Influence of Sediment Type and Exposure Time on Likeness of Colonization Tray and Background Macroinvertebrate Assemblages

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ABSTRACT

Colonization trays containing three sediment types (formulated, Florissant, and pond) were field deployed in a pond and a stream to assess the influence of sediment type, colonization time, and habitat type on benthic macroinvertebrate colonization. Benthos colonizing the trays was compared to ambient fauna to determine similarity. The pond was chosen as the optimal habitat type. By week 12, organisms colonizing trays in each of the three sediment types placed in the pond were not statistically different from Ekman grab samples in regard to both total abundance and abundance of each dominant taxa. The Florissant-filled colonization trays were the most similar to Ekman grab samples. Percent composition of dominant taxa was most similar (89% based on an affinity index) to Ekman grab samples and had the least variation in composition from the Ekman grab samples. The majority of invertebrates in the stream colonized a superficial layer of debris rather than in the sediments originally placed in the trays.

INTRODUCTION

Studies using sediment colonization trays are rarely used to evaluate benthos colonization in soft sediments (Ruth et al. 1994, Hare 1995, Cowell 1984). However, colonization tray studies have addressed several interesting and important questions such as the effect of tray surface area (Ruth et al. 1994) and differences in colonization between littoral and profundal zones (Hare 1995). Comparing colonization of benthos aggregates on contaminated sediment-filled trays to those in the environmental surrounding has provided a useful approach to evaluate simulated disturbances (Cowell 1984, Soster and McCall 1990). To be effective in toxicity studies, the trays containing uncontaminated sediment should exhibit colonization similar to that in the surrounding environment. The main benefits of using colonization trays in toxicological studies are: (1) the ability to dose a smaller amount of sediment and to contain the contaminated sediment, thereby minimizing exposure to the aquatic system, and (2) having control over physical conditions of the sediment such as grain size and total organic carbon.

The interest for evaluating effects of different sediment types arose from studies where sediment-filled trays were used to study the effects of contaminated sediment on benthos (Watzin et al. 1994, Liber et al. 1996, Hare et al. 1994, Flemer et al. 1997). Among these studies, different sediment sources were used as a tray substrate. Watzin et al. (1994) chose an artificially formulated sediment in order to control physical characteristics such as grain size, organic matter content, and pH of pore water. In contrast, Liber et al. (1996), Hare et al. (1994), and Flemer et al. (1997) used natural

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sediment obtained from the habitat used to conduct their colonization experiments.

The objective of our study was to evaluate the potential influences of sediment type and colonization time when using sediment-filled trays to evaluate environmental disturbance responses. This was done by an evaluation of the similarity of benthos colonization in trays containing one of three uncontaminated sediment types (formulated, native pond, and a reference soil from Florissant, MO). These colonization trays were placed in a pond, and a stream pool, over a 12- week period. We specifically sought to determine optimal conditions (sediment type, placement system, and duration) for benthic colonization. Optimal exposure conditions were determined by comparing the benthic assemblages on the trays to those collected from the surrounding habitat.

METHODS AND MATERIALS

Study Sites

Trays were placed in a stream and a pond, located at the Columbia Environmental Research Center (CERC) in Columbia, MO. The 50 m long experimental stream consisting of three sections of riffle, each separated by a pool has previously been described by Fairchild et al. (1987). For this study the middle pool (10m long x 2m wide x 0.30m deep) was used for tray placement. During the 1999 study, average sediment depth in the pool used was 22 cm. The stream was fed by well water (hardness 280 mg/L, velocity over trays 0.02 m/s). A 95% shade cover was placed over the entire stream.

Pond #37, a clay-lined 0.1 ha structure, was described by Fairchild et al. (1992). The study area was a 1x3m area in the center of the pond. To control extensive submersed vegetation growth, primarily *Chara* and *Naja* sp., a 30 m x 30 m, 95% shade cover was placed over the center portion of the pond. The mean pond water depth was 2.5 m. In the 1x3 m study area, the pond was drawn down to 1 m for tray placement and retrieval.

Colonization Trays

Colonization trays were filled with three sediment types: 1) a formulated sediment (low sand, 1.5% TOC) as described by Kemble et al. (1999), 2) an uncontaminated soil (Florissant soil) which has been used as a control sediment in previous toxicity studies (Ingersoll et al. 1996), and 3) sediment collected from the pond (Table 1).

Sediments were filled to the tops of 0.72 L polypropylene trays (15 x 14 cm surface area, 5.4 cm deep). Tray depth of 5.4 cm was chosen due to stream depth limitations, and this was also the biologically relevant layer of interest. Trays were placed into the pond and stream in late April 1999, and three replicate trays of each sediment type were sampled 6 and 12 weeks after placement.

Table 1. Physical characteristics of the three sediment types placed in the colonization trays.

Sediment Type	**Particle Size (%)**		**Organic Carbon (%)**
	sand	silt + clay	
Formulated	14	86	1.5
Florissant	0	100	1.0
Pond	24	76	1.5

Additional side experiments involving variations on the tray design were conducted using the pond sediment placed in the pond habitat. These experiments were done in triplicate and had a colonization time of 12 weeks. One set of replicates had holes drilled in the sides of the trays, and one set had a mesh cover as in (Liber et al. 1996). These modifications were introduced to evaluate whether they would increase colonization. Another replicate set consisted of larger trays (26 x 16 cm surface area, 6 cm deep, 2 L volume), similar in size to previous studies (Liber et al. 1996; Hare et al. 1994).

Evaluation of absence of macroinvertebrates in the native pond sediment

Dry pond sediment was collected during the eight-month period prior to the study when the pond was drained. Sediment was wetted with well water, homogenized, and prepared for placement into the colonization trays. This prepared sediment was also evaluated in four beakers which were placed in a laboratory water bath with renewal of overlaying water to mimic the pond environment. After six weeks, there was no evidence of any benthic organisms. Therefore, drying of the sediment probably eliminated all benthic organisms. All organisms present in the pond sediment-filled trays can be assumed to have colonized during the study and not before the start of the study.

Macroinvertebrate processing

Ekman grabs were collected from both the pond and stream on weeks 6 and 12 of the study. The samplers enclosed a 15 x 15cm surface area. Macroinvertebrates and debris from trays and Ekman grab samples were washed through a 500 μm mesh sieve and preserved in 80% ethanol. Organisms were sorted to major taxonomic groups 1) oligochaetes (Annelida:Oligochaeta), 2) chironomids (Diptera:Chironomidae), 3) other Diptera, 4) molluscs (both Gastropoda and Pelecypoda), 5) Ephemeroptera in the pond habitat, and in the stream habitat, Hirudinea (Annelida: Hirudinea) and, 6) other. The "other" category for pond samples included Tricoptera, Hirudinea, Odonata, Megaloptera, Hemiptera, Coleoptera, and amphipods. The "other" category for stream samples included Trichoptera, Ephemeroptera, Odonata, Megaloptera, Hemiptera, Coleoptera, and amphipods.

Table 2. Mean number of organisms/m^2 (SD) in each treatment compared to the Ekman grab samples during the pond study (n=3). Values within a sampling date followed by a similar letter are not significantly different (p >0.05) across sediment types.

	WEEK 6				WEEK 12			
Organisms	Ekman	Formulated	Florissant	Pond	Ekman	Formulated	Florissant	Pond
Total	58.4(0.2) a	24.1(13.7) b	17.6(9.2) b	5.1(3.1) c	10.0(3.7) a	23.0(20.1) a	14.3(2.9) a	23.3(30.6) a
Oligochaete	39.4(9.6) a	6.5(9.6) b	9.7(8.4) ab	1.3(2.2) b	4.1(2.5) a	10.5(9.9) a	4.6(5.1) a	1.4(2.1) a
Chironomid	8.3(6.7) a	4.0(3.0) ab	1.3(1.5) b	0.8(0.7) b	3.9(21.0) a	0.8(1.0) b	5.7(3.3) a	2.9(2.1) ab
Other Diptera	1.0(0.3)a	3.7(2.4) a	5.1(1.7) a	2.2(1.2) a	0.6(0.3) a	0.6(0.6) a	1.8(2.2) a	0.1(03) a
Mollusca	5.5(5.3) a	10.0(8.3) a	0.5(0.8) b	0.2(0.3) b	0.6(0.6) a	10.6(11.8) a	1.3(1.8) a	18.9(32.8) a
Ephemeroptera	2.1(1.7) a	0 a	0.3(0.3) a	0.6(1.1) a	0.8(1.3) a	0.3(0.3) a	0.9(0.5) a	0 a
Other	2.1(1.0) a	0 b	0.8(0.7) ab	0 b	0.1(0.3) a	0.1(0.3) a	0 a	0 a

Statistical Analysis

Mean benthos colonization (n=3) of each sample type (Ekman grab, formulated, Florissant, and pond) were used in a ranked analysis of variance (ANOVA) procedure. The Tukeys procedure was performed to determine significant differences.

Mean percent composition (n=3) was determined for each major taxonomic group. Percent composition of each major taxonomic groups was ranked from 1 to 6, with 6 being the most abundant. The cumulative differences between the Ekman grab samples and each of the three sediment types were compared. The major taxonomic groups were evaluated with an affinity index (Silver 1975), which is based on the similarity in proportions of the macroinvertebrates colonizing trays compared to those from the Ekman grab samples. All data analyses were performed with the Statistical Analysis System. (SAS 1989)

RESULTS

Pond Colonization

At six weeks, the total numbers of organisms colonized in all three sediment types were statistically lower than in the Ekman grabs. By 12 weeks, colonizing benthos was more similar to that from the Ekman grab samplers. Of the three sediment types, Florissant soil was most similar with no statistical differences in mean total numbers or any individual taxonomic group between Ekman grabs and Florissant soil. Benthos in the formulated and pond sediment differed from the Ekman grabs in regard to only one taxonomic group, the chironomids, which were statistically less abundant in the colonization trays. (Table 2).

Over time the percent composition of dominant taxa in all sediment types became increasingly similar to the composition of the Ekman grab samples. Benthos colonizing the Florissant-filled trays at week 12 was the most similar (89%) to the Ekman grab samples when evaluated with the affinity index (Fig. 1). The same percent compositions of the dominant macroinvertebrates used in the affinity index were ranked and the total variations from the Ekman grab samples were evaluated (Table 3). By week 12 the Florissant samples varied the least from the Ekman grabs in regard to percent composition of colonizing organisms.

The ranked ANOVAs of mean macroinvertebrate colonization at week 12 showed no significant differences between the Ekman grabs and either the standard trays or the three alternative types of trays (side holes, larger, mesh covered) which were filled with pond sediment (Table 4). This was true for the mean total number, and mean total from each taxonomic group present. The mesh-covered trays were most similar to the environmental samples (81%) according to the affinity index, primarily due to the higher percentage of oligochaetes and a reduced number of molluscs in both the Ekman grab samples and mesh-covered trays.

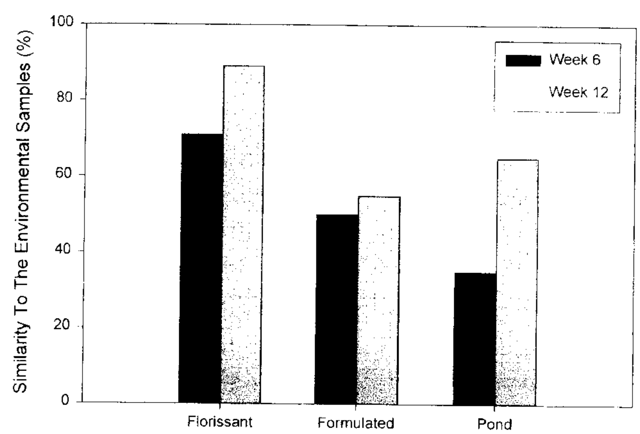

Fig. 1. Similarity based on the percent composition of dominant macroinvertebrates in the three sediment types used in colonization trays compared to the Ekman grab samples comparing weeks 6 and 12 in the pond.

Table 3. Ranked order of means of percent composition of the dominant macroinvertebrates colonized during the pond study comparing three sediment types to the Ekman grab samples (n=3).

WEEK 6	Ekman	Formulated	|Dif|	Florissant	|Dif|	Pond	|Dif|
Oligochaete	6	4	|2|	6	|0|	4	|2|
Chironomid	5	5	|0|	4	|1|	5	|0|
Mollusca	4	6	|2|	3	|1|	2	|2|
Other	3	1.5	|1.5|	2	|1|	1	|2|
Ephemeroptera	2	1.5	|.5|	1	|1|	3	|1|
Other Diptera	1	3	|2|	5	|4|	6	|5|
Total variation			8		8		12

WEEK 12	Ekman	Formulated	|Dif|	Florissant	|Dif|	Pond	|Dif|
Oligochaete	6	5	|1|	5	|1|	4	|4|
Chironomid	5	6	|1|	6	|1|	6	|6|
Other Diptera	4	3	|1|	4	|0|	3	|3|
Ephemeroptera	3	2	|1|	2	|1|	1.5	|1.5|
Mollusca	2	4	|2|	3	|1|	5	|5|
Other	1	1	|0|	1	|0|	1.5	|1.5|
Total variation			6		4		9

Table 4. Mean number of organisms/m^2 (SD) in alternative types of trays filled with pond sediment compared to the Ekman grab samples after 12 weeks in the pond (n=3). Values followed by a similar letter are not significantly different ($p \geq 0.05$) across sediment types.

Organisms	Ekman	Standard	Hole	Large	Mesh
Total	10.0(3.7) a	23.3(30.6) a	9.4(7.0) a	15.4(20.5) a	19.2(8.5) a
Oligochaete	4.1(2.5) a	1.4(2.1) a	3.7(3.2) a	5.2(8.7) a	7.0(5.9) a
Chironomid	3.9(21.0) a	2.9(2.1) ab	2.9(3.3) a	7.8(9.0) a	6.8(1.9) a
Other Diptera	0.6(0.3) a	0.1(0.3) a	1.1(1.5) a	1.0(0.9) a	2.7(1.9) a
Mollusca	0.6(0.6) a	18.9(32.8) a	1.4(1.2) a	1.1(1.5) a	0.6(1.1) a
Ephemeroptera	0.8(1.3) a	0 a	0 a	0.1(0.3) a	0.5(0) a
Other	0.1(0.3) a	0 a	0.3(0.6) a	0.5(0.5) a	1.6(1.5) a

Stream Colonization

At week 6 only one taxonomic group, the oligochaetes, was statistically less abundant in all three sediment types than in the Ekman grab sample. At week 12, there were no statistical differences in regard to both total numbers and all taxonomic groups present (Table 5).

Over time the percent composition of dominant taxa in all sediment types became increasingly similar to the composition of the Ekman grab samples. Benthos colonizing the Florissant-filled trays at week 12 was the most similar (95%) to the Ekman grab samples when evaluated with the affinity index (Fig. 2). The same percent compositions of the dominant macroinvertebrates used in the affinity index were ranked and the total variations from the Ekman grab samples were evaluated (Table 6). By week 12 the formulated samples varied the least from the Ekman grabs in regard to percent composition of colonizing organisms.

In the stream environment the trays became filled with a layer of sediment and debris from the surrounding environment. During sample processing it was observed that the majority of invertebrates were found in this superficial material rather than in the sediments placed in the trays. This layer of debris would cause bias in toxicological studies using experimentally contaminated sediments.

Table 5. Mean number of organisms/m^2 (SD) in each treatment compared to the Ekman grab samples during the stream study (n=3). Values within a sampling date followed by a similar letter are not significantly different (p >0.05) across sediment types.

	WEEK 6				WEEK 12			
Organisms	Ekman	Formulated	Florissant	Pond	Ekman	Formulated	Florissant	Pond
Total	4.3(19.0) a	14.3(10.4) a	15.7(41.4) a	13.3(6.2) a	30.2(26.2) a	12.0(2.0) a	34.0(7.9) a	25.1(22.1) a
Oligochaete	35.2(15.8) a	1.4(0.5) c	8.9(21.9) b	7.1(4.0) b	27.6(26.6) a	8.1(3.4) a	2.89(10.2) a	21.8(20.0) a
Chironomid	0.9(0.4) a	2.7(0.7) a	1.8(1.7) a	1.8(1.7) a	0.6(0.3) a	0.5(0) a	0.3(0.3) a	1.1(1.5) a
Other Diptera	1.3(0.9) a	1.6(1.4) a	0.8(1.0) a	0.8(1.0) a	0.4(0) a	0.6(0.3) a	0.6(0.3) a	0.1(0.3) a
Mollusca	5.0(3.4) a	8.4(9.2) a	4.3(1.2) a	3.2(3.2) a	1.5(1.1) a	2.0(2.3) a	2.7(2.2) a	1.4(2.5) a
Hirudinea	0.4(0) a	0.1(0.3) a	0 a	0.5(0.5) a	0.1(0.3) a	0.8(1.0) a	0.1(0.3) a	0.1(0.3) a
Other	0 a	0 a	0 a	0 a	0 a	0 a	1.3(2.2) a	0.5(0.8) a

Table 6. Ranked order of means of percent composition of the dominant macroinvertebrates colonized during the stream study comparing three sediment types to the Ekman grab samples (n=3).

WEEK 6							
	Ekman	Formulated	|Dif|	Florissant	|Dif|	Pond	|Dif|
Oligochaete	6	4	|2|	6	|0|	6	|0|
Mollusca	5	6	|1|	5	|0|	4	|1|
Other Diptera	4	3	|1|	3	|1|	3	|1|
Chironomid	3	5	|2|	4	|1|	5	|2|
Hirudinea	2	2	|0|	1.5	|.5|	2	|0|
Other	1	1	|0|	1.5	|.5|	1	|0|
Total variation			6		3		4
WEEK 12							
	Ekman	Formulated	|Dif|	Florissant	|Dif|	Pond	|Dif|
Oligochaete	6	6	|0|	6	|0|	6	|0|
Mollusca	5	5	|0|	5	|0|	4	|1|
Chironomid	4	2	|2|	2	|2|	3	|1|
Other Diptera	3	3	|0|	3	|0|	2	|1|
Hirudinea	2	4	|2|	1	|1|	5	|3|
Other	1	1	|0|	4	|3|	1	|0|
Total variation			4		6		6

DISCUSSION

Selection of Optimal Conditions for Colonization Studies

An extensive amount of debris deposited on the surface of the stream trays during the colonization study up to 4 cm by week 12. Although this deposition is common in stream pools (Fairchild et al. 1987), the goal of this study was to evaluate colonization in specific sediment types. It is critical for our toxicological study that benthos be exposed to the sediments placed in the trays. Since most of the colonizing benthos was present in this top layer, not within the sediment in the trays, we conclude that toxicological colonization studies using spiked sediments in trays would be inappropriate in this stream system. However, it is important to note that at week 12 in the stream, benthos colonizing the Florissant-filled trays were very similar (95%) to the environmental samples. Thereby, while these trays do not meet our specific needs, they could be a useful tool in the assessment of stream benthos.

The optimum colonization time in the pond was 12 weeks, and colonization on all three sediment types was similar to the macroinvertebrates collected in the grab samples. A concern with these results is that although there were no statistical differences between the sediment-filled colonization trays and the Ekman grab samples at week 12, that may be the results of the variation among replicates. Since we only used a sample replication of three and considering the observed variations, it would be appropriate to at least double the number of replicates in the future.

The optimal sediment type was the Florissant soil. At week 12 in the pond, benthos colonizing the Florissant-filled trays were the most similar (89%) to the environmental samples. In addition, previous studies have indicated that this sediment works well as a control sediment in toxicity tests (Ingersoll et al. 1996). Therefore, the Florissant soil (a fine silt- and clay- particle size soil obtained from an agricultural area) is recommended as a sediment type in sediment-spiking studies.

Our formulated sediment contained 1.5 % organic carbon, which was higher than that of the Florissant soil (1%). There was evidence of bacterial conditioning in the formulated sediment, which may result in a better substrate for colonization by macroinvertebrates. At week 6, material had turned from white to a dark grey in half of the tray and was black on top. By week 12, the formulated sediment increased in blackness and also appeared to have swelled in the container. The benefit of future studies using an aged or "conditioned" formulated sediment has been suggested previously by Kemble et al. (1999) and may be a useful tool in future colonization studies.

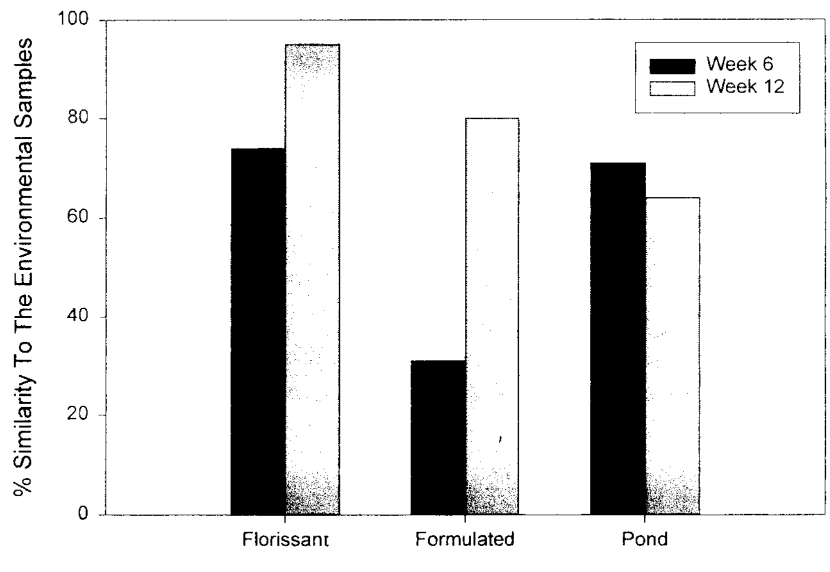

Fig.2. Similarity based on the percent composition of dominant macroinvertebrates in the three sediment types used in colonization trays compared to the Ekman grab samples comparing weeks 6 and 12 in the stream..

Colonization Tray size

Effects of tray size are an important consideration when designing a study (Hare et al. 1995). Desirable attributes of smaller trays include reaching equilibrium faster (Minshall 1988). However, a smaller container size increases the importance of edge effects (Snelgrove et al. 1992). Ruth et al. (1994) evaluated tray size effects with a 6-week colonization study using four tray sizes, and determined that tray size affected both the total abundance and the number of taxa. In our study, the surface area of the standard tray was 210 cm^2. Trays with a surface area of 416 cm^2 were not significantly different in the total number of organisms or numbers of each taxonomic group present. Thereby, it can be assumed that the 210 cm^2 trays are of sufficient size for the pond of interest.

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LITERATURE CITED

Cowell B.C. 1984. Benthic invertebrate recolonization of small-scale disturbances in the littoral zone of a subtropical Florida lake. *Hydrobiologia* 109:193-205.

Fairchild J.F., T. Boyle, W. R. English, and C. Rabeni. 1987. Effects of sediment and contaminated sediment on structural and functional components of experimental stream ecosystems. *Wat Air and Soil Pollut 36:271-293.*

Fairchild J.F., T. W. LaPoint, J.L. Zajicek, M.K. Nelson, F. J. Dwyer, and P.A. Lovely. 1992. Population , community and ecosystem level responses of aquatic mesocosms to pulsed doses of a pyrethroid insecticide. *Environ Toxicol Chem* 11:115-129.

Flemer D.A., B. F. Ruth, C. M. Bundrick, and J.C. Moore. 1997. Laboratory effects of microcosm size and the pesticide chlorpyrifos on benthic macroinvertebrate colonization of soft estuarine sediments. *Mar Environ Res 43:243-263.*

Hare L., R. Carignan, and M.J. Huerta-Diaz. 1994. A field study of metal toxicity and accumulation by benthic invertebrates; implications for the acid-volatile (AVS) model. *Limnol Oceanogr* 39: 1653-1668.

Hare L. 1995. Sediment colonization by littoral and profundal insects. *JNABS* 14:315-323

Ingersoll C.G., P.S. Haverland, E.L. Brunson, T.J. Canfield, F. J. Dwyer, C.E. Henke, N.E. Kemble, D.R. Mount, and R.G. Fox. 1996. Calculation and evaluation of sediment effect concentrations for the amphipod *Hyalella azteca* and the midge *Chironomus riparius*. *J Great Lakes Res* 602-623.

Kemble N.E., F.J. Dwyer, C. G. Ingersoll, T. D. Dawson, and T.J. Norberg-King. 1999. Tolerance of freshwater test organisms to formulated sediments for use as control materials in whole-sediment toxicity tests. *Environ Toxicol Chem* 18:222-230.

Liber K., D.J. Call, T.P. Markee, K.L. Schmude, M.D. Balcer, F.W. Whiteman, and G.T. Ankley. 1996. Effects of acid-volatile sulfide on zinc bioavailability and toxicity to benthic macroinvertebrates: a spiked-sediment field experiment. *Environ Toxicol Chem* 15: 2113-2125.

Minshall G.W. 1988. Stream ecosystem theory: a global perspective. *JNABS* 7:263-288.

Ruth B.F., D.A. Flemer, and C.M. Bendrick. 1994. Recolonization of estaurine sediments by macroinvertebrates: does microcosm size matter? *Estuaries 17:606-613.*

SAS Institute. 1989. SAS User's Guide: Statistics, Version 5 Edition. Cary, NC.
Silver M.W. 1975. The habitat of *Salpa fusiformis* in the California current as defined by indicator assemblages. *Limnol Oceanogr* 20: 230-237.
Snelgrove P.V.R., J.F. Grassle, and R.F. Petrecca. 1992. The role of food patches in maintaining high deep-sea diversity: field experiments with hydrodynamically unbiased colonization trays. *Limnol Oceanogr* 37: 1543-1550.
Soster F.M., and P.L. McCall. 1990. Benthos response to disturbance in western Lake Erie: field experiments. *Can J Fish Aquatic Sci* 47:1970-1985.
Watzin M.C., P.F. Roscigno, and W.D. Burke. 1994. Community-level field method for testing the toxicity of contaminated sediments in estuaries. *Environ Toxicol Chem* 13: 1187-1193.

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