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Burrowing Avoidance Assays of Contaminated Detroit River Sediments, Using the Freshwater Oligochaete *Stylodrilus heringianus* (Lumbriculidae)

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Abstract. The burrowing behavior of *Stylodrilus heringianus* (Lumbriculidae, Oligochaeta) was examined in bioassays, using sediments from suspected areas of contamination in the Detroit River, Michigan (U.S.A.). In assays with control sediments and sediments from a clean Detroit River site, all worms quickly burrowed (<1 hr) and appeared to feed normally over a 96-hr period. In sediments with suspected sediment-bound contaminants, *Stylodrilus* initially burrowed but returned to the surface after a few hours, the time of return apparently dependent on the degree of contamination and length of exposure. The presence of volatile contaminants reduced the initial burrowing response. These observations enhance the possibility of using a *Stylodrilus* burrowing behavior assay to aid in examining suspected areas of sediment contamination in the Great Lakes.

A variety of bioassays are now widely used for estimating the toxicity of effluents, receiving waters, and elutriates (*e.g.*, Microtox™, Bulich and Isenberg 1981; *Ceriodaphnia,* Mount and Norberg 1984; *Selenastrum capricornutum,* Miller *et al.* 1978). However, few assays have been created which assess the effects and potential toxicity of in-place sediment contamination, particularly freshwater sediment contamination and its relationship to the macrozoobenthos community (White 1988).

Keilty *et al.* (1988) recently have described a short-term sediment lethality and avoidance assay using the freshwater aquatic earthworm *Stylodrilus heringianus* (Lumbriculidae). The *Stylodrilus* assay has been used primarily to assess the toxicity of a single sediment-bound compound (endrin) and has not been evaluated as an environmental monitor. *Stylodrilus heringianus* would appear appropriate for environmental assessment of sediment contamination because of its intimate interactions with sediment and because its burrowing behaviors have been quantified and described in detail (Robbins *et al.* 1984; White *et al.* 1987). Further, *Stylodrilus* is the dominant deposit feeding oligochaete of oligotrophic sediments in many northern lakes, especially the Laurentian Great Lakes (Lauritsen *et al.* 1985) where a variety of sediment contaminants exist.

The main objectives of the study were to examine the potential usefulness and sensitivity of the *Stylodrilus* sediment bioassay and to describe *Stylodrilus* behavior patterns in relation to contamination in natural sediments. In this paper, we present assay results, using sediments from four areas of suspected contamination in the Detroit River. Because bioassays by definition are endpoints in themselves (Mount and Norberg 1984), we have attempted to relate the results only to general sediment quality and not to specific sediment structure and chemistry. Deposits along the Detroit River are known to contain a vast array of potentially toxic substances from one of the largest industrial complexes in the world (Chau *et al.* 1985).

Materials and Methods

Control sediments and *Stylodrilus heringianus* were collected from a Lake Michigan depositional area approximately 10 km offshore from Bridgman, Michigan, using a PONAR grab. Water depth was 42 m. Sediments were fine sand with fine-grained silts and clays (Robbins *et al.* 1984). This site has been described in

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detail by White *et al.* (1986a, 1986b). The dominant oligochaete was the oligotrophic *Stylodrilus heringianus,* which occurs throughout much of Lake Michigan's deep depositional areas (Lauritsen *et al.* 1985). Sediments and *Stylodrilus,* with Lake Michigan water, were maintained in a darkened 200 L aquarium at 10°C for several months prior to experimental use. Acclimation of at least one month has been recommended by Keilty (1987).

Detroit River sediments were collected with a PONAR grab at four sites (Figure 1, Table 1), at least three of which (Stations 30, 34, and 53) were suspected to contain a variety of contaminants (Chau *et al.* 1985). Sediments were placed in acid-cleaned glass jars, capped in the field, and kept refrigerated (1°C) in the laboratory. Water also was collected at each site 0.5 m off the bottom and kept refrigerated until used. Tests were run within 48 hr of collection.

Bioassay procedures followed those outlined by Keilty *et al.* (1988). In all assays, approximately 25 ml wet sediment (gently stirred but not sieved) were spooned into 50-ml beakers, and any visible oligochaetes were removed. In the first assay, five replicates were created for each of the four experimental sediments and for the Lake Michigan control sediment. Twenty to 25 ml of matching river or control water (overlying Lake Michigan aquarium water) was gently pipetted into each beaker to limit sediment resuspension. Prepared beakers were placed into an environmental chamber at 10°C, the temperature of the Detroit River at the time of field collections.

Control sediments containing *Stylodrilus* were gently sieved (0.5 mm mesh) to concentrate worms. A fiber-optic light (to prevent unnecessary heating) and a dissecting microscope were used to sort and identify *Stylodrilus.* Ten large (20–30 mm long) *Stylodrilus* were added to each beaker. *Stylodrilus* were added individually to reduce the formation of 'balls' making initial burrowing observations easier (Keilty *et al.* 1988).

Burrowing and visual 'health' observations were made at 0.17, 0.5, 1, 2, 4, 8, 12, 24, 48, 72, and 96 hr. A worm was considered unburrowed if more than an estimated 75% of its body was exposed on the sediment surface. Healthy unburrowed *Stylodrilus* were very active and appeared bright red. Death was defined as an absence of any red coloration (yellow to clear), no response to touch, and an unmistakable degree of body degeneration. At the end of 96 hr, the contents of each beaker was sieved (0.5 mm mesh) to determine the number and health of *Stylodrilus* remaining in the sediment.

Beakers were not covered during the assays to allow natural gas exchange. Dissolved oxygen concentrations in the overlying waters were measured at 0, 12, 48, and 96 hr, using a YSI Model 57A Dissolved Oxygen meter. Dissolved oxygen decreased very slowly in all beakers over the 96-hr period to between 70–80% saturation. As these dissolved oxygen levels should not cause stress to *Stylodrilus* (Robbins *et al.* 1984), water in the beakers was not aerated.

In the second assay, Station 34 sediments were diluted with control sediments. Concentrations were 0 (control), 2, 10, 50, and 100% Station 34 sediment (v:v). Each concentration contained five replicates, and assays followed the protocol given above. A 96-hr LC_{50} determination was made by the Litchfield-Wilcoxon nomographic method (Litchfield and Wilcoxon 1949).

In the third assay, Station 34 sediments were air-dried in an attempt to reduce any volatile contaminants. After drying at room temperature for approximately 96 hr, sediments were rewetted with Station 34 water, gently mixed, and added to four, 50 ml beakers. Assays again followed the protocol given above, except that no additional controls were run.

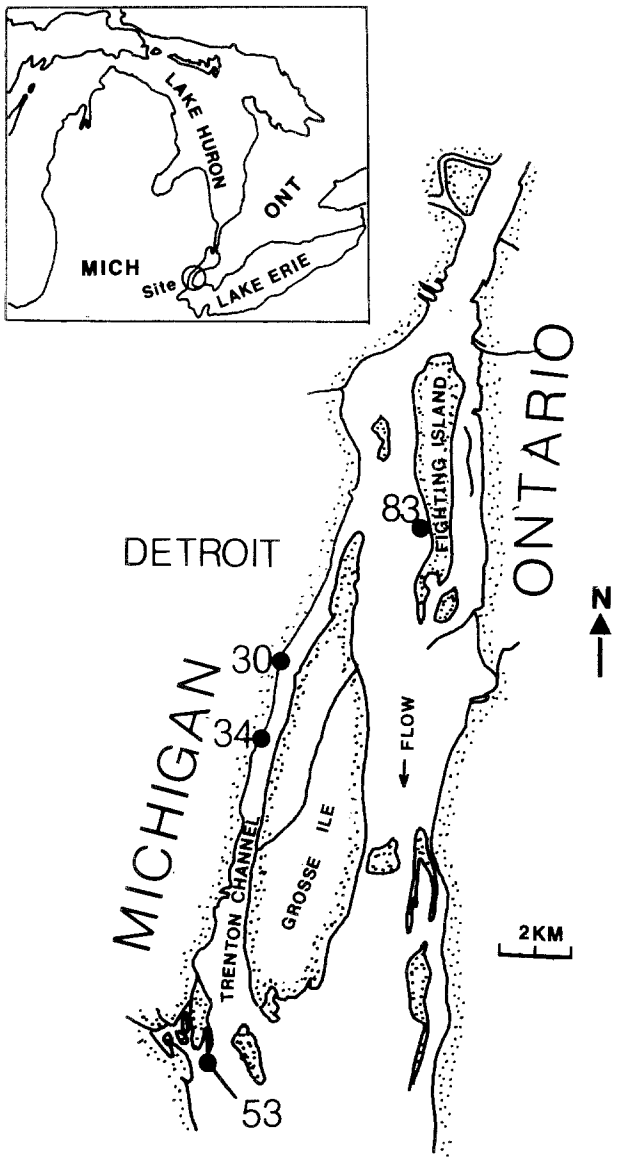

Fig. 1. Map of lower Detroit River showing station locations

Results and Discussion

Detroit River Sediment Assays

Control sediments were typical of off-shore depositional areas in the Great Lakes in being odor-free and containing primarily fine sands and clay with a mixture of various sizes of organic particles (Robbins *et al.* 1984). Although thorough particle analyses have not been completed for the test sediments, some preliminary particle size ranges and qualitative assessments are listed in Table 1. All Detroit River sediments contained some sands and clays. The most variable component was the amount of organic material, which was reflected in

Table 1. Collection location and selected sediment characteristics of control and Detroit River stations. Percent of particles >0.025 mm and >0.063 mm by weight and % total organic carbon (TOC) by weight from an unpublished report by J. DePinto, Clarkson University

Station No.	Latitude/ longitude	Sediment description and composition (>0.250 >0.063 TOC)	Color	Odor at time of collection
30	42°10′19″ 83°09′56″	Silt and fine organic particles with some larger organic debris (8.3 28.9 9.3)	Dark brown	Strong hydrocarbon
34	42°08′51″ 83°10′24″	Silt and fine organic particles (1.2 3.1 10.0)	Black	Very strong hydrocarbon
53	42°04′48″ 83°11′34″	Silty sand with some fine organic particles (4.0 16.9 4.8)	Brown	Very mild hydrocarbon
83	42°12′42″ 83°07′31″	Sandy clay with very few organic particles (3.5 12.0 2.5)	Yellow-brown	No odor
Control	42°00′81″ 86°44′12″	Sandy silt with some fine organic particles (4.3 20.2 3.3)	Brown	No odor

total organic carbon contents that ranged from 2.5% at Station 83 to 10% at Station 34. Sediment compaction was not measured during the assays, but it was not significant enough to have affected burrowing.

In both sets of controls (first and second assays), *Stylodrilus* quickly burrowed and remained burrowed through the 96-hr period (Figures 2 and 7). Most *Stylodrilus* were in the sediment within 0.17 hr, and 99 of 100 had burrowed after 2 hr. Only one worm in the first control set (Figure 2) did not burrow, and it remained alive on the surface throughout the assay. Other *Stylodrilus* occasionally were noted on the surface, but the mean number of unburrowed *Stylodrilus* per replicate was always less than 1.0. There were no mortalities.

Station 83 was located on the west side of Fighting Island (Figure 1) in an area of the Detroit River thought to be away and upstream from most of the more severe areas of contamination (Chau *et al.* 1985). Sediments contained a much greater proportion of larger clay particles than at other test sites or in control sediments. Sediments had no unusual odor at the time of collection (Table 1) or during the assays. Even though high proportions of clay are not typical of the normal *Stylodrilus* habitat (LaDronka 1984), all except one worm had burrowed within one hr, and a mean of less than 1.0 worms per replicate were on the surface at any one time over 96 hr (Figure 3). One death was recorded at 72 hr.

Station 53 was near the mouth of the Detroit River in an area strongly influenced by Lake Erie currents and wave action (Figure 1). Sediments were expected to contain at least some heavy metals, particularly zinc and chromium (Mudroch 1985; Hamdy and Post 1985) and possibly some organic contamination (Oliver and Bourbonniere 1985; Comba and Kaiser 1985). There were no hydrocarbon odors at the time of collection or during laboratory tests. Similar to control and Station 83 burrowing patterns, a mean of more than 8.0 *Stylodrilus* per replicate had burrowed after 0.17 hr, and all worms had burrowed after one hr (Figure 4). However, by 24 hr, a mean of 0.8 worms had returned to the sediment surface, and by 96 hr, the number of unburrowed *Stylodrilus* had increased to a mean of 2.2, showing a moderate behavioral response to some component in the sediment. No mortalities occurred in any of the Station 53 replicates.

Station 30 was located in a depositional area along the west bank of the Trenton Channel. Sediments were suspected of containing a wide variety of metals, chlorinated hydrocarbons, and volatiles (Mudroch 1985; Hamdy and Post 1985; Oliver and Bourbonniere 1985; Comba and Kaiser 1985) and possibly some additional enrichment from the Detroit sewage treatment plant. Sediments were dark and 'oily', and there were distinct hydrocarbon odors during collection and laboratory tests. *Stylodrilus* did not burrow as quickly as in the controls, Station 53, or Station 83 sediments. Through 24 hr, a mean of 1.0 per replicate remained unburrowed (Figure 5). Between 24 and 48 hr, the mean number of unburrowed *Stylodrilus* per replicate increased to 3.4 and increased to 3.8 by 96 hr. A mean of 1.2 *Stylodrilus* per replicate were dead at the end of 96 hr.

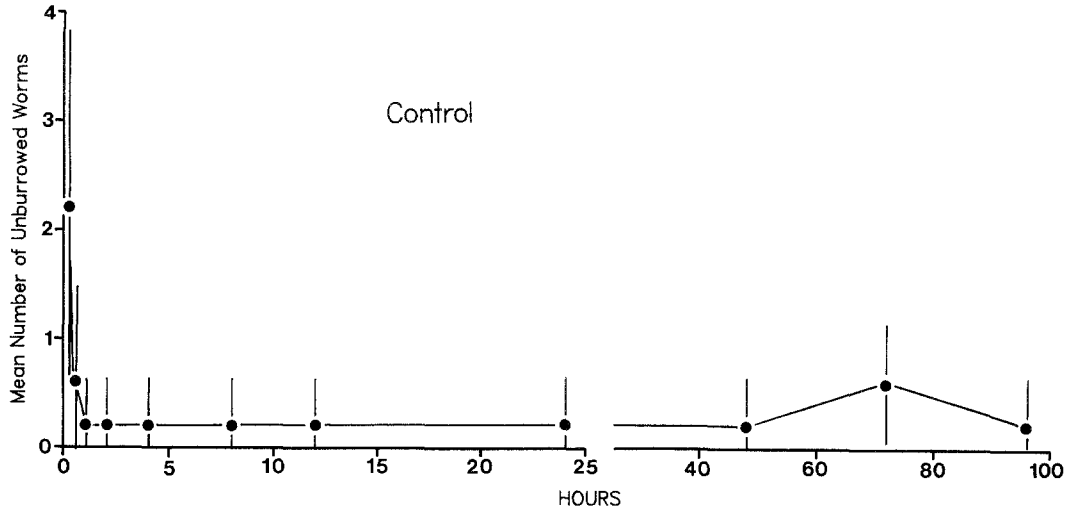

Fig. 2. Mean number and standard error of unburrowed *Stylodrilus* over time in the 96-hr assay using Lake Michigan (control) sediments, 5 replicates, 10 worms per replicate

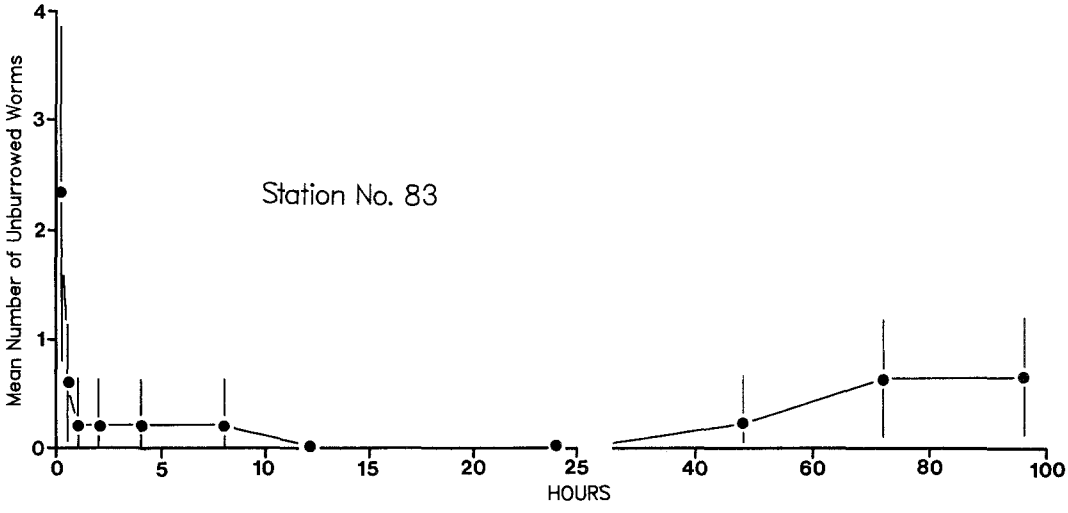

Fig. 3. Mean number and standard error of unburrowed *Stylodrilus* over time in the 96-hr assay using sediment from Detroit River Station 83, 5 replicates, 10 worms per replicate

Similar to Station 30, Station 34 was located in a large backwater area along the western shore of the Trenton Channel. Station 34 sediments were suspected to be the most contaminated of the four sites with potentially high levels of metals, chlorinated hydrocarbons, and volatiles (Mudroch 1985; Hamdy and Post 1985; Oliver and Bourbonniere 1985; Comba and Kaiser 1985). The hydrocarbon odor was very strong at the time of collection and during laboratory tests. A mean of only 3.6 *Stylodrilus* per replicate initially burrowed, and between 12 and 96 hr, a mean of 7.6 worms were on the sediment surface (Figure 6). After 24 hr, most *Stylodrilus* on the surface had lost their distinctive red pigmentation and appeared very unhealthy. After 48 hr, a mean of 7.8 of the *Stylodrilus* on the surface were dead; after 96 hr, a mean of only 0.6 *Stylodrilus* per replicate were alive (two of the original 50 worms were on the surface, and one was buried in the sediment).

Station 34 Sediment Dilution and Drying Assays

In addition to burrowing observations, the dilution series allowed a 96-hr LC_{50} estimate for Station 34 sediments. Results of the control and 100% Station 34 sediment concentration tests did not differ from

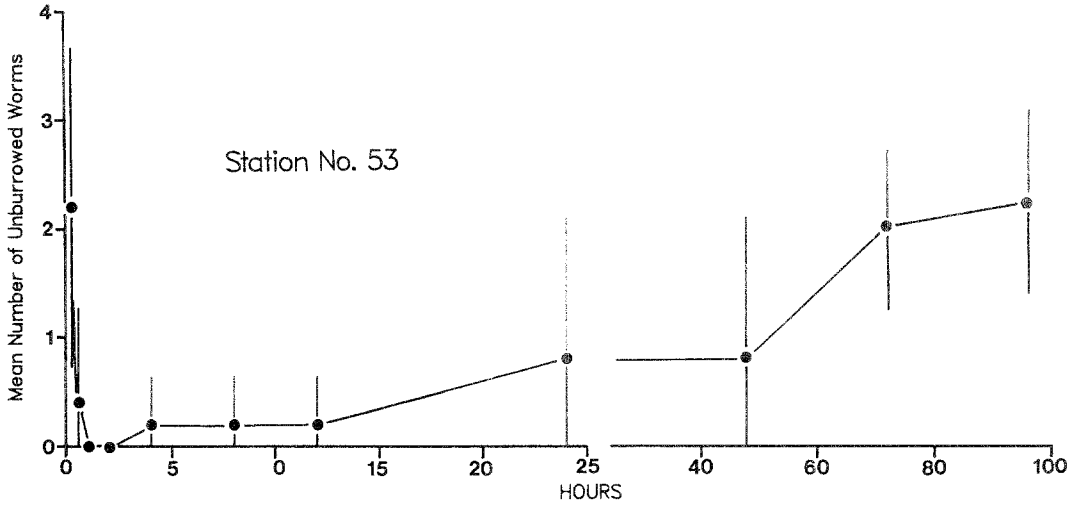

Fig. 4. Mean number and standard error of unburrowed *Stylodrilus* over time in the 96-hr assay using sediment from Detroit River Station 53, 5 replicates, 10 worms per replicate

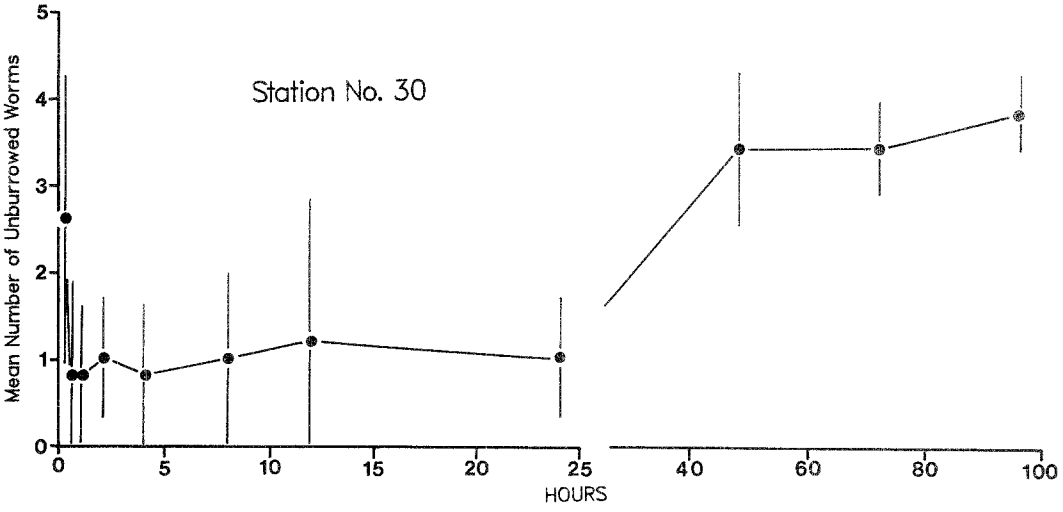

Fig. 5. Mean number and standard error of unburrowed *Stylodrilus* over time in the 96-hr assay using sediment from Detroit River Station 30, 5 replicates, 10 worms per replicate

results in the first assays (Figure 7, see also Figures 2 and 6). In the 100% concentration, less than 2.0 *Stylodrilus* per replicate ever burrowed. A mean of 6.6 of the *Stylodrilus* on the surface were dead after 48 hr, and a mean of 8.4 of the total number of worms per replicate were dead after 96 hr.

Burrowing activities were normal in the 2% and 10% concentrations (Figure 7). A mean of 0.4 *Stylodrilus* per replicate died in the 2% mixture, and a mean of 0.6 died in the 10% mixture. In the 50% mixture, a mean of 5.6 had burrowed after two hr. After eight hr, a mean of 6.6 *Stylodrilus* were on the surface and many were yellowish. Surprisingly, a mean of 7.0 *Stylodrilus* per replicate were burrowed by the end of 12 hr, and most remained in the sediment through 96 hr. Four of 16 *Stylodrilus* on the surface at the end of 48 hr were dead, and mean mortality after 96 hr was 3.8. The 96-hr LC_{50} calculated from these data was approximately equal to a 62% Station 34 sediment concentration (Figure 8).

After air-drying and rewetting the Station 34 sample, sediments were lighter in color, and there were no obvious hydrocarbon odors. Subsequent initial burrowing responses were similar to responses observed in uncontaminated sediments. All *Stylodrilus* had burrowed after four hr (Figure 9). Similarly, after eight hr, only one of 40 *Stylodrilus* was on the surface, yet an additional 13 *Sty-*

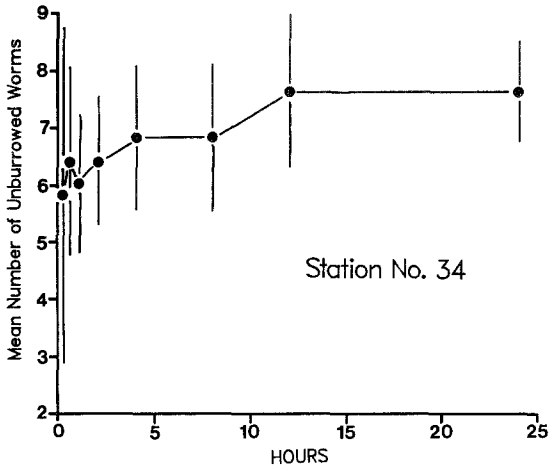

Fig. 6. Mean number and standard error of unburrowed *Stylodrilus* over time in the 96-hr assay using sediment from Detroit River Station 34, 5 replicates, 10 worms per replicate. Points between 24 and 96 hr equal to 24 hr value and are not plotted

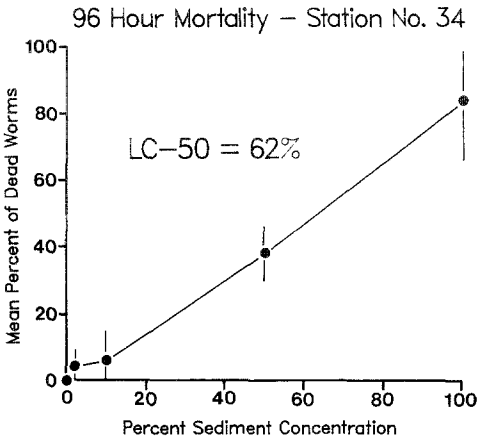

Fig. 8. Mean percent and standard error of *Stylodrilus* mortality at the end of 96 hr in 5 concentrations of Station 34 sediments diluted with Lake Michigan (control) sediment (v:v), 5 replicates, 10 worms per replicate

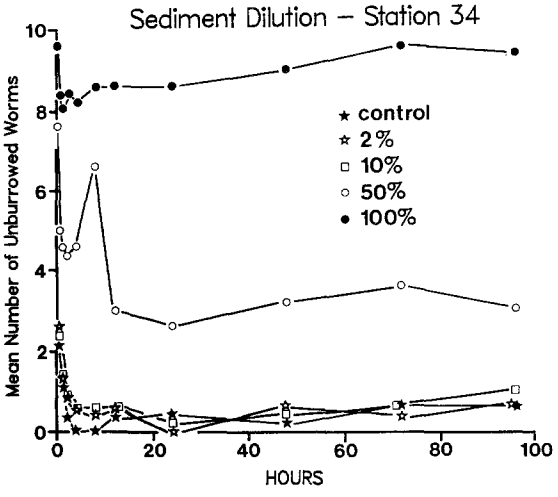

Fig. 7. Mean number of unburrowed *Stylodrilus* over time in a 96-hr assay using various mixtures (v:v) of Station 34 sediment diluted with Lake Michigan (control) sediment, 5 replicates, 10 worms per replicate

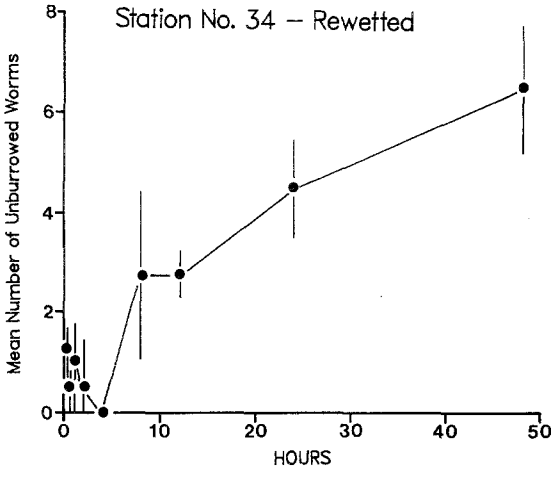

Fig. 9. Mean number and standard error of unburrowed *Stylodrilus* over time in the first 48 hr of the 72-hr assay using dried and rewetted sediment from Detroit River Station 34, 4 replicates, 10 worms per replicate

lodrilus were partially exposed (<25% of body length) on the surface. After 24 hr, a mean of 4.5 *Stylodrilus* were totally exposed on the surface. Several *Stylodrilus* looked unhealthy, but none had died. At 48 hr, a mean of 6.0 were on the surface, and two of the total 40 were dead. At 72 hr, the mean number of *Stylodrilus* dead on the surface had increased to 8.4 per replicate, and the remaining mean of 1.3 worms per replicate on the surface appeared nearly dead. The experiment was stopped at the end of 72 hr; only six of the initial total *Stylodrilus* were alive, none of which appeared healthy.

Burrowing and EC_{50} /LC_{50} Patterns

A variety of burrowing patterns were noted in the sediment assays along with differences in mortality. Combining these data with data from Keilty (1987) and Keilty *et al.* 1988, we have attempted a first assessment of burrowing/mortality patterns in relation to potential sediment contamination (Table 2). Based on Table 2, sediments from Station 83 would be judged non-toxic to *Stylodrilus, i.e.,* levels of metals or toxic organics, if present, were not great enough to stimulate burrowing pattern changes within 96 hr. No mortality occurred in Station 53

Table 2. Levels of sediment contamination as determined from the *Stylodrilus heringianus* burrowing avoidance assays for Detroit River sediments and for data from Keilty (1987) and Keilty *et al.* (1987). Numbers based on 10 worms per assay replicate.

	Mean number of worms on surface			
Level of contamination	Beginning phase 2–8 hr	Ending phase 48–96 hr	% Mortality 96 hr	Detroit River Station
Uncontaminated	<1.0	<1.0	<1.0	Control 83
Sediments alone				
Mild contamination	<1.0	1.0–2.0	<1.0	53
Moderate contamination	<1.0	2.0–5.0	1.0–5.0	
Strong contamination	<1.0	>5.0	>5.0	
Sediments and pore water				
Mild contamination	1.0–5.0	1.0–5.0	<5.0	30
Strong contamination	>5.0	>5.0	>5.0	34

sediments. All *Stylodrilus* burrowed in the beginning phase, but a mean of 2.0 worms per replicate were unburrowed at 96 hr, indicating a mild response, probably due to sediment-bound contaminants. In Station 30 sediments, there was a slight burrowing response in the beginning phase and a high burrowing response in the ending phase, with increased mortality toward the end of 96 hr. This may represent some initial response to pore water contaminants and then a reaction to more tightly bound contaminants.

Station 34 sediment assays yielded strong beginning and ending phase responses and 94% mortality. Responses in the beginning burrowing phase were eliminated by drying and rewetting, suggesting that volatile or pore water contaminants initially present in Station 34 sediments, which acted as an external irritant deterring burrowing, were reduced. However, because *Stylodrilus* returned to the surface and died after the beginning phase in rewetted sediments, the lethal response-producing contaminants apparently were bound to sediments or present in the overlying and pore waters in a non-volatile form. This type of assay provides valuable lethal toxicity data which could be used to compare the relative potential toxicity of different contaminated sediments.

Beyond the general responses provided in Table 2, other behavioral and feeding responses were observed. Normal burrowing and feeding behavior usually resulted in both a mosaic of tunnels through out the sediment, many of which were clearly visible along the sides of the beakers, and in a roughing of the sediment surface (Figure 10). Both tunnels and surface roughing occurred in assays using control and Station 30, 53, and 83 sediments. Although some *Stylodrilus* did burrow in the first

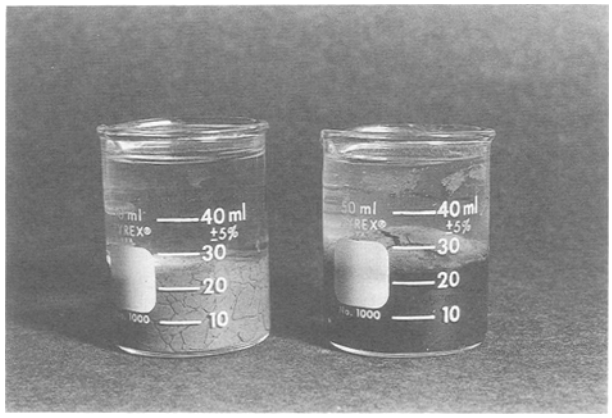

Fig. 10. Photograph of beakers containing Lake Michigan (control) (left) and Station 34 (right) sediments and 10 *Stylodrilus* after 96 hr. Note absence of burrows and surface roughing and the dark coloration of Station 34 sediment.

assay of Station 34 sediments, few tunnels were visible, and the surface remained relatively smooth (Figure 10), suggesting that normal feeding activities did not occur. Feeding channels were present in the rewetted 34 sediment assay, leading to the conclusion that mortality was most likely caused by ingestion of bound or pore water contaminants remaining.

Tubificids are often pictured with their tails protruding some distance above surface fecal mounds (*e.g.*, Mozley and Howmiller 1977; White *et al.* 1986a). Although we have occasionally observed this behavior, a large proportion of the individuals of most Great Lakes tubificid species and *Stylodrilus* remain entirely beneath the sediment surface, except to expel an occasional fecal pellet. In our assays, the classic tail protruding behavior was observed only in response to apparent sediment

contamination. Prior to returning to the sediment surface, about 25% of a worm was exposed as in the classical description, which occurred anywhere from eight hr (rewetted Station 34 sediments) to 72 hr into the assay (Station 53 sediments) and lasted up to 24 hr. Both the extent of visible channels and first stage of the 'unburrowing' process are potential additional response variables to be considered in future assays.

Comparisons With Natural Detroit River Populations and Trophic Indicators

At least a few oligochaetes were present in the sediments collected at each study site. Species were limited, however, to those classified as saprophilic and saprobiontic Tubificidae (Lauritsen *et al.* 1985). *Limnodrilus hoffmeisteri* and *L. claparedianus* were present at Station 30 and were collected there at other times of the year (White, unpub. data). A few *L. hoffmeisteri* and *L. claparedianus* were recovered from Station 34, but no worms had been found in any of our previous collections. Only *L. cervix* was found at Station 53, which also was the only species previously collected there. No oligochaetes were found in Station 83 sediments; however, we had previously collected both *L. hoffmeisteri* and *L. claparedianus*. These data corresponded to recent descriptions of the distribution and abundance of oligochaetes (primarily Tubificidae and Naididae) in the Detroit River (Ontario Ministry of the Environment 1979; Hiltunen and Manny 1982; Thornley and Hamdy 1984; Thornley 1985).

L. hoffmeisteri and *L. claparedianus* have been classified as indicators of organic enrichment. *L. cervix* is an indicator of heavy organic enrichment, while the presence of *Stylodrilus* usually indicates extremely low levels of enrichment (Lauritsen *et al.* 1985). Associations of distributions (particularly for *L. hoffmeisteri* and *Stylodrilus heringianus*) with various in-place contaminants are limited (Chapman and Brinkhurst 1984), but in laboratory studies Chapman *et al.* (1982a, 1982b, 1982c) found that *Stylodrilus* was often less sensitive (using death as the endpoint) than *L. hoffmeisteri* to a variety of contaminants in water. Keilty *et al.* (1988), however, found that *Stylodrilus* was more sensitive than *L. hoffmeisteri* to endrin-contaminated sediments using sublethal behavioral response assays, which is consistent with the proposed trophic status of the two species (Lauritsen *et al.* 1985). These data suggest that a sublethal assay is more sensitive than a lethal assay. Even though Chapman and Brinkhurst (1984), Lauritsen *et al.* (1985), and others have questioned the oligotrophic status of *Stylodrilus,* the results from these tests, along with the fact that some *L. hoffmeisteri* were present in the original sediments, indicate a greater sensitivity of *Stylodrilus* to Detroit River sediment bound contaminants. Further, the ease of separating *Stylodrilus* from other live worms (White *et al.* 1987) makes this an attractive species for bioassay.

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