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# Diffusion of organic and inorganic solutes through macrofaunal mucus secretions and tube linings in marine sediments

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ABSTRACT

Transport models in sediments commonly assume that diffusion occurs through water saturated pore space and that diffusive properties are largely homogeneous and isotropic. The bioturbated zone of marine sediments is characterized by sediment pores filled with mucus gel and criss-crossed by organic membranes that line macrofaunal tubes and burrows. Diffusion experiments utilizing pedal mucus from the naticid snails, *Neverita* (=*Polinices*) *duplicata* and *Euspira* (=*Lunatia*) *heros*, and organic tube linings from the polychaetes *Onuphis jenneri*, *Diopatra cupria*, and *Chaetopterus variopedatus*, demonstrated that the diffusion of both organic and inorganic solutes is inhibited by these common biogenic components. Diffusion of porewater DOC and $Br^-$ tracer through mucus is reduced by factors typically 3–8X relative to free solution. Diffusion rates of DOC and $Br^-$ through mucus and tube linings demonstrate that both charge and size inhibition commonly occur, however, charge discrimination was not observed for a range of inorganic solutes within mucus cements formed by the polychaete *Melinna cristata*. Diffusion of polystyrene sulphonates having varied molecular weights shows that inhibition of diffusion by mucus gel increases regularly with molecular size. No size exclusion or cutoff was observed up to molecular weights of at least 100 kDa. Although increases of solution viscosity by mucus (up to $\sim$ 170 mpoise), could explain solute diffusion inhibition to some extent, size and charge inhibition patterns imply that both mucus and tube linings behave as polyelectrolyte, fibrous meshworks with species specific properties (e.g. open channel patterns) rather than as polyelectrolyte solutions *per se*. The measured diffusion rates of bulk porewater DOC (0.387 $cm^2\ d^{-1}$, 5°C) and of specific polystyrene sulphonates in sea water are substantially higher than predicted by extrapolation from measurements in distilled water, presumably as a result of ionic strength effects on molecular conformations. The transport of solutes, particularly DOC, in the bioturbated zone is greatly complicated by the presence of semipermeable mucus secretions and tube linings. Differential inhibition by biogenic secretions of the transport of specific classes of organic molecules such as exoenzymes, may be especially important to understanding faunal adaptations, processes governing the remineralization of organic matter, and linkages between macrofauna and microbial activities.

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## 1. Introduction

Molecular diffusion of solutes is one of the major transport processes controlling early diagenetic reactions, biogeochemical cycling, and sediment - water exchange in sedimentary deposits (Berner, 1981; Boudreau, 1997). It is commonly assumed that within water-saturated sediments, solutes migrate through fluid-filled interstitial voids, the microgeometries of which are controlled by particle size, shape, and packing structure. The primary differences between diffusion in such a porous medium and in free solution are accounted for in the geometric correction factors porosity ($\varphi$), tortuosity ($\theta$), and pore diameter distributions (Dykhuizen and Casey, 1989). The latter factors are usually incorporated into a single apparent tortuosity. The diffusive flux ($J$) along a coordinate axis $z$, the whole sediment diffusion coefficent ($D_s$), the free solution diffusion coefficient ($D_o$), and concentration gradient ($\partial C/\partial z$) for a solute are thereby formally related through Fick's First Law as:

$$J = -\varphi \frac{D_0}{\theta^2}\left(\frac{\partial C}{\partial z}\right) = -\varphi D_s\left(\frac{\partial C}{\partial z}\right). \tag{1}$$

Under certain conditions, analogy between electrical conductance and diffusion allows for determination of geometric corrections ($\theta^2$) using the relative resistivities of free solution and saturated sediment (Turk, 1976; Maerki *et al.*, 2004). In these instances, $\theta^2 = \varphi F$, where $F$ represents the ratio of saturated sediment to free solution resistivity, or the so-called formation resistivity factor. Values of $D_s$ can also be determined experimentally assuming forms of Eq. (1) (Krom and Berner, 1980; Iversen and Jørgensen, 1993). A critical basic concept in these interpretations for marine deposits is that the physical -chemical properties of overlying water largely define the free solution properties of pore space. It is also implicit in the application of Eq. (1) in most diagenetic models that diffusive properties are homogeneous and isotropic.

Surficial sedimentary deposits are typically inhabited by organisms that continuously secrete organic compounds for purposes such as locomotion, dwelling construction, reproduction, competitive exclusion, or metabolic activity. These biogenic secretions range from free monomeric solutes, to viscous mucus gels, to rigid polymer structures. Exopolymer secretions from fauna and bacteria typically fill pore space, and polysaccharide-rich sheets often line macrofaunal dwelling tubes in the bioturbated zone of marine sediments (Fager, 1964; Frankel and Mead, 1973; Aller, 1983; Chandler and Fleeger, 1984; Bennett *et al.*, 1991; Paterson, 1995; Decho, 2000). These materials alter the physical structure and mass properties of deposits (Rhoads, 1974; Bennett *et al.*, 1991). They also have the potential to affect the physical - chemical properties of pore fluids and to create three dimensional heterogeneity and anisotropy in diffusive properties. It is known, for example, that the fibrous, polymeric linings of macrofaunal tubes can be semipermeable, differentially inhibiting diffusion of small inorganic solutes as a function of charge (Aller, 1983). It is also known that mucus gels can inhibit diffusion of inorganic and organic solutes, and can behave as rigid polyelectrolytes with charge hindrance and

size exclusion properties (Lee and Nicholls, 1987; Desai and Vadgama, 1991; Zhang and Davison, 1999).

Because of the spectrum of molecular size, shape, and charge distributions potentially present in dissolved organic carbon compounds (DOC), any semipermeable diffusive properties of mucus filled pores and the biogenic structures which permeate deposits would have substantial implications for the distribution and transport of diagenetically produced DOC (Aller, 1983). Porewater DOC concentrations can be up to two orders of magnitude greater than those of overlying waters (Krom and Sholkovitz, 1977; Orem *et al.*, 1986; Burdige *et al.*, 1992; Burdige, 2002). Diffusion of DOC out of marine sediments has been measured in situ and in the laboratory, and calculations suggest that the resulting flux is ~ 1–10% of total remineralization, and roughly comparable to organic matter burial fluxes in magnitude (Chen *et al.*, 1997; Alperin *et al.*, 1999; Burdige *et al.*, 1999; Holcombe *et al.*, 2001). Therefore, mechanisms controlling diffusion of DOC are critical to understanding the biogeochemical cycling of sedimentary carbon.

In the present study, we further examine the effects of macrofaunal mucus and tube lining material on the diffusion of solutes, particularly organic solutes, in sedimentary deposits. The movement of bulk natural porewater DOC, a set of polystyrene sulphonates of varied molecular weight, and the inorganic ions: $Br^-$, $NH_4^+$, $SO_4^{2-}$, $Mg^{2+}$, and $Ca^{2+}$ through organic-free porous media, gastropod pedal mucus, and representative macrofaunal tube linings were used to evaluate the existence and magnitude of diffusive retardation, charge hindrance, and size exclusion in these common biogenic matrices. Because diffusion depends in part on solution viscosity (Robinson and Stokes, 1959; Li and Gregory, 1974), the viscosity of gastropod pedal mucus was determined.

## 2. Methods

### *a. Mucus and tube lining sources*

Pedal mucus samples from the naticid gastropods *Neverita duplicata* and *Euspira heros* were obtained by allowing individual animals to crawl on sea water covered glass plates or a bed of glass beds (175 μm mean diameter) for at least 30 minutes. These two macrofaunal species are representative of a widespread coastal North Atlantic group of infaunal carnivores (Kabat, 1990; Weissberger, 1999). Prior to use, glass substrates were acid cleaned in 4N HCl and fired at 450°C to eliminate organic contaminants. Sea water used to wet the beads or glass plates was filtered through 0.2 μm pore size polycarbonate membranes. Mucus was removed from glass plate surfaces by scraping with a stainless steel razor and collecting the gel with a pipet. The presence of mucus on glass beads was verified by visual inspection, cohesive properties of beads, Alcian Blue stain, porosity measurements, and carbohydrate concentration measurements made on subsamples. Mucus was used in experiments or analyses immediately following collection.

The polysaccharide burrow or tube wall linings of the common, infaunal marine polychaetes *Diopatra cuprea*, *Onuphis jenneri*, *Chaetopterus variopedatus*, and *Melinna*

*cristata* were obtained by sieving bulk sediment samples and removing whole tubes, in most cases with living inhabitants. *Diopatra*, *Onuphis*, and *Chaetopterus* tubes were collected from intertidal flats in North Inlet, S. Carolina during November, 1997 and stored at 2°C in sea water until use (~ 2 months). Living *Melinna* were collected from box cores taken in central Long Island Sound, New York during 1999. Individual *Melinna* were allowed to construct tubes composed of glass beads in laboratory microcosms. Tubes were stored at 4°C. In all cases, linings were examined under a microscope before and after experimental measurements for visual evidence of decomposition or any damage during handling which might have compromised their physical integrity. Porosities were calculated from wet/dry ratios assuming a sea water density of 1.02 and an organic matter density of 1.2 g $cm^{-3}$ (Aller, 1983).

*b. Diffusion cell*

A modification of the classic diaphragm diffusion cell was used for all transport experiments (Northrop and Anson, 1929; Robinson and Stokes, 1959; Aller, 1983). Two well-stirred solution reservoirs of known initial compositions were separated by a relatively thin layer of material, in these cases: polycarbonate membrane standards (PCTE filters), burrow wall linings, a plug of clean glass beads, or a plug of mucus-coated glass beads. Diffusion between the reservoirs was monitored as a function of time and used to estimate permeability properties of the separating material. The cell setup was composed of glass, stainless steel, and teflon (Fig. 1). A relatively smaller reservoir, typically containing the more concentrated constituents of interest, was immersed in a larger reservoir. The inner reservoir volume was fixed by sealing with a stopcock assembly. The outer reservoir volume was adjusted to an identical level to eliminate hydraulic pressure gradients, although the fixed inner cell volume prevented bulk flow in any case. Both reservoirs were stirred continuously on either side of the separating material by using magnetically coupled stir bars (teflon coated). Entire cells were submerged in a temperature controlled water bath and maintained at 4–5°C in order to minimize microbial activity.

All diffusion cell components were acid cleaned (4N HCl) and distilled water rinsed before experiments. The matrix under study was secured between the individual reservoirs. Tube lining sections were held directly between two glass plates clamped together by screws. In the case of mucus secretions, glass beads with and without mucus were used to fill a hole in a third glass plate. The resulting plug of beads was covered on each side with a 10 μm pore size polycarbonate filter (Osmonics, manufacturer reported $\theta^2 \sim 1$; $\varphi = 0.079$), and the plate clamped between the reservoirs in the same manner as burrow lining sections. *Melinna* tubes composed of mucus-cemented glass beads were gently split longitudinally and a subcore perpendicular to the wall was taken with a glass tube. The resulting plug from the artificial tube was inserted into a diffusion cell glass plate holder. In addition to clean glass beads, control membranes in the diffusion cells consisted of 10 μm pore size polycarbonate filter membranes and plastic sheet (Saran Wrap, Dow Chemical).

By measuring the concentrations of the solutes under study in the inner and outer

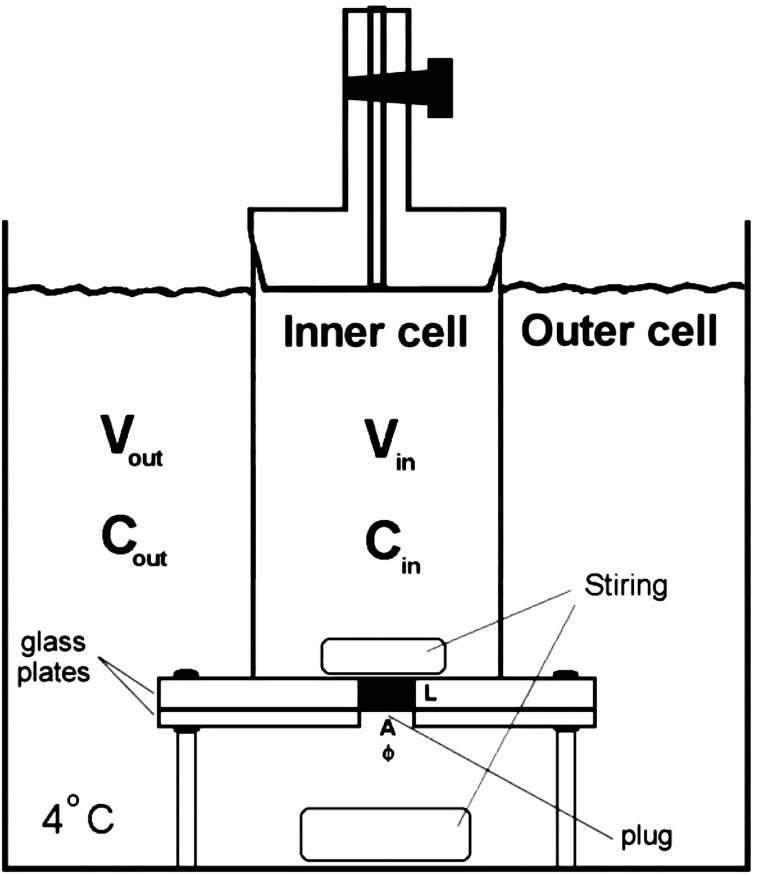


Figure 1. The diaphragm-diffusion cell utilized in experiments (T = 4–5°C). The plug material separating the solution cells depended on experimental treatment (e.g, glass beads, polychaete tube lining). Symbols are as defined in text (Eq. (2)). The magnetic stirrers were teflon coated.

reservoir solutions as a function of time, the time and concentration averaged diffusion coefficient of the separating membrane can be calculated from Eq. (2):

$$\ln\left[\frac{C_{in}(0) - C_{out}(0)}{C_{in}(t) - C_{out}(t)}\right] = \frac{\varphi A D_s}{L}\left[\frac{1}{V_{in}} + \frac{1}{V_{out}}\right]t = mt. \tag{2}$$

where $C_{in}(t)$ = concentration of solute in inner reservoir 1 at time $t$ (mmols dm$^{-3}$)
$C_{out}(t)$ = concentration of solute in outer reservoir 2 at time $t$ (mmols dm$^{-3}$)
$\varphi$ = porosity of the matrix or control plug
$D_s$ = $D/\theta^2$ (as defined in Eq. (1)).
$A$ = area of plug (cm$^2$)
$L$ = the length of the plug (cm)

$V_{in}$ = the volume of the inner solution ($cm^3$)
$V_{out}$ = the volume of the outer solution ($cm^3$)
$t$ = time of sampling (days)

Eq. (2) assumes that once a linear gradient is established, the diaphragm separation behaves as a porous body with an averaged diffusion coefficient corrected for geometric effects as in Eq. (1). A diffusion cell or calibration constant, β, may be calculated from a standard solute with known free solution diffusion coefficient, D, for example, $Br^-$ (Robinson and Stokes, 1959; Cussler, 1997):

$$\beta = \frac{\varphi A}{L\theta^2}\left[\frac{1}{V_{in}} + \frac{1}{V_{out}}\right]. \tag{3}$$

Although Eq. (3) is written to include the entire porous media $\varphi/\theta^2$ geometric correction term, the separation diaphragm or membrane need not be explicitly treated as porous, and parts or all of this term can be included in the definition of a $D$, or $D_s$ as done in Eq. 2. For example, if rather than porous, the cell separation membrane were assumed a continuous phase, then no explicit φ or $\theta^2$ terms would appear in either Eq. (2) or (3), and the measured diffusion coefficient would represent movement within the uniform membrane material.

A total of four different diffusion cells were used throughout this study. The components of each cell were individually numbered, so that A and L in Eqs. (2,3) for each cell remained the same through multiple experiments. The respective reservoir volumes in each cell also remained essentially constant between experimental runs.

*c. Solutes and solutions*

*i. Inorganic solutes.* Bromide was included and monitored in most diffusion runs as a relative calibration standard. In addition, the simultaneous diffusion of $NH_4^+$, $SO_4^{2-}$, $Ca^{2+}$, and $Mg^{2+}$ through *Melinna* glass bead tube walls was examined. For these inorganic solute diffusion experiments, the inner cell solution was 0.2 μm-twice-filtered sea water augmented with 50 mM $Br^-$ (as NaBr) and 12 mM $NH_4^+$(as $NH_4Cl$). The outer solution consisted of artificial sea water (Harrison *et al.*, 1980) having comparable ionic strength (adjusted with $Na^+$, $K^+$, $Cl^-$) but without $SO_4^{2-}$, $Ca^{2+}$, and $Mg^{2+}$.

*ii. Organic solute diffusion experiments.* Natural porewater was centrifuged from surface sediment samples obtained in June and November 1997 from the upper ~30 cm of silt - clay deposits at a well-studied site in central Long Island Sound (Station P or Station NWC, 15 m water depth) (e.g. Aller, 1994). The resulting supernatant was refrigerated overnight to allow precipitation of dissolved Fe and Mn (Orem and Gaudette, 1984), and then filtered through 0.4 μm pore size PCTE filters (Osmonics) and refrigerated at 4°C. The filtered porewater had relatively high total DOC (1.2–1.6 mM) and constituted the inner cell solutions. Before each diffusion run, porewater was re-filtered and NaBr added to a concentration of ~ 250 mM for use in inner cells. The outer cell solutions consisted of

0.1 μm-filtered, UV-oxidized sea water collected from overlying water at the same location as the sediment samples. UV treated water was used within 1 day of oxidation. The pH of inner cell solutions typically ranged from 7.8–8.0 and outer cell 7.6–7.7, generally differing between cells by ~0.2.

DOC diffusion experiments were maintained at 5°C and carried out over periods of 5–8 days. Potential loss of DOC by adsorption or remineralization during experimental runs was examined using control diffusion cells separated by plastic sheet membranes (Saran Wrap, Dow Chemical). Possible production of DOC from remineralization of mucus was checked by monitoring DOC backgrounds in an experiment using UV-oxidized sea water (DOC free) in both inner and outer reservoirs separated by mucus-laden plugs. Spectral absorbance scans (190–820 nm) were also used to qualitatively monitor possible major changes in DOC composition between inner and outer cells.

Molecular size effects on diffusion properties were investigated using commercially available polystyrene sulfonates (Polysciences) having varying chain lengths. The polystyrene sulfonates (PSS) provide a suite of chemically similar compounds differing primarily in size. In experiments with PSS, both inner and outer solutions consisted of 0.2 μm-twice-filtered sea water (~0.4 M $Cl^-$). The inner cell solution was enriched with a particular molecular weight PSS to an initial concentration of several micromolar. All runs took place in the dark, although controls showed that PSS were not photosensitive and concentrations were stable over experimental time scales. PSS diffusion experiments were maintained at 4°C for periods of 4 days.

In all experiments, inner and outer cell solutions were sampled at the beginning and the end of an experimental run, whereas the outer cell solution was serially sampled at least once, and usually several additional times. The calculations of inner cell concentrations at other than initial or final times were based on the outer cell concentration measurements assuming mass balance. Overall mass balances during experiments were checked using directly measured initial and final concentrations in both cells. The volume change of the outer solution during sampling was less than or equal to 1% of the starting volume. Samples were stored refrigerated until analysis at the end of an experiment.

*d. Bulk resistivity factor*

The bulk resistivity of sea water saturated glass beads (175 μm, mean) with and without *Neverita* pedal mucus were measured using a micro resistivity probe (3 mm width; Andrews and Bennett, 1981; Cai *et al*., 1995). Sea water saturated, mucus-laden beads were collected from snail crawling trails and immediately placed into 10 ml glass beakers and covered with sea water. Measurements were made on 9 separate occasions. Triplicate samples were taken on each date and resistivity measured in triplicate on each sample. Mucus-free beads were measured in the same way. Formation or material resistivity factors, $F$, were calculated from the averaged ratio of the resistivity of the saturated beads relative to overlying sea water. Porosities ($\varphi$) of the saturated beads were measured from wet - dry weight ratios and an assumed particle density of 2.6 g $cm^{-3}$.

*e. Viscosity*

The viscosity of *Euspira* mucus was estimated by means of a custom made glass capillary viscometer calibrated with water (Schachman, 1957). A known quantity of wet mucus was collected by micropipet from a plate over which snails had been crawling. The mucus sample was brought to a volume of 1 ml with saline solution and introduced to the viscometer. The relative outflow times ($t$, $t_0$) of solutions from the capillary section of the viscometer, densities ($\rho$, $\rho_0$), and viscosities ($\eta$, $\eta_0$) of mucus and calibration water are related by:

$$\eta/\eta_0 = (t/t_0) \cdot (\rho/\rho_0). \tag{4}$$

Due to the difficulty of obtaining and handling a full milliliter of mucus (e.g, string formation during pipeting), measurements utilizing different dilution volumes of mucus (up to $\sim$ 900 $\mu$l mucus) were made. All measurements were done at 22.5°C. The viscosity of pure mucus was estimated by extrapolation of the outflow times versus volume fractions of mucus samples (Klein, 1985).

*f. Analytical methods*

*i. Inorganic solutes.* Bromide and ammonium were analyzed colorimetrically using phenol red and indophenol blue respectively (Balatre, 1936; Solórzano, 1969; Presley, 1971). Sulfate was analyzed using ion chromatography with background suppression (Dionex 2000; AG4A column). Serial dilutions of sea water were used as standards. The source sea water sulfate concentration was determined gravimetrically as barium sulfate (Presley, 1971). Calcium and magnesium were diluted in lanthanum - 0.1 N HCl and determined using flame atomic absorption spectrophotometry. Analytical precisions were 0.5–1% for bromide, 1.8% for ammonium, 1.00% for sulfate, 1.39% for calcium, and 0.25% for magnesium. These analytical errors corresponded to 0–6% of the change in the difference in concentration across the diaphragm depending on the experiment.

*ii. Organic solutes.* DOC was measured using fluorescence of diffusion cell solutions (Schimadzu RF551). Spectral scans demonstrated optimal response of porewater DOC at an excitation of 350 nm and emission of 445 nm ($\lambda \pm 5$ nm). The relationship of fluorescence response to absolute DOC concentration was calibrated with a series of porewater samples diluted in UV-oxidized sea water. Dissolved organic carbon (DOC) was determined on a Schimadzu high temperature TOC 5000 analyzer (Williams *et al.*, 1993). The resulting correlation gave: DOC ($\mu$M) = 70.46 $\cdot$ (fluorescence) + 46.93, with $r^2$ = 0.9936.

Polystyrene sulfonates are commonly detected by measuring absorbance at 280 nm (Chin and Gschwend, 1991; Burdige and Gardner, 1998). In the present study, we utilized fluorescence response to excitation at 280 nm to ensure high sensitivity at low initial concentrations in the outer cells. Each sulfonate exhibited a slightly different emission signature, although all of the peak emissions were between 361 and 382 nm (Table 1).

Table 1. Emission peaks at excitation 280 nm for polystyrene sulfonates.

| Polystyrene sulfonate MW | Emission peak wavelength (nm) |
|---|---|
| 1,800 | 380.6 |
| 4,600 | 363.8 |
| | 380.0 |
| 18,000 | 381.0 |
| 35,000 | 381.2 |
| 70,000 | 329.8 |

Interference from fluorescence emission by natural sea water (e.g. Chen and Bada, 1992; Coble, 1996) was insignificant. Calibration curves were constructed by obtaining emission spectra of diluted solutions of a compound using an Hitachi F-4500 scanning spectrofluorometer, and comparing both peak height and area under the curve (325–480 nm), both relationships gave very good fits, with typical $r^2$ greater than 0.995 (Fig. 2).

*iii. Characterization of mucopolysaccharides.* The total carbohydrate contents of mucus secretions and tube linings were measured as glucose equivalents using the phenol - sulfuric acid assay (DuBois *et al.*, 1956). Dry samples of known weight were placed in 2 ml of 2.5% NaCl solution and hydrolyzed by addition of concentrated sulfuric acid and phenol (Strickland and Parsons, 1972; Underwood *et al.*, 1995). Absorbance was measured at 485 nm. Dextrose standards (anhydrous D-glucose, Fisher Scientific) were made up in 2.5% NaCl solution and frozen until use.

Carboxyl and sulfur-ester groups on mucopolysaccharides in pedal mucus were stained with the cationic dye Alcian blue at low pH (Troyer, 1980; Horobin, 1988). The dye was prepared by diluting a 0.02% aqueous solution of Alcian blue 8 GX (Sigma) with 0.06% acetic acid (Passow and Alldredge, 1995). Because of dye coagulation, the solution was filtered through a 0.2 μm PCTE filter immediately before use. Mucus-laden and clean glass beads were stained for a few seconds with 1 ml of the stain and rinsed with distilled water. Samples were examined under an optical microscope.

## 3. Results

### *a. Properties of mucus-coated glass beads*

Glass beads over which *Neverita* and *Euspira* crawled were relatively cohesive compared to clean beads, indicating particle binding by mucus. Staining by Alcian blue revealed microscopically visible strands of stained material within the interstices of beads contacted by snails but no stain in clean bead controls. Carbohydrate extractions also demonstrated a consistent shift to higher values after contact with snail mucus secretions, typically measuring $\sim$ 3 mg glucose $g^{-1}$ in control beads and $\sim$10–20 mg $g^{-1}$ in mucus coated beads. The saturated porosity of mucus-coated beads was always systematically higher than clean beads. For example, in porewater DOC experiments the relative

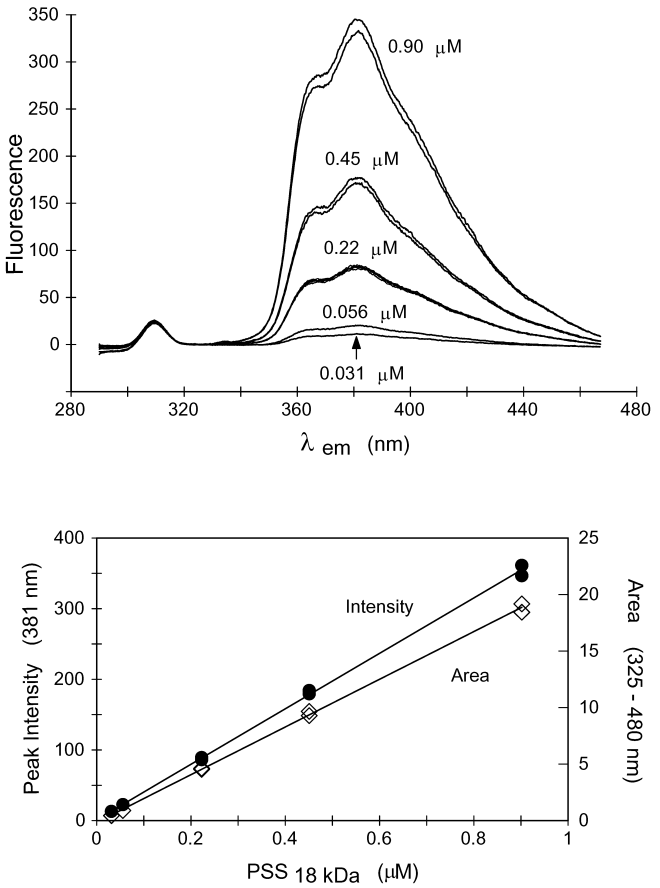


Figure 2. (A.) Example emission spectra as a function of concentration for polystyrene sulphonate (18 kDa), excitation 280 nm. (B) Calibration curve relationships between PSS - 18 kDa concentrations and emission peak intensity at 381 nm (filled circles) (least squares fit, $r^2 = 0.9988$), or fluorescence spectra relative areas integrated from 325–480 nm (open diamonds) (least squares fit, $r^2 = 0.9989$).

porosities were 0.458 ± 0.018 (SE) (clean) and 0.508 ± 0.010 (SE) (mucus-coated), and for the PSS diffusion experiments, porosities were 0.413 ± 0.005 (clean) and 0.456 ± 0.019 (SE) (mucus-coated). The salt-corrected dry/wet weight fraction of 'pipetable' *Euspira* and *Neverita* mucus was 0.0071–0.0186, implying a fractional weight of water ~0.981–0.993. Bead porosities were estimated assuming interstitial solution densities of 1.02.

Resistivity measurements demonstrated that clean and mucus-coated glass beads lie along the same formation resistivity factor relationship with porosity such that $F \sim \varphi^{-1.4}$ (Fig 3). Averaged group porosities in these experiments were 0.398 ± 0.004 (clean) and 0.415 ± 0.003 (SE) (mucus-coated). Thus, the presence of mucus increased porosity and decreased the formation resistivity factor in bead plugs.

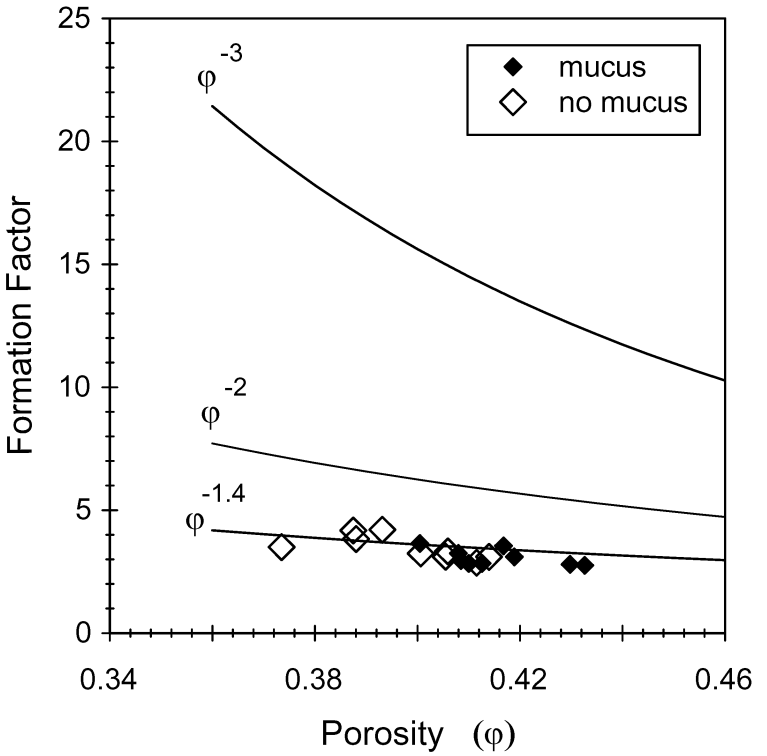


Figure 3. Formation resistivity factors, $F$, as a function of porosity in sea water saturated glass beads (mean diameter $\sim$ 175 μm) with and without mucus. The curves represent functions: $F = \varphi^{-m}$, where $m$ = 1.4, 2, and 3. The presence of mucus increases porosity and decreases $F$.

*b. Viscosity of pedal mucus*

The time it took solutions with different volume fractions of *Euspira* pedal mucus to move through the viscometer was linearly correlated to the mucus volume fraction, giving (in seconds): $t = 0.343 \cdot (\mu l \text{ mucus}) + 35.07$; ($n = 10$, $r^2 = 0.960$, $p < 0.0005$). Mucus was diluted in 2.5% saline solution. It took 1 ml of 2.5% saline solution 35.07 sec to move through the viscometer at 22.5°C. Distilled water with a viscosity of 9.60 mpoise, and $\rho_0 = 0.9976$ g ml$^{-1}$, gave $t_0 = 34.53$ sec. Using Eq. (4) gives $\eta = 107$ mpoise for 100% mucus solution at 22.5°C (with $\rho = 1.015$ g ml$^{-1}$, as per 2.5% saline). Assuming the same temperature dependence as in sea water, correction to 5°C predicts ~170 mpoise for the pedal mucus endmember at the temperature of diffusion experiments.

*c. Diffusion of porewater DOC and $Br^-$*

Serial sampling of the diffusion cells demonstrated that the time dependent concentration changes of porewater DOC and $Br^-$ diffusing through glass beads or tube linings were consistent with Eq. (2), allowing calculations of relative and absolute diffusion coefficients

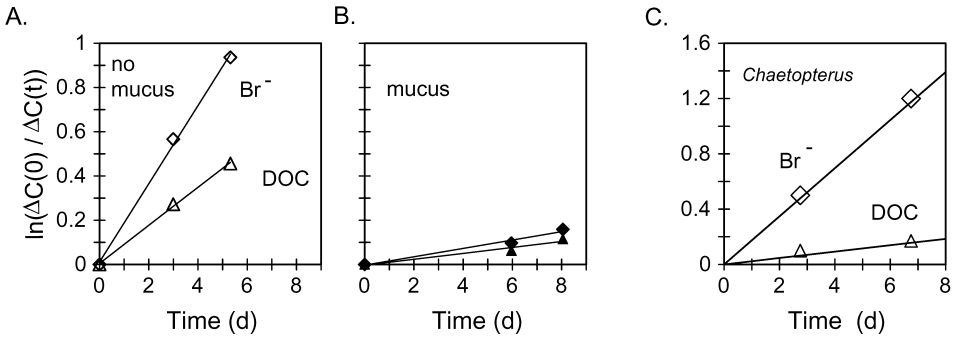


Figure 4. Example diffusion cell concentration difference ratios for DOC and $Br^-$ in sea water as a function of time (Eq. 2) and plug material at 5°C. (A) Clean glass beads. (B) Glass beads coated with *Euspira* pedal mucus. (C) *Chaetopterus* tube lining.

in each case (Fig. 4). No detectable production of DOC from mucus plugs occurred and no detectable loss due to DOC adsorption within the cells were found (Saran Wrap membrane controls) based on fluorescence measurements. No detectable differences in adsorption spectra scans (190–850 nm) of DOC within inner and outer diffusion cells were found. Diffusion rates of both DOC and $Br^-$ were almost always higher through clean glass beads than through mucus-coated beads (Fig. 4A,B). The absolute and relative rates of diffusion of both DOC and $Br^-$ through glass beads were related to the quantity of mucus present as measured by extracted carbohydrate (Fig. 5). In addition to overall direct or inverse correlations, grouped comparisons of mean properties with or without mucus demonstrated that carbohydrate concentrations, bulk DOC diffusion coefficients, $Br^-$diffusion coeffi-

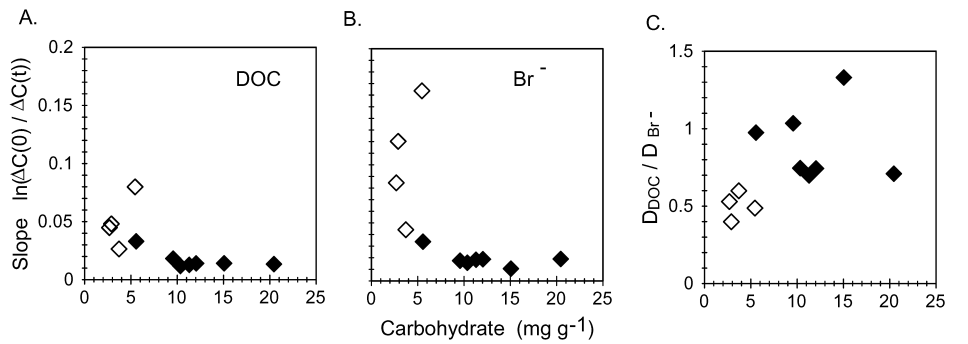


Figure 5. The slopes of the diffusion cell concentration function ($m$, Eq. 2) as a function of the total carbohydrate extracted from glass bead plugs with (closed diamonds) and without mucus coatings (open diamonds). (A) DOC , ($r^2 = 0.42$, $p = 0.031$); (B) = $Br^-$ ($r^2 = 0.39$, $p = 0.039$) (C) Ratio of diffusion coefficients for DOC/$Br^-$ as function of total extracted carbohydrate. A positive relationship is evident ($r^2 = 0.25$; $p = 0.12$), but differences are most strongly expressed by comparing the mean values of diffusion with and without mucus (see text).

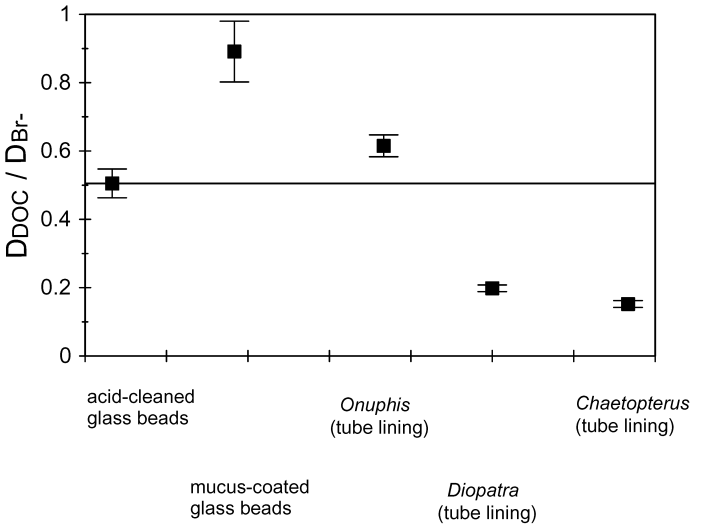


Figure 6. Ratios of diffusion coefficients of porewater DOC/$Br^-$ in clean glass beads, mucus coated glass beads, and tube linings from *Onuphis*, *Diopatra*, and *Chaetopterus.* The horizontal line represents the clean glass bead value as reference ratio.

cients, and the DOC/$Br^-$ diffusion coefficient ratios were statistically different at high probability levels. Two sample *t*-tests (*with* and *without mucus*) assuming unequal variances gave respective two tailed probabilities: $P = 0.0029$ (carbohydrate); $P = 0.063$ (DOC); $P = 0.046$ ($Br^-$); and $P = 0.0043$ (DOC/$Br^-$).

The relative ratios of diffusion coefficients of porewater DOC and $Br^-$ through clean glass beads averaged 0.504 $\pm$ 0.041 (SE) (Fig. 6). Assuming a free solution diffusion coefficient for NaBr at 5°C of 0.768 $cm^2\ d^{-1}$ (Robinson and Stokes, 1959) gives an average bulk DOC diffusion coefficient in sea water of 0.387 $cm^2\ d^{-1}$ (5°C). Although both DOC and Br- diffusion were hindered by the presence of mucus, $Br^-$ was affected to a greater extent, as demonstrated by the increased ratio of DOC/$Br^-$ diffusion coefficients to an average of 0.891 $\pm$ 0.089 (SE) in mucus-laden beads (Figs. 5C, 6). Polychaete tube linings inhibited diffusion of both DOC and $Br^-$ but relative effects were species-dependent and differed from pedal mucus. As in the case of pedal mucus, *Onuphis* tube linings showed a slight tendency to inhibit $Br^-$ transport relative to DOC, however, *Diopatra* and *Chaetopterus* tube linings preferentially inhibited DOC transport (Fig. 6). Microscopic examination of the tubes revealed no evidence of degradation during storage at 2°C; however, it is possible that fresh tube lining material may behave differently. Separate decomposition experiments with *Chaetopterus* tube linings at 22°C demonstrated virtually no decomposition over a several month period under oxygenated conditions (unpublished data).

The effect of mucus or tube lining matrices on specific solute diffusion can be represented by the ratios of diffusion coefficients in free solution to those in the organic

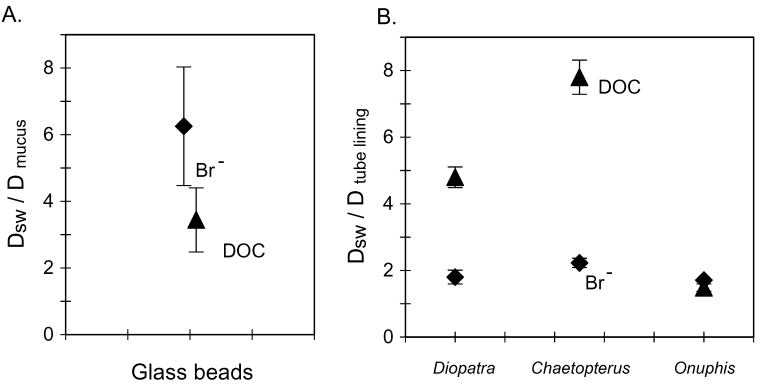


Figure 7. (A) Ratio of average (± SE) free solution diffusion coefficient in sea water to that in mucus gel for DOC and $Br^-$. Transport of both solute types are inhibited, $Br^-$ more so than DOC. (B) Ratio of average (± SE) free solution diffusion coefficient in sea water to those in polychaete tube linings for DOC and $Br^-$. Inhibition of DOC diffusion depends strongly on the source of the tube lining, $Br^-$ less so.

matrix. In the case of glass beads, the ratio of the average diffusion coefficient of a solute in sea water relative to diffusion in mucus can be obtained from the ratio of the respective slopes, $m_{sw}$ and $m_m$, of the function $\ln(\Delta C(0)/\Delta C(t))$ versus time $t$ in the diffusion cell runs with clean beads and mucus-coated beads (Eq. 2). The ratio of slopes with otherwise constant values of β terms (Eqs. 2, 3) gives:

$$\frac{m_{sw}}{m_m} = \frac{\varphi_{sw} D_{sw}}{\theta_{sw}^2} \cdot \frac{\theta_m^2}{\varphi_m D_m} . \tag{5}$$

where the subscripts $m$ and $sw$ indicate sea water filled pore space with and without mucus. The average porosities of clean and mucus laden beads in these experiments were 0.458 ± 0.018 (SE) and 0. 508 ± 0.010 (SE) respectively. Assuming over the porosity range of interest that the resistivity factors are given by $\sim \varphi^{-1.4}$, then $\theta^2 \sim \varphi^{-0.4}$ (Fig 3). The diffusion coefficient ratio in Eq. (5) is then related to the slope ratio by $D_{sw}/D_m \sim 1.16 \cdot (m_{sw}/m_m)$. Because the porosities differ only by ~ 10%, the geometric correction ratio ($\varphi_{sw}\ \theta_m^{\ 2}/\varphi_m\ \theta_{sw}^{\ 2}$) is relatively small and the exact tortuosity - porosity model is not a major determinant of the calculated diffusion coefficient ratio. If the effect of pore space mucus is further incorporated into a hindrance factor, $h$, such that $D_m = hD_{sw}$, then the geometrically corrected slope ratio is $1.16 \cdot (m_{sw}/m_m) = 1/h$. Values of $h$ for pedal mucus were 0.29 and 0.16 at 5°C for DOC and $Br^-$, corresponding to averaged increased diffusion rates in sea water relative to mucus-filled pores of 3.4 ± 0.96 X and 6.3 ± 1.8 X respectively ($1/h$; Fig. 7A). If the mucus mesh itself were treated as a porous material rather than continuous solution phase, then the calculated hindrance factors could be interpreted in terms of geometric parameters comparable to that of the glass beads (e.g., porosity, tortuosity) but scaled to the interstitial space alone.

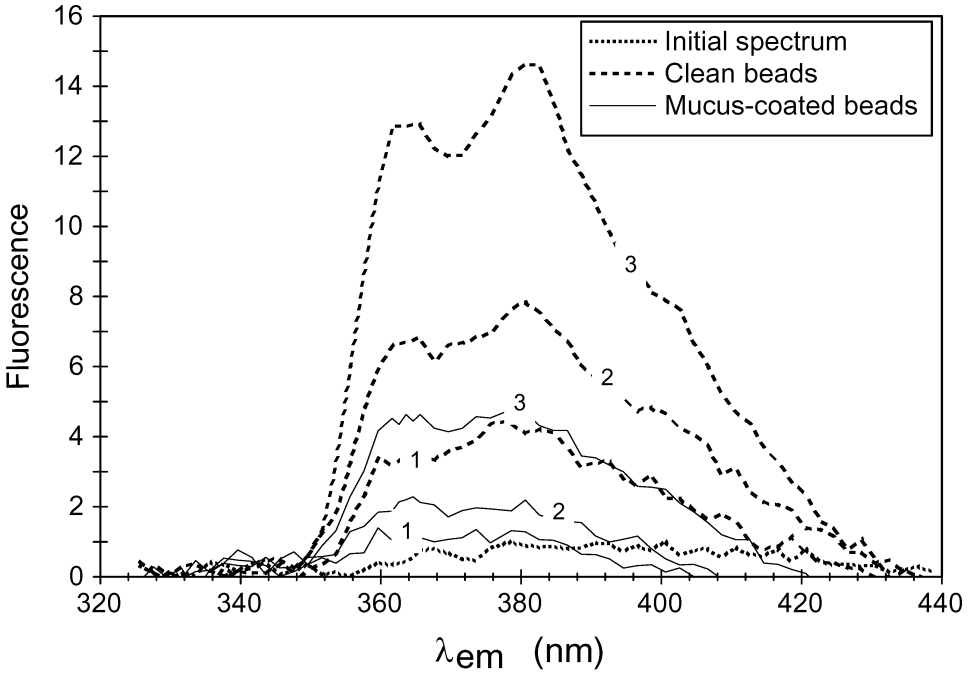


Figure 8. Example emission specta for PSS-18 kDa (Exc. 280 nm) as a progressive function of time in outer cells with diaphram separations of clean glass beads and mucus coated glass beads. The successive times 1, 2, and 3 indicated on the curves represent 27, 51, and 93 hrs respectively. The presence of mucus coatings inhibits diffusion of PSS relative to clean beads.

The proper conceptualization of diffusion coefficients in tube lining matrices is uncertain (Aller, 1983). For the present purposes, linings are assumed to behave as porous frameworks with whole body diffusion coefficients given by: $D_L = D_{sw}/\theta_L^2$, where the subscript $L$ indicates tube lining material. In calculating $D_L$ from diffusion cell constants ($\beta$), measured porosities of $0.812 \pm 0.008$, $0.816 \pm 0.004$, and $0.526 \pm 0.062$ (SE) were used for *Diopatra*, *Chaetopterus*, and *Onuphis* linings respectively. The low measured porosity of *Onuphis* lining material reflects inclusion of agglutinated sand. The values of $D_{sw}$ were taken as 0.768 and 0.387 $cm^2$ $d^{-1}$ for $Br^-$ and DOC. The resulting calculated ratios of $D_{sw}/D_L$ for the lining material demonstrate similar hindrance effects for $Br^-$ transport ($D_{sw}/D_L$ = 1.7–2.2) but greatly different behavior for DOC ($D_{sw}/D_L$ = 1.4–7.8) depending on polychaete species (Fig. 7). A single tortuosity factor cannot account for diffusive properties of all constituents, and the linings of at least some species are semipermeable.

*d. Size dependent diffusion*

The diffusion of PSS through glass beads was hindered by mucus (Fig. 8). There was an inverse relationship between the molecular weight of PSS and respective diffusion

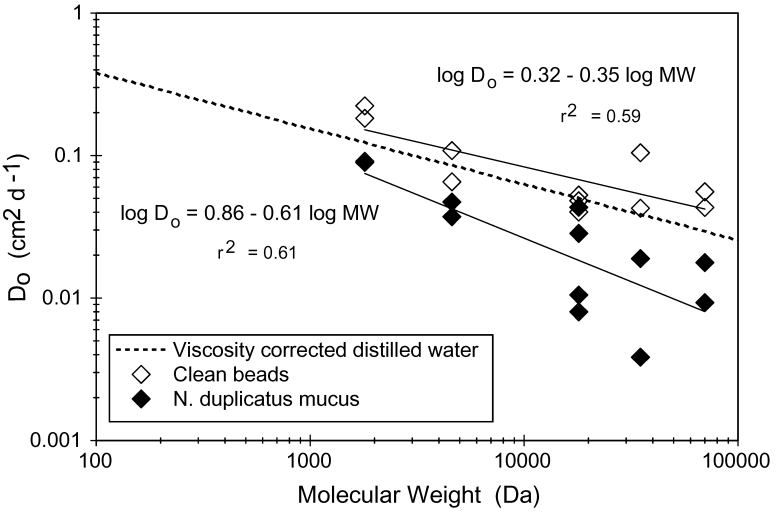


Figure 9. Experimental diffusion coefficients of PSS measured in sea water saturated clean glass beads ($n = 12$) or mucus-coated glass beads ($n = 12$)as a function of PSS molecular weight at 4°C. The general relationship of diffusion coefficients in distilled water corrected to sea water viscosities at 4°C for a range of organic compounds as a function of molecular weight is shown for reference (Burdige, *et al*., 1992). Diffusion coefficients for PSS in sea water are generally higher than predicted from the distilled water relationship corrected for sea water viscosity alone (regressions significantly different, ANCOVA: $\alpha = 0.023$ ($F''1,8''$). Diffusion coefficients through mucus are at least 2X less those in sea water and also decrease more strongly with molecular weight. Linear least square fits are plotted.

coefficients through both clean and mucus-coated glass beads (Fig. 9). Although there is substantial scatter in the data, in the case of clean beads this inverse relationship in sea water largely parallels but tends to lie above the temperature corrected functional relationship between molecular weight and diffusion coefficients for a range of organic molecules in distilled water as compiled by Burdige *et al*. (1992). This latter relationship reflects results of various diffusion experiments using compounds of known molecular weights in distilled water individually corrected to 25°C by assuming the Stokes-Einstein equation. Since our experiments were conducted at 4°C, the relationship was recalculated to this temperature also using the Stokes-Einstein equation (Li and Gregory, 1974):

$$D_{4°C} = \frac{D_{25°C}\eta_{25°C}}{298.15}\frac{277.15}{\eta_{4°C}}. \tag{6}$$

The slope of the logarithmic fit through the diffusion coefficients of the clean bead treatments (−0.35) is slightly closer to the overall slope predicted by the Stokes-Einstein theory (−0.33; Cornel *et al.*, 1986), than the compiled data from distilled water (−0.39; Burdige *et al.*, 1992). In contrast, the relationship between PSS molecular weight and diffusion coefficient through mucus-coated beads, consistently lies well below that found

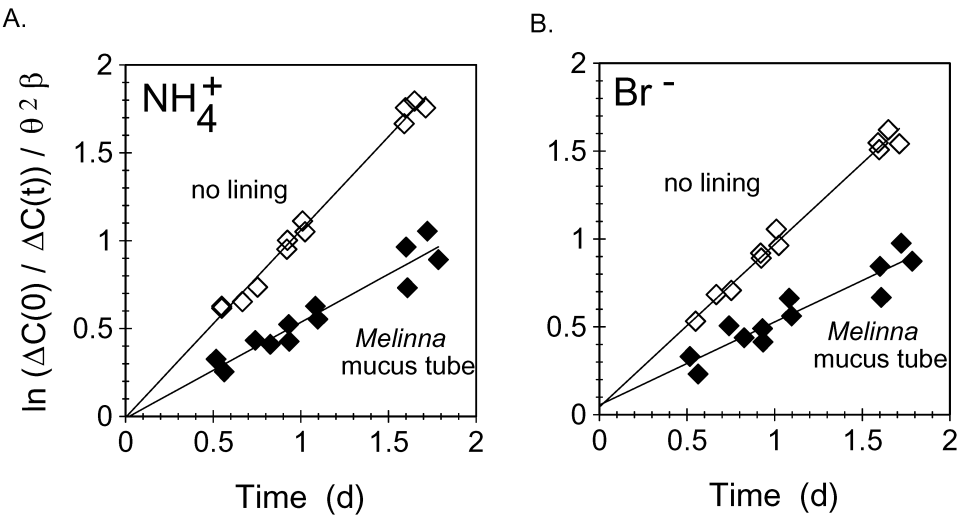


Figure 10. Diffusion cell concentration difference ratios for (A) $NH_4^+$ and (B) $Br^-$ diffusing through PCTE membranes (no mucus) or *Melinna* tube walls in sea water as a function of time (Eq. 2) at 4°C. In these examples, the results from multiple exerimental runs have been normalized by modified cell constants ($\theta^2\,\beta$; Eq. (3) with no tortuosity term) and combined. The respective slopes therefore directly represent the diffusion coefficients $D_s$ for the solutes in each medium ($D_s = D_o$ for PCTE membrane having $\theta^2 = 1$).

for either free solution in sea water or distilled water. The negative slope of the function is also greater in mucus-coated than in clean beads, indicating increasing hindrance with increasing molecular weight in the presence of pedal mucus relative to sea water. At the lower end of molecular sizes, the two calculated PSS relationships (clean, mucus-coated) intersect at a PSS molecular weight of $\sim$ 150, suggesting that this is the lowest limit of the size-based inhibition effect. Around and below this size, the difference in diffusivity through the two matrices is statistically insignificant. The variability of diffusion coefficients through mucus-coated beads at each molecular weight generally increases with increasing size (heteroscedasticity ignored in least squares fit).

*e. Inorganic solute diffusion*

The potential effect of charge and size of inorganic solutes on diffusion through mucus cement in sea water was examined in the artificial tube walls formed by *Melinna* from glass beads in laboratory microcosms. Mucus-cemented tube walls inhibited solute diffusion relative to free solution (Fig. 10). In the case of the representative data shown, the concentration functions (Eq. 2) were normalized to cell constants (Eq. 3) less the tortuosity term so that slopes directly represent the respective diffusion coefficients. The ratio of the slopes corresponds to the ratio $D_{sw}/(hD_m/\theta_m^2)$ as defined earlier. The tortuosity term in Eq. 3 for the no lining case is $\sim$ 1 because standard nontortuous PCTE membranes ($\varphi$ =

0.0785, $L = 0.0054$ cm) were used as controls. If it is further assumed that $\theta_m^2 \sim \varphi^{-0.4}$ (Fig. 3) for the glass beads composing the artificial tube wall, then the hindrance factors, $h$, for each solute can be calculated from the slope ratios as done for pedal mucus (Fig. 11). The calculated mucus hindrance factors differed relatively little between solutes, averaging $h = 0.70 \pm 0.05$ for $Br^-$, $NH_4^+$, $SO_4^{2-}$, $Ca^{2-}$, and $Mg^{2+}$. Excluding $SO_4^{2-}$ ($h = 0.79$), gives an average $h = 0.67 \pm 0.007$. These values are substantially higher than measured for pedal mucus. If the function $\theta^2 = \varphi^{-1}$ commonly found for low porosity sand were assumed (Ullman and Aller, 1982), then the calculated values would be $h \sim 1$, that is, no discernable effect of mucus cement.

## 4. Discussion

### *a. Diffusion through mucus and tube linings*

Diffusion of both organic and inorganic solutes through macrofaunal mucus and organic tube linings is generally inhibited relative to free solution (Figs.4–7, 9). Inhibition can be substantial, decreasing diffusion of bulk DOC and small anions by factors of ~3–8X, as in the case of gastropod pedal mucus and a range of tube linings, or relatively minor, as in the case of inorganic solute diffusion through *Melinna* tube cement. The extent of inhibition is thus a function of organic matrix source and by inference, its composition, concentration (mucus), chemical properties, and physical microstructure. In the case of pedal mucus, the differential behavior of $Br^-$ and DOC demonstrates that solute charge discrimination can be important, at least for anions, as also found for many polychaete tube linings (Aller, 1983) and other mucus gels (Lee and Nicholls, 1987). Oxidation may increase the relative abundance of polar DOC fractions and enhance such charge effects under oxic conditions, such as used in the present experiments, relative to completely anoxic solutions (Orem and Gaudette, 1984). The lack of evidence for charge-related hindrance in mucus cemented tube walls formed by *Melinna*, however, indicates that charge-based hindrance or Donnan exclusion need not necessarily occur. The magnitude of diffusive hindrance observed for a range of solutes in pedal mucus or polysaccharide tube linings, while significant, is far less than the ~ 100X effect inferred for diffusion of $Si(OH)_4$ through mucus gel in marine snow (Brzezinski *et al.*, 1997).

Molecular size is also an important determinant of diffusion behavior through both pedal mucus and polychaete tube linings. The increasing hindrance of polystyrene sulphonate diffusion through pedal mucus as a function of PSS molecular weight is direct evidence of semipermeable properties of mucus based on solute size (Fig. 9). The differential effects on transport of DOC and $Br^-$ through tube linings is further demonstration of size dependent permeability and the relatively increased hindrance of large solutes. Evidence for such an effect is also found in a wide range of cross-linked gel materials including pig gastric mucus (Desai and Vadgama, 1991), specific hydrogels used in DGT and DET techniques(Zhang and Davison, 1999), and is a property used commonly in molecular weight determination by gel filtration or electrophoresis (Rodbard, 1974). A size cutoff or

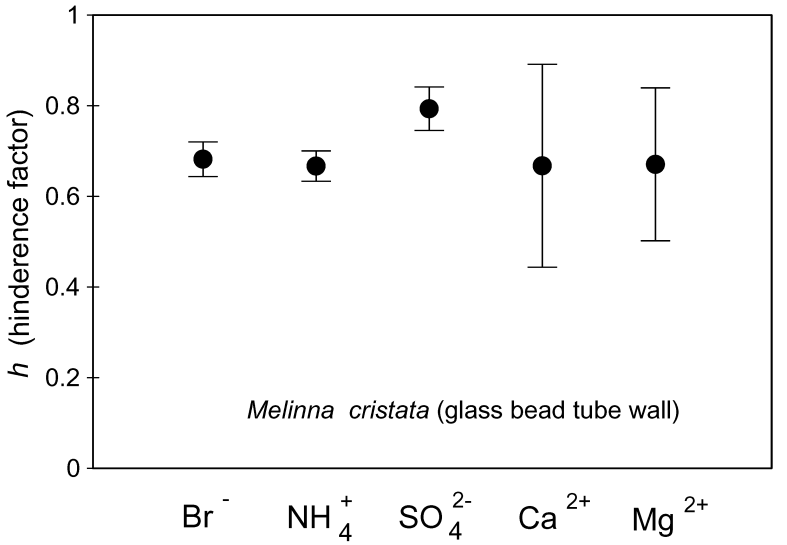


Figure 11. The average inhibition factor, *h*, estimated for *Melinna* mucus cement are 0.67–0.7 and are essentially the same for all inorganic solutes considered, regardless of formal ion charge.

threshold beyond which molecular exclusion occurs was suggested by results in previous studies of diffusion through mucus gels and biofilms (Desai and Vadgama, 1991; Lawrence *et al.*, 1994). No evidence, however, of complete exclusion or threshold filtration property of gastropod pedal mucus was found, at least up to solute molecular weights of ~ 100 kD and corresponding molecular sizes in sea water.

*b. Conceptualization of matrix properties*

The hindrance by pedal mucus of solute diffusion as a function of both solute charge and size indicates that it can behave in part as a charged, rigid framework or crosslinked fiberous gel rather than a polyelectrolyte solution. It seems likely, however, that at low concentrations, or during late stages of gel decomposition, mucus components may be treated as in solution and that the predominant effect on diffusion could then derive from alteration of solution structure as accounted for in viscosity. Given the measured viscosity of pedal mucus of ~ 170 mpoise (5°C), a solution of 10–30% mucus (equivalent gel volume %) could account for the magnitude of diffusive hindrance observed. Viscosity corrections alone, however, cannot account for semipermeable properties, implying that cross linked structure is important.

The apparent increasing variability of measured diffusion coefficients with increasing molecular size as observed for PSS diffusion through pedal mucus, may reflect heterogeneous distribution of mucus or localized domains of gel-forming macromolecules within pore space. Smaller solutes are not as sensitive to such heterogeneity because pores would appear relatively open regardless of the presence or absence of gel framework. Larger

molecules, which interact more strongly with gel structure, should be more constrained by the presence local mucus clusters and thus respond to variable, heterogeneous structure with greater variability in diffusive transport.

Polychaete tube linings also show evidence of properties expected for a charged, porous membrane, including hindrance of solute diffusion as a function of solute charge and size. An unknown fraction of these effects could be due to mucoid secretions from resident bacteria. The effects are apparently species specific with, for example, *Onuphis* tube lining showing relatively greater evidence of charge discrimination and *Diopatra* and *Chaetopterus* showing relatively greater size effects (Fig. 6). For natural sediment particle mixtures, the relation between tortuosity and sediment porosity approximates to $\theta^2 = 1 - \ln(\varphi^2)$ (Boudreau, 1997). Given the estimated porosities of tube linings, tortuosity correction factors of $\sim$ 1.4, 1.4, and 2.3 might be expected for *Diopatra*, *Chaetopterus*, and *Onuphis* linings based on a comparable relationship between tortuosity and porosity in the organic matrix. The measured $Br^-$ hindrance appears to be largely, but not entirely accounted for by such a correction, whereas DOC hindrance is not. These differences presumably reflect the effective size distributions of channel openings within the lining meshworks and charged functional group densities on polymers formed by the different species.

*c. Conceptual models of DOC diffusion in the bioturbated zone*

The measured diffusion coefficients of PSS through clean bead plugs in sea water are apparently elevated relative to those predicted by the relationship for macromolecule diffusion in distilled water compiled by Burdige *et al.* (1992) when corrected only for sea water viscosity and temperature. Porewater DOC in surficial sediments is typically dominated by molecular weight distributions 0.5–3 kDa, and for purposes of calculating diffusive transport of DOC, molecular weights ranging from 1–10 kDa are often assumed (Orem *et al.*, 1986; Chin and Gschwend, 1991; Burdige and Gardner, 1998; Burdige *et al.*, 2004). The averaged diffusion coefficient of DOC at 5°C (salinity = 25 ‰) might therefore be expected to vary between $\sim$ 0.064–0.16 $cm^2\ d^{-1}$ (MW = 10–1 kDa). The measured bulk DOC diffusion coefficient in central Long Island Sound porewater is 0.387 $cm^2\ d^{-1}$ (Fig. 6), substantially exceeding this estimated range. Mackin (1986) measured the diffusion coefficient of bulk DOC extracted from Long Island salt marsh mudflats to be 0.258 $cm^2\ d^{-1}$ (extrapolated from 25° to 5°C), also higher than would be commonly assumed. These higher diffusion coefficients could be explained by smaller average DOC molecular weights of $\sim$0.1–0.3 kDa.

The differences can also be explained by changes in macromolecular conformation as a function of ionic strength and solution composition, for example, the effect of absolute and relative abundance of alkalis and alkaline earths on DOC molecular diameters (e.g. Pasika, 1977; Cornel *et al.*, 1986; Yokoyama *et al.*, 1989; Chin and Gschwend, 1991). Cornel *et al.* (1986) found an increase in diffusivity of humic acids by a factor of $\sim$ 10 when ionic strength is increased from $\sim$ 0.001–1. In the same study they observed a similar but smaller effect for polystyrene sulfonates; however, no effect of ionic strength on diffusion of

uncharged molecules was found. This effect was conclusively attributed to changes in molecular conformation and constriction as charged functional groups are neutralized by ions in solution. Variations in pH have a similar effect: diffusion coefficients vary inversely with pH. Theoretically, if another measure of size were used, such as hydrodynamic radius rather than molecular weight *per se*, the relationship between diffusion coefficients and size should be independent of ionic strength. Thus, the present and previous studies indicate that diagenetic flux calculations using diffusion coefficients for DOC compounds made in distilled water corrected only for viscosity are likely to be in error for particular subsets of DOC, and that ionic strength, solution composition, pH gradients, and the nature of interstitial matter need to be considered.

The semipermeable behavior of both macrofaunal mucus gel and tube linings with respect to both inorganic, but particularly organic solutes, greatly complicates consideration of transport and remineralization reaction processes in the bioturbated zone of marine deposits. The likelihood that sedimentary pores are commonly filled with bacterial and macrofaunal exudates (Decho, 1990, 2000) structurally similar to the mucous secretions used in the present experiments suggests that bulk DOC diffusion coefficients, and those of particular subsets of DOC, may require correction for hindrance before being used in flux calculations. Correlations between readily extractable carbohydrate, or comparable operational measurements, and diffusive hindrance should be explored as a basis for identifying when semipermeability is likely to be significant (Fig. 5).

The possibility that macrofaunal tube linings are semipermeable to DOC means that transport properties are potentially highly heterogeneous and anisotropic as a function of solute group and specific DOC components (e.g., molecular size, charge). The transport regime for small solutes is likely to be entirely different than for large solutes in the bioturbated zone where complex distributions of organic tube linings, mucus secretions, and cemented burrow walls are the rule (Aller, 1983). Size dependent diffusion properties may also significantly alter reaction processes and organic matter preservation by affecting movement or retention of specific enzymes in particular regions. For example, the molecular weights of exoenzymes range from 30–100 kDa (Hoffman and Decho, 2000) and their diffusion away from individual cells, within microbial mats, or around biogenic sedimentary structures must be affected by mucus secretions. It seems likely that retention of exoenzymes near bacterial source cells (Decho, 2000), and impeding the transit of substrate DOC entering proximity to a cell are in fact major adaptive functions of mucoid secretions. The possible increased polarity and size of DOC as oxidation proceeds would further enhance this effect around individual cells, oxidized burrow structures, and at the oxic surficial sediment-water interface (Orem and Gaudette, 1984). Presumably macrofauna can readily manipulate the movement of DOC and subsets of DOC around their burrow structures by judicious use of mucus. Such manipulation of semipermeability and differential molecular diffusion represent another link between benthic macrofaunal and microbial activities, and warrant further detailed investigation (Fig. 12).

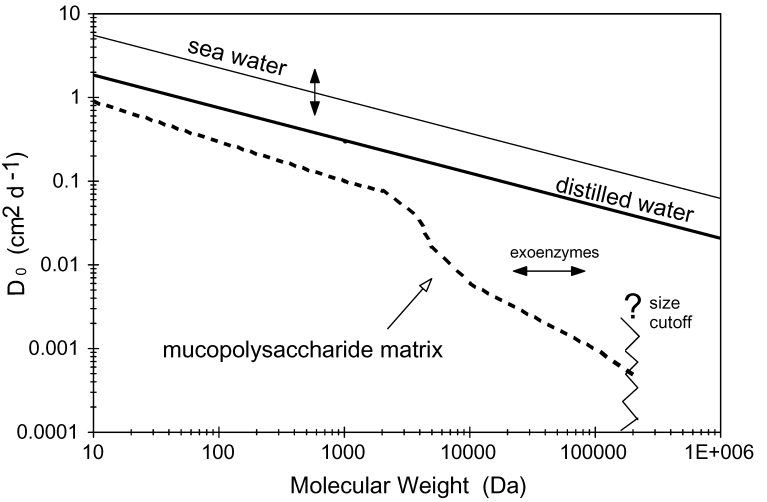


Figure 12. A general conceptual model for DOC diffusion coefficients as a function of molecular weight in sea water, distilled water, mucus gels, and tube linings. The translation to higher values in sea water should apply to charged species and may vary substantially depending on the exact electrolyte composition and DOC compound considered. It is unknown if mucus gels (or tube linings) exhibit size exclusion above molecular weights of ~ 100 kDa. The general size range for classes of functionally important DOC such as exoenzymes implies a major role for mucus gels in dictating enzyme and substrate movement through mucus-rich pore space and adjacent to bacterial cells, within microbial mats, or around biogenic sedimentary structures.

## 5. Conclusions

Macrofaunal mucus secretions and tube linings inhibit the diffusion of both organic and inorganic solutes by factors typically 3–8X lower than in free solution.

In many but not all cases, diffusive hindrance can be a function of both solute charge (anions) and size. Hindrance effects for DOC increase with solute size.

Size and charge effects imply that under some conditions mucus gels and organic tube linings can be treated as charged, porous meshworks with characteristic channel distributions, however, no size cutoff was observed up to at least 100 kDa. Increased viscosity by mucus may also be important.

Diffusion of charged organic solutes is enhanced in sea water relative to patterns predicted by relationships derived from diffusion in distilled water and viscosity dependence, presumably due to changes in the conformation of organic macromolecules in electrolyte solutions.

Semipermeable properties of biogenic secretions potentially greatly complicate movement of solutes, particularly DOC and classes of macromolecules such as exoenzymes, in the bioturbated zone and mucus cemented aggregates generally. Sedimentary exoenzyme - substrate interactions and DOC transport must be viewed within the context of mucoid fabrics.

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