ABSTRACT

Title of Document: INFAUNAL EFFECTS ON PERMEABLE SEDIMENT PROCESSES

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The role of infauna on permeable sediment processes is poorly understood due to methodological limitations and a lack of empirical data. The interactions among porewater flows, sediments, and biogenic structures present a physically and biogeochemically complex sedimentary environment in which traditional measurement techniques and heuristic models are of minimal applicability. Chapter one provides an executive summary of this research. The second chapter describes a field investigation of the impact of the common lugworm and two species of thalassinid shrimp on porewater transport and chemistry in permeable sediments. In this work, novel experimental methods are employed to measure infaunal effects on porewater transport and chemistry. This experiment found differential effects of each taxon on porewater transport and solute chemistry that were highly related to infaunal functional characteristics, and independent of sediment properties.

Results from the field study prompted a laboratory microcosm study of lugworm effects on permeable sediment solute fluxes, presented in chapter three. Flow-through sediment microcosms mimicked tidal draining of intertidal flats and measured the effects of lugworms on sediment biogeochemistry. Lugworms were found to significantly alter solute fluxes as well as stoichiometric ratios from the microcosms. The potential ecosystem consequences of stoichiometric changes to regenerated solutes are explored with a new metric. Finally, chapter four presents a synthesis examination of the infaunal functional attributes important to permeable sediment processes with a multi-site, multi-species field investigation. Head-down deposit feeders were found to have similar effects on advection and chemistry, whereas other infauna had differential effects linked to the composition and morphology of the burrow/tube. The mechanisms by which different infauna may affect permeable sediment properties are discussed, and include consideration of covariates such as organism activity and density.

The results from this research highlight the importance of infauna to permeable sediment processes, while recognizing the limitations of their effects under different physical regimes. Benthic infauna play a significant role in the biogeochemistry of common permeable sediment habitats in coastal and near-shore environments. The results presented herein suggest the loss of large bioturbating infauna from permeable sediments due to human activities may result in significant changes to coastal biogeochemical cycles.

# INFAUNAL EFFECTS ON PERMEABLE SEDIMENT PROCESSES

By

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Dissertation submitted to the Faculty of the Graduate School of the
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# Dedication

This research is dedicated to George and Evelyn Waldbusser, my loving grandparents whose incredibly selfless support, generosity, pride, and strong work ethic provided me with the character needed to accomplish this goal.

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# Chapter 1: Executive Summary: Infaunal Effects on Permeable Sediment Processes

The seafloor of shallow coastal marine and estuarine ecosystems is a complex association of physical, biological, and chemical processes. These shallow water sediment habitats serve as sites of intense organic matter and biogeochemical cycling (Jahnke et al. 2003, Meile and Van Cappellen 2003, Billerbeck et al. 2006). One class of recently recognized sedimentary habitats important to biogeochemical and ecosystem type processes are permeable sediments (Boudreau et al. 2001). Permeable sediments are physically dynamic, sandy habitats found in shelf, near-shore, and inter-tidal locations. Previously, it was believed that these sandier sediments with low standing stocks of organic matter were biogeochemically inactive. Several key studies however illustrated that permeable sediments remineralize organic matter more rapidly than muddy diffusion dominated sediments (Huettel et al. 1996, 1998, Marinelli et al. 1998). Characterizing the factors that modify processes in shallow-water permeable sediments is important because coastal ecosystems are susceptible to changes in benthic biogeochemistry due to anthropogenic impacts (Torgersen et al. 1997, Tyler et al. 2003, Lucea et al. 2005).

Porewater advection is the dominant porewater transport process in permeable sediments, resulting in porewater exchange rates orders of magnitude higher than molecular diffusion alone. On very short temporal (seconds to minutes) and spatial scales (millimeters) diffusion can be a very rapid process, however the divergence in rates of transport between advection and diffusion occurs when the temporal and spatial scales are increased. Two conditions must exist for porewater advection to occur: 1) the

sediment must be permeable to advective flow and 2) a pressure gradient driving flow from high to low pressure must be present. The results of rapid porewater transport through permeable sediments include increased rates of organic matter remineralization (Cook et al. 2007), rapid exchange between pools of reduced porewater and oxidized overlying water (de Beer et al. 2005), and elimination of diffusional gradients in solute concentrations.

Advective porewater flow may erase infaunal associated diffusive gradients, but infauna may modify permeable sediments in other significant ways. Two primary mechanisms by which benthic infauna may affect permeable sediment processes are through modifying sediment permeability or altering porewater pressure gradients. For example, non-local transport of particles by deposit feeders will translocate sediment between depth and the sediment surface and counteract normal compaction processes in sediments (Thayer 1983, Craig et al. 1998). Burrow, tubes, and galleries created by infauna will result in void spaces beneath the sediment surface serving to increase the overall permeability of the sediment (D'Andrea et al. 2002, 2004, Volkenborn et al. 2007). Ingestion and feeding on sediment by infauna results in stripping of organic material, and may result in a reduction of fine material, also increasing permeability (Volkenborn et al. 2007). Alternatively, the creation of impermeable biogenic structures, such as clay lined burrows, may also decrease sediment permeability and result in decreased porewater transport (Waldbusser and Marinelli 2006). Suspension feeding organisms also have the potential to alter sediment permeability through the biodeposition.

Infauna may further affect permeable sediment processes by altering pressure gradients within and acting upon the sediment. Direct pumping of fluid into the sediment has been well studied for its effect on porewater advection and resulting biogeochemistry (Timmerman et al. 2002, Meysman et al. 2006). The creation of biogenic structures both above and below the sediment surface may interact with flow fields to alter pressure gradients. Mounds created by excavating infauna interact with overlying water to generate porewater advection (Munksby et al. 2002). Tubes extending above the sediment surface may also result in passive irrigation of sediments due to the Bernoulli effect (Ray and Aller, 1985, Huettel and Gust 1992, Libelo et al. 1994). Benthic infauna exhibit a diverse array of life history strategies that have the potential to act differentially on sediment permeability and pressure gradients driving porewater advection. An important question is whether these effects are broad scale and promote significant alteration of biogeochemical cycles

The overarching goal of this dissertation research was to examine the role of benthic infauna on permeable sediment processes. This question was examined using three general approaches: 1) examining the extent to which infauna modify advection and biogeochemistry in unmanipulated field environments using a correlative approach, 2) quantifying infaunal effects on permeable sediment fluxes of ecologically important solutes using laboratory microcosms, and 3) examining multiple organisms and sites to identify the specific infaunal attributes that promote sediment biogeochemical alteration

in these habitats. The dissertation includes four chapters, including this introductory chapter. The three remaining chapters are aligned with the three approaches noted above.

Initial field experiments were conducted in False Bay, WA to examine whether two common, co-occurring, and active infaunal taxa have measureable effects on porewater advection and solute concentrations. The arenicolid lugworm polychaete, *Abarenicola pacifica* and two species of thalassinid shrimp (*Upogebia pugettensis* and *Neotrypaea californiensis*) are known to have significant effects on sediment processes, though their feeding modes and life history strategies are different. I hypothesized that the differences in how each taxon interacts with the sediment column and the close spatial proximity of burrows should result in complex and differential effects of each taxon on porewater advection and biogeochemistry. Experimental plots composed primarily of one of the study taxa or mixed communities of both were evaluated for their effects on porewater advection, solute concentrations, and sediment characteristics. Fluorescein-impregnated acrylamide gels were used to infer rates of transport (via loss of the tracer), and acrylamide gel peepers were used to record temporally integrated porewater concentrations of diagenetically important constituents among experimental plots. Laboratory studies evaluated rates of diffusive transport in non-bioturbated sediments for comparative analysis. Results presented in chapter two found: 1) functionally different macrofauna affect rates of porewater advection in permeable sediments, 2) organism effects are not attributable to changes in average measures of sediment granulometry, 3) species composition may further complicate the advective environment and the resulting diagenetic processes, and 4) species effects vary according

to reaction rate kinetics. Species interaction effects on transport are potentially due to inhibition of arenicolid feeding by thalassinid tubes that serve to block sediment fluidization and advective flow. Thus, specific behaviors and interactions among organisms appear to affect transport rates and sediment function in advectively-permeable habitats. The results indicate the importance of integrating behavior, kinetics, and transport into future studies of sedimentary biodiversity and ecosystem function.

The infaunal effects on porewater transport and chemistry found in the previous field investigation led to the question as to whether infauna can affect solute fluxes from permeable sediments. Using flow-through sediment microcosms the effects of the common lugworm (arenicolid polychaete) *Abarenicola pacifica* on permeable sediment solute fluxes were measured and presented in chapter three. Burrow mimics of thalassinid shrimp also were employed to examine effects of these highly impermeable structures on lugworm activity, and consequences to sediment biogeochemistry. This simplified experimental system simulated tidal flushing of an intertidal sandflat and permitted direct measures of *A. pacifica* effects on biogeochemical fluxes. Porewater advection rates were also manipulated to imitate advection rates measured in-situ and evaluate how different physical conditions affect lugworm-biogeochemistry interactions. Fluxes of ammonium, nitrate, phosphate, silicate, and alkalinity were measured by determining the concentration difference between overlying water input and effluent from the flow-though sediment microcosms. Statistically significant results under the low-flow conditions show that arenicolids reduced the flux of ammonium, carbon, phosphate, and alkalinity. The thalassinid burrow mimics had little to no effect on sediment

biogeochemistry. The high-flow conditions appeared to minimize lugworm effects on permeable sediment fluxes. The results provide a context for evaluating the range of advective porewater flows in which infauna influence biogeochemistry. Potential ecosystem consequences of infauna on the ratios of regenerated elements were examined with a new metric (d-value). These d-values calculated the molar distance in graphical space for elemental pairs and are used to evaluate stoichiometric relationships of remineralized solutes in relation to empirical ratios. The stoichiometric evaluations suggest effects of arenicolids on biogeochemical processes in permeable sediments may influence water column nutrient stoichiometry and could have broad ecosystem consequences.

A comparative field campaign investigating the effects of several different common infauna on sediment porewater transport and chemistry identified general effects of functionally similar and dissimilar species. An important research goal is to evaluate the nature, extent, and species-specificity of these effects within realistic porewater advection regimes in different sites. Findings from field studies of three different intertidal flats (False Bay, WA, Cara's Flat, VA, and Debidue Flat, SC), inhabited by six taxonomically different infauna (*Abarenicola pacifica, Upogebia pugettensis, Neotrypaea californiensis, Diopatra cuprea, Balanoglossus aurantiacus, and Onuphis jenneri*) are presented in chapter three. Porewater advection, solute concentrations, bulk sediment granulometry, and density of infauna were measured within experimental plots at each site. Stepwise regression was used to identify independent variables (organism density and granulometry) having the greatest effects on porewater advection and solute

concentrations within sites and develop the best fitting statistical relationships. Different infaunal species at three sites had significant effects on porewater advection and at two sites had significant effects on porewater chemistry. Infaunal density effects on porewater transport or chemistry were statistically confounded with variability in sediment granulometry in some cases. Within site variability of granulometry was found to be small however, and probably not environmentally relevant to transport and reaction processes. Different infaunal species exert significantly different effects on porewater transport and sediment biogeochemistry, while important infaunal attributes appear to be related to feeding strategies and burrow characteristics. Head down deposit feeders increased porewater advection and lowered solute concentrations, while effects of surface feeders appeared to depend more on burrow/tube properties. Additionally, construction of impermeable burrows increased porewater concentrations of several solutes. The infauna functional effects across sites can not be completely disentangled from variability in sediment fines content and hydraulic conductivity. This suggests that infaunal effects on permeable sediment processes may also be context-dependent on sediment properties. Although replication of functional analogs across all sites was not possible in this unmanipulated multi-site experiment, the results do provide evidence of functionally specific effects of infauna on these physically active sediments.

The broader implications of this work suggest that infauna can have important effects on physically active permeable sediments, though these effects will vary with infaunal attributes, interactions among species, and general characteristics of the actual site. Biodiversity effects were not explicitly tested in any of the experiments presented

here, but the significantly different effects of different infauna on porewater transport and chemistry in field studies suggest that infaunal community composition (and other metrics such as biodiversity) may be important variables in permeable sediments. Furthermore, the non-linear effects of infaunal density on porewater solute concentrations in mixed communities in False Bay suggests that community interactions may result in non-linear responses of permeable sediment processes. Likewise, the shift towards "healthier" or lower nutrient regeneration ratios in response to infauna in microcosms suggests that changes to stoichiometric ratios of regenerated nutrients may be another ecosystem effect of infauna on permeable sediment processes. In all a complex picture emerges, though some general patterns are revealed. However, given the complex ways in which infauna may modify porewater transport, solute concentrations, and solute fluxes, it may not be possible to develop simplified models of infaunal effects on permeable sediment processes that are applicable to the broader ecosystem.

# Chapter 2: Macrofaunal modification of porewater advection: The role of species function, species interaction, and kinetics.

## *2.1 Introduction*

Marine sedimentary systems are complex associations of biological, chemical and physical processes that operate on varying spatial and temporal scales. From these collective elements, ecosystem function emerges. The difficulty in understanding intermediate and small scale process complexity of coastal sedimentary systems arises, in part, from current limitations in our characterization of macro-organism interactions with physical and biogeochemical processes (Marinelli & Waldbusser 2005). Studies increasingly point to the importance of species-related differences in activity rates (Boudreau and Marinelli 1994), and density dependent processes (Marinelli and Williams 2003, Lohrer et al. 2004) that create geochemical variability with fundamental ecological significance. For example, keystone species such as urchins or maldanid polychaetes that alter local community structure and small scale sediment geochemistry have broad-scale effects on ecosystem function when integrated over larger scales (Levin et al. 1997, Widdicombe & Austen 1998, Waldbusser et al. 2004). The diagenetic setting, dictated by rates of organic input and internal geochemical cycling (Canfield et al. 1993a 1993b, Thamdrup et al. 1994, and Jahnke & Jahnke 2000), is an additional element of complexity that co-determines the outcome of ecosystem processes (Kristensen et al. 1985). Lastly, physical-biological interactions related to boundary layer dynamics affect interfacial processes (Eckman 1983, Huettel et al. 1998), with significant implications for resource utilization (Taghon et al. 1980), population dynamics (Eckman 1996), and sediment-seawater exchange (Jahnke et al. 2000). Given the diversity of processes and

potential interactions among them, identification of mechanisms that alter system function is crucial to developing predictable relationships between ecosystem structure and function (Levin et al. 2001).

It is increasingly clear that consideration of the geochemical milieu helps elucidate the mechanisms by which biodiversity alters system function. For example, Waldbusser et al. (2004) found that biodiversity effects associated with lower than predicted phosphate fluxes (underyielding) was largely explained by depth integrated oxygen concentrations within the sediment and the effect of oxygen on phosphate adsorption. This relationship was driven by the presence of one active deep dwelling organism in the experimental treatments and therefore may be considered a selection effect (Wardle 1999). The extensive literature on diagenetic and other sedimentary process may readily provide explanatory mechanisms for many of the effects found in biodiversity and ecosystem function studies of sediments. Ongoing debate regarding the nature of biodiversity effects (Kinzing et al. 2001) and lack of congruity among conclusions from sediment diversity experiments (Emmerson et al. 2001, Biles et al. 2002, Bolam et al. 2002) point to the need for a more integrated and thorough investigation of sediment dynamics.

Concurrent with the expansion of biodiversity research in benthic environments has been the increased recognition of permeable sediments (and associated porewater advective flows) as habitats of significant and rapid biogeochemical cycling. As Rocha (2000) argues, the basis for modern diagenetic research has focused on non-permeable

sediments with diffusion and bioirrigation as the primary transport mechanisms. Extant bioirrigation models have successfully captured average geochemical environments inhabited by organisms in diffusive sediments (Guinasso and Schink 1975, Aller 1980, Boudreau 1984). However, these models generally are not suited to permeable sedimentary habitats due to assumptions regarding diffusive transport around organism burrows. A common assumption regarding advectively-dominated environments is the erasing of chemical potential gradients generated by organism burrows, and therefore a dampening or lack of sedimentary organism effects. Rather, the primary importance of infauna in permeable sediments are as creators of topographic features and associated pressure gradients that in turn, drive advective flow (Huettel & Webster 2001, but see D'Andrea et al. 2002, D'Andrea et al. 2004 for counter examples). Furthermore, de Beer et al. (2005) estimated roughly 25% of the exchange between sediment and overlying water on an advective intertidal sand flat was due to bioturbational activities of infauna, emphasizing the potential importance these communities may have on geochemical cycling in permeable sediments. Although recent findings indicate the importance of infauna on sedimentary processes in permeable sediments, it is critical to biodiversity/ecosystem function research that we understand whether functionally different species and interactions among them create ecologically significant variance in permeable sediment processes.

In the current study, we conduct several field experiments in a muddy-sand intertidal flat dominated by three species of two functionally different bioturbating macrofauna, consider the complexity associated with non-diffusion dominated

environments, and the implications for biodiversity/ecosystem studies in terms of species interactions. Our questions in this study are: 1) Do two functionally different bioturbating infauna have different effects on sedimentary processes in this permeable sedimentary habitat, 2) Does the interaction between the two functional types affect the transport of porewater solute distributions, 3) What are the possible mechanisms for any organism effects found on sediment dynamics, and 4) What are the implications for the future of biodiversity and ecosystem function research within permeable sediments, as well as in other habitats?

## *2.2 Methods*

### 2.2.1 Site and Organisms

This study was conducted in False Bay, WA, U.S.A. (Lat = 48.488° , Lon = -123.065°), an intertidal flat approximately one $km^2$ in area during maximum exposure, located on San Juan Island. The tides are mixed semi-diurnal with a maximum 4 m tidal range and daily exposure times of nearly 12 hours during late spring and summer, imparting considerable temperature fluctuations of overlying water and within the sediment (Waldbusser unpublished data). The sediment column in the study area was underlain by an impermeable clay layer roughly 30 cm beneath the sediment surface. All experiments were conducted at the +1 m tidal height (from mean low water).

The study area is relatively pristine and has a diverse infauna, including errant (e.g. nereidid and glycerid polychaetes) and relatively sedentary species (e.g. the bivalve *Macoma* sp. and lumbrinereid polychaetes). However, the dominant taxa are the

lugworm *Abarenicola pacifica* and two species of thalassinid shrimp, *Upogebia pugettensis* and *Neotrypaea californiensis*. Maximum densities of surface features, a proxy for organism density, were roughly 75 fecal mounds or burrow openings $m^{-2}$ for both the arenicolid and thalassinid taxa, respectively (Table 1 and Krager & Woodin 1993). No differentiation could be made between the thalassinid species without destructive sampling, and therefore they are treated as one taxonomic unit.

The arenicolid and thalassinids are characterized by differences in feeding mode and burrowing. *Abarenicola pacifica*, is a head-down deposit feeder with a body length of up to 10 cm. It maintains a mucus-lined, j-shaped tube and feeds indirectly on surface material by fluidizing the sediment above the feeding area at the tube base and subducting surface material downward. Taghon (1988) measured fecal production rates up to $280^{-d}$ (grams of sediment to grams of ash free dry weight worm per day) for *A. pacifica*. A significant body of literature exists on the ecology of arenicolids and the reader is directed to Hobson (1967), Brenchley (1981), Riisgård & Banta (1998), Linton and Taghon (2000) and references therein for further information. The presence and abundance of *A. pacifica* can be verified by characteristic fecal mounds found on the sediment surface next to its well formed tail shaft (Krager & Woodin 1993).

In contrast, thalassinid species create large subsurface galleries with one or more openings to the sediment surface (Nickell & Atkinson 1995). They excavate large volumes of sediment, often suspending fine particles, increasing turbidity of the overlying water, and negatively affecting other organisms (MacGinite 1930, Suchanek

1983, Posey et al. 1991, Pinn et al. 1998, Feldman et al. 2000). Both *Upogebia pugettensis* and *Neotrypaea californiensis* are obligate burrow dwellers, and are considered to be facultative suspension and deposit feeders, respectively (Posey et al. 1991). Further, differences in reproduction and life history strategies seem to allow these sympatric species to co-occur (Dumbauld et al. 1996, Coelho et al. 2000 and references therein).

Experimental plots of areas dominated by the arenicolid, thalassinids, and mixed communities were identified in mid June and maintained until the end of August. Three 0.5 m by 0.5 m plots within three larger blocks were selected by visual inspection of the sediment surface for features characteristic of each taxon. All plots within a block were within 1-2 meters of each other, and blocks were ~50-100 meters apart, and at similar tidal heights. Plots were designated as: 1) arenicolid dominated, 2) thalassinid dominated, or 3) mixed communities of the two taxa. At three intervals over the field season, a series of daily photographs were taken at low tide (23-25 June, 19-22 July, and 4-7 August) to verify these designations, and the persistence of the organisms. Photographic image data, used to estimate the abundances of arenicolids and thalassinids by surface features, were analyzed using Image-J software.

### 2.2.2 Advection and Diffusion Measurements

The potential importance of advective porewater movement within the experimental area was evaluated using a conservative tracer, fluorescein, released from an acrylamide gel diffuser over an outgoing and incoming tide. A 20% acrylamide gel plug (2.54 cm diameter, 5 cm length), containing 1mg $ml^{-1}$ fluorescein was made

according to Browne & Zimmer (2001). A 1 m transect line was established parallel to the long axis of the bay, on a sand bar with a gradual slope, within a site that appeared to lack any obvious surface features that would indicate the presence of large bioturbating infauna. At 1200 hrs the gel was inserted (via core replacement) into the middle of the transect with the center of the gel roughly at 5 cm depth. The gel was then covered with sediment such that the surface was flush with the surrounding area. Starting at 1245 hrs and every hour subsequent, a small volume (~1ml) of porewater was taken at 5 cm depth, along both directions of the linear transect, at three locations: 1, 3, and 5 cm from the gel. The sample was obtained by inserting a canula, attached to a syringe, to the 5 cm depth interval and gently withdrawing fluid at depth. The last sample was taken at 1830 hrs; this was the time the incoming tide had begun to cover the experimental area. We assumed that the major axis of flow would be horizontal, based on the pressure gradient generated by the retention and drainage of porewater within the sediments, though some vertical transport probably occurred. Upon retrieval, all samples were filtered through a 0.45μm filter, and placed in a dark cooler, until analysis on a Turner-Quantech fluorometer. The acrylamide gel plug was left in the sediment for 3 days before retrieval on 26 May 2004, when the porewater sampling was repeated, as above.

To evaluate the relative magnitude of advective versus diffusive transport in these sediments, a lab experiment was conducted to measure tracer movement in a diffusion-dominated environment. Since diffusive transport is the sum of random non-directional Brownian movements resulting in transport down gradient, the vertical or horizontal orientation of the experimental set up is irrelevant on this spatial scale, and thus measures

of vertical diffusion in a controlled experiment can be compared to measures of advective transport measured in-situ in a horizontal direction. A PVC pipe 10 cm in diameter and 30 cm long was capped on the bottom, outfitted with a vertical line of sampling ports drilled at 1.5 cm intervals to a height of 15 cm, and fitted with rubber septa as described in Marinelli et al. (1998). A 20% acrylamide solution with 1 mg $ml^{-1}$ fluorescein was made, poured into the bottom of the PVC pipe, and allowed to polymerize. Sediment from the study site was collected, mixed, and defaunated by allowing the mixture to go anoxic for 2 weeks. Once defaunated, the sediment was carefully added to the pipe to a depth of roughly 10 cm on 9 July 2004 (day 0). The diffusion experiment was kept at constant room temperature and does not reflect the temperature variability found at the site. Temperature changes of surface sediments due to solar heating were roughly $10^{o}$ C at the field site (Waldbusser unpublished data). Using the Wilke-Chang formula to calculate the temperature dependence of changes in the diffusion coefficient of fluorescein (Browne and Zimmer 2001), the free solution diffusion coefficient varied by 0.1 $x10^{-6}$ cm $s^{-1}$ with a $10^{o}$ C temperature change from $10^{o}$ C to $20^{o}$ C. Thus, the consequence of not mimicking field temperatures on diffusive transport is minimal. Porewater samples (~1-2 ml) were taken on days 10, 18, and 25, filtered, and analyzed for fluorescein concentration immediately, as described above.

### 2.2.3 Fluorescein Loss Measurements

To assess differences in rates of porewater advection between plots dominated by functionally different fauna, we deployed acrylamide gels infused with fluorescein in the experimental plots, described above. The fluorescein concentrations remaining in the gels after a given period of time acted as a proxy for relative rates of porewater

advection. We hypothesized that gels in areas of higher advective flows would lose fluorescein faster due to the increased flushing of the surrounding sediment.

Acrylamide plugs (1.1 cm diameter, 9 cm length) were made with a 15% gel (Browne & Zimmer 2001, as above) containing 1 mg $ml^{-1}$ fluorescein. After polymerization, the gels were removed from the cylinders and wrapped individually in a single layer of #75 nitex mesh. On 19 July 2004, five replicate gels were deployed in each experimental plot (described above) 10 cm apart along a 0.5 m transect perpendicular to the axis of advective flow measured previously. The gels were retrieved on 21 July 2004 by taking a 5 cm diameter sediment core around the plug, and then breaking apart the core to obtain the gel plug. Excess sediment was gently wiped from the exterior, and two 5 mm subsections of the plug were taken roughly ~1 cm from each end of the plug. The subsections were placed in pre-weighed sample vials and covered with foil to prevent photo-degradation of fluorescein within the gels. Immediately upon returning from the field, the sample vials were reweighed, and 2.5 ml of D.I. water was added to each vial for back-equilibration of fluorescein out of the gel and into solution. The samples were placed on a shaker table in the dark for 48 hrs. The fluorescein concentration in the back-equilibrated water was then determined via fluorometry and the fluorescein remaining in the gel was corrected based on the dilution factor and the volume of the acrylamide.

### 2.2.4 Sediment Porewater Solutes

Porewater peepers, containing acrylamide gels as solute recorders (modified from Hesslein 1976 and Mortimer et al. 1999), were used to measure depth profiles of

ammonium, phosphate, silicate, alkalinity, and pH at the study site, and evaluate the effects of biologically modified porewater flow on sediment biogeochemistry. Each peeper had ten wells (0.75 cm deep by 3.2 cm wide by 8 mm high) of approximately 2 ml in volume, allowing the measurement of a 10 cm profile from 1 cm below the sediment surface to 11 cm depth. Two milliliters of a 15% acrylamide gel was added to each well. Gels were made with potassium persulfate as an initiator rather than ammonium persulfate, to avoid ammonium contamination (Engstrom & Marinelli in press). After polymerization, the peeper wells were covered with 0.45 μm Magna nylon filter paper and were prehydrated in 30 psu NaCl solution for 5 days prior to deployment in the field on 4 August 2004. Peepers were deployed in the same experimental plots used to measure fluorescein loss but 11 days after these experiments concluded. Three replicate peepers were deployed in each plot within all three blocks, with the narrow edge facing the dominant axis of flow. Peepers were retrieved on 10 August 2004 (6 day deployment), wiped clean of sediment, placed in plastic bags, and refrigerated. Subsequently, the individual gels from each depth interval were removed using clean stainless steel spatulas and latex gloves and placed in 15ml sterile centrifuge tubes containing 8 ml of D.I. water. For back-equilibration, tubes were placed in the dark on a shaker table in a cold room at 10 $^{o}$C for 48 hrs. Solutes and pH were then measured on the back equilibrated solution and corrected for the dilution. Random checks of salinity on the back equilibrated water were done to detect possible evapo-concentration of solutes within the gels either though the course of handling or during deployment in the field.

Calculations were made to verify the response time of the gels to changes in surrounding porewater concentrations. Using free solution diffusion coefficients for ammonium, phosphate, and silicate (Boudreau 1997), the Acrylamide-specific diffusion coefficients were calculated and Bessel series summations were performed as in Browne & Zimmer (2001). Roughly 10% or less of the solute would be present in the gel (75 mm diffusion length based on well depth) after 1 day of equilibration, if exposed to solute-free water. In other words, it would take roughly 24 hrs for the gel to equilibrate within 90% of surrounding concentrations, if those concentrations were constant. Integrating the temporal variability associated with the tidal draining and saturation of these sediments requires an extended sampling. We estimated that a deployment time of 6 days would be sufficient to allow for the gels to accurately record average porewater values; this was true based on prior measures of sediment porewater constituents using direct extraction (Waldbusser unpublished data). Therefore, gels successfully integrated temporal variability in porewater constituents over the deployment period.

### 2.2.5 Chemical Analyses

Analyses of ammonium, phosphate, silicate, and alkalinity were performed on a Smartchem discrete chemical analyzer (Westco Scientific, Danbury, CT). Ammonium was analyzed using a modification of the phenol method as outlined by Koroleff (1976). Phosphate analysis followed a modification of the EPA method 365.2 and Eaton et al. (1995). Silicate was analyzed according to Strickland and Parsons (1972). Alkalinity was determined using the methyl orange method, EPA 310.2. Dissolved inorganic carbon was calculated from the pH and alkalinity measures by dissociation constants using a MATLAB routine (csys.m and equic.m) developed by R.E. Zeebe and D.A.

Wolf-Gladrow (http://www.awi-bremerhaven.de/Carbon/co2book.html). The measurement of pH was conducted using a pH electrode and meter (VWR Scientific model 8000).

### 2.2.6 Sediment Parameters and Measured Permeability

Grain size analysis was conducted on composite samples from each experimental plot using standard sieving techniques and graphical analysis of the cumulative percent distributions following Folk & Ward (1957). Three 3 cm diameter cores of roughly 7 cm deep were taken from each plot and were combined to obtain a composite sample of each plot on 14 August 2004. Composite samples were weighed, dried and re-weighed to calculate porosity [volume pore water/volume (sediment + porewater)]. A value of 2.65 g $ml^{-1}$ was used to correct for the density of quartz, and 1.023 g $ml^{-1}$ for seawater in the calculations. Permeability was calculated by the Rumpf-Gupte equation (Boudreau 1997) using grain size and porosity measures. Sediment organic carbon and nitrogen were determined for three 1 cm diameter surface cores (0.5-1.0 cm deep) taken in each plot using a Carlo Erba-440 Elemental Analyzer.

As an independent permeability measure, one intact sediment core (5.08 cm diameter by ~10 cm high sediment column) was taken in the middle of each plot within one of the three blocks and returned to the laboratory. These cores lacked obvious surface features that would indicate the presence of large bioturbating infauna. Permeability of these intact cores was determined using the falling head permeameter method (Gray 1958). The measured permeability is based on the actual velocity of porewater movement through an intact sediment core (given a certain pressure head),

whereas the calculated permeability uses theoretical considerations and empirically derived relationships between porosity and grain size (Boudreau 1997).

### 2.2.7 Data Analyses

Porewater profiles were depth-integrated using trapezoidal integration. A two-way analysis of variance (ANOVA), with treatment, block, and treatment*block interaction effects, was used to analyze differences in fluorescein loss and integrated porewater data as a function of the dominant taxon/treatment (Arenicolid, Thalassinids, or Mixed). The fluorescein loss and integrated porewater data were transformed to meet the assumptions of normality and homogeneity of variance in the ANOVA tests as follows: fluorescein loss data and porewater data were natural log transformed, and organism abundance data were square root transformed. When no block effect was found, the block effect was dropped from the model, and data were reanalyzed as a one-way ANOVA. A Tukey-Kramer correction was used on the individual t-tests of treatment differences. Statistically-determined outlier values were found and removed from the fluorescein loss analysis in 3 observations of the thalassinid treatment and one observation of the mixed treatment. All statistical analyses were conducted using SAS Version 8.

Two types of post-hoc exploratory regression analyses were conducted to investigate possible density dependence and interaction effects of these two species on variability in sediment porewater constituents. In the analyses, the abundance data obtained from the August photographs were averaged over the three day period. The chemistry data (three replicates per plot) were not averaged for each experimental plot, in

order to reflect spatial variability of porewater within each plot. The original treatment assignments of Arenicolid, Thalassinid, and Mixed were ignored, and the densities of each organism within all plots were regressed against the porewater solute concentrations. We acknowledge the observations are not independent, but point to two reasons why such a method may be appropriate in this exploratory analysis. First, there is a lack of straightforward statistical analysis that may deal with variables that vary on differing spatial scales, such as porewater chemistry and organism abundance. If we are to average up to the largest scale (plots in this analysis) we hide the variance of the porewater chemistry that may be relevant and could be accounted for in a regression analysis. Secondly, standard ANOVA is simply a special case of regression in which measures of a dependent variable are assigned to an ordered categorical independent variable. The determination of a significant slope in such a regression analysis (ANOVA) is the equivalent of a significant treatment effect in a one-way ANOVA. Although our approach is non-traditional, we feel the results provide considerable insight into the data in spite of the limitations of such a post-hoc exploratory analysis.

The first series of post-hoc exploratory analyses were simple linear regressions of each chemical parameter vs. organism density for each species (as determined by surface features). The second series of post-hoc exploratory analyses were multiple linear regressions using each chemical parameter vs. organism density plus a species overlap index (Schoener 1970). Species overlap was calculated by a modification of Schoener's index (1970):

Overlap = 1-|*pa*-*pt*|

where *pa* is the proportion of arenicolids and *pt* is the proportion of thalassinids. Application of a spatial overlap index would account for potential nonlinearities in sediment geochemistry resulting from interactions among infauna. The closer in value the percentages of the two species are, the smaller the absolute difference between them and closer to an overlap value of one. Overlap indices were arcsine transformed because they were proportions. After transformations, all parameters within the data set were standardized (or non-dimensionalized) so each parameter had a mean of 0 and a standard deviation of 1. These values have units of standard deviations and are called standardized deviates or Z-scores (Sokal & Rolf 1969) and are calculated by:

$$Z_i = \frac{X_i - \mu}{\sigma}$$

where $X_i$ is the value of parameter $X$ and observation $i$, $\mu$ is the mean of the measured parameter, $\sigma$ is the standard deviation of the measured parameter, and $Z_i$ is the new standardized value of observation $i$. Regression parameters estimated using Z-scores can be directly compared to each other even if the original observations had different units. Differences in magnitude among the standardized regression parameters can be used to investigate which dependent variables (integrated porewater concentrations) are most influenced by density of arenicolids or thalassinids.

Assumptions (normality, homogeneity of variance) were checked and potential outliers were examined using Cook's Distance, DFBETA values, and studentized residuals (Sokal & Rolf 1969). In all cases 1 to 3 outliers were detected, and in most cases it was the same observation for different solutes, therefore those points were excluded from the regression as true outliers due to overly influential effects on parameter estimates.

## *2.3 Results*

### 2.3.1 Organism Abundance

Results from the photographic surveys indicate the treatment assignments were appropriate and differences among plots remained relatively consistent with time (Table 1). No attempt was made to control or regulate the actual abundance of the two major taxa in the plots throughout the course of these experiments because we wanted to minimize disturbance to the sediment fabric. Therefore, small changes in the relative abundances were expected due to natural variability associated with undisturbed habitats, and minor variability in surface features not directly related to abundance.

### 2.3.2 Advection and Diffusion Experiments

Results from the field measurements of tracer release from a gel diffuser indicate that advective flows are occurring in these sediments over a tidal cycle. Measurable fluorescein concentrations were found 1 cm from the gel source toward the mouth of the bay and slightly down slope 1 hr 45 min from the time of insertion, with a maximum (75 $\mu g\ ml^{-1}$) occurring at 3 hr 45 min from gel insertion (Figure 1). On the opposite side of

the gel, maximum fluorescein concentrations at 1 cm distance reached a peak of only ~2 μg $ml^{-1}$ (data not shown). Thus transport was asymmetric and rapid, likely due to advective processes associated with pressure gradients generated during drainage of the tidal flat. A concern of extracting porewater samples is the possibility of inducing transport via the removal of porewater. Although we cannot unequivocally dismiss some sampling effect of porewater extraction, the directionality of the measured transport is suggestive of advection. The missing section of the curve in Figure 1 (4 hr 45 min from time of gel insertion) was due to little to no extractable porewater in the sediments at 5 cm depth. Three days subsequent to the gel insertion, porewater samples were again taken at 5 cm depth along the same transect as the first sample set (Figure 2). Results confirm a similar pattern of asymmetrical concentration gradients.

A comparison of tracer concentration and transport time in the field to that obtained in the laboratory diffusion experiments confirms the occurrence of advective porewater movement in these sediments. Compared to field data, a similar concentration in the diffusion experiment was found between 10 (5.50 μg $ml^{-1}$ @ 1.5 cm) and 18 days (95.00 μg $ml^{-1}$ @ 1.5 cm) (Figure 3). The difference in time and concentration between advection field experiments and diffusion experiments indicates that advective processes have substantial impacts on porewater transport in this habitat.

### 2.3.3 Fluorescein Loss Experiments

The recovery of the fluorescein-impregnated gels was not completely efficient. For each treatment, there should have been a total of 30 observations (5 surface and 5 deep gel sections per plot in each of 3 blocks). The actual recoverable gel samples for

each treatment were 23, 15, 25 for the arenicolid, mixed, and thalassinid treatments, respectively. Thus, the degrees of freedom were relatively balanced between the arenicolid and thalassinid treatments, but the mixed treatment had fewer observations.

In spite of these difficulties, significant differences were found in the fluorescein loss data ($p < 0.0001$, $F_{2, 58.7} = 14.03$). Fluorescein loss was higher in the arenicolid plots relative to the thalassinid and mixed plots, suggesting that porewater transport was highest in arenicolid regions. No significant effect of depth ($p = 0.2480$, $F_{1, 57} = 1.36$) or block ($p = 0.3018$, $F_{2, 57} = 1.22$) was found, therefore the data were pooled and a one-way ANOVA was conducted with block and depth as covariates. Once again, a significant treatment effect was found, with the arenicolid plots showing significantly less fluorescein remaining in the gels relative to the mixed ($p = 0.0049$, $t_{59.5} = 3.28$) and thalassinid plots ($p < 0.0001$, $t_{57.6} = 5.19$), respectively. No significant difference was found between the mixed and thalassinid plots ($p = 0.5368$, $t_{59.4} = 1.07$) (Figure 4). These differences in fluorescein loss suggest that macrofaunal species composition is an important regulator of the extent of advective transport in permeable sediments. In particular, the arenicolids appear to be greater facilitators of advective transport relative to thalassinids. The lack of difference between deep (~8 cm) and surface (~2 cm) sections of the gel also indicate that these differences are not driven by organism effects on surface topography. Surface topography-driven flows tend to have shallow (3-5 cm) penetration into the sediment column (e.g. Huettel et al. 1998).

### 2.3.4 Sediment Porewater Solutes

The porewater peepers with acrylamide gels appeared to accurately record average porewater solute concentrations (Figure 5), and corresponded well to direct porewater extractions at this site (Waldbusser, unpublished data, Marinelli 1994). Overall trends in the porewater data support differential rates of porewater transport associated with the different taxa in this study. In most cases the depth-integrated porewater concentrations were lower in the arenicolid plots relative to thalassinid plots (Figure 6). However, mixed plots showed considerable variation. For all solutes measured ($NH_4^{1+}$, $PO_4^{3-}$, $Si(OH)_4$, D.I.C., alkalinity, and pH) there was a significant treatment*block interaction in the two-way ANOVA making the interpretation of main treatment effects somewhat difficult (Table 2, Figure 6). Closer examination of the data revealed that in most cases the interaction is driven by significant variation in the mixed treatment across blocks, perhaps associated with differences in abundance of the two organisms in these plots (Table 1). Because of the significant interaction terms in the original two-way ANOVA, the possibility of density-dependent effects (Tables 1 & 2), and kinetic differences in solute reaction rates, post-hoc regression analyses were conducted to explore relationships between organism density and porewater solute concentrations.

Linear regression results support the prediction of both density effects and kinetic effects. Parameter estimates suggest a stronger, negative effect of arenicolid density, and positive effect of thalassinid density, on ammonium and phosphate concentration relative to silicate concentration (Table 3). However, a pattern was detected in the distribution of

the residuals for several of the simple linear regressions. Positive residuals were clustered about intermediate abundances (occurring generally in mixed plots), and negative residuals were found at the extremes (occurring generally in the single species plots). The presence of this non-random pattern in residuals, and failure of the data to meet the Shapiro-Wilkes test for normality, indicates that an additional variable may be needed in the regression analyses.

The results from multiple linear regressions, with overlap index, show better fits than the single linear models in most cases, as indicated by p-values and adjusted $r^2$ of the two parameter model (Table 4). The results also indicate a positive relationship between degree of species overlap and depth integrated porewater concentrations of silicate, ammonium and DIC. This suggests that, while species identity and kinetic effects may contribute to overall porewater concentrations (Table 3), species interaction effects are also operative.

### 2.3.5 Sediment Parameters

Granulometric analysis of composite sediment samples indicated very little difference in sediment grain size, porosity and other measures among the experimental plots (Table 5). This argues against the hypothesis that the effects of the different organisms on porewater transport are related directly to changes in bulk sediment characteristics. In addition, organic C and N measures of surficial sediments among plots also show very little difference (Table 5).

Consistent with the bulk sediment analyses, the calculated permeability from each site shows no clear distinction as a function of experimental treatment, nor is it related to fluorescein loss data (Figure 4 & 7). A simple linear regression between fluorescein remaining in the gels and the calculated permeability for the experimental plots was not significant ($p = 0.6038$, $F_{1, 8} = 0.30$, $r^2 = 0.04$). The porosity measures could be biased toward low values since the sediment samples were taken at low tide and the drainage may have removed some of the water, though this should not affect among-site comparisons. However, the values calculated by loss of weight via drying are very close to earlier porosity measures made of the same area by using direct measurements of changes in volume of dried sediment added to known volumes of water (unpublished data).

Results of the measured permeability using the falling head permeameter experiments of intact sediment cores taken from Block I found the following coefficients of permeability: Arenicolid (26.49 cm $h^{-1}$), Mixed (18.55 cm $h^{-1}$), and Thalassinid (23.60 cm $h^{-1}$). As noted above, these values were based on one core from each site, and therefore do not capture the extent of variability within the experimental plots. Qualitative comparison of the calculated permeability (Figure 7) and the measured permeability (above) show similar patterns in the values between arenicolid and thalassinid plots, but not in the mixed plot.

## *2.4 Discussion*

To understand the effect of biodiversity and community structure on system function in benthic environments, it is important to adopt a mechanistic approach that includes both organisms and processes (Bolam et al. 2002, Reise 2002, and Lohrer et al. 2004). The complex milieu of marine sediments requires investigation of geochemical and physical processes in concert with biological characterization. Geochemical parameters such as organic matter input and reaction rate kinetics, physical parameters such as boundary layer interactions, and sediment granulometry all interact with organism characteristics (behavior, activity rates) and community level processes (density dependence) to determine the ecological landscape. Integration of these features over various scales in space and time determine the emergent ecosystem function.

The results from this study underscore the need for an integrative approach for studies of advectively permeable sediments, an environment that is prominent and biogeochemically significant in coastal and continental shelf habitats (Jahnke et al. 2000, Rocha 2000, Rusch et al. 2001, Jahnke et al. 2003, Reimers et al. 2004). Such environments are characterized by porous sediments with low standing stock but high throughput of organic material and rapid rates of biogeochemical cycling and porewater exchange (Marinelli et al. 1998). We utilized the natural variability of dominant infauna in this permeable sediment habitat to more accurately represent the role of functionally different infauna and their interaction on ecosystem-type processes over previous manipulated experimental systems. An important concern in using unmanipulated naturally occurring infaunal communities is the potential for other larger-scale correlated

parameters to be the drivers of among treatment variability. The spatial proximity of plots, lack of differences in granulometry, and lack of any noticeable pattern in species distributions across the flat all indicate that species distributions (on the scale of the experiment) are not the result of larger scale physical factors that may be confounding treatment effects. In other words, on our scales of measurement, the biology seems to be a causative agent, not responding to our measured parameters. We have shown that: 1) functionally different macrofauna affect rates of porewater advection in permeable sediments, 2) the effects are not attributable to changes in average, vertically-integrated measures of sediment granulometry or other plot specific characteristics that may be due to non-biological effects, 3) species interactions may further complicate the advective environment and the resulting diagenetic processes, and 4) species effects on geochemistry vary according to reaction rate kinetics of particular spoutes (described below).

Previous studies of infaunal effects on permeable sediments have emphasized surface processes related to topographic variation or sediment disturbance (Huettel et al. 1998, D'Andrea et al. 2002). This study emphasizes below-surface processes, including species-specific effects and species interactions. Although D'Andrea et al. (2002) also examined below-surface effects on sediment dynamics, our results suggest a different suite of mechanisms for organism effects on transport. In their study, thalassinids were found to increase organic matter reaction rates in closer proximity to burrows, and to increase flushing rates at depth in the less-permanent sections of the burrow. Our study emphasizes the effects of functionally different species' burrow morphology and feeding

behavior on below-surface advective transport. In addition, below-surface species interactions appear to promote nonlinear relationships between infauna and sediment geochemistry that may form the basis for "biodiversity effects" in sedimentary habitats (Waldbusser et al. 2004).

Surface processes that generate porewater advection include surface gravity waves in shallow water and interactions between surface topography and fluid flow fields (Reimers et al. 2004, Huettel & Gust 1992). Both of these mechanisms are present at False Bay. However, the lack of difference between near surface and deep sections of the gel indicates that transport does not decrease with depth (across the interval we studied, surface to ~10 cm), as is often the case with surface processes affecting the upper 5 cm of the sediment column (Huettel et al. 1998). Examination of the photographic data found roughly 5-7 sand ripple peaks in the sediment surface across a 50 cm transect, corresponding to an average ripple wave length of roughly 10 cm. The shape of the ripples indicates that the dominant flow direction is during the flood tide, counter to the direction of tracer gradient after three days of gel deployment (Figure 2). Observations made during the gel diffuser experiments found that overlying water covers the sediment faster than it can percolate through horizontally due to the pressure gradient. Therefore, the lack of a depth effect on fluorescein loss, the size and shape of the sediment ripples, and the observed direction of tracer transport all indicate that pressure gradients associated with tidal drainage and flooding, coupled with fine-scale variation driven by organisms, are likely the dominant mechanisms driving patterns of advective exchange. Other sources of pressure differentials such as boundary interactions, surface gravity

waves, and thermal convection may also be contributing to rapid transport within these sediments.

The fluorescein loss experiments and sediment permeability analyses suggest that fine scale measurements and consideration of organism behavior may be necessary to capture the mechanisms that promote the observed species differences in advective flow. Finer scale features, such as burrow wall composition or channels associated with feeding and sediment fluffing, are likely to be extremely important in either blocking or facilitating flow; these are not captured by traditional bulk analyses. More advanced measures such as high-resolution CT Scan or ultrasound may be required to reveal these features and their significance to transport in coastal sediments (Wethey & Woodin in review, Solan et al. 2003). Therefore, based on our findings and many prior studies, we must look to organism-specific attributes for discerning mechanism in the differences we found.

We hypothesize that increased flushing rates found in the arenicolid plots compared to thalassinid plots probably relate to differences in motility, feeding, and burrow construction between the two dominant taxa. During feeding, arenicolids fluidize sediment at the base of the feeding area and create localized hot spots of vertical advective throughput (Huettel 1990, Riisgård & Banta 1998, Timmermann et al. 2002, Timmermann et al. 2003). In addition, recent ultrasound measurements (Woodin & Wethey, unpublished data) indicate advective pumping of the area immediately below and surrounding the burrow opening (upper 2-3 cm) during near hourly defecation

events. The effects of vertical advective displacement of particles and fluid by arenicolids may directly or indirectly influence the rate of horizontal transport due to pressure gradients generated through tidal sediment saturation and draining. Arenicolids also appear to move reasonably frequently (Krager & Woodin 1993), perhaps in response to food patches (Woodin, unpublished data), ammonium concentrations (Marinelli, unpublished data) or in relation to life stage (Linton & Taghon 2000). Thus, sediment fluidization and movement may link resources and life history with advective transport. The potential effect of these linkages and their relation to microbial activity, benthic primary production, and nutrient cycling has been noted by Jumars et al. (1990), but little to no empirical evidence exists to verify or nullify these ideas.

In contrast to arenicolids, thalassinids create large feeding galleries where they feed directly in the sediment within the gallery (*Neotrypaea californiensis*), or filter feed by pumping overlying water (*Upogebia pugettensis*). Some investigators have suggested that microbes yielded through "gardening" are also an important food source for thalassinids (Kinoshita et al. 2003, see also Jumars et al. 1990). Thalassinid species are often observed to eject fines (MacGinite 1930, Suchanek 1983, Posey et al. 1991, Pinn et al. 1998, Feldman et al. 2000) or have high pumping rates of burrow water compared to physical processes of tidal exchange (Dworschak 1981) and therefore it should be expected that they would in turn increase the transport rates of porewater within sediments and affect rates of organic matter remineralization (Ziebis et al. 1996, D'Andrea et al. 2002). In contrast to prior studies, our study found no difference in grain size distributions (Table 5) and transport rates were slower in thalassinid areas compared

to arenicolid areas (Figure 4), though true organism-free control plots were lacking. It should also be noted that surface features often found in association with thalassinid burrows were lacking from the experimental area. The redistribution of the fine grained sediment during tidal flows or the dominance of the filter feeding *U. pugettensis* (Griffis & Shuchanek 1991) are two potential reasons sediment mounds associated with burrow openings were not found. Observations from various burrows around the experimental site found that the upper portions of the thalassinid burrows are thickly lined with clay and appear to be impermeable. In addition, pressure sensors placed near thalassinid burrows indicate little to no signal associated with feeding or movement, suggesting the walls are extremely thick (Woodin & Wethey, unpublished data) relative to arenicolid burrow walls (Wethey & Woodin, in review). We suggest that thalassinids in False Bay actually decrease bulk permeability through creation of near-solid structures that serve to interrupt flow. Analogous to pipes running through the sediment column, these near-solid structures may interfere with arenicolid feeding via inhibition of sediment fluidization and subduction.

The use of organism density in our analyses of porewater solutes coupled with kinetic differences among the solutes provides a mechanistic basis for interpreting the complex results obtained. The significant block by treatment interactions in the original two-way ANOVA of porewater data is not surprising, given the differences in relative densities of the two experimental organisms across the mixed treatments (Table 1). Prior investigations of benthic community dynamics and sedimentary functioning used biomass to account for bulk organism effects (Emmerson et al. 2001, Bolam et al. 2002)

but measures of abundance more explicitly account for the effects of burrow surface area on sediment-seawater exchange (Aller 1980) and individual interactions (Marinelli 1994). Furthermore, recent investigators have found density dependent effects on sediment chemistry (Gilbert et al. 2003, Marinelli & Williams 2003, Lohrer et al. 2004) driven in part by kinetic effects. We predicted that, based on kinetics arguments (Aller 1980, Marinelli 1992, Boudreau & Marinelli 1994), ammonium and phosphate should be most sensitive to advective processes facilitated by infauna, and exhibit strong density dependence. Both ammonium and phosphate are produced by organic matter decomposition, and production is not affected by porewater concentration. Such solutes are highly sensitive to the degree of biologically-mediated transport in sediments. Phosphate also is readily adsorbed to particles in the presence of oxygen, so rapid advection of oxic seawater is likely to further decrease phosphate concentrations in the porewater. Conversely, silica dissolution is abiotic, partly controlled by the degree of saturation, and less sensitive to biologically-driven transport. Thus, differences in the effects of infauna on solute concentration may relate in part to interactions between density and reaction rate kinetics, as observed in the regression parameters (Tables 3 & 4).

The inclusion of the overlap parameter also resulted in better model fits, a more detectable density effect, and some congruence with the expected relationship between density, kinetics and solute loss (Table 4). A possible mechanism behind the significant overlap effect may lie in our proposed interaction between arenicolids and thalassinids, where thalassinid burrows act as impermeable objects that restrict the feeding and

fluidizing behaviors of the arenicolids. Posey et al. (1991), and references therein, have shown the negative effects of thalassinids on smaller macrofauna related to bioturbation and/or adult larval interactions. Similar negative effects have been documented with arenicolid feeding and depositional burial of smaller macrofauna (Riisgård & Banta 1998 and refs therein). More importantly to the current study, we present a potential inhibition of arenicolid feeding by thalassinid burrows linking organism behavior and transport mechanisms in sediments possibly cascading into ecosystem functions such as nutrient cycling, microbial dynamics, and benthic primary production. Current models of advective transport include bulk sediment parameters and hydraulic pressure head (Boudreau 1997) and do not reflect this level of complexity. Experiments incorporating the fine scale measures of these processes are required if we are to incorporate biologically complex parameters into current models of elemental cycling in permeable sediments, and into our evaluation of ecosystem services provided by coastal habitats.

Complex associations of the biological, chemical, and physical processes co-act to determine ecosystem function. Our findings illustrate the importance of behavior and ecological considerations in studies of sediment dynamics, and conversely the importance of dynamics and processes in studies of biodiversity and ecosystem function. Developing predictive models of the effects species loss has on the functioning of coastal systems requires a mechanistic, process based approach. Given the broad scope of anthropogenic impacts on many coastal ecosystems, and the well documented changes in the structure of the coastal marine biological community (Levin et al. 2001), integrative studies are critical to understanding and maintaining living resources.

## *2.5 Tables*

**Table 2.5.1- Organism Density.** Density of surface features per $m^2$ from photographic surveys of experimental plots for the three blocks and for two of the three experiment dates. July values correspond to the fluorescein loss experiments, and the August values correspond to the porewater peeper experiments. Values represent number of fecal mounds (arenicolids) and burrow openings (thalassinids). The last column is the ratio of fecal mound to burrow opening for each plot.

| Experimental Plot | Block | Experiment Date | Surface Features Arenicolid | Thalassinid | Ratio (A:T) |
|---|---|---|---|---|---|
| Arenicolid | I | July | 49.33 ± 7.42 | 2.66 ± 1.33 | 18.54 |
| Arenicolid | II | July | 70.66 ± 9.61 | 16.00 ± 4.00 | 4.41 |
| Arenicolid | III | July | 36.00 ± 6.92 | 12.00 ± 0.00 | 3.00 |
| Mixed | I | July | 37.33 ± 2.66 | 21.33 ± 4.80 | 1.75 |
| Mixed | II | July | 20.00 ± 6.11 | 50.66 ± 5.81 | 0.39 |
| Mixed | III | July | 21.33 ± 3.52 | 54.66 ± 2.66 | 0.39 |
| Thalassinid | I | July | 2.00 ± 2.00 | 34.00 ± 6.00 | 0.05 |
| Thalassinid | II | July | 2.66 ± 1.33 | 61.33 ± 9.61 | 0.04 |
| Thalassinid | III | July | 2.66 ± 1.33 | 62.66 ± 8.11 | 0.04 |
| Arenicolid | I | August | 74.66 ± 10.41 | 18.66 ± 2.66 | 4.00 |
| Arenicolid | II | August | 81.33 ± 3.52 | 25.33 ± 9.33 | 3.21 |
| Arenicolid | III | August | 37.33 ± 3.52 | 28.00 ± 4.00 | 1.33 |
| Mixed | I | August | 50.00 ± 10.00 | 28.00 ± 4.00 | 1.78 |
| Mixed | II | August | 25.33 ± 4.80 | 50.66 ± 10.41 | 0.50 |
| Mixed | III | August | 36.00 ± 4.00 | 58.66 ± 3.52 | 0.61 |
| Thalassinid | I | August | 1.33 ± 1.33 | 65.33 ± 11.85 | 0.02 |
| Thalassinid | II | August | 9.33 ± 1.33 | 74.66 ± 13.13 | 0.12 |
| Thalassinid | III | August | 6.66 ± 6.66 | 48.00 ± 6.92 | 0.13 |

**Table 2.5.2- ANOVA Interactions**. F-values for the interaction terms in the two-way ANOVA for block and treatment effects. The interaction is highly significant in all cases except silicate.

| Solute | Effect | DF | F-Value | p-Value |
|---|---|---|---|---|
| *Ammonium* | blk*trt | 4, 18 | 6.89 | 0.0015 |
| *Phosphate* | blk*trt | 4, 18 | 6.57 | 0.0019 |
| *Silicate* | blk*trt | 4, 18 | 4.16 | 0.0148 |
| *D.I.C.* | blk*trt | 4, 18 | 5.95 | 0.0031 |
| *Alkalinity* | blk*trt | 4, 18 | 6.83 | 0.0016 |
| *pH* | blk*trt | 4, 18 | 10.32 | 0.0002 |

**Table 2.5.3- Simple Linear Regression**. Results from the standardized simple linear regression analysis for effects of arenicolid and thalassinid density on integrated porewater solute concentrations. Analyses were performed on standardized data, and therefore the parameter estimates are directly comparable. Asterisks indicate significance at the following levels * = 0.05 ** = 0.01 *** = 0.001. Degrees of freedom for each analysis were between 27 and 24 and dependent on outlier detection and removal.

| **Model=** | **Arenicolid** | | **Thalassinid** | |
|---|---|---|---|---|
| *Solute* | *Estimate* | *Adj. $R^2$* | *Estimate* | *Adj. $R^2$* |
| $NH_4^+$ | -0.698*** | 0.42 | 0.737*** | 0.49 |
| $PO_4^{2-}$ | -0.532*** | 0.37 | 0.678*** | 0.59 |
| $Si(OH)_4$ | -0.412** | 0.24 | 0.456*** | 0.39 |
| DIC | -0.505* | 0.18 | 0.411* | 0.14 |
| Alk | -0.616*** | 0.38 | 0.829*** | 0.68 |
| pH | 0.109 | -0.03 | 0.126 | -0.02 |

**Table 2.5.4- Multiple Linear Regression**. Results from the standardized multiple linear regression analysis inclusive of the overlap index and density effects on integrated porewater concentration. Parameter estimates for both density and overlap are presented for models run with arenicolid density and thalassinid density for each solute. Asterisks indicate level of significance, * =0.05 ** =0.01 *** =0.001.

| **Model=** | **Aren. & Overlap** | | | **Thal. & Overlap** | | |
|---|---|---|---|---|---|---|
| *Solute* | *Aren.* | *Overlap* | *Adj. $R^2$* | *Thal.* | *Overlap* | *Adj. $R^2$* |
| NH4 | -0.972*** | 0.439* | 0.50 | 0.901*** | 0.180 | 0.55 |
| PO4 | -0.684*** | 0.227 | 0.40 | 0.808*** | 0.183 | 0.60 |
| $Si(OH)_4$ | -0.783*** | 0.514** | 0.48 | 0.670*** | 0.329** | 0.58 |
| DIC | -0.690** | 0.647** | 0.29 | 0.766*** | 0.643*** | 0.48 |
| Alk | -0.617** | 0.067 | 0.37 | 0.879*** | 0.114 | 0.68 |
| pH | 0.041 | 0.110 | -0.06 | 0.068 | 0.007 | -0.08 |

**Table 2.5.5- Sediment Properties**. Sediment characteristics from composite samples of all the experimental plots, blocks (I, II, III) and treatments (M=mixed, A=arenicolid, T=thalassinid). Organic carbon and nitrogen are from replicate (3) samples within each plot and in %w/w (± 1 S.D.), standard deviations in organic nitrogen within site was <0.00. Categorical classifications of the sediments are poorly sorted, fine sands that are symmetrical and mesokurtic, from Folk and Ward (1957).

| Measure | I-M | I-A | I-T | II-M | II-A | II-T | III-M | III-A | III-T |
|---|---|---|---|---|---|---|---|---|---|
| Grain (phi) | 2.88 | 2.88 | 2.93 | 2.93 | 2.71 | 2.83 | 3.11 | 2.99 | 3.07 |
| Sorting | 1.78 | 1.78 | 1.78 | 1.91 | 1.77 | 1.84 | 1.88 | 1.79 | 1.86 |
| Skewness | 0.00 | 0.01 | 0.01 | -0.02 | -0.02 | -0.01 | 0.01 | 0.01 | 0.02 |
| Kurtosis | 1.11 | 1.11 | 1.08 | 1.05 | 1.08 | 1.07 | 1.08 | 1.07 | 1.09 |
| Porosity | 0.44 | 0.43 | 0.42 | 0.42 | 0.41 | 0.43 | 0.44 | 0.44 | 0.44 |
| Organic C | 0.17 | 0.21 | 0.21 | 0.19 | 0.18 | 0.21 | 0.18 | 0.20 | 0.17 |
| Org. C S.D. | ± 0.01 | ± 0.09 | ± 0.02 | ± 0.01 | ± 0.01 | ± 0.03 | ± 0.01 | ± 0.06 | ± 0.02 |
| Organic N | 0.02 | 0.02 | 0.03 | 0.03 | 0.02 | 0.03 | 0.02 | 0.02 | 0.02 |

## *2.6 Figures*

**Figure 2.6.1- Porewater Tracer with Tide**. Results from preliminary studies of porewater advection in False Bay sediments. On the left y-axis (solid line), fluorescein concentration at 1cm distance from the gel edge, toward the mouth of the bay, at 5 cm depth in the sediment. The x-axis is time from gel insertion into the sediment. The broken section of the solid line corresponds to the sampling period when no porewater could be extracted from the sediment. The right y-axis (dotted line) is estimated tidal height in False Bay in meters, based on observations and predicted tides in Friday Harbor. The directionality in concentration away from the gel plug down grade and not up indicates the importance of tidally generated pressure gradients in facilitating porewater advection.

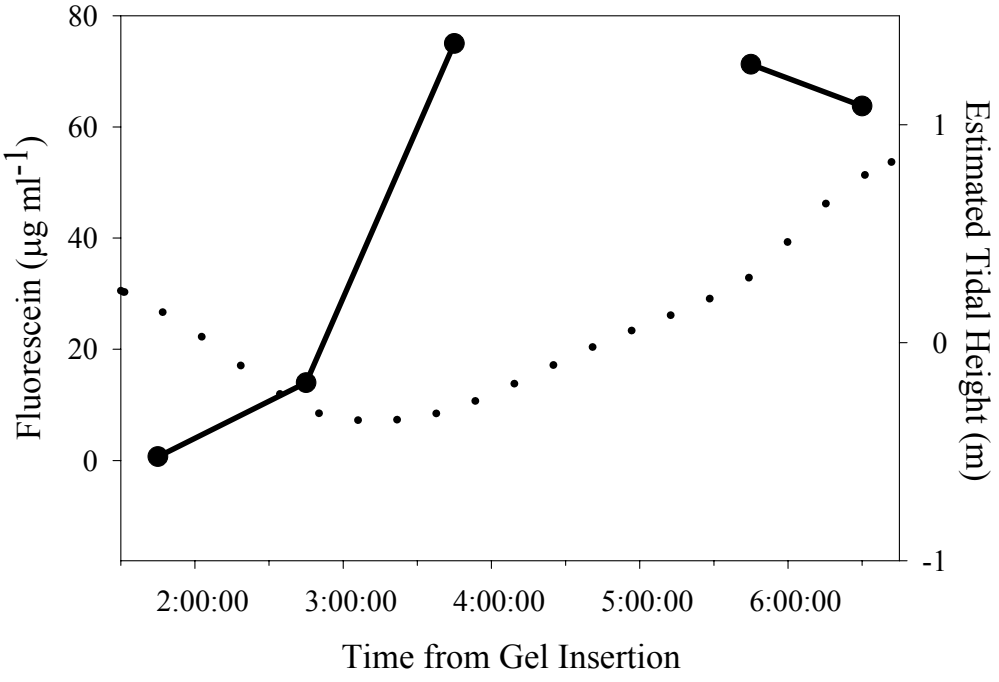


**Figure 2.6.2- Distribution of Tracer in Sediment**. The spatial transect showing fluorescein concentration at 5 cm depth on 26 May 2004, 3 days after the gel was inserted. The peak at 1cm illustrates the effect of tidally-induced pressure gradients on porewater movement and indicates directional (advective) transport. The solid vertical line at x = 0 represents the location of the fluorescein impregnated gel.

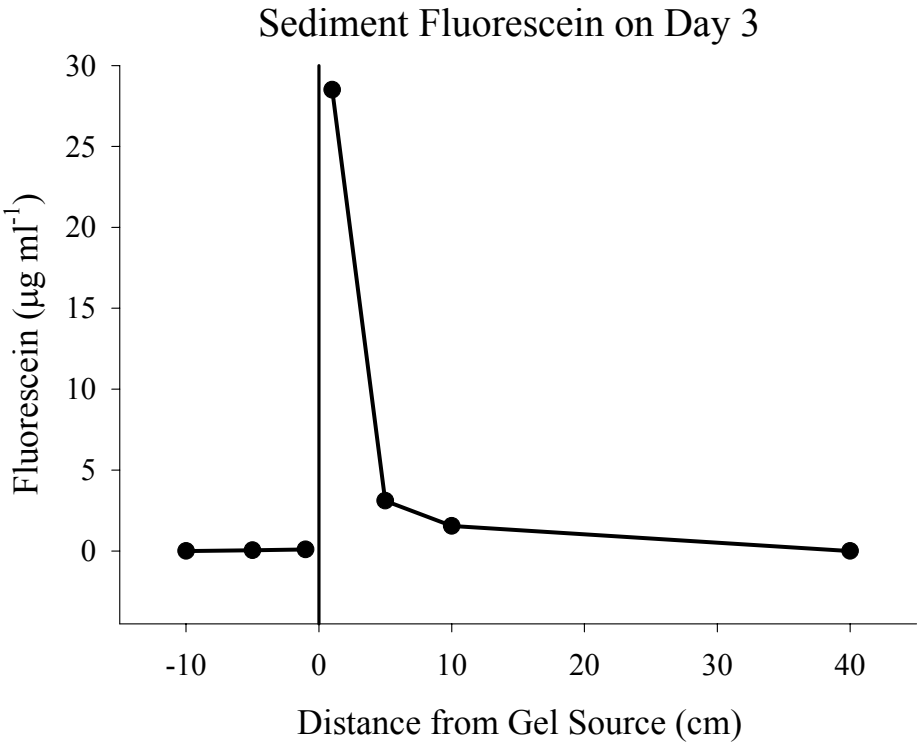


**Figure 2.6.3- Tracer Porewater Profiles**. Time series of fluorescein concentration profiles obtained from diffusion experiment, in a controlled sediment tower with no advective transport. The profiles refer to time from initiation of the experiment. The Stdy. St. line is the theoretical profile at steady state assuming no loss of tracer. This profile was calculated based on the known concentration of tracer in a given volume of gel, volume of sediment and water in the column overlying the gel, and a representative porosity of the sediments in the experiments. Depth 0 is the depth adjacent to the acrylamide gel source.

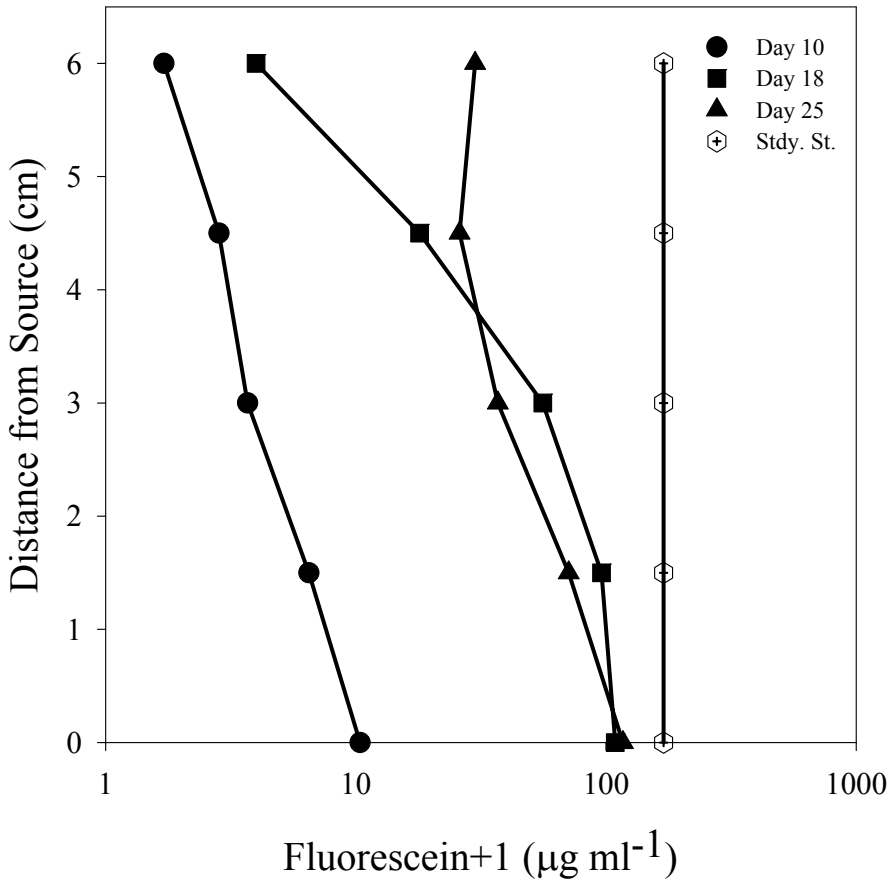


**Figure 2.6.4- Infaunal Treatments on Porewater Advection**. Mean values (± 1 S.E.) of fluorescein remaining in the gels for each treatment (A = arenicolid, M = mixed, and T = thalassinid. Data are pooled for block and depth as no significant effect was found for either factor. Common letters indicate no significant difference between treatments.

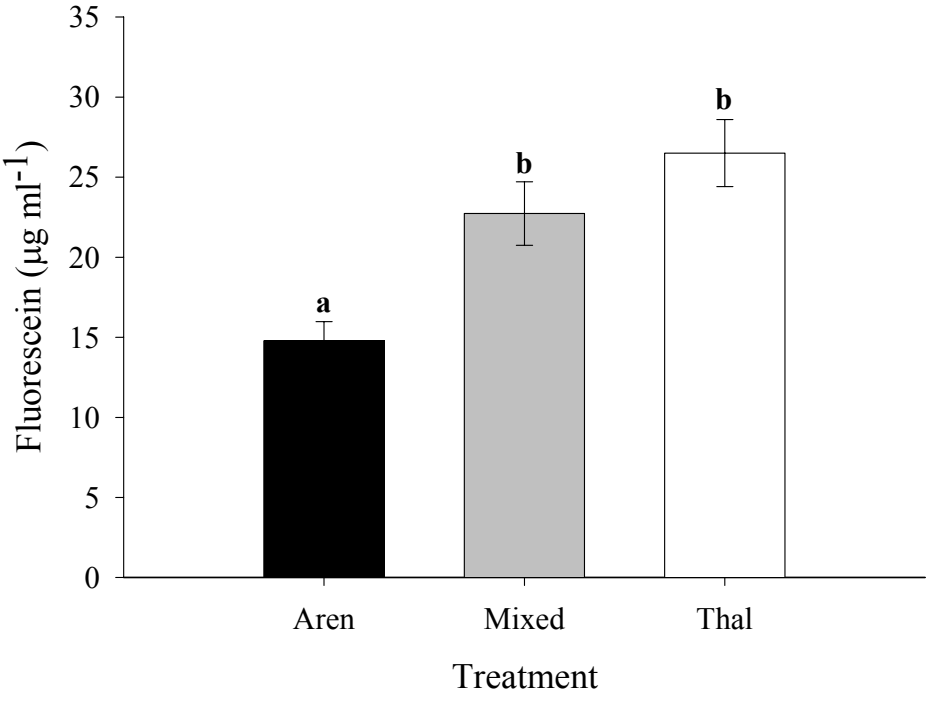


**Figure 2.6.5- Treatment Effects on Porewater Profiles**. Representative porewater profiles from two peeper deployments, one in an arenicolid plot (filled symbols), and one in a thalassinid plot (open symbols). Profiles of ammonium (circle), phosphate (square), and silicate (triangle) are shown.

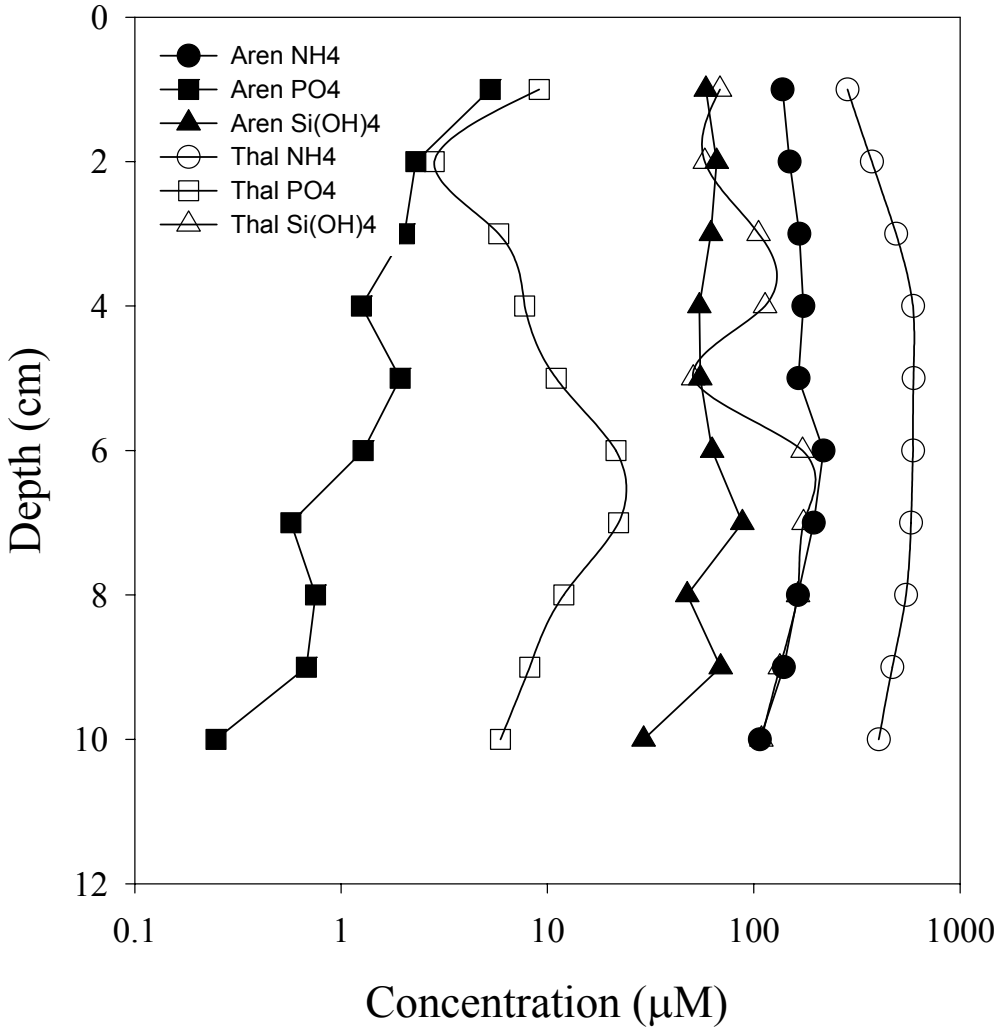


**Figure 2.6.6- Integrated Porewater Concentrations by Treatment**. The calculated least square means and standard errors for depth- integrated porewater profiles of each plot within each block. The x-axis refers to block number, and the y-axis is the depth integrated porewater concentration (μmol $cm^{-2}$), note scales are different. Ar = Arenicolid, Mi = Mixed, and Th = Thalassinid.

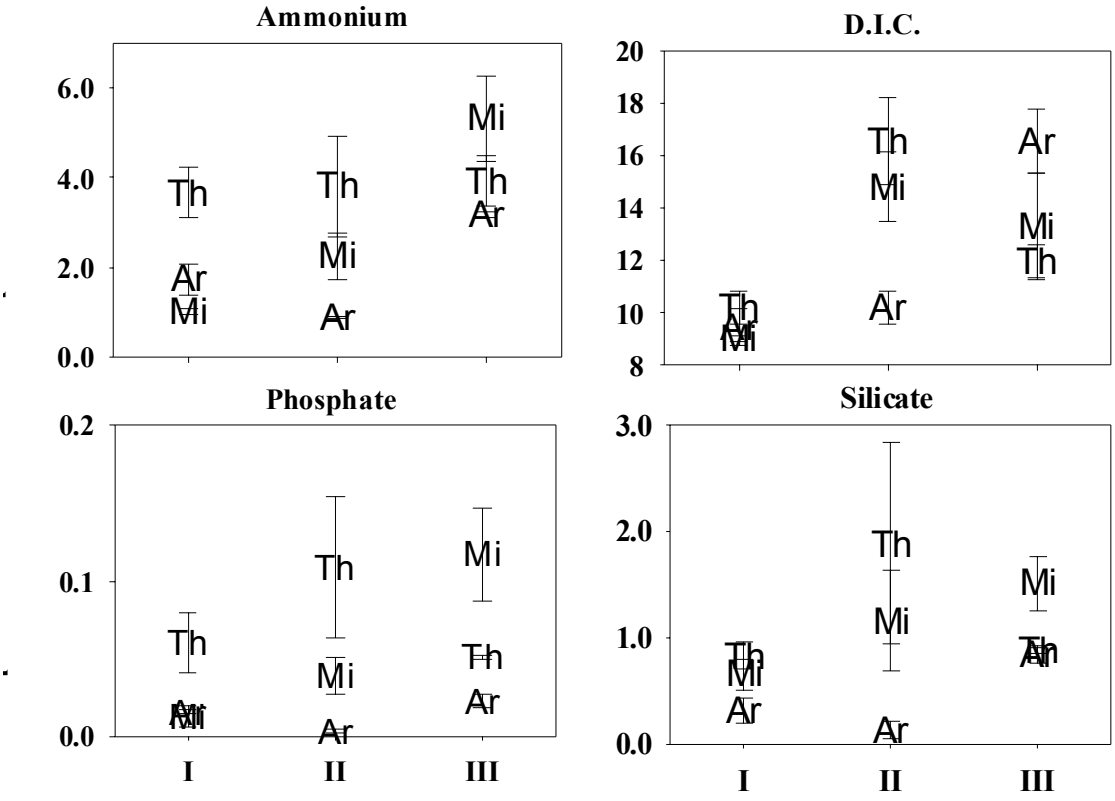
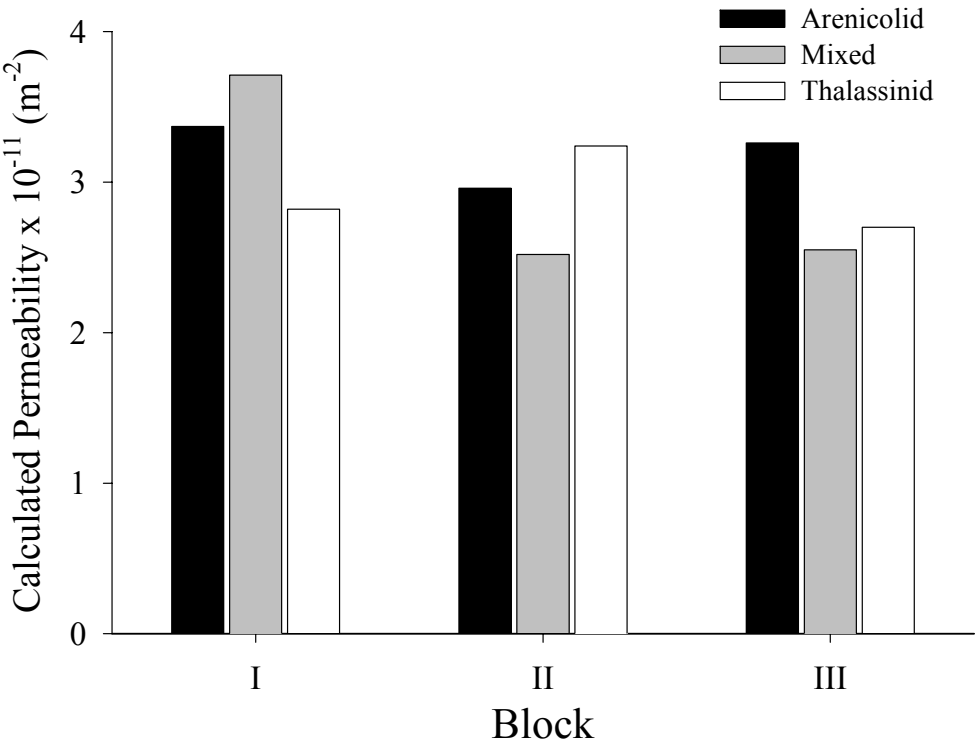


**Figure 2.6.7- Sediment Permeability within Experimental Plots**. Calculated permeability by the Rumpf-Gupte equation for composite samples from each plot among blocks.



# Chapter 3: Macrofaunal influences on permeable sediment fluxes: Species effects, stoichiometric relationships, and environmental consequences.

## *3.1 Introduction*

Coastal and estuarine sediments influence water column nutrients and elements through solute fluxes from remineralized organic matter (Balls 1994, Nixon et al. 1996, Beck and Bruland 2000). Porewater advection within coastal, permeable sediments has been recognized as a process that can alter ecosystem-wide solute regeneration rates (Marinelli et al. 1998, Boudreau et al. 2001, Jahnke et al. 2003). Many highly-productive estuarine and coastal habitats consist of sediment types that are advectively permeable with low organic matter standing stocks but high rates of remineralization (Boudreau et al. 2001, Middelburg et al. 2005) It is well established that benthic infauna modify transport-reaction processes within muddy (diffusive dominated) sediments in complex and ecologically important ways (Marinelli and Williams 2003, Waldbusser et al. 2004, Thrush et al. 2006). Permeable sediments however, due to their dynamic nature, present significant challenges to collecting empirical data (Berg et al. 2003, Precht et al. 2004, Polerecky et al. 2005) that may be used to verify and expand theories of animal-sediment relationships in physically active sediments. Subsequently the effects of infauna on permeable sediment functioning have been relatively unexplored (D'andrea et al. 2002, Nogaro et al. 2006, Waldbusser and Marinelli 2006).

In permeable sediments, irrigation and other activities by macro-infauna causes bioadvection though the sediment column and alters biogeochemical processes (Huttel

1990, Timmermann et al. 2002, Meysman et al. 2005). However, the consequences of bioadvective flow, relative to flows imposed by tidal draining and wave pumping, are poorly understood. Infaunal effects on permeable sediment solute fluxes should have ecological relevance to coastal elemental cycling, when bioadvection is comparable to rates of physically driven porewater advection. These two different transport processes, bioadvection and physically drive porewater advection, will often occur simultaneously, but are fundamentally very different. The net effect of interacting transport processes on sediment biogeochemistry will likely be different than from either process in isolation. The interaction of bioadvective and physically-driven flows could: 1) increase the total volume of oxic and suboxic zones within the sediment, 2) increase the residence time of porewater within the sediment, and 3) result in upward pumping of porewater from the sediment-water interface due to irrigation. Specific effects on biogeochemical processes may include stimulation of sub-oxic processes such as denitrification, and alteration of oxygen sensitive adsorption/desorption processes such as those associated with phosphoric minerals. Reaction rate kinetics and the balance of reaction and transport are also likely to be affected, leading to changes in nutrient regeneration rates relative to Redfield Ratio organic matter. These types of stoichiometric imbalances have significant consequences to ecosystem dynamics by altering the relative availability of limiting nutrients (Sterner and Hessen 1994, Sterner and Elser 2002, Turner 2002).

Characterizing functional infauna effects on sediment reaction-transport processes provides the basis for understanding how changes to benthic community composition may affect coastal biogeochemical cycling. Models of animal-sediment biogeochemical

relationships often characterize infaunal effects as "bulk" or "average" properties that do not capture dynamic behavior, although experimental studies in diffusive-dominated sediments suggest these species-specific and population-level effects are important to sedimentary processes (Waldbusser et al. 2004, Mermillod-Blondin et al. 2005, Norling et al. 2007). Benthic-pelagic coupling is tight in coastal permeable sediments where organic matter is rapidly remineralized and advective throughput is high (Boudreau et al. 2001, Middelburg et al. 2004). Therefore, quantifying the relationship between infaunal species and biogeochemical processes within permeable sediments provides an important mechanistic bridge between infaunal communities and the coastal ecosystem. This relationship is particularly important given the ecosystem-engineering behavior of active bioturbating infauna, such as the common lugworm, arenicola.

We examined the role of the ubiquitous lugworm, *Abarenicola pacifica,* on sedimentary fluxes of ecologically and diagenetically important solutes within an experimental permeable sediment habitat. The objectives of this study were to: 1) verify and expand on previous field experiments showing the importance of infaunal behavior and species interactions on intertidal permeable sediment functioning, 2) examine whether species-specific effects are expressed differently at two representative porewater advection rates, and 3) examine the broader ecological implications of infaunal activity and interactions in permeable sediments through examination of solute regeneration stoichiometry. We used a simple flow-through microcosm system to simulate tidally driven porewater movement within intertidal sediments. Additionally we develop a novel

metric (molar distance "d") to quantitatively assess stoichiometric relationships of solute exchange that may be applied more broadly to evaluate ecosystem functioning.

## *3.2 Methods*

We established a simplified intertidal community in laboratory microcosms, analogous to the upper intertidal sandflat of False Bay, described by Waldbusser and Marinelli (2006). The dominant large bioturbators in the False Bay study area are the arenicolid *Abarenicola pacifica* and two species of thalassinid shrimp *Upogebia pugettensis* and *Neotrypaea californiensis* (Waldbusser and Marinelli 2006). The ecology and behavior of these taxa have been reviewed extensively elsewhere (e.g. Riisgård and Banta 1998, Nickell and Atkinson 1995). Briefly, arenicolids are active head-down deposit feeders that fluidize sediment during feeding, while thalassinids excavate and live in extensive burrow networks, typically irrigating only their burrow lumen during deposit- or filter feeding. The two taxa occur in dominant patches or mixed assemblages in the upper intertidal reaches of False Bay. Thalassinids create relatively thick clay lined burrows in the upper 10-20 cm of the sediment column, the same depth range inhabited by the arenicolids. In contrast to thalassinids, arenicolid burrows are mucus lined. A previous field investigation at False Bay found that thalassinid burrows may decrease the effect of arenicolid bioturbation on sediment porewater profiles due to structural type effects of thalassinid burrows (Waldbusser and Marinelli 2006).

### 3.2.1 Microcosms

Flow-through microcosms were constructed using 20 cm diameter by 18 cm tall plastic buckets (Fig. 1). A nitex mesh glued into place two cm above the base of the

bucket was used to retain sediment while creating a sediment free dead-space at the bottom of the microcosm, allowing overlying water to percolate through the sediment column and out the bottom. This system therefore provided an integrated measure of the biogeochemical processes occurring within the sediment column via difference between the overlying water and effluent from the microcosms. Microcosms were kept in a water bath at Friday Harbor Laboratories (Fig. 1-A) that was continuously flushed with running ambient seawater from Friday Harbor (T = $10^{o}$ C and S = 32). The siphon pressure from the bottom of the microcosm pulled overlying water through the container and sediment column (Fig. 1-B). After passing through the sediment and the 60 μm nitex mesh, porewater drained to the 2 cm dead-space beneath the sediment (Fig. 1-C), and flowed through the outlet tubing (4.8 mm I.D.) (Fig. 1-D). To control the rate of flow a screw-type pinch valve (Fig. 1-E) was used in conjunction with adjusting the pressure head. Water samples were timed, weighed, and converted to volume with appropriate corrections for salinity and temperature to provide a measure of flow rate, later corrected to exchange rate. Two 300 watt halogen lamps were suspended roughly 0.5 m above the water surface to mimic 12 hr day/night cycles and provide light for benthic photosynthesis. A relatively uniform light input was measured as ~150 μmol $s^{-1}$ $m^{-2}$ at the water surface.

### 3.2.2 Sediment and Organisms

Sediment and arenicolid polychaetes were collected on 17 May 2005 from False Bay, San Juan Island, WA (Lat = 48.488°, Lon = -123.065°) and returned to the Friday Harbor Laboratories, University of Washington, where the laboratory experiment was

conducted. Sediment characteristics from the collection area are: grain size = 133 μm, porosity = 0.43, and % (wt/wt) organic carbon = 0.19 (from Waldbusser and Marinelli 2006, see Table 1). Layers of sediment from the upper 3-5 cm were placed directly into the microcosms in the field to minimize disturbance to sediment structure. This sediment layering was done until a sediment column of roughly 12 cm was reached in each microcosm. Previous studies suggest that this portion of the sediment column is regularly flushed by advective porewater flows (Billerbeck et al. 2006, Waldbusser and Marinelli 2006). Microcosms were transported back to the lab and placed in a flow-through seawater bath. *Abarenicola pacifica* were collected from the same intertidal area as the sediment. Individuals were placed gently in a bucket of seawater and returned to the laboratory where they were examined for injuries. Whole undamaged individuals were selected and randomly divided into groups of five. Worm groups were collectively wet weighed, and groups were added to the appropriate microcosms within 36 hrs of the field collection, as described below. Activity of arenicolids was monitored by recording the number of fresh fecal coils observed on the sediment surface in each microcosm during sampling periods (described below). The physical presence of thalassinid burrows was simulated with a clear, rigid plastic tubing (wall thickness ~2 mm), roughly 1 cm in diameter and 15 cm in length, open on both ends, and filled with sediment to the level of the sediment-water interface. These tubes were placed in flowing seawater for three days prior to use and scrubbed before adding to the microcosms. Five mimic burrows were inserted into the sediment in a circular pattern, at a distance roughly half of the radius of the microcosm and at a slight angle from vertical in each replicate "mimic" microcosm (below). The mimics simulated the structural component of thalassinid burrows in the

field in that they allowed water to flow through the sediment within the tubes but no exchange was possible across the tube walls. The mimics were used to overcome the difficulties associated with collecting intact individuals and maintaining them in containers much smaller than their extensive burrow networks.

### 3.2.3 Experimental Design

A total of 11 microcosms were partitioned among four experimental treatments as follows: Arenicolid (3), Mimic (2), Arenicolid & Mimic (3), and a Sediment-only Control (3). The following abbreviations are used to denote each treatment Arenicolid (A), Mimic (M), Arenicolid & Mimic (AM) and Control (C). The Mimic was replicated only twice due to space limitations in the overlying water bath. These densities of worms or mimics were used to reflect the densities found in False Bay (Krager and Woodin 1993, Waldbusser and Marinelli 2006).

Previous studies of porewater advection in False Bay demonstrated that the tidal draining of porewater was a dominant physical force of porewater movement (Waldbusser and Marinelli 2006). Therefore, we designed our experiments to reproduce tidal draining as the dominant physical mechanism inducing porewater water advection. The "low flow" scenario used in these experiments reflected porewater advection rates in False Bay (Waldbusser and Marinelli 2006) and other intertidal systems (De Beer et al. 2005, Billerbeck et al. 2006). We also used a higher rate of flow ("high flow") to represent more physically active sediments such as continental shelf sands (Reimers et al. 2004) and very active tidal flats (De Beer et al. 2005). Using two flow rates allowed us to evaluate the relative importance of infaunal effects in varying physical regimes. The

"low flow" and "high flow" rates used in the microcosms were 0.5 ml $min^{-1}$ and 5.0 ml $min^{-1}$, respectively. These flow rates translate to exchange rates of 20 L $m^{-2}$ $d^{-1}$ or 200 L $m^{-2}$ $d^{-1}$ for the low and high flows, respectively and vertical porewater velocities of 0.2 cm $hr^{-1}$ or 2.0 cm $hr^{-1}$ (corrected for porosity). At time = 0 the low flow rate was set, worms were added, and the flow was maintained at a constant rate for a 32 hr acclimation period. After the acclimation period, a 4 hr no flow, 8 hr low flow regime was established for 48 hrs to simulate tidal effects on porewater movement. During this period, simultaneous effluent samples from all microcosms were taken three times over each 8 hr flow period at 2, 5, and 8 hrs after initiating flow. After 48 hrs of low flow/no flow regime, the high flow rate was established. The high flow rate was held constant for the remainder of the experiment, and effluent samples were taken at 2, 4, 6, 24, and 36 hrs following the initiation of the high flow rate. Overlying water samples were also taken at all sample points.

### 3.2.4 Chemical Analyses

After sample collection, pH was measured using a standard pH probe and meter calibrated to three points with pH standards of 4, 7, and 10. Immediately thereafter, samples were filtered using a 0.45 μm filter, and placed in a $4^{o}$ C refrigerator. Within one week of collection, all samples were analyzed for alkalinity, ammonium, nitrate, phosphate, and silicate on a SmartChem discrete chemical analyzer (Westco Scientific, Danbury CT) using modifications of the following methods: Alkalinity- E.P.A. 310.2, Ammonium- Koroleff 1976, Nitrate- E.P.A. 353.3, Phosphate- E.P.A. 365.2 and Eaton et al. 1995, and Silicate- Strickland and Parsons 1972. Dissolved inorganic carbon (DIC)

was calculated from alkalinity and pH using dissociation constants (Zeebe and Wolf-Gladrow 2001). Fluxes were calculated using the following formula:

$$J_a = E \times ([a_{out}] - [a_{in}])$$

where $J_a$ is the flux of solute a (in mmol $m^{-2}$ $d^{-1}$), E is the exchange rate (in L $m^{-2}$ $d^{-1}$), and $[a_{out}]$ and $[a_{in}]$ are the concentrations (in mmol $L^{-1}$) of solute a in the effluent and overlying water, respectively. The exchange rate (E) was calculated as follows:

$$E = \frac{F}{A}$$

where F is the flow rate of the effluent in L $d^{-1}$ and A is the sediment surface area in $m^2$. Overlying water pH values were missing, so we were unable to calculate dissolved inorganic carbon (DIC) in the overlying water and therefore we lack DIC flux by difference in concentration, as was done with the other solutes. Therefore, we present the DIC values as a release rate, the effluent concentration multiplied by the exchange rate from above. This value does not account for the difference between the overlying water and effluent concentrations (reaction), and simply accounts for the flow rate and effluent concentration, not the extent of reaction, as do the flux measures described above.

### 3.2.5 Stoichiometric Values

In order to better quantify effects of infauna on nutrient regeneration ratios we developed a novel quantitative metric. The metric, similar to a regression residual in two dimensions, provides one value that accounts for the two-dimensional difference between an observation (point) and a prescribed relationship (line), in this case the Redfield Ratio.

The measured concentrations of two (or potentially more) solutes are treated as axes in geometric space with units of concentration or mass (Fig. 2). The perpendicular distances of the points or observations from the prescribed line are determined by the Law of Cosines, and provide a measure of deviation from an empirical/prescribed relationship in molar distance, referred to as d-value. The d-value is sensitive to changes in either or both solute concentrations. As calculated, the d-value is the summed minimum concentration needed to bring an observation to a prescribed relationship. Therefore, if both values change in the same ratio as the prescribed line, a smaller overall change in transport/reaction processes is needed to minimize the d-value than if each concentration changed independently. Additionally, geometric relationships in molar space are less susceptible to statistical and numerical problems of ratios.

For each stoichiometric relationship considered (C:N, N:P, C:P, Si:N), the perpendicular distance (d) of all observations from the prescribed line was determined (Fig. 2). Redfield Ratio organic matter (106:16:1 for C:N:P) was used as the empirical ratio for all pairs except Si:N, where 1:1 was used (Brzezinski 1985). For example the d-value of C:N was determined as:

$$d = \Delta C \times \sin(\theta)$$

where d is the molar distance (in μmol), θ is the angle (in radians) where the hypotenuse of the right triangle crosses the prescribed line, and ΔC is the difference between the measured carbon concentration and the predicted carbon concentration based on measured nitrogen concentrations and Redfield Ratio. Concentration differences (or legs

of the triangle) were calculated based on the difference between measured and stoichiometrically predicted values (Fig. 2). The calculated molar distance (d) was then multiplied by the ratio of the absolute value and actual value of one of the legs of the triangle, thereby indicating which side of the prescribed line an observation is on. The sign of the d-values therefore determines whether there is a subsidy or deficiency in one element relative to the other, based on expected Redfield stoichiometry.

### 3.2.6 Data Analyses

Porewater velocity data were analyzed to ensure that porewater advection was consistent among treatments throughout each of the two flow regimes. A one-way ANOVA was conducted with flow as the dependent variable and treatment as the independent variable. Individual treatment differences were determined using t-tests with Tukey's adjustment for multiple comparisons. Assumptions of normality and heteroscedascity of residuals were checked by Shapiro-Wilke's statistic and Hartley's F-max test, respectively. Solute flux data and stoichiometric d-values (molar distance) were analyzed with a repeated measures ANCOVA to determine organism effects on both solute fluxes and effluent stoichiometry. The high flow data lacked enough replication over time to conduct the proper data analysis, therefore, no data analyses were run on the high flow data. Separate analyses were run with each solute or stoichiometric pair (e.g. C:N, N:P, C:P, and Si:N) as independent variables, treatment as dependent variable, and time as the covariate. Solute and stoichiometric data were log transformed, and a spatial-power covariance structure was used in the repeated measures analyses to account for the unequal spacing of measures over time. Assumptions of ANCOVA were checked as described above, and in all cases the data were heteroscedastic. Therefore, the variance was partitioned by

treatment to account for the unequal variance. For most of the solutes in the flux analyses, and all the stoichiometric comparisons, the ANCOVA assumptions of normality were violated. In these instances the Fisher-Pittman permutation test (10,000 permutations) was used to produce distribution-free probabilities for the fixed effects in the ANCOVA and the treatment differences in the Tukey's t-tests (Edgington 1995). When a significant treatment by time interaction was detected, the predicted values of each treatment over time were plotted to visually ensure that the interaction occurred between two treatments that were not statistically different from each other. All data analyses were conducted using SAS Version 8.

## *3.3 Results*

### 3.3.1 Porewater Velocity

Within each flow regime, the microcosms maintained relatively consistent flow rates among the replicate microcosms (Fig. 3). The mean (± 1 S.D.) vertical porewater velocities for all microcosms by flow rate were 0.24 ± 0.05 cm $hr^{-1}$ and 2.10 ± 0.19 cm $hr^{-1}$ for the low and high flows, respectively. Mean vertical porewater velocities (cm $hr^{-1}$) for each treatment with standard deviations for the low flow regime were: A = 0.25 ± 0.04, AM = 0.26 ± 0.06, M = 0.23 ± 0.05, and C = 0.23 ± 0.06. The high flow regime porewater velocities were: A = 2.17 ± 0.12, AM = 2.25 ± 0.19, M = 1.93 ± 0.09, and C = 2.00 ± 0.13. Analysis of variance of porewater velocity by treatment showed no statistical difference among the low flow treatments ($F_{3,117} = 1.75$, P = 0.1606), while differences were detected among the high flow treatments ($F_{3,51} = 15.10$, P = <0.0001) (Fig. 3). Tukey's t-test detected statistical differences in the high flow experiments as

follows: A > C ($t_{51}$ = 3.52, P = 0.0050) and M ($t_{51}$ = 4.31, P = 0.0004); AM > C ($t_{51}$ = 5.02, P = <0.0001) and M ($t_{51}$ = 5.65, P = <0.0001). Thus, microcosms containing arenicolids had significantly higher flow rates than microcosms not containing arenicolids during the high flow regime.

### 3.3.2 Fecal Production

Fecal production, assessed as number of new coils per sampling period, was very similar between the two animal treatments throughout the course of the experiment (Fig. 4) varying from 0-4 new fecal mounds at each observational period. These data indicate that the burrow mimics had little to no effect on arenicolid feeding activity and defecation frequency. There was considerable within treatment variability, although error bars have been excluded for presentation purposes. Interestingly, during the low flow regime, fecal mound production in each treatment seemed to follow a 24 hr cycle coinciding with the extreme tides in the False Bay mixed semi-diurnal tidal cycle.

### 3.3.3 Solute Fluxes

Although fluxes of solutes were variable over the course of this experiment (Fig. 5), significant effects of arenicolid were found. In general, these effects were greatest at low porewater advective flow and diminished at higher advective flow (Table 2). The presence of arenicolid worms significantly lowered fluxes of ammonium, phosphate, DIC (release rate), and alkalinity relative to non-animal (C and M) treatments during the low flow regime (Table 2 & 3). There was little difference between the two arenicolid treatments (A and AM), or between the non-animal treatments (C and M) (Tables 2 & 3). This finding suggests that the mimics did not influence the effects of arenicolids on

sediment biogeochemical processes, nor did the mimics themselves affect sediment biogeochemistry. The permutation tests did not change the inferences from the original statistical analyses. All treatment by time interactions occurred between non-animal treatments (Control and Mimic), or animal treatments (Arenicolid and Arenicolid & Mimic), therefore, the differences between animal and non-animal treatments are valid.

The increase in flow resulted in increased fluxes and decreased differences among treatment fluxes (or effluent concentrations) (Table 2, Fig. 5), illustrating that effects of arenicolids are minimized under increased porewater flow conditions. The effects of arenicolids were not consistent for all solutes; ammonium fluxes in the C and M treatments during the low flow regime and all high flow treatments were very similar, while low flow A and AM treatment fluxes were almost an order of magnitude lower than the C and M low flow treatments (Table 2). Nitrate fluxes increased an order of magnitude with a 10-fold increase in porewater velocity, irrespective of treatment. Conversely, silicate fluxes were highly variable over the course of the experiment. Within the low flow regime A and AM treatments promoted net silicate uptake whereas C and M treatments promoted net silicate dissolution. Under high flow, silicate uptake occurred in all treatments, with the highest uptake in the C and M treatments. Phosphate fluxes followed a similar pattern within the low and high flow regimes, A and AM treatments typically reduced fluxes of phosphate by a factor of two relative to C and M treatments. A and AM treatments also decreased DIC release rates by roughly a factor of two relative to C and M in the low flow regime, while release rates were similar in all treatments in the high flow regime. Finally, alkalinity fluxes followed a pattern similar to

ammonium where low flow A and AM treatments had lower fluxes than all other treatments of both flow rates. The overall variance in fluxes among treatments is also noteworthy. In general, the A treatment had the smallest variance in flux rates, followed by the AM treatment, with the C and M treatments had the largest variance in flux rates (Table 2).

### 3.3.4 Stoichiometry

Arenicolids had differential effects on solute fluxes, relative to non-animal treatments) that led to changes in the stoichiometry of solutes returned to the water column by sediment biogeochemical processes. Stoichiometric effects were evaluated using molar distance on the low flow data only because arenicolid effects were most pronounced in this flow regime, and we lacked replication in the high flow data (Fig. 6 & 7, Table 4). Statistical differences were detected between A, AM (animal) and C, M (non-animal) treatments for three of the four stoichiometric molar distances calculated (C:P, N:P, Si:N) (Table 4, Fig. 7). Furthermore, statistical differences were found between the A and AM treatments for Si:N and N:P molar distances. Significant treatment by time interactions were also detected for these molar distances, and therefore, the between-animal treatment differences should be viewed cautiously. Other significant treatment by time interactions occurred only between the C and M treatments or between the A and AM treatments. In the case of N:P, C:P, and N:Si molar distances, A and AM treatments were characterized by stoichiometric molar distances closer to zero (or nearer to the prescribed relationship), while C and M treatments were characterized by a greater deviation from zero. In total, the stoichiometric consequences of arenicolids on

remineralized solutes to the overlying water are: 1) decreasing the phosphorous relative to nitrogen and carbon and 2) decreasing nitrogen relative to silica.

## ***3.4 Discussion***

The experiments presented here coupled with field studies (D'andrea et al. 2002, De Beer et al. 2005, Waldbusser and Marinelli 2006) and modeling exercises (Timmermann et al. 2002, Meysman et al. 2006) advance a mechanistic understanding of infaunal effects on permeable sediment functioning. Methodological limitations currently inhibit direct measures of infaunal effects on permeable sediment fluxes in the field. Therefore, laboratory and modeling studies are needed to quantify biological effects on permeable sediment processes and the potential ecosystem consequences. The simple yet novel experimental system we constructed simulated advective porewater movement associated with tidal flushing and draining of intertidal sediments, while providing control of vertical porewater velocity. Furthermore, we used a simplified "community" consisting of one species, and burrow mimics from a second species. Within our experimental microcosms, arenicolids created statistically significant changes to several solute fluxes, while the differential effects on specific solutes created stoichiometric changes in elemental regeneration ratios. The changes in biogeochemistry that we measured in this system represent an integrated effect of all arenicolid activities on permeable sediment processes, and provide a starting point to estimate the potentially large scale effects of this common infauna on important ecosystem services such as nutrient cycling.

Permeable sediments are significant contributors to the cycling of organic matter and nutrients in coastal environments (Marinelli et al. 1998, Jahnke et al. 2005, Billerbeck et al. 2006), and therefore factors that cause variability in permeable sediment fluxes have potential ecosystem scale effects. Given the low standing stocks of organic matter found in these sandier environments, and the difficulties in measuring flux and reaction rates have limited understanding of their biogeochemical significance. Recent studies have allowed quantification of fluxes from permeable sediment habitats, and comparison to organically rich muddy sediments (Table 5). Although the techniques among studies vary, one should note flux rates between permeable and diffusive sediments are within the range of values of each other. Furthermore, the range of flux values measured in this study, due to the presence or absence of arenicolids, is wide compared to full range of values in the studies listed in Table 5, especially for ammonium, phosphate, and silicate. The arenicolid induced variability in flux rates highlights the importance of these organisms and possibly other infauna on altering rates and pathways of sediment biogeochemical processes as found in diffusion dominated sediments (Andersen and Kristensen 1988, Lohrer et al. 2004, Waldbusser et al. 2004).

The measured flux rates of various solutes in the microcosms were within the range of values measured in other permeable sediment systems (Table 5). The most appropriate values to compare to this study are those of Billerbeck et al. 2006, as this study specifically looked at tidal seepage, the process that was simulated in our experimental microcosms. Our measured fluxes of ammonium, silicate, and phosphate are similar to those measured in-situ by Billerbeck et al. 2006. Interestingly, the relatively

low N:P ratios found in our microcosms (Figure 6), were also observed in-situ by Billerbeck et al. (2006) in their seepage water. As we argue below, they also note that coupled nitrification-denitrification or phosphate release from mineral phases with anoxic porewater are likely mechanisms for this enhanced phosphate release relative to nitrogen. The C:P ratios in the microcosm effluent (Figure 6) indicate that at least in the non-arenicolid treatments, phosphate desorption is the likely mechanism, while in the arenicolid treatments it is likely a combination of denitrification and increased phosphate adsorption.

A comparison of measured porewater advection rates with arenicolid irrigation rates suggests that arenicolids exert significant force on the total porewater flow within permeable sediments. Measured porewater advection and arenicolid bioirrigation rates compiled from previous studies and scaled to a modeled lugworm areal domain (400 $cm^2$ from Meysman et al. 2006) are shown in Table 6 (A, B). These calculations suggest that arenicolid irrigation may increase porewater flow by 10% to over 100% of the advective porewater flow within intertidal flats. Although we lacked direct measures of arenicolid irrigation in our study, we applied the scaled values from prior studies for comparison with our experimental system (Table 6-C). The calculations suggest that lugworms could increase total porewater flow within the microcosms by a factor of 10 in the low flow regime. Furthermore, field arenicolid densities are often greater than one individual per 400 $cm^2$ (Krager and Woodin 1993, Waldbusser and Marinelli 2006), potentially magnifying the bioirrigation effect. The interaction of the upward irrigation through the arenicolid feeding funnel and downward tidal draining likely creates additional variability

in the extent of sediment oxygenation. The irrigation may also make available remineralized nutrients in the overlying water, if the porewater velocity from upward irrigation exceeds downward transport via tidal draining, and the vertical length scales are short enough to overcome dispersive effects. Virtually no effects of the worms were found on sediment biogeochemistry under high flow conditions, even though bioirrigation may have doubled total porewater flow. The lack of worm effects in the high flow experiments may be due to effects of flow on behavior and irrigation, lower residence time of porewater in the sediment, or interactions among these factors. The above calculations do however provide a mechanistic and quantitative basis for the effect of arenicolids (and potentially other infauna) on permeable sediment processes.

Thalassinid burrow mimics did not alter arenicolid effects on biogeochemical fluxes, indicating that the structural component of the burrows do not modify arenicolid behavior significantly. The mimics were intended to represent the physical properties of the clay lined burrows found in False Bay, and therefore other possible effects of the thalassinid burrows such as exchange across the burrow wall and hot-spots of microbial activity were not captured in our experimental system. Although fluxes between the arenicolid (A) and arenicolid & mimic (AM) treatments did not differ statistically (Table 3), there was a general trend of diminished effect of arenicolids on solute fluxes in the AM treatment relative to A treatment. The lack of an effect of mimics may be due to container effects, the simplified geometry of our mimics relative to real thalassinid burrows, or other properties of natural burrows and organisms that our mimics did not adequately replicate. Although our previous field studies have found that thalassinid

burrows appeared to alter arenicolid effects on porewater concentrations (Waldbusser and Marinelli 2006), our experimental system of porewater advection is also rudimentary. Flow rates in our laboratory simulations were relatively constant, whereas in-situ porewater advection (driven by other physical processes in addition to tidal draining) are likely more tortuous creating variable residence times of porewater within the sediment. This variation may lead to local differences in porewater solute concentrations among patches of fauna that could not be captured in our experimental system.

The significantly large decrease in nitrogen (~4 mmol $m^{-2}$ $d^{-1}$), dissolved inorganic carbon (>20 mmol $m^{-2}$ $d^{-1}$), and phosphate (~1 mmol $m^{-2}$ $d^{-1}$) release in the low flow, arenicolid treatments highlights the potential ecosystem consequence of these common infauna (Tables 2 & 3). Uptake via primary production and loss via dentrification are likely sinks of the ~4 mmol $m^{-2}$ $d^{-1}$ of nitrogen ($NH_4^+$ & $NO_3^-$). If the roughly 4 mmol $m^{-2}$ $d^{-1}$ decrease of DIN flux in the arenicolid treatments was attributed to arenicolid-enhanced denitrification, this would match estimates of continental shelf denitrification rates (Seitzinger and Giblin 1996). While arenicolid irrigation would increase total porewater flow though permeable sediments, we suggest that the heterogeneity in porewater movement created by complex irrigation patterns (Meysman et al. 2006, Wethey and Woodin 2005, Wethey unpublished data) is also an important mechanism affecting biogeochemical processes. Arenicolid bioturbation coupled with porewater advection would presumably increase the: 1) size of the sub-oxic zone as shown by Timmermann et al. (2006), 2) residence time of a porewater parcel draining through the sediment, particularly in the area encompassing the feeding funnel, and 3)

upward transport of porewater. The upward movement of porewater in the feeding funnel flowing against the downward movement of tidally draining porewater would result in a net increase in the time a parcel of porewater is retained within the sediment column. Rao et al. (in press) have shown in experimental sediment columns that residence time of porewater within permeable sediments may be an important factor in rates of denitrification. Increased porewater residence time coincident with the injection of overlying oxygenated water at depth should enhance nitrification, typically the rate-limiting step in coupled nitrification-denitrification (Jenkins and Kemp 1984, Kemp et al. 1990). Structures such as the semi-permeable tail shaft in the arenicolid burrow may also serve as important microzones for microbial transformations in permeable sediments, as they are in diffusion-dominated sediments (Aller 1988, Kristensen 1988). Furthermore, Huttel (1990) measuring porewater profiles of nitrogen species in sandy sediments found that arenicolids increase nitrate relative to ammonium, suggesting that the presence of arenicolids stimulates nitrification. Our experimental results coupled with the previous studies noted above suggest that arenicolid-stimulated denitrification in permeable sediments is likely and may be an important ecosystem consequence of bioturbation.

The net uptake or sink of nutrients in our arenicolid treatments may also be explained by phosphate (or ammonium) adsorption (Krom and Berner 1980, Sanudo-Wilhelmy et al. 2004), or primary production. Although we lack direct measurements that would allow us to differentiate the sink(s) of nitrogen and phosphorus, the balance between loss via biogeochemical processes versus primary production does have important ecosystem scale consequences. The reduction in DIC release of greater than 20

mmol $m^{-2}$ $d^{-1}$ from treatments with arenicolids relative to those without may indicate a 50% increase of microphytobenthic production when compared to field measurements in False Bay (Pamatmat and Fenton 1968) and other intertidal flats (Underwood and Kromkamp 1999). Previous investigators have shown a stimulatory effect of bioturbators on benthic primary production (Bianchi and Rice 1988) or nutrient uptake in the absence of light (Marinelli 1992) in diffusion-dominated sediments. This potential stimulation of primary production is likely due to the upward transport of porewater to the sediment surface, and it is also possible that some portion of the upwardly transported solutes are lost to the overlying water. If arenicolids stimulate benthic primary production in this permeable sedimentary system, an increase in labile food availability to these deposit-feeders and the rest of the intertidal flat community may result. While this postulate is speculative and warrants further investigation, our findings suggest potentially important links among bioturbators, porewater advection, nutrient cycling, and benthic primary production in permeable sediments.

The differential infauna effects on specific biogeochemical pathways in permeable sediments leads to imbalanced elemental regeneration ratios. We developed a new metric to determine the "molar distance" of our observations from empirical elemental relationships in geometric space (Fig. 2 & 7). This analog to a geometric mean regression residual overcomes statistical and interpretive problems associated with the use of ratios and carries units of concentration. Ratios fail to capture information about the absolute values of the two numbers; rather they only capture relative concentrations to each other. There is utility in ratios for this reason, but when investigating aspects of

marine ecosystems such as nutrient cycling, ratios may also hide important characteristics of the system. An example of this can be seen when comparing ratios from different ecosystems (Table 7). The d-values represent a combined change to the system that is needed to bring the two values closer to a prescribed line, in this case Redfield nitrogen:phosphorous, rather than simply the relative concentrations. For example, High Venice Lagoon has an N:P ratio of 48, compared to Redfield (16) this appears to be incredibly imbalanced, but note the small concentrations of both N (2.40) and P (0.05) and small d-value (0.10) therefore only a small absolute change in concentration is needed in either nutrient to bring it back in line with Redfield. In other words, only small variation in the rates of processes controlling concentrations of these two nutrients are needed to move it from a N:P ratio of 48 to 16. Additionally in Table 7, several systems have an N:P ratio of roughly 5.00 to 5.75, while their d-values range from -0.51 to -2.51 reflecting more accurately the change in concentrations needed to move the system closer to Redfield. Ratios therefore do not capture an important aspect of marine ecosystem dynamics, the absolute changes in concentrations that may move the system from limited in one nutrient, to limited in another. Whereas d-values provide a useful metric that captures a measure of how "far" a system may be from a prescribed relationship, which may be much more relevant when considering nutrient regeneration in coastal ecosystems.

To further illustrate the utility of the d-value over straight ratios, we have plotted two pair of values for two hypothetical elements in Figure 8. The figure illustrates that the d-values capture a level of information that is not apparent in the ratios. In this

example, observations with x, y values of 4, 13 and 28, 89 have a similar ratio of 0.3, but the distance of those values relative to an empirical relationship, (1:1 ratio) is quite different having d-values of 6.4 and 43.1, respectively. In other words, values of a given ratio will fall upon a line of a slope different than the empirical 1:1 line, and therefore the ratio will remain constant as x, y values increase, but the distance of a given observation will deviate further from the empirical line. Thus, a larger increase or decrease in given processes regulating the concentration of the elements would be required to bring the observation inline with the empirical relationship. Similarly, two observations may have the same d-value, while the ratio is very different, as in figure 8. This illustrates that the actual change in some process required to bring these two observations inline with the empirical relationship is similar, even though the ratios are dramatically different.

The mean molar ratio (and standard deviation) are provided below the x-axis on Fig. 7 for each stoichiometric pair, for comparison. The ratios alone suggest that differences between arenicolid and non-arenicolid treatments are not very large for Si:N or N:P. However, the molar distance values (d) indicate that a much smaller change in the silica:nitrogen balance is needed to move the arenicolid treatments across the "0" or prescribed line relative to non-arenicolid treatments. Therefore, we believe molar distance may be an important metric used in conjunction with ratios to examine stoichiometric relationships in benthic-pelagic exchange and other ecological applications. Molar distances may also be calculated for multiple dimensions or elements in geometric space thereby overcoming another limitation of simple ratios.

We have calculated molar distances (d) for 4 different stoichiometric pairs (Fig. 7) that have potentially important ecosystem consequences (Table 4). Several investigators have shown that the values of Si:N > 1 provide diatoms a competitive advantage over dinoflagellates, while values < 1 tend to slow diatom growth and give dinoflagellates a competitive advantage (Brzezinski 1985, Rahm et al. 1996, Turner et al. 1998). A diatom versus dinoflagellate community has potential implications for the occurrence of harmful algal blooms, the structure of marine food webs, and the rate and timing of organic matter deposition to the sediment surface (Officer and Ryther 1980, Cloern 2001). Our experiment showed that arenicolids promoted Si:N d-values > 1 in fluxes from sediments, which would favor diatoms over dinoflagellates (Fig 7). Therefore given the strong coupling between sediment and overlying water, the loss of arenicolids may promote water column stoichiometry favoring dinoflagellates over diatoms. Our experiments, coupled with other recent studies, illustrate the potentially important consequence of bioturbator loss in permeable sediments to coastal ecosystem dynamics (e.g.Volkenborn and Reise 2007), a concern in coastal environments subject to anthropogenic degradation via eutrophication or dredging.

In conclusion, our experiments illustrate the important ecological role infauna may play in biogeochemical exchange between permeable sediments and overlying water. Benthic fluxes in shallow coastal areas are sufficient to modify water column composition of ecologically important solutes (Nixon et al. 1996, Beck and Bruland 2000, Sohma et al. 2001). Therefore, if infaunal species modify solute fluxes in ecologically relevant ways then infaunal species loss or replacement may have large

"bottom up" consequences on coastal and estuarine ecosystem functioning. Anthropogenic disturbances to permeable sediment infaunal communities may have indirect effects on elemental cycling and alter ecosystem function, as in diffusion-dominated sediments (Solan et al. 2004). Our results build upon other recent studies in diffusion-dominated sediments showing the important role infaunal community structure plays in remineralization of biogenic elements (e.g. Biles et al. 2003, Covich et al. 2004, Michaud et al. 2005). The extrapolation of results from microcosm studies to larger scale ecological phenomenon is not without pitfalls, although these comparisons are needed if we are to understand the potential consequences of benthic species loss/change on coastal ecosystem functioning.

## *3.5 Tables*

**Table 3.5.1- Sediment Properties**. Sediment granulometry of the study site where experimental sediment was collected. Values are means (± 1 S.D.) across 9 sites from composite samples within 0.5 $m^{-2}$ plots. Note the low variability in parameters. Methods and data originally published in Waldbusser and Marinelli (2006).

| Measure | Value | Identification |
|---|---|---|
| Grain (phi) | 2.92 ± (0.120) | *Fine Sands* |
| Sorting | 1.82 ± (0.050) | *Poorly Sorted* |
| Skewness | 0.00 ± (0.014) | *Symmetrical* |
| Kurtosis | 1.08 ± (0.020) | *Mesokurtic* |
| Porosity | 0.43 ± (0.012) | |
| Organic C % (w/w) | 0.19 ± (0.017) | |
| Organic N % (w/w) | 0.02 ± (0.005) | |

**Table 3.5.2- Average Solute Fluxes**. (Next page) Mean flux (mmol $m^{-2}$ $d^{-1}$ ± 1 σ) for each treatment and flow regime, and general effect of arenicolids on fluxes. DIC is release rate rather than flux (see text for explanation). Letters in the parenthesis indicate low (L) or high (H) flow. Positive values indicate release from sediments and negative values indicate uptake by sediments. Arenicolid effects are denoted as (▼) decreases flux, (▲) increases flux, (n/c) no change, and (n/s) indicates a change that is not statistically significant during the low flow regime. *The high flow fluxes were calculated excluding the first three sampling periods as this represented a transitional phase that was quite different than the values measured later, and therefore the general arenicolid effects in the high flow regime are based on observation rather than statistical tests.

| **Treatment (flow)** | $NH_4^+$ | $NO_3^-$ | Si(OH)4 | $PO_4^{3-}$ | DIC | Alkalinity |
|---|---|---|---|---|---|---|
| *Arenicolid (L)* | 0.35 (0.15) | -0.40 (0.12) | -0.24 (0.10) | 0.01 (0.06) | 32.47 (4.91) | 3.62 (2.01) |
| *Aren & Mimic (L)* | 0.64(0.58) | -0.46 (0.12) | -0.33 (0.16) | 0.17 (0.26) | 40.19 (11.28) | 10.22 (8.09) |
| *Control (L)* | 3.59 (1.17) | -0.43 (0.09) | -0.01 (0.17) | 0.90 (0.25) | 53.53 (9.68) | 28.84 (11.27) |
| *Mimic (L)* | 4.02 (1.80) | -0.42 (0.12) | 0.07 (0.26) | 1.05 (0.38) | 64.00 (14.90) | 33.64 (12.38) |
| *Arenicolid (H)* | 3.92 (0.53) | -4.62 (0.47) | -0.78 (0.73) | 2.79 (0.69) | 330.89 (18.58) | 45.54 (11.17) |
| *Aren & Mimic (H)* | 4.84 (0.61) | -4.45 (0.72) | -0.48 (1.15) | 3.53 (1.10) | 353.33 (41.64) | 58.73 (28.43) |
| *Control (H)* | 2.54 (0.58) | -4.62 (0.49) | -2.47 (2.66) | 5.75 (1.04) | 301.15 (21.32) | 40.65 (13.00) |
| *Mimic (H)* | 3.17 (0.16) | -4.36 (0.42) | -0.98 (2.60) | 6.27 (0.65) | 301.44 (26.44) | 46.70 (25.58) |

| **General Arenicolid Effect** | $NH_4^+$ | $NO_3^{2-}$ | $Si_2OH_4$ | $PO_4^{3-}$ | DIC | Alkalinity |
|---|---|---|---|---|---|---|
| Low Flow Regime | ▼ | n/c | ▲(n/s) | ▼ | ▼ | ▼ |
| High Flow Regime** | ▲ | n/c | ▼ | ▼ | n/c | n/c |

**Table 3.5.3- Treatment Effects on Fluxes**. p-values for fixed effects from the ANCOVA and for organism treatment comparisons of low flow flux data. Asterisks denote p-values obtained using permutation tests as explained in the text. Treatment abbreviations as follows: A= Arenicolid, AM= Arenicolid & Mimic, C= Control, and M= Mimic. Bold values indicate significance at $\alpha = 0.05$.

| **Fixed Effects** | *$NH_4^+$ | $NO_3^-$ | $Si(OH)_4$ | *$PO_4^{3-}$ | *DIC | Alk |
|---|---|---|---|---|---|---|
| *Treatment* | **0.0001** | 0.2946 | 0.3641 | **0.0001** | **0.0054** | **0.0007** |
| *Time* | **0.0001** | 0.6581 | **<0.0001** | **0.0110** | 0.3397 | 0.4319 |
| *Treatment x Time* | **0.0103** | 0.0744 | 0.2010 | **0.0009** | 0.3447 | **0.0066** |
| **Treatment Diffs.** | | | | | | |
| *A* ***vs.*** *AM* | 0.1201 | 0.7140 | 0.3646 | 0.1126 | **0.0322** | 0.1253 |
| *A* ***vs.*** *C* | **0.0001** | 0.9726 | 0.2476 | **0.0001** | **0.0001** | **0.0001** |
| *A* ***vs.*** *M* | **0.0001** | 0.9976 | 0.2103 | **0.0001** | **0.0001** | **<0.0001** |
| *AM* ***vs.*** *C* | **0.0001** | 0.6855 | 0.0878 | **0.0002** | **0.0079** | **0.0390** |
| *AM* ***vs.*** *M* | **0.0001** | 0.5974 | 0.0885 | **0.0001** | **0.0007** | **0.0128** |
| *C* ***vs.*** *M* | 0.9398 | 0.9855 | 0.9775 | 0.4554 | 0.1883 | 0.9990 |

**Table 3.5.4- Treatment Effects on Stoichiometry**. p-values for fixed effects from the ANCOVA and for organism treatment comparisons of stoichiometric molar distances (d) for the low flow regime only. Treatment abbreviations as follows: A= Arenicolid, AM= Arenicolid & Mimic, C= Control, and M= Mimic. Bold values indicate significance at $\alpha = 0.05$.

| **ANOVA Fixed Effects** | C:N | Si:N | N:P | C:P |
|---|---|---|---|---|
| *Treatment* | 0.1082 | **0.0001** | **0.0001** | **0.0001** |
| *Time* | **0.0076** | **0.0113** | 0.2978 | 0.0751 |
| *Treatment x Time* | 0.4337 | **0.0050** | **0.0292** | 0.6167 |
| **Treatment Comparisons** | | | | |
| *A* ***vs.*** *AM* | 0.1370 | **0.0491** | **0.0197** | **0.0204** |
| *A* ***vs.*** *C* | 0.0730 | **0.0001** | **0.0001** | **0.0001** |
| *A* ***vs.*** *M* | **0.0001** | **0.0001** | **0.0001** | **0.0001** |
| *AM* ***vs.*** *C* | 0.8646 | **0.0001** | **0.0001** | **0.0001** |
| *AM* ***vs.*** *M* | 0.4856 | **0.0001** | **0.0001** | **0.0001** |
| *C* ***vs.*** *M* | 0.2323 | 0.8563 | 0.7030 | 0.2910 |

**Table 3.5.5.- Comparison of Flux Measurements**. Measured flux values for various solutes from permeable sediment systems, and diffusion dominated systems. The values from Asmus et al. 2000 (subtidal) are compiled from nine studies of various systems along the Atlantic coast of the U.S.A. and are all subtidal with depths less than 10 m. The Asmus et al. (2000) tidal flat values are compiled from six studies in Europe. All flux values are in mmol $m^{-2}$ $d^{-1}$.

| Permeable Sediment | Habitat | $NH_4^+$ | $NO_3^-$ | $Si(OH)_4$ | $PO_4^{3-}$ | TCO2/DIC |
|---|---|---|---|---|---|---|
| D'Andrea et al. 2002 | Tidal Flat | ~3 to 7 | - | - | - | 20 to 140 |
| Billerbeck et al. 2006 | Tidal Flat | 1.10 to 7.60 | - | 0.14 to 1.70 | 0.28 to 2.50 | - |
| Cook et al. 2007 | Tidal Flat | - | - | - | - | 32 to 120 |
| Billerbeck et al. 2007 | Tidal Flat (coarse) | ~0 | ~-0.15 | ~-0.05 | -0.005 | -2.64 to 0.83 |
| Billerbeck et al. 2007 | Tidal Flat (fine) | -0.02 to 0.90 | ~-0.10 | 0.10 to 0.18 | 0.08 | -1.49 to 5.50 |
| This Study (low flow) | Tidal Flat | 0.35 to 4.02 | -0.40 to -0.46 | -0.33 to 0.07 | 0.01 to 1.05 | 32.47-64.00 |
| Marinelli et al. 1998 | Continental Shelf | -0.33 to 1.42 | - | -0.14 to 6.50 | - | - |
| Ehrenhauss et al. 2004 | Continental Shelf | -0.33 to 0.54 | -0.20 to 1.16 | 0.22 to 0.30 | 0.00 to 0.06 | - |
| Jahnke et al. 2005 | Continental Shelf | 2.45 | - | 0.62 | - | 17.7 |
| **Diffusive Sediment** | | | | | | |
| from Asmus et al 2000 | Shallow Subtidal | -0.13 to 1.58 | -0.67 to 0.25 | 0.04 to 1.04 | -0.03 to 0.29 | - |
| Cook et al. 2004 | Shallow Subtidal | 0.10 | -0.04 | - | - | 20-180 |
| Serpa et al. 2007 | Shallow Subtidal | 0.10 to 0.25 | - | - | 0.003 to 0.01 | - |
| from Asmus et al 2000 | Tidal Flat | -0.29 to 0.37 | -0.49 to 0.36 | -2.64 to 1.49 | -0.01 to 0.04 | - |
| Serpa et al. 2007 | Tidal Flat | 0.50 to 1.00 | - | - | 0.02 to 0.08 | - |
| Billerbeck et al. 2007 | Tidal Flat | -0.01 to 0.60 | -0.15 to 0.10 | 0.20 to 0.40 | 0.05 | 2.67 to 3.35 |
| Murray et al. 2006 | Sand | ~7.20 | ~1.44 | - | ~2.88 | - |
| | Mud | ~7.80 | ~2.16 | - | ~0.96 | - |
| | Marsh | ~6.60 | ~4.08 | - | ~0.72 | - |

**Table 3.5.6- Advection and Bioirigation Rates**. Estimates of advective and bioirrigation effects from: **A.** Field studies of advective porewater exchange scaled to a modeled arenicolid areal domain of 400 $cm^2$ from (Meysman et al., 2006), **B.** Laboratory and modeling studies of arenicolid irrigation rates, and **C.** Estimates from this study of the low and high flow rates and arenicolid pumping based on 5 worms per microcosm pumping at 1 ml $min^{-1}$ $worm^{-1}$, scaled to the areal domain (the microcosms were ~346 $cm^2$). It is important to note, in the field, often several worms may occupy the modeled areal domain of 400 $cm^2$.

| **A. Physically Driven Advection** | **Advection Rate (ml $min^{-1}$ $domain^{-1}$)** |
|---|---|
| Billerbeck et al. 2006 (North Sea, intertidal) | ~0.1 |
| Le Hir et al. 2000 (Humber Estuary, intertidal) | 0.6 |
| De Beer et al. 2005 (Wadden Sea, intertidal) | 4.4 to 13.9 |
| Reimers et al. 2004 (Atlantic Bight, shelf) | 6.9 to 38.8 |
| **B. Arenicolid Pumping Rates** | **Bioirrigation Rate (ml $min^{-1}$ $worm^{-1}$)** |
| Timmermann et al. 2002 | 0.18 to 1.02 |
| Meysman et al. 2006 | 0.30 to 1.30 |
| Riisgård et al. 1996 | 1.5 |
| Wells 1949 | 4.5 |
| **C. Estimates from Microcosms** | **Microcosm Rates (ml $min^{-1}$ $domain^{-1}$)** |
| Advective flow (Low or High flow) | 0.6 or 5.8 |
| Bioirrigation (5 worms at 1ml $min^{-1}$) | 5.0 |

**Table 3.5.7- Comparison of Stoichiometric Ratios**. Comparison of nitrogen, phosphorous, stoichiometric ratios, and d-values for several different marine ecosystems. Nitrogen and phosphorous concentrations are in μmol $L^{-1}$ and ranked from lowest to highest N:P ratio. Note that d-values vary considerably from ratios. Nitrogen and Phosphorous data from Boynton et al. 1982.

| System | N | P | N:P | d |
|---|---|---|---|---|
| Roskeeda Bay, Ireland | 0.40 | 2.20 | 0.18 | -2.17 |
| Pamlico River, NC | 1.50 | 8.00 | 0.19 | -7.89 |
| Narrangansett Bay, RI | 0.60 | 1.60 | 0.38 | -1.56 |
| Upper Chesapeake, MD | 5.00 | 6.00 | 0.83 | -5.68 |
| Beaufort Sound, NC | 0.50 | 0.50 | 1.00 | -0.47 |
| Bedford Basin, Nova Scotia | 0.60 | 0.50 | 1.20 | -0.46 |
| Chincoteague Bay, MD | 3.20 | 2.50 | 1.28 | -2.30 |
| Peconic Bay, NY | 1.90 | 1.30 | 1.46 | -1.18 |
| W. Wadden Sea, Netherlands | 3.00 | 2.00 | 1.50 | -1.81 |
| E. Wadden Sea, Netherlands | 4.00 | 2.50 | 1.60 | -2.25 |
| Mid-Patuxent River, MD | 4.20 | 2.30 | 1.83 | -2.03 |
| S.E. Kaneohe Bay, HI | 1.00 | 0.50 | 2.00 | -0.44 |
| St. Margarets Bay, Nova Scotia | 1.10 | 0.50 | 2.20 | -0.43 |
| Cen. Kaneohe Bay, HI | 0.80 | 0.30 | 2.67 | -0.25 |
| Long Island Sound, NY | 1.50 | 0.50 | 3.00 | -0.41 |
| Up-Patuxent River, MD | 10.00 | 2.00 | 5.00 | -1.37 |
| L. San Francisco Bay, CA | 20.60 | 3.80 | 5.42 | -2.51 |
| Barataria Bay, LA | 4.60 | 0.80 | 5.75 | -0.51 |
| U. San Francisco Bay, CA | 11.50 | 2.00 | 5.75 | -1.28 |
| Victoria Harbor, British Columbi | 11.50 | 2.00 | 5.75 | -1.28 |
| Mid-Chesapeake Bay, MD | 4.50 | 0.60 | 7.50 | -0.32 |
| Baltic Sea | 1.30 | 0.10 | 13.00 | -0.02 |
| Loch Etive, Scotland | 1.10 | 0.06 | 18.33 | 0.01 |
| Vostok Bay, Russia | 1.00 | 0.05 | 20.00 | 0.01 |
| Duwwamish River, WA | 60.00 | 3.00 | 20.00 | 0.75 |
| Hudson River, NY | 5.00 | 0.16 | 31.25 | 0.15 |
| High Venice Lagoon, Italy | 2.40 | 0.05 | 48.00 | 0.10 |
| Apalachiocola Bay, FL | 10.00 | 0.10 | 100.00 | 0.52 |

## *3.6 Figures*

**Figure 3.6.1- Illustration of Microcosm Setup**. Cartoon of the experimental flow-through microcosm system. Letters denote the following: (A) overlying water bath, (B) microcosm and sediment column, (C) dead-space below sediment column, (D) outlet tubing, (E) screw-pinch valve used to regulate porewater flow through microcosm, (F) the effluent point of collection from the microcosms. Timed collection of effluent occurred across all 11 microcosms simultaneously while timing sample period, then each sample was weighed in order to calculate flow rate at each sampling period.

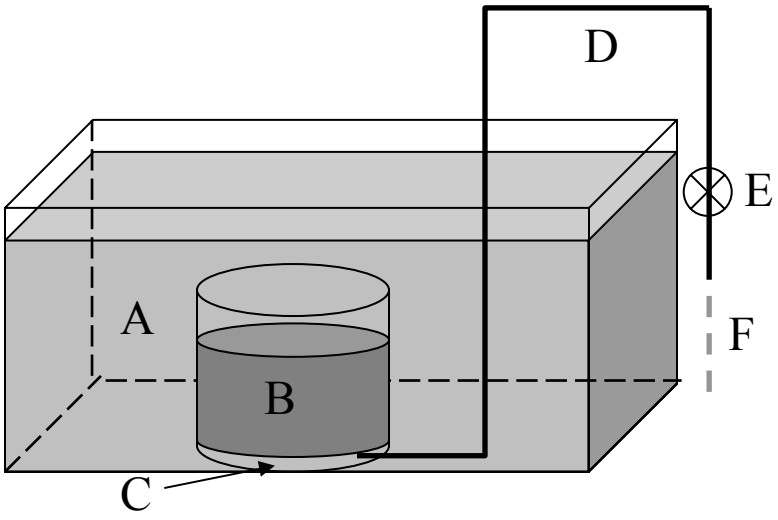


**Figure 3.6.2- Illustration of d-value Calculation**. Graphical illustration of the calculations used to determine d-values for the stoichiometric relationships in the case of C:N (not to scale). Solute concentrations are represented by the x (DIN) and y (DIC) axes. Measured concentration is indicated by $N_i$ and $C_i$, while the expected value based on known stoichiometric relationships are $N_e$ and $C_e$. The length of a perpendicular line from the observation (obs) to the theoretical line was calculated by simple geometric relationships (as shown), providing a distance with the units of mass or μmol in this case. The angle θ reflects the slope of the Redfield relationship between C and N, or any other stoichiometric relationship (a constant); therefore if a change occurs in one solute and not the other the change in the d-value will reflect the different scales of variability between the two elements/solutes.

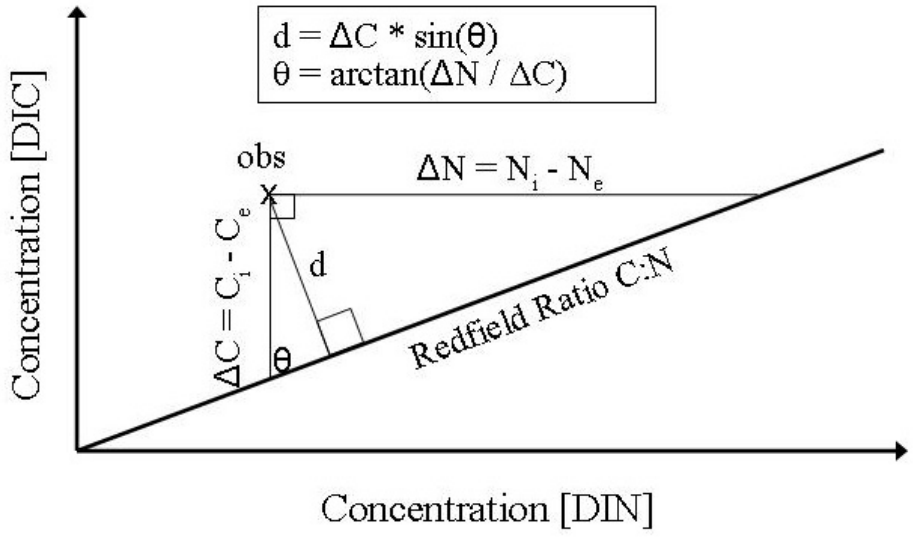


**Figure 3.6.3- Porewater Velocity in Microcosms**. The mean vertical porewater velocity as calculated from the rate of effluent discharge, surface area of the sediment within the microcosm, and porosity of the sediment. Error bars are ± one standard deviation. The light grey bars and left y-axis represent the velocities during the low flow experiments, while the dark grey and right y-axis are the high flow values. Treatment groups are along the x-axis, with A&M representing the Arenicolid and Mimic treatment. Treatment differences are indicated by different letters in the high flow regime (no differences found in the low flow treatments).

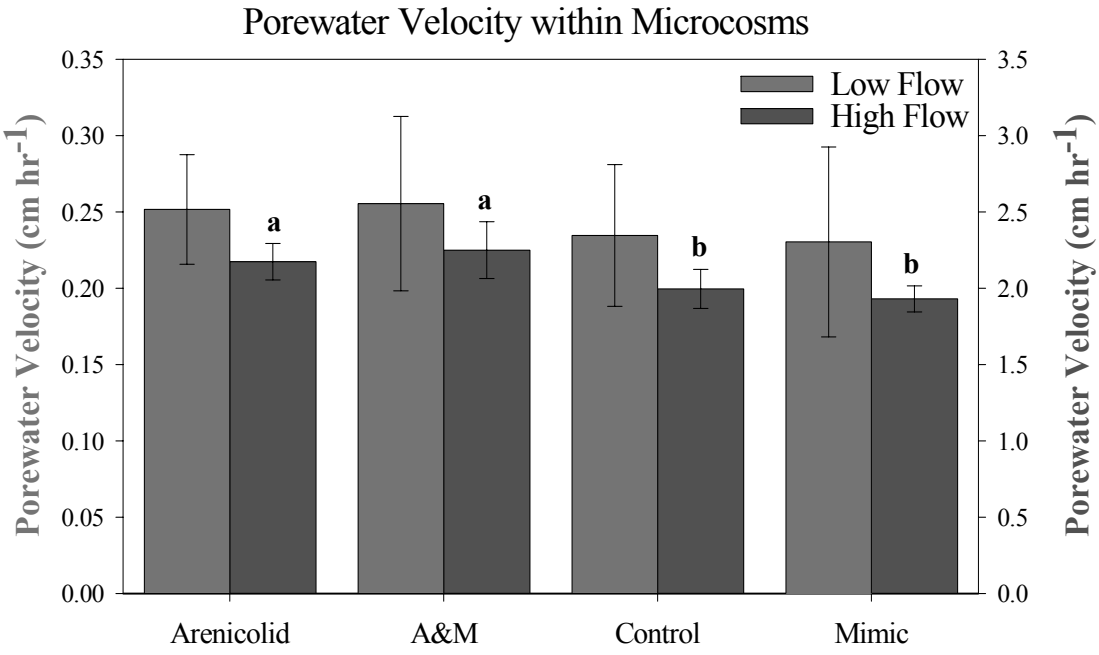


**Figure 3.6.4- Fecal Production and Tide**. Mean number of new fecal mounds per treatment at each sampling period, variance excluded for illustrative purposes. The tidal height from Friday Harbor is overlain to illustrate the coincidence of feeding activity in the laboratory microcosms, with the tidal cycle in the field. The False Bay tides are offset approximately 1 hour behind those of Friday Harbor.

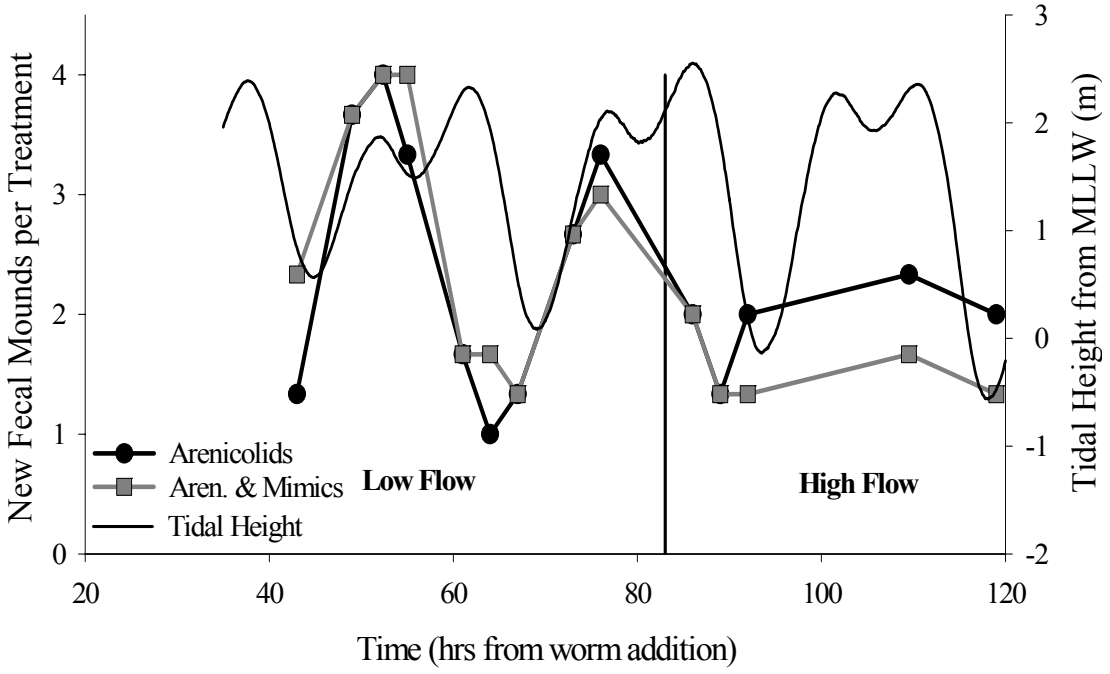


**Figure 3.6.5- Effluent Concentrations**. Time series plots of ammonium (A) and silicate (B) effluent concentrations over the course of the entire experiment for all replicates. The non-shaded/shaded sections along the x-axis represent the day/night cycle, beneath the light cycle is listed the flow regime. Changes to the flow regime are demarcated by the bold vertical lines, low flow was roughly 0.5 ml $min^{-1}$, and high flow was roughly 5.0 ml $min^{-1}$.

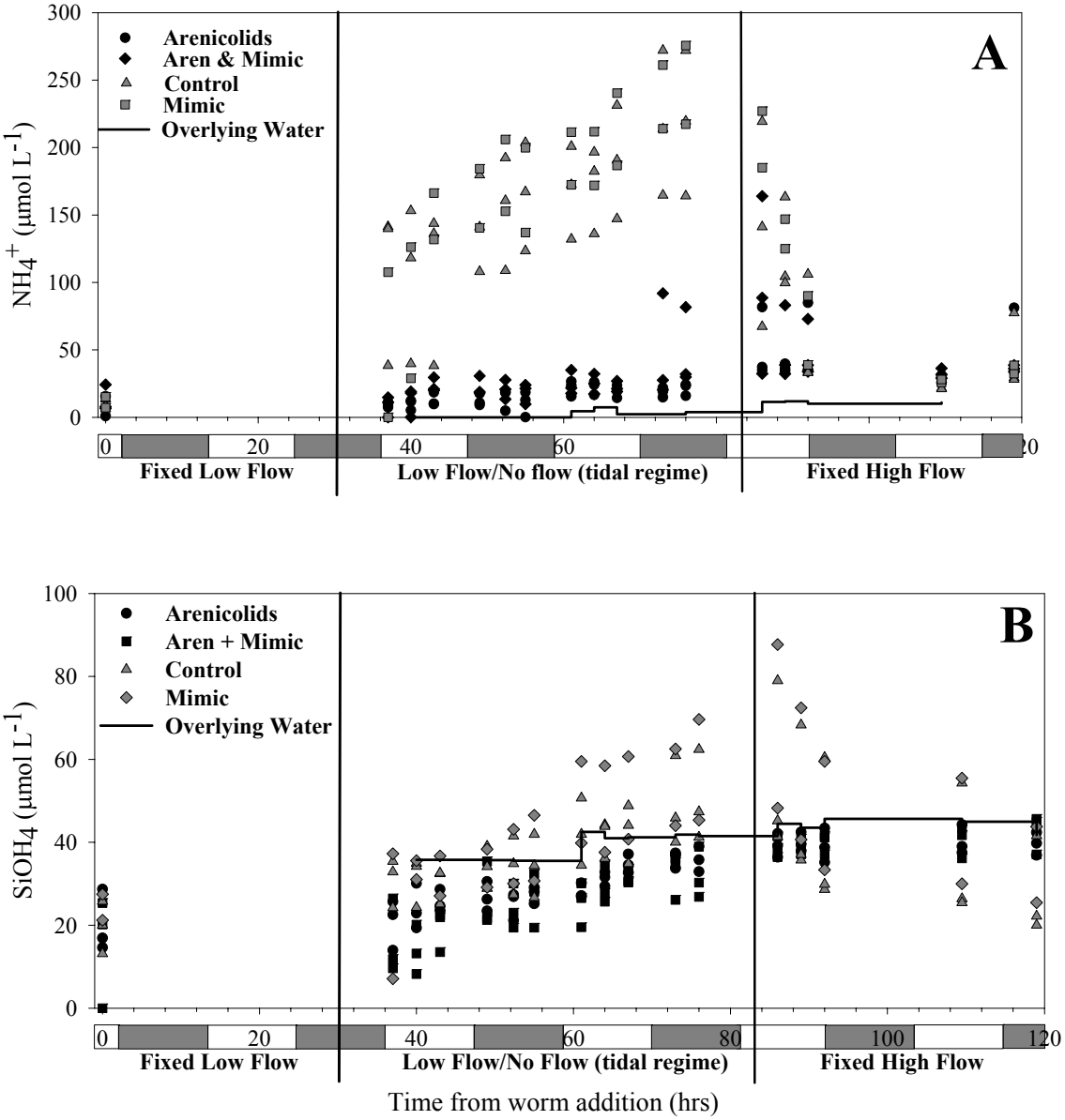


**Figure 3.6.6- Effluent Stoichiometry**. Stoichiometric plots of the low flow effluent concentrations for C:N (upper left), N:P (upper right), C:P (lower left), and Si:N (lower right). The line on each graph represents the Redfield Ratio line, with the exception of Si:N. The Si:N line represents the 1:1 line denoting the elemental ratios that promote diatom production (silica surplus), or dinoflagellete (nitrogen surplus). Note the separation of the animal (black symbols), and non-animal (grey symbols) treatments as well as the large difference in variance between these two groups. Note differences in scale among the four plots.

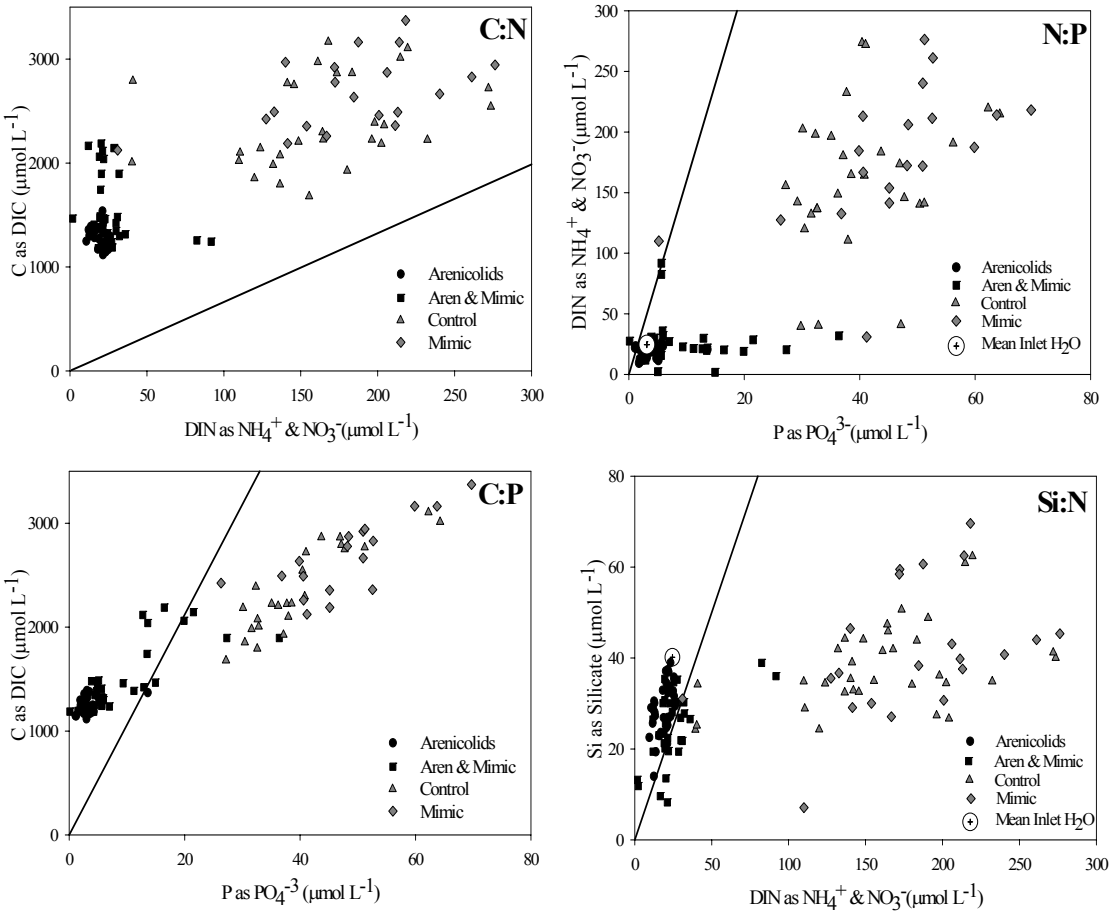


**Figure 3.6.7- Treatment effects on d-values**. Box plots of the d-values (molar distance) of observations from the theoretical ratio calculated as noted in the text and Figure 2. The lower and upper bounds of the box represent the 25th and 75th percentile of the data. The lower and upper error bars represent the 10th and 90th percentile, and dots represent values outside the 10th or 90th percentile. The median is represented by the horizontal line, and the mean by the asterisk. Values presented on the x-axis below the treatment names are the mean and (standard deviation) of the effluent ratios for comparison of stoichiometric ratios to d-values. Note differences in scale among the four plots.

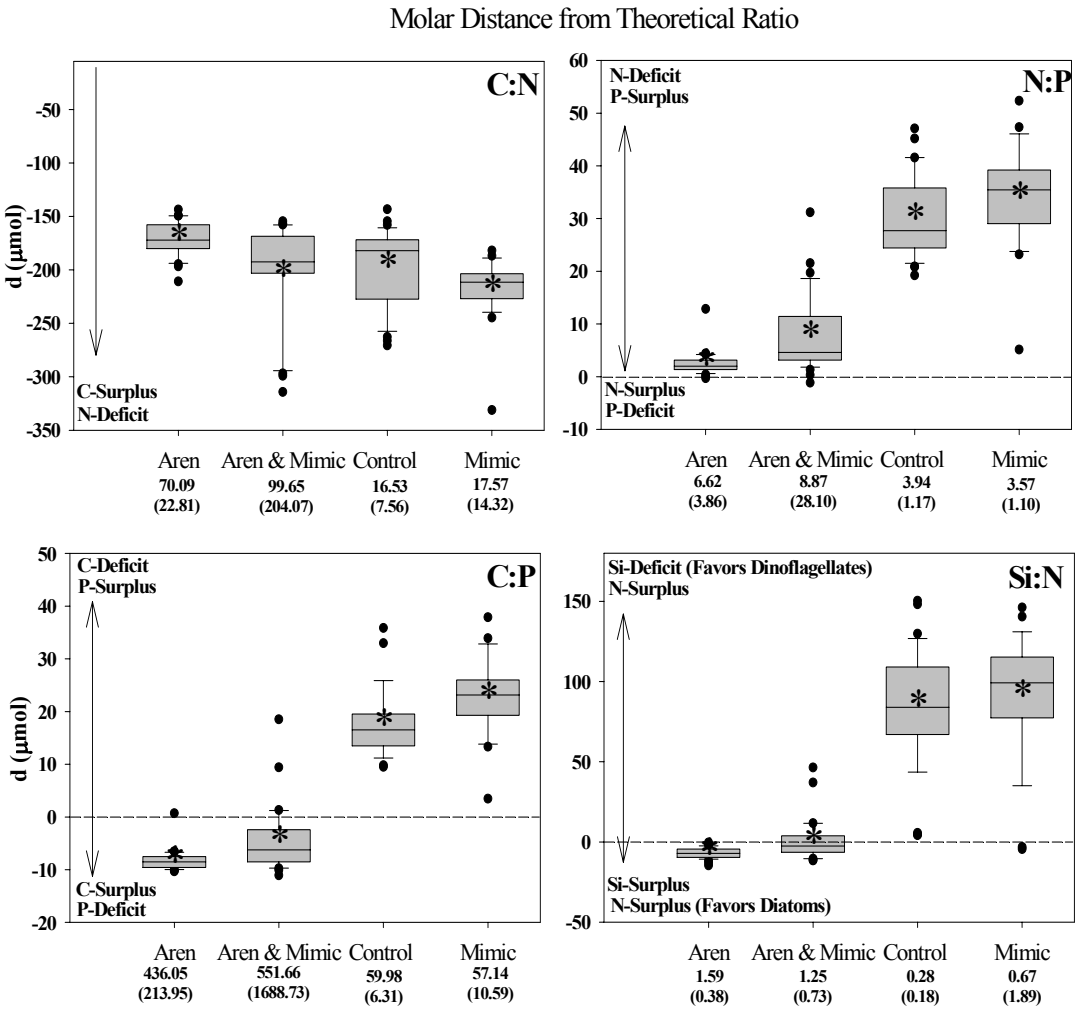
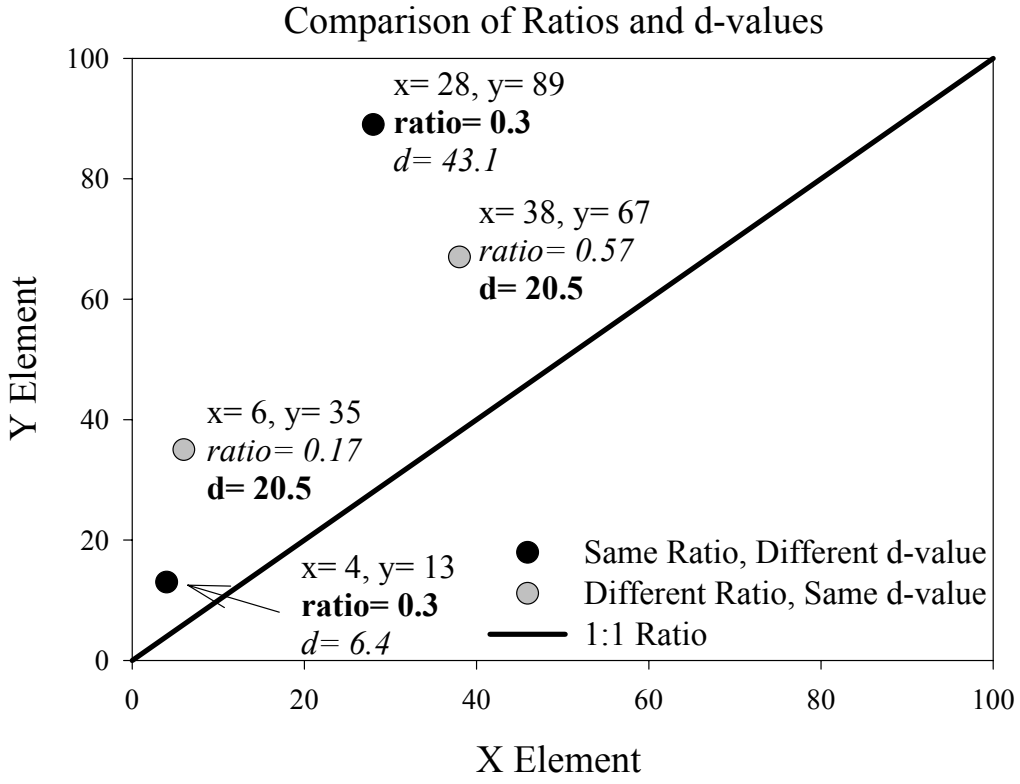


**Figure 3.6.8- Comparison of Ratios and d-values**. Selected observations from a randomly generated data set of two hypothetical elements having an empirical stoichiometric relationship of 1:1. The points in black have the same stoichiometric ratio, but different d-values, as noted on the graph. The points in grey have the same d-value, but different stoichiometric ratios. In total, the d-values capture a level of information not captured by the ratios. The d-values provide a value that more closely represents the magnitude of change required in various processes to bring an observation inline with the empirical relationship. In other words, the d-value better represents the deviation of an observation from a prescribed relationship, in real units, compared to ratios.



# Chapter 4: Infaunal Functional Groups and Important Attributes to Permeable Sediment Processes: A Multi-site, Multi-species Investigation

## *4.1 Introduction*

Intertidal and shallow subtidal coastal sediments host a diverse community of infauna, including many that alter the sediment column in complex and often non-intuitive ways. Most infaunal studies in permeable sediments have focused on species of the ubiquitous arenicolid polychaete family (e.g. Huettel et al. 1990, Timmerman et al. 2002, Meysman et al. 2006, Volkenborn et al. 2007), although a handful of studies have examined other species (Jones and Jago 1993, D'Andrea et al. 2002, 2004, Nogaro et al. 2006, Mermillod-Blondin and Rosenberg 2006, Waldbusser and Marinelli 2006). Recent modeling (Meysman et al. 2005, Timmerman et al. 2006), laboratory (Wethey and Woodin 2005), and field efforts (Volkenborn et al. 2007) involving arenicolids have advanced our understanding of bioirrigation in permeable sediments. However, using a representative bioturbator in a diverse benthic community may mask other processes that are significant to the broader ecosystem. Estuarine and coastal ecosystems are sensitive to changes in benthic processes (Torgersen et al. 1997, Meile and Van Cappellen 2003, Tyler et al. 2003, Lucea et al. 2005) and the extensive modification of sediments by infauna implies that biogeochemical processes altered by different infauna may affect system wide biogeochemistry (Waldbusser et al. 2004). Therefore, an important goal for marine sediment research is to determine the infaunal attributes that modify sediment biogeochemical processes. One approach to this complex problem is to characterize

infaunal behaviors and assign functional groups according to similar effects on sediment processes.

Applying functional groups in permeable sediments may require a different approach than traditionally used for diffusion dominated sediments. Traditional functional groupings in marine sediments have used feeding modes or guilds (Fachauld and Jumars 1979, Hutchings 1998) or particle mixing effects (Pearson 2001, Biles et al. 2002, Gerino 2003). However D'Andrea et al. (2004) found no direct effect of traditional functional groupings on particle mixing in permeable sediments. Gerino (2003) suggested using an inverse approach for functional groups: first identifying key processes, then grouping organisms based upon their impact on given processes. The physically active nature and role of porewater advection in permeable sediments makes the use of an inverse approach to functional groups in permeable sediments potentially very effective.

Within permeable sediments, there are several "key" processes that alter physical and chemical dynamics; the rapid movement and exchange of porewater is a key process that differentiates permeable from diffusive sediments. Permeable sediments permit porewater advection to occur when pressure gradients are applied that force water from high to low pressure. Currents and surface gravity waves create flow interactions with sediment surface topography that result in oscillating or constant pressure gradients capable of inducing porewater advection (Reimers et al. 2004). Larger scale pressure gradients may also drive porewater advection through tidal flats (Le Hir et al. 2000,

Billerbeck et al. 2006), submarine groundwater aquifers (Burnett et al. 2003), or beneath tidal marshes (Jahnke et al. 2003). The advection of porewater through sediments increases organic matter remineralization rates, moves particulates through the sediment, stimulates biogeochemical processes, and rapidly removes metabolites relative to diffusive sediments (e.g. Huetttel et al. 1996, 1998). The basic parameters that regulate porewater advection are the permeability of the sediment ($k > 2 \times 10^{-11}$ $m^{-2}$) and the presence of a pressure gradient. Therefore, the two primary mechanisms by which infauna may modify porewater advection (and resulting biogeochemistry) are through modifying the permeability of the sediment or pressure gradients (through pumping or structure/flow interactions) acting on the sediment.

Infaunal organisms have diverse life history strategies that include myriad behaviors and biogenic sediment structures, many of which alter sediment granulometry (e.g. Rhoads and Young 1970) or have the potential to alter or create pressure gradients (e.g. Huettel and Gust 1992, Wild et al. 2005). The scale of these infaunal effects is localized to areas immediately surrounding a burrow, feeding funnel, or other structure created through infauna activity. Many of these localized effects increase permeability and counter normal sediment compaction processes (Craig et al. 1998). Infauna may have broad scale effects if their density and activity is large enough to modify a significant proportion or critical region of the sediment column. Volkenborn et al. (2007) have shown that populations of arenicolids maintain the permeability of intertidal sediments relative to areas where the arenicolids have been excluded. Abundant smaller organisms can also have substantial effects on permeable sediments; D'Andrea et al.

(2004) found that amphipods were responsible for significant sediment mixing and creating void spaces in the presence of several larger, less abundant species. A combination of organism attributes and their density ultimately determine whether infaunal communities influence porewater advection and chemistry over broad scales, through modifying permeability and pressure gradients. Characterizing key functional attributes of infauna in permeable sediments provides a basis for predictive animal-sediment models to quantify the potential effects of benthic species loss or replacement on important coastal processes. Therefore, applying an inverse approach to functional groups (Gerino 2003) in permeable sediments, infauna may be grouped as porewater advection enhancers or inhibitors through sediment permeability modification and pressure gradient modification.

A multi-site, multi-species investigation of infaunal effects on porewater advection and biogeochemistry was initiated to examine differential effects of infauna on permeable sediments. The primary questions were: 1) What infaunal traits affect fluid transport and sediment biogeochemistry? 2) How do these traits interact with other environmental variables? To address these questions, I compared the effects of different infaunal species on porewater advection and biogeochemistry both within and across permeable sediment sites. Sediment differences across sites were evaluated to determine broader scale controls on permeable sediment processes, and couch infaunal effects in the proper context. Infaunal attributes found to affect permeable sediment processes were identified and the potential mechanisms linking attributes to transport and reaction processes were explored.

## *4.2 Methods*

### 4.2.1 Sites and Organisms

This study was conducted at three tidal flats in the U.S.A.: False Bay located on San Juan Island, Washington (lat = 48.489, lon = -123.066), Cara's Flat located in the coastal bays area of Virginia (lat = 37.591, lon = -75.616), and Debidue Flat located east of Georgetown, South Carolina (lat = 33.335, lon = -79.167). All three sites are well flushed with coastal ocean waters over daily tide cycles, consist of fine sands, and contain relatively low organic matter. Resident bioturbating benthic infauna create distinct surface features visible at low tide when each of the flats are exposed (figure 1). These large bioturbating organisms are significant habitat modifiers through their burrowing, irrigation, and feeding activities evident on the sediment surface.

*False Bay, WA, USA*- The tidal flats of False Bay are located within a semi-enclosed bay that drains fully during the lower of the semi-diurnal tides. The average daily tidal range is ~2.3 m. False Bay is adjacent to the Georgia Strait of the Pacific Northwest Passage, and is subject to strong seasonal storms during the winter months. Within False Bay, the tidal flats comprise a series of sand bars, pools, and tidal creeks creating greater topographic relief over shorter distances than the other two sites described below. Sediment ripples had a wavelength of roughly 8 cm during the time of this study. The sample area is located near the head of the bay on a homogenous shallow sand bar subject to 12 hr plus exposure times.

The arenicolid polychaete *Abarenicola pacifica* and two species of thalassinid shrimp *Upogebia pugettensis* and *Neotrypaea californiensis* are found in high densities on this flat. These two taxonomic groups will be referred to as arenicolids and thalassinids, respectively. The thalassinids are treated as one taxonomic unit, given their functional similarities including the creation of relatively impermeable burrows/galleries over large areas of intertidal and subtidal sediments(Kinoshita et al. 2005, Papaspyrou et al. 2005). They may filter or deposit feed. (Posey et al. 1991, Ziebis et al. 1996, Pinn et al. 1998). The arenicolid is an active head-down deposit-feeder that pumps overlying water through its tail shaft into the sediment fluidizing sediment in the feeding funnel (Huettel 1990, Riisgard and Banta 1998, Meysman et al. 2006). This irrigation activity allows the arenicolids to subduct and feed on fresh organic matter, resulting in localized areas of increased permeability (Jones and Jago 1993).

*Cara's Flat, VA, USA*- This flat was recently formed when a storm cut through the barrier island roughly seven years ago. The flat is adjacent to a tidal salt marsh that has been slowly encroaching across the flat from south to north (pers. comm. M. Lukenbach). A gradient in sediment characteristics exists from finer grained muddy sediments adjacent to the marsh, to sandy sediments adjacent to the small creek where the breach in the barrier island was formed. Along this gradient is also a gentle slope from the higher muddy/marsh areas to the sandy sediment closer to the creek of roughly 50 cm over 200 m in distance. The sediment surface is relatively flat over the plot scales of 0.25 $m^2$. Only a few poorly formed ripples in the sediment were found at this site, ripples that were noted in some plots were typically 1 cm in wavelength. The spring tidal range at

this location is roughly 1.5 m. The spring tides are noted here because these are the tides that coincided with sampling.

In the fine-sands area of Cara's Flat (closer to the creek than marsh), the dominant infauna are the hemichordate *Balanoglossus aurantiacus* (referred to as hemichordate) and onuphid polychaete *Diopatra cuprea* (referred to as diopatra). *B. aurantiacus* is a large deep dwelling deposit feeder that inhabits a u-shaped tube. It uses multiple feeding funnels to subduct surface material in the anterior end of the burrow to depth, and creates new feeding funnels over tidal cycles to days (Duncan 1987). Unconsolidated fecal mounds are produced at the position of their tail shaft (figure 1). The hemichordates are also known for their production of bromylphenols as chemical defense (Kicklighter et al. 2004). *Diopatra cuprea*, the onuphid polychaete commonly known as the junk worm, is a surface feeding omnivore (Myers 1972, Brenchley and Tidball 1980). It lives in a leathery parchment tube with various shell fragments, algae, and other materials glued to its burrow. The top of the tube (tube cap) extends several centimeters from the sediment surface into the overlying water. The tube cap is particularly impermeable, given the thick parchment tube and additional material glued there by the worm.

*Debidue Flat, SC, USA*- This is a well studied intertidal flat located in a shallow tidal creek network on North Island, adjacent to North Inlet (Grant 1981, D'Andrea et al. 2002). Tides are heavily influenced by the wind direction. Most of the area has fringing tidal marshes, situated between a barrier island and North Island. The upper 2-3 cm of sediment on the flat regularly migrates over a few days with the changing tides (Grant

1981). Ripples and changes to surface topography were noted during experiments, as movement of ripples is observed in consecutive daily sediment surface photographs. The surface ripples were not organized as well as False Bay in terms of long perpendicular ripples, indicating that the flow direction is probably more variable at Debidue Flat relative to False Bay. Wavelengths in sediment features were approximately 20 cm in length. The spring tidal range in this area is 1.7 m.

There are two general areas on Debidue Flat, a lower muddier area with a diverse infaunal community, and a sandier higher section dominated by the onuphid polychaete *Onuphis jenneri* (referred to as onuphis). Studies were focused on the sandier upper and mid-level portions of the flat where both onuphid polychaetes, *O. jenneri* and *Diopatra cuprea* are found. *O. jenneri* is a surface deposit feeder, smaller in size than diopatra. It builds tubes that extend several centimeters from the sediment surface; onuphis burrows are thin, flimsy, and made from sand grains embedded in a polysaccharide matrix. The diffusional characteristics of these burrows have been characterized (Aller 1983, Hannides et al. 2005) and found to be more permeable then diopatra burrows (Aller 1983).

### 4.2.2 Sampling Design

The sampling design at each site varied slightly according to site characteristics and previous measurements made, although the general methodology and comparisons within sites are similar. The experimental plots consist of 50 x 50 cm quadrants, marked by wooden dowels inserted into the sediment on the corners of each plot. The biggest difference among the three study sites is how the experimental plots were situated. In

False Bay, a block design was used to locate plots of varying densities of arenicolids and thalassinids within close (< 5 m) proximity of each other. This resulted in nine sites that were used for both the porewater advection and chemistry measurements. Plots were chosen that were dominated by arenicolids, thalassinids, or a mixture of both, and these three treatments were applied within 3 blocks, resulting in 9 total plots. The original analyses may be found in Waldbusser and Marinelli (2006). Since there were no block effects, all data were pooled and analyzed with regression (detailed below), with organism density and sediment properties as independent variables, and measures of porewater advection or solute concentrations as dependent variables. The porewater advection measurement was conducted 19 July 2004 to 21 July 2004, and porewater solutes were measured from 4 August 2004 to 10 August 2004, within the same experimental plots.

Experimental plots at Cara's Flat also consisted of three blocks and three plots within each spatially explicit block. As above, no differences in sediment characteristics were found across blocks, and therefore, data were pooled and analyzed by regression analyses. All the plots within a block at Cara's Flat were also within meters of each other, and plots were selected based on the abundance of either hemichordates or diopatra. The same plots were used for both the porewater advection and chemistry measurements. Advection measurements were made 22 July 2005 to 24 July 2005, and porewater solutes were measured 17 August 2005 to 22 August 2005.

Experimental plots at Debidue Flat were arranged in transects across the flat, with each experimental plot roughly within a meter of the next plot. The advection measurements were arranged in two different transects, one across the sandy area and one across a slightly less sandy area higher in diopatra density. Both transects had eight experimental plots. As no statistically different sediment properties were found between these two transects, the data were pooled for the regression analyses. Porewater solutes were only measured in the sandy area, with one transect of nine sites. The porewater solute and advection measurement sites were different due to the fact that the porewater solutes were measured one year after the advection measurements and the site markers would not last the winter. Porewater advection measurements were made 16 October 2005 to 18 October 2005, and porewater solutes were measured 10 July 2006 to 16 July 2006.

### 4.2.3 Organism Density

The densities of infauna within each experimental plot were estimated by replicate photographs of each plot and enumeration of surface features (tubes, fecal coils, burrow openings) associated with different infauna (Figure 1) (Krager and Woodin 1993, Widdicombe et al. 2003, Waldbusser and Marinelli 2006). A photo-quadrant, the same size as each plot (50 cm x 50 cm), was used to take daily photographs during each deployment of fluorescein gels and porewater peepers (both described below). In the case of the fluorescein gels which were only deployed for 2 days, photographs were taken the day before the deployment to ensure at least 3 days of photographs were taken. Each surface photograph was then inspected manually and distinct digital markers were placed on each type of feature. The software package Image-J was used to produce scaled maps

of the burrow features, as well as count, and record the spatial coordinates, of each feature. The counts were then averaged over the days that photographs were taken to determine average densities of each organism within each plot. More than a year after the initial image processing, several images from each site were reviewed and counts redone manually for quality control. Additionally, plots that had considerable day to day variance were re-examined. Densities were adjusted for Debidue Flat because the migration of sand ripples caused previously exposed tube caps to be flush with the sediment surface. This resulted in only a moderate increase in densities of *Onuphis jenneri* that were previously counted as "other".

### 4.2.4 Advection Measurements

The rates of porewater advection within each experimental plot were inferred by quantifying loss of a tracer from replicate gel diffusers inserted into the sediment. This method is described fully in Waldbusser and Marinelli (2006), and will be reviewed briefly here. Tracer is released to the surrounding sediment from a gel via diffusion. As advection occurs next to and around the gel, it removes the tracer that was diffused out of the gel, creating a steeper concentration gradient, and therefore larger loss of tracer from the gel. Acrylamide gel plugs made following (Browne and Zimmer 2001) and 1 mg $ml^{-1}$ of fluorescein were cast in 1.1 x 9 cm cylinders. Gels were inserted into the sediment and buried approximately 1-2 cm below the sediment surface. Within each plot 5 replicate gels were aligned perpendicular to the slope of the sediment surface. The exact spatial coordinates of the gels within the plot were recorded so that they could be retrieved with minimal sediment disruption at the end of the experimental period.

The acrylamide gels were deployed during spring tides at Cara's Flat and Debidue Flat or exceptionally good semi-diurnal tides at False Bay (typically every two weeks) for 48 hrs. After the 48 hr deployment, the gels were extracted from the experimental plots at low tide. Any remaining sediment was gently wiped from the gel surfaces. A 5 mm subsection was taken 1 cm from both the near surface and deep ends of the gel. These sub-sections were placed into pre-weighed 5 ml sample vials, weighed again, and diluted with 2.5 ml of deionized water. Any gels that were exposed on the sediment surface or had been damaged while they were deployed in the sediment were noted. During the back equilibration, all samples were kept in a 4$^{o}$ C refrigerator. Back equilibrations of the gels were conducted for 48 hrs - 5 days, and during back equilibration, sample vials were either manually agitated or kept on a shaker table. After the back equilibration period, the fluorescein concentration in the deionized water was determined on either a Turner Designs fluorometer, or an Agilent high performance liquid chromotographer (HPLC) equipped with a fluorescence detector and calibrated to at least 5 standard solutions of fluorescein. The concentration of the fluorescein in the gels ($Fluor_{gel}$) was then determined from the dilution factor of the gel volume and diluent by:

$$Fluor_{gel} = \left( \frac{Gel_V + Dil_V}{Gel_V} \right) * Fluor_{sample}$$

where $Gel_V$ is the volume of the gel section, $Dil_V$ is the volume of diluent, and $Fluor_{sample}$ is the fluorescein measured in the back equilibrated sample.

### 4.2.5 Porewater Solutes

Porewater peepers (Hesslein 1976) were used to determine time averaged porewater concentrations of ammonium (by Koroleff 1976), nitrate (by EPA 353.3), phosphate (by Eaton et al. 1995), silicate (by Strickland and Parsons 1972), alkalinity (by EPA 310.2), pH (standard potentiometric electrode), dissolved inorganic carbon (DIC), and calcite saturation state. These methods are described fully in Waldbusser and Marinelli (2006), and therefore the methodology will be reviewed briefly here. The calculation of DIC and saturation state from alkalinity and pH was conducted using the co2sys.exe program (Lewis and Wallace 1998). All chemical analyses were run on a Smartchem discrete chemical analyzer (Westco Scientific). The porewater peepers were constructed of PVC plastic and have a sampling depth range of roughly 10 cm. Each peeper had ten wells, each was roughly 0.75 cm deep, 3.2 cm wide, and 0.8 cm high, resulting in a volume of roughly 2 ml. The wells of the peeper were filled with 15% acrylamide gel polymerized with potassium persulfate, rather than ammonium persulfate to prevent ammonium contamination of samples (Engstrom and Marinelli 2005). The acrylamide gels within the peeper wells were allowed to polymerize overnight and were then placed in a sodium chloride solution of 30 ppt, in order to prevent ionic imbalance between porewater and the hydrated acrylamide.

The peepers were hydrated for 5 days prior to deployment in the field. Before deployment, peepers were affixed with 0.45 μm Magna Nylon filter paper in a shallow water bath to prevent trapped air bubbles behind the filter paper. Three replicate peepers were deployed in each experimental plot within the three sites, oriented such that the

narrow edge of the peeper was perpendicular to the relief of the flat. Peepers were deployed for 5-6 days, and daily photographs of each plot were taken during the deployment. Upon retrieval, peepers were extracted from the sediment, wiped clean of sediment, quickly placed in zip lock bags in a cooler until returned to the laboratory. Once in the lab, peepers were kept in a $4^{o}$ C refrigerator while the gels from each peeper were extracted and placed into individual pre-weighed sample vials. All gels were extracted from the peepers within 24 hrs. Gels and sample vials were weighed again, and 8 ml of deionized water was added to each sample to back equilibrate solutes within the gels for 48-72 hrs. Spot checks of salinity were conducted on the back equilibrated water to ensure that peepers had not evapo-concentrated solutes, and that the back equilibration period was sufficient to equilibrate the gels and water.

### 4.2.6 Granulometry

Individual sites were sampled differently for granulometry. Three 3 cm diameter cores were taken and combined for each experimental plot in False Bay and Debidue Flat, while separate triplicate cores for each plot were taken for Cara's Flat, with surface and deep subsections of each core. Cores were taken to a depth of roughly 10-15 cm. Sediment samples were placed in sealed containers, in the dark, and frozen within 12 hrs of collection. Porosity was measured by loss of mass by drying at $60^{o}$ C. Samples were weighed, dried for 24 hrs, re-weighed, then dried for subsequent 24 hr periods until there was no change in mass. After a stable mass measurement was made, the sample was placed on a sieve shaker for 15 min. with a distribution of five sieves from 0 to 3.5 phi and a pan. From these measurements, percent fine content is material passing the 3.5 phi

sieve. The mass of sediment remaining on each sieve was weighed and granulometric properties were determined by the methods of Folk and Ward (1957). Several representative sediment samples were sub-sampled for organic carbon and nitrogen after drying by high temperature combustion and elemental analysis (EPA #440.0 1997).

### 4.2.7 Statistical Analyses

To determine the relative roles of infauna and sediment properties on transport and reaction processes in permeable sediments, stepwise multiple regression analyses were employed. This technique can be used to eliminate correlated variables and identify multiple statistically significant independent variables acting on one dependent variable. All data were averaged within each experimental plot, and these within-plot averages were used for all regression analyses at a given site. Differences in near surface versus deep sections of the fluorescein gels were determined by analysis of variance (ANOVA). If a significant difference was found, separate regressions for the surface and deep sections of the gels were conducted. In order to determine organism effects and the role of sediment properties, organism density and sediment granulometric variables at each site were treated as independent variables in regression analyses. The response or dependent variables in the regression analyses were the fluorescein remaining in the gels (a proxy for porewater advection), or integrated porewater solute concentrations at each site.

The stepwise regression provides best-fit combinations of independent variables for one to the total number of variables in the regression. For these analyses a maximum

of five variables were put into the model for the selection process. The stepwise regression then runs all possible combinations of variables and provides the best fitting models based on user defined selection criteria. Adjusted r-squared, mean square error (MSE), and Mallow's cp index were used to identify the best possible model with fewest possible variables by maximizing adjusted r-squared and minimizing MSE and Mallows cp. Collinearity of independent variables can be a problem with this technique, whereby variability is overly reduced by fitting a model that has correlated variables. Therefore, collinearity was checked by the condition index of the independent variables (calculated by Eigen values), using 5 as a value that requires further investigation. With a condition index below 5 the variables in the model are not artificially lowering MSE because of correlated variables.

Once the best fitting independent variables were determined with stepwise regression, the regression (simple or multiple depending on selection process) with only these parameters was run, and statistical significance was evaluated for each variable in the model. If a variable chosen with the stepwise regression was not significant in the multiple regression model, then the variable was removed from the model, and the model was re-run. Natural log transformations were made on variables as needed in order to meet assumptions. Once a suitable model was determined for each regression, overly influential data points were evaluated by studentized residuals (> 2) and Cook's distance (> 1). If overly influential data points were found, these were examined, removed, and the regression was re-run without these points. If no change in the results was found due to removal of the point, it was left in the regression because the presence or absence of

the point did little to change the inference of the analysis. In some cases removing one data point created a second overly influential data point; these were not removed from subsequent analyses. No more than one data point was removed from any regression analysis run. All statistical analyses were conducted using SAS software version 8.

## *4.3 Results*

### 4.3.1 Across Site Differences

Differences in granulometry, porewater solute concentrations, and porewater advection were measured among the three sites (table 1 & 2). Differences in sediment, biogeochemistry, and physical flows are potentially important covariates with organism effects at these three muddy-sand intertidal habitats. The primary differences among sites in sediment characteristics were in grain size, percent fines, and hydraulic conductivity (calculated from porosity and grain size by Carmen-Kozeny Equation) (table 1). The larger fines content in False Bay was likely due to clay particles given the clay lens found roughly 30 cm beneath the surface sediment layer (Waldbusser and Marinelli 2006). There was no relationship between percent fines and organic carbon at False Bay, as was found at Cara's Flat (figure 2), further suggesting the high fines content at False Bay is dominated by clay rather than organic matter. Although organic matter can be bound to clay particles, the data suggests that at False Bay there is an abundance of clay relative to organic matter. The percent fines decrease by roughly an order of magnitude across each site from False Bay to Cara's Flat to Debidue Flat (table 1). Differences in grain characteristics and hydraulic conductivity would suggest that the highest overall porewater advection rate occurs at Debidue Flat, followed by Cara's Flat, then False Bay.

In fact, False Bay is subject to the highest integrated rate of porewater advection, followed by Debidue Flat, then Cara's Flat, as measured by the tracer gels (figure 3). It should be noted that the tracer gels measure an integrated porewater advection over time. Therefore, it is possible that intermittent high energy effects could alter granulometry, yet not result in a greater time averaged porewater advection rate. Differences in porewater advection across sites appears to be driven by tidal range (figure 3) while bulk sediment properties do not reflect overall physical porewater flows.

Porewater solute concentrations (of select solutes) also appear to be driven by tidal range (figure 4). The grand means of silicate, DIC, and ammonium within each site vary with site differences in tidal range, with increased tidal range increasing DIC ($r^2$ = 0.73) and ammonium ($r^2$ = 0.99) concentrations, and decreasing silicate concentrations ($r^2$ = 0.96). Additionally, grand means of saturation state ($r^2$ = 0.70), alkalinity ($r^2$ = 0.97), and pH ($r^2$ = 0.47) increase with increasing percent fines content of sediment across sites (figure 5).

### 4.3.2 Within Site Infaunal Effects

Within all three sites infaunal effects on porewater advection were found (table 3) and within two sites infaunal effects on porewater solute concentrations were found (table 4). The head-down deposit feeders at False Bay and Cara's Flat significantly decreased solute concentrations, where as the thalassinids at False Bay significantly increased solute concentrations (table 4). The onuphid polychaete *Onuphis jenneri* at Debidue Flat significantly increased porewater advection (lower fluorescein concentration in gels), yet this effect did not result in changes to porewater solute concentrations. The

taxonomically similar onuphid polychaete *Diopatra cuprea* did not have significant effects on porewater advection at Debidue Flat or Cara's Flat (table 3). Individual site and organism effects are examined in greater detail below.

*False Bay, WA*-Within False Bay, there was no significant difference in fluorescein concentrations between the surface and deep sections of the tracer gels (ANOVA $p = 0.6780$, $F_{1,16} = 0.18$). Due to significant correlation between arenicolid and thalassinid density in False Bay, separate stepwise regressions were run with arenicolid and thalassinid density in two different models inclusive of sediment variables in each. No statistically significant models of thalassinid density effects (alone and in combination with sediment variables) on fluorescein were found. For the arenicolid stepwise regression a two parameter model with arenicolid density and grain size was found to be the best model variability in fluorescein gel concentrations ($p = 0.0043$, $F_{2,8} = 15.51$, adj $R^2 = 0.78$). The results from the two parameter model indicate that increasing arenicolid density decreases fluorescein concentrations (increasing advection) (table 3, figure 6) and increasing grain size increases fluorescein concentrations (decreasing advection). The range in grain size values was roughly 30 μm, resulting in a coefficient of variance (CV) of 0.04%. The relationship between increasing grain size and increasing fluorescein concentration indicates that a larger grain size is decreasing porewater advection.

Statistically significant effects of both arenicolid and thalassinid density on porewater solute concentrations were detected with simple linear regression. No significant differences were detected in sediment parameters in the original analyses of

this data set (Waldbusser and Marinelli 2006), and variance in sediment parameters were very small (Table 1). Therefore, simple linear regressions were run for each organism density separately on each solute, resulting in separate analyses of arenicolid and thalassinid densities on porewater solute concentrations. For both groups of regressions, concentration data were natural log transformed. Arenicolid density significantly reduced porewater concentrations of: ammonium ($p = 0.0179$, $t_{1,8} = 3.08$, adj $R^2 = 0.51$), phosphate ($p = 0.0078$, $t_{1,8} = 3.69$, adj $R^2 = 0.61$), silicate ($p = 0.0095$, $t_{1,8} = 3.54$, adj $R^2 = 0.59$), alkalinity ($p = 0.0475$, $t_{1,8} = 2.49$, adj $R^2 = 0.37$), and DIC ($p = 0.0222$, $t_{1,8} = 2.92$, adj $R^2 = 0.49$). Thalassinid density significantly increased porewater concentrations of: ammonium ($p = 0.0284$, $t_{1,8} = 2.75$, adj $R^2 = 0.45$), phosphate ($p = 0.0013$, $t_{1,7} = 5.68$, adj $R^2 = 0.82$), silicate ($p = 0.0116$, $t_{1,7} = 3.58$, adj $R^2 = 0.63$), alkalinity ($p = 0.0004$, $t_{1,7} = 7.21$, adj $R^2 = 0.88$), DIC ($p = 0.0001$, $t_{1,7} = 2.92$, adj $R^2 = 0.92$), Ωaragonite ($p = 0.0290$, $t_{1,7} = 2.86$, adj $R^2 = 0.51$), and Ωcalcite ($p = 0.0290$, $t_{1,7} = 2.86$, adj $R^2 = 0.51$). The denominator degrees of freedom are lower in several of the thalassinid regressions because an overly influential data point was found and removed. As noted previously (Waldbusser and Marinelli 2006), the densities of arenicolids and thalassinids in False Bay are negatively correlated, so differential organism effects on porewater chemistry are suggestive. Overall, the results show differential infaunal influence on solutes that are subject to different formation and uptake processes in permeable sediments.

*Cara's Flat, VA*- Fluorescein concentrations were significantly lower in deep sections of the gels relative to surface gel sections (ANOVA $p = 0.0002$, $F_{1,17} = 23.56$), indicating increased rates of porewater advection ~10 cm deep in the flat relative to near the

sediment surface. The difference in deep fluorescein concentrations relative to near surface indicated separate regression analyses should be run for organism and sediment effects on surface and deep gel fluorescein concentrations. The stepwise regression for near surface gel fluorescein concentrations best fit a two parameter model with percent fines and hemichordate density, however it was not significant. A simple linear regression of hemichordate density on near-surface fluorescein concentrations was not significant either ($p = 0.2528$, $F_{1,8} = 1.55$, Adj. $R^2 = 0.06$). The stepwise regression for fluorescein remaining in the deep gel sections found that a three parameter model including percent fines, hemichordate and diopatra densities was the best at explaining variance in deep gel fluorescein concentrations. When running the three parameter model for the deep section of the gels, only hemichordate density was significant in explaining variance in the fluorescein remaining in the gels. A simple linear regression was run with hemichordate density on deep fluorescein gel concentrations and was significant ($p = 0.0080$, $F_{1,8} = 13.42$, Adj. $R^2 = 0.61$) (figure 6). These results indicate that hemichordates had a significant effect on increasing porewater advection at depth, no effect on near sediment surface advection, and these effects were independent of sediment characteristics. No significant effects of diopatra density on fluorescein gel concentrations in deep or near-surface gel sections were found. It should also be noted that maximum densities of hemichordate and diopatra in Cara's Flat (~15 and 16 per $m^2$, respectively) were much lower than arenicolid and thalassinid (~ 75 and 60 per $m^2$, respectively) densities in False Bay.

The stepwise regressions for porewater solutes identified a two parameter model with hemichordate and percent fines as the best fitting model explaining variance in solute concentrations. Solute concentrations of nitrate ($p = 0.0462$, $F_{2,7} = 6.05$, Adj. $R^2 = 0.59$), phosphate ($p = 0.0010$, $F_{1,7} = 35.86$, Adj. $R^2 = 0.83$), and silicate ($p = 0.0072$, $F_{1,7} = 15.97$, Adj. $R^2 = 0.68$) were significantly affected by hemichordate density and percent fines. Percent fines was not significant in the two parameter model explaining variance in phosphate and silicate concentrations. Therefore percent fines was dropped for subsequent analyses, and a simple linear regression was run for hemichordate density effects on phosphate and silicate porewater concentrations. Hemichordate density significantly decreased phosphate and silicate concentrations (figure 7). In the significant two-parameter model explaining variability in nitrate, nitrate concentrations decreased with increasing hemichordate density ($p = 0.0183$, $t_{1,7} = 3.45$) and increased with increasing percent fines ($p = 0.0494$, $t_{1,7} = 2.58$). In the significant models for phosphate, silicate, and nitrate, there was an overly influential data point as determined by studentized residuals and Cook's distance. This data point was therefore removed. The final degrees of freedom for all F and t-tests reflect this above.

*Debidue Flat, SC*- Fluorescein concentrations were significantly greater in the near-surface sections of the gels than the deep sections by one-way ANOVA ($p = 0.0045$, $F_{1,31} = 9.43$), as was seen in Cara's Flat. Therefore, separate stepwise regressions were run for the surface and deep sub-section fluorescein concentrations of the gels. The stepwise regression determined that a two parameter model with onuphis density and porosity was the best for explaining variance in the amount of fluorescein remaining the gels. A

significant model was fit for both the near surface gels ($p = 0.0001$, $F_{2,15} = 19.00$, adj $R^2 = 0.71$) and the deep gels ($p = 0.0090$, $F_{2,15} = 6.92$, adj $R^2 = 0.44$), though only porosity was statistically significant in the deep gel section model. In the near-surface fluorescein gels model, both onuphis density and porosity were highly significant, but it is important to note that the variance in porosity was quite small, with a range of 0.42 to 0.45. This would indicate that although the porosity is statistically significant, it probably is not environmentally relevant in terms of a degree of change that would actually impact porewater transport. The porosity term was left in the model due to the statistical findings. More interesting was that the onuphis density had a significant effect on fluorescein remaining in the near surface gels (or porewater transport).

The porewater chemistry study at Debidue Flat was restricted to the sandier upper section of the flat (dominated by onuphis) due to the significant effect of onuphis on porewater advection determined by the fluorescein gels. The porewater chemistry study was conducted the season after the fluorescein study, and therefore, the same sites could not be maintained throughout the winter. No sediment granulometry samples were taken during the porewater study because the samples from the previous season indicated very little variability in any of the values (Table 1). A simple linear regression of onuphis density on porewater solute concentrations found no significant effects of density on any of the solutes measured (ammonium, nitrate, phosphate, silicate, DIC, pH, alkalinity, or saturation state).

## *4.4 Discussion*

The results from this multi-species, multi-site investigation illustrate that different attributes of infauna are important to permeable sediment processes. The total number of species utilized in this study was small, but nevertheless provides a comparative context. Within the current study, natural variability of infaunal density across the scale of meters was used as an explanatory variable for within site differences in porewater advection and solute concentrations. Using an undisturbed sedimentary habitat and naturally occurring infaunal densities is a powerful approach to examine infaunal effects on permeable sediment function, though controlling for variability in sediment parameters is difficult. In spite of this difficulty, statistically significant effects of infauna on porewater advection were measured at three sites (table 3), and porewater chemistry at two sites (table 4). In most cases the within-site variability in sediment parameters is very small (table 1), and not likely to be environmentally relevant. These results suggest that certain functional characteristics may have similar effects on permeable sediment processes, and therefore, it may be possible to define new functional groups that link infaunal attributes, porewater advection, and solute dynamics.

### 4.4.1 Functional Groups and Infaunal Attributes

Functional characteristics or groups have long been used to find similarities in benthic infauna (e.g. Fauchald and Jumars 1979, Hutchings 1998, Pearson 2001) that can help explain interactions (Woodin 1976) as well as processes. Recent work has focused on the link between functional groups and ecosystem processes in sediments (Biles et al. 2002, Gerino et al. 2003, Widdicombe and Austen 2003, Michaud et al. 2005, Caliman et al. 2007, Norling et al. 2007). In muddy diffusion dominated sediments, traditional

feeding guild functional groups or bioturbator groups would classify the study organisms as: 1) head-down deposit feeders/conveyer belt (arenicolid and hemichordate), 2) surface deposit feeders/inverse conveyer belt (diopatra and onuphis), and 3) gallery diffuser (thalassinids). The results from this study only partially support these groupings. In particular the two head-down deposit feeders have strong similarities, while the surface deposit feeders have different effects (tables 3&4). Therefore, infauna in the current study could be tentatively grouped as: 1) advection enhancers by permeability and pressure gradient modification (arenicolids and hemichordates), 2) advection enhancers by pressure gradient modification (onuphis), and 3) advection inhibitors by permeability modification (thalassinids and potentially diopatra). Although diopatra has similar feeding strategies to onuphis, the lower permeability of its tubes may classify it as an advection inhibitor. The groupings outlined here should not replace current functional groups, but rather help to refine groups for their specific effects on permeable sediments. The broader applicability of these functional groups and possible basis for a predictive framework may be examined by identifying the mechanisms linking infaunal attributes to permeable sediment processes.

The head-down deposit feeders (advection enhancers) feed by subducting surface material in a feeding funnel, translocating sediment particles vertically on non-local scales between the sediment surface and deeper within the sediment. Ingestion of surface sediment by head-down deposit feeders requires significant pumping of overlying water through the sediment to subduct surface material for high ingestion rates (Timmerman et al. 2002, 2006, Meysman et al. 2005, 2006). This behavior is found in other head-down

feeders with dead-end burrows (such as maldanids), or relatively unconsolidated anterior burrow openings (such as hemichordates). Subduction and mixing of sediment decreases sediment compaction and increases the permeability of sediment on localized spatial scales in the burrow/feeding funnel/fecal mound (Jones and Jago 1993, Craig et al. 1998, Wild et al. 2005). Localized increases in permeability would result in localized increases in porewater advection under the same pressure gradient compared to other non-mixed sediments. Additionally, dense populations of arenicolids have been shown to strip organic rich fine material (Wild et al. 2005, Volkenborn et al. 2007), and create an "open bed" for advective exchange. The maximum population sediment ingestion rates for the organisms in this study are roughly 0.35 L sediment per $m^2$ per day for the arenicolids in False Bay (estimated from Linton and Taghon 2000), and almost 2.0 L of sediment per $m^2$ per day for the hemichordates in Cara's Flat (estimated from Duncan 1981, Thayer 1983). These calculations and results of this study suggest that extensive sediment gut passage, direct irrigation associated with feeding, and mixing of sediment are important mechanisms by which the head-down deposit feeders increase sediment permeability, and therefore transport/reaction processes, in similar ways (tables 3&4, figures 6&7). The lack of large covariance in bulk sediment parameters and organism density by either head-down deposit feeder is surprising given the sediment turnover rates for each population. This may be due to a mismatch between fine scale sediment alteration and bulk sediment measurements. Regardless, head-down deposit feeders appear to be an important functional group to permeable sediment processes as flow enhancers.

The surface deposit feeders (advection enhancer and non-affecter) are not actively feeding at depth, nor fluidizing the sediment column in order to subduct fresh organic matter. Onuphis and diopatra do cause non-local particle transport by ingesting sediment at the sediment surface and defecating at depth. This feeding mode results in a very localized effect on particles and given the sedentary nature of both species within their tubes their bioturbation potential is small (Swift 1993). Additionally both diopatra and onuphis tube caps extend several centimeters above the sediment surface, though their burrows differ in permeability and morphology. Projecting tube caps into the overlying water interact with strong tidal flows, creating pressure gradients that may drive porewater advection within the upper sediment layers (Huettel and Gust 1992). Onuphis burrows are thin and made of mucus bound sand grains which are more permeable than the leathery tubes of diopatra based on porosity of burrow walls (Aller 1983, Hannides et al. 2005). The morphology of tube caps is quite different in these taxonomically similar species. Onuphis tube caps are oriented vertically whereas diopatra tube caps are usually curved over and down towards the sediment surface (figure 1), with significant attachment of algae and other debris restricting the opening. Although the bioturbation potential of this group is small, the interaction of burrows and tube caps with overlying water currents is another mechanism by which these tube building surface deposit-feeders may affect permeable sediment processes by modifying pressure gradients.

The porewater advection enhancer onuphis increasing porewater flow in the upper sediment of Debidue Flat was the only measurable effect of the surface deposit feeders (table 3). Lack of porewater solute effect may be due to the lower reactive material at

Debidue Flat (table 1), and highlights the potential for context dependency of infaunal effects across sites. Onuphis increased porewater advection may be due to direct irrigation of their burrows, their higher density (than diopatra), and more permeable burrow walls. It is also possible that passive irrigation (Ray and Aller 1985, Libelo et al. 1994, Munksby et al. 2002) of these burrows is responsible for increased porewater advection. Passive irrigation can exceed active ventilation rates of even intense irrigators (Libelo 1994). Any infaunal tube-building species having burrow openings above a permeable sediment surface has the potential to create an "open bed" due to pressure gradients generated by the Bernoulli effect (Libelo et al. 1994). Infaunal tubes above the sediment surface have different orientations (figure 1). Difference in tube cap morphology could be a mechanism for the onuphis effect relative to diopatra on porewater advection, though density and differences in tube composition are likely to play a role also. More work is needed to examine tube effects on passive irrigation, but evidence suggests that tube building species with vertically oriented tube caps and dead ended burrows may be advection enhancers due to passive irrigation.

Results from this study indicate that thalassinids are advection inhibitors due to their effects on solutes such as silicate and phosphate (table 4). Both silicate and phosphate concentrations in porewater are sensitive to mixing with overlying water, and therefore are sensitive to changes in porewater advection/exchange (discussed further below). The presence of impermeable burrows within the sediment column should act to decrease porewater advection by creating obstacles that impede flow, lowering permeability. Galleries of thalassinids create large open spaces beneath the sediment

surface and could act as channels for porewater or overlying water flow. The impermeable nature of the burrow linings suggests that there is little advective exchange across burrow walls, while overlying water may drain through galleries during low tide. Waldbusser and Marinelli (2006) suggest that thalassinid burrows in the upper sediment column act as impermeable barriers to porewater flow and irrigation by arenicolids. Therefore, obstacle building thalassinids, species that create impermeable tubes, and other permeability lowering species such as suspension feeders (through biodeposition) represent a third group or mechanism by which infauna may affect permeable sediment processes; through decreasing sediment permeability. Little evidence of porewater flow inhibitors in permeable sediments has been found experimentally or otherwise, and this relatively unexplored theme is potentially an important infaunal effect on permeable sediments.

An important question is whether changes in transport (table 3, figure 6) have measureable effects on porewater chemistry and sediment-seawater exchange. Phosphate and silicate are sensitive to irrigation and play an important role in coastal biogeochemical cycles. Active ventilation of burrows can maintain silicate porewater concentrations below equilibrium values (Marinelli 1992, 1994), and within permeable sediments silica is typically more undersaturated relative to diffusion dominated sediments (Ehrenhauss and Huettel 2004). The advective flow enhancers (with the exception of onuphis) lowered porewater silicate concentrations, while the flow inhibitor thalassinid increased porewater silicate (table 4, figure 7). The difference between the onuphis and other flow enhancers is probably linked to differences among sites in

reactive organic material and fines content (table 1), and differences in how each species interacts with the sediment resulting in changes to sediment properties.

Phosphate is sensitive to irrigation as silicate is, and should respond similarly to infaunal modification of porewater advection. Variability of porewater phosphate is driven in large part by the interaction of phosphate, porewater oxygen, and minerals (Sundby et al. 1992). Oxygen causes phosphate to strongly adsorb to particles and can precipitate to form several different minerals. Infauna can reduce porewater phosphate due to increasing porewater oxygen in diffusion dominated sediments (Widdicombe and Austen 1998, Waldbusser et al. 2004, Michaud et al. 2006). The advection enhancing organisms decreased porewater phosphate (except onuphis) and the advection inhibitor (thalassinid) increased porewater phosphate (table 4, figure 7). Interestingly, the per individual effect of hemichordate relative to arenicolid on lowering phosphate concentrations is an order of magnitude greater, and nearly a factor of four greater on lowering silicate (from regression slopes in figure 7). The larger per individual impact of the hemichordate relative to the arenicolid is likely due to size dependent sediment ingestion rates (Cammen 1979) and more importantly the calculated difference in population sediment throughput above. This potential relationship between sediment throughout and equilibrium type solute concentrations suggests a possible allometric relationship linking ingestion rates of the head-down feeders and sediment biogeochemistry. Species functionality, activity, and other components of biodiversity appear to be ecologically important to biogeochemical cycles in permeable sediments, while this study also suggests these relationships are context dependent across sites.

### 4.4.2 Within Site Granulometry

Bulk sediment measurements of parameters such as porosity, percent fines, or grain size provide valuable information about the physical environment at a given site and the context in which animal-sediment interactions manifest. The fine-scale modification of sediment properties by infauna, such as sediment fluidization in arenicolid feeding funnels (e.g. Jones and Jago 1993, Craig et al. 1998), is often not captured in typical bulk grain analyses. Bulk sediment properties within some sites covary with infaunal density, though the variability in bulk grain parameters was typically small (table 1) and probably not sufficient to drive changes in transport and chemistry. Within False Bay, the arenicolid effect of increasing porewater advection was confounded by a statistically significant negative effect of increasing grain size on porewater advection (table 3). The range of values in False Bay grain size was 30 μm; theoretical work would predict a positive effect on porewater advection with increasing grain size. The arenicolids in False Bay may be causing a small loss of fines (as seen in Volkenborn et al. 2007) and increasing the mean grain size. Within Cara's Flat, the decrease in porewater nitrate with increasing hemichordate density was statistically confounded by a barely significant effect of nitrate increase with increasing fines ($p = 0.0494$, $t_{1,7} = 2.58$). Percent fines content in the flat ranged from 0.64 to 1.49%. The positive relationship between fines and nitrate may be related to increased reactive substrate (figure 2). Additionally geochemical hotspots of the hemichordate burrows and increased fines interacting with elevated flushing of the sediment due to bioirrigation may increase suitable habitat for denitrifying bacteria. However, the exact mechanisms by which hemichordates and fines content are affecting nitrate in the porewater cannot be

determined from the current study. Lastly, increases in porosity at Debidue Flat were found to decrease porewater advection (table 3) but the range of porosity measured was only 0.42 to 0.45 and again likely not relevant. Although there were some statistical effects of granulometry, the relative changes within sites in these values were very small and probably not environmentally relevant.

### 4.4.3 Across Site Granulometry

The functional group effects suggested by this data may be entangled with general across site differences, and therefore, the environmental effects cannot be fully disengaged from the differential effects of functional groups. Differences in sediment properties can vastly alter the effects of similar organisms. For example, Jones and Jago (1993) noted corophium amphipods increased permeability in sandy sediments, while Meadows and Tait (1989) found that corophium amphipods decreased permeability in muddy sediments. The primary difference among sites in this study is the two order of magnitude change in fines content from False Bay to Cara's Flat to Debidue Flat (table 1). The lowest percent fines at Debidue Flat correspond with the smallest organism effects on porewater solute concentrations (tables 3&4). However, the order of magnitude decrease in fines content at Cara's Flat does not translate into significantly lower effects of hemichordates relative to arenicolids on porewater chemistry (figure 6). Unfortunately only one species was located at two different sites, and two functional groups (as defined in the current study) located at only two sites each, making detailed statistical analyses across sites impossible. The results however still highlight important infaunal attributes to permeable sediment processes. Across site differences is one

difficulty in using undisturbed sites and unmanipulated infaunal communities in studies of animal-sediment interactions.

It is clear that different functional groups of organisms follow large-scale distributional patterns related to sediment/physical properties (e.g. Pearson and Rosenberg 1978). This relationship may be used to focus efforts on habitats where certain functional groups will be found. For example, within the study area of Debidue Flat, no head-down deposit feeders were found, likely due to the lower of fines content and organic matter (table 1). Hydraulic conductivity was also highest at Debidue Flat, but still over an order of magnitude below theoretical limits of efficient bio-pumping, $10^{-4}$ m $s^{-1}$ (Meysman et al. 2006). Therefore, the effects of head-down deposit feeders on permeable sediment processes are likely limited to a range of permeable sediment habitats having higher fines and lower hydraulic conductivity. Qualitative in nature, this result suggests that infaunal effects in physically dynamic environments are context dependent, but also limits the possible organism-habitat combinations that need to be quantified.

Broad across site patterns in porewater advection and biogeochemistry are likely effects of hydrology, local tidal regime, geology, and a suite of other regional factors. These potential drivers of local transport and biogeochemical processes are important to note in order to understand the limits to infaunal effects on sediment parameters. General across site trends in advection and solute concentrations in this study appear to be driven by tidal range (figure 3&4) and to a lesser degree by percent fines on some solutes (figure

5). Although the functional group effects are potentially blurred by the across site trend in fines content, this does not negate the findings of this study. Rather, it illustrates the importance of further studies examining these relationships in-situ, and the need to develop a more encompassing view of permeable sediment functioning to include both broad scale drivers and the integrated localized effects of infauna across plot-wide scales.

### 4.4.4 Conclusions

Traditional functional groups related to feeding mode or guild may not be adequate to capture relevant mechanisms by which infauna modify permeable sediment processes. Grouping organisms based on their effects on permeable sediment processes, in particular porewater advection, simplifies complex infaunal behavior and provides a framework for predictive models. Infauna affect permeable sediments by two primary mechanisms, modifying sediment permeability and modifying pressure gradients. An alternative functional classification scheme for infauna in permeable sediments would therefore be a two tier system of classifying first porewater enhancers and inhibitors and then the identifying mechanisms as sediment fabric modification and/or pressure gradient modification. This classification scheme is presented to help refine functional groups in permeable sediments, identify potentially important groups of infauna, provide a framework for future modeling work.

The head-down deposit feeders in this study are porewater advection enhancers that modify pressure gradients due to irrigation, and modify fine-scale sediment properties through sediment ingestion. The effects of the surface deposit feeders and gallery diffusers are variable and appear to be related to morphological differences of

biogenic structures. As flow inhibitors the thalassinids decrease permeability due to the impermeable burrows beneath the sediment surface. Advection enhancing effects of onuphis relative to diopatra are likely due to the more permeable onuphis burrows, and possibly passive irrigation of their burrows by pressure gradient modifications.

Current models of animal-sediment interactions should be expanded to capture infaunal alteration of sediment permeability and modification of pressure gradients. The differential infaunal effects on permeable sediments measured in this field study support the need for these expanded models. More empirical data are also needed so models may be parameterized to better quantify the fine-scale modification of sediment permeability and porewater advection. General site characteristics will also dictate the nature of animal-sediment interactions in permeable sediments, suggesting important context dependent relationships between infauna and sediment processes. Finally, the measured differential effects of infauna on permeable sediment processes in this study provide continuing evidence of the potentially important consequence of benthic species loss on coastal sediment biogeochemical cycling.

## *4.5 Tables*

**Table 4.5.1- Sediment Properties of Experimental Sites.** Grand means among sites of sediment characteristics. Bold values indicate characteristics that were variable across site, and numbers in parentheses indicate the number of samples from each site. *From D'Andrea et al. 2002.

| | **False Bay, WA (9)** | | **Cara's Flat, VA (54)** | | **Debidue Flat,SC (16)** | |
|---|---|---|---|---|---|---|
| | μ | ±σ | μ | ±σ | μ | ±σ |
| ***Grain (phi)*** | **2.92** | 0.12 | **2.35** | 0.10 | **2.44** | 0.03 |
| ***Grain (um)*** | **133.08** | 11.05 | **197.50** | 13.34 | **185.29** | 4.10 |
| **% Fines** | **17.44** | 4.03 | **1.12** | 0.51 | **0.17** | 0.04 |
| *Porosity* | 0.43 | 0.01 | 0.39 | 0.02 | 0.44 | 0.01 |
| ***Hydraulic Conductivity*** | **$2.43\times10^{-6}$** | **$3.14\times10^{-7}$** | **$3.25\times10^{-6}$** | **$6.75\times10^{-7}$** | **$4.69\times10^{-6}$** | **$4.31\times10^{-7}$** |
| *Sorting* | 1.82 | 0.05 | 1.39 | 0.04 | 1.38 | 0.02 |
| *Skewness* | 0.00 | 0.01 | 0.00 | 0.01 | -0.01 | 0.02 |
| *Kurtosis* | 1.08 | 0.02 | 1.10 | 0.01 | 1.09 | 0.01 |
| *Organic C* | 0.19 | 0.02 | 0.11 | 0.04 | *0.04 | n/a |
| *Organic N* | 0.02 | 0.01 | 0.01 | 0.01 | n/a | n/a |

**Table 4.5.2- Average Porewater Concentrations.** Grand means of porewater solute concentrations, averaged across all peepers and depths. All values are in mmol L-1 except for alkalinity in meq L-1, and saturation state and pH are dimensionless.

| | False Bay, WA | | Cara's Flat, VA | | Debidue Flat, SC | |
|---|---|---|---|---|---|---|
| | μ | ±σ | μ | ±σ | μ | ±σ |
| ***Ammonium*** | 313.14 | 195.95 | 120.00 | 35.50 | 168.67 | 29.19 |
| ***Nitrate*** | n/a | n/a | 6.13 | 5.54 | 1.92 | 1.66 |
| ***DIN*** | n/a | n/a | 126.13 | 36.60 | 170.58 | 28.82 |
| ***Phosphate*** | 5.52 | 7.51 | 11.05 | 9.08 | 0.93 | 1.52 |
| ***Silicate*** | 101.73 | 86.03 | 280.78 | 72.88 | 204.24 | 110.75 |
| ***Ph*** | 6.92 | 0.19 | 6.78 | 0.14 | 6.19 | 0.11 |
| ***Alkalinity*** | 2513.73 | 823.21 | 1677.36 | 301.47 | 1445.04 | 506.13 |
| ***DIC*** | 2759.97 | 844.58 | 1854.17 | 330.15 | 2197.17 | 728.47 |
| ***Calcite Sat.*** | 0.44 | 0.29 | 0.28 | 0.11 | 0.05 | 0.03 |
| ***Aragonite Sat.*** | 0.28 | 0.19 | 0.19 | 0.07 | 0.03 | 0.02 |

**Table 4.5.3- Fluorescein Regression Results.** Arrows indicate direction of a statistically significant effect ($\alpha = 0.05$). Numbers in parentheses indicate range of measured values for the variable. The first p-value is the probability for the given variable, and the "p-value model" is the probability for the full model.

| Site/Model | Parameter | p-value | Adj. $R^2$ | p-value model |
|---|---|---|---|---|
| **False Bay "Best Fit"** | **Arenicolid** (▼ 2-70 $m^{-2}$)<br>**Grain Size** (▲ 117-153 μm) | **0.0025**<br>**0.0084** | **0.78** | **0.0043** |
| False Bay Thalassinid | Thalassinid | 0.0819 | 0.28 | 0.0819 |
| Cara's Flat Surface | Hemichordate | 0.2528 | 0.06 | 0.2528 |
| **Cara's Flat Deep** | **Hemichordate** (▼ 0-15 $m^{-2}$) | **0.0080** | **0.61** | **0.0080** |
| **Debidue Surface** | **Onuphis** (▼ 8-79 $m^{-2}$)<br>**Porosity** (▲0.42-0.45) | **0.0096**<br>**0.0051** | **0.71** | **0.0001** |
| **Debidue Deep** | **Porosity** (▲0.42-0.45) | **0.0005** | **0.59** | **0.0005** |

**Table 4.5.4- Regression Results for Porewater Chemistry.** Direction of triangles indicate the direction of a significant effect of organism density on specific solute ($\alpha$ = 0.05), n/s = not significant, and n/a = measurements of solute not available. Numbers in the parentheses are the adjusted $R^2$ for the model and p-value for the variable. Some of the models included sediment parameters in addition to the organism densities based on model selection criteria.

| | **False Bay** | | **Cara's Flat** | | **Debidue Flat** |
|---|---|---|---|---|---|
| | *Arenicolid* | *Thalassinid* | *Hemichordate* | *Diopatra* | *Onuphis* |
| **Ammonium** | ▼(0.51, 0.0179) | ▲(0.45, 0.0284) | n/s | n/s | n/s |
| **Nitrate** | n/a | n/a | ▼(0.59, 0.0462) | n/s | n/s |
| **DIN** | n/a | n/a | n/s | n/s | n/s |
| **Phosphate** | ▼(0.61, 0.0078) | ▲(0.81, 0.0013) | ▼(0.83, 0.0010) | n/s | n/s |
| **Silicate** | ▼(0.59, 0.0095) | ▲(0.62, 0.0116) | ▼(0.68, 0.0072) | n/s | n/s |
| **pH** | n/s | n/s | n/s | n/s | n/s |
| **Alkalinity** | ▼(0.37, 0.0475) | ▲(0.87, 0.0004) | n/s | n/s | n/s |
| **DIC** | ▼(0.48, 0.0222) | ▲(0.91, 0.0001) | n/s | n/s | n/s |
| **Ω Aragonite** | n/s | ▲(0.50, 0.0290) | n/s | n/s | n/s |
| **Ω Calcite** | n/s | ▲(0.50, 0.0290) | n/s | n/s | n/s |

## *4.6 Figures*

**Figure 4.6.1- Surface Features of Infauna.** Representative images of surface features associated with the experimental species in this study, A- *Abarenicola pa*cifica, B- Thalassinid, C- *Diopatra cuprea*, D- *Balanoglossus aurantiacus*, and E- *Onuphis jenneri*.

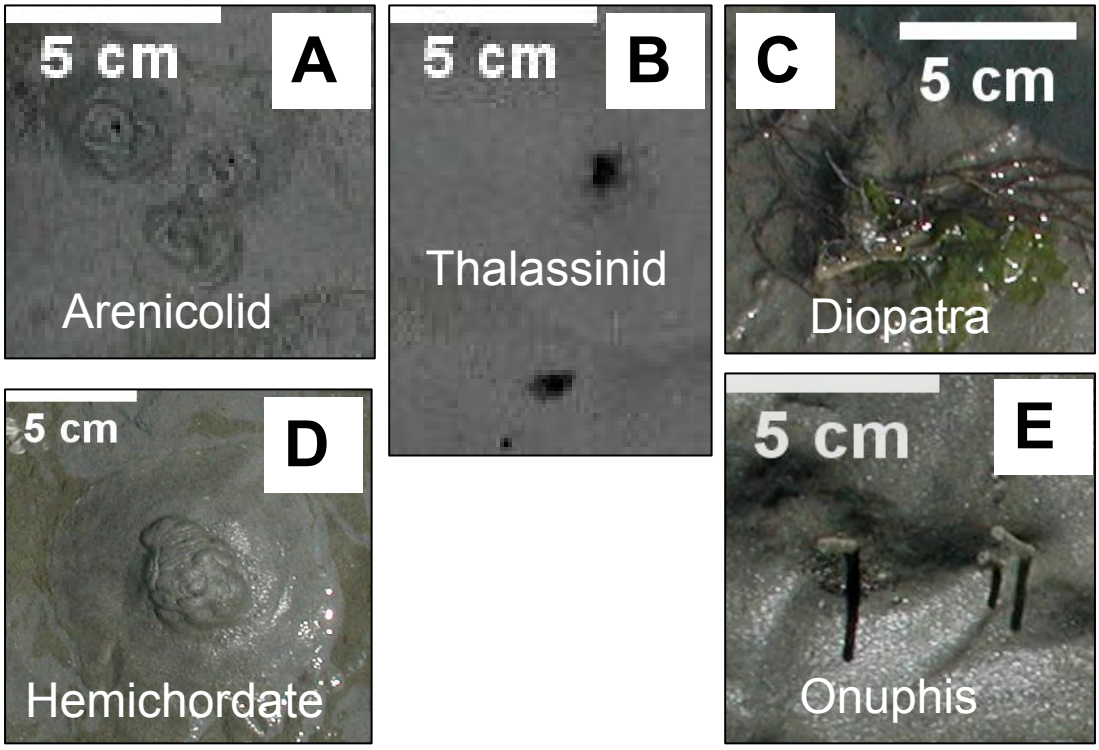


**Figure 4.6.2- Organic Matter and Percent Fines.** Organic content versus percent fines at False Bay (grey) and Cara's Flat (black). Note the order of magnitude difference on the x-axis between False Bay and Cara's Flat. There was no significant relationship between organic C and fines at False Bay, where as the relationship was highly significant at Cara's Flat.

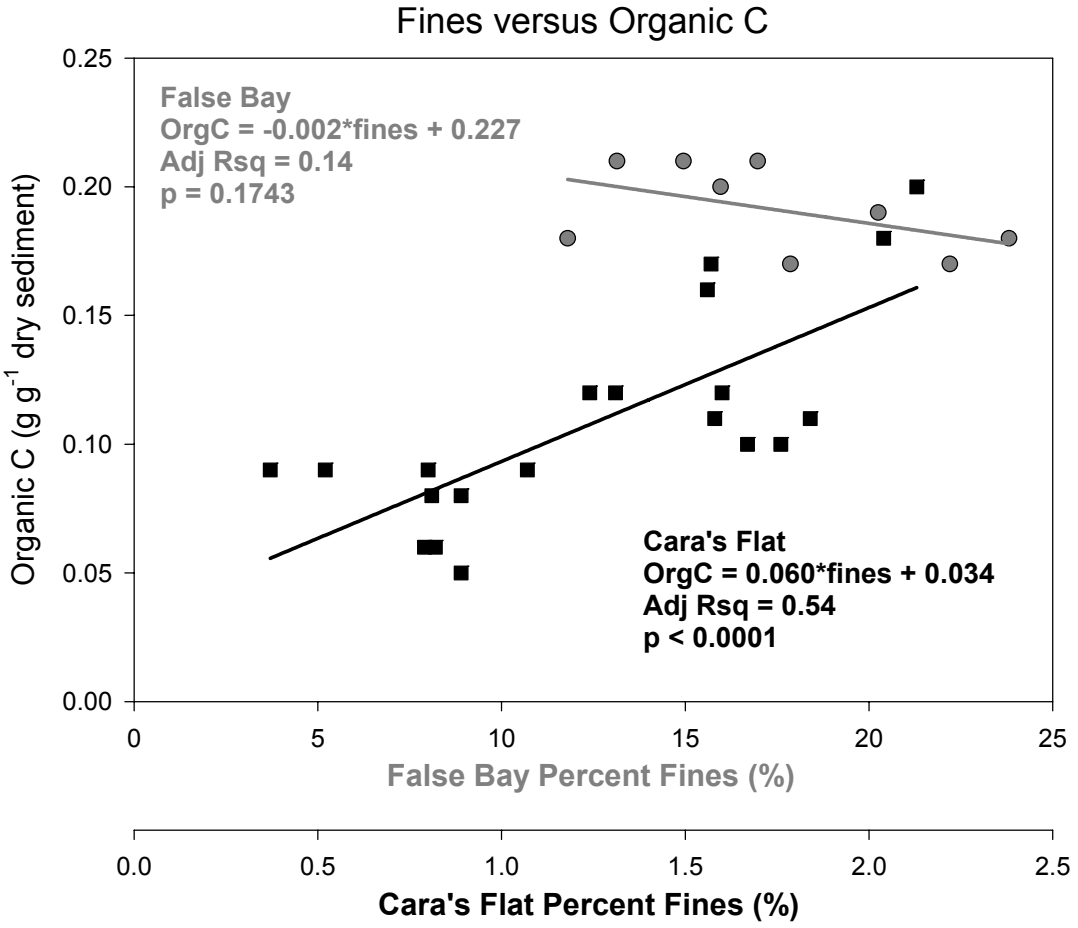


**Figure 4.6.3- Fluorescein in Gels and Tidal Range.** The grand mean fluorescein remaining in gels plotted on top of tidal range at each experimental site. Error bars are standard deviations. Fluorescein remaining in the gel is the inverse of porewater advection, less fluorescein indicates higher rates of porewater advection. Surface and deep fluorescein refers to the near surface and deep subsections of the gels.

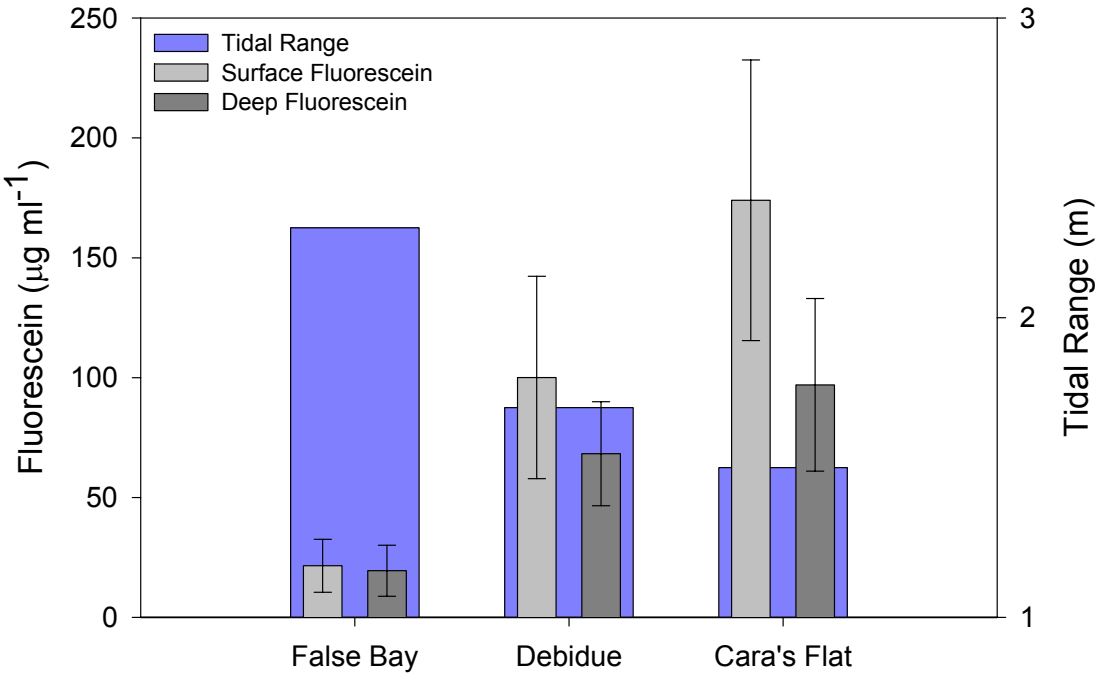


**Figure 4.6.4- Solute Concentrations versus Tidal Range.** Plots of grand mean solute concentrations for silicate, ammonium, DIC, and pH versus tidal range for each experimental site. Left y-axis is concentration (grey) and right y-axis is tidal range (blue), and error bars are standard deviations.

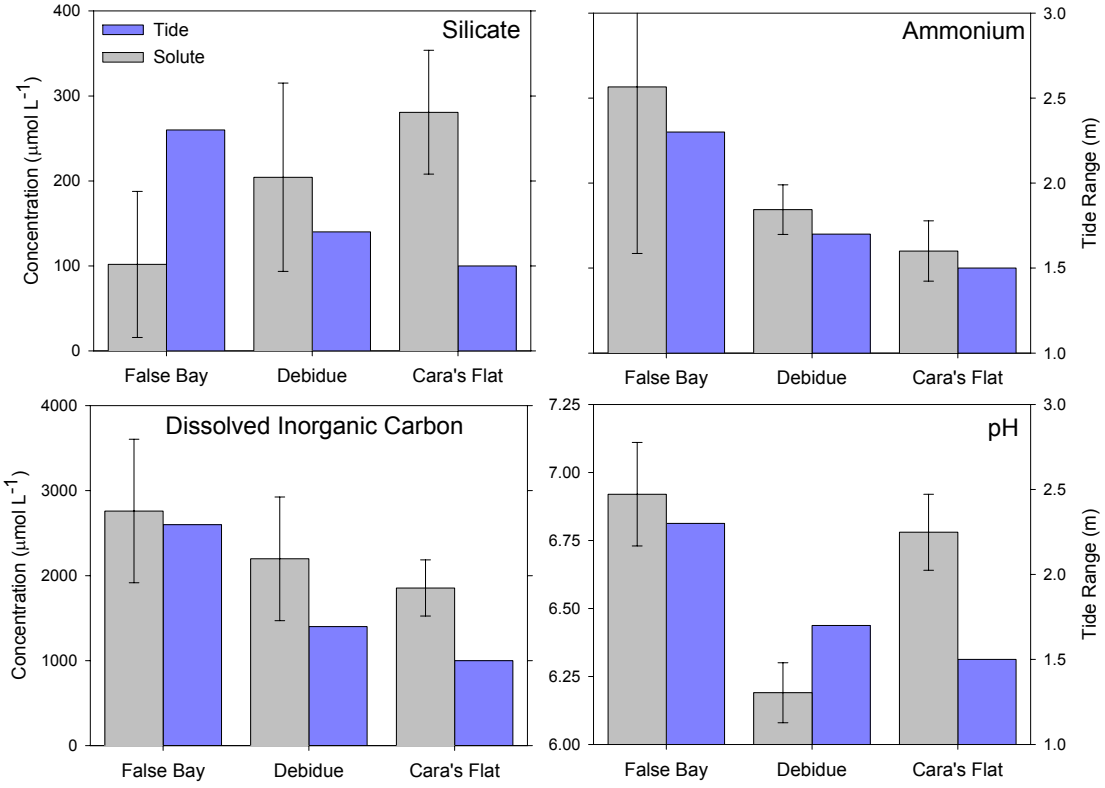


**Figure 4.6.5- Solute Concentrations versus Percent Fines.** Plots of grand mean solute concentrations for pH, alkalinity, saturation state, and ammonium versus percent fines for each experimental site. Solute concentrations are plotted on the right y-axis, percent fines is plotted on the left y-axis and on a log scale. Percent fines is plotted in blue and mean solute concentration in grey.

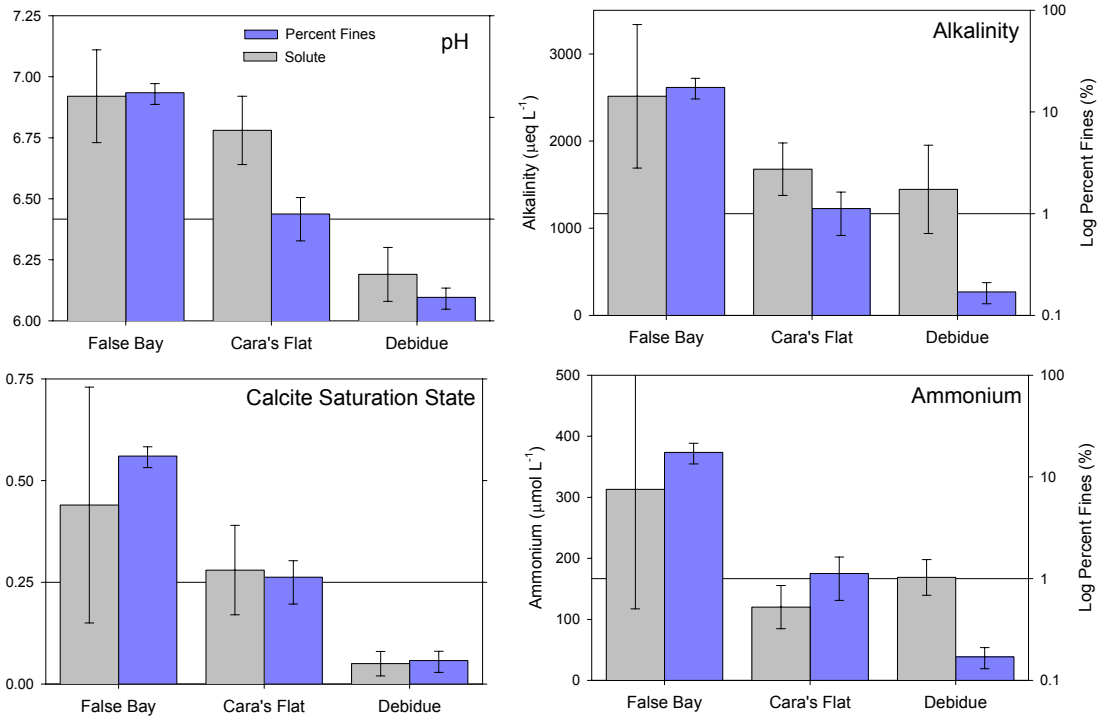


**Figure 4.6.6- Fluorescein in Gels versus Organism Density.** Fluorescein remaining in gels versus arenicolid and hemichordate densities. The fluorescein concentrations in the arenicolid versus fluorescein plot (circles) are average of deep and surface gel sections. The fluorescein concentrations in the hemichordate regression (squares) are for the deep gels only. Note the different scales on the x-axis, and the slopes from the significant regression analyses next to each line. The slopes are the per organism effects on fluorescein remaining in the gels for each species and site.

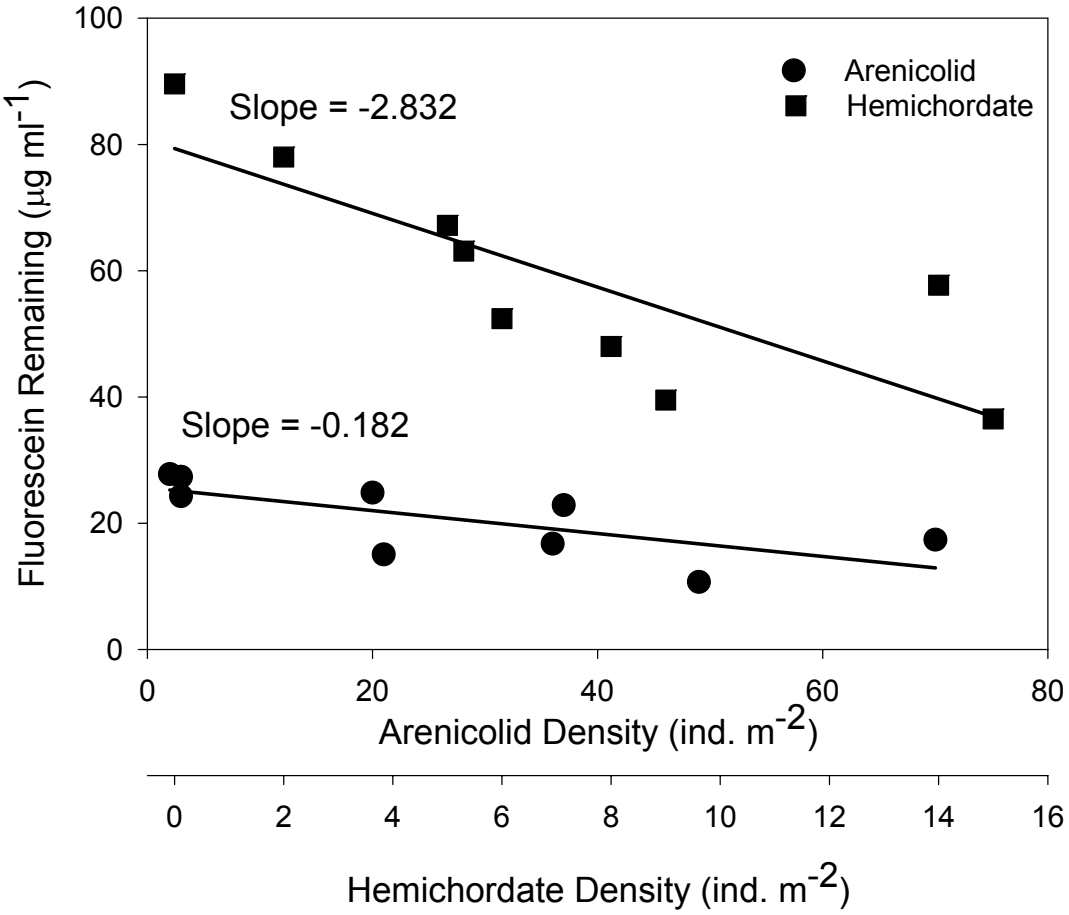
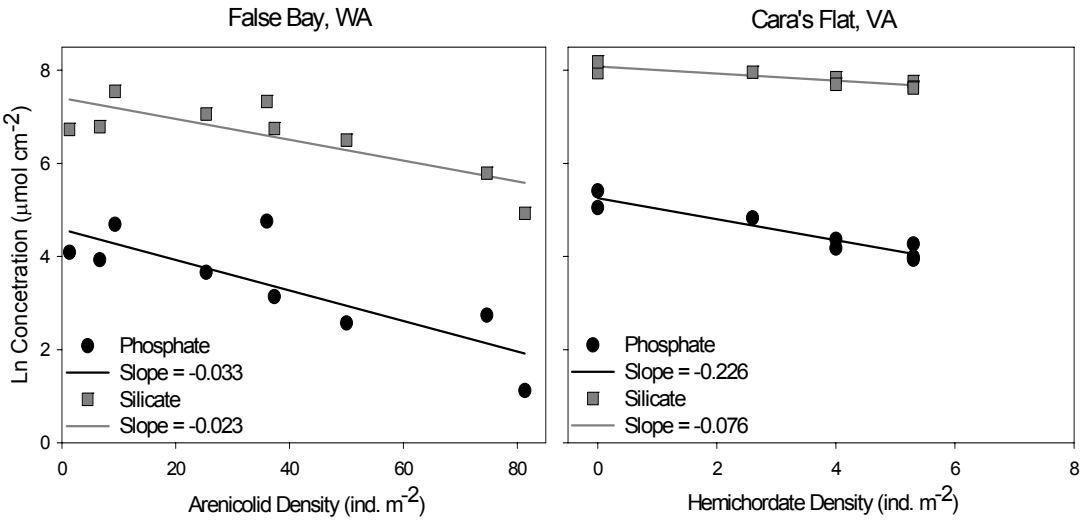


**Figure 4.6.7- Phosphate and Silicate versus Organism Density.** Integrated concentration of phosphate (black circles) and silicate (grey squares) plotted against arenicolid and hemichorate density (left and right panels, respectively). The concentrations are natural log transformed, and note the order of magnitude difference in the x-axes. The slopes for each significant regression are noted in the figure legend. The slopes are the per individual effect of each species on solute concentrations.



# Appendix

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