

# The Effects of Salinity on Nitrogen Losses from an Oligohaline Estuarine Sediment

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**Abstract** Benthic respiration, sediment–water nutrient fluxes, denitrification and dissimilatory nitrate reduction to ammonium (DNRA) were measured in the upper section of the Parker River Estuary from 1993 to 2006. This site experiences large changes in salinity over both short and long time scales. Sediment respiration ranged from 6 to 52 mmol m<sup>-2</sup> day<sup>-1</sup> and was largely controlled by temperature. Nutrient fluxes were dominated by ammonium fluxes, which ranged from a small uptake of -0.3 to an efflux of over 8.2 mmol N m<sup>-2</sup> day<sup>-1</sup>. Ammonium fluxes were most highly correlated with salinity and laboratory experiments demonstrated that ammonium fluxes increased when salinity increased. The seasonal pattern of DNRA closely followed salinity. DNRA rates were extremely low in March, less than 0.1 mmol m<sup>-2</sup> day<sup>-1</sup>, but increased to 2.0 mmol m<sup>-2</sup> day<sup>-1</sup> in August. In contrast, denitrification rates were inversely related to salinity, ranging from 1 mmol m<sup>-2</sup> day<sup>-1</sup> during the spring and fall to less than 0.2 mmol m<sup>-2</sup> day<sup>-1</sup> in late summer. Salinity appears to exert a major

control on the nitrogen cycle at this site, and partially decouples sediment ammonium fluxes from organic matter decomposition.

**Keywords** Benthic respiration · Benthic fluxes · Salinity · Ammonium · Denitrification · Dissimilatory nitrate reduction to ammonium (DNRA) · Estuary · Sediments

## Introduction

Sediments play a major role in controlling the cycling and availability of nitrogen in estuaries (Nixon 1981; Boynton and Kemp 1985; Giblin et al. 1997; Hopkins et al. 1999). The decomposition of organic matter in sediments produces ammonium that can be released into the overlying water and used in support of primary production. However, not all of the ammonium produced reaches the overlying water. Substantial amounts of ammonium can be nitrified in near-surface sediments. While some of the nitrate produced may diffuse to the overlying water, the majority is often lost as nitrogen gas via denitrification (Seitzinger et al. 1988). Quantitatively, the loss of nitrogen via this coupled nitrification/denitrification pathway is high, but can vary greatly among estuaries. Seitzinger (1988) compared a number of estuaries and found that denitrification removed from 15–70% of the inorganic nitrogen regenerated from decomposing organic matter in sediments. Estuarine denitrification rates are suppressed by low water column oxygen concentrations because the source of nitrate to support coupled nitrification/denitrification is from the oxidation of ammonium (Seitzinger 1990; Cornwell et al. 1999).

Nitrate present in the water column may also be removed through direct denitrification. A number of studies have shown that rates of direct denitrification are often correlated

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with water column nitrate concentration (Kemp et al. 1990; Rysgaard et al. 1995; Jensen et al. 1996). The relative importance of direct versus coupled denitrification varies greatly between estuaries (e.g. Jensen et al. 1996; Nielsen et al. 2001; LaMontagne et al. 2002) and sometimes even between different locations within the same estuary (Tobias et al. 2003).

Sediments in estuaries are subjected to changing physical conditions that occur on a range of time scales. In temperate regions, temperature, salinity, and bottom water oxygen concentrations nearly always exhibit significant seasonal trends. Conditions may also oscillate rapidly over daily, or even hourly, time scales due to changes in the tidal regime or freshwater discharge. While the influence of temperature and oxygen changes in the water have been examined in some detail, the potential effects of salinity changes on the nitrogen cycle in the sediment have only recently been appreciated (Seitzinger et al. 1991; Rysgaard et al. 1999; Fear et al. 2005).

The salinity of the environment affects the release and N cycling processes in sediments in a number of ways. First, salinity may change the amount of ammonium released from sediments by physicochemical mechanisms. In sediments, the adsorption of ammonium is strongly dependent upon salinity (Boatman and Murray 1982; Gardner et al. 1991). In zones of the estuary where salinity varies, ammonium can effectively be stored in the sediments during low salinity conditions and released as the estuary becomes more saline. This mechanism alters the timing of benthic N release but may not greatly change the magnitude of benthic N fluxes over the annual cycle (Weston et al. 2010). Changes in the timing of ammonium flux can be quite significant, however, because the effect is greatest when river discharge and watershed N loading are lowest. Hence, salinity-enhanced ammonium flux can contribute to summertime algal blooms (Holmes et al. 2000).

Salinity changes may also alter the magnitude of benthic N release through a number of physiological mechanisms. By directly affecting nitrifiers, denitrifiers, or both, salinity changes may alter the efficiency by which nitrogen is lost via coupled nitrification/denitrification. Seitzinger et al. (1991) suggested that freshwater sediments have higher denitrification rates than marine sediments because the higher ammonium adsorption capacity of low salinity sediments allows the bacteria to more effectively nitrify the ammonium. Differences in porewater sulfide concentrations associated with salinity differences may also affect microbial efficiency. Sulfide concentrations in coastal marine sediments are normally higher than those in freshwater and sulfide has been shown to inhibit nitrification (Joye and Hollibaugh 1995). However, there is conflicting evidence in the literature about the direct effects of salinity, with some studies finding evidence of a direct

inhibitory effect (Rysgaard et al. 1999) while others found that both nitrification and denitrification rates increased when salinity was increased (Magalhaes et al. 2005).

Another mechanism by which denitrification rates may be affected by salinity is by altering rates of dissimilatory nitrate reduction to ammonium (DNRA), a process that may compete with denitrification for nitrate. Some studies have shown that as salinity increases, sulfate reduction also increases, which may favor DNRA over denitrification (Brunet and Garcia-Gil 1996; An and Gardner 2002). The balance of this competition of dissimilatory processes for nitrate has important implications for DIN release to the water column, because in DNRA, the end product, ammonium, remains biologically available within the estuary. Under some circumstances DNRA can become a major sink for nitrate produced within the sediments or from the overlying water (Tobias et al. 2001; Christensen et al. 2000; Bonin et al. 1998; An and Gardner 2002; Gardner et al. 2006), surpassing denitrification as a removal process for nitrate.

We examined the influence of salinity changes on benthic nitrogen cycling at a single site in the upper Parker River Estuary of Plum Island Sound. The effects of daily, seasonal, and inter-annual salinity variations on benthic fluxes were examined over a 13-year period. Detailed measurements of denitrification and DNRA were made over one annual cycle. In a companion paper, the importance of ammonium adsorption to nitrogen dynamics at this and other locations in the estuary is reported and modeled (Weston et al. 2010). Data on potential nitrification and the diversity of ammonium-oxidizing bacteria at this site were reported previously (Bernhard et al. 2005, 2007).

## Methods

**Study Site** The study was conducted in the upper reaches of the Plum Island Sound Estuary, in northeastern MA, USA (<http://www.mbl.edu/PIE>). The study site, P22, was located approximately 22 km upstream from the mouth of Plum Island Sound, and 2 km below the Parker River dam, in the oligohaline region of the estuary. At this location, the estuary is narrow, surrounded by extensive tidal marshes. Water depth in the center of the channel ranges from just over 1 m at low tide to more than 4 m at high tide. This site is characterized by large seasonal changes in salinity driven by river discharge. Discharge from the Parker River, monitored by the USGS (<http://www.usgs.gov>, station 01101000), ranges from zero during some summer months to over  $30 \text{ m}^3 \text{ s}^{-1}$  during peak discharge periods. Hydrodynamics are described in Vallino and Hopkinson (1998).

**Sampling Times** Station P22 has been sampled repeatedly since June 1993 (Table 1). A detailed description of this

**Table 1** Water column and sediment data from the flux studies

| Date       | Salinity<br>psu | T<br>°C | NO <sub>3</sub> <sup>-</sup><br>μM | %C  | C/N  |
|------------|-----------------|---------|------------------------------------|-----|------|
| 18-Jun-93  | 2               | 22      | 0.2                                | 2.4 | 18.7 |
| 20-Sep-93  | 18              | 16      | 5.4                                | 4.2 | 17.7 |
| 6-Dec-93   | 0               | 5       | 14.1                               | 5.1 | 17.7 |
| 5-Apr-94   | 0               | 9       | 10.6                               | 4.3 | 20.6 |
| 25-Jul-94  | 10              | 22      | 1.1                                | 4.9 | 19.6 |
| 23-Jul-96  | 0               | 18      | 9.7                                | 5.6 | 16.3 |
| 21-Aug-96  | 1               | 23      | 0.0                                | NA  | NA   |
| 10-Sep-96  | 4               | 19      | 0.0                                | 3.2 | 14.3 |
| 23-Mar-99  | 0               | 4       | 13.0                               | 3.4 | 12.9 |
| 3-May-99   | 0               | 15      | 1.2                                | 3.6 | 12.3 |
| 23-Jun-99  | 2               | 22      | 0.1                                | 4.2 | 15.5 |
| 9-Aug-99   | 12              | 21      | 0.2                                | 3.2 | 15.1 |
| 05-Oct-99  | 0               | 15      | 7.6                                | 4.0 | 14.5 |
| 02-May-00  | 0               | 14      | 3.1                                | NA  | NA   |
| 18-Sep-00  | 1.5             | 18      | 6.3                                | NA  | NA   |
| 24-Apr-01  | 1               | 10      | 10.1                               | 3.4 | 12.4 |
| 28-Aug-01  | 10              | 23      | 0.7                                | 3.5 | 13.5 |
| 22-Apr-02  | 1               | 11      | 7.6                                | 3.8 | 13.3 |
| 09-Sep-02  | 14              | 21      | 0.5                                | 3.6 | 15.7 |
| 14-Apr-03  | 2               | 8       | 18.0                               | 2.7 | 13.9 |
| 29-Sep-03  | 2               | 19      | 4.8                                | 3.3 | 14.8 |
| 19-Apr-04  | 0               | 14      | 9.1                                | 4.5 | 22.5 |
| 14-Sep-04  | 0               | 19      | 9.0                                | 4.4 | 17.1 |
| 12-Apr-05  | 0               | 11      | 17.1                               | 3.7 | 11.9 |
| 12-Sep-05  | 10              | 23      | 0.1                                | 3.4 | 13.4 |
| 19-Apr-06  | 1.5             | 14      | 9.9                                | 4.0 | 14.8 |
| 12-Sept-06 | 4               | 18      | 2.2                                | 3.7 | 15.0 |

Salinity, temperature, and water column nitrate are from water column samples taken at low tide when the cores were collected. Sediment % C (weight percent) and C/N (atomic ratio) were taken at the same time as the fluxes except during 1999. Sediment data from 1999 is from cores taken within 1–2 weeks of the flux sampling. Data is the average of the top 2 cm

NA denotes times where the data is not available

and three other sites in the estuary from the first 2 years of sampling (June, September, and December, 1993 and April and July, 1994), including sediment porewater profiles, nutrient and oxygen fluxes, and sediment carbon, nitrogen and chlorophyll inventories, are presented in Hopkinson et al. (1999; note that in this paper the station was called P2 but has since been renamed P22). The site was visited again during the summer of 1996 (July, August, and September), most intensively in 1999 (March, May, June, August, and October), and subsequently twice a year; during April or May (high-discharge conditions), and August or September (low-discharge conditions), as part of the routine LTER monitoring program (Table 1). During 1999, additional

measurements were made of dissolved and exchangeable ammonium in sediments at this and other sites along the estuary (Weston et al. 2010).

**Water Column Measurements** Concurrent with all flux core collections, temperature and salinity were measured in the field and water column samples were collected for nitrate analysis. During 1999, a continuous record of temperature, salinity, and oxygen was collected at half-hour intervals. From March 18 through July 22 data were logged with a Hydrolab DataSonde 4, and from July 22 through November 11 with a YSI 6600. Salinity and oxygen calibrations were checked at least every 3 weeks and corrected as needed. Fouling of the probes during a low-discharge period between August and September resulted in the loss of oxygen data and sometimes salinity and temperature data as well. The salinity pattern was filled in using a hydrodynamic model (Vallino and Hopkinson 1998), anchored by the existing continuous record and a field measurement made on September 6.

**Benthic Respiration and Nutrient Fluxes** The methods used were the same as those described in Hopkinson et al. (1999). Large cores (15 cm diameter) were taken subtidally at low tide, transported to the laboratory, and placed in an incubator at ambient temperature. Triplicate cores were collected in 1993–1994 and duplicate cores were collected thereafter until 2005–2006.

Flux measurements representing these low tide conditions (i.e., the lowest daily salinity) were made in all years. In some years of the study, additional flux measurements were made under conditions approximating high tide, and additional cores were taken for denitrification measurements during 1999 and several of the other sampling times (see below).

Cores were incubated using filtered water (20 and 1 μm cartridge filters) from the site. Cores were sealed with tops containing magnetic stirrers and gently stirred throughout dark incubations. Oxygen was monitored using an Orbisphere O<sub>2</sub> meter and incubation duration was determined by the time required for the oxygen to fall 2–5 ppm but remain above 50% saturation (6–48 h). Samples of overlying water were taken for nutrients at least five times during the incubation period. Ammonium was analyzed using the Solorzano (1969) technique, nitrate + nitrite samples were immediately frozen and later run on a rapid flow analyzer (Alpkem RFA-300), and phosphate was preserved with trace-metal-grade acid and analyzed within 1 week using the method of Murphy and Riley (1962).

At the beginning and end of the incubation period, samples were taken into 60-ml glass BOD bottles and saved for measurements of total dissolved inorganic carbon (DIC) and oxygen. DIC samples were preserved with mercuric chloride and analyzed with a high precision coulometric

TCO<sub>2</sub> analyzer. Sediment–water fluxes were calculated based on the slope of linear regressions fitted to concentration changes of nutrients, DIC or oxygen. We scaled the dark incubations to daily rates. We believe this is justified because light penetration is severely limited by the very turbid water at this site and because previous whole-system <sup>15</sup>NO<sub>3</sub> additions carried out just downstream demonstrated that benthic micro-algae were not important in N cycling (Holmes et al. 2000; Hughes et al. 2000).

*Measurements to Examine the Effect of Salinity on Nitrogen Exchange* In October 1999, May and September 2000, and April and August 2001, our traditional low-tide flux measurements were expanded to include additional cores incubated under conditions of higher salinity. For these experiments, surface water was collected from a location downstream of the study site chosen to represent the maximal seaward end of the tidal excursion and thus the water that would be present at the site at high tide. In October 1999, the water was taken quite far downstream and was 14 (psu) higher than the upstream low tide water. For the subsequent four experiments, the salinity difference between the low- and high-tide incubations was standardized; in these experiments, high-tide water was collected from a site where it was 8 psu higher than ambient low tide salinity. Fluxes were measured as described above.

In May of 2000, the flux experiment was extended to 1 week to examine the longer-term effect of salinity on nitrogen release and to validate a model of salinity diffusion and nitrogen exchange (Weston et al. 2010). After the routine flux incubation, which under early season, cold temperatures lasted about 48 h, the cores were uncapped and gently stirred to prevent hypoxia. Ammonium measurements were taken at day 5 and the overlying water was changed to prevent ammonium build up. The cores were resealed and ammonium and oxygen concentrations were measured at approximately 12-h intervals for the next 2 days to determine if the respiration rates or ammonium fluxes had changed over the course of the incubation.

Changes in porewater salinity and ammonium that occurred over the period of the extended flux measurement were also examined. Initial porewater salinity and ammonium profiles were measured in replicate small cores (6 cm diameter), taken concurrently with the flux cores. Final profiles after the 7-day incubation were obtained by sub-coring the flux cores with the smaller diameter (6 cm) core tubes.

#### Denitrification Measurements by the Isotope Pairing Method

Denitrification measurements were made during all six flux experiments conducted between March 1999 and May

2000. For these experiments, four additional cores were taken for the measurement of denitrification by isotope pairing method (IPM; Nielsen 1992). Cores were incubated as described above with two cores receiving additions of 15 μM <sup>15</sup>NO<sub>3</sub><sup>-</sup> to the overlying water while two cores received 45 μM <sup>15</sup>NO<sub>3</sub><sup>-</sup>. Measurements of nitrate immediately before and after the <sup>15</sup>NO<sub>3</sub><sup>-</sup> additions were taken to determine the ratio of <sup>15</sup>NO<sub>3</sub><sup>-</sup>/<sup>14</sup>NO<sub>3</sub><sup>-</sup> in the overlying water. Samples from the overlying water were taken for the determination of <sup>29</sup>N<sub>2</sub> and <sup>30</sup>N<sub>2</sub> at the beginning, middle, and end of the incubation period to check that time courses were linear.

Samples for dissolved gases were taken from the overlying water by siphoning the water into evacuated glass bulbs fitted with gas tight valves (Emmerson et al. 1999). After the final sample was taken, the sediment was slurried with the overlying water, covered and allowed to settle for 5 min. The slurry was sampled by siphoning into glass bulbs as before. During March and May of 1999, all the bulbs contained 100 μL of a saturated HgCl<sub>2</sub> solution which had been dried in the bulbs prior to evacuation. Denitrification rates calculated from the slurry samples were four to ten times higher than rates calculated from the overlying water samples. Subsequent tests showed that the amount of HgCl<sub>2</sub> used in the bulbs was probably not sufficient to eliminate microbial activity in the slurry samples, and therefore the resulting data were compromised. The amount of HgCl<sub>2</sub> was increased to 1 ml for the slurry samples in all subsequent experiments.

Sample processing for trapping and isotopic analysis of the N<sub>2</sub> in the bulbs was modified from Emmerson et al. (1999) as follows. After the majority of water was removed from the bulbs, they were attached one at a time to a vacuum line, and immersed in a dry ice/ethanol bath to freeze out the remaining water. The gas from the bulb was cryogenically trapped on silica gel in a glass tube that also contained copper oxide. The tube was flame-sealed, removed from the vacuum line, and combusted at 350°C overnight to remove oxygen. Gas samples were introduced to a Finnegan mass spectrometer by breaking the tubes directly into the sample stream of the instrument.

In 2004, sample processing for denitrification measurements was simplified after the acquisition of a membrane inlet mass spectrometer (MIMS). Denitrification measurements were made on the same cores that were incubated for respiration and nutrient fluxes. On the morning following the completion of those incubations, the headspace water was replaced and <sup>15</sup>NO<sub>3</sub><sup>-</sup> was added. Nitrate additions were increased over previous years such that one core of each pair received 45 μM <sup>15</sup>NO<sub>3</sub><sup>-</sup> while the other received 90 μM <sup>15</sup>NO<sub>3</sub><sup>-</sup>. Water samples for gas analyses were siphoned into 12 ml Exetainer™ vials and preserved with

10  $\mu\text{l}$  saturated  $\text{HgCl}_2$  solution. Samples were analyzed on the MIMS (Kana et al. 1998). Water and  $\text{CO}_2$  from the gas sample were cryogenically trapped and oxygen was removed by an in-line copper reduction tube heated to  $600^\circ$  in a tube furnace prior to the gases entering the mass spectrometer (Eyre et al. 2002).

Denitrification was calculated using the equations of Nielsen (1992). These are probability equations that depend on the assumption of uniform mixing of the  $^{15}\text{NO}_3^-$  label throughout the  $\text{NO}_3^-$  pool. The production of  $^{15}\text{N}_2$  ( $^{15}\text{D}$ ) from the added  $^{15}\text{NO}_3^-$  was calculated from the production rate ( $p$ ) of  $^{29}\text{N}_2$  and  $^{30}\text{N}_2$ :

$$^{15}\text{D} = p^{29}\text{N}_2 + p2 \times (^{30}\text{N}_2)$$

The formation of  $\text{N}_2$  from unlabeled nitrate, including nitrate produced by in situ nitrification was calculated as:

$$^{14}\text{D} = (p^{29}\text{N}_2)/2 \times p(^{30}\text{N}_2) \times ^{15}\text{D}$$

The total denitrification (both from ambient  $^{14}\text{NO}_3^-$  and from the added  $^{15}\text{NO}_3^-$  tracer) is equal to:

$$D^{\text{tot}} = ^{15}\text{D} + ^{14}\text{D}$$

The total denitrification driven by both labeled and unlabeled nitrate in the overlying water can be calculated from  $^{15}\text{D}$  and the isotopic enrichment of nitrate during the incubation ( $\epsilon$ ):

$$D_w^{\text{tot}} = ^{15}\text{D}/\epsilon$$

In situ rates of coupled nitrification/denitrification ( $D_n$ ) and direct denitrification ( $D_w$ ) can then be calculated:

$$D_n = D^{\text{tot}} - D_w^{\text{tot}} \quad \text{and} \quad D_w = D_w^{\text{tot}} - ^{15}\text{D}$$

The calculation of denitrification using isotope pairing equations is affected if anaerobic ammonium oxidation (anammox) is a significant process in the sediments. However, measurements have shown that anammox accounts for only a trivial percent of the  $\text{N}_2$  production at this site (Koop-Jakobsen and Giblin 2009).

**DNRA** Measurements of the dissimilatory reduction of nitrate to ammonium were made on the same cores used for isotope pairing. A 400-ml sample of the slurry was taken and immediately frozen at  $-80^\circ\text{C}$  to halt microbial activity. Combined porewater ammonium and exchangeable ammonium were extracted from thawed samples by adding 2 N KCl to a subsample that was then shaken, centrifuged, and filtered. The resulting ammonium concentration was measured and a portion of the extract was used for the subsequent analysis of  $^{15}\text{NH}_4^+$  using the method of Holmes et al. (1998). The in situ rate of DNRA was calculated using the relative rates of  $D_{14}$  and  $D_{15}$  calculated from the isotope

pairing equations for denitrification as detailed above and the production ( $p$ ) rate of  $^{15}\text{NH}_4$  (Risgaard-Petersen and Rysgaard 1995):

$$\text{DNRA} = p^{15}\text{NH}_4 \times (D_{14}/D_{15})$$

**C/N of Mineralization** In October of 1999 and in June of 2000, sediment samples were taken to measure the DIC and ammonium produced during mineralization. Cores (6.5 cm) were taken near the flux cores and sectioned into 2-cm intervals in a glove bag under a nitrogen atmosphere. Sections were sealed into centrifuge tubes and subsets were harvested at 24 h intervals over 4 days. For each depth interval, total DIC and ammonium (dissolved + exchangeable) was measured. Due to the small sample size, DIC was measured by GC. In October, data after the first two time-periods were extremely variable so only two time-points were used. The rate of DIC production was calculated using a linear regression.

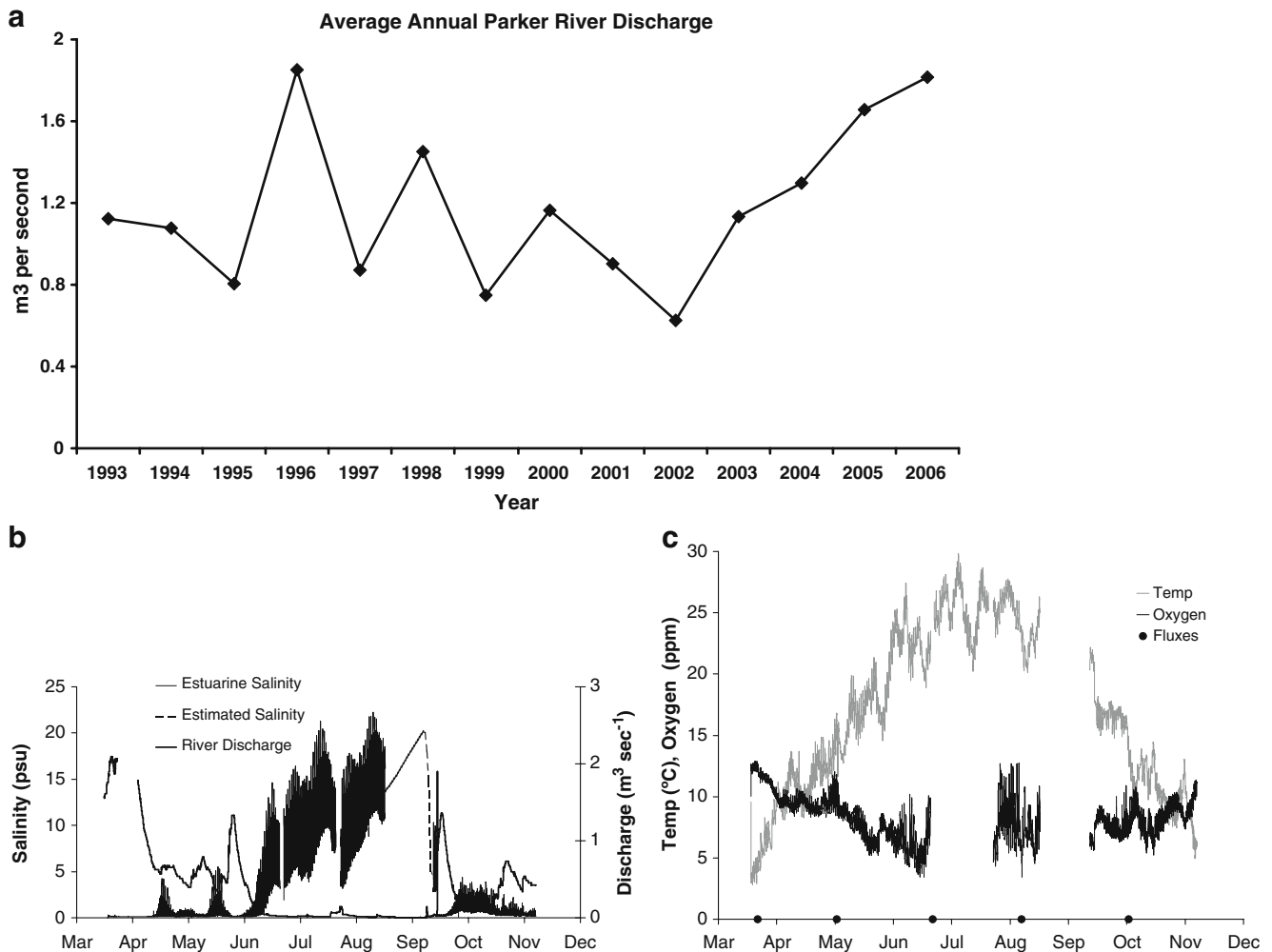
**Sediment Porosity and C/N** Sediment porosity and C/N values were determined on replicate separate cores taken at the same time as the flux cores as described in Hopkinson et al. 1999, although the sampling interval varied from 0.5 to 2 cm over the course of the study. Porosity was determined using standard techniques and organic carbon and nitrogen was measured on a Perkin Elmer 2400 CHN elemental analyzer following carbonate removal.

## Results

### River Discharge and Water Column Properties

Discharge during the 1993–2006 study period exhibited nearly the full range of conditions observed on the Parker River since the river was gauged in 1945. The highest annual discharge over the study period was over  $1.85 \text{ m}^3 \text{ s}^{-1}$  in 1996, which was one of the highest annual discharges on record (Fig. 1a). Annual discharge in 2006 was also high and 2006 was characterized by the largest spring flood on record. The lowest discharge during the study occurred during 2002 and was just under  $0.63 \text{ m}^3 \text{ s}^{-1}$ . This was the third lowest annual discharge in the USGS record.

The detailed records of discharge, temperature, and salinity collected in 1999 illustrate a typical seasonal pattern for the upper estuary. Discharge was high in the spring and autumn and low during the summer, ranging from a maximum of  $2.0 \text{ m}^3 \text{ s}^{-1}$  in mid March to  $<0.01 \text{ m}^3 \text{ s}^{-1}$  during much of the summer (Fig. 1b). Salinity showed large variations due to changes in freshwater discharge and the tide. Salinity was close to zero during the period of high discharge but began



**Fig. 1** **a** Average annual discharge of the Parker River 1993–2006. Data are from USGS (<http://www.usgs.gov>, station 01101000). **b** Discharge from the Parker River during 1999 and the salinity at the study site, P22 in the estuary. Due to fouling data were lost during part

of the summer and mean daily salinity was estimated from a hydrodynamic model. **c** Temperature and oxygen from the same site as **b**. Dates for the 1999 flux study are shown on the X axis

to rise once discharge fell below  $1 \text{ m}^3 \text{ s}^{-1}$ . While there was an overall pattern of increasing salinity over the spring and summer as freshwater discharge decreased, there were also strong patterns due to semi-diurnal and bi-monthly (spring and neap) tidal cycles. Temperature at the site showed a strong seasonal pattern varying from  $3^\circ\text{C}$  on March 19th just after the logger was deployed, to nearly  $30^\circ\text{C}$  in early July. Diurnal changes in temperature were generally smaller than  $2^\circ\text{C}$  (Fig. 1b).

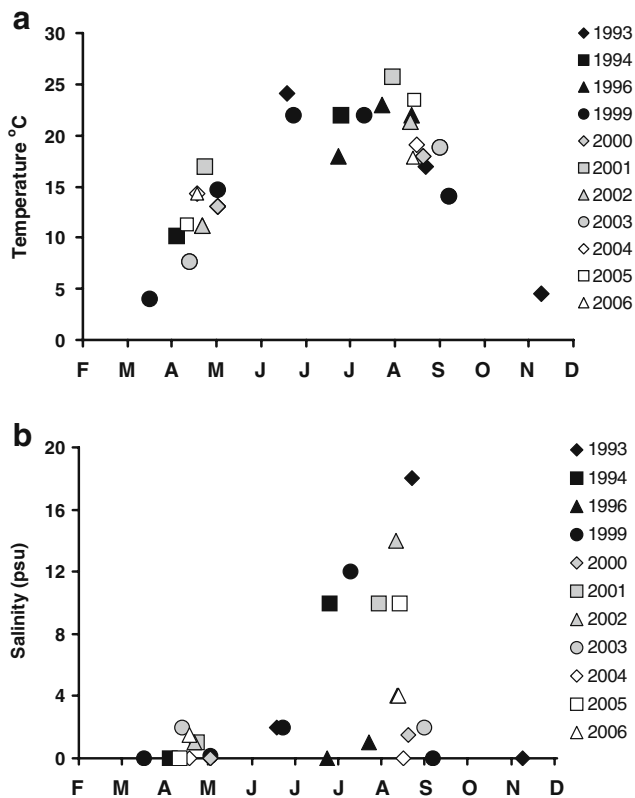
Dissolved oxygen concentrations showed large diurnal swings due to photosynthesis and respiration, especially during the summer, but the estuary did not exhibit any persistent hypoxia (Fig. 1c). Similar results were found in late summer in this estuary in 1996 (Holmes et al. 2000). Monthly oxygen transects also indicate that hypoxia in this region is not typical.

Temperature measurements made during low tide sampling from 1993–2006 show that inter-annual variability in

temperature at the site is fairly small and not closely tied to discharge (Table 1; Fig. 2a). In contrast, salinity shows very large inter-annual variation even between summers when runoff is typically low (Table 1; Fig. 2b). During some summers, such as 1996, large rain events due to mid-summer convective storms, or remnants of tropical storms, lead to significant runoff events and an atypical freshening of the estuary. As a result of this large variability, temperature and salinity are only weakly correlated for the 27 flux measurements ( $r=0.48$ ,  $p<0.012$ ).

#### Benthic Fluxes

Sediment oxygen ( $\text{O}_2$ ) uptake over the study period varied from 6 to  $52 \text{ mmol m}^{-2} \text{ day}^{-1}$  (Table 2; Fig. 3), which are typical rates for mesotrophic temperate estuaries (reviewed in Nixon 1981; Boynton and Kemp 2008).  $\text{O}_2$  uptake rates were significantly related to temperature ( $R^2=0.63$ ,  $p<$



**Fig. 2** Temperature and salinity at low tide for each of the 27 fluxes from 1993–2006

0.00001), but not to salinity ( $R^2=0.10$ ,  $p<0.10$ ). Total DIC release showed a larger range, 6.7 to 95 mmol m<sup>-2</sup> day<sup>-1</sup> (Table 2) and over the whole data set averaged 14% higher than the oxygen fluxes. Like oxygen uptake, DIC release was significantly related to temperature ( $R^2=0.22$ ,  $p<0.012$ ) but not salinity ( $R^2=0.10$ ,  $p<0.61$ ), although the relationship with temperature was weaker than for oxygen fluxes. As previously discussed (Hopkinson et al. 1999), DIC fluxes are influenced not only by metabolism but also by seasonally changing DIC fluxes driven by porewater salinity gradients. Therefore, in this study, we have assumed that O<sub>2</sub> fluxes more accurately reflect sediment metabolism given the salinity variations at our site.

Dissolved inorganic nitrogen fluxes were dominated by ammonium. Ammonium fluxes ranged from a slight uptake, -0.3 mmol m<sup>-2</sup> day<sup>-1</sup> to an efflux of over 8.2 mmol m<sup>-2</sup> day<sup>-1</sup> (Table 2; Fig. 4). Ammonium fluxes were uniformly low in the spring but showed extremely large inter-annual variation during summer and autumn (Fig. 4). In contrast to benthic metabolism, patterns of ammonium fluxes could not be explained by temperature ( $R^2=0.086$ ,  $p<0.14$ ), nor were they related to sediment oxygen uptake ( $R^2=0.06$ ,  $p<0.32$ ). Instead, ammonium fluxes could be significantly related to the salinity of the overlying water ( $R^2=0.24$ ,  $p<0.009$ ), although much of the variation in ammonium

fluxes remained unexplained. Including both temperature and salinity in the regression did not improve the fit.

There was a net efflux of nitrate from the sediments during some incubations, while in others, the sediments took up nitrate (Table 2). On average, nitrate fluxes were very small, ranging from a net efflux of 0.5 mmol m<sup>-2</sup> day<sup>-1</sup> to a net uptake of 0.5 mmol m<sup>-2</sup> day<sup>-1</sup>. Nitrate fluxes were positively related to temperature ( $R^2=0.20$ ,  $p<0.019$ ) and negatively related to nitrate concentrations in the overlying water ( $R^2=0.20$ ;  $p<0.018$ ), but showed no significant relationship with salinity ( $R^2=0.26$ ,  $p<0.79$ ).

Similar to nitrate, phosphate was sometimes released from sediments and at other times taken up from the overlying water (Table 2). Benthic phosphate fluxes ranged from an uptake of -1.23 mmol m<sup>-2</sup> day<sup>-1</sup> to a release of 0.29 mmol m<sup>-2</sup> day<sup>-1</sup> and were not related to either temperature or salinity ( $p<0.95$  and 0.64, respectively).

The atomic ratio of oxygen uptake to DIN release (O/N) is often used to determine the potential importance of denitrification in sediments (Nixon et al. 1976). If the organic material being decomposed has the composition of fresh phytoplankton and if all of the metabolism is ultimately reflected by oxygen uptake, an atomic O/N ratio of 13.25 to 17 is expected (Nixon et al. 1976). While other things may alter this ratio (see Discussion in Groffman et al. 2006), a ratio substantially higher than expected suggests that mineralized nitrogen is being lost through denitrification. High O/N ratios are commonly reported in marine and estuarine sediments (Nixon et al. 1976; Banta et al. 1995; Giblin et al. 1997; reviewed in Boynton and Kemp 2008). At our site, on two occasions, the O/N was negative due to DIN uptake but for the rest of the samples, ranged from 4.8 to over 3,000 (Table 2). In the spring, the ratio was always higher than 20, but mid-summer and fall values were often lower. Values considerably less than that expected for the decomposition of phytoplankton demonstrate that nitrogen efflux was not driven simply by metabolism. A simple regression between O/N and salinity yielded a non-significant negative relationship (excluding the negative values and one extreme value (August 2001,  $R^2=0.048$ ,  $p<0.31$ ,  $n=24$ ).

#### Effect of Salinity on Nitrogen Exchange

When cores were incubated at both high and low salinities (Fig. 5; Table 3) we saw no significant differences in respiration between treatments. In contrast, ammonium fluxes were always higher under the more saline conditions (Fig. 5). In both the 2000 and 2001 spring incubations and in Oct. 1999, ammonium flux under the higher salinities was two to four times that of the sediments incubated with the lower salinity water. In September 2000 and August 2001, average ammonium fluxes from sediments incubated

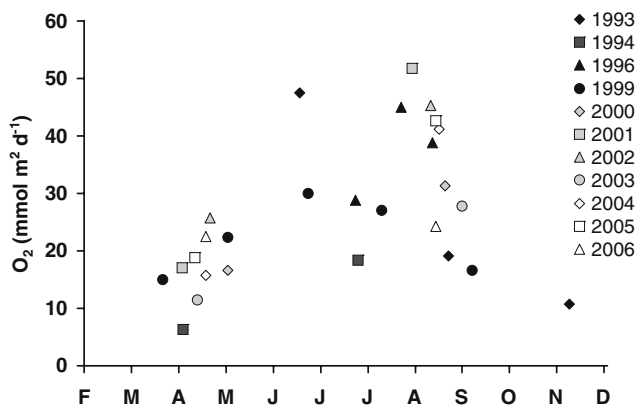
**Table 2** Oxygen, dissolved inorganic carbon (DIC), ammonium, nitrate and phosphate fluxes ( $\text{mmol m}^{-2} \text{day}^{-1}$ ) from the site incubated with overlying water taken at low tide

| Date      | O <sub>2</sub> |      | DIC  |      | NH <sub>4</sub> <sup>+</sup> |     | NO <sub>3</sub> <sup>-</sup> |      | PO <sub>4</sub> <sup>-3</sup> |      | O/N    |
|-----------|----------------|------|------|------|------------------------------|-----|------------------------------|------|-------------------------------|------|--------|
|           | Mean           | SE   | Mean | SE   | Mean                         | SE  | Mean                         | SE   | Mean                          | SE   |        |
| 18-Jun-93 | 47.5           | 1.8  | 95.0 | 27.9 | 8.2                          | 0.4 | 0.50                         | 0.02 | -0.16                         | 0.05 | 10.9   |
| 20-Sep-93 | 19.1           | 0.1  | 25.3 | 1.6  | 8.0                          | 0.2 | -0.24                        | 0.00 | -0.03                         | 0.00 | 4.8    |
| 6-Dec-93  | 10.8           | 0.4  | 15.5 | 1.3  | 1.7                          | 0.2 | -0.17                        | 0.03 | 0.06                          | 0.01 | 12.7   |
| 5-Apr-94  | 6.4            | 0.9  | 9.5  | 0.5  | 0.4                          | 0.0 | 0.02                         | 0.03 | 0.02                          | 0.00 | 28.4   |
| 25-Jul-94 | 18.4           | 1.7  | 19.6 | 1.4  | 5.6                          | 0.3 | -0.08                        | 0.01 | 0.08                          | 0.01 | 6.6    |
| 23-Jul-96 | 28.8           | 0.6  | 18.6 | 0.6  | 2.0                          | 0.5 | 0.48                         | 0.04 | 0.10                          | 0.04 | 23.0   |
| 21-Aug-96 | 45.0           | 4.0  | 35.5 | 3.2  | 0.6                          | 0.3 | 0.06                         | 0.01 | 0.17                          | 0.07 | 126.5  |
| 10-Sep-96 | 38.9           | 1.5  | 13.7 | 2.3  | 3.0                          | 0.1 | 0.03                         | 0.02 | 0.03                          | 0.07 | 25.8   |
| 23-Mar-99 | 15.0           | 0.5  | 15.6 | 3.6  | 0.7                          | 0.3 | -0.10                        | 0.05 | -0.07                         | 0.03 | 45.8   |
| 3-May-99  | 22.4           | 1.6  | 30.4 | 7.0  | 1.1                          | 0.6 | 0.10                         | 0.08 | 0.03                          | 0.02 | 36.8   |
| 23-Jun-99 | 30.1           | 0.9  | 37.2 |      | -0.3                         | 0.0 | 0.08                         | 0.02 | -0.12                         | 0.09 |        |
| 9-Aug-99  | 27.1           | 2.0  | 46.8 | 0.7  | 2.2                          | 1.0 | 0.01                         | 0.01 | -0.04                         | 0.02 | 24.3   |
| 5-Oct-99  | 16.7           | 0.7  | 22.3 | 2.1  | 2.2                          | 0.4 | -0.19                        | 0.08 | -1.23                         | 0.90 | 15.4   |
| 2-May-00  | 16.6           | 0.8  | 46.7 | 1.9  | 1.5                          | 0.1 | -0.06                        | 0.05 | 0.26                          | 0.22 | 22.2   |
| 18-Sep-00 | 31.4           | 1.3  | 21.2 | 3.7  | 3.5                          | 1.7 | -0.48                        | 0.17 | 0.10                          | 0.08 | 17.8   |
| 24-Apr-01 | 17.1           | 1.7  | 20.3 | 4.8  | 0.8                          | 0.3 | -0.10                        | 0.01 | -0.02                         | 0.01 | 44.8   |
| 28-Aug-01 | 51.7           | 1.7  | 38.1 | 14.8 | 0.0                          | 0.2 | 0.01                         | 0.09 | -0.14                         | 0.10 | 3721.1 |
| 22-Apr-02 | 25.7           | 1.6  | 41.1 | 1.1  | 2.2                          | 0.4 | -0.43                        | 0.06 | 0.00                          | 0.04 | 23.7   |
| 9-Sep-02  | 45.2           | 13.0 | 22.4 | 4.7  | 6.0                          | 3.8 | 0.13                         | 0.33 | 0.29                          | 0.13 | 14.8   |
| 14-Apr-03 | 11.4           | 0.3  | 6.7  | 0.8  | 0.4                          | 0.2 | -0.35                        | 0.06 | -0.01                         | 0.03 | 61.4   |
| 29-Sep-03 | 27.9           | 6.3  | 22.5 | 4.5  | 3.4                          | 0.8 | -0.35                        | 0.24 | 0.05                          | 0.01 | 16.6   |
| 19-Apr-04 | 15.7           | 0.6  | 10.5 | 0.0  | 0.6                          | 0.0 | -0.22                        | 0.04 | -0.08                         | 0.09 | 53.1   |
| 14-Sep-04 | 41.2           | 13.8 | 33.9 | 13.7 | 1.6                          | 0.9 | 0.13                         | 0.01 | -0.05                         | 0.04 | 49.0   |
| 12-Apr-05 | 26.0           | 1.9  | 30.2 | 4.1  | 2.2                          | 0.5 | -0.6                         | 0.09 | 0.15                          | 0.09 | 23.9   |
| 12-Sep-05 | 42.7           | 4.6  | 30.6 | 5.5  | -0.1                         | 0.1 | -0.03                        | 0.01 | -0.26                         | 0.08 |        |
| 19-Apr-06 | 22.5           | 0.9  | 19.0 | 2.3  | 0.4                          | 0.1 | -0.25                        | 0.02 | 0.01                          | 0.01 | 287.7  |
| 12-Sep-06 | 24.3           | 1.6  | 21.9 | 2.4  | 1.3                          | 0.6 | -0.17                        | 0.02 | 0.00                          | 0.00 | 45.1   |

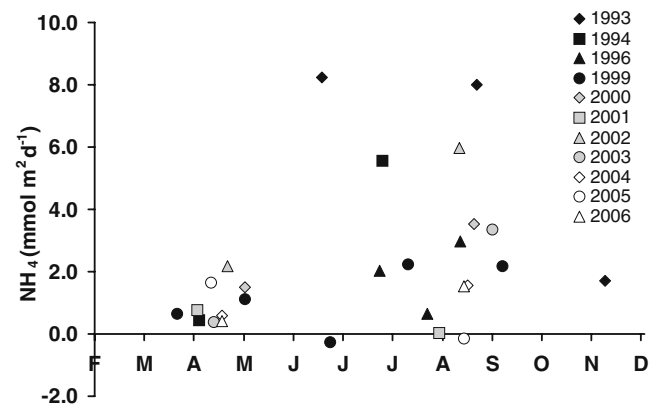
Negative values for the ratios are omitted. See text for details  
 O/N is the ratio of the atomic ratio of the oxygen uptake to the DIN efflux

at the higher salinity were also higher than those of the low salinity but the differences were not significant. The O/N flux ratios for sediments incubated with higher salinity water were also lower than when sediments were incubated with low salinity water (shown for May only; Table 3).

Sediments that were held under two different salinity regimes for an extended (6 days) incubation (May 2000) showed sustained differences in NH<sub>4</sub><sup>+</sup> fluxes but no differences in respiration (Table 3). The initial difference in ammonium flux was large: cores incubated with the higher

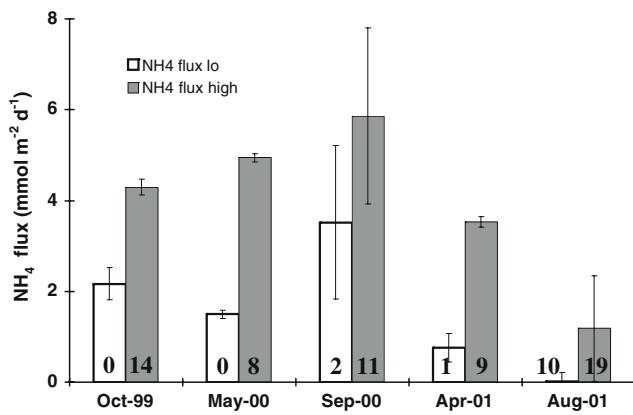


**Fig. 3** Oxygen uptake at P22 for each flux measurement



**Fig. 4** Ammonium flux from the sediments at P22 over the study





**Fig. 5** Ammonium fluxes from cores incubated with water taken from the site at low tide and (*flux lo*) and water collected further downstream where the salinity was higher (*flux high*). The salinity that the cores were incubated at is shown on the bars

salinity water exhibited fluxes that were more than three times higher than those incubated with low tide water and had O/N ratios below 8. After 6 days, ammonium fluxes had significantly decreased in these cores but were still nearly twice those of sediments held at low salinity. The ammonium fluxes of cores held at low salinity did not change significantly and always had O/N flux ratios above 20.

#### Denitrification

Results of isotope pairing experiments must demonstrate that the assumptions of the method were addressed. In their review of the method, Steingruber et al. (2001) concluded that the necessary conditions would be met if (a) the calculated rates of in situ denitrification ( $D_{14}$ , from unlabeled  $\text{NO}_3^-$ ) are independent of the label concentration, (b) rates of denitrification attributable to the added label ( $D_{15}$ ) increase linearly with the concentrations of the additions, and (c) production rates of  $^{29}\text{N}_2$  and  $^{30}\text{N}_2$  are constant. All three of these criteria were met in our core incubations.

Rates calculated from water column samples as compared to slurry samples showed that the water column

measurements underestimated the absolute rate of denitrification although the seasonal pattern was identical (Fig. 6a). On average, rates were 80% higher from the slurries. Because the slurry samples were compromised for March and May 1999, denitrification is shown from only the overlying water for these months; these are underestimates but they retain the seasonal pattern.

Ambient denitrification rates ( $^{14}D$ ) in 1999 showed a strong seasonal pattern, with the highest rates, about  $1 \text{ mmol m}^{-2} \text{ day}^{-1}$ , in the spring and fall and the lowest rates,  $<0.2 \text{ mmol m}^{-2} \text{ day}^{-1}$ , in summer (Fig. 6a). Denitrification rates in 1999 (using data from the overlying water only) were negatively related to salinity ( $R^2=0.59$   $p=0.074$ ) although the regressions were not statistically significant.

The importance of salinity can also be seen by comparing years with different salinity patterns. A similar seasonal denitrification pattern was observed in 2005, which, like 1999, had typical seasonal changes in salinity. During this year, denitrification rates were significantly lower in September than in April, although rates during both months were lower than we observed in 1999 (Fig. 6b). In contrast, in 2004, the seasonal pattern in salinity was not typical; near record spring discharges and frequent summer storms kept salinities in the upper estuary near 0 psu. During this year, we did not see as dramatic a difference in denitrification rates between spring and late summer as in other years (Fig 6b).

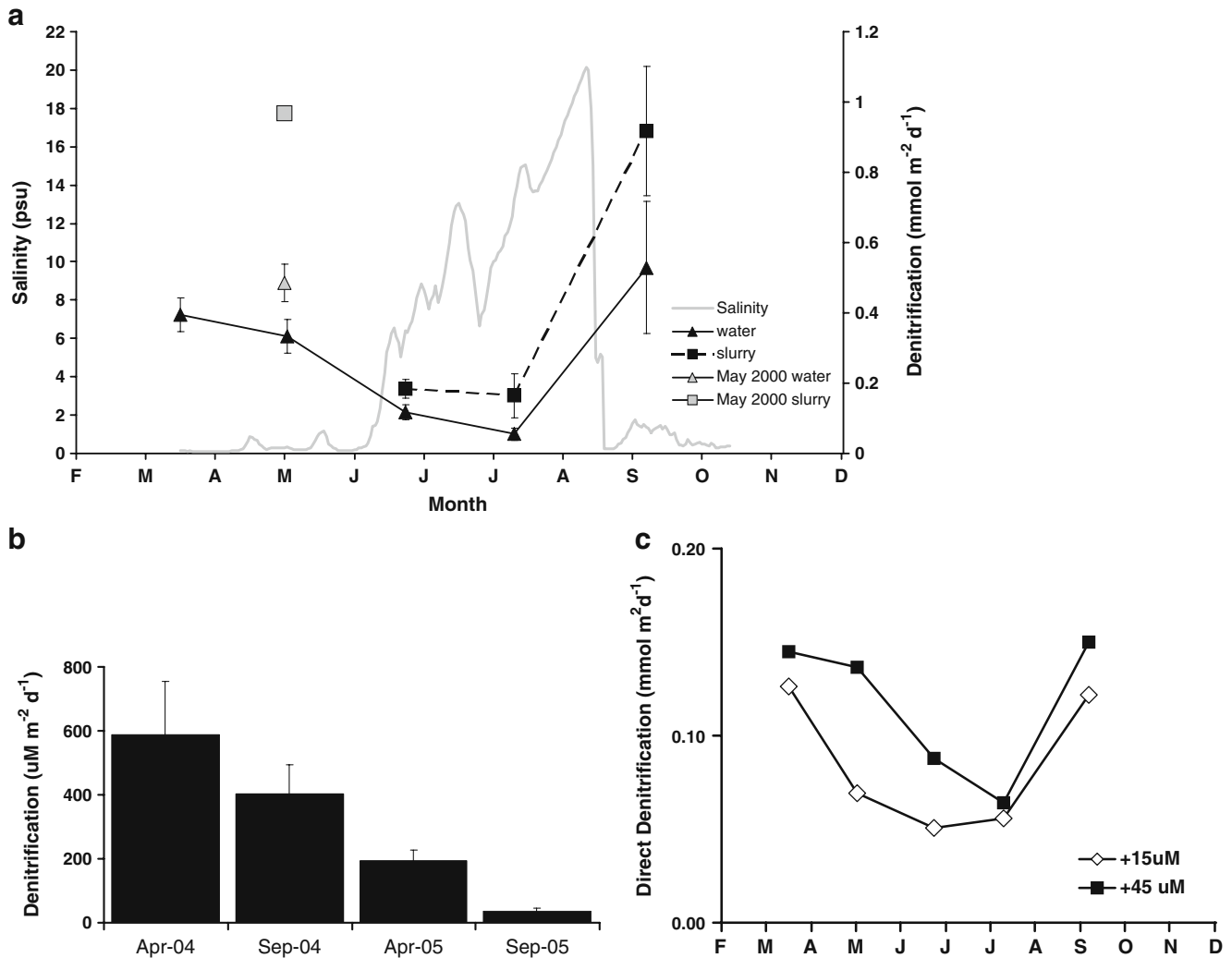
Adding nitrate always stimulated direct denitrification but the relative increase changed over the season (Fig 6c). Under conditions of equivalent nitrate availability, denitrification potential, estimates as total (ambient + added labeled nitrate) direct denitrification ( $D_w^{\text{tot}}$ ) was nearly three times greater in the spring and the fall when salinity was low than at the end of summer. Hence, although nitrate stimulated denitrification, the effect was small compared to other factors so that in the overall data set there was no significant relationship between overlying water nitrate concentration and ( $D_w^{\text{tot}}$ ).

Using the isotope pairing data we can partition the ambient denitrification between coupled ( $D_n$ ) and direct

**Table 3** Oxygen and ammonium fluxes from cores incubated with ambient low salinity water (0 psu) and water from an upstream station where the salinity was higher (8 psu)

|          | Salinity<br>PSU | O <sub>2</sub><br>mmol m <sup>-2</sup> day <sup>-1</sup> | O <sub>2</sub><br>SE | NH <sub>4</sub> <sup>+</sup><br>mmol m <sup>-2</sup> day <sup>-1</sup> | NH <sub>4</sub> <sup>+</sup><br>SE | O/N  |
|----------|-----------------|--|----------------------|--|------------------------------------|------|
| 2-May-00 | 0               | 16.6   | 0.8                  | 1.5  | 0.1                                | 22.2 |
| 8-May-00 | 0               | 18.9   | 1.0                  | 1.8  | 0.3                                | 20.7 |
| 2-May-00 | 8               | 17.9   | 1.6                  | 4.9  | 0.1                                | 7.3  |
| 8-May-00 | 8               | 18.2   | 0.5                  | 3.8  | 0.3                                | 9.5  |

Fluxes were measured on May 2, the cores were held for 6 days and fluxes were measured again on May 8. Nitrate fluxes on May 2 were very low. No nitrate fluxes were available for May 8 so O/N ratios are calculated based upon ammonium alone. See text for details

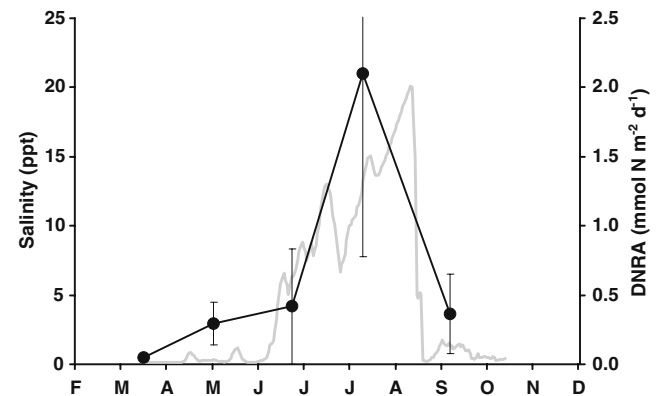


**Fig. 6** **a** Denitrification and salinity at P22 during 1999 and May of 2000. *Filled triangles* represent denitrification rates calculated from the overlying water during 1999 and are shown to illustrate the seasonal pattern. *Filled squares* show the rates calculated from slurries (no slurry data are available for March and May in 1999). *Open*

*symbols* are the water and slurry data from 2000. **b** Denitrification rates from April and September 2004 and 2005. **c** Direct denitrification rates calculated from the <sup>15</sup>NO<sub>3</sub> added to the overlying water. Nitrate was added at two concentrations, 15 and 45 μM

denitrification ( $D_w$ ). In our studies, coupled nitrification/denitrification was always the dominant process. The highest contribution of direct denitrification was during March when it was responsible for approximately 12% of the N<sub>2</sub> flux. This was also the period when nitrate concentrations in the overlying water were highest (12.7 μM). During the late spring and summer when nitrate concentrations were below 1.5 μM, more than 97% of the denitrification was from coupled nitrification/denitrification. Direct denitrification rose again to contribute 7% of the total denitrification in October as nitrate concentrations in the overlying water reached 8 μM.

**Dissimilatory Nitrate Reduction to Ammonium** In contrast to denitrification, DNRA showed a positive relationship with salinity (Fig. 7,  $R^2=0.988$ ,  $p<0.002$ ,  $n=5$ ). Rates



**Fig. 7** Changes in salinity and rates of dissimilatory nitrate reduction to ammonium (DNRA) during 1999

were extremely low in March, less than  $0.1 \text{ mmol m}^{-2} \text{ day}^{-1}$  but increased throughout the summer to a high of  $2.0 \text{ mmol m}^{-2} \text{ day}^{-1}$  in August. Variability was also greatest in August. Rates fell again in October to less than  $0.4 \text{ mmol m}^{-2} \text{ day}^{-1}$ . Seasonal patterns in DNRA were not significantly related to temperature ( $R^2=0.36$ ,  $p<0.29$ ,  $n=5$ ).

*The C/N of Mineralization and Sediment C/N* Our two mineralization experiments showed that the ratio of  $\text{CO}_2$  produced to N mineralized under anoxic conditions varied with season and sediment depth. The average C/N mineralization ratio for the top 10 cm was 9.4 in October whereas it was considerably lower, 6.6, in June (data not shown). The lowest C/N values were typically seen in the surface sediments and were highest at 4–6 cm.

Sediment %C (Table 1) showed considerable temporal variation although this may also reflect spatial variability at this site that receives episodic inputs of marsh debris which is not uniformly distributed. There was no clear seasonal pattern, with %C ranging from 2.4% to 5.1%, but averaging 3.8%. Sediment C/N values were variable also but did not show a seasonal change that could account for the changes in the flux O/N ratios (Table 2). Bulk sediment C/N was always higher than C/N from mineralization and ranged from 12.3 to 22.5.

## Discussion

### Sediment Fluxes

Ultimately, sediment respiration rates in estuaries and shallow coastal systems are controlled by the organic matter supply to the sediments (Nixon 1981; Kemp and Boynton 1981; reviewed in Hopkinson and Smith 2004). Other factors, such as temperature, the oxygen content of the overlying water, and the activities of benthic fauna can alter the timing and the efficiency with which organic matter is mineralized. Over the 13-year period of this study, there were substantial inter-annual differences in respiration. Temperature explained 79% of the variation in respiration over a single annual cycle (1999) but when data from all years were combined, temperature explained only 63% of the variation. These variations are undoubtedly driven by inter-annual differences in organic matter inputs from the adjacent marsh and/or from phytoplankton. Despite the variability, benthic respiration, and thus overall metabolism, followed a clear seasonal pattern reflecting changes in temperature.

A number of factors control the rate of DIN release from sediment; most importantly the supply of mineralized N and the extent to which mineralized N is lost through

processes such as coupled nitrification/denitrification. In many estuaries, there is an increase in respiration as a result of the deposition of the spring bloom but due to relatively high rates of denitrification, DIN release may be quite low (e.g., Boynton and Kemp 1985; Banta et al. 1995). Later in the summer, there may be a much closer relationship between respiration and DIN release (Boynton and Kemp 1985). While seasonal differences in denitrification will affect the relationship between respiration and DIN release, many researchers still find an overall significant correlation between the two processes (Banta et al. 1995; Giblin et al. 1997; Boynton and Kemp 2008).

At this site, DIN release consists almost entirely of ammonium. Ammonium fluxes were extremely variable and, in contrast to most other studies, we found no significant correlation between respiration and ammonium release, nor was ammonium release related to temperature. A number of processes may contribute to variability in the ammonium flux and thereby shift O/N ratios above or below that expected from a close coupling to organic matter decomposition (i.e., benthic respiration). These include changes in the amount of mineralized N that is denitrified, changes in DNRA, changes in the C/N of the material being mineralized, and changes in physicochemical exchange of  $\text{NH}_4^+$  in sediments.

In spring, O/N flux ratios were higher than would be expected from decomposition, suggesting that denitrification might explain the uncoupling of DIN release and respiration at this time of year. High spring rates of denitrification are consistent with data from other systems (Jensen et al. 1988; Banta et al. 1995) and are supported in our study by direct measurements. Later in the year, O/N ratios were frequently near, or even below that expected from decomposition of fresh phytoplankton and were highly variable. Low ratios could be caused by decomposition of a nitrogen-rich (low C/N) material present during late season. However, this is not supported by our C and N mineralization data that showed higher C/N ratios in the fall. Also, the C/N of the sediment does not suggest that there was an N-rich substrate present late in the year, in fact, the C/N values of sediments were often lowest in the spring (Table 1).

Salinity appears to play a major role in controlling both the inter-annual variability and the seasonal pattern in ammonium fluxes at this site. Short-term experiments in which salinity of the overlying water of paired cores was altered demonstrated that increases in salinity can drive large changes in ammonium flux in the absence of any change in the sediment mineralization rates but the effects appears to be greatest when salinity is low. Our extended experiments showed a sustained effect of salinity on  $\text{NH}_4^+$  fluxes, which persisted throughout the 6-day incubation (Table 3). These results suggest the effect of changing salinity on DIN fluxes may be larger during the spring

season, when  $\text{NH}_4^+$  stores in the sediment have built up over the winter. Later in the season, once the salinity has increased and stored sediment  $\text{NH}_4^+$  pools have been flushed out, further increases in salinity may only have minor effects on DIN fluxes.

Although the regression analysis of ammonium flux on salinity suggests that salinity might not be the major driver of ammonium release, a simple regression does not take into account the time lags due to the diffusion of salts into and ammonium out of porewater, or the non-linear relationship of ammonium adsorption and salinity (Weston et al. 2010). However, the impact on salinity on N fluxes in this system is clearly demonstrated by using a simple mechanistic numerical model, which does incorporate these time lags and salinity dependent adsorption values (see Weston et al. 2010). With this model, it is possible to describe seasonal changes in porewater ammonium profiles with great accuracy based on seasonal changes in salinity (Weston et al. 2010). The model also predicts the ammonium flux of experimental cores and is able to recreate the seasonal pattern of ammonium fluxes (Weston et al. 2010) with much more confidence than the simple regression relationship we use here.

#### Denitrification and DNRA

Boynton and Kemp (2008) reviewed estuarine denitrification and found that most rates fall between 0.25 and 2.40  $\text{mmol N m}^{-2} \text{day}^{-1}$ , with over 40% of the measured rates exceeding 1.20  $\text{mmol N m}^{-2} \text{day}^{-1}$ . Denitrification measured at our oligohaline site in spring and fall was in the low end of this range, reaching a maximum of about 1.00  $\text{mmol N m}^{-2} \text{day}^{-1}$ . Summer rates, however, averaged less than 0.20  $\mu\text{mol N m}^{-2} \text{day}^{-1}$ , lower than rates observed in most estuaries. The summer rates at this site were also an order of magnitude lower than those observed in the nearby saline Rowley River, which also drains into Plum Island Sound (Tobias et al. 2003). Very low summer rates have been reported from a few other estuarine systems. For example, Nielsen et al. (1995) reported rates of only 0.10 to 0.20  $\text{mmol N m}^{-2} \text{day}^{-1}$  during summer in Norsminde Fjord, when water column nitrate was absent.

High spring rates of denitrification observed in other studies are often driven by direct denitrification that is supported by high concentrations of water column nitrate (e.g. Nielsen et al. 1995; Rysgaard et al. 1995). In contrast, direct denitrification was of minor importance in our system, likely due to the fact that nitrate concentrations in the overlying water are relatively low, even in spring.

It is difficult to account for the very low summer rates we observed compared to other estuaries. In some systems, low summer denitrification rates are observed due to oxygen limitation, and/or sulfide inhibition of nitrifiers

and denitrifiers (e.g., LaMontagne et al. 2002; An and Gardner 2002; Fear et al. 2005; Boynton and Kemp 2008). However, neither of these explanations seems to completely explain our findings. Oxygen concentrations in the overlying water at our site were usually above 5 ppm, suggesting that oxygen limitation should not have been severe enough to drastically reduce coupled nitrification/denitrification. Also, although we did not measure sulfides routinely during this study, sulfide concentrations in the top 0–4 cm of the sediments at this site are generally low (Hopkinson et al. 1999).

We believe the large salinity changes that occur over the season may be an important factor limiting coupled denitrification in this system, especially during the summer and fall. During 2004, when salinity did not change notably over the season, we did not observe nearly as large a decrease between spring and summer denitrification rates compared to other years. One mechanism by which salinity may be altering denitrification rates is by changing the supply rate of nitrate from nitrification. Bernhard et al. (2005, 2007) found that potential nitrification had a salinity optimum around 10 psu during all times of the year at P22 but rates were significantly reduced at seawater salinities. Accordingly, there was a seasonal pattern in rates of potential nitrification, with lower rates in summer when salinities exceeded the optimum. In addition, populations of ammonium-oxidizing bacteria (nitrifiers) were smaller in summer than in spring. Previous work (Mondrup 1999) had also found that nitrification rates were lower when salinities were higher at this site. These findings suggest that salinity may act primarily on the nitrifiers. However, our experiments showed that denitrification rates were reduced in summer, even when nitrate was supplied during the incubations, demonstrating that salinity can act on denitrifiers as well ( $D_w$ ; Fig 6c).

Other studies have looked for an effect of salinity on nitrification and denitrification but the results have been inconsistent. Fear et al. (2005) found no relationship between salinity and denitrification in the Neuse River Estuary but factors other than salinity, including oxygen and nitrate concentrations in the water column, varied along the transect they studied. Similarly, Nielsen et al. (2001) found no consistent patterns between salinity and denitrification in Randers Fjord. However, Rysgaard et al. (1999) found clear evidence for an inhibitory effect of salinity on denitrification. In contrast, nitrification and denitrification increased with salinity in rocky biofilms in the Douro River Estuary (Magalhaes et al. 2005).

Another possible explanation for the lower denitrification rates in summer may be that DNRA is serving as the preferred nitrate sink under summer conditions. Our data suggest that the importance of DNRA increases as the estuary becomes more saline and by summer, rates of

DNRA rates greatly exceeded denitrification rates (Fig. 7). Although DNRA has historically been ignored in coastal sediments, a number of studies have now shown that DNRA can equal or exceed denitrification and that there can be a seasonal switch in their relative importance. Bonin et al. (1998) found maximal rates of denitrification in the spring while DNRA peaked in October. Tobias et al. (2001) observed high DNRA rates relative to denitrification in salt marsh sediments, and DNRA varied with the pattern of seasonal ground water flow. In a number of Texas estuaries DNRA rates also equal or exceed denitrification rates (An and Gardner 2002; Gardner et al. 2006) with DNRA rates rising with salinity. However, it could not be determined if salinity was driving the switch to DNRA, as hypoxia and sulfide concentrations increased in conjunction with salinity. Recently, Dong et al. (2009) reported the relative importance of DNRA increased toward the seaward end of the Colne Estuary. They attributed this pattern to changes in overlying nitrate concentration and suggested DNRA is favored under conditions of lower nitrate. Nitrate concentration may also play an important role in determining pathways, but in our system the relative importance of denitrification increased in late summer and autumn when salinity was reduced, even though nitrate concentrations were still very low.

What is supporting DNRA in the Parker River system during the summer? Calculated rates of ambient DNRA reach their maximum at the end of summer when nitrate concentrations in the estuary are extremely low and potential nitrification rates and the population of ammonium oxidizers are at their lowest levels (Bernhard et al. 2005, 2007). The rates of DNRA are two to five times higher than can be supported by nitrification, even if we assume that the highest rates of potential nitrification (Bernhard et al. 2007) occur in situ. Other investigators have reported that DNRA can be supported by intracellular nitrate stores, such as those accumulated by S oxidizing bacteria (Schulz and Jørgensen 2001; Sayama 2001) but in this situation a very long storage time would be required.

Another possibility is that the method we used overestimates DNRA. The assumption that the production of  $^{14}\text{N}/^{15}\text{N}$  measured for denitrification is the same for DNRA may not hold if the zone of DNRA extends below the zone of nitrification. In this case, the added nitrate could stimulate DNRA. However, we did not routinely see higher DNRA in the higher nitrate treatment. Another possibility is that potential nitrification rates may not reflect the nitrification rates of the whole in situ community.

However, we do not believe that the seasonal pattern of DNRA can be explained by methodological problems. It is clear that the fate of the added  $^{15}\text{NO}_3$  shifts seasonally. So, while there may be issues with the calculation of the ambient DNRA rates, the changing fate of the added nitrate indicates a major shift in nitrate reducing pathways over

the season. Why salinity appears to shift the relative importance of these two nitrate removal processes warrants further investigation.

## Summary

Sediments in the upper Parker River Estuary show large seasonal and inter-annual variations in the flux of ammonium to the overlying water, and in rates of denitrification and DNRA. These changes in N cycling processes do not appear to be driven by temperature or respiration but are linked to changes in the salinity of the overlying water. Lower salinities, such as those that usually occur in the spring and in fall, favor high rates of denitrification, low rates of DNRA, and low rates of ammonium fluxes. In contrast, higher salinities, which typically reach their maximum in late summer, are associated with low rates of denitrification, and maximal values of DNRA and ammonium fluxes. Changes in salinity are thus of central importance in regulating the form, fate, and eventual removal of DIN from the oligohaline region of the Parker River Estuary.

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