance factor, was 50 μmol N m⁻² h⁻¹. It is interesting, though inconclusive, that the N₂ : Ar gradient measured in July may have been higher than in October, despite anoxia being present in the mid bay region. This suggests that the southern Bay region may exhibit substantial rates in summertime.

There are no reported direct measurements of denitrification for the southern region of the Chesapeake Bay main stem. This section of the Chesapeake Bay has significant populations of burrowing worms, unlike the mid and upper bay regions, and their effect on nitrification has been noted for this part of the Bay (Mayer et al. 1995; Cowan and Boynton 1996). Denitrification is influenced significantly by the presence of macrofauna (Pelegri et al. 1994) and it would be expected that spatial heterogeneity of burrowing organisms would complicate a sampling strategy for measuring denitrification from sediment samples. This points to one advantage of the open system method of measurement as it spatially integrates meter to kilometer scale heterogeneity in denitrification flux.

Relating the denitrification flux to the remineralization flux allowed us to estimate the denitrification efficiency for the system. Denitrification efficiency is defined here as the fraction of remineralized nitrogen that is converted to nitrogen as N₂. Remineralization of nitrogen was estimated from the change in oxygen concentration along the spatial gradient. In October, bottom water oxygen exceeded 35% of air saturation at all stations,

indicating a predominance of aerobic remineralization processes. The pycnocline was below the euphotic zone, preventing photosynthetic oxygen input into the bottom water. (Secchi depths were 10–20% of the depth at the pycnocline in this study, consistent with previous evaluations [Smith and Kemp 1995].) O_2 concentration along the spatial gradient exhibited a linear decrease from km 0 to 144 at a gradient of $-1.1 \text{ mmol } O_2 \text{ m}^{-3} \text{ km}^{-1}$ (Fig. 6). This corresponded to a volumetric flux of $-0.40 \text{ mmol } O_2 \text{ m}^{-3} \text{ h}^{-1}$ using the assumed residual current velocity.

The volumetric flux in the bottom water was assumed to depend on only two processes: respiration in the water column and sediment oxygen demand. This relationship can be expressed as $R_{\text{system}} = \text{SOD}/z + R_w$, where R_{system} is the measured rate of oxygen concentration change in the water, SOD is the sediment oxygen demand expressed as an areal flux, z is the height of the water layer, and R_w is the rate of respiration within the water layer. R_{system} was determined from the gradient measurements to be $-0.40 \text{ mmol } O_2 \text{ m}^{-3} \text{ h}^{-1}$. SOD was measured in this study at km 0 and 144 using sediment core oxygen flux measurements. Although this method included the measurement of blank water oxygen flux, which should be a direct measurement of R_w , use of that data for R_w proved inappropriate for the following reason. Volumetric oxygen fluxes in the blank water were 18 and 4.5 times higher at km 0 and 144, respectively, than the estimated flux from the gradient analysis. We attribute those elevated fluxes to the experimental protocol used in the core flux experiments. The protocol included a pre-equilibration period during which the overlying water, including blank water, was gently bubbled in a common reservoir. It is likely that there was a greater amount of suspended particulate matter and dissolved organic matter in this water caused by those manipulations prior to capping the chambers. This would promote higher rates of aerobic respiration in the chambers compared to the activity in ambient water, so the water column respiratory fluxes were considered biased in our study and not suitable for direct comparison to the open water measurements.

SOD measurements were not influenced by this affect because the water flux was subtracted from the total flux (water + SOD) measured in the sediment cores, as by standard practice. SOD averaged $-0.94 \text{ mmol } O_2 \text{ m}^{-2} \text{ h}^{-1}$ at km 0 and $-1.3 \text{ mmol } O_2 \text{ m}^{-2} \text{ h}^{-1}$ at km 144 (Table 1). The contribution of these fluxes to the overlying bottom water (SOD/ z) was $-0.21 \text{ mmol } O_2 \text{ m}^{-3} \text{ h}^{-1}$ at km 0 and $-0.30 \text{ mmol } O_2 \text{ m}^{-3} \text{ h}^{-1}$ at km 144. The average of those fluxes was 64% of the volumetric oxygen uptake flux measured in the open system,

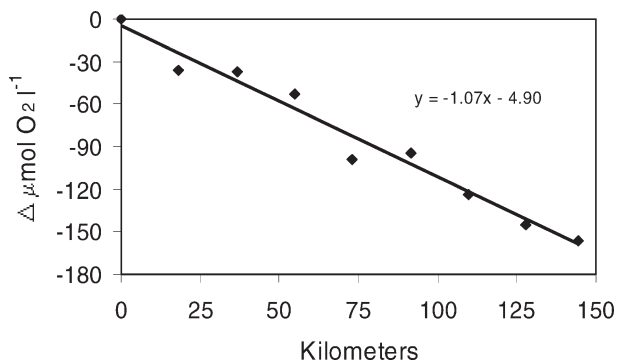


Fig. 6. Change in O_2 concentration with distance in bottom water samples.

which included SOD and bottom water column respiration. By difference, we estimated that approximately two-thirds of the oxygen demand was associated with the sediments and one-third ($0.15 \text{ mmol } O_2 \text{ m}^{-3} \text{ h}^{-1}$) was associated with water column respiration.

Our indirect estimate of R_w can be compared to direct measurements of bottom water respiration in an annual cycle study of the Chesapeake Bay by Smith and Kemp (1995). We applied their temperature relationship derived from near-monthly measurements of R_w at km 0 and 144 (their LB and MB stations, respectively) to our study parameters and obtained estimated R_w of $-0.6 \text{ mmol } O_2 \text{ m}^{-3} \text{ h}^{-1}$ at km 0 and $-0.3 \text{ mmol } O_2 \text{ m}^{-3} \text{ h}^{-1}$ at km 144. Cited precision of the temperature regression model was $\pm 50\%$ for 96% of the values (Smith and Kemp 1995). At both sites, R_w determined from the gradient and SOD flux calculation was approximately 30% of the rate determined by the Smith and Kemp regression equations. This is outside the 95% confidence limits of the regression model, though we caution that there is unresolved error in our estimates and there may be interannual variability, which is notable in the Chesapeake Bay system (Malone 1992), that would not be included in the Smith and Kemp (1995) regression source data.

From the preceding analysis, we can directly address the question of the significance of the denitrification pathway in remineralization (respiratory) processes within the system. For this calculation, we used the areal oxygen consumption rate as a measure of remineralization. Implicit in this flux is remineralization associated with reoxidation of anaerobic respiratory products (e.g., reduced iron, manganese, sulfur) along with remineralization by aerobic organisms. Remineralization is also associated with denitrification (NO_3^- respiration), so total remineralization was approximated by

the sum of oxygen consumption and denitrification. Oxygen uptake was converted to nitrogen remineralization assuming a molar respiration quotient (O₂ : CO₂) of 1 and a carbon : nitrogen (C : N) molar ratio of remineralized biomass of 6.6. For the whole system (sediment plus deep water), remineralization of nitrogen, estimated from O₂ flux, occurred at a rate of 264 μmol N m⁻² h⁻¹ (i.e., [1740 μmol O₂ m⁻² h⁻¹/1 O₂ : C]/6.6 C : N) compared to a denitrification flux of 73 μmol N m⁻² h⁻¹. The percentage of denitrification to the total nitrogen remineralization flux (O₂ plus NO₃⁻ respiration) was 22%. Since denitrification is presumed to have occurred in the sediments only, and approximately two-thirds of the remineralization (oxygen consumption) in the bottom layer system was associated with the sediments, the denitrification efficiency of sediment-associated processes was 30%.

The previous calculations assumed denitrification was supported by existing NO₃⁻. In fact, there was little NO₃⁻ in the bottom water in the source region (<0.5 μM at km 0) and elevated NO₃⁻ in the down-flow end (16 μM at km 144) indicating that net nitrification was occurring consistent with previously documented nitrification activity in the Chesapeake Bay (McCarthy et al. 1984; Horrigan et al. 1990; Kemp et al. 1990). N₂ production was likely supported partly by in situ coupled nitrification-denitrification (Jenkins and Kemp 1984). The maximum oxygen demand for nitrification can be estimated by the sum of net nitrification and coupled nitrification-denitrification. Assuming the theoretical 2 O₂ consumed per NO₃⁻ produced, the net nitrification oxygen demand was 21% of the total oxygen consumption (2 × 16 ΔμM NO₃⁻/156 ΔμM O₂). Reducing the areal oxygen consumption flux by 21% and accounting for oxygen consumption due to nitrification that directly supported denitrification (2 × 73 μmol N m⁻² h⁻¹) yields a revised estimate of O₂ consumption associated with remineralization of 1229 μmol O₂ m⁻² h⁻¹ (i.e., 1740 μmol O₂ m⁻² h⁻¹ × 0.79 - 146 μmol O₂ m⁻² h⁻¹). Using this revised estimate and recalculating denitrification efficiency as in the previous paragraph, we obtain an upper limit estimate of efficiency. For the whole system (sediment plus water), the denitrification efficiency was 28%, whereas the denitrification efficiency was 37% for the sediment alone.

The possibility that some of the N₂ was produced by anaerobic ammonium oxidation (ANAMMOX) can not be ruled out, although the moderately eutrophic Chesapeake Bay system does not provide optimal habitat for ANAMMOX relative to heterotrophic denitrification (Risgaard-Petersen et al. 2004; Engstrom et al. 2005) and consequently we

have referred to N₂ production as denitrification in this study. ANAMMOX, to the extent that it was occurring, would alter the efficiencies because one of the two N₂ atoms comes from ammonium (NH₄⁺), rather than both atoms coming from NO₃⁻. Half as much O₂ would be consumed to support ANAMMOX as for heterotrophic denitrification and the efficiency of fixed nitrogen removal would be between the low and high estimates given above.

The flux data from core chamber experiments could also be used to estimate the denitrification efficiency for sediment-only processes. The average efficiencies at the two stations fell in the vicinity of the 30% estimate from the gradient analysis: 24% at km 0 and 29% at km 144. An alternative approach was to estimate the efficiency using measured sediment nitrogen fluxes, including the total flux of NH₄⁺, N₂-nitrogen, and NO₃⁻. Estimated efficiencies based on net N fluxes were 43 ± 12% at km 0 and 37 ± 12% at km 144.

Eyre and Ferguson (2002) compared several shallow Australian lagoons characterized by different benthic trophic structures and observed a decrease in denitrification efficiency with increasing sediment heterotrophic activity (except in a lagoon with significant seagrass coverage). The denitrification efficiency estimates (using oxygen stoichiometry) for our study fell in the range of 20–30%, whereas the efficiency based on dissolved inorganic nitrogen + N₂ fluxes was near 40%. These results align well with the relationship reported in Eyre and Ferguson (2002), if we assume a 1 : 1 stoichiometry between O₂ flux (reported in our study) and CO₂ flux (reported in Eyre and Ferguson). This would suggest that the estuarine system under measurement here could be characterized as moderately eutrophic, which is consistent with general assessments of the Chesapeake Bay.

Our ability to measure denitrification from measurements of ambient N₂ concentration was aided by the two layer circulation that effectively prevented the lower water mass from ventilating with the atmosphere. In the regions south and north of the study area, mixing frequently occurs throughout the water column. N₂ excess in the water caused by sediment denitrification would be diminished as some of it would be lost to the atmosphere. Given the results here, we can do a first order estimate of the gradient we might expect in regions outside the study reach and provide an assessment of the suitability of the method when two-layer flow is absent.

We are interested in the magnitude of the N₂ excess in a well mixed water column when the N₂ flux into the water column from denitrification is c. 70 μmol N m⁻² h⁻¹ (=35 μmol N₂ m⁻² h⁻¹) and

exchange with the atmosphere happens under typical ambient conditions. The gas exchange rate across the air interface of an open body of water can be described as $dC/dt = K_a[C_s - C]/z$, where K_a is the gas exchange coefficient ($m\ h^{-1}$), C_s is the solubility concentration ($mol\ m^{-3}$), C is the measured concentration ($mol\ m^{-3}$), and z is the average depth (m) of the water body. We can ignore z to make the units consistent with the preferred denitrification flux units, which are on an areal (m^2) basis. At steady state, the air-water gas exchange rate equals the rate of denitrification and we can set $K_a[C_s - C] = 35\ \mu mol\ N_2\ m^{-2}\ h^{-1}$. The concentration gradient depends on K_a , which depends on environmental factors, in particular wind velocity. A reasonable estimate of K_a for the Chesapeake Bay is $0.06\ m\ h^{-1}$ corresponding to an average wind velocity of $5\ m\ s^{-1}$ (Wanninhof et al. 1985). The concentration gradient at steady state would be on the order of $0.6\ \mu mol\ N_2\ l^{-1}$ or c. 0.1% above the solubility concentration. This sensitivity analysis is valid even when the assumed gradient is elevated by a factor of 2–3, which would correspond to a minimum air exchange coefficient with minimal wind forcing.

The expected small (c. 0.1%) excess N_2 caused by the biological flux is on the same order as physical effects that cause disequilibrium with respect to solubility. Natural water bodies are subject to surface temperature fluctuations that can cause 1.5–2.5% changes in N_2 per degree Celsius and c. 0.15% change in the $N_2:Ar$ ratio per degree Celsius under equilibrium conditions. Excess N_2 (relative to Ar as well as absolute amount) can arise from breaking wave action and bubble collapse in the surface water (Emerson et al. 1991). Bubble injection can elevate N_2 relative to Ar on the order of 1% in natural waters. Nonsteady state conditions add additional uncertainty to the assignment of causes of N_2 excess in sampled water. In our study, we observed N_2 excess of 0.3–0.5% relative to Ar and relative to solubility equilibrium in surface water. This elevation in N_2 is within the range expected for solubility-driven processes. We conclude that a minor biological contribution to the excess N_2 would not be resolvable using the present technique for fluxes of a magnitude observed in this water body if the water column in contact with the sediments is also substantially in contact with the atmosphere.

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