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Sediment Preference in the Asiatic Clam (*Corbicula fluminea*) and Viviparid Snail (*Campeloma decisum*) as a Response to Low-Level Metal and Metalloid Contamination

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Abstract. Sediment preference experiments were performed with the asiatic clam (*Corbicula fluminea*) and viviparid snail (*Campeloma decisum*) to determine the potential use of clam and snail behavior as a response to low-level metal and metalloid contamination. Three sediment types with varying levels of metal contamination were paired in various combinations. Clams and snails were placed in aquaria along the interface between the sediment types. Daily location and burial status were noted for two weeks. Clams spent significantly more days in the uncontaminated sediment when paired with one of the contaminated sediments. Snails spent more days in contaminated sediments when paired with the uncontaminated sediment, but none of these differences was statistically significant. Clams moved fewer days in tanks with the two most contaminated sediment types. Burrowing of snails was relatively unaffected by sediment treatments. The behavior of clams was more sensitive than the behavior of snails to sediment metal contamination. Consequently, clam behavior appears to be a better behavioral indicator of metal contamination.

Because of the small size of many freshwater streams, endemic biota are vulnerable to inputs of chemical pollutants. Benthic organisms are especially vulnerable as many chemicals concentrate in sediment. However, benthic organisms that are mobile may avoid deleterious effects of the contaminated sediment by moving to less contaminated sites.

Many studies have focused on the effects of contaminated sediment on the burrowing behavior of benthic organisms. The depth to which juvenile hard clams (*Mercenaria mercenaria*) burrowed in oiled sediment was significantly shallower than in control sediment and the time taken to burrow beneath the surface was also longer in oil-contaminated sediment (Olla *et al.* 1983). Using a marine bivalve (*Macoma balthica*), an increase in sediment heavy metal concentration increased the time for 50% of the population to burrow (McGreer, 1979). Above a threshold of 5.8 μg/g copper added to dry sediment, the time for 50% of littleneck clams (*Protothaca staminea*) to burrow increased logarithmically with increasing sediment copper concentration (Phelps *et al.* 1983).

Contaminated sediments can be avoided by benthic organisms if given the choice of a less contaminated or uncontaminated sediment. Phoxocephalid amphipods (*Rhepoxynius* spp.) avoided sediments contaminated with domestic sewage or spiked with trace metals (Oakden *et al.* 1984). *Corophium volutator* avoided sediment treated with mercury when paired with control sediment (Erdem and Meadows, 1980). A marine bivalve (*Macoma balthica*) significantly avoided sediment contaminated with high concentrations of heavy metals when paired with control sediment (McGreer 1979). Avoidance of pesticide contaminated sediment in the field has been suggested in the flounder (*Platichthys flesus*) (Møhlenberg and Kiørboe 1983).

This study was the first of two studies evaluating the effect of metal and metalloid contaminated sediment on mollusc behavior and the potential use of behavior as an indicator of contaminated sediment. The purposes of this study were to: (1) compare sediment preference and burrowing behavior between sediments with varying levels of metal and metalloid contamination in the freshwater asiatic clam (*Corbicula fluminea*) and viviparid snail (*Campeloma decisum*), and (2) evaluate the potential use of either the asiatic clam or viviparid snail as indicator species for sediment metal contamination.

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Materials and Methods

Organism Collection and Maintenance

Clams were collected from Lower Three Runs Creek (LTR) on the U.S. Department of Energy's Savannah River Site (SRS) near Aiken, SC. Snails were collected from Upper Three Runs Creek (UTR), another stream on the SRS, using a wire net (Allison 1942). Clams ranged from 32 to 41 mm in shell length with a mean (±SD, n = 160) of 35.2 ± 1.9 mm. Snails ranged from 12 to 29 mm in length and 0.33 to 3.76 g in wet weight with means (±SD, n = 160) of 18.4 ± 4.3 mm and 1.28 ± 0.80 g, respectively. Clams and snails were immediately placed in a 520-L tank (Living Stream™ model LSW-700) that received a continuous flow of UTR water. Clams and snails were held under a 12h-light:12h-dark cycle. Clams and snails were held for less than three wks before use; assays were not performed simultaneously, which accounts for differences in sediment and water quality between assays.

Labeling Clams and Snails

To identify individuals, clams were labeled using numbered pieces of styrofoam tied to nylon line and glued to each clam shell using a waterproof epoxy. This floating label was necessary because many clams buried themselves completely. A unique color pattern was painted on the tip of each snail shell with waterproof paint so that individual snails could be identified.

Sediment Collection and Setup

Sediment was collected from three locations on the SRS and was collected to a depth of no more than 15 cm, from areas swept clear of large amounts of organic matter and plant debris to minimize variability among sediment types. One location where sediment was collected was Lower Three Runs Creek (LTR), which receives water from Par Pond, a site reported to have very low concentrations of dissolved and suspended metals (Newman *et al.* 1985). Sediment was also collected from Steed Pond (STD). Steed Pond received effluent from M-area, a facility which produced extruded aluminum-clad, depleted-uranium reactor targets (Pickett *et al.* 1987). The waste effluent formerly generated by M-area operations contained hydroxide precipitates of Al, U, Ni, Pb and other metals (Pickett *et al.* 1987). The final location of sediment collection was from a drainage creek below ash settling basins at the 400D coal fired power plant (ASH) (Site C, Newman *et al.* 1985; Alberts *et al.* 1985). This site has high concentrations of certain metals and metalloids in dissolved and particulate phases (Alberts *et al.* 1985). Sediments were taken to the laboratory immediately after collection and refrigerated until being placed in the aquaria; sediment was thoroughly mixed before being placed in aquaria. Samples were taken for metal analyses (Table 1), particle size analyses and organic carbon determination (Table 2) and immediately frozen in polyethylene bags until being analyzed.

For the clam assay, different sediment types were placed on the bottom of 20-L, glass aquaria (49 cm long × 26 cm wide × 29 cm high) to a depth of 6 cm in the following left-right combinations: LTR/LTR (4), LTR/ASH (2), ASH/LTR (2), LTR/STD (2), STD/LTR (2), STD/ASH (2) and ASH/STD (2), with the number of aquaria for each paired sediment combination in parentheses. This design included a total of 16 aquaria. Aquaria containing both "control" sediments (LTR/LTR) were used so that behavior independent of paired sediment types could be determined. For the snail assay, different sediment types were placed on the bottom of 5-L plastic trays (34 cm long × 21 cm wide × 9 cm high) to a depth of 1 cm, in the same combinations as those described for the clam assay. The interface between sediment types in an aquarium or plastic tray ran lengthwise, dividing the tank in half. Before sediment was added to each aquarium or plastic tray, colored gravel was added at a concentration of 20% (v/v) and thoroughly mixed. The percentage of different colored gravel in each side was quantified at the conclusion of the assay to determine the extent of mixing which occurred between sediments during the assay. Each aquarium was filled with UTR water and allowed to settle for 24 h before the addition of the test organism.

Preference Bioassay

The preference assay began when 10 clams or snails per aquarium (160 total) were placed along the interface between the two sediment types. Clams were placed foot end down into the sediment, with valve openings facing the same direction. Snails were placed operculum (shell opening) down, all facing the same direction. Clams were placed in the sediment so that 50% of the shell was buried, whereas snails were placed on the surface of the sediment. The initial location of each clam or snail was recorded. Because *C. fluminea* are primarily suspension feeders, 25-cm air stones were placed along two sides of each aquarium for the clam assay to circulate and aerate the water. Clams were fed daily a powdered, 50:50 (w/w) mixture of Tetramin™ fish flakes (Tetrawerke, Germany) and Purina trout chow at a rate of 0.02 g · clam^{-1} · day^{-1}. Snails were not fed during the 2 week assay, because snails presumably obtained sufficient food from the sediment. The location and burial status of each clam or snail was noted daily for two weeks. Clams and snails were considered buried if greater than 50% of their shell was beneath the sediment. Half of the water in each aquarium was replaced daily with UTR water. Both preference assays were performed under a 12-h light:12-h dark cycle.

Water Quality

Water samples were collected in 60 ml polyethylene bottles from each aquarium or plastic tray at the beginning (Day 1) and end (Day 14) of the assay for total alkalinity, other major anions (Cl^-, NO_3^-, SO_4^{2-}), major cations (Na^+, K^+, Mg^{2+}, Ca^{2+}), and trace element (Al, As, Cd, Cr, Cu, Ni, Se, U, Zn) analyses. Samples for alkalinity were refrigerated immediately after collection and analyzed within 48 h. Samples for major anion and cation analysis were passed through Sepak™ C18 reverse phase columns and refrigerated until being analyzed. Samples for trace element analyses were filtered through 0.45 μm membrane filters, acidified to $pH < 2$ with distilled nitric acid and stored at room temperature until analyzed.

Total alkalinity was measured by potentiometric titration using an Orion model 901 research ionalyzer and a model 81-02 Ross combination electrode. Major anions were measured with a Dionex 4020i ion chromatograph with a conductivity detector and an HPIC-AS4A separator column. Major cations were measured for the clam assay using a Dionex 4020i ion chromatograph with a conductivity detector and an HPIC-CG3 separator column. Major cations were measured for the snail assay using an acetylene/air flame on a 180-80 Hitachi Polarized Zeeman Atomic Absorption Spectrophotometer. Samples for dissolved metal analyses were analyzed by General Engineering Laboratories (Charleston, SC) using graphite furnace atomic absorption spectroscopy (As and Se) or inductively coupled plasma (ICP) spectrometry (Al, Cd, Cr, Cu, Ni, U and Zn).

Water temperature, dissolved oxygen, pH and conductivity were measured during both assays every three days using the Hydrolab Environmental Data Systems Unit (Model SVR2-SU). Dissolved oxygen was not measured during the snail assay because of the shallow water (≈5 cm) and presumably high gas exchange.

The UTR water used in the clam assay had the following characteristics (mean ± SD; n = 80 for temperature, D.O., pH, and specific conductance; n = 32 for alkalinity, major cations and anions): temper-

Table 1. Total element content (μg/g dry sediment) in LTR, STD, and ASH sediment and the percentage in each fraction using a sequential extraction technique. An asterisk (*) indicates that the mean metal concentration was significantly greater ($\alpha = 0.05$) than the mean of the "control" sediment (LTR). "ND" indicates that no metal was detected from that fraction. The number in parenthesis is one standard deviation around the mean and n = 3 for all samples

		Clam assay			Snail assay		
Element	Fraction	LTR	STD	ASH	LTR	STD	ASH
Al (μg/g)		429.8	562.6	376.7	359.0	780.6*	204.0
		(3.6)	(185.3)	(29.0)	(38.1)	(41.6)	(8.1)
	#1 (%)	7	34	7	1	1	1
	#2	7	6	11	13	24	21
	#3	2	3	1	5	9	4
	#4	21	20	36	29	39	43
	#5	5	5	4	5	4	3
	#6	1	1	1	1	1	1
	#7	57	31	40	46	22	27
As (μg/g)		0.19	0.23	1.69*	0.19	0.26*	0.95*
		(0.01)	(0.03)	(0.41)	(0.02)	(0.03)	(0.01)
		NOT DETECTED IN ANY FRACTIONS			NOT DETECTED IN ANY FRACTIONS		
Cd (μg/g)		<0.02	<0.02	0.06*	<0.02	<0.02	0.05*
				(0.01)			(0.01)
	#1 (%)	ND	ND	31	NOT DETECTED IN ANY FRACTIONS		
	#2	ND	ND	46			
	#3	ND	ND	ND			
	#4	ND	ND	ND			
	#5	ND	ND	ND			
	#6	ND	ND	23			
	#7	ND	ND	ND			
Cr (μg/g)		0.71	1.08*	0.62	1.77	2.21	0.57
		(0.05)	(0.15)	(0.05)	(0.74)	(0.39)	(0.07)
	#1 (%)	ND	ND	ND	ND	ND	23
	#2	16	7	33	17	28	21
	#3	ND	27	7	4	8	2
	#4	ND	ND	ND	8	29	33
	#5	36	38	25	16	12	8
	#6	4	3	2	ND	ND	ND
	#7	44	25	33	55	23	13
Cu (μg/g)		0.30	0.71*	0.65*	0.33	1.21*	0.96*
		(0.01)	(0.23)	(0.10)	(0.02)	(0.05)	(0.05)
	#1 (%)	ND	30	ND	ND	ND	ND
	#2	44	9	23	53	50	63
	#3	9	5	8	16	12	12
	#4	13	4	7	ND	ND	ND
	#5	28	9	12	7	8	9
	#6	3	1	5	ND	ND	ND
	#7	3	42	45	24	30	16
Ni (μg/g)		0.30	7.36*	0.95	0.30	21.9*	1.05
		(0.01)	(1.52)	(0.20)	(0.01)	(1.1)	(0.05)
	#1 (%)	ND	43	27	39	16	23
	#2	19	7	20	39	22	22
	#3	ND	3	2	ND	10	3
	#4	14	24	31	ND	24	30
	#5	ND	17	ND	4	17	2
	#6	8	2	3	ND	1	ND
	#7	59	4	17	18	10	20
Se (μg/g)		<0.05	<0.05	0.15*	<0.05	<0.05	0.19*
				(0.02)			(0.01)
		NOT DETECTED IN ANY FRACTIONS			NOT DETECTED IN ANY FRACTIONS		

Table 1. *Continued*

		Clam assay			Snail assay		
Element	Fraction	LTR	STD	ASH	LTR	STD	ASH
U (µg/g)		<0.09	13.71* (5.85)	<0.09	<0.09	27.8* (3.9)	<0.09
	#1 (%)	ND	23	ND	ND	36	ND
	#2	ND	7	ND	ND	34	ND
	#3	ND	40	ND	ND	14	ND
	#4	ND	5	ND	ND	8	ND
	#5	ND	7	ND	ND	ND	ND
	#6	ND	1	ND	ND	ND	ND
	#7	ND	17	ND	ND	8	ND
Zn (µg/g)		2.35 (0.44)	2.11 (0.55)	2.51 (0.70)	1.94 (0.25)	3.35* (0.16)	2.38* (0.23)
	#1 (%)	49	27	36	ND	13	ND
	#2	13	5	32	16	31	25
	#3	5	2	2	2	3	1
	#4	1	8	4	ND	ND	ND
	#5	22	13	14	3	3	2
	#6	6	5	10	ND	ND	ND
	#7	4	40	2	79	50	72

Table 2. Sediment characteristics of Lower Three Runs Creek (LTR), Steed Pond (STD), and ash basin (ASH) sediments. The numbers in parentheses are one standard deviation around the mean and n = 3

	Clam assay			Snail assay		
Variable	LTR	STD	ASH	LTR	STD	ASH
Percent organic carbon	0.15 (0.06)	0.04 (0.01)	0.06 (0.02)	0.10 (0.06)	0.15 (0.02)	0.04 (0.02)
Percent course sand (1–<4 mm)	1.3 (0.2)	3.9 (0.3)	12.5 (0.4)	12.7 (1.1)	4.8 (0.3)	16.8 (0.4)
Percent medium sand (0.25–<1mm)	82.6 (0.9)	80.8 (0.4)	58.7 (0.4)	58.4 (1.2)	72.4 (0.5)	59.6 (0.8)
Percent fine sand (0.062–<0.25 mm)	12.9 (0.6)	10.0 (0.6)	18.3 (0.4)	20.7 (1.6)	12.3 (0.5)	15.6 (1.5)
Percent silt and clay (<0.062 mm)	3.2 (1.6)	5.3 (0.9)	10.5 (0.3)	8.2 (1.6)	10.5 (0.7)	8.0 (0.8)
Median particle size (mm)	0.36 (0.01)	0.37 (0.01)	0.34 (0.01)	0.33 (0.03)	0.34 (0.01)	0.37 (0.02)

ature, 18.05 ± 1.40°C; D.O., 9.16 ± 0.31 mg/L; pH, 6.34 (median), 5.97–6.47 (range); specific conductance, 33.0 ± 3.0 µmhos/cm; total alkalinity, 3.87 ± 1.43 mg $CaCO_3$/L; Cl^-, 2.18 ± 0.34 mg/L; SO_4^{2-}, 2.15 ± 0.85 mg/L; Na^+, 2.38 ± 0.27 mg/L; K^+, 0.69 ± 0.21 mg/L; Mg^{2+}, 0.73 ± 0.27 mg/L; Ca^{2+}, 2.79 ± 0.55 mg/L. The UTR water used in the snail assay had the following characteristics (mean ± SD; n = 80 for temperature, pH, and specific conductance; n = 32 for alkalinity, major cations and anions): temperature, 17.13 ± 2.62°C; pH, 6.23 (median), 5.92–6.47 (range); total alkalinity, 3.44 ± 0.93 mg $CaCO_3$/L; Cl^-, 2.41 ± 0.36 mg/L; NO_3^-, 0.48 ± 0.36 mg/L; SO_4^{2-}, 2.26 ± 1.52 mg/L; Na^+, 1.93 ± 0.61 mg/L; K^+, 0.58 ± 0.22 mg/L; Mg^{2+}, 0.36 ± 0.04 mg/L; Ca^{2+}, 2.90 ± 0.46 mg/L. Samples analyzed for anions, cations, and dissolved metals were judged acceptable using procedure blanks, sample spikes and replicate analyses.

Statistical Analyses

The effects of sediment type, opposite sediment type and side of tank on the percentage of days spent in a particular sediment type were determined by analysis of variance (ANOVA) using the Statistical Analysis System program (SAS 1988). The Student-Newman-Keuls procedure was used to determine differences in percentage of days spent in a particular sediment type, daily movement, percentage of days moved and percentage of days buried by clams and snails among different sediment treatments. The Student-Newman-Keuls procedure was also used to determine differences in metal concentrations among different sediment types. When sediment concentrations were below detection limit, the Kruskal-Wallis test was used to test for significant differences between those concentrations below the detection limit and those concentrations above the detection limit. An α of 0.05 was used in all tests to indicate significance.

Sediment Analyses

Particle size analysis was performed on each of the three sediment types. Approximately 25 g of sediment was put onto a 63 µm sieve and wet sieved until water passing through was clear. The sieve with sediment was dried in an oven at 122°C. The dried sediment sample was weighed and the fraction less than 63 µm was determined by the difference. The remaining dry sediment was shaken through a standard sieve series and the weight of each fraction was determined. Organic carbon content was determined on each sediment by a modified Walkey-Black titration (Gaudette *et al*. 1974). In both assays, sediment characteristics were similar in both percent organic carbon and particle size (Table 2). Overall, median particle size among different sediments was very similar, even though there were differences in the proportions of different size classes of sands among sediments (Table 2).

Nitric acid digests ("total metal") were performed on each of the three sediment types. Two to three g sediment samples were freeze-dried for 24 h and digested for 6 h at 65°C in 10 ml of concentrated nitric acid. Extracts were diluted to 25 ml with deionized water. Samples were analyzed for metals and metalloids by General Engineering Laboratories (Charleston, SC) using ICP (Al, Cd, Cr, Cu, Ni, U, and Zn) or graphite furnace atomic absorption spectroscopy (As and Se) and expressed as μg metal per gram of dry sediment. In the clam assay, STD sediment was significantly more contaminated with Cr, Cu, Ni, and U, whereas ASH sediment was significantly more contaminated with As, Cd, Cu, and Se compared to the "control" sediment (LTR) (Table 1). In the snail assay, STD sediment was significantly more contaminated with Al, As, Cu, Ni, U, and Zn, whereas ASH sediment was significantly more contaminated with As, Cd, Cu, Se, and Zn compared to the "control" sediment (LTR) (Table 1).

Sequential extractions were also performed on sediment samples to estimate the fractionation of metal and metalloids. One to 1.5 g samples of wet sediment were collected in triplicate before addition to the aquaria and frozen in 50 ml Oak Ridge tubes until extractions were performed. Sequential extractions were performed on wet sediment samples using a modified version of the method described in Miller *et al*. (1986). Samples were shaken by a Burrell Model 75 wrist-action shaker. Notional fractions and extracts used were as follows: (1) "exchangeable" (30 ml 0.50M calcium nitrate), shaken 16 h; (2) "acid soluble" (30 ml of 0.44M acetic acid + 0.1M calcium nitrate), shaken 8 h; (3) "manganese-oxide occluded" (30 ml 0.01 M hydroxylamine hydrochloride + 0.1M nitric acid), shaken 0.5 h; (4) "organically bound" (30 ml 0.1M sodium pyrophosphate), shaken 24 h; (5) "amorphous iron-oxides" (30 ml 0.175 M ammonium oxalate + 0.1M oxalic acid), shaken 4 h in the dark; (6) "crystalline iron-oxides" (15 ml 0.15M sodium citrate + 0.05M citric acid + 0.5 g sodium dithionate/10 ml of extract), shaken 0.5 h at 50°C; and (7) "residual" (10 ml concentrated nitric acid) heated at 65°C for 6 h, diluted to 25 ml with deionized water. Following centrifugation, all supernatants were transferred to a 60 ml polyethylene bottle. All extracts except numbers 5 and 7 were acidified with concentrated distilled nitric acid to $\text{pH} < 2$. Samples were analyzed by General Engineering Laboratories (Charleston, SC).

Results

Water Quality

Water quality of UTR water was similar for both the clam and snail assays. Nitrate was not measured during the clam assay, although nitrate levels were believed to be similar to those obtained in the snail assay. There were no significant differences in dissolved trace element concentrations among the different paired sediment treatments; therefore, data from all treatments were combined. In the clam assay, water collected from all aquaria had the following mean (±SD, $n = 32$) dissolved elemental concentrations: Al, 116.0 ± 55.2 μg/L; As, <5.0 μg/L; Cd, <2.0 μg/L; Cr, <2.0 μg/L; Cu, 39.2 ± 14.6 μg/L; Ni, 4.66 ± 1.53 μg/L; Se, <5.0 μg/L; U, 8.16 ± 4.47 μg/L; Zn, 34.1 ± 10.0 μg/L. In the snail assay, water collected from all aquaria had the following mean (±SD, $n = 32$) dissolved elemental concentrations: Al, 55.8 ± 23.1 μg/L; As, <5.0 μg/L; Cd, <2.0 μg/L; Cr, <2.0 μg/L; Cu, 33.9 ± 6.36 μg/L; Ni, 3.20 ± 4.01; Se, <5.0 μg/L; U, <10.0 μg/L; Zn, 23.6 ± 3.96.

Sediment Analyses

Elements bound to sediments were generally found in only a few of the fractions following the sequential extraction procedure (Table 1). Those detected in fraction #7 (hot nitric acid digest) were not included in the fractionation descriptions since this was a residual digest to extract any metals not extracted in previous fractions.

If we assume that elements associated with earlier extracts are more bioavailable, bioavailability information can be derived from the fractionation data (Table 1). For those elements detected in all three sediments, the fractionation data suggest the following in terms of bioavailability. Aluminum and chromium were more bioavailable in STD and ASH sediments than in LTR sediment. Overall, there were no noticeable differences in copper bioavailability among sediments. Nickel was more bioavailable in STD and ASH sediments when compared to LTR sediment in the clam assay, whereas nickel was about equally bioavailable in all sediments from the snail assay. Zinc was equally bioavailable among sediments from the clam assay, and not very bioavailable among sediments from the snail assay. Conclusions regarding the bioavailability among sediments could not be derived from the fractionation data from certain metals (As, Cd, Se, and U) because they were either not detected in any fractions or detected in only one sediment.

The mixing between different sediment types was determined with colored gravel. During both assays, the majority of mixing at the interface between different sediment types occurred within the first 2 cm on either side of the sediment interface. Because of this mixing, clams or snails found in this "mixing zone" during the assay were not counted as being in either sediment.

Avoidance/Preference Assay

In the clam assay, sediment ($p = 0.0017$), the opposite sediment ($p = 0.0072$) and side of the tank ($p = 0.0001$) had significant effects on the percentage of days spent in a particular sediment type (Table 3). There was no significant interaction effect between sediment and opposite sediment ($p = 0.3037$) (Table 3). Clams spent significantly more days in LTR sediment (75%) when paired with ASH sediment (25%) (Figure 1). Although not statistically significant, clams also spent more days in LTR sediment (55%) when paired with STD sediment (45%) and in STD sediment (57%) when paired with ASH sediment (43%) (Figure 1). Clams also significantly preferred the left side of the test aquarium (86%) when compared to the right side (14%). No clam mortality was noted during the two week assay.

In the snail assay, only the side of the tank ($p = 0.0007$) had a significant effect on the percentage of days spent in a particular sediment type. Neither sediment ($p = 0.3254$) nor the opposite sediment ($p = 0.3738$) had a significant effect (Table 3). There was no significant interaction effect between sediment and opposite sediment ($p = 0.6216$). Although none of these

Table 3. Summary of ANOVA results from clam and snail preference assays. Dependent variable: Percentage of days in a certain sediment type

Clam preference assay

Source	DF	Sum of squares	F value	Pr > F
Model	7	41590	34.01	0.0001
Error	24	4193		
Corrected Total	31	45783		
Sediment	2	2938 (Type I)	8.41	0.0017
Opposite sediment	2	2135 (Type I)	6.11	0.0072
Sed. × Oppos. Sed.	2	438 (Type I)	1.25	0.3037
Side of tank	1	36079 (Type I)	206.51	0.0001

Snail preference assay

Source	DF	Sum of squares	F value	Pr > F
Model	7	3024	2.94	0.0225
Error	24	3523		
Corrected Total	31	6547		
Sediment	2	345 (Type I)	1.18	0.3254
Opposite sediment	2	301 (Type I)	1.03	0.3738
Sed. × Oppos. Sed.	2	142 (Type I)	0.49	0.6216
Side of tank	1	2235 (Type I)	15.23	0.0007

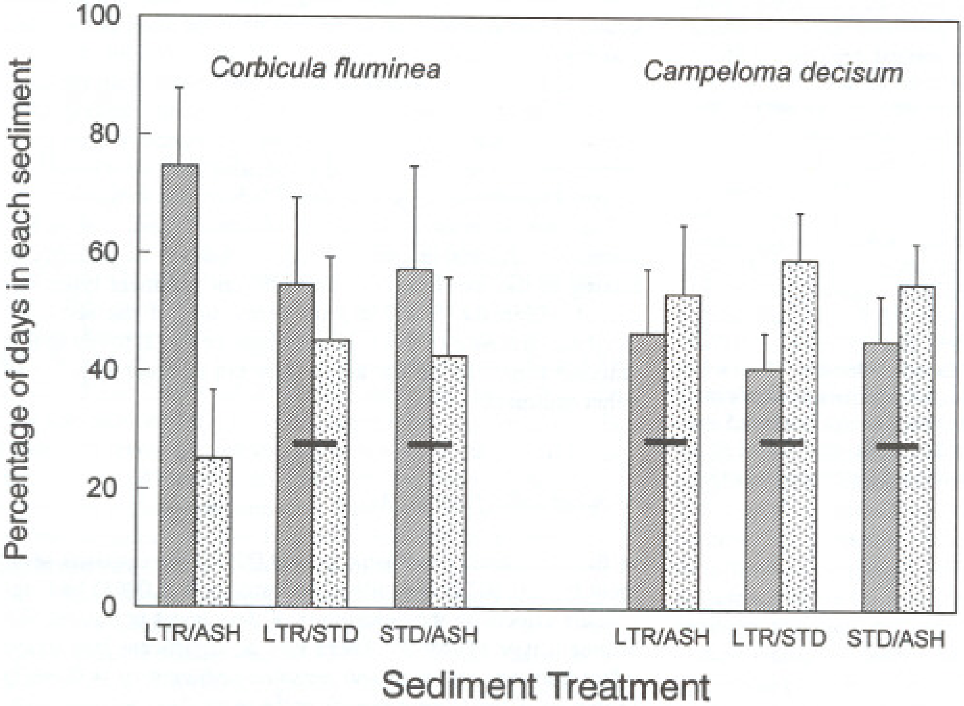

Fig. 1. Mean percentage of days spent in each sediment type by clams or snails in the paired sediment preference assay at different paired sediment treatments. Error bars denote one standard error around the mean. Within one sediment treatment, means without overlapping horizontal bars are significantly different ($\alpha = 0.05$) within each paired sediment treatment as determined with the Student-Newman-Keuls procedure

differences were statistically significant, snails spent a greater percentage of days in ASH sediment (53%) when paired with LTR sediment (47%), STD sediment (59%) when paired with LTR sediment (41%), and ASH sediment (55%) when paired with STD sediment (45%) (Figure 1). Snails also had a significant preference for the left side (57%) of the test aquarium when compared to the right side (43%). No snail mortality was noted during the two week assay.

Effect of Sediment Treatment on Behavior

Sediment treatment had no significant effect on the daily movement of either clams or snails. Overall, snails moved about 10 cm per day while clams moved about 1.2 cm per day.

Clams moved significantly fewer days in the ASH/STD tanks (65%) than in the LTR/LTR (75%), ASH/LTR (71%), or STD/LTR (73%) tanks (Figure 2). Snails moved a significantly higher percentage of days in STD/LTR tanks (94%) than in the LTR/LTR (90%), ASH/LTR (86%) or ASH/STD (86%) tanks (Figure 2). In general, snails moved a higher percentage of days when compared to the clams.

Sediment treatment had no significant effect on the percentage of days buried by clams. Overall the clams were buried about 90% of the days. Snails were buried a significantly lower percentage of days in ASH/LTR tanks (91%) than in the ASH/STD tanks (99%), but not a significantly lower percentage than in the LTR/LTR (96%) or STD/LTR (97%) tanks. In general, snails were buried a higher percentage of days when compared to the clams.

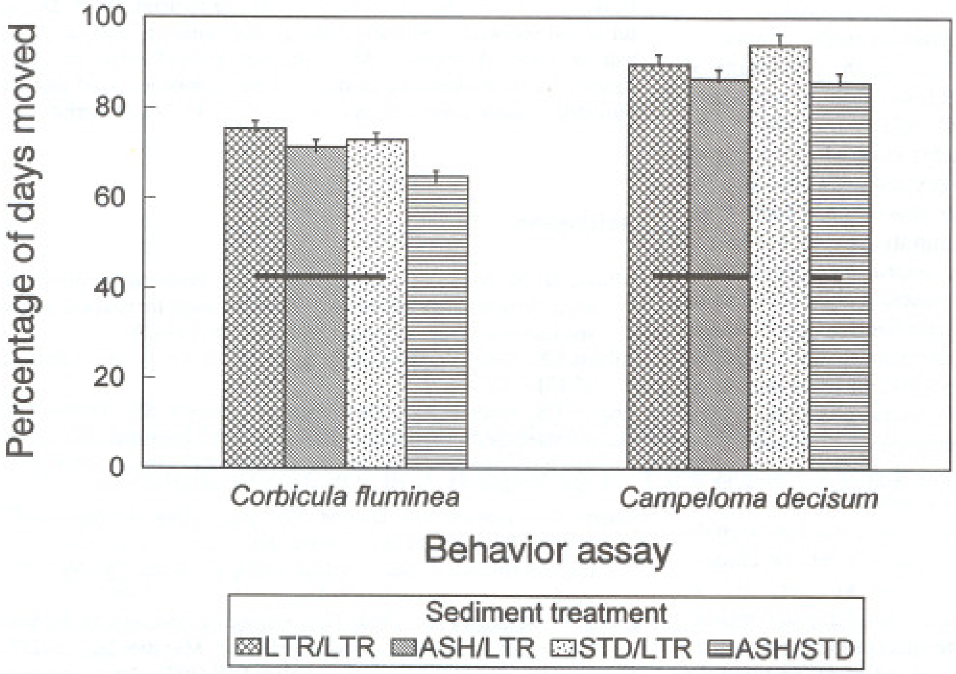

Fig. 2. Mean percentage of days moved by clams and snails at different paired sediment treatments (LTR/LTR, ASH/LTR, STD/LTR and ASH/STD). Error bars denote one standard error around the mean. Means without overlapping horizontal bars are significantly different ($\alpha = 0.05$) as determined with the Student-Newman-Keuls procedure. The number of clams per paired sediment treatment was forty

Discussion

The element levels found in sediments from the present study were consistent with what was expected. Sediment from below the ash settling basin (ASH) had elevated levels of As and Se, which was consistent with previous studies on the Savannah River Site (Cherry *et al.* 1979a, 1979b). The sediment collected from Steed Pond (STD) had elevated levels of Ni and U, reflecting known metal releases into Steed Pond via Tim's Branch (Pickett *et al.* 1987). The sediment collected from LTR contained low levels of all metals analyzed, suggesting that LTR has received little or no contamination from these metals. These low levels of contamination would be supported by an earlier study (Alberts *et al.* 1985). While none of these sediments was highly contaminated, they were all similar in both organic carbon content and particle size, which allowed us to determine the effect of sediment element contamination and not some other characteristic of the sediment (organic carbon). There were minor differences in percent organic carbon and median particle size among sediments, although these differences were probably biologically insignificant. Overall, the results indicated that both STD and ASH sediments were more contaminated than LTR sediment. The fractionation data suggested that more elements were bioavailable in STD and ASH sediment than in LTR sediments.

The results from the present study showed that asiatic clams (*Corbicula fluminea*) preferred uncontaminated sediment (LTR) when paired with one of the contaminated sediments (ASH). The avoidance of element contaminated sediment has been shown in previous studies using benthic organisms (McGreer, 1979; Oakden *et al.* 1984; Erdem and Meadows 1980). Because both sediment and the opposite sediment significantly affected location, clams not only preferred one sediment but avoided the opposite sediment. Although these results clearly demonstrate avoidance of contaminated sediment, multiple factors likely determine location in sediment as evidenced by the significant preference for the left side of the tank.

In contrast to clams, a freshwater viviparid snail (*Campeloma decisum*) showed no significant sediment preference. In fact, the snails had a general but statistically insignificant preference for contaminated sediments (STD and ASH) when paired with uncontaminated sediment (LTR). As *C. decisum* feed on organic debris and sediment associated microflora (Newman and McIntosh 1982), differences in food content between different sediment types could have affected sediment preference. If snails were feeding within the sediment, one sediment type could contain more food (organic debris) and therefore snails may spend more time in that particular sediment type. Even though snails did not significantly prefer one sediment type over another, the feeding ecology suggested that sediment preference may be affected more by sediment food content than by sediment metal concentration. In contrast, *C. fluminea* obtained a smaller proportion of their food from the sediment, which suggested that their sediment preference could be less dependent on the food content of the sediment and more dependent on other factors (contamination).

Both clams and snails had a significant preference for the left side of the test tank when compared to the right side, regardless of the sediment type. This effect may have been due to the orientation toward or away from an external stimulus in the laboratory, e.g., light or temperature. However, much effort was made to reduce directional stimuli by randomly placing tanks within the laboratory and covering the window. The tendency for animals to prefer the left side of the tank over the right side could be controlled if certain measures are taken prior to initiating a behavioral assay. External stimuli (light and temperature) need to be uniform throughout the room where the experiment is run. External factors that could affect behavior need to be determined prior to starting a behavior study. If animals are orienting to one side over another, these factors can be controlled and the experimental design structured to quantify such external factors. Even though both clams and snails preferred the left side of the test tank, this was accounted for in the experimental design and sediment preference was still discernable. Because of external factors that affected behavior, good experimental design was critical to obtaining usable results.

Sediment treatment also had an effect on the percentage of days moved by clams. Clams moved fewer days in tanks containing both contaminated sediments (ASH/STD) when compared to other paired treatments. Phelps *et al.* (1983) reported that above 5.8 μg/g copper added to dry sediment, the time for 50% of littleneck clams (*Protothaca staminea*) to burrow increased with increasing copper concentrations. The burrowing activity of juvenile hard clams (*Mercenaria mercenaria*) was found to decrease in sediment contaminated with oil (Olla *et al.* 1983). Whereas sediment contamination had no significant effect on percentage of days buried in the present study, percentage of days moved by clams was significantly decreased in tanks containing both contaminated sediments (ASH, STD). Therefore, in the present study, percentage of days moved by clams was a more useful indicator of sediment contamination than percentage of days buried or daily movement.

Even though contaminated sediment levels did not strongly affect clam behavior, higher concentration levels or chronic exposure in the field could decrease burrowing activity. A decrease in activity by *C. fluminea* in the field due to contaminated sediment could make them more susceptible to predation. Pearson *et al.* (1981) reported that oiled sediment inhibited the burrowing activity of the littleneck clam leading to increased predation by the Dungeness crab (*Cancer magister*).

In contrast to clams, snail behavior showed no apparent relationship between the degree of sediment contamination and burrowing behavior. These results also support the relative insensitivity of *C. decisum* behavior as an indicator of sediment contamination when compared to *C. fluminea*. Even though clam behavior appears to be a more sensitive indicator of sediment metal contamination than snail behavior, they both were sensitive to external factors in the laboratory as evidenced by the preference for the left side of the aquaria. Controlling external stimuli in the laboratory when using both *C. fluminea* and *C. decisum* for behavioral assays would be important for reducing directional movement external to the assay. Further tests are necessary to determine the relative sensitivity of both *C. fluminea* and *C. decisum* and to determine their usefulness in future behavioral tests.

The results support the continued use of behavioral responses as sublethal indicators of sediment contamination. However, it also suggests that control and quantification of other influences such as tank side are critical in studies using low levels of contamination. While the ecological importance of avoidance behaviors in the field cannot be determined solely from assays such as the present study, these behaviors may be useful in explaining the distribution of benthic organisms in polluted areas. Avoidance assays should be useful in future studies which attempt to assess the environmental impact of contaminated sediment on the benthic communities.

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