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POPULATION-SPECIFIC TOXICITY RESPONSES BY THE FRESHWATER OLIGOCHAETE, *STYLODRILUS HERINGIANUS*, IN NATURAL LAKE MICHIGAN SEDIMENTS

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Abstract – Sediment reworking rate, mortality and organism dry weight were measured for *Stylodrilus heringianus* in laboratory microcosms. The experiments were designed to identify potential population-specific response differences to mixed (stirred to obtain a more uniform particle size distribution over depth) and unmixed (passively settled) microcosm sediments. Lake Michigan sediments and worms were collected offshore Benton Harbor, Michigan and Grand Haven, Michigan.

The mixed Benton Harbor sediments were toxic to *S. heringianus* collected from Grand Haven, whereas there were no significant differences in measured responses between mixed and unmixed sediment microcosms for Grand Haven-collected worms exposed to Grand Haven sediments or Benton Harbor-collected worms exposed to Benton Harbor sediments. Note that the mixing of sediments resulted in increased availability of contaminants sorbed to the fine sediment fraction.

Because contaminant and oligochaete population density data suggest that Grand Haven sediments are less contaminated, the population-specific response suggests that *S. heringianus* may adapt to the low level long-term stressful conditions (chemical or otherwise). Results also suggest caution and consideration of the history of test organisms in the design and interpretation of toxicity tests.

Keywords – Ecotoxicology Sediment reworking Gamma scan system Oligochaete mortality Oligochaete weight loss

INTRODUCTION

Oligochaetes, a major benthic component of lacustrine systems [1], ingest subsurface sediments and convey them to the sediment–water interface as fecal pellets [2–4]. As a result of this conveyor-belt/sediment reworking activity, sediment bound xenobiotics are often encountered in higher surface concentrations than would be predicted from deposition rates [5–7]. The most novel of current approaches for examining sediment reworking processes uses a gamma scan system to quantify oligochaete feeding rates [4,6]. This method measures the burial rate of sediment fractions caused by feeding activity (the reworking rate) by monitoring the burial of a surface applied submillimeter layer of sediment–sorbed ^{137}Cesium in laboratory microcosms [4,6]. Comparisons between contaminated and uncontaminated sediments, using reworking rates, postexperimental worm mortalities and organism dry weights as response variables, afford an extremely sensitive indication of toxicity to oligochaetes from relatively long-term (approx. 50 d), low level contaminant exposures [6,7].

Previous gamma scan/toxicity studies conducted in our laboratory [6–8] had used the oligotrophic Lumbriculid oligochaete, *Stylodrilus heringianus*, and its surrounding sediments, collected from Lake Michigan off Benton Harbor, Michigan. In these earlier experiments, particle distributions and toxicant exposures to oligochaetes were determined in sediment microcosms when microcosm sediment–contaminant–lake water slurries were poured and allowed to settle passively [6].

In a recent effort to create a more uniform exposure over depth to a sediment bound toxicant (p,p'-DDT), microcosm sediment slurries were permitted to settle as usual, then overlying water was removed, sediments were mixed to uniformity and the water gently returned. For field collection convenience (the home port of the research vessel is Grand Haven), *S. heringianus* obtained from the Grand Haven (generally considered less contami-

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nated than Benton Harbor [9]) region of Lake Michigan were used, whereas the sediments were from a large Benton Harbor stock supply.

It became apparent from reworking rates and the general appearance of control cells (no DDT) that the "remixed" Benton Harbor sediments were toxic to the worms collected from Grand Haven (T.J. Keilty, unpublished data). Three important questions raised by this observation were addressed. (a) How did remixing make previously innocuous sediments toxic? (b) Would remixing of sediments result in a toxic response by *S. heringianus* in general? (c) Were remixed Benton Harbor sediments toxic only to *S. heringianus* collected from Grand Haven?

MATERIALS AND METHODS

Overview of experimental design

Three experiments—each of approximately 42 d duration—were conducted using the gamma scan system (described below). In experiment 1, oligochaetes collected from Grand Haven were exposed to sediments collected from Benton Harbor. In experiment 2, both sediments and worms collected from Grand Haven were used and in experiment 3, both sediments and worms collected from Benton Harbor were used. Six glass microcosms (5.5 cm × 3.5 cm × 30 cm), each containing approximately 200 g dry weight sediment and 75 *S. heringianus*, were used in each experiment. In each experiment, sediments in three microcosms were allowed to settle passively, whereas sediments in the other three were remixed. Measured response variables were reworking rates, and worm mortality and dry weight after termination of each test.

Collection sites

Benton Harbor sediments were collected in October 1983, with a Ponar grab 10 km offshore of the Donald C. Cook Nuclear Plant at a water depth of 42 m (Fig. 1). To create a uniform sediment for many future experiments, sediments were allowed to dry at room temperature and thoroughly mixed by passing them through a 0.25 mm mesh sieve. Sediments were "reconstituted" as needed with lake water and a few milliliters of sieved fresh Benton Harbor sediment (sediment from tank used to house most recently collected worms) to provide an active bacterial flora. Benton Harbor worms were collected at the sediment collection site annually or biannually to date. All worms were maintained for at least one month prior to use in 200 liter aquaria in the dark at 10°C.

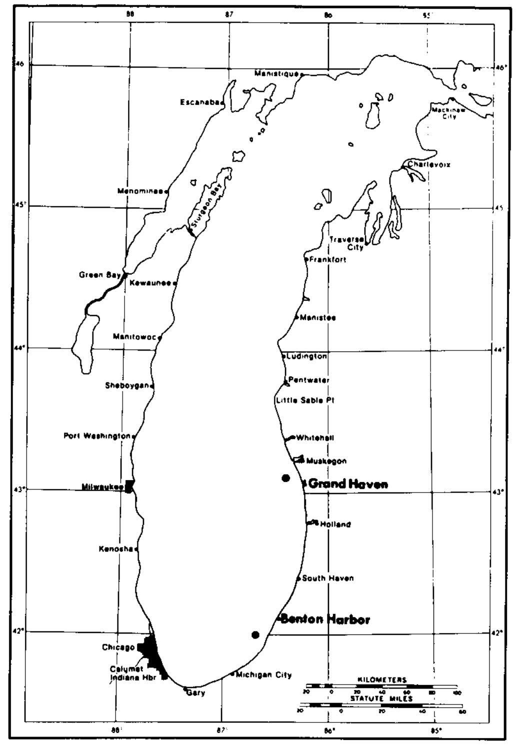

Fig. 1. Offshore Grand Haven and Benton Harbor sediment and worm collection locations in Lake Michigan.

Grand Haven sediments and worms were collected in October 1988, with a Ponar grab approximately 10 km northwest of Grand Haven, Michigan, in 45 m of water (Fig. 1). Wet sediments were stored at 4°C for approximately one month and sieved (0.5 mm mesh) prior to use.

Gamma scan system

The basic gamma scan system has been described in detail previously [6,10]. For these experiments, an upgraded automated (PC computer driven) system was used. The system consisted of a well-collimated NaI scintillation detector supported on a mechanically driven elevator/platform capable of precise, repeatable movements in both *x* and *y* axes. The apparatus was used to vertically scan experimental microcosms contained in an aquarium filled with aerated epilimnetic Lake Michigan water maintained at 10°C. The entire aquarium was covered with black plastic to keep out light. Prior to the addition of worms, a submillimeter layer of the gamma-emitting radioisotope ^{137}Cs adsorbed on illite clay particles was pipetted onto the sediment surface of each microcosm. A multichannel analyzer system was used to isolate counts from the 0.662 MeV energy peak of the ^{137}Cs. The top 1 to 3 cm of each microcosm was scanned in mm increments (600 s counts/mm) approximately twice daily after the addition of oligochaetes. Depths of the peak position of gamma activity in each microcosm were calculated by fitting count data to a Gaussian distribution [2,4,9] and plotted vs. time.

Microcosm preparation

In experiments 1 and 3, 2 liters of lake water and approximately 1 ml of fresh Benton Harbor sediment were added to dried Benton Harbor sediment (approximately 1,200 g dry weight total) in a 4 liter beaker and the mixture was stirred for 24 h. After settling 72 h, overlying water was decanted off and enough fresh water added to thoroughly remix the sediment. Each of six microcosms was then filled with the slurry. To assure a consistent distribution of particles between microcosms, about 100 ml of the slurry was added to each microcosm at a time, restirring the slurry in between, until all were full. In experiment 2, presieved wet Grand Haven sediments were treated in the same manner.

Unmixed sediment microcosms were allowed to settle without any further manipulation for about one month at 4°C in the dark. Mixed sediment cells settled for 14 d under the same conditions, then overlying water was aspirated off. A long-handled stainless spatula was then used to thoroughly mix and redistribute the sediment particle size fractions into a more uniform vertical column. The overlying water was then returned by gently pipetting it along microcosm walls. The resultant cells were then permitted to settle an additional 16 d at 4°C in the dark.

After settling, a submillimeter layer of ^{137}Cs was pipetted onto the sediment surface in each microcosm. Cells were then placed into the aquarium and allowed to equilibrate to 10°C for 1 d. Initial positions of ^{137}Cs activity were determined prior to the addition of 75 worms to each microcosm. Worms were removed from culture tanks and added to microcosms in lots of 15 to minimize any potential size bias [6]. Microcosms were scanned to determine depths of peak gamma activity approximately twice per day for the duration of all experiments.

Postexperimental measures

At the termination of experiments, microcosm sediments were gently sieved (0.25 mm mesh) to recover oligochaetes for percent mortality and dry weight determinations. Worms were teased clean of debris and all adhering sediment particles with forceps under a dissecting microscope. Worms from each microcosm were separated into three roughly equal lots and dried at room temperature in a desiccator before weighing.

Additionally, prior to experiment 1, both unmixed (1) and mixed (2) cells were prepared as described above to determine the vertical porosity and organic carbon distributions in microcosms without oligochaetes. Only one unmixed cell was created because similar data existed from previous experiments [6,7]. After the settling period, cells were frozen in a dry ice–alcohol bath after [6]. Entire frozen sediment "plugs" were sectioned in 1 cm intervals with a fine-blade hacksaw and each interval was measured for porosity (wet wt. − dry wt./wet wt. × 100) and organic carbon (percent ashfree dry weight). One mixed and one unmixed microcosm from experiments 2 and 3 were treated in an equal fashion to examine oligochaete influence on particle–organic carbon redistribution.

Mean reworking rates, worm weights and worm mortality in unmixed vs. mixed sediment microcosms in each experiment were compared using one-way ANOVA on the statistical package SAS (SAS Institute, Cary, NC). Differences were considered significant if $p < 0.05$. Reworking rates were calculated by dividing the slope of the ^{137}Cs burial data by 75 (number of worms added) and expressing it in terms of cm/worm/h.

RESULTS AND DISCUSSION

Reworking rates

In experiment 1, sediment reworking by Grand Haven *S. heringianus* in mixed Benton Harbor sediment was significantly lower than in unmixed sediment (Table 1, Fig. 2). However, no significant differences between reworking rates in mixed vs. unmixed sediments were observed in experiments 2 and 3 (Table 1, Figs. 3 and 4).

Oligochaete reworking rates measured in all sediments except the mixed Benton Harbor with Grand Haven worms (experiment 1) were consistent with previous control rate measurements for *S. heringianus* [3,6,11]. For example, using the same Benton Harbor sediments with Benton Harbor *S. heringianus*, [6] observed rates for control cells ranged from 0.86 to 1.39 × 10^{-5} cm h^{-1} $worm^{-1}$, whereas observed rates in all three experiments ranged from 0.61 to 1.11 × 10^{-5} cm h^{-1} $worm^{-1}$. However, the mean reworking rate for the mixed Benton Harbor sediment with Grand Haven

Table 1. Postexperimental mean (SD = standard deviation) percent worm mortalities, worm dry weights (mg) and sediment reworking rates (cm h^{-1} $worm^{-1}$ $\times 10^{-5}$) for experiments 1-3

	Reworking rate (cm h^{-1} $worm^{-1}$ $\times 10^{-5}$) Mean (SD)	Mortality (%) Mean (SD)	Dry weight (mg) Mean (SD)
Experiment 1[a]			
Mixed	0.17 (0.10)*	50.9 (40.0)*	0.489 (0.088)*
Unmixed	0.78 (0.14)	3.5 (4.1)	0.604 (0.064)
Experiment 2[b]			
Mixed	0.61 (0.03)	3.3 (4.6)	0.602 (0.083)
Unmixed	0.94 (0.11)	0.0 (0.0)	0.662 (0.045)
Experiment 3[c]			
Mixed	0.89 (0.11)	0.0 (0.0)	0.802 (0.061)
Unmixed	1.11 (0.06)	0.0 (0.0)	0.791 (0.087)

The asterisk (*) indicates a significant difference between mixed and unmixed sediment types ($p < 0.05$).
[a]Benton Harbor sediment, Grand Haven worms.
[b]Grand Haven sediment, Grand Haven worms.
[c]Benton Harbor sediment, Benton Harbor worms.

worms was 0.17×10^{-5} cm h^{-1} $worm^{-1}$. Because of the gamma scan system's ability to quantify subtle alterations in oligochaete feeding behavior in response to toxic contaminants (such as endrin, [6]), it appears that the Grand Haven worms responded to a toxicant or suite of toxicants made available by mixing the Benton Harbor sediment.

Mixing alone resulted in a small but insignificant reduction in reworking rate, as demonstrated in experiment 2 (Grand Haven sediment and worms). However, the reduced rate was still six times that of the rate observed from the mixed Benton Harbor sediment and Grand Haven worms. The slight reduction in the reworking rates from the mixed sediment microcosms (this was also observed in experiment 3) was likely caused, in part, by the increased compaction (approximately 1.5 cm relative to unmixed sediments) of microcosm sediments after mixing.

In experiment 3 (Benton Harbor sediments and worms), reworking rates from unmixed and mixed microcosms were not significantly different; and again, the reworking rate in mixed sediments was slightly lower than the rate in unmixed sediments. Therefore, reworking data alone suggest that toler-

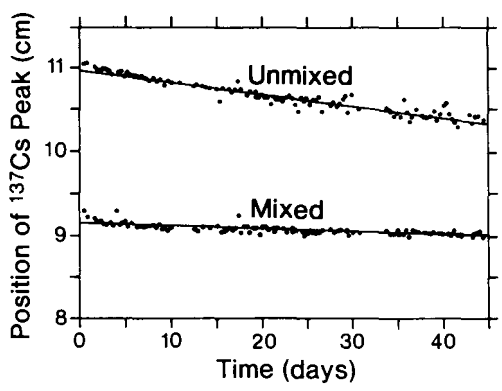

Fig. 2. Mean depths ($n = 3$) of ^{137}Cesium plotted vs. time (d) in mixed and unmixed sediment microcosms using Benton Harbor sediments and Grand Haven worms.

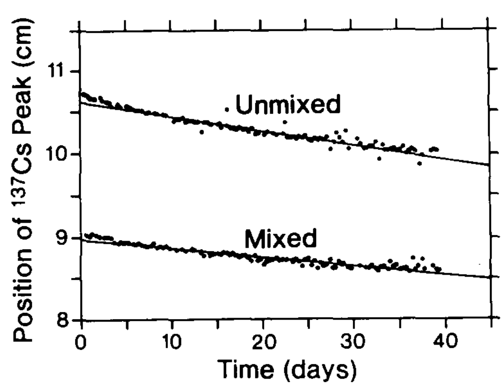

Fig. 3. Mean depths ($n = 3$) of ^{137}Cesium plotted vs. time (d) in mixed and unmixed sediment microcosms using Grand Haven sediments and Grand Haven worms.

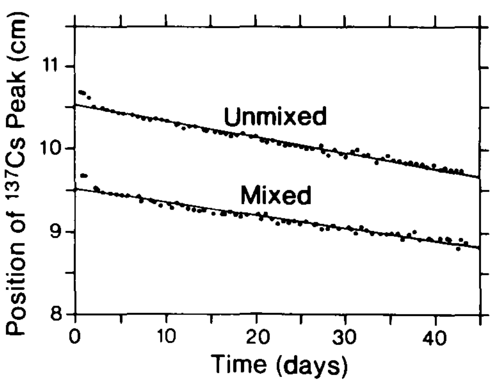

Fig. 4. Mean depths ($n = 3$) of ^{137}Cesium plotted vs. time (d) in mixed and unmixed sediment microcosms using Benton Harbor sediments and Benton Harbor worms.

ance to some contaminant(s) in Benton Harbor sediments is significantly lower for Grand Haven collected *S. heringianus* than for *S. heringianus* collected from Benton Harbor.

Worm mortality

Postexperimental worm mortality was negligible in mixed and unmixed sediments in all experiments except for mixed sediments in experiment 1, where it averaged 50.9% (Table 1).

Consistent with previous gamma scan toxicity experiments [6,7], observed worm mortality at the termination of all three experiments was negligible where normal reworking activities were evident (0 to 3.5 percent, Table 1). However, in the mixed Benton Harbor sediment in experiment 1, where reworking by Grand Haven *S. heringianus* was greatly impaired, worms suffered a 50.9 (±40.0) % level of mortality. Based on previous work, the high variability in mortality can be attributed to the lower (relative to worm dry weight and reworking rate) sensitivity of the mortality measurement as a response variable in subacute oligochaete toxicity test formats [6,8,12].

Worm weights

Postexperimental worm dry weights were consistent with the reworking and mortality data. In experiment 1, mean dry weights of the surviving Grand Haven oligochaetes in mixed Benton Harbor sediments were significantly lower than dry weights of Grand Haven worms in unmixed Benton Harbor sediments. There were no significant differences between worm weights from mixed vs. unmixed in experiments 2 and 3 (Table 1). Therefore, the weight reduction observed in experiment 1 suggests that normal feeding behavior was altered and the alteration was consistent with other observed weight reductions in previous sediment toxicity tests [6,7].

Dry weights of Benton Harbor worms in general are significantly higher than dry weights of Grand Haven worms. This may be because the Benton Harbor area has a higher flux of organic carbon to the sediments, resulting in more available food resources (as well as contaminants) to the Benton Harbor population [13,14]. In previous studies [6,7], slight but significant increases in body weight were observed for *S. heringianus* exposed to low levels of endrin (approx. 20 ng/g dry wt. sediment), indicating some potential stimulatory effects for exposures to low contaminant levels. It is possible that the Benton Harbor population has adapted to the low level ambient contamination, while exploiting the relatively nutrient rich sediments.

Microcosm sediment porosity and particle distribution

In unmixed Benton Harbor sediments uninfluenced by oligochaete reworking, the upper 3 cm had a greater water content than mixed sediments (Fig. 5). The most pronounced disparity was observed in the top centimeter (70.2 vs. 40.7% in unmixed, mixed, respectively), whereas water contents were nearly equal below 3 cm.

In microcosm sediments reworked by oligochaetes, water content profiles were more similar, yet the uppermost cm of the unmixed sediments

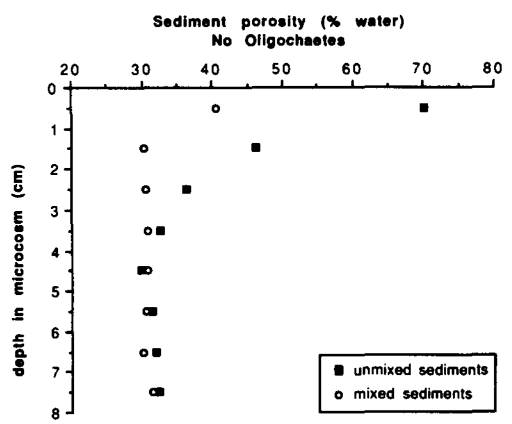

Fig. 5. Vertical percent water content data for unmixed and mixed microcosm sediments without oligochaetes (Benton Harbor sediment).

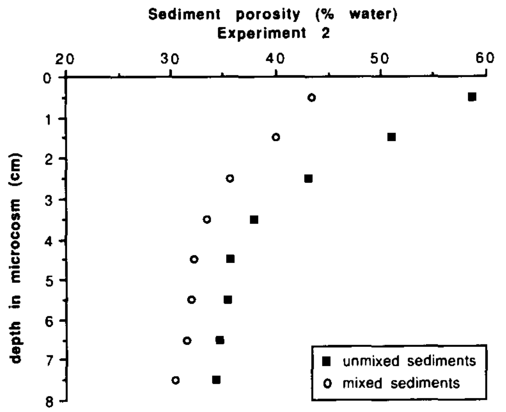

Fig. 6. Vertical percent water content data for unmixed and mixed microcosm sediments from experiment 2 (Grand Haven sediments and worms).

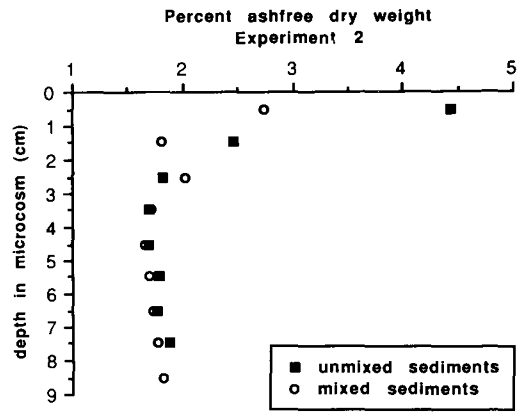

Fig. 8. Vertical distribution of organic carbon as percent ashfree dry weight for unmixed and mixed microcosm sediments from experiment 2 (Grand Haven sediments and worms).

was only about 55% water, and the top cm of the mixed sediments rose slightly to about 44%. Lower depth strata were once again similar (Figs. 6 and 7).

Trends in organic carbon profiles (as percent ashfree dry weight) in both mixed and unmixed microcosms were similar to trends in sediment porosity distributions (Figs. 8 and 9).

Differences in porosity and particle distribution between unmixed and mixed microcosm sediments are prominent in the upper 3 cm, well within the feeding zone of *S. heringianus* [2,7]. By creating a more uniform, more compact particle distribution in the mixed microcosms, it is possible that the normal feeding pattern is disrupted resulting in a stressful condition. In experiment 2 using Grand Haven sediments and worms, the reworking data suggest a slight stress; however, reworking data from experiment 3 using Benton Harbor sediments and worms do not support this hypothesis. The small, yet consistent, higher reworking rate observed in unmixed microcosm sediments is more likely caused by a slightly greater overall compaction (approx. 1-2 mm) of unmixed microcosm sediments during an experiment. Although not quantified, and therefore not incorporated into reworking rate

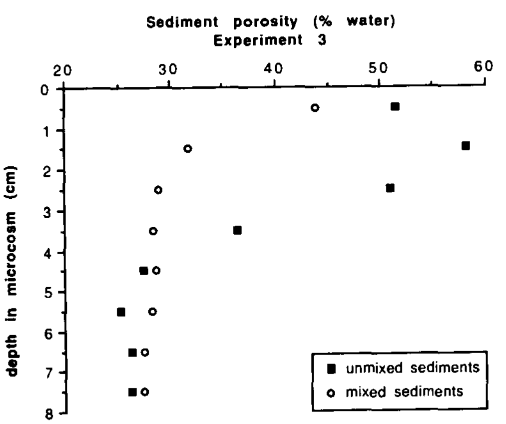

Fig. 7. Vertical percent water content data for unmixed and mixed microcosm sediments from experiment 3 (Benton Harbor sediments and worms).

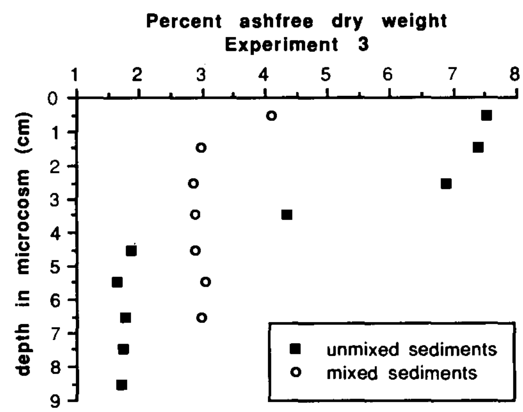

Fig. 9. Vertical distribution of organic carbon as percent ashfree dry weight for unmixed and mixed microcosm sediments from experiment 3 (Benton Harbor sediments and worms).

calculations, this would account for an overestimation of reworking in unmixed sediments similar in magnitude to the observed difference.

Exposure of benthos to sediment-associated contaminants occurs by two routes; from the interstitial water and by ingestion of sediment particles [15]. Because the oligochaetes selectively ingest fine, bacteria-laden organic particles [16–18], exposure by ingestion would be greater than predicted from bulk sediment contaminant concentrations, because hydrophobic organic contaminants sorb preferentially to the organic fraction of the sediment. The reduction in reworking rate, worm dry weight and increase in mortality observed in the mixed sediments from experiment 1 may be caused by the creation of a more uniform vertical distribution of resident contaminants in the Benton Harbor microcosms. This uniform distribution may have made highly contaminated particles associated with the surface of the passively settled cells more available for ingestion by oligochaetes in the mixed cells. And, if more contaminants are available overall throughout the entire feeding zone in the mixed microcosms, then the uptake by the interstitial water should also be greater. However, the exact uptake mechanism remains elusive and will require further study.

Ecotoxicological considerations

Results from all three experiments and available sediment contamination and organism density data for the Benton Harbor and Grand Haven sampling locations suggest that the oligotrophic *S. heringianus* possesses a sufficient genetic plasticity or acclimative ability to develop some tolerance to habitat perturbations. Although a complete spectrum of contaminants is not available for both locations, comparative polycyclic aromatic hydrocarbon data exist [9]. Off Benton Harbor, combined sediment concentrations of anthracene, phenanthrene, fluoranthene, pyrene, chrysene and benzo[*a*]pyrene were found to be approximately seven times that of concentrations off Grand Haven (approx. 700 ppb vs. 110 ppb [9]).

Additionally, previous oligochaete population density work suggests that the ratio of *S. heringianus* to total tubificidae (generally considered tolerant of eutrophic to mesotrophic conditions) may be a useful indicator of lake trophic status [14], particularly for identifying the subtle changes inherent in a lake's evolution from oligotrophic to mesotrophic status (higher ratios would be indicative of more oligotrophic waters). In a 1981 benthic study [19], ratios of *S. heringianus* to total tubificidae were found to be 2.73:1 and 1.39:1 for offshore Grand Haven and Benton Harbor areas, respectively. These ratios are consistent with the limited contaminant data, but perhaps more importantly, they may indicate that the fewer *S. heringianus* individuals relative to the tubificids found off Benton Harbor may be a more stress-tolerant population than the population found where conditions are more consistent with the species' oligotrophic nature. Taken in conjunction with the experimental results, it would appear that a true difference in stress-tolerance exists between the two populations.

CONCLUSIONS

Sediment toxicity can be assessed from the mosaic of measured response variables generated when using oligochaetes and the gamma scan system. In this instance, using reworking rates, worm mortalities and dry weights, it is clear that mixed Benton Harbor sediment is toxic to *S. heringianus* native to Grand Haven, but not to *S. heringianus* native to Benton Harbor. Such a population-specific response suggests that the species may be able to adapt to low level, long-term stresses (chemical or otherwise), and the results underscore the need to consider the collection environment when natural populations are collected for toxicity assessments.

Further, these studies suggest that contaminants, perhaps both nutrients and xenobiotics, focused in the depositional basin offshore Benton Harbor, have the potential to alter the ecological balance. Our data (although suggestive and not cause–effect) are the first evidence that the low levels of contaminants in the open lake are affecting any of the resident organisms.

Using a matrix of responses to demonstrate the effect of sediment-associated contaminants permits an improved definition of potential effects and the sensitivity to detect population specific responses.

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