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Timothy H. Shepherd  
*University of South Carolina*

Stephen E. Stancyk  
*University of South Carolina*

Timothy J. Shaw  
*University of South Carolina*

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# EFFECTS OF THE BURROWING BRITTLESTAR, *MICROPHIOPHOLIS GRACILLIMA* (ECHINODERMATA: OPHIUROIDEA), ON THE FLUX OF LITHIUM, AN INERT TRACER, ACROSS THE SEDIMENT-WATER INTERFACE

Timothy H. Shepherd<sup>a, d</sup> Stephen E. Stancyk<sup>a, b</sup> Timothy J. Shaw<sup>a, c</sup>

<sup>a)</sup> Marine Science Program and Belle W. Baruch Institute for Marine Biology and Coastal Research, University of South Carolina, Columbia, South Carolina 29208, USA

<sup>b)</sup> Department of Biological Sciences, University of South Carolina, Columbia, South Carolina 29208, USA

<sup>c)</sup> Department of Chemistry, University of South Carolina, Columbia, South Carolina 29208, USA

<sup>d)</sup> current address: University of Georgia, Marine Institute, Sapelo Island, Georgia 31327, USA

**ABSTRACT** Burrowing and ventilation activities of infaunal organisms have been shown to affect geochemical processes in sediments and at the sediment-water interface. Although burrowing brittlestars are dominant in many benthic environments, their role in these processes is poorly known. We tested the effect of the amphiuroid brittlestar, *Microphiopholis gracillima*, on the flux of lithium ion from the sediment to the overlying water by using sediment cores with false bottoms for continuous flow of a  $\text{Li}^{+1}$ -seawater solution. Brittlestars at densities of 300 and 600 individuals  $\text{m}^{-2}$  caused a twofold increase in the rate that Li was transported through the sediment. Density of brittlestars appeared to have no effect on the flux of  $\text{Li}^{+1}$  from the sediment, indicating a possible threshold beyond which density increases do not influence fluxes of solute from the sediment.

## INTRODUCTION

The effect of infaunal organisms on sediment characteristics has been well documented (Rhoads 1974, Rhoads and Boyer 1982, Aller 1982). Through their burrowing, feeding and ventilation activities, infauna can modify physical properties of the sediment such as shear strength, sorting of grain size, and porosity (Rhoads 1974, Rhoads and Boyer 1982, Aller and Aller 1992). They can also influence the flux or exchange of dissolved chemicals such as nutrients or pollutants between the sediment and overlying water (Lerman 1977, Berner 1976, Aller 1978, Luedtke and Bender 1979, Emerson et al. 1984, Marinelli 1992). Fluxes can be an order of magnitude or more over those expected for molecular diffusion alone (Aller 1982, Benoit et al. 1991, Marinelli 1994), and can influence sediment chemistry by introducing oxygen to the sediments and removing sediment solutes like ammonia and sulfides (Aller 1982, Emerson et al. 1984). Quantification of organism influence on flux is important for understanding nutrient dynamics and the fate of pollutants that enter the sediments (Luedtke and Bender 1979, Aller 1982, Emerson et al. 1984, Rutgers van der Loeff et al. 1984, Benoit et al. 1991, Marinelli 1994).

Despite extensive recent research on the effect of infaunal organisms on fluxes of dissolved chemicals across the sediment-water interface much remains to be learned. Most research has involved polychaetes or bivalves, and there is little information on how species-to-species interactions or particular combinations of organisms affect the flux (Aller and Yings 1985, Marinelli 1992). There are

many important infaunal organisms whose influences on fluxes have not been examined. One such group includes burrowing ophiuroids in the family Amphiuroidae.

Amphiuroid brittlestars live with their central disc burrowed several centimeters into muddy or sandy sediments, with one or more arm tips extended to the sediment surface for feeding and ventilation (Hyman 1955, Thomas 1962, Woodley 1975). Ventilation is performed by undulation of the arms and contraction or pumping of the disc (Hyman 1955, Woodley 1975, Pentreath 1971). Amphiuroids have a world-wide distribution (Hyman 1955), and can be found from the intertidal zone to depths of several hundred meters in the oceans (Thomas 1962). They may occur in densities as high as 3000 individuals  $\text{m}^{-2}$  (Josefson 1995, Valentine 1991, Duineveld and Van Noort 1986, Bowmer and Keegan 1983) which has led to their use as dominants or co-dominants in the definition of many benthic marine communities (Thorson 1957). The species used in this study, *Microphiopholis gracillima* (Stimpson) (= *Amphipholis gracillima*, Thomas 1962, Hendler et al. 1995), occurs from Bermuda and Virginia to Brazil and is common along the southeastern coast of the United States (Singletary 1980). *M. gracillima* creates its burrows by removing sediment from depth and depositing it at the surface at burrow openings; and burrows are of a semi-permanent nature (Thomas 1962, Stancyk unpublished data).

The purpose of this investigation was to examine how *M. gracillima* influenced the flux of  $\text{Li}^{+1}$ , an inert tracer, from the sediment. We tested the hypotheses that a) the presence of brittlestars would increase the rate of  $\text{Li}^{+1}$

transport through the sediments and b) the rate of  $\text{Li}^{+1}$  transport would increase as brittlestar density increased. Lithium is used because of its small size, which causes hydration of the ion and reduces its reactivity. Lithium ion is rarely exchanged for the common sodium ion in sediments (Cocco et al. 1978).

## MATERIALS AND METHODS

*Microphiopholis gracillima* and sediment were collected from a subtidal mud flat in North Inlet, Georgetown, SC (37°20'N; 70°10'W) on 8 October 1995. In North Inlet *M. gracillima* has a density of 34-56 animals  $\text{m}^{-2}$  (Pape-Lindstrom et al. 1997). *M. gracillima*, separated from the sediment in the field by gently sieving, were placed in plastic bags with seawater for transport to Columbia, SC. In the lab, brittlestars were anesthetized with 35‰  $\text{MgCl}_2$  in a 1:1 solution with seawater, and 60 intact, healthy brittlestars were separated into four groups of 5 and four groups of 10 brittlestars. They were held in aquaria under experimental conditions until being placed into experimental cores.

In the lab, sediment was processed by wet sieving through a 1 mm mesh screen to remove large shells and macrofauna. The sediment was then mixed by hand, and two 13 liter (L) portions were separated and placed into plastic buckets to settle overnight. Overlying water was then removed, and 260 ml of a 10‰  $\text{Li}^{+1}$  stock solution (stock solution was made by dissolving 61.08g of  $\text{LiCl}$  into a liter of water) was mixed into each bucket for a nominal concentration of 200 mg  $\text{Li}^{+1} \text{L}^{-1}$  sediment. After sitting for 24 h in the  $\text{Li}^{+1}$  solution, sediment was mixed again by hand and added to cores to create a 10 cm column of sediment in each core.

Sediment cores were made of clear acrylic plastic (inner diameter = 14.6 cm; wall thickness = 32 mm). False bottoms were created by placing 70m Nitex® screen between the core wall and a PVC ring approximately 2.5 cm tall, which held the screen tautly in place 2.5 cm above the base of the core (Wilson-Finelli 1996). Once the PVC ring and Nitex® screen were in place, two holes were drilled on opposite sides of the false bottom to allow a flow-through of a  $\text{Li}^{+1}$ -seawater solution. Two holes were also drilled on the upper portion of the core so that the overlying water could be flushed with natural seawater when samples were not being taken. A clear PVC stopcock was threaded into one hole to control the flow of seawater into the core. Plexiglass squares (7 in. x 7 in.) were affixed to the base of the cores with silicone sealant.

When the silicone had dried, twelve cores were set on a table with the false bottoms connected in a series by

tubing, so that water could flow from the false bottom of one core to the next. After the twelve cores were assembled and connected with the tubing, they were partly filled with seawater, and air bubbles were removed from the screens creating the false bottoms. Once air bubbles were removed, silicone sealant was placed along the core edge at the false bottom, and a Gelman® extra-thick glass fiber filter (diameter 142 mm) was placed on top of the screen to keep sediment from falling into the false bottom. The seawater was then drained down to just above the filter, and the  $\text{Li}^{+1}$ -containing sediment was slowly added to each core under constant mixing until it reached the desired level. After settling for 24 h sediment was added or removed to create a sediment column of 10 cm. One liter (approximately 6 cm) of seawater was then added on top of the sediment for the overlying water. Cores then had aerators added to overlying water and were covered with plastic wrap to reduce evaporation. A 7 L reserve (open and unaerated) of a  $\text{Li}^{+1}$ -seawater solution was made up with 6.685 L of seawater and 0.315 L of 10‰  $\text{Li}^{+1}$  stock solution for a nominal concentration of 450 ppm of  $\text{Li}^{+1}$ . With the cores connected in a series, the first core (core 1) had the  $\text{Li}^{+1}$ -seawater pumped into the false bottom from the reserve with a peristaltic pump at a rate of  $11.9 \pm 0.7 \text{ ml min}^{-1}$ ; the last core (core 12) had the  $\text{Li}^{+1}$ -seawater pumped (same pump) out of the false bottom back into the reserve. The chambers were completely set up and running on 26 October 1995.

Because  $\text{Li}^{+1}$  was added to the sediment, some time was necessary to allow the sediment to equilibrate and establish a concentration gradient with the reserve concentration of  $\text{Li}^{+1}$  at the sediment base (approximately 400 ppm) and a much lower concentration in the overlying water. The overlying water concentration of  $\text{Li}^{+1}$  was kept low by flushing the overlying water daily when samples were not being taken. Flushing of the overlying water was performed by running seawater from a 20 L carboy to each core individually through the inflow stopcock and out by way of a larger outflow opening into a bucket to be discarded. During times of sampling the overlying water was not flushed, but the seawater solution flowing through the false bottoms flowed continuously due to the small volume of the false bottoms (=500 ml). Cores did not have brittlestars during the period that the sediment was equilibrating. Samples of the overlying water were taken repeatedly between 8 November 1995 and 19 December 1995 to determine if a concentration gradient had stabilized.

Brittlestars were added to randomly designated cores on 22 December 1995. Treatments included controls (no brittlestars), 5 brittlestars per core (300  $\text{m}^{-2}$ ), and 10 brittlestars per core (600  $\text{m}^{-2}$ ) with four replicates each.

Because all cores were linked in a series, treatments were arranged in a randomized block design, so that each treatment occurred once per three cores, to control for a possible decrease of  $\text{Li}^{+1}$  from the reserve as water passed through the series of 12 cores.

Brittlestars were given 23 days to establish burrows before samples were taken. The temperature during sampling was  $24.8 \pm 0.8^{\circ}\text{C}$  with the salinity at 33‰. On 14 January 1996 three 1 ml samples of the overlying water were taken from each core every 12 h for 120 h. Samples were then diluted to a volume of 20 ml with deionized water for analysis of  $\text{Li}^{+1}$ . Samples from cores 8 and 9 were rediluted due to high concentrations of  $\text{Li}^{+1}$ . Core 8 had a total dilution factor of 200; core 9 had a dilution factor of 80. The reserve was sampled every 24 h: three 1 ml reserve samples were diluted to a volume of 200 ml. All samples were analyzed for  $\text{Li}^{+1}$  with a Perkin-Elmer 5100PC flame atomic absorption spectrometer (Gieskes et al. 1991). The calibration curve was created from standards of 1, 2 and 3  $\text{mg Li}^{+1}\text{L}^{-1}$  with all samples diluted within this range. Linearity of the curve was assisted from the corresponding  $R^2$ , and calibration curves with an  $R^2$  greater than 0.99 were used to determine  $\text{Li}^{+1}$  concentration.

Analysis of  $\text{Li}^{+1}$  concentration data was performed in SAS using an analysis of covariance with time as the covariate (SAS Institute Inc. 1982). The model was used to obtain the rate of change in the  $\text{Li}^{+1}$  concentration (slope) into the overlying water by treatment and the standard deviations around the treatment slope. Treatment slopes were then compared using 95% Bonferoni-corrected confidence intervals.

## RESULTS

During the time that the sediment was relaxing, the reserve was losing water at a rate of approximately 100 ml  $\text{day}^{-1}$ . On 2 November, 6 L of a 400 ppm  $\text{Li}^{+1}$ -seawater solution (nominal concentration) were added to the reserve. The reserve lost a little more water, but stabilized in early December at a volume of 4.7 L. The reason for the loss of water is unknown, but may have been caused by evaporation in the cores, with the reserve water replacing the lost overlying water.

During the time that the flux was being measured, the reserve had a slow steady loss of  $\text{Li}^{+1}$  from 247 to 214  $\text{mg Li}^{+1}\text{L}^{-1}$ . This corresponds to a loss rate of  $-0.24 \text{ mg Li}^{+1}\text{L}^{-1} \text{ h}^{-1}$ . A mass balance calculation revealed that 95% of the  $\text{Li}^{+1}$  lost from the reserve was accounted for by the increase in the cores. The change in  $\text{Li}^{+1}$  concentration in the reserve had no significant effect on the model used in SAS.

Figure 1 shows the change of  $\text{Li}^{+1}$  over time in cores grouped by treatment. All  $\text{Li}^{+1}$  values were standardized by

subtracting the mean  $\text{Li}^{+1}$  concentration in the overlying water of each core at time zero from all observations within a core. Actual starting and ending  $\text{Li}^{+1}$  concentrations are shown in Table 1. In general, the brittlestars increased the flux of  $\text{Li}^{+1}$  across the sediment-water interface by a factor of 2.5 - 3.5 times over the rate observed in the controls (0.29 to 0.21 vs. 0.08  $\text{mg Li}^{+1} \text{ h}^{-1}$ ).

There was some variation within treatments. In the control cores, the flux of  $\text{Li}^{+1}$  varied from 0.02 to 0.15  $\text{mg Li}^{+1} \text{ h}^{-1}$ , and cores 6 and 7 had much higher fluxes than cores 2 and 11 (0.10 & 0.15 vs. 0.04 & 0.02  $\text{mg Li}^{+1} \text{ L}^{-1}$ ), but they could not be eliminated as outliers (Figure 1).

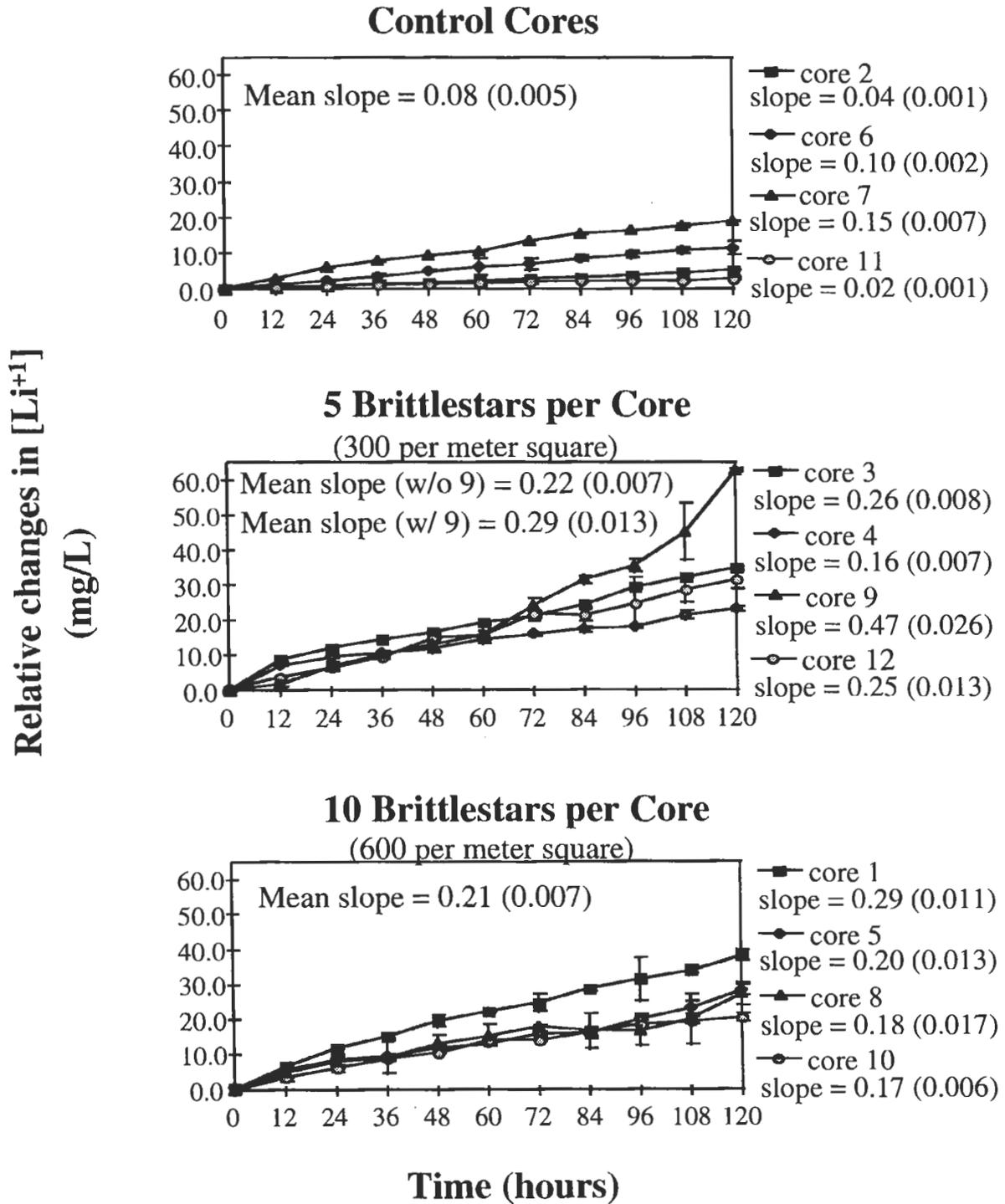
Cores containing brittlestars had, on average, considerably higher fluxes than control cores. The 5 brittlestar treatment had a mean flux of 0.29  $\text{mg Li}^{+1} \text{ h}^{-1}$ . Core 9 was unusual, with an increasing slope in the last half of the experiment and an extremely high flux of 0.47  $\text{mg Li}^{+1} \text{ h}^{-1}$ . When core 9 is excluded, the mean flux drops from 0.29 to 0.22  $\text{mg Li}^{+1} \text{ h}^{-1}$  (Figure 1). The 10 brittlestar treatment had a mean slope of 0.21  $\text{mg Li}^{+1} \text{ h}^{-1}$ . Three of the cores (5, 8, and 12) grouped together very nicely, but core 1 had a slightly higher flux (Figure 1).

Figure 2 is a graph of the mean treatment slopes. Because of the unusual size and shape of its slope, core 9 was excluded from this graph and the rest of the analysis. Figure 2 shows that the brittlestars caused a 2.7-fold increase in the flux of  $\text{Li}^{+1}$  across the sediment-water interface. When 95% Bonferoni-corrected confidence intervals are compared, there is a significant difference in the control from the brittlestar treatments, but no difference when the density of brittlestars is changed from 300 to 600 brittlestars  $\text{m}^{-2}$  (Table 1).

## DISCUSSION

This study demonstrated that burrowing brittlestars had a significant effect on the flux of  $\text{Li}^{+1}$  across the sediment-water interface. Brittlestars in natural densities significantly increased the rate of  $\text{Li}^{+1}$  transported out of the sediment by 2-3 times over controls (0.21 or 0.22 vs. 0.08  $\text{mg Li}^{+1} \text{ h}^{-1}$ ; Figure 2). This significant increase in  $\text{Li}^{+1}$  transport falls within reported values of organism effects on fluxes across the sediment-water interface (Table 2).

One explanation for the unexpected variation among control cores is that the sediments were not fully equilibrated in cores 6 and 7. Another possible explanation for the high fluxes in control cores 6 and 7 could be the existence of slight variations in the core height. The PVC rings used to create the false bottoms were cut using a band saw, and the rings were not exactly the same height. This caused some of the cores to sit slightly lower than others when sediment



**Figure 1.** The relative change in concentration of  $Li^{+}$  in the overlying water over time in cores grouped by treatment. Values were standardized by the subtraction of the  $Li^{+}$  concentration at time zero for each core. Error bars indicate the standard deviation of the three replicate measures at each sampling period. The slope for each core is given in the legend as  $mg Li^{+} h^{-1}$  (standard deviation).

OPHIUROIDS AND SEDIMENT FLUX

**TABLE 1**

**Relevant values, with cores grouped by treatment. The lithium flux rate of the cores with their associated standard error are given. A negative flux rate means that lithium was fluxed out of the sediment. The starting and ending Li<sup>+</sup> concentrations (mg/L) for each core are listed. Treatment mean flux is given with the 95% Bonferoni-corrected confidence interval.**

Controls	Flux rate of Li <sup>+</sup> (mg/hr)	Std. Err.	Starting-Ending Li <sup>+</sup> concentration	Porosity
Core 2	-0.04	0.001	2.8-8.2	0.45
Core 6	-0.10	0.002	6.7-18.0	0.45
Core 7	-0.15	0.007	18.0-36.8	0.47
Core 11	-0.02	0.001	9.5-12.5	0.43
Average Bonferoni	-0.08 -0.07-(-0.09)	0.005		
Five brittlestars				
Core 3	-0.26	0.008	8.3-43.0	0.46
Core 4	-0.16	0.007	8.5-31.5	0.51
Core 9	-0.47	0.026	29.1-91.5	0.46
Core 12	-0.25	0.013	29.7-60.7	0.47
Average	-0.29	0.013		
Average Without core 9 Bonferoni	-0.22 -0.20-(-0.24)	0.007		
Ten brittlestars				
Core 1	-0.29	0.011	24.8-62.4	0.46
Core 5	-0.20	0.013	13.3-41.3	0.46
Core 8	-0.18	0.017	68.0-95.3	0.46
Core 10	-0.17	0.006	19.1-39.3	0.47
Average Bonferoni	-0.21 -0.19-(-0.23)	0.007		0.46

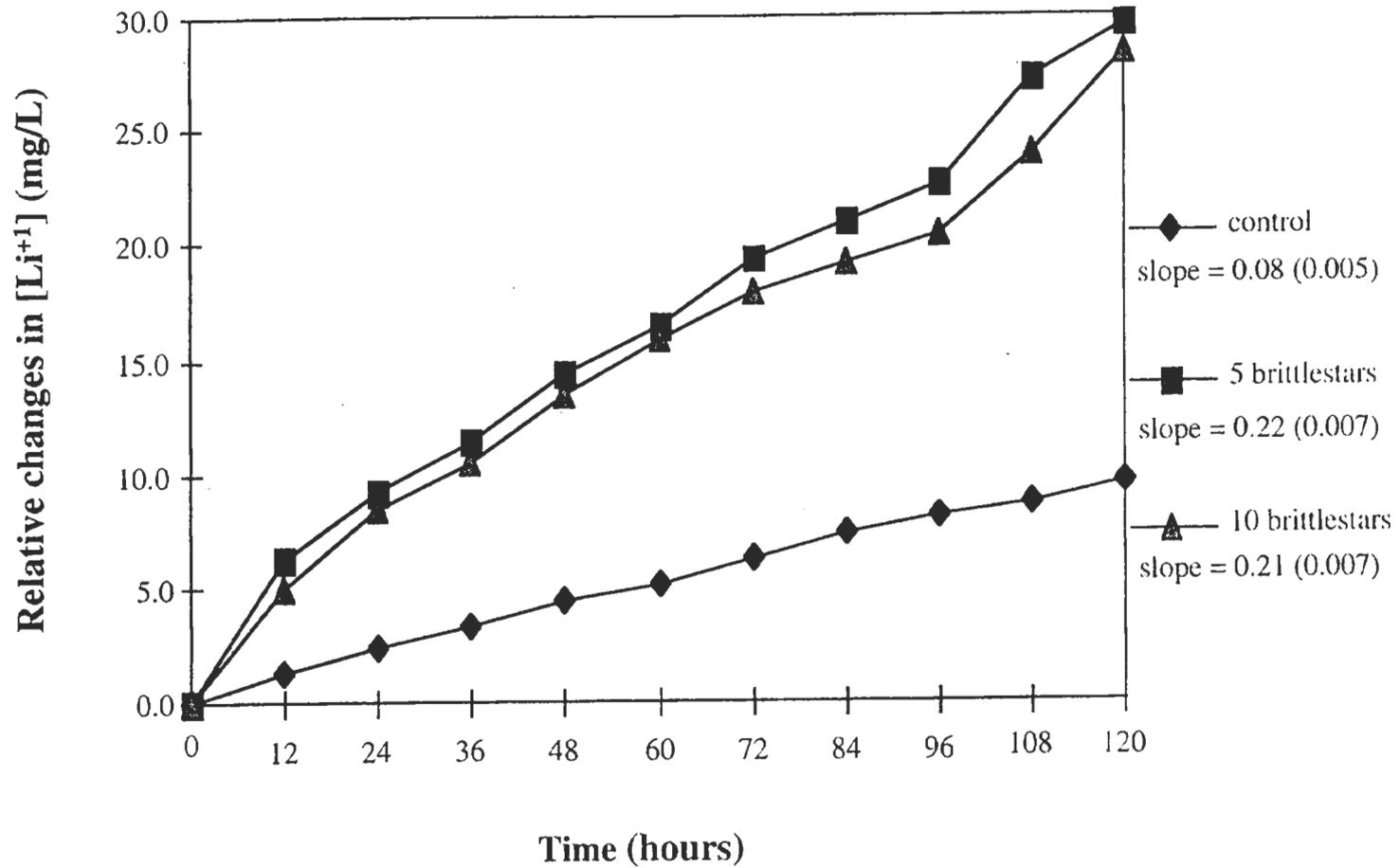


Figure 2. Average slopes for all treatments based on the values standardized by the subtraction of the Li<sup>+</sup> concentration at time zero for each core. The slope for each treatment is given as mg Li<sup>+</sup> hr<sup>-1</sup> (standard deviation)

TABLE 2

Comparison of literature values of measured flux over flux predicted by molecular diffusion. Controls *in situ* were not always possible, so that the observed flux due to organisms was compared to the flux-based calculations of molecular diffusion in sediments (see Berner 1976, Lerman 1977 and Aller 1982 for discussions on calculating fluxes across the sediment-water interface). Note that differences in flux rates will vary depending on the chemistry of the compound or tracer studied (modified from Benoit et al. 1991).

Laboratory or Field Setting	Species or Location	Observed Flux/ Predicted Flux	Source
Laboratory	<i>Yoldia limatula</i>	1.4	Aller 1978
Laboratory	<i>Heteromastus filiformis</i> , <i>Macoma balthica</i> , <i>Tellina texana</i>	2-5	Aller and Yingst 1985
Field	Po delta lagoon, Italy	3-20	Barbanti et al. 1992
Field	Mystic River, CT, USA	13-30	Benoit et al. 1991
Field	Puget Sound, WA, USA	3-5	Emerson et al. 1984
Field	Gulf of Mexico, TX, USA	8-10	Filipek and Owen 1980
Field	Long Island Sound, CT, USA	5	Goldhaber et al. 1977
Field	Hudson River estuary, NY, USA	2-3	Hammond et al. 1977
Laboratory	<i>Eupolyornia</i> <i>heterobranchia</i>	≤2.4	Marinelli 1994
Field	Buzzards Bay, MA, USA	0.2(winter) 8 (summer)	Martin and Sayler 1987
Field	Narragansett Bay, RI, USA	6	McCaffrey et al. 1980
Field	Gullmarsfjorden, Sweden	2-10	Rutgers van de Loeff et al. 1984
Laboratory	<i>Microphiopholis</i> <i>gracillima</i>	5-10	This study

column height and water volume were held constant. A core that sat lower than other cores would have an increased head pressure from the other cores due to their higher water level. Because the cores were interconnected through the false bottoms, the head pressure would exert a pressure at the base of the sediment column, forcing the  $\text{Li}^{+1}$ -seawater solution to be pushed up into the sediments. With no organisms to remove the forced influx of  $\text{Li}^{+1}$  from the sediment, the core would not be at steady state. This problem could be solved by using a multi-channel peristaltic pump so that each core would have a separate push/pull system, thus removing variance due to interconnections.

The flux of  $\text{Li}^{+1}$  increased dramatically in the overlying water in core 9 (a 5 brittlestar treatment) during the last half of the experiment (Figure 2). In this case, one or more brittlestar(s) probably established a burrow at the base of the sediment column, setting up a channel for  $\text{Li}^{+1}$  to pass easily from the false bottom to the overlying water. *Microphiopholis gracillima* commonly burrows to a depth of 10 cm (Singletary 1980), which was the height of the sediment columns used in this experiment, but we have seen them extend arm burrows to 20 cm in a core with a 20 cm sediment column.

Interestingly, the doubling of density from 300 to 600 brittlestars  $\text{m}^{-2}$  did not change the rate that  $\text{Li}^{+1}$  was moved across the sediment-water interface (5 brittlestars, 0.22  $\text{mg Li}^{+1} \text{ h}^{-1}$ ; 10 brittlestars, 0.21  $\text{mg Li}^{+1} \text{ hr}^{-1}$ ; Figure 2). This is in contrast to two *in situ* studies, Rutgers van der Loeff et al. (1984) and Barbanti et al. (1992), which reported a positive relationship between the density of organisms and the flux of nutrients across the sediment-water interface.

Although an increase in the transport of  $\text{Li}^{+1}$  was expected with increasing density of brittlestars, the fact that there was no difference was not a complete surprise. In examining infaunal effects on sediment dynamics, Aller (1982) created a 3-dimensional model based on a centrally irrigated burrow and the surrounding sediment. The model showed that the distance between burrows affected the flux of solutes across the sediment-water interface and predicted that crowding in high densities would reduce the irrigation requirements of infauna due to the lower concentration of sediment-derived solutes such as ammonia in the surrounding sediments. Based on Aller's model, the brittlestars in this experiment could have benefited from the irrigation of the other brittlestars, thereby reducing each individual's need for ventilation at higher densities. The results imply that there is a threshold density above which the flux would remain constant even when brittlestar numbers are increased. A test of this hypothesis will require data on densities below 300  $\text{m}^{-2}$ .

There are a number of areas where future research is needed to examine the role of the benthos on fluxes across the sediment-water interface. In particular, the existence of a threshold density above which fluxes are stabilized could have a significant impact on flux models of dissolved chemicals in areas populated by infauna such as burrowing brittlestars. Predictions of nutrient fluxes, nutrient production rates and fate of pollutant transfers could be affected (Aller 1982, Emerson et al. 1984, Marinelli 1992). Emerson et al. (1984) suggested that infaunal organisms could affect the mobility of trace metals (Cu and Cd) by the removal of sulfides from the sediment with irrigation of the burrows. But environmental managers need to know if such processes vary with infaunal density or not.

In conclusion, this experiment showed that amphipod brittlestars significantly increased the flux of Li across the sediment-water interface 2.75 times over control cores. Increasing the density from 300 to 600 brittlestars  $\text{m}^{-2}$  had no effect on the flux of  $\text{Li}^{+1}$ , leading to a hypothesis that a threshold density exists beyond which higher densities will not increase the rate that solutes are moved from the sediments.

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