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# Recruitment responses of benthic infauna to manipulated sediment geochemical properties in natural flows

by Steven J. Engstrom<sup>1</sup> and Roberta L. Marinelli<sup>1,2</sup>

## ABSTRACT

Recent studies have shown that local variation in surface sediment geochemistry can have significant effects on recruitment rates of benthic invertebrates. Experiments presented here tested (1) the utility of manipulating surface porewater concentrations using spiked polyacrylamide gels and (2) the recruitment responses of the polychaete *Arenicola cristata* and the bivalve *Mercenaria mercenaria* to manipulated ammonium concentrations in realistic flows provided by a straight flume and an annular flume. Data show that successful manipulation of sediment porewater ammonium concentration is feasible in flowing waters, i.e., overlying waters remained relatively free of ammonium while manipulated concentration levels were maintained within the upper sediment layers where new recruits explore. Thus, over short experimental periods, ammonium signals can be independently modified while variables such as grain size, organic content and flow are held constant. Responses of new recruits varied as a function of experimental condition. In straight flume trials, the manipulation of ammonium concentration was successful, and within the range of concentrations tested, retention of *Mercenaria* was significantly reduced at the highest ammonium level. However, retention of *Arenicola* was uniformly low among all treatments and no significant ammonium response was detected. In annular flume runs, overall ammonium concentrations were higher than in straight flume runs, but were consistent with the intended manipulation. *Mercenaria* retention was uniformly low, and no differences in retention, as a function of ammonium concentration, were found. However, for *Arenicola*, significant differences were observed, with highest retention in lower ammonium environments. These results suggest that ammonium, isolated from other cues, plays a significant role in determining recruitment patterns, with variation in recruit responses related to signal strength.

## 1. Introduction

Soft-sediment benthic systems are physically, chemically, and biologically diverse environments that play an important role in global elemental cycling. Infaunal organisms greatly modify transfers of carbon, nitrogen and other important elements, as a function of activity rate (Marinelli and Boudreau, 1996), density (Gilbert *et al.*, 2003) and diversity (Waldbusser *et al.*, 2004). Thus, from both an ecological and geochemical standpoint, it is important to understand the processes that determine benthic community structure and

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resultant interactions. Competition, predation, and disturbance are important and long-recognized processes that regulate community composition. More recently, the effects of recruitment success and the role of supply-side ecology on community composition have received attention (e.g., Gaines and Roughgarden, 1985). These factors exert significant control over the abundance of organisms and thus, the possibility that interactions will occur.

Both intrinsic factors related to larval life histories and extrinsic parameters related to physical, chemical and biotic events, affect recruitment patterns and overall species success. For example, intrinsic factors such as a lengthy planktonic larval stage may facilitate dispersal over large distances (Crisp, 1974), allowing greater gene flow and interconnectivity between communities, avoidance of predators, and/or disruption of parasitism or disease cycles (Strathmann, 1993). Alternatively, benthic dispersal provides for localized adaptation to community forcing functions, stronger links between adults and recruits but an increased vulnerability to disturbance and disease (Woodin, 1976). Moderate dispersal distances, on the order of tens of m, may allow recruits to span a range of environments, and facilitate a type of "bet hedging" (Strathmann *et al.*, 2002). This may be particularly advantageous for recruits that require a diverse environment for growth and/or utilize a different spatial niche than the adult, or perhaps disadvantageous if recruits are susceptible to mortality originating from adult conspecifics (Andre and Rosenberg, 1991; Woodin and Marinelli, 1991).

Extrinsic factors such as large-scale, dynamic physical processes in coastal and estuarine systems affect larval transport and distribution (Ekman, 1967; Scheltema, 1974; Levin, 1986) and for planktonic larvae, clearly influence recruitment success. The degree to which physical transport processes on smaller scales determine invertebrate recruitment has been more controversial (Butman *et al.*, 1988a, b; Olafsson *et al.*, 1994). There is a general consensus that patterns of deposition and erosion may be influenced by small-scale physical processes operating at the boundary (e.g., Eckman, 1983; Butman, 1987), however, recruit behavior clearly plays a role. For example, some new recruits are able to adjust their position and rate of movement in the presence of a dynamic flow environment (Butman *et al.*, 1988a; Woodin *et al.*, 1995). Deliberate timing of vertical swimming behavior can enable benthic organisms to travel appreciable distances with tidal currents (Shanks, 1995) and against predominant flows within an estuary. Finally, some evidence suggests that larvae settle and test substrates when flow is minimal, such as at slack tide, and thus avoid unnecessary energy expenditure (Whitlatch and Osman, 1998; Gross *et al.*, 1992). It is most likely that recruitment success is a result of an increase in favorable opportunities arising from active settlement behavior in addition to the chance that passive deposition will place an individual into a suitable environment.

Chemical cues in overlying waters, in porewaters, and at substrate surfaces also affect site selection (Pawlik, 1992; Turner *et al.*, 1994; Tamburri *et al.*, 1996) over a range of spatial scales. For sediments, large-scale patterns in availability of oxygen are a major covariate of infaunal distribution (Thorson, 1966; Pearson and Rosenberg, 1978; Diaz and

Rosenberg, 1995). However, community-wide processes can also affect meso-scale patterns as well. Through bioturbation and irrigation, infauna modify the particulate environment and create steep geochemical gradients over spatial scales of mm to cm and temporal scales of days to weeks (e.g., Meyers *et al.*, 1988; Marinelli, 1994). These processes impact sediment stability (Rhoads, 1974), sediment-seawater exchanges (Aller, 1982) and the overall character of the ecosystem (Suchanek, 1983). Recent studies (e.g., Woodin *et al.*, 1993, 1995, 1998; Marinelli and Woodin, 2002) have shown that local variation in the chemical environment, facilitated by biogenic disturbance, affects recruitment decisions of newly settled juveniles (new juveniles). Other investigations show that factors related to organic content (Butman and Grassle, 1992; Hardege *et al.*, 1998) and associated biogeochemical interactions (Cuomo, 1985; Dubilier, 1988; Raimondi, 1990; Fitt and Coon, 1992; Rodriguez *et al.*, 1995) can also be important. Given the high spatial variation in geochemical properties in sediments, and the strong relationships between organic matter content, diagenetic properties, and space, it is highly likely that local variation in recruitment patterns are strongly influenced by local geochemical processes evident during the surface exploratory phase of new recruits.

Below we describe manipulative experiments that examine the coupled effects of geochemical and physical processes on recruitment patterns in soft sedimentary environments. We developed a method for manipulating surface chemical properties by producing polyacrylamide gel diffusers with a known amount of geochemical solute and embedding the gels beneath a layer of sediment. This provided a predictable manipulation of a single substance within the range of natural variation that may result from biogenic disturbances or elevated organic inputs, while holding all other variables constant. In previous studies, the recruitment responses to geochemical factors has been integrated across a suite of environmental variables, confounding organic composition, diagenetic processes, and hydrodynamics (e.g., Butman and Grassle, 1992; Marinelli and Woodin, 2002). The experiments described below provide insights into the recruitment response of settling larvae in an environment characterized by a single, dominant chemical cue in a system that realistically simulates natural hydrodynamic conditions. By examining recruitment in the presence of flow, behavioral responses that exploit hydrodynamic conditions can be discerned. Furthermore, with successful manipulation of a specific solute known to modify recruitment patterns, while holding other geochemical parameters constant, the recruitment response to the manipulated substrate can be identified. The following null hypotheses were tested:

(H<sub>0</sub>): Experimental manipulation of sediment geochemical properties will not produce a significantly different chemical environment.

(H<sub>0</sub>): In a hydrodynamic environment, recruiting individuals will not be differentially retained in geochemically-manipulated sediments.

## 2. Methods

We chose to target ammonium in sediment porewaters as a solute cue because (1) ammonium is a natural byproduct of organic matter oxidation-reduction reactions, (2) ammonium concentrations are frequently elevated by disturbance (Aller, 1994) and (3) prior studies (Woodin *et al.*, 1998; Marinelli and Woodin, 2004) have shown a negative relationship between ammonium concentration in surface sediment porewaters and recruitment responses of new juveniles.

### *a. Gel diffuser trials*

A series of experiments were initiated to determine the diffusive characteristics of acrylamide gels and their utility as a source of ammonium to surface sedimentary layers. Specifically we examined (1) the time course of diffusion of ammonium from a bare (no overlying sediment layer) gel to an overlying water column (the diffusive flux), using different ammonium concentrations in gels; (2) the effect of sediment layer thickness on ammonium flux, and (3) the utility of ammonium persulfate vs. potassium persulfate as a crosslinking agent. This latter trial was initiated because the ammonium persulfate was a significant ammonium source and a no-ammonium control was desired. Polyacrylamide gels were made according to Browne and Zimmer (2001) with acrylamide concentrations ranging from 8–15% (w/v). For most experiments, crosslinking of the acrylamide gel was initiated with 0.05% ammonium persulfate (APS; w/v, final) and catalyzed with 0.05% tetramethylethylenediamine (TEMED; v/v final). Potassium persulfate (KPS) was substituted for ammonium persulfate (APS) in the potassium persulfate trials. In both cases, polymerization occurred in less than 1 h. These trials are described below.

To characterize the time course of ammonium release from bare gels to the sediment surface and overlying waters, and to test the effect of ammonium concentration within gels on the flux, diffusion experiments were carried out with gels containing three different ammonium concentration spikes: 0, 500, and 2,000  $\mu\text{M}$  ammonium. Ammonium spikes were accomplished by addition of ammonium sulfate to distilled de-ionized (DDI) water. For each concentration, three replicates were prepared, each consisting of 100 ml of gel poured into 250 ml, sterile specimen containers and allowed to set. Subsequently, 100 ml of DDI water was gently pipetted on top of the gel to create an overlying water (OLW) column of 4 cm, and allowed to incubate for 300 min. The OLW was stirred every 15 min to prevent the buildup of a solute gradient in the water column and prevent feedback between water column concentrations and the acrylamide gel. At 60-min intervals, 3 ml samples were withdrawn, filtered (0.2  $\mu\text{m}$  PTFE filters) and placed in sterile containers. The samples were then refrigerated until analysis for ammonium concentration, usually within 48 h, according to the method of Koroleff (1976). *Flux* is a measure of mass ( $m$ ) per unit area ( $A$ ) per unit time,  $t$ , (Eq. 1).

$$\text{Flux} = m/(A * t). \quad (1)$$

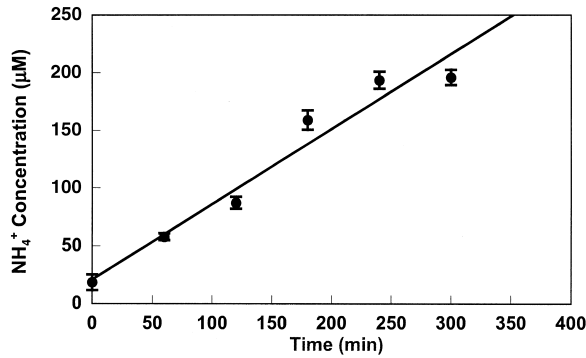


Figure 1. Change in overlying water NH<sub>4</sub><sup>+</sup> concentration over time in bare gel (no sediment) experiments using 2,000 µM NH<sub>4</sub><sup>+</sup> spiked APS gel. Samples taken every 60 min (*n* = 3). Regression fit to a linear function (*F* = 199.02, *R*<sup>2</sup> = 0.9549, *p* < 0.001).

Since concentration of a solute is measured as mass per unit volume, flux can thus be determined by multiplying concentration change ( $\Delta C$ ) change over time (*t*) by water column height (*L*) (Eq. 2).

$$Flux = \Delta C * L / t. \tag{2}$$

Thus, the flux of ammonium across the gel surface was calculated by linear regression of change in concentration (*C*) vs. time (*t*), normalized to the height of the water column (*L*). Preliminary experiments suggested that a linear relationship between concentration and time was appropriate for the relatively short duration of these experiments (Fig. 1). Because the amount of overlying water removed for each sampling was relatively small, the data were not adjusted to correct for variation in water column height due to the sampling procedure.

To test the effect of sediment layer thickness on ammonium flux from gels, a second set of experiments was conducted using 2,000 µM acrylamide gels topped with sediments of varying thickness. For these experiments, gels were created with a higher concentration (15%) of acrylamide, which produced a firmer layer for support of the sediment column. The setup was similar to the previous experiment but with 50 ml of gel. Sediment consisted of seawater-soaked (>24 h), moderately sorted (mean grain size = 287 µm) store-bought sandbox sand (hereafter referred to as play sand) that was gently placed on top of the gel to achieve a thickness of either 1 or 3 cm. Two controls were attempted: a bare gel (no sediment cover) and a gel covered with a plastic disk (to prevent diffusion) overlain by 1 cm of sediment. Approximately 150 ml of DDI water was added to produce a water column 5.5 cm in height. Four replicates were used for each treatment. The sampling/stirring regime was similar to that described for the previous experiment.

Gels produced with no-ammonium DDI water solutions released substantial concentrations of ammonium due to the ammonium persulfate (APS) crosslinker (see Results).

While this is advantageous for having an ammonium source, it presents a difficulty for establishing a no-ammonium gel control. A preliminary trial was conducted to test the utility of using potassium persulfate (KPS) as a cross-linking agent. APS (0.05%) gels were made with DDI water (no added ammonium sulfate) or 2,000  $\mu\text{M}$  ammonium sulfate-spiked DDI; KPS (0.05%) gels were made in DDI water with no added ammonium. Approximately 50 ml of gel were poured into each container to which 135 ml of DDI water was added after the gels had set. The OLW was stirred every 30 min. Filtered (0.2  $\mu\text{m}$ ) overlying water samples were taken at 0, 105, 280, and 380 min, stored and analyzed as described above. Three replicates were prepared for each treatment.

Acrylamide gels produced with KPS took slightly longer to set but had the same consistency as APS gels. Flux of ammonium out of the KPS gels was negligible (0.098–0.0156  $\mu\text{m cm}^{-2}\text{min}^{-1}$ ), while significantly higher flux rates were found for the 0  $\mu\text{M}$  (2.5–0.042  $\mu\text{m cm}^{-2}\text{min}^{-1}$ ) and 2,000  $\mu\text{M}$  (3.6–0.049  $\mu\text{m cm}^{-2}\text{min}^{-1}$ ) treatments containing APS. Acrylamide gels made with KPS, however, were not used in further tests because concentrations of potassium added by KPS (here calculated to be 0.0037 M) were similar in magnitude to natural potassium concentrations in seawater (0.01 M, Drake *et al.*, 1978). Thus, gels made with seawater and additional potassium provided an elevated  $\text{K}^+$  concentration that might affect vital functions, such as chemoreception, of settling juveniles. Control procedures were modified to consist of sediment only or sand placed on top of gels with no incubation time (see below).

#### *b. Straight flume experiments*

In these experiments, the response of organisms to chemically manipulated sediment surfaces was tested in a small, straight channel flume. We used *Arenicola cristata* and *Mercenaria mercenaria* because they are ecologically and/or economically important species with a cosmopolitan distribution along the eastern shore of the United States. The polychaete *Arenicola cristata* is a subsurface deposit feeder and significant bioturbator that serves as a food source for many benthic feeders, including commercially important demersal fish. The bivalve *Mercenaria mercenaria* (hard clam) is a suspension feeder that lives in muds and muddy sands and has commercial importance. Both species have a short dispersal phase and must return to benthic habitats similar to those from which they were released.

Egg-cases of *Arenicola cristata* were collected from Bly Creek in the North Inlet estuary, Georgetown, SC (79° 09' W, 33° 19' N) in April, 2001. The gelatinous mass was maintained in aerated seawater in the lab and upon release, larvae were transferred to small glass bowls. After approximately 7 d, the larvae had grown to six setigers (roughly 600  $\mu\text{M}$ ). At this stage, individuals will burrow into sediments and begin to build tubes (Woodin *et al.*, 1995). Thus we define 6-setiger arenicolids as new juveniles ready to adopt a benthic existence. Larval *Mercenaria mercenaria* were obtained from SeaPerfect Aquafarms, Charleston, SC. The cultures were kept in aerated seawater and fed *Isochrysis galbana*. Depending on temperature, larval *Mercenaria* will lose the velum and adopt a

benthic existence approximately 10–20 d after spawning. Only organisms that had lost the velum within the previous 24 h were selected for experiments, and we define these as new juvenile *Mercenaria*. To ensure that the individuals were competent to burrow, and that the test sediment was acceptable, preliminary acceptability tests were performed on six setiger arenicolids and *Mercenaria* that had just lost the velum. The new juveniles were carefully pipetted onto field-collected sediment (obtained from Bly Creek, SC) and aged play sand (soaked in aerated, oxygenated seawater for 48 h) and their exploratory and burrowing behaviors were observed through a dissecting microscope. In particular, the time spent exploring the surface prior to initiation of burrowing was quantified. Previous observations have indicated that short exploratory times, and rapid burrowing, are indicative of an acceptable habitat, whereas long exploratory times, or failure to burrow, are taken as rejection of the habitat (Woodin *et al.*, 1995; Marinelli and Woodin, 2002). The observation period was 6 min (Woodin *et al.*, 1998). Preliminary observations indicated that both *Arenicola* and *Mercenaria* juveniles burrowed into aged play sand and field sediments within short time frames (less than 1 min. for more than 4 out of 7 individuals of each species tested), as observed in acceptable sediments in previous studies (Woodin *et al.*, 1998; Marinelli and Woodin, 2002).

*i. Description of the straight flume.* The straight flume is a gravity-fed, nonrecirculating flume (Woodin *et al.*, 1995) with dimensions of 1.26 m by 10 cm. Water flow is facilitated by a header tank kept at constant volume and is regulated by a control valve. Water entering the working section passes through straighteners (a hexagonal matrix of straws), which help smooth the flow. A circular hole (diameter 5.8 cm) in the working section 75 cm downstream allows placement of cores into the flume such that core surfaces were flush with the flume bottom. For all trials, the flume was filled to a depth of 1.8 cm. While this experimental regime may not simulate a fully-developed marine bottom boundary layer, it is likely similar to flow through shallow tidal creeks (common habitats for worms and bivalves), and it provides a hydrodynamic means for new recruits to depart from an unacceptable site.

Results of preliminary trials (below) indicated that 12% acrylamide gels made with ammonium persulfate provided a source of ammonium to the overlying water. When incubated for 6 h with a thin (1 cm) sediment over-layer, ammonium concentrations in the porewater created a distinct surface signature (mean ammonium concentration  $414 \pm 78 \mu\text{M}$ ). At the same time, gels that were covered with fresh sediment but allowed no incubation time had very little ammonium (mean ammonium concentration  $30 \pm 69 \mu\text{M}$ ) at the sediment surface. Thus, the addition of ammonium sulfate, and the presence or absence of an incubation period, allowed the establishment of three treatments: Low ammonium (0  $\mu\text{M}$  ammonium sulfate gel with 1 cm sediment and no incubation period), Intermediate ammonium (0  $\mu\text{M}$  ammonium sulfate gel with 1 cm sediment and 6 h of incubation time, hereafter referred to as Medium); and High ammonium (6,000  $\mu\text{M}$  ammonium gel with 1 cm of sediment and a 6 h incubation time). For these experiments,



acrylamide gels were made with artificial seawater (DDI water with Instant Ocean salts) with addition of ammonium sulfate to the desired concentration level.

*ii. Preparation of experimental cores.* Approximately 60 ml of acrylamide gel were poured into each experimental core. Cores consisted of a plexiglass annulus (inner diameter = 5.8 cm; height = 4 cm for the low, and 8 cm for the medium and high), with a removable plunger (a solid PVC disk with 2 O-rings). After the gels had set, 0.75 cm of aged play sand was added to all Medium and High ammonium cores, followed by addition of 100 ml of 3  $\mu\text{m}$ -filtered seawater (salinity of 25 ppt). These cores were allowed to equilibrate for at least 6 h. At the conclusion of the incubation period for Medium and High ammonium cores, the overlying water was removed and replaced four times with fresh, 3  $\mu\text{m}$  filtered natural seawater. For the Low ammonium cores, a small amount of artificial seawater was added to the surface of the uncovered gel to keep it moist. Immediately before use in flume trials, the water was removed and a layer of aged sand was added to match the thickness in the medium and high cores. Subsequently, seawater was added and replaced as in the Medium and High ammonium cores. For all cores, the water column height just prior to flume trials was 2 cm.

*iii. Experimental protocol.* Because straight flume experiments could involve only one core at a time, trials were run within short (1 h) blocks of time, with each block containing a Low, Medium, and High core trial (randomly distributed) for a total of eight experimental blocks. For each of the Medium and High trials, filtered samples (0.2  $\mu\text{m}$ , <1 ml) of the overlying water (OLW) column were taken to establish ammonium concentrations immediately after placement of the core in the flume bed but prior to the addition of new juveniles. Since there was little OLW for the Low ammonium trial, no sample was taken prior to adding new juveniles.

For each trial, new juveniles (75 *Arenicola* and 50 *Mercenaria*) were collected and transferred to a small holding dish containing 20 ml of 3  $\mu\text{M}$  filtered seawater. An experimental core was inserted into the bottom of the drained flume so that the water column of the core was slightly (several mm) below the flume bottom surface. The dish containing both species of new juveniles and a small volume of water was gently transferred to the overlying water of the core, such that no disruption of the sediment surface, and no overflow of water onto the flume channel occurred. The new juveniles were then allowed to settle in still water for 5 to 7 min. Although both species of test organisms were added at the same time, the likelihood of interaction among individuals is quite small. Assuming *Mercenaria* is approximately a 250  $\mu\text{m}$  disc and *Arenicola* is a 600  $\mu\text{m}$   $\times$  200  $\mu\text{m}$  rectangle, the area taken up by the new juveniles is less than 0.2  $\text{cm}^2$ , whereas the area of the sediment surface in the core is  $>26 \text{ cm}^2$ . Thus, organisms were sparsely distributed over a comparatively broad area. Subsequent to addition of new juveniles, a sample of the overlying water in the core was taken to determine if water column ammonium concentrations changed as a result of the addition process. The sample was filtered through a 0.2  $\mu\text{m}$  filter, transferred to a clean vial, and refrigerated prior to analysis.

After organisms were allowed to settle, flow was initiated and the flow speed was adjusted to  $3.0 \pm 0.5 \text{ cm s}^{-1}$ . This flow speed was chosen because it represents hydrodynamic conditions found in environments inhabited by the study species. Once flow was established, the core plunger was gently pushed upward until the sediment surface was flush with the flume bottom. The sediment surface was exposed to flow for 3 min. Flow was turned off, the flume was then drained, and a porewater sample ( $<0.7 \text{ ml}$ ) was taken from the top 5 mm of sediment, using a syringe fitted with a 16 gauge needle. The sample was filtered ( $0.2 \text{ }\mu\text{m}$ ), transferred to a clean vial, and refrigerated. The entire 0.75 cm layer of sediment above the gel surface was removed, preserved with buffered formalin, and stained with Rose Bengal for subsequent enumeration of juveniles. The process was repeated eight times for each of three treatments.

*iv. Analysis.* Water samples were returned to the Chesapeake Biological Laboratory and analyzed for ammonium within 5 d as previously described. The sediment samples were transferred from a formalin solution to ethanol and then sorted using an Olympus SZH-10 zoom dissecting scope to determine the number of *Arenicola* and *Mercenaria* juveniles remaining in the sediment. Ammonium concentration data were tested for normality and homogeneity of variance and having met these assumptions, were analyzed as an unreplicated randomized block design by analysis of variance (ANOVA) using SAS v8.0 (SAS Institute, Cary, NC, 1999). Treatment (Low, Medium, High) was considered a fixed effect whereas Block was considered a random effect. Retention of new juveniles onto the manipulated sediment surface was proportional (juveniles retained relative to juveniles added) and as such, the data were transformed using an arcsin transformation. However, the transformed data violated the assumption of homogeneity of variance and thus, analysis of variance could not be used. Consequently, the data were analyzed using a Jonckheere-Terpstra nonparametric ordered alternatives test, where an observed response is evaluated relative to a predicted response, and where deviation from the null hypothesis is expected in a particular direction (Hollander and Wolfe, 1973). In this experiment, we predicted that retention rates would be higher in Low ammonium sediments, and lower in High ammonium sediments.

*c. Annular flume experiments*

A second set of experiments testing *Mercenaria* and *Arenicola* responses to ammonium-spiked sediments was conducted using an annular flume. The annular flume provided a more realistic bottom boundary layer and allowed simultaneous testing of treatments and replicates. Due to differences in the timing of larval availability, separate trials for each species were run.

The flume (see Porter, 1999 for a more detailed explanation) consisted of a circular (180 cm outer diameter) channel 20 cm wide and 20 cm deep. The channel flow was driven by contact on the water surface with a plexiglass ring 10 cm across, thus covering half of the channel width. The ring was attached to a rigid support system driven by a variable

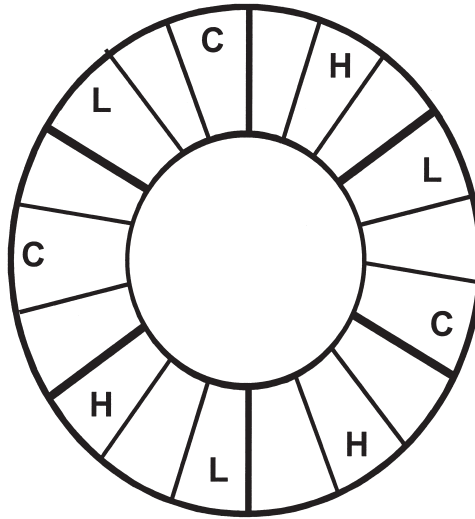


Figure 2. Diagram of the section setup within the annular flume. Replicate treatments (Low and High for *Mercenaria*, Control, Low and High *Arenicola*) were randomly assigned to test sections. For all trials, flow was in a clockwise direction, and partitions were covered by a smooth sediment layer. Diagram not drawn to scale.

speed DC motor. Six removable resin-coated wood and fiberglass trays were made to fit snugly into the flume on the bottom of the channel. The trays measured 18 cm across with walls approximately 3–4 cm high. Each tray was subdivided into three equal ( $430 \text{ cm}^2$ ) sections by two plastic walls 3 cm high, giving a total of 18 sections. The sections held a volume of approximately 2 L and were used to accommodate acrylamide gels of varying ammonium concentration. Of the 18 sections, either six (*Mercenaria* experiment) or nine (*Arenicola* experiment) were assigned as test sections and the remaining sections were not used (Fig. 2). All sections were arranged to alternate between test sections and unused sections, so that no two test sections were directly adjacent to one another in the flume.

To characterize the fluid dynamic environment, velocity profiles were measured with a SonTek Micro ADV (acoustic Doppler velocimeter). The flow profiles were measured at 15 cm from the inner wall of the flume channel (channel width = 20 cm), rather than 10 cm from the inner wall (center of channel) due to the position of the driving ring. Profiles were measured at a ring speed of 4.5 RPM, at 1 mm intervals above the sediment surface to 12 mm, and at 3 mm intervals until freestream velocity was reached (Fig. 3). The position of the ADV and sample volume was controlled with a micromanipulator. The height of measurement above the sediment water interface was determined using a combination of measurements of the signal-to-noise ratio and a direct measurement of the ADV probe tip above the sediment surface, according to Finelli *et al.* (1999).

Given the relatively large area of the flume and the grain size of the experimental sediment, we asked whether advective processes might affect porewater flows, particle

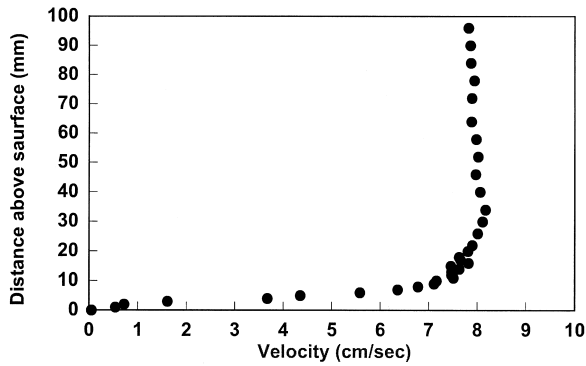


Figure 3. Velocity profile above the sediment water interface (SWI) at ring speed 4.5 RPM as measured by a SonTek® MicroADV. Measurements taken every 1 mm below 12 mm from SWI, and every 3 mm above 12 mm, with vertical position controlled by micromanipulator. Mean freestream velocity calculated to be  $7.79 \pm 0.21 \text{ cm s}^{-1}$ .

transport, and ammonium concentrations within the annulus. Advection of porewater can arise from pressure differences driven by surface gravity waves or topographical variation in the sediment surface (e.g., Huettel *et al.*, 1996). Since flow within the flume was smooth and relatively free of secondary circulation artifacts (<7.2% secondary circulation, see results), and the bed was smoothed prior to each trial, pressure differences at the sediment surface were minimal. As a qualitative test, rhodamine WT dye was added to wet sediment and then placed in the annular flume and smoothed. The sediment surface was observed for release of dye during normal flow. Very little dye was released from the sediment surface, suggesting that advection was not occurring. Similarly, no significant bedload transport was observed at the ring speed used in the experiments. These observations, and high ammonium measured within the bed relative to the low ammonium measured within the water column (see below), suggest advective processes did not control porewater flow and that current velocities did not erode particles. Thus, departure of new juveniles from a site is more likely to be a result of behavior rather than fluid forcing.

*i. Sediment characteristics.* Field sediments used in the annular flume experiments were collected from Tom s Cove, VA ( $73^{\circ} 23' \text{ W}$ ,  $37^{\circ} 56' \text{ N}$ ), sieved ( $500 \mu\text{m}$ ) and stored in running seawater (see below). Grain size distribution of both field sediments and play sand (determined according to Folk and Ward, 1957) were similar (mean grain size =  $290 \mu\text{m}$  and  $287 \mu\text{m}$ ) with sediment types categorized as well to moderately sorted (Std. dev. = 0.5987 and 0.7289), medium sands tending toward coarser grains (skewness = 0.1970 and  $-0.0389$ ) for field sediment and play sand, respectively. Thus, both sediment types were physically similar and likely to have similar effects on the hydrodynamic environment near the sediment-water interface.

*ii. Preliminary trials.* To determine whether ammonium buildup in the flume water column occurred when gels were embedded in trays, several preliminary flux tests were conducted. Two acrylamide gel solutions were made, one with a 2,000  $\mu\text{M}$  ammonium solution at a salinity of 28 ppt and one with filtered (3  $\mu\text{m}$ ) seawater and no additional ammonium, also at a salinity of 28 ppt. The control gel (750 ml) was poured into a downstream section of a test tray, and the 2,000  $\mu\text{M}$  gel was poured (750 ml) into the upstream section of the same test tray. Once the gels had set, 1.5 cm of play sand was added on top and smoothed with a trowel. All remaining upstream sections were filled with play sand (no gel) and the entire bed was smoothed. The flume was then filled with 22°C, seawater with a salinity of 28 ppt to create a water column 15 cm high (roughly a total of 150 L) and allowed to equilibrate for 5 h in still water. Water column samples were taken roughly 10 cm above the sediment water interface, over the 2,000  $\mu\text{M}$  gel treatment section, filtered, and stored as previously described. The flume was drained until the water level was flush with the sediment surface. Porewater samples (1 ml) were taken as previously described for the three different substrates within test trays (no gel below sediment, 2,000  $\mu\text{M}$  gel below sediment, and 0  $\mu\text{M}$  gel below sediment). The flume was then refilled with seawater and another water column sample was taken and processed as described previously. The flume was then turned on at a speed of 7 to 8  $\text{cm s}^{-1}$  (4.5 RPM) for 2 h. At the conclusion of the flow period, a post-flow water column sample was taken. Subsequently, the flume was drained, and post-flow porewater samples were taken within each experimental section, as described previously. This process was repeated once more after the flume ran for an additional 2 h at 4.5 RPM. Sampling before and after flow established if ammonium concentrations were affected by the addition of water to the flume channel, the flow, and the presence of the treatments. No significant change in concentration was observed in the overlying water across all flume refills ( $F_{1,2} = 0.368$ ,  $p > 0.05$ ) over a 2 h interval, thus accumulation of ammonium in the water column was negligible. This suggests that water column ammonium affects on new juveniles due to the flume filling process was unlikely. Although higher gel concentrations were used for recruitment trials, the duration of the experiment was shorter and appreciable buildup did not occur (see Results).

*iii. Annular flume protocols.* The seawater used in annular flume experiments was filtered water from the Patuxent River, MD, USA (salinity of 13 ppt, 18°C), brought to room temperature and raised to a salinity of 21 ppt (*Mercenaria* experiment) or 28 ppt (*Arenicola* experiment) with Instant Ocean salts (Aquarium Systems, Inc.). The water was aerated and stored in a tank (~150 L) until the start of the experiment. The experimental sediments consisted of a mixture of field-collected sediment (from an intertidal location at Tom's Cove, VA, 73° 23' W, 37° 56' N) that had been sieved (500  $\mu\text{m}$ ) and stored in running seawater, and play sand (as in previous trials). Prior to each trial, both the field sediment and play sand were rinsed with prepared seawater. Rinsing alleviated high ammonium concentrations that evolved during sediment storage.

*iv. Mercenaria experiment.* Larval *Mercenaria mercenaria* (8 d old) were obtained from Cherrystone Aqua-farms, Cheriton, VA. Cultures were fed *Isochrysis galbana* and monitored continually to determine when the transition from a swimming to a benthic form (loss of velum) occurred. Individuals that had shed the velum within the previous 24 h (new juveniles) and were actively moving by use of their foot were counted (50 per experimental replicate) and separated into holding dishes. Before the trials, new juveniles were assessed for burrowing times and sediment-acceptability as in the straight flume experiments. Preliminary sediment-acceptability tests indicated that unmanipulated field sediment provided a suitable settlement environment. Of the ten representative individuals tested, all burrowed in less than 2 min.

Due to the time required to sort competent *Mercenaria* new juveniles, only three experimental replicates of each of two treatments (Low and High ammonium) were completed for each trial. Three replicates of each treatment were randomly assigned to the test sections within the flume. The treatments consisted of two acrylamide (12%) gel concentrations (0  $\mu\text{M}$ , and 6,000  $\mu\text{M}$ ). The gels were poured into each section (approximately 2 cm deep) and allowed to set for at least 8 h. Once set, the trays were put into the flume. The trays without gels were filled with 4 cm of play sand whereas the test sections were filled with 2 cm of play sand. Then, the entire flume bottom surface was covered with approximately 1.0 cm of field sediment. All seams between trays, sections, and the wall of the flume were smoothed with a trowel so that no obstructions to flow occurred. The flume was then slowly filled with prepared seawater to a depth of 4 cm and allowed to equilibrate for a minimum of 6 h, to allow ammonium to diffuse toward the sediment surface. After the equilibrium time, an overlying water column sample was taken from the flume (pre-organism addition OLW), and then the water was drained to the same level as the sediment surface. One porewater sample (pre-organism addition PW) was taken near the center of each test section. The flume was then filled to a depth of 4 cm.

To add new juveniles to the test sections, plastic cylinders (6 cm diameter, 9 cm high) were gently placed onto the sediment surface at the center of each test section (Fig. 4A). Individuals from the holding dishes were then gently pipetted into the water column within the plastic cylinders, using a minimal amount of water so that a large pressure head was not established. Sediment was not resuspended during the addition phase. The flume was slowly filled until the water height outside and inside the cylinders was equal. The cylinders were then removed and the water column was raised to a height of 15 cm (Fig. 4B). A sample of the water column was taken for analysis of ammonium concentration. The dishes that held the new juveniles were later checked under a dissecting scope for any *Mercenaria* remaining, i.e., that were not added to the test sediment.

Once the flume was filled, flow was initiated and maintained for 8 min. This time period is approximately ten times the average observed exploration time for new recruits of several bivalve and polychaete species prior to burrowing in acceptable sediments (Marinelli and Woodin (2002) and references therein). It therefore provided sufficient time for evaluation of sediment-acceptability and movement away from the site if sediments

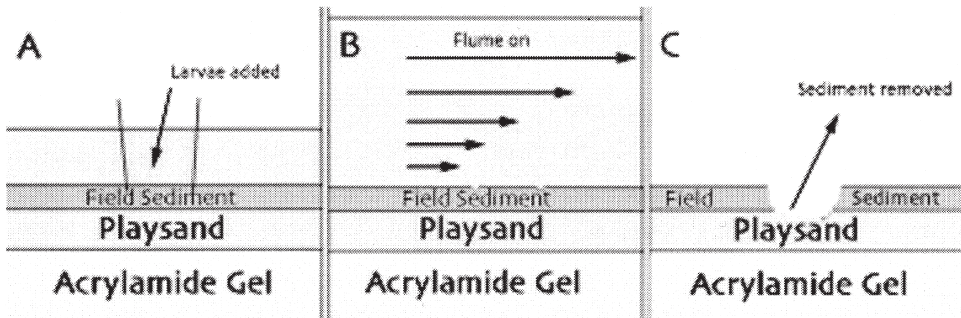


Figure 4. Diagram of new juvenile addition, flow initiation, and sampling sequence. Acrylamide gel was poured into flume sections. Store-bought sand (play sand) and then field sediment was added on top. The flume was filled halfway, and then the new juveniles were added to the sediment surface by pouring them into a plastic cylinder that encompassed a region of the sediment surface and allowing them to settle onto the surface for up to 35 min (A). The flume was then filled to a depth of 15 cm and flow turned on to 4.5 RPM for 8 min (B), with a mean freestream velocity of  $7.8 \pm 0.28 \text{ cm s}^{-1}$ . The flume was then drained and porewater samples taken just below the sediment surface using a needle and syringe and then filtered ( $0.02 \mu\text{m}$ ). The sediment where new juveniles were added was extracted, preserved, stained and later sorted for remaining individuals (C).

were deemed unacceptable. At the conclusion of the flow period, a water column sample (for ammonium analysis) was taken, and the flume was drained until the water level was flush with the sediment surface (Fig. 4C). Subsequently, porewater ammonium samples were taken in the test sections, and processed as previously described. The sediment where competent new juveniles were added was sampled to a depth of 2 cm, preserved in buffered formalin, and stained with Rose Bengal. The entire experimental procedure, with freshly prepared acrylamide gels, was performed two additional times for a total of three trials.

Water column and porewater samples were refrigerated immediately and analyzed for ammonium concentration within 24 h of collection. The sediment samples were transferred to ethanol and then sorted under a dissecting scope to count number of individuals in the sample. This experiment was treated as a randomized complete block design with replication, with Treatment as a fixed effect and Trial as a random effect. Because the new juvenile data were treated as proportional, these data were transformed using an arcsin function. The ammonium data were log-transformed. Both data sets were tested for normality and homogeneity of variance and, having met these assumptions, were analyzed with ANOVA (SAS v.8.0, SAS Institute, Cary, NC, 1999).

*v. Arenicola experiment.* Egg masses of *Arenicola cristata* were obtained from Tom's Cove, Assateague, VA ( $73^{\circ} 23' \text{ W}$ ,  $37^{\circ} 56' \text{ N}$ ), at low tide on the 27<sup>th</sup> of May, 2003. They were transported to the Chesapeake Biological Laboratory and reared as described above,

to the 6-setiger stage where transition to a benthic existence occurs. Less than 6 h before the start of each trial, 75 new juveniles, were placed into each of nine dishes. Representative individuals were chosen from the juvenile population and tested for competency and burrowing behavior as previously described. Sediment-acceptability tests indicated that rinsed (2 to 3 times) field sediment (Tom's Cove, VA, see above) was moderately acceptable. Of the eight arenicolids tested, five burrowed in under 2 min; the other three did not burrow in the observation period (>2 min).

Three experimental trials were performed over 3 d. For each trial, nine test sections and nine unused sections were established with no two test sections directly adjacent in the flume. The treatments consisted of (1) Control (field sediment with no underlying gel), (2) Low (0  $\mu\text{M}$  acrylamide (12%) gel with play sand and field sediment on top, which conforms with the procedures used in the *Mercenaria* experiments described previously and (3) High (6,000  $\mu\text{M}$  gel with play sand and field sediment on top). Within each flume run, treatments were replicated in triplicate and were randomly assigned to test sections. Field sediment with no gel was used as a control because previous efforts to limit ammonium flux from acrylamide gels (e.g., covering with plastic or use of KPS) were unsuccessful. Due to this failure, a behavioral response to the presence of the acrylamide, in the absence of ammonium could not be evaluated experimentally. We consider such a response unlikely as the acrylamide is tightly bound, and the gel layers were significantly deeper than new juveniles burrow—thus direct contact was unlikely. A control without acrylamide provides insight into the recruitment response associated with natural field sediments and relatively low ammonium concentrations.

Sediment trays were prepared as described previously. The procedure for preparing the flume, and adding individuals, followed the methods described above for the *Mercenaria* experiment, except for the number of treatments per trial (2 for *Mercenaria*, 3 for *Arenicola*) and the number of individuals (50 *Mercenaria*, 75 *Arenicola*) per replicate. Water column and porewater samples, and sediment samples were handled as described above for the *Arenicola* experiment. The new juvenile data were arcsin transformed, and the ammonium data was log-transformed prior to analysis. The data were tested for normality and homogeneity of variance and, having met these assumptions, were analyzed by ANOVA as a randomized complete block design with replication (SAS v.8.0, SAS Institute, Cary, NC, 1999), with Treatment as a fixed effect and Trial as a random effect.

### 3. Results

#### *a. Gel diffusion trials*

Ammonium was released from all treatments in the bare gel experiments due to the presence of ammonium persulfate as a cross linking agent (all treatments) and the addition of ammonium sulfate as a spike (500 and 2,000  $\mu\text{M}$  treatments). Higher fluxes were associated with the magnitude of the ammonium spike (Fig. 5). In all cases, diffusion of



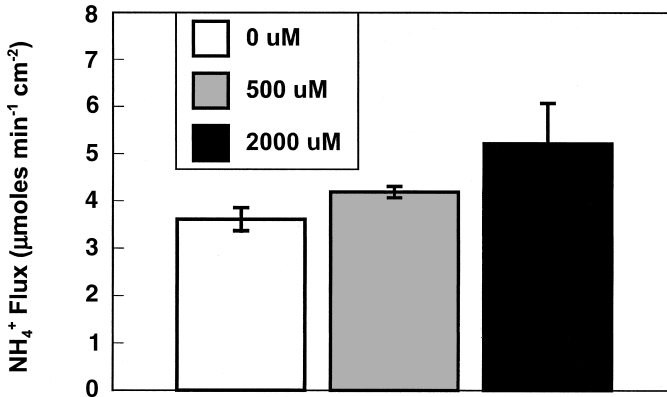


Figure 5. Flux of  $\text{NH}_4^+$  from bare (no sediment) acrylamide gels into overlying water. Gels were produced with 0, 500, and 2,000  $\mu\text{M}$  ammonium sulfate solutions. Flux was calculated from change in overlying water concentration over 300 min, and water column volume normalized to gel surface area. Bars are mean values  $\pm$  one standard deviation ( $n = 4$ ).

ammonium from the gel (surface area = 28.3  $\text{cm}^2$ ) to the overlying water was linear (e.g., see Fig. 1), and variability among replicates was quite low. Fluxes were significantly different between the 0  $\mu\text{M}$  concentration gel treatment and the 500 and 2,000  $\mu\text{M}$  gel treatments (Fig. 5; ANOVA:  $F_{2,6} = 7.46$ ,  $p = 0.0236$ ).

Diffusion of ammonium was greatly reduced when the gels were covered with a layer of sediment (Fig. 6; ANOVA:  $F_{3,11} = 918.76$ ,  $p < 0.0001$ ). Results support the prediction that as sediment depth increases, the diffusional distance also increases, and over short

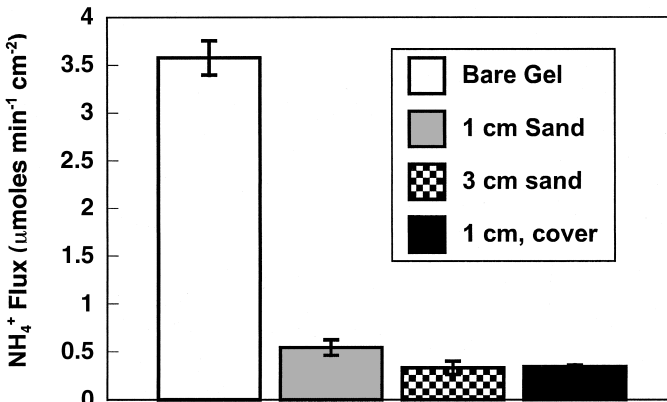


Figure 6. Flux of  $\text{NH}_4^+$  from acrylamide gels covered with varying depths of sediment on top of gels ( $n = 3$ ). Gels were produced with 2,000  $\mu\text{M}$  ammonium sulfate solutions with APS crosslinker and covered with 1 and 3 cm of play sand. Acrylamide gels used in the third treatment were covered with plastic cellophane and then 1 cm of play sand. Bars are mean values  $\pm$  one standard deviation ( $n = 3$ ).

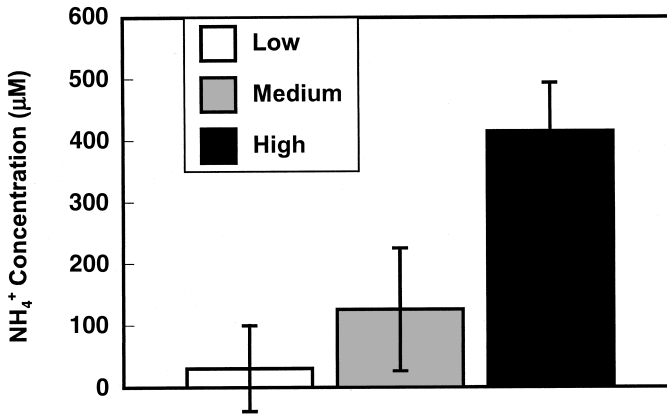


Figure 7. Porewater  $\text{NH}_4^+$  concentrations taken from top 0.5 cm of sediment surface for predesignated Low, Medium, and High treatments in the straight flume experiments. Bars are mean values  $\pm$  one standard deviation ( $n = 8$ ).

incubation times, fluxes are reduced. Comparative tests (data not shown) suggest that ammonium adsorption onto play sand is greater than in field-collected sediment. Therefore, some ammonium adsorption onto play sand particles may have further reduced ammonium flux to the overlying water. Although the plastic layer between sediment and gel reduced fluxes of ammonium slightly, the plastic appeared to be somewhat permeable to ammonium and as such, a gel control could not be established.

#### b. Straight flume experiments

Ammonium concentrations in water samples taken above the test sediments both prior and subsequent to addition of new juveniles of both *Arenicola* and *Mercenaria* were relatively low (mean  $< 3.0 \mu\text{M}$  for Medium and  $< 8.4 \mu\text{M}$  for High) and within the range of water column concentrations for the North Inlet (Marinelli and Woodin, 2004). This suggests the disturbance of porewater associated with the addition of new juveniles was minimal. Samples of the incoming water used within the flume indicated ammonium concentrations were near zero (mean  $< 0.001 \mu\text{M}$ ). However, sediment porewater concentrations in Low, Medium and High concentration cores were elevated relative to the overlying water and were significantly different from one another (Fig. 7; ANOVA treatment effect  $F_{2,13} = 21.82$ ,  $p < 0.0001$ ; block effect  $F_{7,13} = 0.82$ ,  $p = 0.5859$ ). These results suggest that the ammonium manipulation of the sediment surface via acrylamide gels in flowing water was successful, and that overlying water concentrations were never contaminated with high ammonium. Concentrations of porewater ammonium for the Medium and High treatment cores were more than an order of magnitude higher than the Low treatment cores, but still within the range of naturally occurring ammonium concentrations in the upper sediment layers where juveniles explore.

Results from the Jonckheere-Terpstra nonparametric ordered alternatives test indicate a

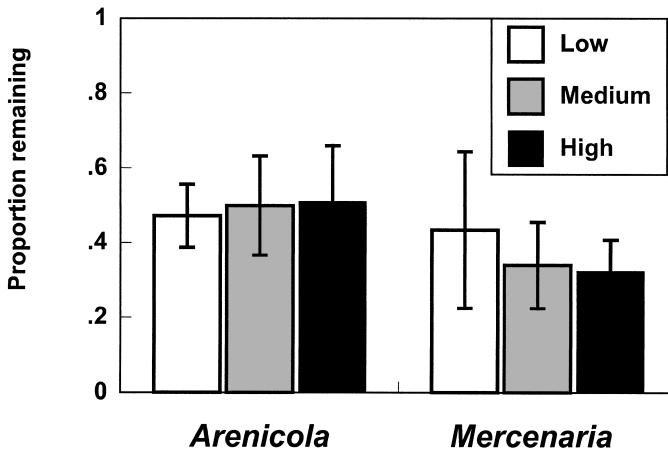


Figure 8. Proportion of juvenile *Arenicola* and *Mercenaria* individuals remaining in Low, Medium, and High  $\text{NH}_4^+$  concentration treatments compared to the total number added prior to flow for Straight Flume Experiments. Bars are mean values  $\pm$  one standard deviation.

significant difference ( $p = 0.0046$ ) in retention of *Mercenaria* among treatments but no difference for retention of *Arenicola* (Fig. 8). For both *Mercenaria*, and *Arenicola*, mean retention of new juveniles was low (approximately 30%) perhaps due to insufficient organic coating on the play sand or other factors related to the substrate. However, for *Mercenaria*, the retention pattern followed the predicted response for avoidance, i.e., higher retention for Low ammonium sediments, and low retention in High ammonium sediments. The response from *Arenicola* was not consistent with avoidance of high ammonium sediments, as in previous experiments (Woodin *et al.*, 1998). For *Arenicola*, factors associated with play sand may have been more significant than the ammonium signal, prompting an overwhelming avoidance response to the substrate.

### c. Annular flume experiments

*i. Flow characterization.* Flow profiles yielded information concerning flow velocity, turbulence intensity, and shear velocity. Freestream velocities ( $z \geq 12$  mm from sediment surface) averaged  $7.79 \pm 0.21 \text{ cm s}^{-1}$ . Below 12 mm, flow decreased to near zero just above the bed, following a typical log-linear relationship between distance from bed and velocity (Fig. 3). Although annular flumes often have secondary circulation and do not strictly obey a log-linear relationship, a detailed characterization of this flume (Porter, 1999) indicated secondary circulation of  $<7.2\%$  at the flow speeds used in this experiment.

Shear velocity ( $u^*$ ) was calculated from the relationship between shear stress ( $\tau_b$ ) and the water density ( $\rho$ ), (assumed to be 1.028 at  $\sigma = 28$ ; Eq. 3).

$$u^* = \sqrt{(\tau_b/\rho)} \quad (3)$$

Shear stress ( $\tau_b$ ) was calculated from the relationship between the drag coefficient ( $C_D$ ), water density ( $\rho$ ), and mean freestream velocity ( $U$ ;  $7.79 \text{ cm s}^{-1}$ ; Eq. 4) as described in Porter (1999):

$$\tau_b = \rho C_D U^2 \quad (4)$$

The drag coefficient was estimated using the friction factor ( $f$ ) and the relationship  $C_D = f/8$ . The friction factor is obtained from a Moody Diagram (Daily and Harleman, 1966), which relates ( $f$ ) as a function of the Reynolds Number ( $Re$ ; Eq. 5) and the Relative roughness ( $R_r$ ; Eq. 6) of a surface. The Reynolds number ( $Re$ ) is defined as:

$$Re = 4hU/\nu \quad (5)$$

where ( $\nu$ ) is the kinematic viscosity of water ( $1.0 \times 10^{-6} \text{ m}^2 \text{ s}^{-1}$ ). Relative roughness ( $R$ ) is defined as:

$$R_r = k_s/4h \quad (6)$$

where ( $k_s$ ) is roughness element height and ( $h$ ) is water column height. Mean grain size of the field sediment (0.03 cm) was assumed to be a maximum roughness element height ( $k_s$ ) and the water column height ( $h$ ) was the same throughout all tests at 15 cm. Relative roughness ( $R_r$ ) was thus found to be 0.005 and the calculated Reynolds number ( $Re$ ) was  $4.41 \times 10^7$ . From the estimate of the friction factor ( $f$ ) at 0.0205, obtained from the Moody Diagram,  $C_D$  was calculated to be approximately 0.00256,  $\tau_b$  was 0.1551, thus  $u_*$  was  $0.394 \text{ (cm s}^{-1}\text{)}$ . Shear velocities in this range indicate that drag on particles was not sufficient to cause significant erosion on the sediment surface, and that organisms remaining on the surface among sediment grains would not encounter drag forces that would remove them from the exploratory site. The possibility exists, however, that organisms modify their position to enhance drag and promote advection from the site (Woodin *et al.*, 1995).

*ii. Mercenaria experiment.* Manipulation of porewater ammonium concentrations was successful. Differences between pre- and post-addition overlying waters, and pre- and post-addition porewater samples within a treatment were much smaller than porewater concentrations across treatments (Fig. 9) and confirm that any release of ammonium from the sediments to the water column was minimal and rapidly dissipated. There were significant differences in porewater ammonium concentration among treatments (Fig. 10; ANOVA, treatment effect  $F_{1,14} = 17.3$ ,  $p = 0.0010$ ; Fig. 10, mean Low concentration =  $472 \text{ }\mu\text{M}$ , mean High concentration =  $921 \text{ }\mu\text{M}$ ), as well as a significant trial effect ( $F_{2,14} = 31.7$ ,  $p < 0.0001$ ). The concentrations in these trials were considerably higher than in previous test runs, and also differed among each other, perhaps because of variation in the desorption of ammonium during the extensive field-sediment rinsing process. Analysis of the sediment samples indicated that retention rates of *Mercenaria* for all treatments were surprisingly low (overall mean =  $0.0755 \pm 0.0758$  proportion of individuals found upon

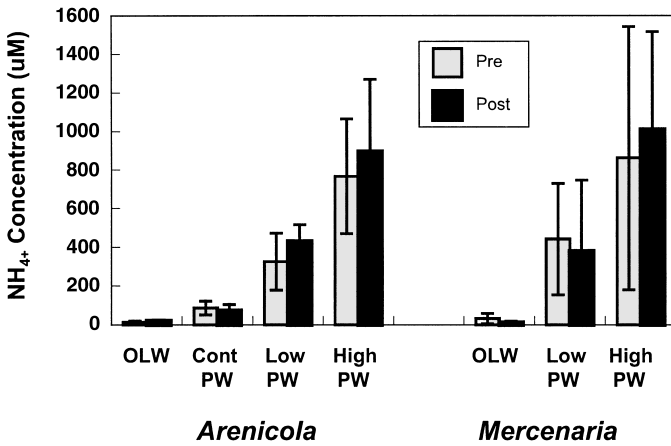


Figure 9. Test of the effect of new juvenile addition to overlying water (OLW) and porewater (PW) NH<sub>4</sub><sup>+</sup> concentrations for each treatment in *Mercenaria* (Low, High) and *Arenicola* (Control, Low, High) annular flume experiments. Pre- and post-addition samples were not significantly different from each other for each treatment. Bars are mean values ± one standard deviation (*n* = 3).

sediment sorting compared to initially added) and no treatment or trial effect was found (treatment  $F_{1,14} = 2.51, p = 0.1355$ , trial  $F_{2,14} = 0.41, p = 0.6743$ , Fig. 11). It is possible that the porewater ammonium concentrations (for example: trial 3 mean Low concentration = 714 µM, High concentration = 1,760 µM) found in all treatments caused rejection of the habitat, i.e., that all treatments were above a threshold for rejection by *Mercenaria* of a recruitment site.

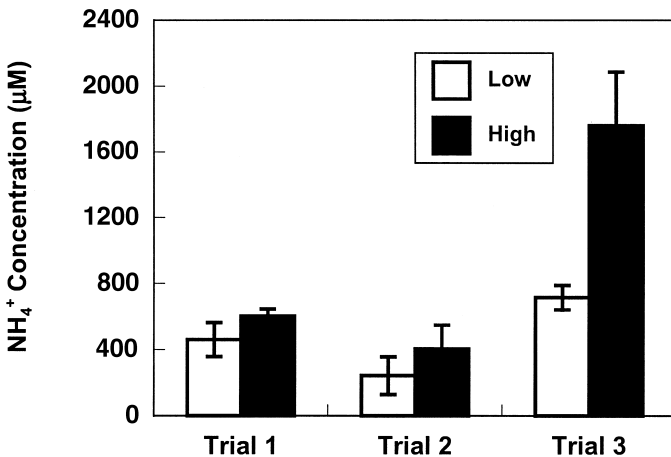


Figure 10. Porewater NH<sub>4</sub><sup>+</sup> concentrations for Low and High ammonium concentration treatments for each *Mercenaria* trial in the annular flume experiments. Bars are mean values ± one standard deviation (*n* = 3).

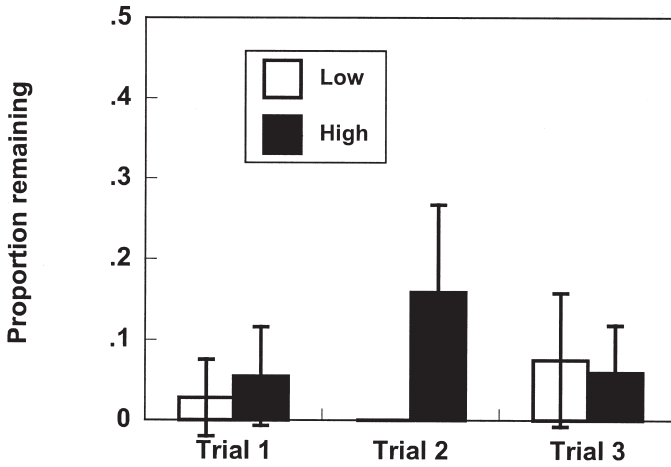


Figure 11. Proportion of *Mercenaria mercenaria* found in Low and High  $\text{NH}_4^+$  concentration treatments for all trials at end of experiment compared to number initially added. Bars are mean values  $\pm$  one standard deviation ( $n = 3$ ).

*iii. Arenicola experiment.* Ammonium concentrations in water samples (mean of two replicates per trial) taken in the overlying water in the flume both prior and subsequent to addition of new juveniles were not significantly different ( $F_{1,4} = 7.708$ ,  $p = 0.3156$ ). Similarly, differences between pre-addition and post-addition porewater samples from each treatment were much smaller than treatment differences (Fig. 9). Therefore, the treatment effects were much greater than the effects of addition and or other potential artifacts, and ammonium contamination in the water column was not a concern. Sediment porewater concentrations indicate that the ammonium manipulation of the upper sediment layers was successful, with highest concentrations in the High treatment, which were significantly greater than the Low treatment, which was also greater than the Control (Fig. 12: ANOVA: treatment effect:  $F_{2,22} = 49.61$ ,  $p < 0.0001$ ). No trial effect was observed (Fig. 12: ANOVA  $F_{2,22} = 1.62$ ,  $p = 0.7061$ ). Multiple comparisons of the least square treatment means of the porewater concentration data revealed that the Control, Low, and High treatments were significantly different from each other ( $H > L > C$ ). Ammonium concentrations in the Control treatment averaged  $87.02 \pm 35.37 \mu\text{M}$  ammonium, suggesting that the rinsed, but further unmanipulated field sediments contained notable amounts of ammonium, possibly originating from breakdown of organic matter in sediments during the lengthy storage period.

Arenicolid juveniles also responded to the imposed treatments (Fig. 13; ANOVA treatment effect  $F_{2,22} = 12.23$ ,  $p = 0.0003$ ), and multiple comparisons tests indicated higher retention in the Control and Low treatments relative to the High treatments. There also was a significant trial effect (ANOVA: trial effect  $F_{2,22} = 11.71$ ,  $p = 0.0003$ ), suggesting that while the pattern of the response did not vary over trials, the strength of the

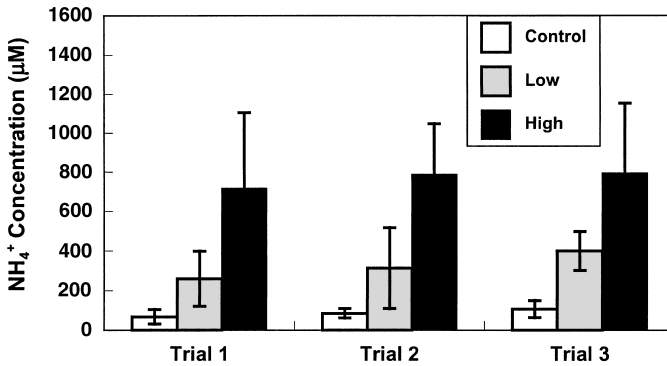


Figure 12. Porewater  $\text{NH}_4^+$  concentrations for Control, Low, and High  $\text{NH}_4^+$  concentration treatments for each *Arenicola* trial. The control treatment had no acrylamide gel beneath the sediment surface. Bars are mean values  $\pm$  one standard deviation ( $n = 3$ ).

response did. Thus, new juveniles appeared to consistently leave the substrates with the highest ammonium levels. As sediment type, grain size, and flow were held constant it appears that retention was driven by surficial ammonium concentration.

#### 4. Discussion

Recruitment is an important process structuring community development and interactions in the benthos. The success of individuals is enhanced if they can distinguish favorable from unfavorable sites and make recruitment decisions rapidly. Investigations of settling infauna have shown that organisms alter their recruitment choices when signals

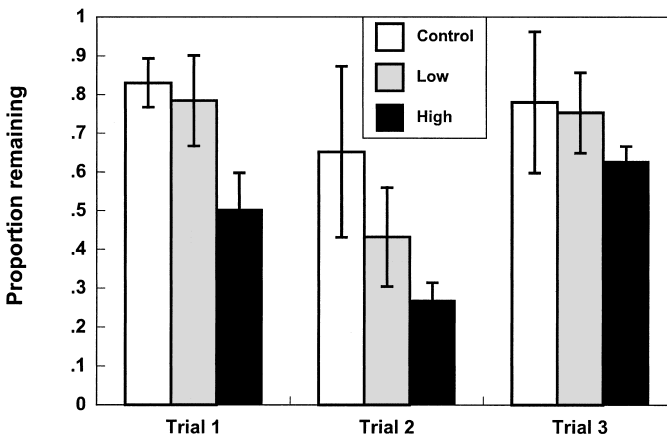


Figure 13. Proportion of *Arenicola cristata* found in Control, Low, and High  $\text{NH}_4^+$  concentration treatments for all trials at end of experiment compared to number initially added. Bars are mean values  $\pm$  one standard deviation ( $n = 3$ ).

related to disturbance (Woodin *et al.*, 1998) and food concentration (Butman *et al.*, 1988a) are encountered. We have argued that surface geochemical properties can provide important disturbance signals, as indicated by paired observations of recruit behavior and numerical models of transient geochemical properties (Woodin *et al.*, 1998; Marinelli and Woodin, 2002). As suggested by Strathmann *et al.* (2002), avoidance of disturbance and other adult-related sources of mortality (e.g., predation) may be an important adaptation to local dispersal. Given their relatively short dispersal phases, and their responses to modified surface properties, *Arenicola* and *Mercenaria* recruitment is consistent with this hypothesis.

The current study advances previous empirical studies through the development of a technique for manipulation of porewater chemistry while holding other variables constant. This approach allows evaluation of the importance of dissolved geochemical recruitment cues isolated from common covariants of geochemistry such as grain size and organic content. It also tests recruitment response in the presence of flow, allowing evaluation of the time scale of recruit responses and the likely mechanism of departure from a site.

#### *a. Porewater manipulations*

The research described herein demonstrates that acrylamide gel diffusers offer a steady, predictable, and reproducible means of manipulating one solute while holding other factors in the sediment matrix constant. Ammonium embedded within acrylamide gel successfully produced elevated porewater ammonium concentrations (Fig. 5), which could be sustained during experimentation. Over short incubation times, increasing the diffusional distance by adding a sediment layer decreased both the flux and the concentrations in porewater (Fig. 6), providing yet another technique for controlling concentration. Spiking of gels with ammonium sulfate led to higher porewater concentrations and higher fluxes than unspiked gels, although spiked sediments remained within the concentration ranges found in normal coastal sediments (e.g., Table 1 in Woodin *et al.*, 1998). The diffusional and controlled-release characteristics are particularly useful for sediments because concentrations within the surrounding matrix are constantly shifting due to bioturbational activities. The ability to isolate substances allows further identification of important cues associated with diagenetic processes in sediments, irrespective of grain size and organic content.

Porewater concentrations achieved through the manipulation were found to be different from each other within both straight flume and annular flume experiments. However in the annular flume trials, low, and control treatment concentrations were higher than expected (Figs. 10 and 12), due to difficulties associated with the overlying sediment column. The storage of field sediment in tall buckets may have resulted in significant ammonium buildup. Further, mixing of the sediment prior to use in the flume may have enhanced organic matter breakdown, and thus ammonium production as well as ammonium desorption (e.g., Aller, 1994). These processes likely contributed to higher and more variable signals and in the case of *Mercenaria*, variability in the recruitment response (below).



b. *New juvenile retention data*

i. *Mercenaria* experiments. In the straight flume experiments, *Mercenaria* showed a negative response to elevated porewater concentrations of ammonium. These findings argue that within the range of ammonium concentrations tested, lower ammonium habitats are indeed acceptable. In the annular flume experiments, ammonium concentrations were substantially higher across all treatments, and *Mercenaria* retention was uniformly low. The new juveniles chosen for each of the trials in this experiment had shed the velum (pediveligers), and likely departed the sediment surface via advection using local flow. Because all new juveniles used were competent to establish an adult existence, differences in the recruitment response were likely a result of the comparatively high ammonium levels in both Low and High treatments, and as opposed to juvenile age. Unfortunately, a no-gel control was not used in this experiment, and thus we cannot rule out the possibility that other factors related to the larval culture, or the sediment properties, were operative.

Other studies have found long exploratory times, and habitat rejection, for *Mercenaria* on disturbed sediment surfaces irrespective of ammonium concentration (Marinelli and Woodin, 2004). It is possible that *Mercenaria* may be responding to another environmental parameter such as organic content (not measured in this study) or another covariate of disturbance that is not directly associated with ammonium. Ambiguity in *Mercenaria* recruitment responses to sediment conditions has been noted in other studies as well. For example, Butman *et al.* (1988b) showed that under realistic flow conditions similar to this study ( $u^* = 0.30 \text{ cm s}^{-1}$  Butman *et al.* 1988b,  $u^* = 0.39 \text{ cm s}^{-1}$  current study), sediments with high organic content elicited higher recruitment of *Mercenaria* than abiotic glass beads of similar grain size. Bachelet *et al.* (1992) attempted to repeat the experiments performed by Butman *et al.* (1988b) but had difficulty interpreting the *Mercenaria* response. They reported that no sediment selection was found for *Mercenaria* in their short-term (4 h), still water experiments, perhaps due to difficulties associated with establishing competency of a large number of individuals within a short experimental time period. For the annular flume experiments we had similar experimental constraints, as preparation of the flume bed was time intensive. This effort, relative to the rapidity of *Mercenaria* metamorphosis, forced us to limit the number of experimental manipulations in the annular flume experiments.

ii. *Arenicola* experiments. Data from the straight flume experiment described above indicate no difference in response to sediments with elevated ammonium concentrations (Fig. 8) relative to control sediments. This response is likely due to use of an unacceptable substrate in the straight flume experiment. The aged play sand appeared to be moderately acceptable to arenicolids in preliminary trials although burrowing times in natural sediment are often more rapid, on the order of seconds (Marinelli and Woodin, 2002) relative to minutes as observed in this study. In the annular flume experiments where natural sediments were used, overall retention of *Arenicola* was higher than in the straight flume experiments, regardless of ammonium concentration on the surface. Thus, play sand

may have prompted an avoidance response that superceded any response to ammonium levels.

When natural sediments were used in the annular flume, new juvenile retention on surfaces with elevated ammonium was consistently lower than in control sediments, supporting the hypothesis that ammonium serves as a negative cue (Fig. 13). The fact that differences in retention of arenicolids were found when all other factors were held the same (flow environment, sediment type, etc.) suggests that the new juveniles were responding to elevated porewater ammonium concentrations. As with *Mercenaria*, the rejection response for *Arenicolids* involved departure from the site of deposition, over relatively short (minutes) time frames. These findings support previous assertions that failure to burrow is an indication of habitat rejection, and that flowing water facilitates departure from unsuitable sites.

### c. *The gel technique*

The acrylamide gel technique has significant promise as a method for manipulating local chemical environments in sediments, while holding other factors constant, and determining the ecological consequences of the manipulation. However, parameters such as the amount and type of sediment cover were important components within our experimental system. While ammonium concentrations were significantly different as a function of treatment, the absolute levels were often higher than anticipated, most likely due to sediment manipulation. In addition, other factors related to sediment quality appeared to be operative, as indicated by the apparent dislike of aged play sand as indicated by low retention rates. Our findings suggest it is important to evaluate both sediment type and the effect of sediment manipulation during the placement of gels, so that unintended effects can be discerned, and adequate controls can be devised. With sufficient testing, an appropriate experimental environment can be developed both in the laboratory (as described above) and in the field (Marinelli *et al.*, 2004).

### d. *Significance of post-settlement choice*

For many species settling on hard substrate environments, recruitment choice is critical due to the permanence associated with attachment. Gosselin and Qian (1996) reported greater than 50% mortality for cohorts of the barnacle *Balanus glandula* during the first 4 d of settlement, with the majority of deaths during the first day. They argued that organisms able to detect sources of mortality, and choose the location and timing of settlement accordingly, will have a higher probability of recruitment success. Our findings confirm that these arguments hold for infauna as well. That is, exploratory behavior, sensory processes, and active choice are important, as indicated by rapid responses to biogeochemical cues linked to disturbance/mortality, even in the presence of flow. The persistence of these traits for the infauna is particularly interesting because many species are capable of post-settlement migration as juveniles and adults (e.g., Gunther, 1992; Armonies, 1994;

Stocks, 2002). Clearly the ability to distinguish sites at an early age is critical regardless of mobility at later stages in life.

In a study of settlement of the intertidal barnacle *Tesseropora rosea*, Caffey (1985) suggested that the spatial and temporal predictability of disturbance processes affecting new recruits, and subsequent mortality, is far less certain than for established adult populations. Few studies quantify the spatial and temporal fidelity of disturbance events in soft sediments, although some (e.g., Krager and Woodin, 1993) suggest a moderate degree of predictability. Clearly, the scale dependent nature of disturbance and recolonization (Gunther, 1992) will have a strong impact on determining the relationship between recruitment success and adult population structure. Findings from the present study support the idea that spatial heterogeneity in geochemical conditions associated with disturbed surfaces can lead to differential patterns in settlement. The significance of spatial scales of geochemical disturbance to recruitment choice remains relatively unexplored.

## 5. Conclusions

Many studies have shown the existence of cues in nature and there is evidence that benthic species can perceive and respond to substances that are associated with favorable or unfavorable environments (see review by Rodriguez *et al.*, 1993; Pawlik, 1992; Woodin, 1991). Previous studies have linked ammonium concentrations with disturbance and suggest that avoidance of disturbed areas may enhance recruitment success. However, diagenetic processes, physical factors, and bioturbational events combine to produce a matrix of signals that often covary. This study confirms that ammonium, in isolation from other porewater solutes, is an important recruitment cue. It also underscores the importance of intrinsic and extrinsic factors as drivers of recruitment success. Intrinsic factors include behaviors associated with site selection, and extrinsic factors include physical processes that dictate both delivery to, and departure from, a settlement site.

While numerous studies have shown that biological communities affect the chemical environment, this study underscores the effect of the local chemical environment on recruitment patterns and community ecology. Studies that can separate and evaluate the importance of these diverse geochemical signals will significantly advance our understanding of ecological processes in soft sediment systems. Acrylamide gel diffusers are an excellent technique to further test these relationships in sedimentary environments both in the laboratory (this study) and field (Marinelli *et al.*, 2004). Finally, the conduct of experiments in flows similar to the natural environment affirms the importance of fluid motion as a partner in the efforts of new recruits to migrate to other sites. Thus, hydrodynamic, diagenetic, and ecological processes in combination affect recruitment patterns and overall success.

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