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Nitrification potentials of benthic macrofaunal tubes and burrow walls: effects of sediment NH_4^+ and animal irrigation behavior

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ABSTRACT: We examined the natural variation of nitrification potentials (NPs) of surface sediments and macrofaunal tubes and burrow walls in relation to sediment NH_4^+ level, season, and macrofaunal species. NP (the ability of a unit of sediment to oxidize NH_4^+ when NH_4^+ and O_2 are not limiting) is an index of the abundance and activity of nitrifying bacteria which we measured in slurries with the chlorate block technique (nmol NO_2^--N produced g^{-1} dry weight sediment h^{-1}). The NP of the tubes of the polychaete *Loimia medusa* was positively related to sediment NH_4^+ (KCl-extractable) concentration at 3 sites where tubes were collected in June 1990 (Spearman rank correlation coefficient $r_S = 0.90$, $p = 0.03$), as was the NP of surface (0 to 1 cm) sediment ($r^2 = 0.92$, $p = 0.002$). The degree to which tube NP exceeded the NP of surface sediment was, however, negatively associated with sediment NH_4^+ ($r_S = -0.84$, $p = 0.05$). Tube NP of *L. medusa* did not vary significantly with date (February, April, and June 1990). Tubes or burrow walls of *Macoma balthica* (bivalve), *Leptocheirus plumulosus* (amphipod), and the polychaetes *Macroclymene zonalis*, *Pectinaria gouldii*, *L. medusa*, and *Diopatra cuprea* had NPs significantly greater (2 to 20 times) than that of adjacent sediment from the same depth interval, indicating that these species stimulated nitrification. Except for burrows of *M. balthica*, the NPs of these structures were significantly ($p \leq 0.05$) greater (1.5 to 61 times) than that of surface sediment. The duration of macrofaunal irrigation activity, but not irrigation rate, was positively associated ($r_S = 0.72$, $p = 0.01$) with the enhancement of NP in tubes and burrow walls relative to surface sediment. These findings indicate that macrofaunal tubes and burrows tend to be sites of enhanced NP and that this enhancement varies among species due to variations in irrigation behavior. The NP of macrofaunal structures also varies among sites in relation to sediment NH_4^+ concentrations.

KEY WORDS: Nitrification potential · Benthic macrofauna · Tubes · Burrows · Ammonium · Irrigation

INTRODUCTION

Benthic macrofauna can significantly increase rates of nitrification (oxidation of NH_4^+ to NO_3^-) in sediments (Kristensen et al. 1991, Mayer 1992, Henriksen et al. 1993). Nitrification is performed chiefly by a specific group of chemoautotrophic bacteria that require O_2 as well as NH_4^+. Consequently, nitrification is generally restricted to a narrow zone of surface sediment where O_2 is present, typically the top 1 to 6 mm (Revsbech et al. 1980, Henriksen et al. 1981, Rasmussen & Jørgensen 1992). In organically rich sites, rates of nitrification are often limited by the depth of O_2 penetration (Henriksen & Kemp 1988, Kemp et al. 1990). By irrigating their tubes and burrows, macrofauna oxygenate additional sites for nitrification. Moreover, studies have shown that macrofaunal tubes and burrow walls have higher potential rates of nitrification than surface sediment, suggesting that these structures may provide a better environment for nitrifying bacteria (Blackburn & Henriksen 1983, Henriksen et al. 1983, Kristensen et al. 1985). The role of macrofauna in nitrification, and the underlying factors affecting this relationship, are important to understand because nitrification is a key

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link between nitrogen mineralization and the potential loss/export of nitrogen from marine and estuarine systems via denitrification (Seitzinger 1988, Kemp et al. 1990).

Although a variety of macrofaunal taxa stimulate nitrification (e.g. Aller et al. 1983, Henriksen et al. 1983), the effect of macrofauna on nitrification in the field is largely unknown due to methodological problems of measuring nitrification rates in sediment containing macrofauna. For example, use of the acetylene block technique (Sloth et al. 1992) is hindered by the fact that acetylene increases NH_4^+ excretion rates of macrofauna (Kristensen et al. 1991). Use of the nitrapyrin (N-serve) block technique is restricted to sediment with uniform distributions of macrofauna (Henriksen & Kemp 1988). In addition, nitrapyrin's effect on NH_4^+ excretion rates of macrofauna is unknown. Its use on sediment in the Bering Sea with uniformly distributed populations of bivalves or amphipods indicated, however, that a substantial portion of nitrification (25 and 65%, respectively) occurred in macrofaunal burrows (Henriksen et al. 1993). In methods involving the addition of $^{15}NH_4^+$, there are problems with determining the isotopic ratio of NH_4^+ at the actual sites of nitrification and with ensuring that the isotopic ratio of NH_4^+ in the surface nitrification zone equals that in nitrification zones associated with macrofauna.

A useful method for examining the macrofaunal effect on nitrification in natural sediments involves the measurement of nitrification potentials (NPs) of animal tubes or burrow walls and that of surface sediment. NP is the ability of a sediment mass (or volume) to oxidize NH_4^+ when NH_4^+ and O_2 are not limiting. Measurements of NP provide an index of the abundance and activity of nitrifying bacteria. NP cannot, however, be equated with biomass of nitrifying bacteria because NP on a per cell basis can vary seasonally and with the availability of O_2 (Smorczewski & Schmidt 1991). Measurements of NP are easily made and estimated rates of nitrification calculated from NP, oxygen penetration, and *in situ* temperature generally agree well with measured rates of nitrification (Henriksen et al. 1981). For a few species, the effect of macrofauna on *in situ* rates of nitrification has been estimated from the NPs of their tubes or burrow walls and that of surface sediment, along with measurements or estimates of animal density and O_2 penetration depths (Blackburn & Henriksen 1983, Kristensen et al. 1985, Mayer 1992). These studies indicated that more than 25% of benthic nitrification may occur in macrofaunal tubes and burrows.

Little is known about the factors regulating the NP of macrofaunal tubes and burrow walls or the resultant difference in NP between these structures and surface sediments. In this study, our goal was to investigate the variations in the NP of macrofaunal structures and surface sediments that are associated with sediment NH_4^+ concentrations, changes in season, and macrofaunal species. We expected that the NP of macrofaunal structures and sediments would be strongly affected by the availability of NH_4^+ in sediments and the irrigation activities of different species (which regulate O_2 availability in tubes and burrows).

METHODS

General approach. The effects of NH_4^+ availability and season on the NP of macrofaunal tubes and surface (0 to 1 cm) sediments were examined for natural sediment samples from Chesapeake Bay, USA, containing the polychaete *Loimia medusa*. All measurements were made in 1990. The effect of NH_4^+ availability in sediments was examined in June by determining the relationship between the NP of *L. medusa* tubes and sediment NH_4^+ concentration for samples collected from 3 estuarine sites (for details see 'Field sites' below) expected to differ in NH_4^+ availability. Tubes were analyzed from VIMS Beach (VB, n = 4), York River (YR, n = 2) and Wolf Trap (WT, n = 1) (see below for site descriptions). At each site, we measured the NH_4^+ concentration (KCl-extractable) of sediment at the surface (0 to 1 cm) and for the depth interval occupied by *L. medusa* tubes (0 to 13 cm; Mayer 1992). The measurements of NH_4^+ concentration and NP of surface sediment were made on subsamples of 2 replicate cores of bulk sediment. The effect of season was evaluated at a single site (VB) from the variability of NP of *L. medusa* tubes collected in February, April and June (n = 3, 3 and 4, respectively). The NP of surface sediment was measured on 2 replicate cores of bulk sediment in February and June.

To examine the variation in the NP of macrofaunal structures among macrofaunal species employing a range of irrigation behaviors, we measured the NP of tubes or burrow walls of the amphipod *Leptocheirus plumulosus*, the anemone *Ceriantheopsis americanus*, the bivalve *Macoma balthica* and the polychaetes *Macroclymene zonalis*, *Pectinaria gouldii*, *Loimia medusa*, and *Diopatra cuprea*. Tubes, burrow walls and sediment samples were collected in June from 4 different estuarine sites [VB, YR, WT and PK (Poropotank); see below]. NP measurements were also made for bulk surface (0 to 1 cm) sediments and for sediments in the depth interval occupied by each species (adjacent sediment). To further assess the effect of macrofaunal irrigation behavior on NP, we compared our results with those reported in the literature for 4 other species. These include the amphipod *Corophium volutator* (Henriksen et al. 1983), the bivalve *Mya arenaria*

(Henriksen et al. 1983), and the polychaetes *Lanice conchilega* (Henriksen et al. 1981, Blackburn & Henriksen 1983) and *Nereis virens* (Kristensen et al. 1985).

For species with large tubes or burrows, we also examined the variability of NP within individual structures to determine the necessity of sampling whole structures for accurate determination of their NP.

Field sites. Sampling was conducted at 4 sites in Chesapeake Bay. Much of this research was conducted at VB, an intertidal sandflat near the mouth of the York River estuary with sediment of fine to medium sands (Skrabal 1987), 37° 14' 85" N, 76° 30' 05" W. The second site, YR, is a muddy sediment site in the lower portion of that estuarine tributary, 37° 14' 59" N, 76° 9' 82" W (depth = 8 m). The third site, PK, is a muddy sediment site in the upper York River, 37° 26' 69" N, 76° 43' 81" W (depth = 3 m). The fourth site, WT, which is characterized by silty sand, is located in the mainstem of Chesapeake Bay, 37° 16' 06" N, 76° 09' 81" W (depth = 12 m).

Tubes of *Loimia medusa* (n = 4 in June as well as tubes collected for examining effect of season) and *Diopatra cuprea* (n = 5) were collected at VB. Additional tubes of *L. medusa* (n = 2) and 1 tube of *Ceriantheopsis americanus* were collected at YR. The burrow walls of *Leptocheirus plumulosus* [pooled samples, r (replicates) = 4] and oxidized sediment surrounding *Macoma balthica* (n = 2) were collected at PK. Tubes of *Macroclymene zonalis* (pooled samples, r = 4) and *Pectinaria gouldii* (n = 5) as well as additional tubes of *L. medusa* (n = 1) and *C. americanus* (n = 1) were collected at WT. Samples for *D. cuprea* were collected in 1989; samples for the other species were collected in 1990.

Sampling techniques and sediment processing. At the subtidal sites, sediment was collected with a U.S. Naval Electronics Laboratory spade box corer (20 × 30 cm, maximum depth of penetration = 60 cm). To collect 2 bulk sediment samples per site, we subcored (core i.d. = 2.5 cm) 2 replicate box cores. After the removal of a side plate, box cores were dissected to locate animal tubes and burrows which were identified and then sampled. Tube and burrow wall samples were placed into preweighed centrifuge tubes. At the intertidal site, tubes of *Diopatra cuprea* and *Loimia medusa* were excavated by hand or were extracted from sediment collected with a hand corer. Two replicate cores (i.d. = 2.5 cm) for bulk sediment samples were collected by hand. Samples (macrofaunal tubes or burrow walls and cores of bulk sediment) were held on ice until processed (≤12 h) to minimize changes in their NP or NH_4^+ content.

We distinguished the structures inhabited by benthic animals as either tubes or burrows and sampled them differently because of differences in their structural integrity. 'Tubes' are well-defined structures that remain intact when carefully removed from sediment, whereas 'burrows' have walls that readily disintegrate upon handling. As a result, the outer boundary of burrow walls is not clearly defined. Because we were interested in burrow walls as sites for nitrification, the strong color difference between the oxidized portion of the walls (light brown) and the adjacent reduced sediment (black) was used to operationally define the boundaries of burrow walls for sampling; only the light brown portion of burrow walls was sampled.

Tubes were carefully extracted from the sediment, and animals were removed. Tubes were submerged in a pan of water to detach loose sediment clinging to tubes, and then were gently blotted with paper towels. Only intact tubes were used for measurements of NP. After tube length was measured, large tubes were cross-sectioned into segments of about equal length to examine within-tube variation of NP. The small size of tubes collected from juvenile *Macroclymene zonalis* required pooling several tubes (5 to 10) for each sample. For most species, additional tubes were collected in a similar fashion for measurement of water content (to convert NP to a per gram dry weight basis; see below). For *Ceriantheopsis americanus*, large tubes were divided lengthwise to permit measures of NP and water content on subsamples from the same tube.

The oxidized walls of burrows inhabited by *Leptocheirus plumulosus* and the oxidized sediment surrounding *Macoma balthica* were sampled with a small spatula. Care was taken to collect oxidized material evenly along the entire length of the amphipod burrows or the body of *M. balthica*. Adjacent anoxic sediment was collected along the same depth interval 0.25 to 1.0 cm away from the boundary between oxic and anoxic sediment because bulk sediment at PK was riddled with amphipod burrows (>15000 ind. m^{-2}; Mayer 1992).

In the laboratory, bulk sediment cores were sliced into the following depth intervals: 0–1 cm (surface sediment), 1–3, 3–5, 5–9, 9–13, and 13–17 cm. Each section was divided into 3 subsamples for analysis of NP, KCl-extractable NH_4^+, and sediment water content (or into 2 subsamples when NH_4^+ was not measured). NPs of bulk sediment were used to represent the NP of sediment adjacent to tubes/burrows except at PK. For each species, average NP of adjacent sediment was calculated for either the depth interval covered by tubes or for the entire 17 cm interval sampled when tubes penetrated deeper than 17 cm (*Diopatra cuprea, Ceriantheopsis americanus*).

Measurement of NP. NP was measured in aerobic slurries with surplus NH_4^+ by the chlorate block technique (Belser & Mays 1980, Henriksen & Kemp 1988). Chlorate blocks the final conversion of NO_2^- to NO_3^- so that NP is measured as the production rate of NO_2^- per

unit sediment. The chlorate block technique was used because of the high sensitivity and ease of measuring NO_2^- by hand. After determination of sample wet weight, filtered (1.2 µm) station water and 10 mM NH_4Cl (final concentration 1 mM) and 1 M $NaClO_3$ (final concentration 10 mM) were added to the 50 ml centrifuge tubes in which slurries were incubated. Final incubation volume was fixed at 25 or 40 ml. Sediment samples generally varied in size between 2 and 3 g wet weight. After a 30 min preincubation period on a shaker table, each slurry was centrifuged, subsampled (7 ml) for initial concentrations of NO_2^-, resuspended and then returned to the shaker table. Slurries were incubated in the dark at room temperature (23 to 25°C) on a shaker table for 12 to 18 h before final sampling. The supernatant samples were filtered (glass-fiber filter, GF/F) and frozen for subsequent analysis of NO_2^-. Nitrite was analyzed by the azo dye method described in Parsons et al. (1984).

NPs were calculated as the amount of NO_2^- produced per gram dry weight of sample (sediment, tube or burrow material) per hour (nmol N g^{-1} dw h^{-1}). Rates were normalized to dry weight (versus volume) because of the difficulty in accurately determining the volume of animal tubes; our main objective was to examine the NP of tubes and burrow walls as well as compare them to the NP of sediments. Rates expressed in those terms are also ecologically relevant because nitrifying bacteria in sediments are generally associated with particles (Fenchel & Blackburn 1979). Dry weight of samples was determined from sample wet weight and the water content of similar material.

For large tubes or burrows that were sampled in sections to examine within-structure variation in NP, we used the following equation to calculate the NP of whole structures, NP_T (nmol N g^{-1} dw h^{-1}), from the weighted average of the NP of sections, NP_i (nmol N g^{-1} dw h^{-1}), with section weights W_i (g dw): $NP_T = (\sum W_i NP_i)(\sum W_i)^{-1}$.

Sediment NH_4^+ levels. The concentration of interstitial plus exchangeable NH_4^+ in sediment was measured using KCl extraction (Blackburn & Henriksen 1983). A volume of 10 ml of 1 N KCl was added to about 5 g wet sediment, and sediment and KCl were mixed by shaking. After a short extraction period (about 30 min), the samples were centrifuged, and the supernatant was removed, filtered (GF/F) and frozen. Ammonium concentration per cm^3 wet sediment was calculated from the water content of sediment by assuming a dry sediment bulk density of 2.5 g cm^{-3} for muddy sediment sites (Aller & Benninger 1981) and 2.65 g cm^{-3} for sandy sites (Allen 1985). Ammonium was analyzed by the phenol hypochlorite-indophenol blue method of Solorzano (1969) describ-ed in Parsons et al. (1984).

Statistical analyses. All measurements are reported as means ± SD. Standard deviations of ratios (e.g. NPs of tubes per NP of surface sediment) were calculated with regard to propagation of error (Meyer 1975). ANOVA was used to test for statistical differences among 3 or more means (values for different sites or dates) after data passed tests for normality. When ANOVA indicated a significant difference ($p < 0.05$), the Student-Newman-Keuls multiple range test (SNK) was used to compare means. Student's *t*-test was used to test, for each species, whether the NP of tubes or burrows was significantly greater than the NP of adjacent and surface sediment. We evaluated the relationship between NP of surface sediment and sediment NH_4^+ concentration with regression analysis. We used Spearman rank correlation analysis with correction for ties (Siegel 1956) to test for significant relationships between 2 variables when the use of nonparametric statistics was more appropriate (e.g. one of the variables was not necessarily normally distributed or was the ranking of species according to categories of irrigation rate or time spent irrigating). It was also used to test for a positive association between tube NP and ambient NH_4^+ level due to within-site variation in NH_4^+.

RESULTS

Sediment NH_4^+ effects on NP

Average NH_4^+ concentrations in the 0 to 13 cm depth interval (in which tubes resided) differed significantly (ANOVA: $p = 0.002$; SNK: $p < 0.05$) among all 3 sites where *Loimia medusa* tubes were collected (YR > WT > VB) whereas only the YR site differed significantly (ANOVA: $p = 0.02$; SNK: $p < 0.05$) from the other 2 sites in terms of the NH_4^+ concentrations at the sediment surface (Table 1). The 10-fold increase in sediment NH_4^+ for the 0 to 13 cm depth interval between VB and YR was accompanied by a significant (Student's *t*-test: $p = 0.02$), 30-fold increase in NP of *L. medusa* tubes.

Table 1. Sediment NH_4^+ concentrations (KCl-extracted, n = 2) at sites where samples were collected for examination of effect of sediment NH_4^+ on NP of both *Loimia medusa* tubes and surface sediment. Means ± SD. Values within a column with the same letter are not significantly ($p < 0.05$) different

Site	Conc. (nmol NH_4^+ cm^{-3})	
	0-1 cm	0-13 cm
York River	373 ± 86^A	699 ± 146^A
Wolf Trap	110 ± 29^B	202 ± 3^B
VIMS Beach	54 ± 37^B	71 ± 5^C

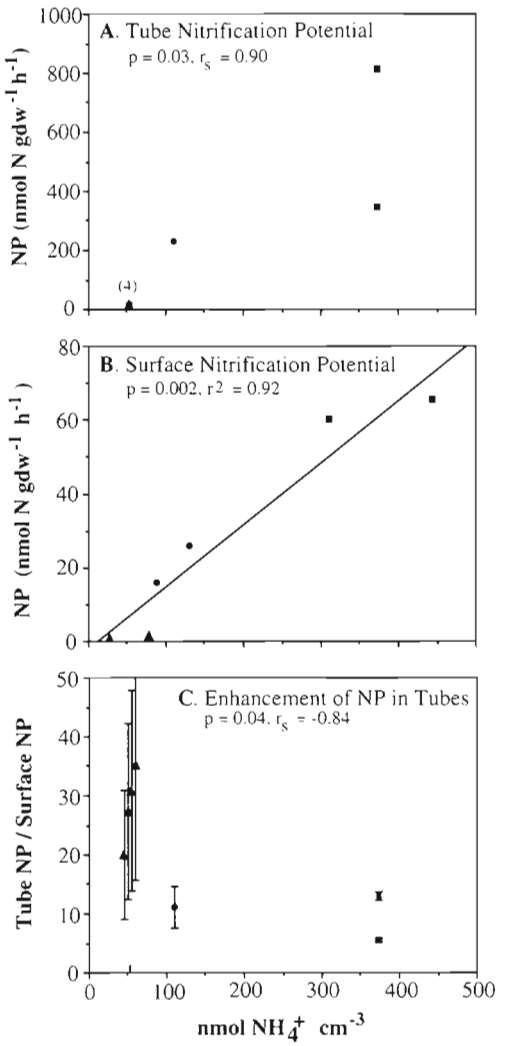

Fig. 1. Effect of ambient sediment NH_4^+ concentrations (KCl-extracted, 0 to 1 cm) on nitrification potentials (NP, nmol N g^{-1} dw h^{-1}) of *Loimia medusa* tubes, surface sediment (0 to 1 cm), and the enhancement of NP in tubes relative to surface sediment (tube NP/surface NP). Symbols identify collection site: VB (▲), WT (●), YR (■). r_S = Spearman rank correlation coefficient. (A) NPs for individual *L. medusa* tubes (VB, n = 4; WT, n = 1; YR, n = 2) versus average (n = 2) NH_4^+ at site. (B) Surface NP versus NH_4^+ concentration for individual cores. (C) Enhancement of NP in tubes relative to surface sediment versus NH_4^+ at site. Means ± SD of ratio (given variation in NP of surface sediment at each site). Points at VB are staggered for clarity; actual location indicated on *x*-axis

The NP of individual *L. medusa* tubes (n = 7) was positively associated with the average sediment NH_4^+ concentrations for both the 0 to 1 cm (Spearman rank correlation: p = 0.03; Fig. 1A) and 0 to 13 cm (relationship about identical in appearance to Fig. 1A) depth intervals. Similarly, the NP of surface sediment varied in a positive, approximately linear relationship with NH_4^+ concentration of surface sediment (p = 0.002; Fig. 1B). At all 3 sites, the NP of *L. medusa* tubes was greater than that of surface sediment. The degree to which the NP of tubes exceeded that of surface sediment was, however, negatively associated (Spearman rank correlation: p = 0.04) with the average NH_4^+ concentration of surface sediment where tubes were collected (Fig. 1C).

Given the observed relationship between NP of *Loimia medusa* tubes and the concentration of NH_4^+ in sediments (Fig. 1A), we also tested for a similar relationship for pooled data of 7 macrofaunal species whose tube/burrow NP exceeded that of adjacent or surface sediment (see Table 3). There was, however, no detectable association between the NP of macrofaunal tubes and burrow walls (mixed species, varying number of replicates per species) and the average NH_4^+ concentration of surface sediment where structures were collected (Spearman rank correlation: p = 0.12; Fig. 2A). In addition, there was no detectable association between the degree to which tube or burrow wall NPs exceeded surface sediment NP and surface sediment NH_4^+ level (p = 0.09, Fig. 2B).

Seasonal variations in nitrification potential

There were no significant differences among the NPs of tubes of *Loimia medusa* collected at the intertidal site (VB) in February, April and June (Table 2). The average NP for all of the tubes collected at this site was 17.6 ± 3.7 nmol N g^{-1} dw h^{-1}. The NP of *L. medusa* tubes was significantly greater than that of adjacent and surface sediment (Student's *t*-test: p < 0.01). Unlike the NP of tubes, the NP of surface sediment varied greatly between dates (Table 2). The NP of *L. medusa* tubes was 361 times greater than that of surface sediment in February, whereas it was only 28 times greater in June.

Variation of NP within tubes

The tubes or burrows of *Ceriantheopsis americanus*, *Diopatra cuprea*, *Loimia medusa* and *Macoma balthica* were large enough to permit examination of variation in NP within individual structures. Only the tubes of *L. medusa* and *D. cuprea* demonstrated substantial within-structure variation in NP. For both species, the amount of variation differed among individuals. For *D. cuprea*, the maximum NP within each tube was 3 to 43 (mean = 20) times greater than the minimum. The tubes of *L. medusa* exhibited substantial within-tube variation in NP on all dates they were collected (Table 2). The variation was greatest in June when the maximum NP within each tube was 5 to 24 (mean = 14)

Table 2. Seasonal variation in nitrification potentials (NP, nmol N g^{-1} dw h^{-1}) of *Loimia medusa* tubes and surface (0 to 1 cm) sediment (n = 2) at site VB. Variation in NP within individual tubes reported as the ratios of the maximum NP of sections in the tube (Max. NP_i) to both the NP of the whole tube (NP_T) and to the minimum NP of sections in the tube (Min. NP_i). Means ± SD. na: data not available

Month	Temp. (°C)	No. tubes	NP_T	$\frac{\text{Max. } NP_i}{NP_T}$	$\frac{\text{Max. } NP_i}{\text{Min. } NP_i}$	Surface NP
February	8	3	15.9 ± 2.8	2.6 ±0.2	9 ± 4	0.04 ± 0.01 (0.07 ± 0.01)[a]
April	13	3	17.7 ± 4.3	2.0 ± 0.8	4 ± 1	na
June	26	4	18.8 ± 4.3	2.6 ± 1.1	14 ± 8	0.67 ± 0.37 (1.02 ± 0.56)[a]
Pooled	–	10	17.6 ± 3.7	2.4 ± 0.8	9 ± 6	–

[a]Surface NP in nmol cm^{-3} h^{-1}

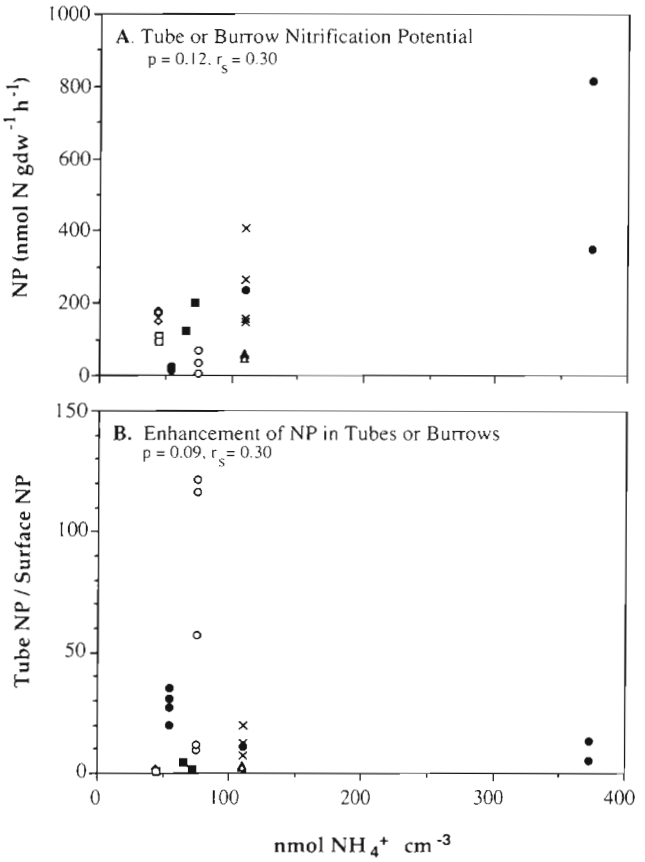

Fig. 2. Influence of ambient sediment NH_4^+ concentrations (KCl-extracted, 0 to 1 cm) on both (A) the nitrification potential (NP, nmol N g^{-1} dw h^{-1}) of macrofaunal tubes or burrows and (B) the degree of enhancement of NP in tubes or burrows relative to surface (0 to 1 cm) sediment for species stimulating nitrification. Species were identified as follows: *Diopatra cuprea* (○, n = 5); *Loimia medusa* (●, n = 7); *Leptocheirus plumulosus* (◇, n = 4, pooled samples); *Macoma balthica* (□, n = 2); *Macroclymene zonalis* (△, n = 4, pooled samples); *Nereis virens* (■, n = 2, mean for site); and *Pectinaria gouldii* (×, n = 5) (see references in Table 3). r_S = Spearman rank correlation coefficient

times greater than the minimum. Although the range in NP within individual *L. medusa* tubes varied seasonally, the maximum NP within the tubes was generally about 2.5 times greater than the overall tube NP (Table 2).

To examine the pattern of NP within tubes, NPs of tube sections were expressed as proportions of the maximum value within each tube. Only tubes of *L. medusa* demonstrated a consistent pattern of NP with position in tube. For tubes sampled in June (n = 4), NPs of tube sections were significantly associated (Spearman rank correlation: $p < 0.01$) with location within the tube (Fig. 3). The asymmetry in tube NP was associated with an asymmetry in tube thickness (weight per cm length); the thicker side had the higher NP. No consistent pattern of within-tube variation in NP was detected for *L. medusa* tubes collected in February (not shown) or April (Fig. 3).

Variation in NPs with macrofaunal species

For the 7 species of macrofauna examined, the NP of tubes and burrow walls ranged from 1.6 to 581 nmol N g^{-1} dw h^{-1} (Table 3). Tubes of the anemone, *Cerianthopsis americanus*, had the lowest NP whereas tubes of *Loimia medusa* had both the second lowest and the highest average NP because of the variation in tube NP between sites (Table 3). The variation in tube NP between individuals was high (CV > 40%) for *Pectinaria gouldii*, *L. medusa* and *Diopatra cuprea*, even for structures collected at the same location (Table 3). The CV was highest for tubes of *D. cuprea* (86%).

The NP of tubes or burrow walls was significantly greater (Student's *t*-test: $p < 0.05$, 2 to 200 times) than that of adjacent sediment (Table 3) for the amphipod *Leptocheirus plumulosus*, the bivalve *Macoma balth-*

ica and the polychaetes *Macroclymene zonalis*, *Pectinaria gouldii*, *Loimia medusa* and *Diopatra cuprea*. We did not calculate a ratio for *P. gouldii* because its vertical mobility precluded attempts to assign individuals to a particular depth interval, but the NP of its tubes exceeded that of surface sediment (which exceeded NP of other sediment depths). The 100-fold range in the enhancement of NP in tubes or burrows of these species relative to adjacent sediment (expressed as the ratio between the 2 values) was larger than the 30-fold range in the NP of tubes and burrows (Table 3). The ratio between the NP of tubes or burrows and that of adjacent sediment was lowest for *M. zonalis* and, on average, was highest for *D. cuprea*. In contrast to the species discussed above, the NP of *Ceriantheopsis americanus* tubes was lower than that of adjacent sediment (12% of adjacent sediment NP).

The NP of tubes or burrow walls was significantly greater ($p < 0.05$; 1.5 to 61 times) than that of surface sediment for 5 of the 6 species whose tube/burrow NP exceeded that of adjacent sediment (Table 3). For the 1 exception, *Macoma balthica*, the NP of burrow walls about equaled that of surface sediment. Tubes of *Diopatra cuprea* had the highest but most variable enhancement of NP relative to that of surface sediment.

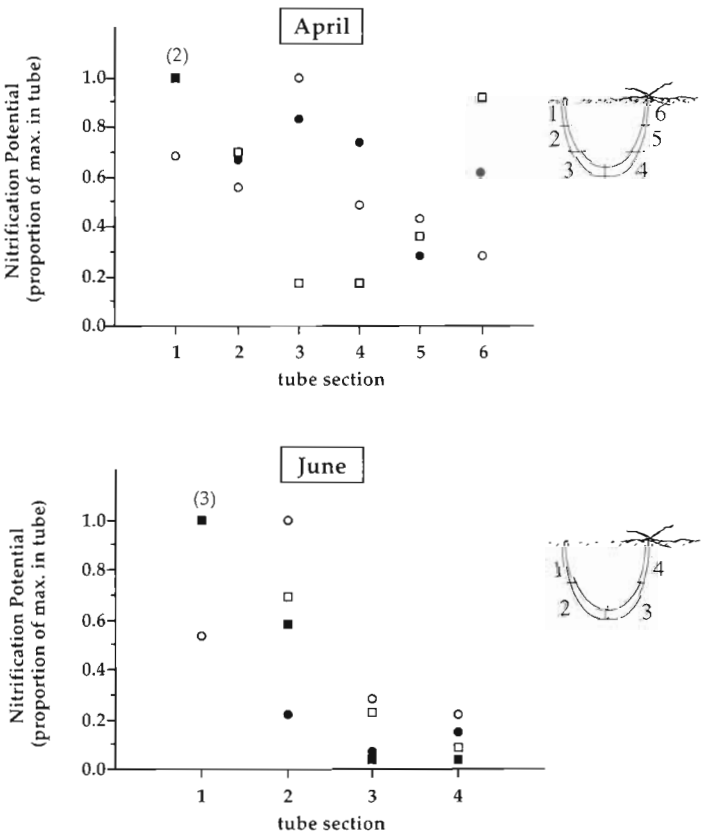

Fig. 3. Pattern of variation in nitrification potential (NP, nmol N g^{-1} dw h^{-1}) within individual tubes of *Loimia medusa* collected in April (n = 3) and June (n = 4) at site VB. Plotted NPs of tube sections expressed as proportion of the maximum value within each tube. Individual tubes represented by different symbols. Numbers over symbols indicate number of identical data points. In June, NPs of sections were associated with their position within the tube (Spearman rank correlation: $p < 0.01$, $r_S = 0.81$)

Effects of macrofaunal irrigation on NP

Among the species examined in this or previous studies (cited in Table 3), the NP of tubes or burrow walls was greater than that of adjacent or surface sediment for macrofauna that introduce O_2 to subsurface sediment (Table 3). To examine the degree to which species irrigation behavior influences the NP of tubes or burrow walls and the enhancement of NP in these structures relative to surface sediment, we categorized all macrofauna according to the rate at which they pumped water when irrigating (Table 3). Because most infaunal animals irrigate intermittently (Kristensen 1988), we also classified species according to the amount of time spent irrigating (Table 3). General categories were used for several reasons. First, species were classified according to reports in the literature where data were collected under diverse conditions. Secondly, in some cases, values for close relatives were used when information for a particular species was unavailable. Finally, for many species, irrigation behavior is very flexible; irrigation rate and time spent irrigating vary in response to changes in temperature, and concentrations of O_2, sulfide, and food (Mangum & Burnett 1975, Mangum 1976, Kristensen 1983, 1989, Woodin & Marinelli 1991). Categories for irrigation rate include: (1) zero (no direct irrigation); (2) low irrigation rates (<10 ml h^{-1}); (3) intermediate irrigation rates (25 to 120 ml h^{-1}); and (4) high irrigation rates (*Nereis virens* is distinguished from the rest by its irrigation rate of approximately 180 ml h^{-1}; Kristensen et al. 1991). Categories for time spent irrigating include: (1) zero; (2) low (≤30%); and (3) high (≥50%). *Pectinaria gouldii* was not classified due to lack of information.

Spearman rank correlation analysis was used to determine whether tube NP or the enhancement of NP in tubes increased with species irrigation rate or the time species spend irrigating. The NP of tubes or burrow walls [mean for each data set (site or date) for each species] was not significantly associated

Table 3. Species comparison of nitrification potentials (NP) of macrofaunal tubes and burrow walls and their enhancement relative to NP of both surface (Surf., 0–1 cm) and adjacent (Adj., depth interval occupied by tube/burrow) sediment. Species categorized according to irrigation rate (ml water h^{-1} during irrigation: Z = zero; L = low, <10; I = intermediate, 25–100; H = high, >150) and duration (% time spent irrigating: Z = zero; L = low, ≤30; H = high, >50) based on literature values (reported in parentheses). Generally reported mean ± SD, but reported range of means when data available for more than 1 site or date. na: data not available

Species	Tube or burrow NP (nmol N g^{-1} dw h^{-1})	NP ratios[a]		$[NH_4^+]$[b] (nmol cm^{-3})	Irrigation behavior[c]		Source
		Tube:Surf.	Tube:Adj.		Rate (ml h^{-1})	Duration (%)	
Polychaeta							
Diopatra cuprea	36 ± 31	61 ± 54*	200 ± 172*	75 ± 9	I (68)	H (>50)	This study
Lanice conchilega	179 to 292	15 to 20	na	na to 820	I (38–100)	H (60–100)	Blackburn & Henriksen (1983)[d,e]
Loimia medusa	19 to 581	9 to 28*	11 to 28*	54 to 373	I (38–100)	H (60–100)	This study (June)
Macroclymene zonalis	54 ± 7	2.6 ± 0.9*	2.3 ± 0.3*	110 ± 29	L (2)	L (<5)	This study
Nereis virens	124 to 203	1.7 to 4.1	na	65 to 72	H (180)	L (20–28)	Kristensen et al. (1985)[d,f]
Pectinaria gouldii	249 ± 106	11.9 ± 6.4*	na	110 ± 29	na	na	This study
Amphipoda							
Corophium volutator	29	2.7	2.7	na	I (60)	H (>50–100)	Henriksen et al. (1983)[d,g]
Leptocheirus plumulosus	164 ± 6	1.5 ± 0.4*	4.6 ± 1.1*	45 ± 12	I (60)	H (>50–100)	This study
Bivalvia							
Macoma balthica	100 ± 9	0.9 ± 0.2	9.8 ± 0.9*	45 ± 12	Z	na	This study
Mya arenaria	27	2.2	4.2	na	Z	na	Henriksen et al. (1983)[d,g]
Anthozoa							
Ceriantheopsis americanus	1.6 ± 0.4	0.06 ± 0.05	0.12 ± 0.08	110 to 373	Z	Z	This study

[a] Calculated propagation of error for SD of ratios (Meyer 1975)

[b] KCl-extracted NH_4^+ of sediment, 0–1 cm depth interval except for *L. conchilega* (0–2 cm)

[c] For actual species or related taxa (*L. medusa*, *M. zonalis*, *L. conchilega*, *L. plumulosus*). Compiled from Dales (1961), Mangum (1964, 1976), Sassaman & Mangum (1974), Aller & Yingst (1978), Foster-Smith (1978), Henriksen et al. (1983), Kristensen (1988), Kristensen et al. (1991), Winsor et al. (1990), Woodin & Marinelli (1991)

[d] To convert NP values expressed as cm^{-3} to g^{-1} dw, we used dry bulk density of 2.65 g cm^{-3} for sand (Allen 1985)

[e] Sediment NP from Henriksen et al. (1981). Estimated tube NP from rate of NO_3^- production in seawater at *in situ* temperature and $[NH_4^+]$ by correcting for higher temperature of NP assay (22°C) with Q_{10} = 2.5 (Hansen et al. 1981)

[f] Estimated KCl-extractable NH_4^+ from interstitial NH_4^+ provided by Kristensen (pers. comm.)

[g] Values for burrow wall NP read from figures

* Tube NP significantly greater (Student's *t*-test, $p < 0.05$) than NP of surface or adjacent sediment

with species irrigation rate ($p = 0.07$; Fig. 4A) or the percentage time species spent irrigating ($p = 0.21$; Fig. 4B). The enhancement of NP in tubes or burrow walls relative to that of surface sediment was not associated with species irrigation rate ($p = 0.21$; Fig. 4C) However, there was a significant, positive association between the enhancement of NP in tubes or burrows and the time species spent irrigating ($p = 0.01$; Fig. 4D)

DISCUSSION

Previous investigations have reported 6 macrofaunal species whose tube or burrow NP exceeds that of adjacent or surface sediment. Of the 7 additional species examined in the present study, 6 stimulated nitrification in their tubes or burrows (i.e. tube or burrow NP > NP of adjacent sediment). The NP of macrofaunal tubes or burrow walls must exceed that of surrounding sediment from the same depth interval to prove that nitrifying bacteria grow (i.e. nitrification occurs) in these structures, because measurable NP is commonly found in sediment below the narrow zone of oxygenated surface sediment. Nitrifying bacteria transported downward by physical disturbance or bioturbation can survive without O_2 for a prolonged period (Hansen et al. 1981). Of the 6 additional species found to stimulate nitrification in their tubes or

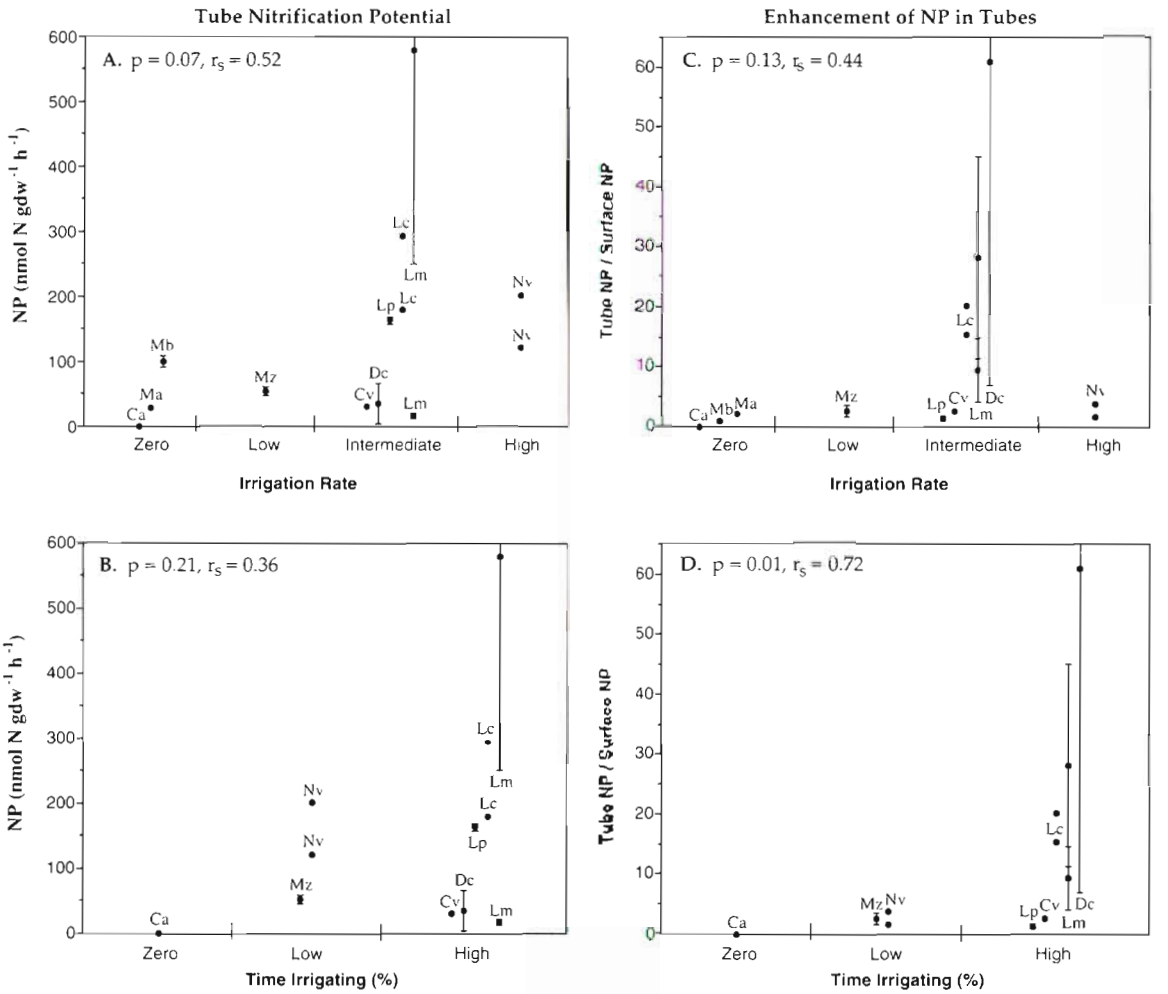

Fig. 4. Influence of irrigation behavior of macrofaunal species on both the nitrification potential (NP, nmol N g^{-1} dw h^{-1}) of macrofaunal tubes or burrows and the degree of enhancement of NP in tubes or burrows relative to surface (0 to 1 cm) sediment (r_S = Spearman rank correlation coefficient). Species are identified as follows: (Ca) *Ceriantheopsis americanus*; (Cv) *Corophium volutator*; (Dc) *Diopatra cuprea*; (Lc) *Lanice conchilega*; (Lm) *Loimia medusa*; (Lp) *Leptocheirus plumulosus*; (Mb) *Macoma balthica*; (Mz) *Macroclymene zonalis*; (Ma) *Mya arenaria*; (Nv) *Nereis virens* (see references in Table 3). Means ± SD (except SD not available for Cv, Lc, Ma, Nv). Presentation of species within each category is staggered for clarity. (A & C) Effect of irrigation rate (when irrigating): Zero; Low (<10 ml water h^{-1}); Intermediate (25 to 100 ml water h^{-1}); High (>150 ml water h^{-1}). (B & D) Effect of time spent irrigating: Zero; Low (≤30%); High (>50%)

burrows, 5 appeared to provide a better environment for nitrifying bacteria than surface sediment (NP tube > NP surface sediment; Table 3). While variable between species, different environments, and individuals, the degree to which NP of tubes and burrow walls exceeds that of surface sediment can be substantial; 4 of the 13 species now examined had tube NPs greater than 10 times the NP of surface sediment: *Pectinaria gouldii*, *Lanice conchilega*, *Loimia medusa* and *Diopatra cuprea* (in order of increasing enhancement).

The lack of seasonal variation in the NP of *Loimia medusa* tubes collected at the intertidal site (Table 2) suggests that macrofaunal tubes can be relatively stable sites for nitrifying bacteria. Actual rates of nitrification, however, may vary seasonally due to changes in temperature and the availability of NH_4^+ and O_2. The contrasting lack of stability in the NP of surface sediments (Table 2) suggests that whatever factors limited the NP of surface sediment in February (perhaps disturbance by winter storms and ice) were relatively unimportant in the environment provided by macrofaunal structures. It also suggests that macrofaunal structures may be especially important sites for nitrification when nitrification at the sediment surface could be limited by low NP.

Before discussing the significance of the enhancement of NP in macrofaunal structures relative to surface sediment or the possible regulation of NP by sediment NH_4^+ and macrofaunal irrigation behavior, we should discuss a few points concerning methodology. First, the substantial variation (14- to 20-fold) in NP among different sections of individual tubes of *Loimia medusa* and *Diopatra cuprea* indicates that only whole tubes or complete sampling of burrow walls should be used to accurately assess the NP of macrofaunal tubes or burrows. Second, we may have slightly underestimated the NP of our samples because the chlorate block can be incomplete (87% versus 100% efficient; Smorczewski & Schmidt 1991). This fact should not, however, affect our findings or their implications because they depend upon comparisons between the NP of samples (e.g. tubes and adjacent sediment) which were measured by the same technique. Although we did include data from other studies in our examination of the relationships between species irrigation behavior and the effect of macrofauna on NP, one of the studies (Kristensen et al. 1985) also used the chlorate block method. For the studies that did not use chlorate, the extent of the underestimation of NP is similar to the extent to which our measurements would have been increased by a slightly greater incubation temperature (23 to 25°C versus 22°C).

Effect of sediment NH_4^+ levels on NP

Ammonium availability appears to play a substantial role in the regulation of NP because the NP of both *Loimia medusa* tubes and surface sediment increased significantly with sediment NH_4^+ concentration (Fig 1). The fact that the highest and second lowest reported values of tube or burrow NP were for tubes of the same species collected from different NH_4^+ environments (Table 3, Fig. 2A) underscores the sizable role that NH_4^+ availability in sediment plays in determining the NP of macrofaunal structures. Results of a few other studies also suggest that NH_4^+ availability influences NP of animal tubes or burrow walls (Blackburn & Henriksen 1983, Kristensen et al. 1985, Henriksen unpubl. in Henriksen & Kemp 1988).

Considering the fact that nitrification requires NH_4^+, regulation of NP by NH_4^+ availability is not surprising. This relationship, however, has not been widely recognized. Limitation at the sediment surface of both NP (Fig. 1B) and actual rates of nitrification (Mayer 1992) by NH_4^+ availability could be responsible for the relatively greater NP of macrofaunal tubes and burrow walls (Kristensen et al. 1985, Henriksen & Kemp 1988, this study). Although we did not measure NH_4^+ levels in macrofaunal tubes or burrow walls, NH_4^+ should be more available in these structures than at the sediment surface because of increases in sediment NH_4^+ concentration with depth (Berner 1980, this study), and excretion of NH_4^+ by macrofauna. In addition, higher rates of NH_4^+ production in tubes or burrow walls, associated with enhanced bacterial and protozoan activity (Alongi 1985, Reichardt 1988), would also increase NH_4^+ availability for nitrifying bacteria in tubes/burrows. The negative association between NH_4^+ concentration at the sediment surface and the ratio of tube NP to NP of surface sediment (Fig. 1C) also suggests the importance of NH_4^+ availability to the enhancement of NP in macrofaunal structures. In other words, the benefit to nitrifying bacteria from residing in macrofaunal tubes or burrows versus surface sediment appears to be smaller in higher NH_4^+ environments, where NH_4^+ limitation of nitrification at the sediment surface would be less acute. The degree to which NP of *Lanice conchilega* tubes (Henriksen et al. 1981, Blackburn & Henriksen 1983) and *Nereis virens* burrow walls (Kristensen et al 1985) exceeded that of surface sediment was also smaller in higher NH_4^+ environments.

Variation of NP within tubes

The pattern of decreasing NP along the length of *Loimia medusa* tubes collected in June (Fig. 3) suggests that a similar gradient existed in the availability of the

substrate controlling nitrification along the length of the tube. Previous observations of worm orientation relative to tube thickness (Mayer pers. obs.) and terebellid irrigation behavior (Dales 1961) suggest that the general pattern of water flow in these U-shaped tubes was with water entering at the side with the higher NP. Because O_2 is consumed by macrofaunal and microbial respiration as water passes along the length of the tube, the tube entrance would be the most oxygenated. Therefore, the availability of O_2 probably regulated the pattern of NP within individual *L. medusa* tubes at the intertidal site in June. The fact that the degree and pattern of variation in NP within individual *L. medusa* tubes varied seasonally (Table 2, Fig. 3) suggests that the relative importance of O_2 in regulating NP within these tubes also changed seasonally.

Effect of macrofaunal irrigation behavior

Clearly, species differences affect the NP of macrofaunal structures. In the cases of *Leptocheirus plumulosus* and *Macoma balthica*, *Macroclymene zonalis* and *Pectinaria gouldii*, and *Loimia medusa* and *Ceriantheopsis americanus*, the NP of macrofaunal structures from the same collection site differed between species (Table 3). The effect of sediment NH_4^+ environment on NP of tubes and burrow walls, however, complicates species comparisons (across sampling sites) of the quality of macrofaunal structures as sites for nitrifying bacteria. The ratio of tube or burrow NP to NP of surface sediment provides a better index for comparing species. It indicates the degree of enhancement of NP in tubes and burrows relative to surface sediment and also appears to successfully reduce variation associated with collection site (probably because tube NP and NP of surface sediment both increase with sediment NH_4^+; Fig. 1). In the case of *L. medusa*, the ratio reduced the 30-fold variation in tube NP by a factor of 10. In addition, the ratio appears more sensitive to species differences; the variation among species in enhancement of NP in tubes and burrows relative to surface sediment was twice the variation in tube or burrow NP (Table 3).

The large variation in macrofaunal irrigation behavior could be responsible for a major portion of the variation among species in the enhancement of NP in these structures via its effect on the supply of O_2 in these locations. Given the fact that O_2 availability in tubes and burrows is generally lower than at the sediment surface (Kristensen 1988), the supply of O_2 in tubes and burrows probably limits the degree to which other traits make them better environments for nitrifying bacteria than the sediment surface. The enhancement of NP in tubes or burrow walls relative to surface sediment appeared to be positively related to macrofaunal irrigation behavior; the structures exhibiting the greatest enhancement of NP were inhabited by strong irrigators, i.e. macrofauna with at least intermediate irrigation rates and irrigating their tube or burrow more than half of the time (Table 3 & Fig. 4).

Enhancement of NP in tubes or burrows is not, however, determined solely by irrigation behavior. The bivalves *Macoma balthica* and *Mya arenaria* increased the NP of their burrows relative to NP of adjacent sediment (Table 3) even though these species do not advect water through sediment (Winsor et al. 1990). A thin layer of sediment surrounding these bivalves is oxidized (Henriksen et al. 1983, Mayer pers. obs.) apparently via diffusion of O_2 across the organism's body wall. The tendency for such a diffusive release of O_2 to be relatively slow probably accounts for the fact that the NP of sediment surrounding these bivalves was equal to or just slightly greater than that of surface sediment. Sassaman & Mangum (1974) predicted that for *Ceriantheopsis americanus*, the non-irrigator whose tube showed no enhancement of NP relative to adjacent sediment, diffusion of O_2 across the body wall would be insignificant.

Of the 2 main traits comprising macrofaunal irrigation behavior, rate and duration, the latter appears to explain a greater fraction of the variation among species in the enhancement of NP in tubes and burrows relative to surface sediment. For the species examined, the enhancement of NP in tubes and burrows was positively associated with the percentage time species spent irrigating, but not with species irrigation rate (Fig. 4). The overriding importance of the duration of irrigation activity is demonstrated dramatically by the low enhancement of NP in burrow walls of *Nereis virens* despite the species' high irrigation rate (Table 3, Fig. 4C). The O_2 level in *N. virens* burrows approaches that at the sediment surface when the polychaete is irrigating, but *N. virens* spends only 20 to 30% of its time irrigating and O_2 is consumed shortly (5 to 10 min) after the polychaete stops (Kristensen 1985, 1989). The lack of a positive association, in this study, between species irrigation rate and the enhancement of NP in macrofaunal structures does not mean that irrigation rate is unimportant. Enhancement of NP might be greatest at intermediate irrigation rates (see below) or it might increase with irrigation rate as long as the animals spend an adequate amount of time irrigating. A larger data set including more species with high irrigation rates is needed to evaluate these 2 possibilities.

Irrigation exports NH_4^+ from tubes and burrows as well as importing O_2. At highest rates of water turnover, the availability of NH_4^+ in tubes and burrows might be depressed sufficiently to limit the benefit to nitrifying bacteria of residing in these structures rela-

tive to surface sediment. This mechanism might explain why 2 strong irrigators, *Corophium volutator* and *Leptocheirus plumulosus* (water turnover rate estimated at ≥1 min^{-1}), demonstrated only mild enhancement of NP in the walls of their burrows (burrow NP just 2 to 3 times NP of surface sediment; Table 3, Fig. 4).

Average rate of water turnover in tubes or burrows might provide the best 'single' species trait for predicting the availability of O_2 in tubes and burrows and consequently for predicting species differences in the enhancement of NP in these macrofaunal structures. It integrates both irrigation rate and duration and also accounts for species differences in the size of tubes and burrows. Measurements of irrigation rates and duration as well as rates of water turnover in burrows and tubes were beyond the scope of this study (a preliminary survey of the effects of species differences and sediment NH_4^+ availability on the NP of macrofaunal tubes and burrows), but should be utilized in future studies investigating factors regulating the NP of macrofaunal tubes and burrows.

In conclusion, we find that macrofaunal tubes and burrows tend to be sites of greatly enhanced NP compared to both adjacent anaerobic sediments and oxidized surface sediments. This enhancement varies among species, apparently due to variations in irrigation behavior. The NP of macrofaunal structures also varies among sites in relation to sediment NH_4^+ concentrations.

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