

Behavioral responses of a phoxocephalid amphipod to organic enrichment and trace metals in sediment

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ABSTRACT: Phoxocephalid amphipods, *Rhepoxynius* spp., avoided sediments mixed with domestic sewage, or with concentrations of trace metals (zinc and cadmium) which are characteristic of marine sediments at waste-water discharge sites. The amphipods burrowed and swam from fine sands enriched with 15 % sewage, and from sewage-contaminated sediment collected near the White's Point outfall in southern California, USA. They also suffered high (> 50 %) mortality in sediment inoculated with high levels of zinc (610 $\mu\text{g g}^{-1}$) or cadmium (190 $\mu\text{g g}^{-1}$); swam and burrowed from sediments with high and moderate concentrations of either element; and burrowed from sediments with relatively low concentrations of either element. These responses indicate that behavioral avoidance of contaminated sediment may be important in maintaining population patterns of these phoxocephalids, which are often rare or conspicuously absent around large waste discharges.

INTRODUCTION

Marine bottom communities around large waste discharges often are highly modified, and characterized by the absence of certain groups of benthic invertebrates, particularly crustaceans and echinoderms (Filice, 1959; Pearson and Rosenberg, 1978; Word, 1979). Among burrowing crustaceans, phoxocephalid amphipods appear to be especially sensitive to waste discharge and sewage-enriched habitats (Word, 1979). Because of its apparent sensitivity, one species, *Rhepoxynius abronius*, has been the focus of recent efforts to develop a bioassay that might aid in regulating the quality of waste discharges (Swartz et al., 1979; Swartz et al., 1982).

The causes of the low abundance of phoxocephalid amphipods near waste discharges have not been determined. While bioassay studies often concern the lethal effects of sediment contaminants only, many organisms may avoid substrates that cause high mortality. Although habitat selection among amphipods is well known (Meadows and Campbell, 1972), this behavior has not been related to population changes around waste discharges. Nor has it been determined whether phoxocephalids avoid sediments that have been modified by sewage disposal. Such avoidance could be in

response to either organic enrichment or elevated concentrations of metals in the sediment.

The possible role of habitat selection was explored in simple laboratory experiments in which phoxocephalids (*Rhepoxynius* spp.) were given a choice between settling in contaminated or uncontaminated sediments. The specific objectives of the experiments were to determine if phoxocephalids (1) avoid sediments enriched with organic waste; and/or (2) avoid sediments containing elevated concentrations of trace metals (zinc and cadmium). Zinc and cadmium were selected for these experiments because both of these potentially toxic elements are enriched in the sediment perturbed by ocean outfall discharges (Förstner and Wittmann, 1981).

METHODS

Experimental animals and sediments were collected in Monterey Bay, California, at a water depth of 15 m by SCUBA divers using acid-cleaned plastic buckets. The sediment was passed through a 0.5 mm Nytex screen to remove macrofauna, then held in running sea water. *Rhepoxynius* spp. were collected from the screen residues with a pipette, and were held in aquaria for at least 24 h before use. Due to the difficulty

of identifying live phoxocephalids to species, mixtures of approximately 80 % *Rhepoxynius abronius* – previously identified as *Paraphoxus epistomus* (Oliver et al., 1980, 1982) – and 20 % *R. fatigans* – formerly *P. daboius* (op. cit.) – were used. Final identifications were performed after the experiment was concluded.

Aliquots of the Monterey Bay sediment were enriched to 1, 5 and 15 % by volume with sewage from the digestion tank of the Watsonville municipal treatment plant. This sewage was relatively high in organics, and relatively low in heavy metals ($0.08 \mu\text{g Zn g}^{-1}$; $0.005 \mu\text{g Cd g}^{-1}$) and pesticides (unpubl. data, California Water Quality Control Board, Central Coast Region).

Sewage-enriched sediment was also collected in the field with a Van Veen grab at JWPCP Station C-6 (Hershelman et al., 1981), which is 0.5 km from the Los Angeles County Sanitation District's marine discharge at White's Point. This sediment, which was relatively high in organics, heavy metals ($930 \mu\text{g Zn g}^{-1}$; $27 \mu\text{g Cd g}^{-1}$), and pesticides (Word and Mearns 1979), was frozen until use.

Metal-enriched sediment was prepared by adding $\text{Zn}(\text{NO}_3)_2$ or CdCl_2 to wet, sieved (median $\phi = 3.2$), sand from Monterey Bay and homogenizing it for 30 min prior to the test period. The concentrations of zinc ($31 \pm 5 \mu\text{g g}^{-1}$) and cadmium ($0.11 \pm 0.03 \mu\text{g g}^{-1}$) in the untreated Monterey Bay sediment, which was used as the control sediment, were relatively low; and the additions made the metal concentrations representative to field levels in severely, moderately and slightly polluted habitats. Untreated sediment was processed in the same manner as the enriched sediments. Treatments were generally replicated 3 times.

The concentrations of zinc and cadmium in the sediments were measured after a 0.5 N HCl extraction (Flegal, 1983) by atomic absorption spectrophotometry using a Perkin-Elmer 603 with a deuterium arc background corrector. The 0.5 N HCl extraction provided a measure of the relatively labile, biologically available metal concentrations of the sediments, and is similar to the dilute HCl extractions utilized by Agemian and Chau (1976), Cross et al. (1976), Malo (1977), Luoma and Bryan (1981), and Rice et al. (1981).

The biologically available concentrations of zinc and cadmium in the treated sediments were essentially the same as their near total concentrations. The near total concentrations (measured with an aqua regia digestion) of those elements in the untreated Monterey Bay control sediments were relatively low ($0.11 \mu\text{g Cd g}^{-1}$, $31 \mu\text{g Zn g}^{-1}$), and 23 % of the cadmium and 45 % of the zinc in them were extracted with 0.5 N HCl. This lability of added metals is illustrated in Table 1, which shows the concentrations of the treated sediments decreased from 6 to 40 % during the 72 h experiments.

Table 1. Biologically available (0.5 N HCl extractable) concentrations of metal-enriched sediments at the start and finish of the 72 h experiments on the behavioral responses of *Rhepoxynius* spp. to elevated concentrations of zinc and cadmium in sediments

Metal	Elemental concentration ($\mu\text{g g}^{-1}$)		Percent loss
	Initial	Final	
Zinc	613	490	20
	200	175	12
	188	175	7
	163*	163	7
	51	48	6
Cadmium	190	143	25
	5.8	4.1	29
	5.6	4.1	27
	6.5*	3.9	40
	.05	<.05	

* 24 h incubation

All test containers and plumbing were PVC. Before each experiment, the sediment bowls were acid cleaned and flushed in running sea water for 3 h. Experiments were run on an acid-cleaned plastic water table enclosed in a polyethylene tent. A uni-directional flow-through water system with filtered water from Monterey Bay was used, so temperature and salinity were at ambient levels.

Each experiment involved a choice between 2 types of sediment. Bowls were divided in half by a removable plastic barrier, with one half containing experimental sediment and the control half containing untreated sediment. Sediment was added to the experimental bowls to a depth of 3 cm; a cylindrical screen of 0.5 mm Nytex which reached above the water surface was fitted around the outside of the dish to prevent amphipod escape; and the dish was placed on the water table. Test containers were oriented on the water table so that both sediments had the same water flow, and control dishes were placed upstream to prevent possible contamination from effluent desorbed from the enriched sediments. After 15 min, 1 cm surface cores were taken for elemental analysis of the initial elemental concentrations, and amphipods were added to the bowls. Experiments ran for 72 h, which is the optimal period for observing habitat selection behavior without possible starvation effects (Oakden, 1981). The amphipods on the sediment surface were then removed; sediment cores were taken for analysis of the final elemental concentrations; and the sediment from each half was sieved to remove all remaining amphipods.

Two variations of this basic design were used. In the first variation, 10 amphipods were placed in each sediment type, and no barrier was placed between the 2 sediments. Those amphipods were able to burrow

laterally between the 2 sediment types. In the second variation, 15 amphipods were added to the contaminated sediment only, which was physically separated from the control sediment by a 3.5 cm high plastic barrier. Those amphipods, therefore, had to leave the sediment and swim to get to the other sediment.

RESULTS

Responses to sewage-enriched sediments

Amphipods burrowed away from sewage-enriched sediments at most concentrations (Table 2). Avoidance was greater at higher levels of enrichment. There was

Table 2. *Rhepoxynius* spp. Distribution in 72 h, 2-choice preference experiments with sewage-enriched sediments and untreated sediments

Enriched sediment	Distribution of <i>Rhepoxynius</i> spp.*		
	Enriched sediment	Untreated sediment	Dead
1 % sewage	18	19	2
5 % sewage	11	27*	2
15 % sewage	1	26*	7
White's Point	3	31*	2

* Distribution of *Rhepoxynius* spp. in untreated sediment is significantly greater ($p < 0.05$, chi-square test) than in sewage-enriched sediment

no measurable preference or avoidance of sediment enriched with only 1 % sewage. Amphipods also burrowed away from sediment collected near the White's Point outfall in southern California, which had a high organic content, and also differed in grain size, metal content and organic composition from the control sediment from Monterey Bay. There was also significantly ($p < 0.05$, chi-square test) increased mortality of amphipods in the most sewage-enriched sediment (15 %) relative to the other sediments. These treatments were each replicated twice, and because there was no significant difference between replicates, data from the replicates were combined in Table 2.

When the amphipods could not burrow away from sewage-enriched sediment, they swam away from it (Table 3). This response contrasted with that in the control experiment, where *Rhepoxynius* spp. did not swim away from untreated sediment (Table 3). Because there was uneven replication of the swimming experiments, the results are presented in Table 3 as the mean number of amphipods per experimental condition, rather than the total number in all replicates, as in Table 2.

Table 3. *Rhepoxynius* spp. Distribution in 72 h sediment avoidance experiments in which organisms were initially placed in sewage-enriched sediment, and had to swim over a barrier to untreated sediment

Substrate	Mean distribution of <i>Rhepoxynius</i> spp.			
	No. remaining in enriched sediment	No. swimming to untreated sediment	Number dead	Number of replicates
Enriched sediment				
15 % sewage	6	8*	1	2
White's Point	6.4	5.3*	3.3	3
Control sediment	14	0	1	1

* Number of *Rhepoxynius* spp. swimming from sewage-enriched sediment to untreated sediment is significantly greater ($p < 0.05$, chi-square test) than in control experiment

Responses to metal-enriched sediments

Amphipods burrowed away from fine sand with elevated zinc concentrations (Table 4). There was over 50 % mortality in sediments with the highest zinc concentration ($610 \mu\text{g g}^{-1}$), and all surviving amphipods

Table 4. *Rhepoxynius* spp. Distribution in 72 h, 2-choice preference experiments with untreated sediment, and sediment enriched with either zinc or cadmium

Metal	Concentration of enriched sediment ($\mu\text{g g}^{-1}$)	Distribution of <i>Rhepoxynius</i> spp.*		
		Enriched sediment	Un-treated sediment	Dead
Zinc	613	0	24*	29
	200	7	45*	6
	51	19	39*	1
	control	54	61	3
Cadmium	190	0	9*	47
	5.8	6	37*	13
	< 0.05	29	31	0
	control	54	61	3

* Distribution of *Rhepoxynius* spp. in untreated sediment is significantly greater ($p < 0.05$, chi-square test) than in metal-enriched sediment

were in the untreated sediment after 72 h. Mortality was much less (10 %) in less enriched ($200 \mu\text{g g}^{-1}$) sediment, and 12 % of the amphipods were found living in it after 72 h. There was still a 2:1 preference among the amphipods for the untreated sediment over sediment enriched with only $51 \mu\text{g g}^{-1}$ of zinc,

although the mortality in that experiment (2 %) was comparable to that of the controls (3 %).

Burrowing avoidance of cadmium was similar to that of zinc (Table 4). At the highest concentration ($190 \mu\text{g g}^{-1}$), there was 84 % mortality after 72 h, and the surviving amphipods were all in the untreated sediment. At $5.8 \mu\text{g g}^{-1}$, mortality was lower (23 %), and there was still a significant ($p < 0.05$, chi-square test) preference for the untreated sediment. However, there was no mortality or significant difference in the distribution of amphipods in sediments with the lowest enrichment level ($< 0.05 \mu\text{g g}^{-1}$).

Amphipods also actively swam to avoid sediments with intermediate levels of zinc and cadmium when they could not burrow away from it (Table 5). Seventy

Table 5. *Rhepoxynius* spp. Distribution in 72 h sediment avoidance experiments in which organisms were initially placed in sediment with elevated concentrations of zinc ($188 \mu\text{g g}^{-1}$) or cadmium ($5.6 \mu\text{g g}^{-1}$), and had to swim over a barrier to untreated sediment

Substrate	Distribution of <i>Rhepoxynius</i> spp.		
	No. remain- ing in enriched sediment	No. swim- ming to untreated sediment	Num- ber dead
Enriched sediment			
Zinc ($188 \mu\text{g g}^{-1}$)	13	30*	2
Cadmium ($5.6 \mu\text{g g}^{-1}$)	7	20*	18
Control sediment	37	8	0

* Number of *Rhepoxynius* spp. swimming from metal-enriched sediment and remaining in the untreated sediment is significantly greater ($p < 0.05$, chi-square test) than in the control experiment

percent of the amphipods placed in sediment containing $188 \mu\text{g g}^{-1}$ of zinc swam to the untreated sediment. Forty percent of the amphipods placed in sediment containing $5.6 \mu\text{g g}^{-1}$ of cadmium died, and 44 % swam away from it. Both of these results are significantly ($p < .05$, chi-square test) different from the controls, where amphipods only swam across the barrier occasionally.

DISCUSSION

Phoxocephalid amphipods actively avoided laboratory sediment enriched with sewage, cadmium, and zinc. They showed a similar avoidance of organically-enriched fine sand containing Watsonville sewage, which has relatively low concentrations of zinc and cadmium, and sewage-enriched sediment collected near the White's Point discharge, which has relatively high concentrations of pollutants. They also avoided

sediment enriched with relatively moderate and low levels of zinc or cadmium, and they experienced relatively high mortality in sediments with higher concentrations of those metals.

Amphipods burrowed and swam from sediments containing sewage or elevated concentrations of trace metals. Because phoxocephalids are well-developed burrowers that swim infrequently both in aquaria and in the field (Oliver et al., 1980; Slattery, in press; Oakden, in press), swimming is probably a more extreme avoidance behavior than burrowing (Oakden, in press). Perhaps in field conditions, amphipods leave the sediment more quickly and swim longer distances after contacting a relatively undesirable sediment.

Among the phoxocephalids, there is considerable interspecific variation in behavior. The species of *Rhepoxynius* that we studied live at shallower depths than those at the White's Point discharge. However, several deep-water species occur around that site in sediments with relatively high levels of trace metals that were avoided by *Rhepoxynius* spp. in our laboratory experiments. For example, *Heterophoxus oculatus* is found around the White's Point discharge in sediments with as much as $19 \mu\text{g g}^{-1}$ cadmium and $530 \mu\text{g g}^{-1}$ zinc (unpubl. data, Los Angeles County Sanitation District), and *Rhepoxynius bicuspidatus* is found at the Orange County discharge in sediments with $6 \mu\text{g g}^{-1}$ cadmium and $108 \mu\text{g g}^{-1}$ zinc (unpubl. data, Orange County Sanitation District, 1981). Species of phoxocephalids inhabiting different water depths and sedimentary environments have different sediment preferences (Oakden, in press), and may avoid different concentrations of trace metals as well.

Behavior studies have shown that benthic invertebrates, including phoxocephalids, will inhabit non-optimal sediment if no other choice is available (Meadows and Campbell, 1972; Erdem and Meadows, 1980; Oakden, in press). The White's Point and adjacent Hyperion discharges are the largest sewage outfalls on the California coast, and they impact a broad region of continental shelf. Because the entire Santa Monica Bay and Palos Verdes Peninsula section of the southern California coast ($\approx 70 \text{ km}$) have anthropogenically elevated metal levels (Word and Mearns, 1979), phoxocephalids may select suboptimal sediment of necessity.

However, there is no information about the sublethal effects of these choices. Our general understanding of the sublethal effects of waste water discharges on the benthos is fragmented (Eagle, 1981), and the sublethal effects of pollutants on critical aspects of life history, such as mating behavior, pheromone detection, and early development of young, have not been examined.

The levels of zinc and cadmium used in our experiments were similar to concentrations found at various

distances from a large waste discharge. However, we manipulated only 2 trace metal concentrations, and did not examine their potential synergistic effects. Therefore, our experiments do little to unravel the complexity of effects that occur when a large number of trace metals and other pollutants accumulate in sediments by waste water discharges (Luoma, 1977). It was nonetheless surprising to us that the phoxocephalids respond so dramatically to relatively low enrichments of a single trace metal.

It is difficult to link the behavior observed in laboratory experiments to the large-scale (many km²) population and community patterns around waste discharges. Based solely on our experiments, we cannot eliminate habitat selection as a process influencing the distribution and abundance of phoxocephalid amphipods around a large waste discharge such as White's Point. Because phoxocephalids avoid sediments enriched with either organic matter or relatively low levels of trace metals in the laboratory, they may avoid similar enriched sediments in the field. Despite the presence of biological mechanisms for detoxifying pollutants (Bryan, 1976), our studies indicate that sublethal effects of these substances on habitat selection may be as important in establishing and maintaining field populations as the mortality caused by a relatively polluted substrate.

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