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Development of a Bioaccumulation Test Method with the Amphipod *Leptocheirus plumulosus*

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PURPOSE: This technical note provides information on the procedures and approach utilized in the development of a bioaccumulation test method using the amphipod *Leptocheirus plumulosus*.

BACKGROUND: Dredged material evaluations routinely require the use of sediment bioaccumulation tests to determine the potential for sediment-borne contaminants to bioaccumulate and to evaluate the risk posed to higher trophic levels. Marine/estuarine bioaccumulation test species include field-collected *Macoma nasuta* and *Nereis virens*, as well as other less frequently used bivalves and polychaetes. Utilizing field-collected organisms in bioaccumulation tests is problematic. Field-collected organisms may be subject to seasonal availability (e.g., available in the summer but not in the winter). They may also display varying levels of responsiveness (e.g., survival, variation in uptake rates, etc.) due to temporal changes in the field such as natural or anthropogenic background contaminant exposure (American Society for Testing and Materials (ASTM) 2007). Laboratory cultured organisms, such as *Leptocheirus plumulosus*, are reared in a controlled environment where environmental conditions are closely controlled and monitored, eliminating many of the issues associated with the use of field-collected organisms. The time required to reach steady state is also a concern with some species. Steady-state body residues of most hydrophobic organic compounds (HOCs) of concern in *M. nasuta* are typically approached many weeks beyond the standard 28-day duration of sediment bioaccumulation tests (Boese et al. 1997). The failure to reach steady state within the standard 28-day time frame requires an estimation of steady-state residues using conversion factors which introduce uncertainty into bioaccumulation estimates (U.S. Environmental Protection Agency (USEPA) and U.S. Army Corps of Engineers (USACE) 1998). The metabolic disposition of contaminants in an organism is also important when selecting a bioaccumulation test species. *N. virens* and other polychaetes biotransform PAHs efficiently and therefore are poor indicators of the bioaccumulation potential of these compounds (McElroy 1990; Driscoll and McElroy 1996; Jorgensen et al. 2005).

Recently developed micro methods for tissue analysis require much less tissue mass compared to traditional tests while providing comparable detection limits (Jones et al. 2006; Millward et al. 2007). A smaller mass requirement allows for the exploration of the use of smaller invertebrate species, such as *L. plumulosus* (Figure 1), for use in sediment bioaccumulation tests. *L. plumulosus* is routinely used as a model organism for conducting acute and chronic sediment toxicity tests (USEPA 1994; 2001). It is easily cultured and is readily available from aquatic test organism vendors. Preliminary experiments conducted at the ERDC using sediment-spiked compounds revealed that *L. plumulosus* bioaccumulates HOCs at steady-state concentrations during short sediment exposures (i.e., one week or shorter) and inefficiently metabolizes PAHs. These attributes warrant the development of a standard bioaccumulation test method for use with this

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species. Additionally, the feasibility of utilizing amphipods for evaluating bioaccumulation potential has been established by researchers who have used them for evaluating PCB body burdens in both the laboratory and the field setting (Millward et al. 2005; Cho et al. 2007). The availability of a bioaccumulation test method using *L. plumulosus* would result in improved evaluation of dredged material bioaccumulation potential compared to current available methodology due to reduced sediment and labor requirements and improved predictive value resulting from elimination of uncertainties associated with deriving steady-state body residues using multipliers and loss due to efficient biotransformation.

OBJECTIVES: The specific objective of this study was to develop a 28-day bioaccumulation test method with *L. plumulosus* by evaluating various test parameters to determine the optimum test or exposure conditions in a controlled environment. Variables were evaluated concurrently to determine the optimum level of each that results in the highest survival and mass (i.e., meeting or exceeding minimum analytical requirements) at test termination. High survival and tissue mass at the end of sediment exposure are the principle objectives in bioaccumulation test method development since the primary endpoint is measurement of tissue residue levels. High quality chemical residue analysis requires the maximum amount of tissue recovery that can be obtained.

Figure 1. The estuarine amphipod *Leptocheirus plumulosus.*

METHODS: Parameters evaluated as part of the test method development process included two levels of organism density, water exchange frequency, and ration level. Specifically, treatment levels included starting densities of 25 or 50 organisms per chamber, once weekly or three times weekly water renewal, and no feeding and feeding (20 mg of Tetramarin® fish food per beaker on Tuesdays and Fridays). The food ration level selected was based on results obtained in previous preliminary studies conducted at the ERDC. This feeding level demonstrated good survival with no fouling of the sediment surface. Treatment levels were designated as follows:

Nomenclature: Density-water exchange frequency-feeding regime (1=fed; 0 = not fed)			
25-1-0	25-3-0	50-1-0	50-3-0
25-1-1	25-3-1	50-1-1	50-3-1

The test method procedures utilized with *L. plumulosus* are generally similar to the bioaccumulation methods described for *N. virens* and *M. nasuta* in the ocean and inland testing manuals (USEPA and USACE 1991; 1998). The day prior to test initiation, adult test organisms (1-2 mm in size) were isolated from in-house culture sediment by passing the culture sediment through a 2-mm and a 1-mm sieve stack. Organisms retained on the 1-mm sieve were used in the study while organisms retained on the 2-mm sieve and passing through the 1-mm sieve were discarded or returned to culture. Organisms were placed in bowls of clean Instant Ocean® seawater and observed to ensure they were healthy. Once organisms were isolated and their level of health established, 200 mL (3 cm) of clean control sediment was added to five replicate 1-L beakers for each treatment level. The control sediment was collected from Sequim Bay, WA and contained a

TOC concentration of 0.64% with a wet to dry weight ratio of 3 to 1. Sediment was overlain with 20‰ synthetic seawater (Crystal Sea, Marine Enterprise, Intl., Baltimore, MD) and placed in an environmental chamber. The temperature of the environmental chamber was set at 23°C and the light cycle was set at 16 hr light:8 hr dark. Trickle flow aeration was provided to each beaker.

The following day overlying water quality parameters (dissolved oxygen, pH, salinity, and ammonia) were measured on one replicate of each treatment level. Depending on treatment level, 25 or 50 adult *L. plumulosus* were counted and placed into the appropriate test beakers. Feeding and water exchanges were conducted during the course of the study according to the study design. Fifty percent water exchanges were conducted on Wednesdays for beakers requiring one renewal and on Mondays, Wednesdays, and Fridays for beakers requiring three renewals. Water quality parameters (dissolved oxygen, pH, temperature, and salinity) were measured on Wednesdays prior to water exchange and organisms were fed on Wednesdays following the scheduled water exchange.

At day 28, water quality parameters (dissolved oxygen, pH, temperature, salinity, and ammonia) were measured on the overlying water from each beaker. The sediment from each beaker was then passed through a 500-µm sieve and organisms retained were enumerated and placed in clean seawater and allowed to purge sediment from their guts for approximately 4 hr (Millward et al. 2005). The amphipods were then placed in pre-tared vials for mass determination. Statistical analyses of the survival and growth endpoints within treatment levels were conducted using a t-test with α=0.05.

Optimum conditions for the test method were determined by selecting the highest organism density with the lowest number of water exchanges and lowest feeding level that did not result in significant decreases in survival or growth. A high density is desirable to ensure adequate tissue mass for tissue residue analysis. A low water exchange frequency minimizes the loss of contaminants from the exposure system due to the flux of contaminants from the sediment to the overlying water. A low feeding ration level minimizes the expected reduced exposure from the addition of supplementary food and also minimizes the effect of adding additional organic carbon to the exposure system.

Results and Discussion: Test survival and biomass results are summarized in Tables 1 and 2. The effects of density, water exchange frequency, and ration level on survival and growth are presented in Figures 1-6.

Table 1. *L. plumulosus* survival after 28 days.

Treatment	Mean	STDEV	CV	n
Control	98.0%	4.5%	4.6%	5
25-1-0	54.4%	8.3%	15.2%	5
25-1-1	64.0%	25.3%	39.5%	5
25-3-0	56.0%	2.8%	5.1%	5
25-3-1	68.0%	17.2%	25.3%	5
50-1-0	29.6%	9.2%	31.1%	5
50-1-1	74.4%	7.9%	10.7%	5
50-3-0	41.6%	12.3%	29.5%	5
50-3-1	72.8%	8.3%	11.4%	5

Table 2. *L. plumulosus* total replicate biomass (mg) after 28 days.

Treatment	Mean	STDEV	CV	n
Control	170.27	20.46	12.0%	5
25-1-0	39.68	8.15	20.5%	5
25-1-1	103.48	48.73	47.1%	5
25-3-0	40.27	5.56	13.8%	5
25-3-1	121.77	21.69	17.8%	5
50-1-0	52.50	26.40	50.3%	5
50-1-1	197.06	19.95	10.1%	5
50-3-0	45.82	11.23	24.5%	5
50-3-1	179.74	46.89	26.1%	5

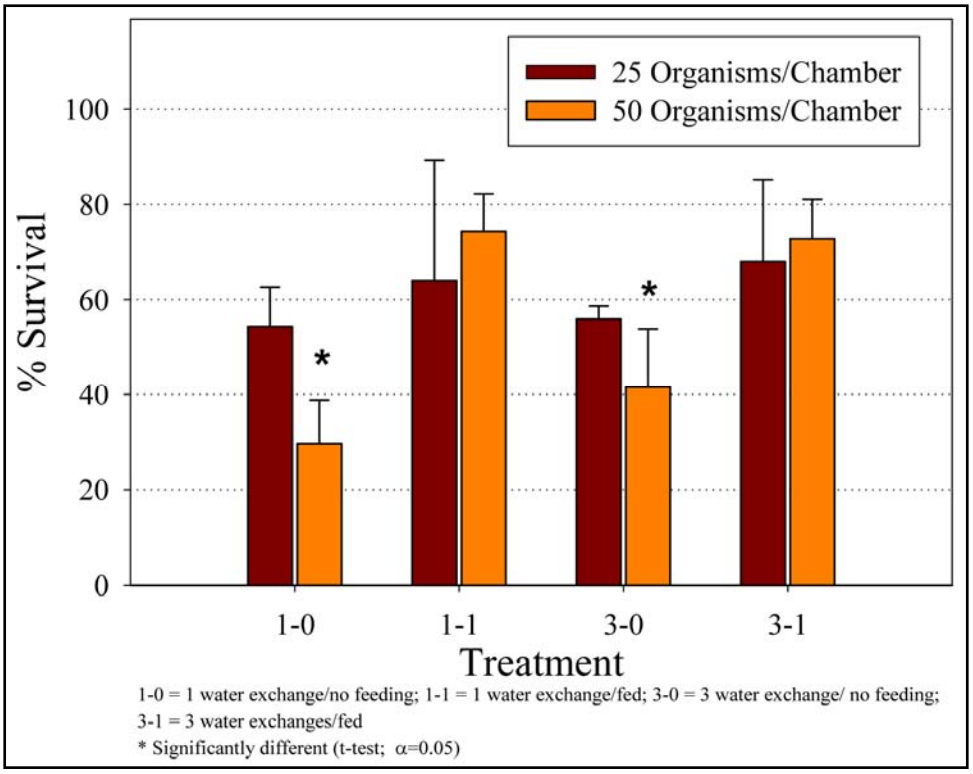

Figure 1. Effect of density on *L. plumulosus* 28-day survival.

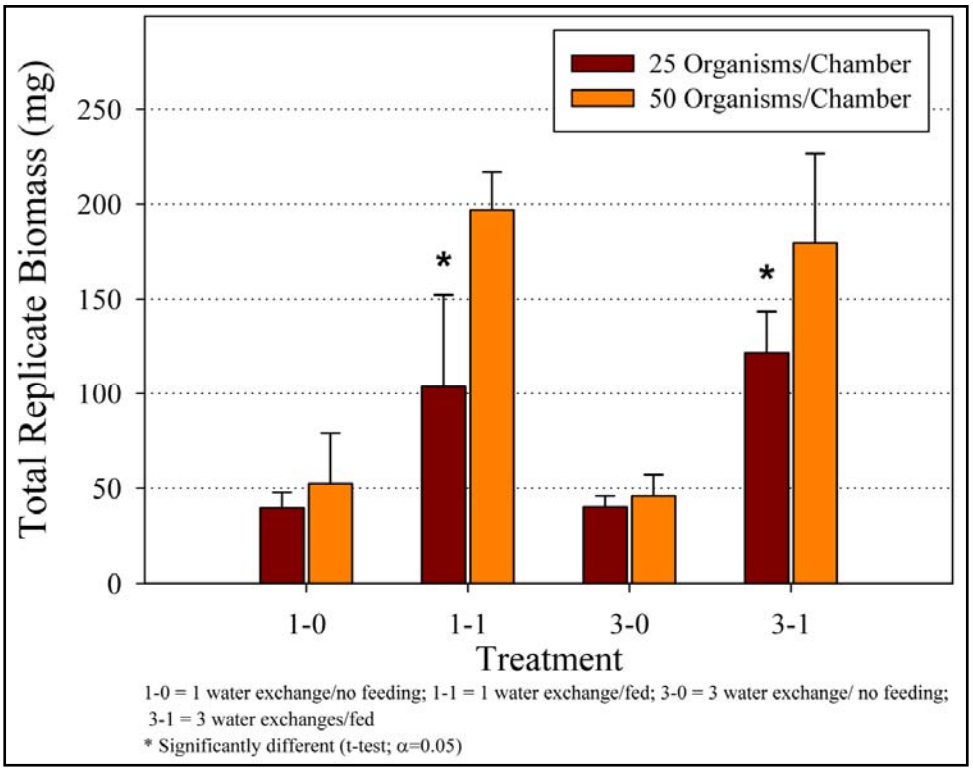

Figure 2. Effect of density on *L. plumulosus* 28-day growth.

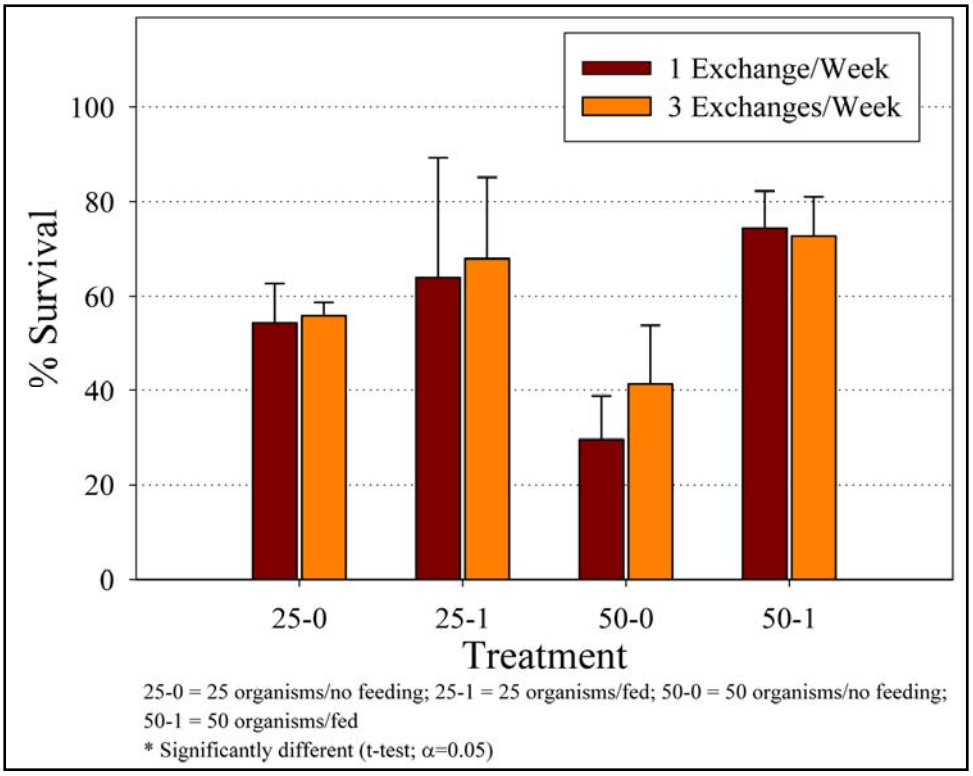

Figure 3. Effect of water exchange frequency on *L. plumulosus* 28-day survival.

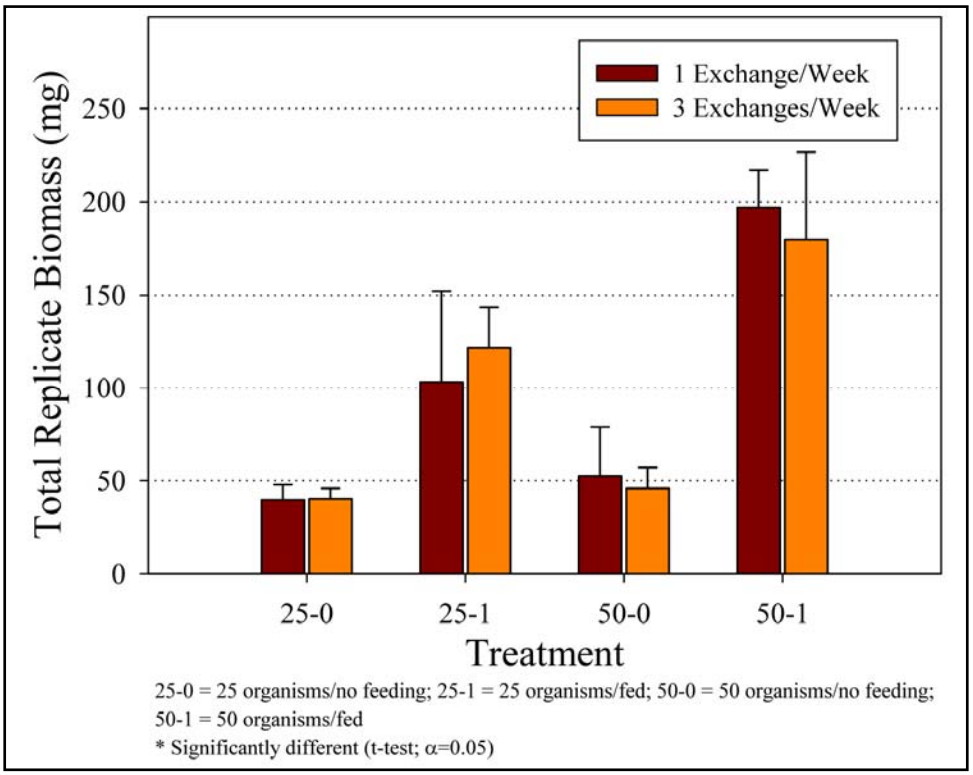

Figure 4. Effect of water exchange frequency on *L. plumulosus* 28-day growth.

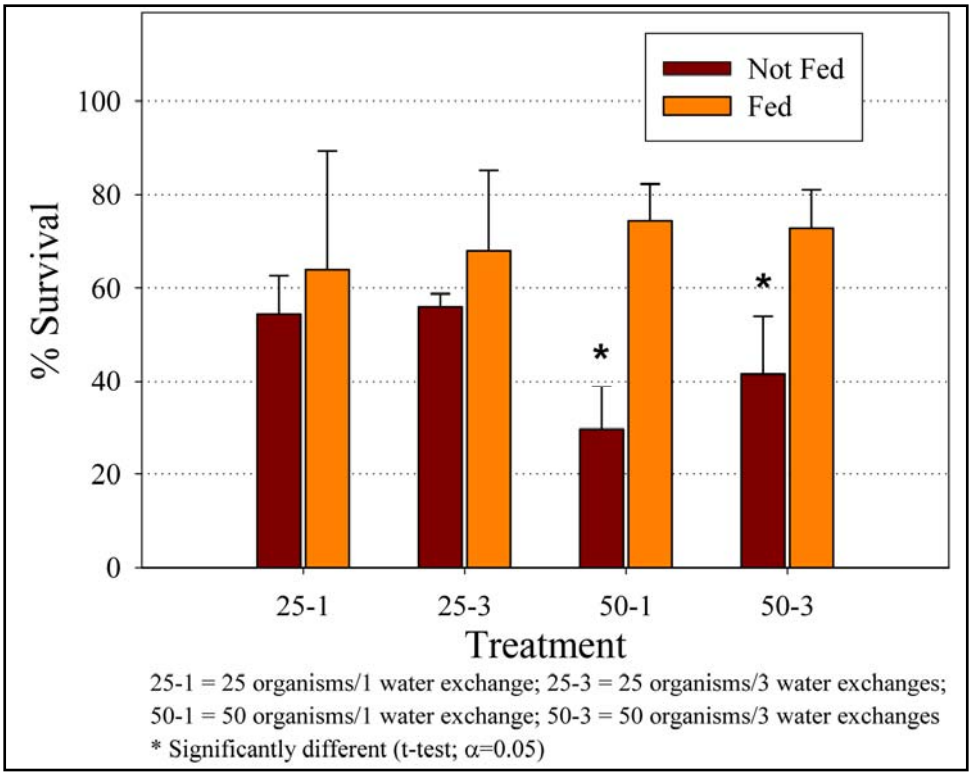

Figure 5. Effect of ration level on *L. plumulosus* 28-day survival.

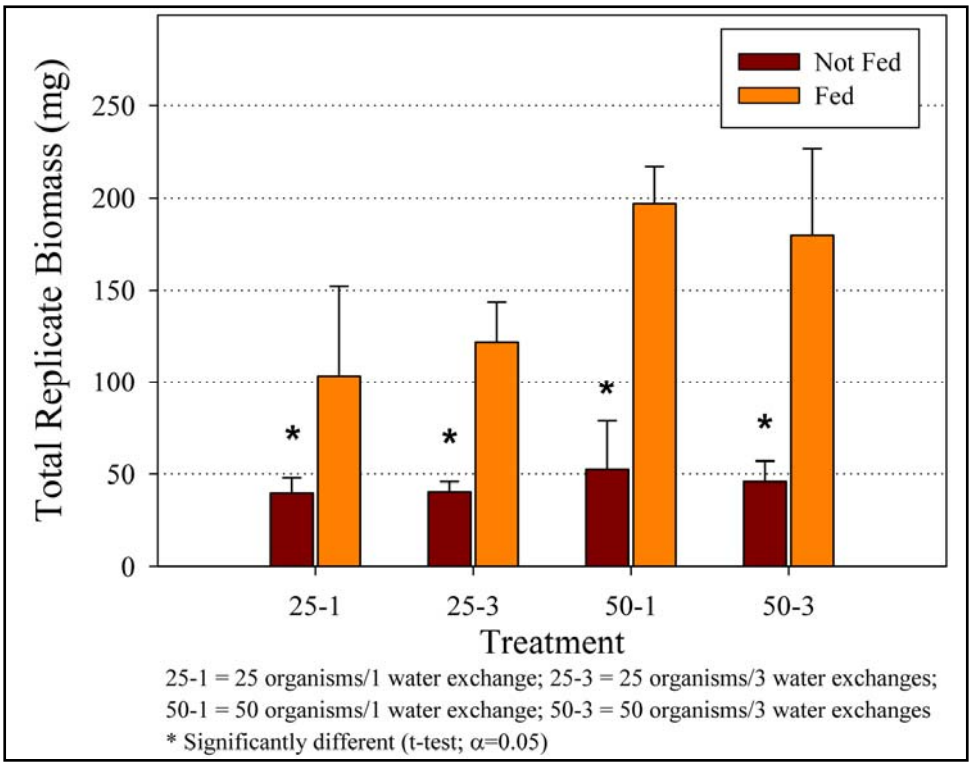

Figure 6. Effect of food ration level on *L. plumulosus* growth.

Significant density effects on survival and growth were observed in no-feeding treatments (Figure 1). A significant density effect on growth was observed in the highest density, where water exchanges were conducted three times weekly and the organisms were fed (Figure 2). Water exchange frequency had no effect on survival or growth (Figures 3 and 4). No feeding resulted in significantly lower survival in the high density treatment and lower growth for all treatment combinations (Figures 5 and 6). In summary, the survival and growth endpoints were primarily impacted by feeding levels, which were exacerbated by higher density levels. The only exception was the pure density effect observed in the highest density, where water exchanges were conducted three times weekly and the organisms were fed.

The treatment combination providing optimum survival and growth was a density of 50 organisms per chamber with once-weekly water exchange and twice-weekly feeding. This combination provides tissue mass well exceeding the mass requirements for PAH and PCB micro method analysis (ca 100 mg). These conditions were selected for further development of the *L. plumulosus* bioaccumulation test method.

Test conditions proposed for the 28-day bioaccumulation test method with *L. plumulosus* are summarized in Table 3. A general test activity schedule is provided in Table 4.

Table 3. Test conditions for *L. plumulosus* 28-day sediment bioaccumulation test method.

Parameter	Condition
Chamber size	1 Liter beaker
Replicates per treatment	5
Organisms per replicate	50
Organism size	Passing through a 2-mm sieve and retained on a 1-mm sieve
Salinity	20‰
Sediment depth	3 cm (~200 mL)
Temperature	23°C
Light cycle	16:8 L:D
Water renewal	Once weekly
Feeding	20 mg/chamber twice weekly (Tues and Fri)
Aeration	Trickle flow
Water Quality Measurements	Dissolved oxygen, pH, temperature and salinity in one replicate per treatment at test initiation and all beakers at test breakdown

Table 4. Daily activity schedule for conducting for *L. plumulosus* 28-day sediment bioaccumulation test method.

Day	Activity
-1	Isolate amphipods for conducting bioaccumulation test. Add sediment to test chambers, place chambers into exposure system.
0	Measure overlying water quality (pH, temperature, salinity, dissolved oxygen and ammonia). Transfer the appropriate number of amphipods to each exposure chamber. Collect a subset sample of amphipods for residue analyses.
1-27	Observe test organism behavior daily. Record test chamber temperature daily. On Wednesdays measure overlying water quality (pH temperature and salinity), perform a 50% water exchange and place 20 mg of Tetramin® in each replicate test chamber.
28	Measure overlying water quality (pH, temperature, salinity, dissolved oxygen and ammonia) in each test chamber. End the study by passing the sediment through a 500-µm sieve and collecting the surviving amphipods. Allow the amphipods to purge their guts for 2-4 hr. Determine the mass of the surviving amphipods.

SUMMARY AND RECOMENDATIONS: Evaluation of *L. plumulosus* as a model organism for determining the bioaccumulation potential of contaminants in dredged material demonstrated the viability of a test method with this organism. The use of *L. plumulosus* in conjunction with available micro methods for PAHs and PCBs as well as select standard chemistry approaches (e.g., metals) will result in a substantial cost savings due to much lower sediment volume requirements and labor required to conduct the study.

Future work will focus on determining the timeframe required to reach steady state in *L. plumulosus*, which could result in a decrease in the duration of the test and further cost savings. Future work will also be conducted to compare the bioaccumulative endpoints such as steady-state body residues and biota sediment accumulation factors (BSAFs) between *L. plumulosus* and the frequently used standard bioaccumulation test methods using *N. virens* and *M. nasuta* (and other organisms as deemed appropriate) to determine relative responsiveness of the *L. plumulosus* method as compared to these standard approaches.

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