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Sediment Toxicity in Reach 15 of the Upper Mississippi River

Technical Report to the Illinois Department of Natural Resources

Center for Aquatic Ecology

Denise B. Stoeckel, K. Douglas Blodgett, and Richard E. Sparks

Illinois Natural History Survey
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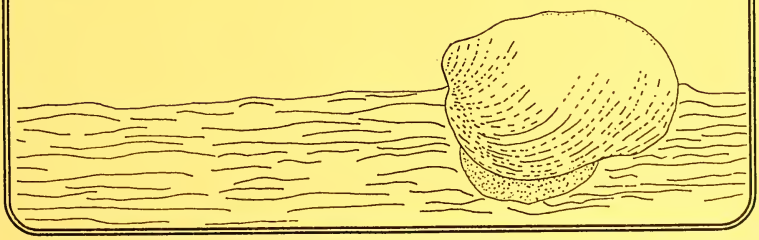

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DISCLAIMER

The findings, conclusions, and views expressed herein are those of the researchers and should not be considered as the official position of the Illinois Department of Natural Resources.

ACKNOWLEDGMENTS

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ABSTRACT

The goal of this project was to determine whether Reach 15 of the Upper Mississippi River contained any toxic sediments that would threaten the native mussel refuges within the reach. Toxicity assays were conducted on porewaters collected from sediments of three sites of Reach 15 during 1994 and 1995: near ALCOA, Sylvan Slough, and Campbell's Slough. *Ceriodaphnia dubia* acute toxicity and survival/reproduction 7-day assays were performed on porewaters of collected sediments in the first year. Results from the first year indicated sediments collected from the ALCOA site were toxic. Work in the second year consisted of *C. dubia* survival/reproduction 7-day assays and a mussel filtering assay (MFA) that measured filtering rates of a native mussel, the deertoe (*Truncilla truncata*). In Year 2, chronic toxicity was detected in sediments from Sylvan Slough and Campbell's Slough, but not in sediments collected from the ALCOA site. The differences in the location of toxic sediments within Reach 15 from Year 1 to Year 2 indicates that the sources and deposition sites for toxicants may vary from year to year. We concluded that toxic sediments did occur within Reach 15 of the Upper Mississippi River, especially within Sylvan Slough, home to the federally-endangered Higgin's-eye pearly mussel, *Lampsilis higginsi*. The mussel filtering assay used in this project proved to be an inexpensive tool for the assessment of sediment toxicity to native mussels.

1. Introduction

Reach 15 of the Upper Mississippi River contains one of seven mussel refuges established by the Illinois Department of Natural Resources, formerly the Illinois Department of Conservation, and is home to the federally-endangered Higgin's-eye pearly mussel, *Lampsilis higginsi* (Blodgett and Sparks 1987a, b, and c). The refuges were established in 1988 to protect endangered or threatened mussels, to provide a source of native mussel species to repopulate other areas, and to serve as unharvested reference areas for comparison with harvested areas.

A massive die-off of native mussels occurred in the Upper Mississippi River from 1981 through 1986 (Blodgett and Sparks 1987a, b, and c). Large numbers of two economically important mussels species, the threeridge (*Amblema plicata*) and the washboard (*Megalonaias gigantea*), died in Reaches 14 and 15 of the Mississippi River (Fritz 1983; Blodgett and Sparks 1987c). Subsequent research to identify the causes of this die-off was unsuccessful (Sparks *et al.*, 1990) and the reason for the die-off remains a mystery.

This document reports the results of sediment toxicity studies performed using *Ceriodaphnia dubia* to determine whether there were any toxic sediments that may threaten the refuges, including any toxicity that might linger from the 1981 to 1986 die-offs. Toxicity assays were conducted on sediment porewaters collected from Reach 15 of the Mississippi River during 1994 and 1995. *Ceriodaphnia dubia* acute toxicity and survival/reproduction 7-day assays were performed on porewaters of collected sediments in the first year. Although short-term toxicity studies using a standard reference organism, *C. dubia,* were the only tests planned, findings from the first year of the study (1994) provided enough evidence to warrant further studies. Work in the second year consisted of *C. dubia* survival/reproduction 7-day

assays and a mussel filtering assay (MFA). The MFA tested for toxicity of the porewaters by observing filtering rates of a native mussel, the deertoe (*Truncilla truncata*).

2. Methods

Ceriodaphnia dubia survival/reproduction assays and 48-hour toxicity assays were used the first year to detect sediment toxicity in Reach 15.

2.1. Materials and Methods

2.1.1 Sediment and Porewater Collection Procedures

This study required the collection of sediment from which we extracted porewater for use in the assays. It is important to collect sediment in a manner that disturbs the collection site as little as possible so as to collect a representative sample and increase the likelihood that laboratory experiments conducted with the collected sediments or porewaters simulate experiments conducted *in situ*, i.e., at the site of collection (ASTM 1991). The collection of sediments with a Ponar grab was considered the most efficient means of collecting a sample with minimum disturbance of the collection site. Sediment samples were collected from designated sample sites within Reach 15 of the Mississippi River (Table 1, Figure 1) with a

Sample Site	River Mile
Campbell's Slough	490.2
ALCOA	489.2
Sylvan Slough	485.0

Table 1. Locations of sediment collection sites in Pool 15 given in Mississippi River Miles upstream from the Ohio River. Sample site locations in this table correspond to sample site locations in Figure 1. Sediment samples were collected at these locations using a Ponar grab.

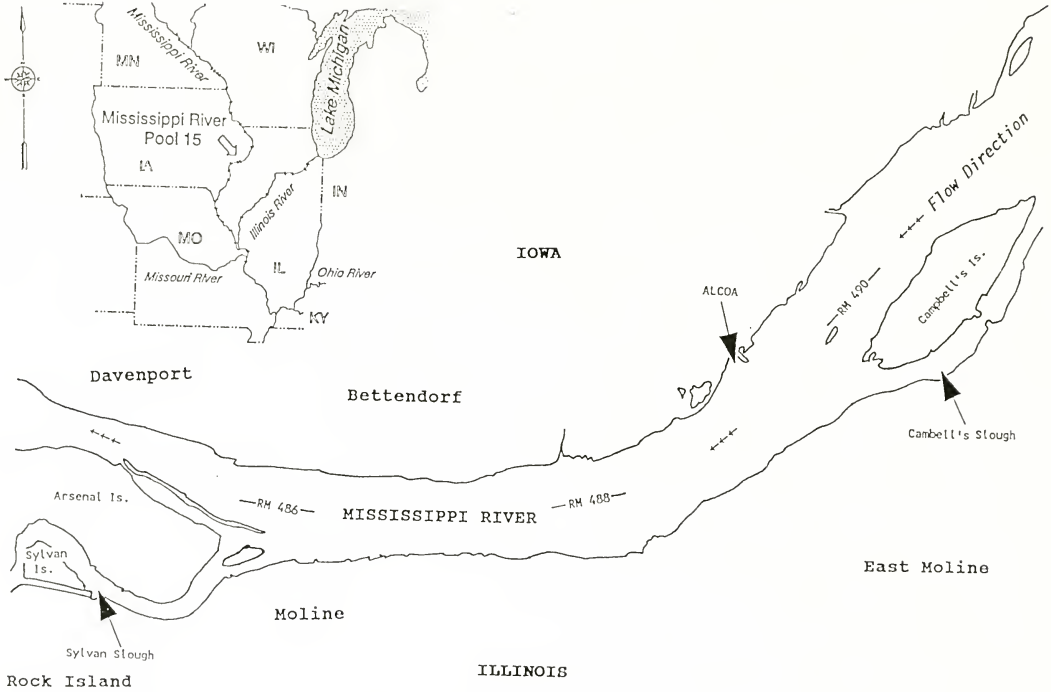


Figure 1. Location of sediment sampling sites within Reach 15 of the Mississippi River. Sites (inidicated by the black arrows) were identified in tocixity tests by the name of the nearest land site or waterway. Campbell's Slough = RM 490.2; ALCOA = RM 489.2; Sylvan Slough = RM 485.30.

Ponar grab. Collected sediment was placed in prewashed high-density polypropylene ice chests (Coleman®) and then covered with river water with no or minimum air space. Sediments were stored in the ice chests for less than two weeks before testing.

Porewater was extracted from the collected sediments by centrifugation. Sediment (approximately 250-300 g) was placed in high-density polypropylene centrifuge bottles and spun for 30 minutes at 4,000 X g and 4°C. The collected porewater was placed in prewashed 1-L cubitainers with no head space and stored at 4°C for a maximum of one week. Prior to testing, porewaters were poured through a 110-µm mesh screen to remove any large particulates and extraneous organisms (i.e., copepods and midge larvae) and allowed to reach test temperature (20 ± 1°C).

2.1.2. Control Solutions

Standard reconstituted water (pH = 7.6-8.0, hardness = 160-180 ppm $CaCO_3$, alkalinity = 110-120 ppm $CaCO_3$) (Marking and Dawson 1973) was used as the control solution in sediment toxicity tests conducted in the first year. Standard reconstituted water was made as needed. Filtered creek water was used to culture organisms used in the assays both years of the study and as a control solution for the second year of work. Filtered creek water was obtained by filtering Quiver Creek water (Havana, IL) through a sand filter. Dissolved oxygen, pH, hardness, alkalinity, conductivity, and temperature of the control solution were measured and recorded before testing.

2.2. Organisms

2.2.1. *Ceriodaphnia dubia*

Starter cultures of *C. dubia* were obtained from the Environmental Research Laboratory

of the United States Environmental Protection Agency (USEPA) at Duluth, Minnesota. One half of the starter culture was placed in a 20-L aquarium containing 5 L of hard standard reconstituted water and the other half of the culture was placed in a similar aquarium containing 5 L of filtered creek water. These cultures were fed a 1:1 mixture of yeast-cerophyll-trout chow solution (YCT) and *Selenastrum capricornutum* according to *C. dubia* (NETAC 1992a, 1992b). Culture water was renewed twice weekly, and cultures were exposed to an approximate 16h:8h light:dark cycle.

Approximately one week before testing, a styrofoam brood board was set up by removing 60 neonates (< 24 h) from each of the cultures and placing each one into a 30-ml plastic cup containing 25 ml hard standard reconstituted water. Brood board cultures were fed 0.1 ml YCT and 0.1 ml *S. capricornutum* solution daily.

2.2.2. *Truncilla truncata*

Deertoe mussels, *T. truncata*, were collected from Reach 15 of the Mississippi River (RM 492.1) on December 1, 1994, and maintained in the laboratory at Forbes Biological Station, Havana, IL. Tubs containing clean sand in a flowing water system (unfiltered creek water, 4-7°C) were used as holding containers. Mussels were fed ½ teaspoon (2.4 ml) of algal suspension (Diet B, Coast Oyster Company, 12951 Del-Red Road, Suite 195, Bellevue, Washington 98005-2628) every other day and exposed to an approximate 16h:8h light:dark cycle. The mussels were held for one month prior to testing to acclimate them to the laboratory setting.

Mussels were acclimated to test temperature (20°C) by placing three sets of 10 mussels in separate 20-L aquaria containing approximately 18 L of continuously aerated filtered creek water. The aquaria were placed in an incubator where the ambient temperature was raised 3°C

every other day. Mussels were fed as described above. One-half volume (approximately 9 L) of aquarium water was replaced with fresh, 20°C, filtered creek water every other day. Mussels were allowed to remain at 20°C for a minimum of two days before testing.

2.3 Toxicity Assays for Year 1

2.3.1. *Ceriodaphnia dubia* Acute Toxicity Assays

Fifteen milliliters of porewater solution were placed in 30-ml plastic cups. Neonates were collected from the brood boards and placed in a glass beaker containing 100 ml of culture water. A subsample of ten neonates was removed from the beaker and then placed in one of the 30-ml test cups. A new subsample of neonates was collected for each test cup. Death was defined as the cessation of movement of appendages, a motionless organism at the bottom of the cup, or as failure of an organism to move after gentle prodding. Cups were observed for mortality at 24 h and 48 h, and dead organisms were removed.

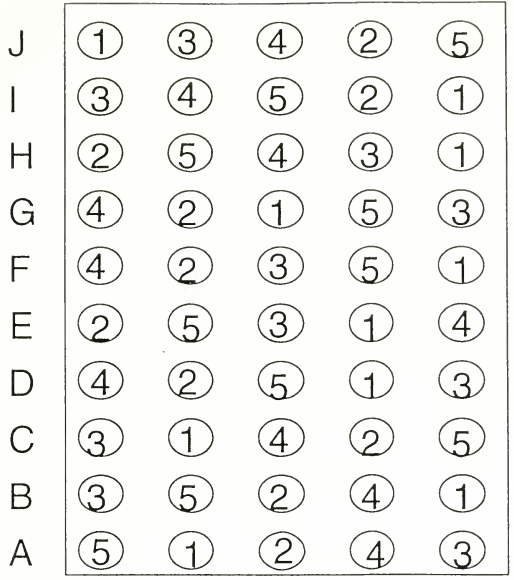
2.3.2. *Ceriodaphnia dubia* Survival / Reproduction Assays

A modified *C. dubia* Survival and Reproduction Test Method (USEPA 1989) was used to assess the chronic toxicity of remaining volumes (approximately 925 ml) of each porewater after conducting the acute toxicity assays. Two to three days before the test, the test brood boards consisting of 60 brood cups were set up with one egg-bearing female each. The day that 12 or more brood cups had eight or more young (< 24 h old), the test was started.

Two control solutions were used in the chronic test. The first was titled "control" and consisted of hard standard reconstituted water. The second was titled "storage control" and consisted of hard standard reconstituted water stored in the ice chests for the same duration as the sediments. The purpose of the storage control was to assess for any possibile toxic plasticizers.

solvents, or other chemicals that would have leached from the ice chests used for sediment storage. Five liters of hard standard reconstituted water were stored in a large ice chest, and then 1 L was collected for centrifugation at 4,000 X g at 4°C. The water was stored in a 1-L cubitainer with no head space at 4°C for no more than 1 week.

Temperature, dissolved oxygen (D.O.), pH and conductivity of each porewater solution were measured and recorded prior to the start of the test. When the D.O. was below 8 ppm O_2 for any solution, it was aerated for 1 hour. Ten 10-ml volumes of each porewater solution were poured into separate 30-ml plastic cups and placed in a brood board (test board). Ten brood cups that contained eight or more young were chosen to provide neonates for the test. Using one brood cup at a time, one neonate was placed in each of the five treatment cups in one row in the test board. Different brood cups were used for each row on the test board. After the addition of neonates, the cups on the test board were placed in random order according to a random diagram obtained from USEPA (1989) (Figure 2). After the addition of the neonates 0.1 ml YCT and 0.1 ml *S. capricornutum* solution were added to each cup. Test solutions were renewed daily by placing 10 ml of fresh porewater solution in new 30-ml plastic cups. Temperature, pH, D.O. and conductivity were measured before the addition of the porewater to new test cups. The adults were removed from each test cup with a Pasteur pipette, and placed in a clean test cup containing fresh porewater solution. We recorded the status of the adult (alive or dead), and counted the number of young produced. Old test solutions and cups were discarded. The adults were fed 0.1 ml YCT and 0.1 ml *S. capricornutum* solution daily after solution renewals. Tests ran until at least 60% of the surviving females in the control solutions had produced at least three broods or until the porewater solutions were used up.



Key:	
Rep	Solution
1	Control
2	Treatment-Control
3	Sylvan slough
4	ALCOA
5	Campbell's Slough

Roman numerals denote columns; capital letters denote rows.

Figure 2. Random cup test board used for the *C. dubia* survival/reproduction assays. Initially, test and control solutions were poured into individual cups across each row. Then cups were rearranged according to the random cup test board above. Five neonates from one female were then placed in individual cups across one row. Each row, therefore, represented one replicate for each test solution.

2.4. Toxicity Assays for Year 2

2.4.1. *Ceriodaphnia dubia* Survival / Reproduction Assays

Procedures for the *C. dubia* survival/reproduction assays were the same as above using porewaters from the sediments collected the second year of the study.

2.4.2. Mussel Filtering Assay (MFA)

A mussel filtering assay was used to assess chronic toxicity of the collected porewaters in the second year of the study. The mussel filtering assay is based on the premise that the ability of bivalves to filter particles from water is impaired by the presence of pollutants (Anderson *et al.* 1978; Sparks *et al.* 1981; Aldridge *et al.* 1987; Sparks and Sandusky 1983; Sparks *et al.* 1992;).

Filtering rates were determined by measuring the ability of native deertoe mussels (*Truncilla truncata*) to filter yeast from a suspension of known concentration. In this study, one mussel was placed in each of five replicate 1000-ml beakers containing 500 ml test solution (i.e., porewaters or control solutions). Because of the limited amount of porewater collected from Campbell's Slough, only three replicates were used. Mussels were exposed to porewaters and control solution using a static-renewal procedure where porewaters and control solutions were exchanged at 24 hours of exposure. The static-renewal procedure was used to prevent the build up of metabolic wastes and the depletion of dissolved oxygen in replicate beakers. After exposure, we tested individual mussels for filtering ability by placing them in a yeast suspension (10 mg/L yeast in distilled water) and allowing them to filter for 4 h. The mussels were removed from the beakers and rinsed with deionized water to remove any particulate matter. A "yeast control" beaker consisting of 10 mg/L yeast suspension with no mussels was used to determine

the change in yeast concentration due to the settling of the yeast during the 4-h filtering period of the study. Yeast concentrations in all vessels (including the yeast control) were measured indirectly by measuring absorbance of a 3-ml sample at 580 nm (Aldridge *et al.* 1987) using a spectrophotometer (Milton Roy Spectronic 301, Milton Roy Analytical Products Division, Rochester, New York 14625) at the beginning (e.g., initial yeast concentration) and end of the 4-h filtering period (final yeast concentration). Filtering rates of the treatment were then determined by the following equation (Sparks and Dillon 1993):

$$Fr = (Y_0 - Y_4) - Y_C \cdot Wt^{-1} \cdot h^{-1} \qquad \text{eqn. 1}$$

where:

Y_0	=	Initial yeast concentration (mg yeast · L water^{-1})
Y_4	=	Yeast concentration after 4 h filtering period (mg yeast · L water^{-1})
Y_C	=	Difference in yeast concentration in yeast control (mg yeast· L water^{-1}).
Wt	=	Dry weight of mussel (g)
h	=	Time (4 h)
Fr	=	Mussel filtering rate (mg yeast · g mussel^{-1}· h^{-1})

Initial trials of the mussel filtering assay resulted in negative filtering rates for individual mussels exposed to Sylvan Slough porewaters. All filtering rates were also below 1 mg yeast • g dry wt^{-1}· mussel^{1} · h^{-1}. Since filtering rates are calculated as the difference between initial and final yeast concentrations (eqn. 1), a negative filtering rate would indicate an increase in solids (e.g., yeast or some extraneous organic material) in the test beaker. This is possible since some mussels were seen to expel fecal material while in the yeast solution.

Negative filtering rates could have also been caused by differing settling rates of yeast in the vessels. Yeast in vessels containing mussels will not settle at the same rate as yeast in the yeast control vessels because of the filtering activity of the mussels. As mussels siphon the yeast

solution into and out of their bodies the water in the vessel was mixed, causing much of the yeast to remain in suspension. Therefore, the loss of yeast from the vessels containing mussels would be due largely to the intake of yeast by the mussels, and loss of yeast due to settlement and/or sorption to glassware would have had a minimal effect on the yeast concentration. Modifications were made to the methods including rinsing the mussels with deionized water and patting them dry before placement in the yeast suspension. Also, after investigating the source of variation in the data, we decided to remove the change in concentration in the yeast control from equation 1. This change resulted in the following equation:

$$Fr = (Y_0 - Y_4) \cdot Wt^{-1} \cdot h^{-1} \qquad \text{eqn. 2}$$

where:
- Y_0 = Initial yeast concentration (mg yeast · L water^{-1})
- Y_4 = Yeast concentration after 4 h filtering period (mg yeast · L water^{-1})
- Wt = Dry weight of mussel (g)
- h = Time (4 h)
- Fr = Mussel filtering rate (mg yeast · g mussel^{-1}· h^{-1})

The removal of the yeast control from the calculation made it possible to compare filtering rates obtained from mussels exposed to porewaters to the filtering rates of mussels exposed to filtered creek water (control solution) (Table 2). Figure 3 summarizes the steps of the modified mussel filtering assay used in the study.

	Source	1	2	3	4	5	Mean
With Yeast Controls	Control	0.01	0.14	0.14	0.05	0.18	0.10
	Treatment	-0.03	-0.08	-0.03	0.01	0.05	-0.02
Without Yeast Controls	Control	1.93	2.48	2.55	2.55	3.11	2.53
	Treatment	1.56	1.70	1.37	2.19	3.18	2.00

Table 2. Comparison of filtering rates of individual mussels exposed to Sylvan Slough porewaters as determined with the change in yeast control concentration (eqn. 1) and without the change in the yeast controls (eqn. 2). Filtering rates have units of mg yeast · g dry wt^{-1}· mussel·h^{-1}. The negative filtering rates were considered not to be "realistic" of "true" filtering rates. Positive filtering rates resulted after eliminating the change in yeast concentration from the yeast controls from the equation.

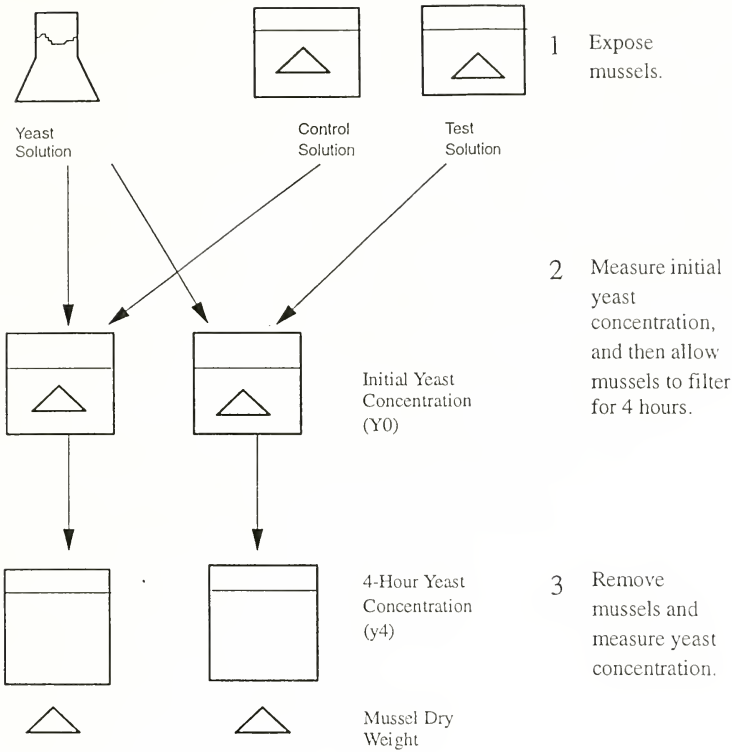


Figure 3. Schematic drawing representing the steps of the mussel filtering assay. Diagram depicts the modification made to original assay of Sparks and Dillon (1993) where the yeast-control was removed. The removal of the yeas-control resulted in more realistic filtering rates for the mussels in our study.

2.5. Statistical Analysis

Acute *C. dubia* toxicity test results were analyzed by Fisher's Exact Test (USEPA 1989). Data collected from the *C. dubia* Survival / Reproduction Assays were analyzed by Fisher's Exact Test to obtain an indication of lethal toxicity, and reproductive data were analyzed according to methods for statistical analysis of *C. dubia* reproduction data (USEPA 1989). Normality of the data was tested by Shapiro-Wilk's test of normality, followed by Bartlett's test for homogeneity of variance (USEPA 1989). Once normality of the data was established, and variances of the means were found to be homogenous, the effect of the porewater solutions on reproduction was determined by Dunnett's Procedure (USEPA 1989). Mussel filtering assay results were expressed as the percent decline in filtering rates from the control value. The treatment results (i.e., filtering rates measured in porewaters) were divided by the control results (i.e., filtering rate measured in filtered creek water), and then 1 was subtracted from the quotient. A negative value indicated inhibition of filtering rates by the porewaters (chronic toxicity), a positive value indicated stimulation of filtering by the mussels, and 0 indicated no response (Sparks *et al.* 1992). Mussel filtering rates were also analyzed by a one-way ANOVA to determine if significant differences between treatments and controls could be detected.

3. Results

3.1. Year 1

3.1.1. *Ceriodaphnia dubia* Acute Toxicity Assay

Because of high mortality in the control solution (12 %), this test was considered invalid and no statistical analyses were performed (Table 3). *Ceriodaphnia dubia* were fed the morning of

the test before placing the organisms in the test solutions. The organisms were not fed during the 48-h test period. Mortality in the control and storage control solutions may have been caused by the lack of food during the test period. The three porewater solutions, although filtered through a 110-µm-mesh screen, may have contained fine particulate matter which could have provided a temporary food source for the organisms, thus reducing mortality.

	% Mortality	
Sample	24 h	48 h
Control	2	12*
Storage control	2	6
Sylvan Slough	0	2
ALCOA	0	0
Campbell's Slough	2	2

Table 3. Mortality data for the *Ceriodaphnia dubia* acute toxicity tests of selected porewaters of Reach 15. Mortality in the control mussels was higher than 10 % (as indicated by the asterisk, *) making this test invalid.

3.1.2. *Ceriodaphnia dubia* Survival / Reproduction Assay

Porewater for the *C. dubia* survival / reproduction assay was depleted after six days. No adult mortality was recorded in control, storage control or Sylvan Slough porewater solutions, but one adult death occurred in each of the ALCOA and Campbell's Slough solutions. Adult death was recorded on Day 4 of the experiment for ALCOA, and on Day 7 for Campbell's Slough. Differences between observed mortality in the storage control and porewater solutions, and in the control solutions were not significantly different (Fisher's Exact Test, $p < 0.05$). Therefore, it was concluded that the porewaters were not acutely toxic.

A total of 146, 188, 143, 24, and 127 young were produced by adults in the control, storage

control, Sylvan Slough, ALCOA, and Campbell's Slough solutions, respectively (Table 4). One-way t-tests performed among respective porewater and storage control solutions, and the control solution (USEPA 1989), determined that the ALCOA porewater solution significantly ($p < 0.05$) reduced production of young by the *C. dubia* adults (Tables 4 and 5).

Source	df	Sum of Squares	Mean Square	F value	p value
Between	4	1493.72	373.43	25.5075	0.000
Within	45	658.6	14.64		
Total	49	2152.32			

Table 4. ANOVA table for Dunnett's Test of Significance for the reproduction data of the Survival/Reproduction *C. dubia* test. A significant difference was detected between the production of young by organisms exposed to replicate series of porewater solutions ($p < 0.05$). The mean square value for Between replicate series (373.43) was larger than the mean square value for Within replicate series (14.64) indicating that most of the variance in the data was found between the replicate series, or porewater solutions. This indicates that the significant effect detected could be attributed to the effect of the porewaters on the organisms, and not on experimental error.

Solution	t-value
Storage control	-2.46
Sylvan Slough	0.18
ALCOA	7.13*
Campbell's Slough	1.11

Table 5. Calculated t-values for number of young produced in each porewater solution tested against the control solution to determine significant effects of porewater on reproduction of *C. dubia*.
*Organisms exposed to ALCOA sediment porewaters produced significantly fewer young (45 df, $p = 0.05$, $t_2 = 2.225$) compared to organisms exposed to the control solution and other porewaters.

3.2. Year 2

3.2.1 *Ceriodaphnia dubia* Survival / Reproduction Assay

Only the porewater from Sylvan Slough sediments was available for the *C. dubia* survival / reproduction assay. Experimental error and poor health of the laboratory cultures prevented testing the remaining porewaters. The results from the *C. dubia* assays using Sylvan Slough

porewaters are presented in Table 6. Although minimal acute mortality occurred, reproduction by both control and treatment organisms was extremely low. Neither group of organisms produced young until the seventh day of the test indicating poor health of the culture, and invalidity of the test results. Therefore, no information concerning the toxicity of the sediments of Pool 15 was gained from the *C. dubia* survival / reproduction assay during the second year of the study.

Source	No. of Adults	% Mortality	# Young / Adult
Sylvan Slough	9	1	1.55
Control	9	1	1.44

Table 6. Results from *C. dubia* survival / reproduction assay from the second year of the study. Although adult mortality was minimal in both tests, the production of young by the adults was less than two young per adult. The decreased reproduction by the adults indicated that the lab culture was in poor health. No conclusive information regarding the toxicity of Sylvan Slough sediments could be obtained from this test.

3.2.2 Mussel Filtering Assay

Mean filtering rates of *T. truncata* exposed to sediment porewaters and control solutions are given in Table 7. One-way ANOVA indicated mussels exposed to Campbell's Slough porewater exhibited significantly lower filtering rates compared to control mussels. Average filtering rates in Sylvan Slough and ALCOA porewaters were not significantly different from corresponding average control filtering rates.

There was a 53% decline in filtering rates in mussels exposed to Campbell's Slough porewaters and a 21% decline in filtering activity in those mussels exposed to Sylvan Slough porewaters. Personnel detected an odor resembling petroleum from Campbell's Slough

sediments, indicating a possible source of toxicity. Mussels exposed to porewater collected from ALCOA sediments displayed a small, insignificant increase in filtering activity (1%) (Table 7).

	Filtering Rates		One-way ANOVA		
Porewater	Control	Treatment	F value	p value	% Diff. from Control
Sylvan Slough	2.53 (SE=0.17, N=5)	2.00 (SE=0.29, N=5)	2.96	0.16 n.s.	-21
Campbell's Slough	1.88 (SE=0.25, N=3)	0.88 (SE=0.07, N=3)	12.80	0.01**	-53
ALCOA	0.79 (SE=0.02, N=2)	0.80 (SE=0.02, N=3)	2.17	0.32 n.s.	+ 1

Table 7. Mean filtering rates (mg yeast · g dry wt mussel^{-1}· h^{-1}) for mussels exposed to Sylvan Slough, Campbell's Slough and ALCOA site porewaters.

Filtering rates of mussels exposed to Sylvan Slough and ALCOA sediment porewaters were not significantly different (indicated by n.s.) from those mussels exposed to the control solution (filtered creek water). Mussels exposed to Campbell's Slough sediment porewaters exhibited significantly lower (indicated by **, $p < 0.05$) filtering rates compared to mussels exposed to the control solution.

4. Discussion

In the first year of the study we detected chronic sediment toxicity in porewaters collected from the ALCOA site. Those porewaters significantly reduced the reproductive rate of *Ceriodaphnia dubia*. Since chronic toxicity was detected in sediment from that location, a study involving infaunal organisms (i.e., deertoe mussel, *Truncilla truncata*) was conducted during the second year to determine if the chronic toxicity observed in *C. dubia*, a laboratory animal, could also be detected with an infaunal organism. Unfortunately, because of poor health of the culture and experimental error, no direct comparison of sediment toxicity was accomplished between parallel tests involving *C. dubia* and *T. truncata* during the second year.

Observation of the toxicity assays conducted both years of the study provided some information concerning the quality of the sediments in Reach 15. In year one, organisms exposed to ALCOA porewaters displayed the effects of chronic toxicity and in year two, chronic toxicity responses were observed in organisms exposed to Campbell's Slough and Sylvan Slough sediment porewaters. Since the sediments used in the studies were collected approximately one year apart, we expected to see some difference in the results. Sediment toxicity episodes may be brief and infrequent, allowing organisms to colonize between episodes (Sparks and Dillon 1993). Also, toxic hot spots, as well as benthic organism distribution are known to be patchy (Sparks *et al.* 1992; Sparks and Dillon 1993).

The mussel filtering assay used in this study indicated mussel filtering rates were affected by porewaters from Reach 15 of the Upper Mississippi River. Although we can assume a 53% decrease in filtering rate indicates a chronic toxic effect, there is no information on the effect of a decrease in filtering rate on the survival of a mussel species, such as a threshold filtering rate

below which mussels lose weight and eventually die. We need to determine such threshold filtering rates for critical mussel species.

Although porewater has been recognized as a suitable "surrogate" for assessing the toxicity of whole sediments, researchers have not found a suitable extraction method that does not alter the physical and chemical properties of the porewaters in the "natural state" (Carignan *et al.* 1985; Ankley *et al.* 1994). Also, the problems associated with the collection of sediment porewaters (e.g., time and amount of sediment required to provide sufficient volumes of porewater for testing) warrants the use of whole sediment studies. Recently, the U.S. Environmental Protection Agency (1994) approved a sediment testing intermittent-renewal (STIR) system for invertebrate sediment toxicity testing. The STIR system enables the maintenance of acceptable water quality parameters (e.g., dissolved oxygen and temperature) by automatically renewing overlying water in sediment tests at rates ranging from 1 to 21 volume renewals per day. The STIR system reduces the labor associated with the renewal of overlying water by hand and affords a gentle exchange of water that results in virtually no sediment suspension. This method is simple and inexpensive to assemble and operate.

We recommend testing sediments of Reach 15 and sediments of remaining high quality mussel refuges in the Upper Mississippi and Illinois rivers, using the species and test procedures previously mentioned. Tests conducted in this study indicate that chronic toxicity is present in the sediments of Reach 15. By comparing sediments from other mussel refuges to Reach 15 sediments, we can determine if sediment toxicity is caused by local non-point pollution sources along Reach 15 or by upstream sources.

Macroinvertebrates are continuous indicators of environmental quality. The composition of

benthic macroinvertebrate communities reflects changes in the physical and chemical condition within a reach over time. Biological monitoring is based on the fact that different species react to pollution in different ways. Pollution-sensitive organisms, such as mayflies, freshwater mussels and clams, are more susceptible than others to the effects of many physical or chemical changes in a river. Pollution-tolerant organisms such as midge larvae and aquatic worms can cope with adverse conditions more easily. Based on the chronic nature of sediment toxicity detected in Reach 15 of the Upper Mississippi River, a program involving the monitoring of benthic macroinvertebrates over time is recommended. A biomonitoring program would be relatively inexpensive compared to a water or sediment chemistry monitoring program. Monitoring would consist of collecting benthic macroinvertebrates from randomly selected sites in Reach 15 at 3-month intervals. Identification and enumeration of the organisms could be done in the field allowing for rapid collection of information. A change in the benthic community composition from a diverse collection of pollution-sensitive and pollution-tolerant organisms to a relatively homogenous collection of predominantly pollution-tolerant organisms would alert managers to a problem potentially affecting a native mussel bed. Action could be taken immediately to identify the source of pollution and possibly alleviate the problem.

An innovative monitoring tool, The Mossel Monitor (Delta Consult, The Netherlands) has been recently introduced (Kramer *et al.* 1989) and consists of a tube containing zebra mussels permanently attached to a platform with electrical sensors that detect the opening and closing of the shells. Zebra mussels, as well as other mussels, open their shells for respiration and feeding most of the time under normal environmental conditions. Similar monitoring devices have been developed at North Texas State University (Waller *et al.* 1995) Under times of stress, such as

during the presence of a pollutant in the water or sediment, mussels close their shells for extended periods. The Mossel Monitor would be placed in an mussel bed, along with a microcomputer, power supply, and recorder in a waterproof housing. The electronic components can also be housed separately on shore. Data could be downloaded from The Mossel Monitor hourly or daily, and analyzed for any changes in zebra mussel shell movement. This equipment could also be set up to automatically alert a manager and to trigger a water sampler to collect a sample of water when the alarm is given. Factors causing the stress response could be identified by subsequent chemical analysis.

In order for any monitoring program to be successful, it is necessary for the work to be continuous. If biomonitoring is selected as a route of action for the protection of Illinois' native mussels refuges, it should be supported by a long-term commitment of funds for personnel and equipment.

5. Conclusions

1. Toxic sediments did occur within Reach 15 of the Upper Mississippi River. These sediments exhibited chronic, rather than acute toxic effects on deertoe mussels (*Truncilla truncata*) and waterfleas (*Ceriodaphnia dubia*).

2. Organisms exposed to porewaters extracted from sediments of Sylvan Slough of Reach 15 displayed a toxic response indicating chronic toxicity. Sylvan Slough is an Illinois native mussel refuge and home to the federally-endangered Higgin's-eye pearly mussel, *Lampsilis higginsi*.

3. The mussel filtering assay, with modification, is a good method of detecting sediment

toxicity using native mussel species.

4. A continuous monitoring program should be developed for the mussel beds of the Upper Mississippi River to prevent another massive mussel die-off in the future. ✓ recommendation

6. Recommendations

The tests results of this study determined that toxic conditions do occur in the sediments of Reach 15 of the Mississippi River. These findings warrant environmental monitoring of the sediments of Reach 15. To insure populations of native mussels continue to exist in our river systems, extensive monitoring programs are needed (Havlik and Marking 1987). Therefore we recommend the following:

1. Monitor the sediments of Reach 15 and other Illinois mussel refuges with recently approved sediment toxicity methods and species.

Recently, the U. S. Environmental Protection Agency announced the acceptance of the two assays as standard protocols for the determination of sediment toxicity: the *Hyalella azteca* 10-day survival test and the *Chironomus tentans* 10-day survival and growth test. Since chironomid larvae are commonly found in the sediments of large rivers, sediment toxicity tests using *C. tentans*, rather the *C. dubia*, would be more appropriate for any future sediment toxicity projects for Reach 15 of the Upper Mississippi River.

2. We also recommend standardizing the mussel filtering assay and then using this procedure as an economical screening tool for the assessment of toxic sediments in Reach 15 of the Upper Mississippi River.

Although recommendation 1 above would be the best way to determine the presence of sediment pollution in Reach 15, the mussel filtering assay used in this project is an economical alternative. The mussel filtering assay could provide useful information needed to conserve native mussels. The mussel filtering assay, using infaunal mussel or clam species, has shown promise as a valuable screening tool to assess the quality of sediments to infaunal organisms (Sparks and Dillon 1993). The method needs to be calibrated with established laboratory tests (e.g., midge growth assay).

3. *In situ*, continuous biological monitoring devices, such as the Mossel Monitor or similar devices (Waller *et al.* 1995) should be used to monitor water quality of the Upper Mississippi River.

The assays conducted during this study indicated that some sediments in Reach 15 of the Upper Mississippi River are toxic to the benthic fauna. We recommend a continuous monitoring program, such as the use of the Mossel Monitor, that measures biological variables to detect pollution pulses traveling down the river through Reach 15. The use of the Mossel Monitor would allow a manager to detect potential pollution problems and identify likely sources of the problem before another disaster, like the mussel die-off of 1981 through 1986, occurs again.

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APPENDIX A

SYLVAN SLOUGH SEDIMENT TOXICITY
MUSSEL FILTERING ASSAY

Data associated with mussel filtering assay using Sylvan Slough sediment porewaters.

Table 1. 48 hour mortality and water quality data for Truncilla truncata exposed to Sylvan Slough pore water.

Mussel # CONTROL	Hour	Dead/ Alive	D.O.	pH	T°
1	24	A	7.30	8.41	20
2	24	A	5.60	8.29	20
3	24	A	6.80	8.40	20
4	24	A	6.80	8.45	20
5	24	A	6.80	8.44	20
1	48	A	5.10	8.37	20
2	48	A	4.55	8.29	20
3	48	A	5.70	8.41	20
4	48	A	6.20	8.48	20
5	48	A	5.90	8.46	20
TEST					
1	24	A	6.50	7.95	20
2	24	A	6.70	8.00	20
3	24	A	4.70	7.75	20
4	24	A	6.00	7.91	20
5	24	A	6.50	8.01	20
1	48	A	5.70	8.21	20
2	48	A	5.90	8.30	20
3	48	A	3.35	8.07	20
4	48	A	5.40	8.25	20
5	48	A	5.70	8.35	20

Table 2. Determination of the yeast concentration curve to use for mussel filtering assay.

Yeast	Absorption (550 nm)		
(mg/L)	Rep. #1	Rep. #2	Average
0	0.000	0.000	0.000
2	0.003	0.003	0.003
4	0.005	0.005	0.005
6	0.007	0.007	0.007
8	0.009	0.009	0.009
10	0.011	0.011	0.011
12	0.013	0.013	0.013
14	0.014	0.014	0.014

Table 3. Regression statistics associated with the standard yeast curve for the Sylvan Slough porewater assays.

Statistic	Value
Constant	-0.68
Std Err of Y Est	0.50
R Squared	0.99
No. of Observations	8.00
Degrees of Freedom	6.00
X Coefficient(s)	991.15
Std Err of Coef.	38.23

Figure 1. Standard yeast concentration curve for Sylvan Slough porewater assays.

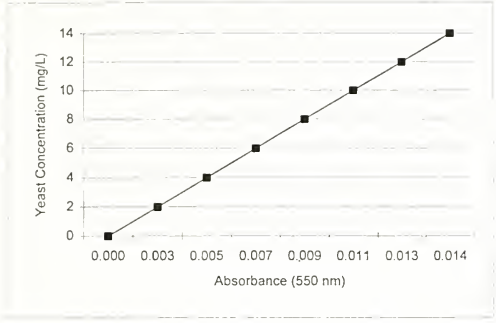

Table 4. Zero (0) hour water quality for the pore water exposure of the mussel filtering assay.

Hour	D.O. (mg/L)	pH	Temp (°C)	Conductivity (mho)	Hardness (ppm)	Alkalinity (ppm)
0	808	7.4	17.3	0.44	276	221

Table 5. Yeast concentrations in yeast control, control, and test vessels for the mussel filtering assay.

TIME: 0 h	Vessel #	Abs. (550 nm)	Conc. (mg/L)	Conc. (mg)
Yeast	1	0.012	11.21	5.61
Control	2	0.013	12.20	6.10
	3	0.014	13.19	6.60
	4	0.015	14.19	7.09
	5	0.014	13.19	6.60
Control	1	0.012	11.21	6.10
	2	0.013	12.20	6.60
	3	0.014	13.19	6.60
	4	0.014	13.19	6.60
	5	0.014	13.19	5.61
Test	1	0.012	11.21	5.61
	2	0.012	11.21	6.10
	3	0.013	12.20	6.60
	4	0.014	13.19	7.09
	5	0.015	14.19	0.00

Table 5. (Continued)
TIME: 4 h

Yeast	1	0.007	6.26	3.13
Control	2	0.007	6.26	3.13
	3	0.008	7.25	3.62
	4	0.008	7.25	3.62
	5	0.009	8.24	4.12
Control	1	0.006	5.27	2.63
	2	0.004	3.28	1.64
	3	0.005	4.27	2.14
	4	0.007	6.26	3.13
	5	0.004	3.28	1.64
Test	1	0.007	6.26	3.13
	2	0.008	7.25	3.62
	3	0.008	7.25	3.62
	4	0.008	7.25	3.62
	5	0.008	7.25	3.62

Table 6. Wet and dry weghts of mussel tissue and shells used in the determination of mussel filtering rates.

Control Mussel #	Pan (g)	Pan + Wet Tissue (g)	Wet Tissue (g)	Pan + Dry Tissue (g)	Dry Tissue (g)	Shell(g)
1	1.00	3.19	2.19	1.38	0.39	11.68
2	0.99	3.84	2.85	1.44	0.45	13.24
3	0.99	3.87	2.88	1.43	0.44	13.44
4	0.99	3.09	2.10	1.33	0.34	15.42
5	0.99	3.63	2.64	1.39	0.40	15.17
Test						
1	0.99	3.16	2.17	1.39	0.40	14.00
2	0.99	2.95	1.96	1.28	0.29	13.90
3	0.99	3.69	2.70	1.45	0.45	13.89
4	0.99	3.22	2.23	1.33	0.34	14.92
5	1.00	2.71	1.71	1.27	0.27	17.22

Table 7. Determination of the change in yeast concentration in yeast control vessels.

Yeast Control	0 Hours Conc. (mg)	4 Hours Conc. (mg)	Change in Yeast Concentn.	Average Change in Yeast Control
1	5.61	3.13	2.48	2.87
2	6.10	3.13	2.97	
3	6.60	3.62	2.97	
4	7.09	3.62	3.47	
5	6.60	4.12	2.48	

Table 8. Determination of mussel filtering rates of control and test mussels using the yeast controls.

Control	0 Hours Conc. (mg)	4 Hours Conc. (mg)	Change in Yeast Conc. (mg)	Average Change in Yeast Control (mg)	Dry Mussel Tissue (g)	Filtering Rate*
1	5.61	2.63	2.97	2.87	0.39	0.01
2	6.10	1.64	4.46	2.87	0.45	0.14
3	6.60	2.14	4.46	2.87	0.44	0.14
4	6.60	3.13	3.47	2.87	0.34	0.05
5	6.60	1.64	4.96	2.87	0.40	0.18
Test						
1	5.61	3.13	2.48	2.87	0.40	-0.03
2	5.61	3.62	1.98	2.87	0.29	-0.08
3	6.10	3.62	2.48	2.87	0.45	-0.03
4	6.60	3.62	2.97	2.87	0.34	0.01
5	7.09	3.62	3.47	2.87	0.27	0.05

*Filtering rate = mg yeast/ g dry mussel tissue/hour

Table 9. Determination of mussel filtering rates of control and test mussels without the yeast controls.

Control	0 Hours Conc. (mg)	4 Hours Conc. (mg)	Change in Yeast Conc. (mg)	Dry Mussel Tissue (g)	Filtering rate*
1	5.61	2.63	2.97	0.39	1.93
2	6.10	1.64	4.46	0.45	2.48
3	6.60	2.14	4.46	0.44	2.55
4	6.60	3.13	3.47	0.34	2.56
5	6.60	1.64	4.96	0.40	3.11
Test					
1	5.61	3.13	2.48	0.40	1.56
2	5.61	3.62	1.98	0.29	1.70
3	6.10	3.62	2.48	0.45	1.37
4	6.60	3.62	2.97	0.34	2.19
5	7.09	3.62	3.47	0.27	3.18

*Filtering rate = mg yeast/g dry mussel tissue/h

Table 10. F-test statistics comparing mussel filtering rates for mussels exposed to control solution and Sylvan Slough porewaters.

Statistic	Control	Sylvan Slough
Mean	2.53	2.00
Variance	0.18	0.53
Observations	5.00	5.00
df	4.00	4.00
F-value	2.96	
P(F<=f) one-tail	0.16	
F Critical one-tail	4.11	

APPENDIX B

CAMPBELL'S SLOUGH SEDIMENT TOXICITY
MUSSEL FILTERING ASSAY

Data associated with mussel filtering assay data using Campbell's Slough sediment porewater.

Table 1. 48 hour mortality data for *Truncilla truncata* exposed to Campbell's Slough porewater.

Mussel # CONTROL	Hour	Dead/Alive	D.O.	pH	T°
13	24	A	5.40	8.00	20
47	24	A	6.40	8.14	20
34	24	A	6.30	8.10	20
13	48	A	5.00	7.78	20
47	48	A	6.40	7.93	20
34	48	A	5.60	7.90	20
TEST					
22	24	A	5.00	7.75	20
23	24	A	5.20	7.81	20
3	24	A	5.60	7.83	20
22	48	A	5.50	7.75	20
23	48	A	5.60	7.75	20
3	48	A	5.60	7.77	20

Table 2 Determination of the yeast concentration curve to use for mussel filtering assay

mg/L	Rep #1	Rep #2	Average
0	0.000	0.000	0.000
2	0.003	0.003	0.003
4	0.005	0.005	0.005
6	0.007	0.007	0.007
8	0.009	0.009	0.009
10	0.011	0.011	0.011
12	0.013	0.013	0.013
14	0.014	0.014	0.014

Table 3 Regression statistics associated with the standard yeast concentration curve for the Campbell's Slough porewater assays.

Statistic	Value
Constant	-0.68
Std Err of Y Est	0.50
R Squared	0.99
No. of Observations	8.00
Degrees of Freedom	6.00
X Coefficient(s)	991.15
Std Err of Coef.	38.23

Figure 1. Standard yeast concentration curve for Campbell's Slough porewater assay.

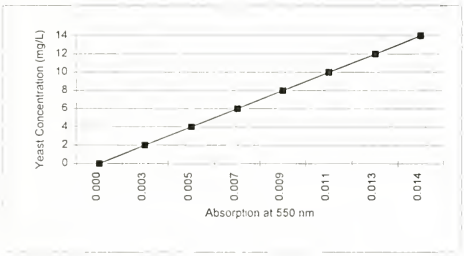

Table 4. 0 hour and 48 hour water quality for the pore water exposure of the mussel filtering assay.

0 hour	D.O	T°	pH			
Control	8.2	18	8.0			
Test	8.7	19	7.3			
48 hour				Hardness	Alkalinity*	Conductivity*
Control	8.0	18	8.2	197	286	0.44
Test	8.2	18	7.5	217	280	0.46

* Alkalinity measured as mg CaCO3/L, Conductivity measured as mho

Table 5 Yeast concentrations in yeast control and test vessels for the mussel filtering assay

TIME 0 h	#	Abs. (550 nm)	Conc. (mg/L)	Conc. (mg)
Yeast	1	0.012	11.21	5.61
Control	2	0.011	10.22	5.11
	3	0.012	11.21	5.61
Control	1	0.012	11.21	5.61
	2	0.011	10.22	5.11
	3	0.012	11.21	5.61
Test	1	0.011	10.22	5.11
	2	0.011	10.22	5.11
	3	0.012	11.21	5.61
TIME 4 h				
Yeast	1	0.008	7.25	3.62
Control	2	0.009	8.24	4.12
	3	0.009	8.24	4.12
Control	1	0.004	3.28	1.64
	2	0.004	3.28	1.64
	3	0.008	7.25	3.62
Test	1	0.008	7.25	3.62
	2	0.008	7.25	3.62
	3	0.008	7.25	3.62

Table 6. Wet and dry weights (g) of mussel tissue and shells used in the determination of mussel filtering rates.

Mussel # Control	Pan (g)	Pan + Wet Tissue (g)	Wet Tissue (g)	Pan + Dry Tissue (g)	Dry Tissue (g)	Shell (g)
1	0.99	4.88	3.89	1.48	0.49	17.19
2	0.99	4.33	3.34	1.47	0.48	21.94
3	0.99	4.53	3.54	1.56	0.57	19.76
Test						
1	1.00	4.39	3.40	1.48	0.48	17.61
2	0.99	4.52	3.52	1.45	0.46	16.81
3	0.99	4.53	3.54	1.46	0.47	19.94

Table 7. Determination of the change in yeast concentration in yeast control vessels.

Vessel #	0 Hours Conc. (mg)	4 Hours Conc. (mg)	Change in Yeast Conc (mg)	Average Change in Yeast Control (mg)
1	5.61	3.62	1.98	1.49
2	5.11	4.12	0.99	
3	5.61	4.12	1.49	

Table 8 Determination of mussel filtering rates of control and test mussels using the change in yeast concentration in the yeast controls.

Control	0 Hours Conc. (mg)	4 Hours Conc. (mg)	Change in Yeast Concentn	Average Change in Yeast Control (mg)	Dry Mussel Tissue (g)	Filtering Rate*
1	5.61	1.64	3.96	1.49	0.49	1.26
2	6.10	1.64	4.46	1.49	0.48	1.56
3	6.60	3.62	2.97	1.49	0.57	0.65
Test						
1	5.11	3.62	1.49	1.49	0.48	0.00
2	5.11	3.62	1.49	1.49	0.46	0.00
3	5.61	3.62	1.98	1.49	0.47	0.26

*Filtering Rate = mg Yeast/g dry mussel tissue/h

Table 9. Determination of mussel filtering rates of control and test mussels without the use of the change yeast concentration in the yeast control vessels

Control	0 Hours Conc. (mg)	4 Hours Conc. (mg)	Yeast Conc. (mg)	Dry Mussel Tissue (g)	Filtering Rate*
1	5.61	1.64	3.96	0.49	2.01
2	6.10	1.64	4.46	0.48	2.34
3	6.60	3.62	2.97	0.57	1.31
Test					
1	5.11	3.62	1.49	0.48	0.78
2	5.11	3.62	1.49	0.46	0.81
3	5.61	3.62	1.98	0.47	1.05

*Filtering Rate = mg Yeast/g dry mussel tissue/h

Table 10. F-test statistics comparing mussel filtering rates for mussels exposed to Control solution and Campbell's Slough pore waters.

Statistic	Control	Campbell's Slough
Mean	1.88	0.88
Variance	0.28	0.02
Observations	3.00	3.00
df	2.00	2.00
F	12.80	
P(F<=f) one-tail	0.07	
F Critical one-tail	9.00	

APPENDIX C

ALCOA SEDIMENT TOXICITY
MUSSEL FILTERING ASSAY

Data associated with mussel filtering assay using ALCOA sediment pore waters.

Table 1. 48-hour mortality data for Truncilla truncata exposed to ALCOA sediment pore water.

Mussel # CONTROL	Hour	Dead/Alive	D.O.	pH	T°
1	24	A	6	8.3	19
2	24	A	5.1	8.21	19
3	24	A	7	8.45	19
1	48	A	5.1	8.23	19
2	48	A	6.3	8.38	19
3	48	D	6.8	8.52	19
TEST					
1	24	A	4.7	19	NO
2	24	A	4.2	19	DATA
3	24	A	4.8	19	
1	48	A	4.85	19	NO
2	48	A	5.1	19	DATA
3	48	A	5.3	19	

Mussels used in 48-h toxicity tests were not used in mussel filtering assay.

Table 2. Determination of the yeast concentration curve for use in the mussel filtering assay.

Yeast	Absorption (550nm)		
(mg/L)	Rep. #1	Rep. #2	Average
0	0	0	0
2	0.002	0.002	0.002
4	0.004	0.005	0.005
6	0.006	0.006	0.006
8	0.008	0.008	0.008
10	0.011	0.011	0.011
12	0.013	0.013	0.013
14	0.015	0.015	0.015

Table 3. Regression statistics associated with the standard yeast curve for the ALCOA pore water studies.

Statistic	Value
Constant	0.14
Std Err of Y Est	0.30
R Squared	1.00
No. of Observations	8.00
Degrees of Freedom	6.00
X Coefficient(s)	922.64
Std Err of Coef.	21.42

Figure 1. Standard yeast concentration curve for ALCOA pore water assays.

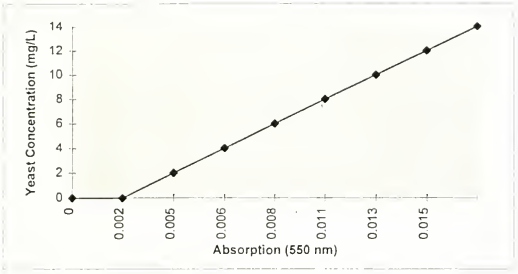

Table 4. Water quality parameters of porewater monitored at 0-hour and 48-hour of the mussel filtering assay.

0 h	D.O.	T°	pH			
Control	8.5	17	8.28			
Test	9	16	7.85			
48 h				Hardness	Alkalinity	Conductivity
Control	8.1	19	8.45	254	175	0.41
Test	8.1	18	7.8	228	197	0.4

Alkalinity measured as mg CaCO3/L; Conductivity measured as mho.

Table 5. Yeast concentrations in yeast control, control, and test vessels for the mussel filtering assay.

TIME: 0 h	Mussel #	Abs. (550 nm)	Conc. (mg/L)	Conc. (mg)
Yeast	1	0.009	8.44	4.22
Control	2	0.01	9.36	4.68
	3	0.01	9.36	4.68
Control	1	0.009	8.44	4.22
	2	0.009	8.44	4.22
	3	0.01	9.36	4.68
Test	1	0.009	8.44	4.22
	2	0.009	8.44	4.22
	3	0.009	8.44	4.22
TIME: 4 h				
Yeast	1	0.006	5.67	2.84
Control	2	0.007	6.60	3.30
	3	0.007	6.60	3.30
Control	1	0.005	4.75	2.38
	2	0.002	1.98	0.99
	3	0.001	1.06	0.53
Test	1	0.005	4.75	2.38
	2	0.006	5.67	2.84
	3	0.006	5.67	2.84

Table 6. Wet and dry weights (g) of mussel tissue and shells used in the determination of mussel filtering rates.

Mussel # CONTROL	Pan (g)	Pan + Wet Tissue (g)	Wet Tissue (g)	Pan + Dry Tissue (g)	Dry Tissue (g)	Shell (g)
1	0.99	4.26	3.26	1.56	0.56	22.07
2	0.99	6.56	5.57	2.04	1.05	20.39
3	NO DATA					
TEST						
4	0.99	4.73	3.73	1.55	0.55	19.66
5	0.99	3.92	2.93	1.41	0.42	22.53
6	1.00	4.45	3.46	1.46	0.46	23.48

Table 7. Determination of the change in yeast concentration in yeast control vessels.

Vessel #	0 Hours Conc. (mg)	4 Hours Conc. (mg)	Change in Yeast Conc. (mg)	Average Change in Yeast Control
1	4.2208101	2.8368546	1.3839555	1.38
2	4.6821286	3.2981731	1.3839555	
3	4.6821286	3.2981731	1.3839555	

Table 8. Determination of mussels filtering rates of control and treatment mussels using the yeast controls.

Mussel # CONTROL	0 Hours Conc. (mg)	4 Hours Conc. (mg)	Change in Yeast Conc. (mg)	Yeast Conc. (mg)	Dry Tissue Weight (g)	Filtering Rate
1	4.22	2.38	1.85	0	0.56	0.00
2	4.22	0.99	3.23	-1.11E-16	1.05	-2.64E-17
3	NO DATA		0.00			
TEST						
1	4.22	2.38	1.85	0.00	0.55	0.00
2	4.22	2.84	1.38	0.00	0.42	0.00
3	4.22	2.84	1.38	0.00	0.46	0.00

Filtering Rate = mg yeast/g dry tissue/h

Table 9. Determination of mussel filtering rates for control and treatment mussels without the use of the yeast control.

Mussel #	0-Hours Conc. (mg)	4-Hours Conc. (mg)	Change in Yeast Conc. (g)	Dry Tissue Weight (g)	Filtering Rate
Control					
1	4.2208101	2.3755361	1.845274	0.56477	0.816825
2	4.2208101	0.9915806	3.2292295	1.04987	0.768959
3	NO DATA		0		
Test					
1	4.2208101	2.3755361	1.845274	0.55414	0.832494
2	4.2208101	2.8368546	1.3839555	0.41645	0.830805
3	4.2208101	2.8368546	1.3839555	0.46426	0.745248

Filtering Rate = mg yeast/g dry tissue/h

Table 10. F-test statistics comparing mussel filtering rates for mussels exposed to Control solution and ALCOA porewaters.

Statistic	Control	ALCOA
Mean	0.792892402	0.8028493
Variance	0.00114558	0.00248914
Observations	2	3
df	1	2
F	2.172819457	
P(F<=f) one-tail	0.315177089	
F Critical one-tail	8.526315786	