

A CULTURE-BASED ASSESSMENT OF THE EFFECTS OF CHLORPYRIFOS ON MULTIPLE MEIOBENTHIC COPEPODS USING MICROCOSMS OF INTACT ESTUARINE SEDIMENTS

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Abstract—Meiobenthos occur at densities of $>10^6/m^2$ in almost every uncontaminated estuarine sediment of the world, yet few studies have explored their utility for a multiple-species chronic bioassay of sediment contaminant effects. A new approach using laboratory-reared, whole-sediment meiobenthic microcosms was developed to holistically test the sublethal effects of chlorpyrifos on benthic copepod survival, age structure, and reproduction. Entire sediment-dwelling meiofaunal communities from a pristine estuarine preserve were cultured (i.e., net population gains were achieved in controls) for at least a full generation to the known 96-h copepod adult 25% lethal concentration and larval 50% lethal concentration of sediment-associated chlorpyrifos (21–33 $\mu g/kg$ sediment). As an internal bioassay control, we added and cultured 100 gravid benthic copepods (*Amphiascus tenuiremis*) in each microcosm to assess the exact rather than relative effects of chlorpyrifos on population growth and dynamics. All but one copepod species reproduced and was cultured successfully for at least 21 d using these techniques. We found no chlorpyrifos effects on total meiobenthic copepod densities, but the predominant naturally occurring copepod *Microarthridion littorale*, known to be a major prey item of many juvenile fish, was significantly reduced in some chlorpyrifos-spiked microcosms. Other naturally occurring copepods were either unaffected or their growth was enhanced in the presence of chlorpyrifos. *Amphiascus tenuiremis*, known to be adversely affected at this concentration in 96-h static bioassays, increased dramatically in every microcosm. Species-specific responses of endemic copepods were masked when combined into the more commonly used coarse taxonomic category of total copepods.

Keywords—Mesocosm Meiofauna Sediments Chlorpyrifos

INTRODUCTION

Benthic fauna of almost every aquatic ecosystem in the industrialized world are chronically exposed to a wide variety of pollutants associated to one degree or another with sediment compartments [1–3]. This is especially true in estuaries, where muddy sediments also provide an important nursery habitat for many bottom-feeding juvenile fishes and the vast numbers of meiobenthos upon which the fish feed. The ecological and trophic importance of meiobenthos in estuarine ecosystems is well documented [4], as is the preferably oxidized nature of their sediment microhabitat; $>98\%$ of all meiobenthic copepods live in the 1- to 2-cm oxic zone of muddy sediments [5,6]. Few species can survive anoxic conditions beyond minutes to hours. This meiofaunal microhabitat preference puts this community at high risk to pollutants that are most concentrated and bioavailable in surficial layers of sediments. Compared with macrobenthos, the toxic effects of contaminants on meiobenthic communities generally, and meiobenthic copepods specifically, remain largely unknown [6,7]. This is so in spite of the established importance of meiobenthos in marine food webs and large-scale sediment processes [4,6,8].

Giesy and Hoke [9] proposed that the ideal sediment bioassay should be rapid, simple, replicable, inexpensive, standardized, sensitive, discriminatory, ecologically relevant, relatable to field effects, and useful in developing regulatory

standards. Single-species in vitro bioassays with meiofauna meet many of these criteria. However, the ecological relevance and predictiveness of single-species bioassays can be determined only with a controlled “whole-community” multiple-species approach. The ideal mesocosm- or microcosm-based risk assessment approach should provide a greater degree of environmental realism than can be achieved in simple single-species, single-phase in vitro bioassays [10]. This approach must also provide acceptable precision across replicates and accuracy with regard to contaminant-related effects. Large mesocosms often provide a high degree of environmental realism, but they are expensive to build and maintain and difficult to replicate with precision. Smaller microcosms are much easier to build, maintain, and replicate at a higher level of precision. The extensive meiofaunal literature [7,11,12] would argue that for sediment studies, a meiofaunal microcosm of only 100 cm^2 is equivalent to much larger (e.g., 1–5 m^2) macrofaunal mesocosms in both scale and numbers of species and individuals that can be tested. Yet the man-hours, expense, and maintenance are far less, so levels of replication (and statistical rigor) can be much higher. Most of the information we have to date on the effects of sediment-associated contaminants on meiofaunal survival and population growth has been acquired from in vitro “spiked-sediment” and sediment-dilution bioassays with single species. In the present study we took advantage of the proven amenability of meiobenthos for sediment culture [7,13–15] and developed a laboratory-based, whole-sediment microcosm bioassay for sediment contaminant effects on the life cycles of multiple meiobenthic species.

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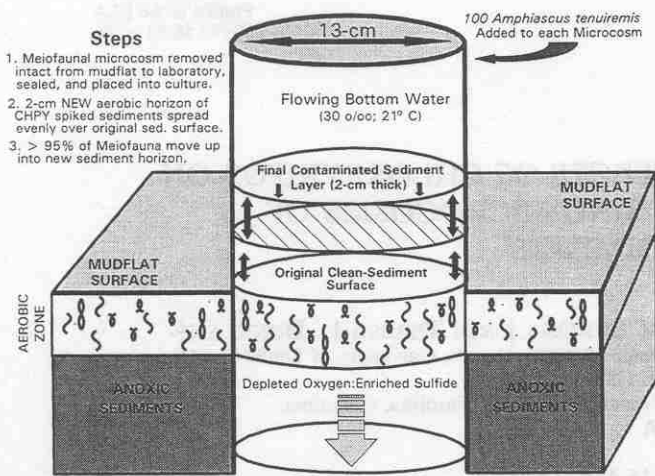


Fig. 1. Schematic diagram of meiofauna microcosm design and approach used in this study. CHPY = chlorpyrifos; sed = sediment.

Our objectives were to construct and test multiple time and space replicated sets of 1,750-cm³ flow-through microcosms containing intact, undisturbed field sediments and their associated meiobenthic taxa (6,000–10,000 individuals per microcosm); to conduct experiments with resident meiofauna exposed to sublethal concentrations of a common, high-hazard, organophosphorous pesticide, chlorpyrifos (CHPY), which is used extensively for insect control in coastal regions of the southeastern United States [16]; and to determine the exact population response of a sentinel benthic copepod, *Amphiascus tenuiremis*, when cultured in CHPY-spiked microcosms. Chlorpyrifos, *O,O*-diethyl-*O*-(3,5,6-trichloro-2-pyridyl) phosphorothioate, with trade names Dursban® and Lorsban®, is a potent acetylcholinesterase inhibitor used to control a variety of insect pests. It is very toxic to aquatic invertebrates and fishes and exhibits moderate persistence in natural systems [17,18]. Its concentration has been reported to be as high as 245 ng/g wet sediment in Buzzard's Bay, Massachusetts, USA [16].

MATERIALS AND METHODS

Meiofauna and test sediments were collected from the North Inlet Estuary, Georgetown, South Carolina, USA. North Inlet is as pristine a natural salt marsh preserve as is known, and the sediments there have extremely low to undetectable levels of pesticides, heavy metals (<2 ng Cd/kg-sediment), polycyclic aromatic hydrocarbons, and polychlorinated biphenyls (PCBs) (e.g., <3.5 ng PCB/kg sediment, T. Bidleman, unpublished data). Therefore, our control microcosms and fauna were preexposed to no or negligible pollutants. Eight cylindrical sediment blocks (13 × 30 cm) were cored from an undisturbed 1-m² area of a North Inlet mudflat during low tide. Meiofauna often exhibit heterogeneous spatial dispersion [5]; thus, only a 1-m² area was cored to maximize the chances for acquiring good microcosm replicability in species composition and density. Thin-walled beveled acrylic cylinders (Fig. 1) were inserted 10 cm into the mudflat. Sediments surrounding the base of each cylinder were then excavated, and a locking plastic plate milled precisely to fit the inner cylinder diameter was attached to the core bottom. Entire blocks were lifted intact from the mudflat, preserving natural redox profiles, biogenic structure, etc., and covered with plastic petri dishes. Core blocks were made leakproof by immersing the bottom of each

core into a petri dish filled with uncured silicone rubber, then allowing the rubber to harden, and were finally transported to the laboratory.

All eight sealed microcosm blocks (Fig. 1) were connected to a drip-through, artificial seawater system (5- to 6-h turnover rate; 30 ppt, 21°C, 12:12 light:dark cycle; after Chandler [14]) within 3 h of collection and allowed to acclimate for 3 d before further manipulation or disturbance. Gentle seawater flow (1–2 ml/min) was directed into each microcosm via 20-ga polyethylene tubing and removed by Nitex mesh-covered drain ports. Nitex mesh (50 μm) was used to prevent the loss of swimming meiofauna by seawater outflow. After 3 d, all visible errant macrofauna (>98% *Nassarius [Illyanassa]* snails) were removed with forceps to eliminate or minimize macroinvertebrate enclosure effects.

On day 3 after collection, two cores (=microcosms) were randomly sacrificed to estimate initial abundances of the taxa (see below for meiofauna extraction procedures). Into two microcosms (control), a 2-cm-thick flocculent surface layer (45 g < 0.125 mm sediments) of homogenized, CHPY-free sterile sediments was added. Into the other four microcosms (treatment), identical sediments with CHPY spikes were added to the surface. These sediment additions elevated the depth of the original redox potential discontinuity (RPD) layer up to the base of the newly added homogeneous flocculent layers (i.e., at approx. 2 cm depth for North Inlet muds). Because >95% of all meiofauna prefer the oxic horizon, this sediment addition ensured movement of each microcosm community into the sediment layer that we could control. In preliminary trials and earlier studies [14], clean flocculent sediment deposition caused no meiofaunal mortality, caused the complete meiofaunal community to move up into the sediment layer, and eliminated almost all of the within-microcosm spatial variability in sediment structure and contaminant dispersion. Because the purpose of this research was to test the utility of meiofaunal microcosms for replicable culturing of meiofauna under stress, identical experiments were replicated three times in June, July, and September 1995. Treatment sediments were spiked with CHPY at measured values of 21 to 33 ng/g CHPY. This concentration bracketed the a priori known 96-h 20 to 25% lethal concentration (LC 20–25) for adult *Amphiascus tenuiremis* and the 50% lethal concentration (LC50) for nauplii [19]. Spiking methods followed Green et al. [19], with a 30-ng CHPY/g dry sediment concentration targeted by volume/volume CHPY addition to a 12% sediment slurry under vortexing. Sediments were allowed to mix for 1 h, then held at 4°C for 48 h before use.

Once the added 2 cm of sediment settled completely (4–6 h), we then added 100 ovigerous females (approx. one female per square centimeter of microcosm surface) of the cultured meiobenthic copepod *A. tenuiremis* to the resident meiofaunal community in each microcosm. *Amphiascus tenuiremis* does not occur in the sediments tested but thrives in these sediments and is occasionally found as a rare species in the North Inlet ecosystem [20]. Therefore, by knowing the number of *A. tenuiremis* added, we could determine exact mortality, net population growth, and resultant population structure at the end of each exposure [21–23]. Relative changes in those parameters were measured for all other microcosm copepods after CHPY exposure (i.e., relative to mean densities at time zero and to controls). Also, because *A. tenuiremis* was the only copepod of the family Diosaccidae in these microcosms and this family has distinct crablike nauplii, it allowed assessment

of naupliar larval success in addition to the other more traditionally studied benthic copepod life-history categories (i.e., copepodites, adults by sex).

Microcosms were cultured on 25 ml of a 1:1:1 mixture of frozen algae, *Dunaliella tertiolecta* (Butcher), *Phaeodactylum tricorutum* (Bohlin), and *Isochrysis* aff. *galbana* (isol. Haines T-iso), every fourth day for up to 21 d. Initially, 1 L of each algal species was homogenized and concentrated by centrifugation at 3,490 relative centrifugal force and frozen until ready for use. On each feeding day, three 50-ml aliquots were thawed and split between the six microcosms (a total of 6×10^7 algal cells per microcosm). Aliquots of sediment were taken for chemical analyses within 2 h of sediment addition (i.e., initial) and at the end of each experiment (i.e., final).

The entire meiofauna community (i.e., complete statistical population) was collected from each microcosm by aspirating off (down to the anoxic RPD layer) the top two cm of sediment into an aspiration flask. Collected sediment was then fixed in buffered formalin, stained with rose bengal, and sieved through a 63- μm sieve. The fraction retained was placed into a cylindrical sample-splitting chamber [24]. Each microcosm sample was split into eight equal parts. Four parts were randomly selected, sorted, and counted. In experiment I and part of experiment II, meiofauna were classified to nematodes, each life stage and species of harpacticoid copepod, and other taxa. In experiment III, only copepods were enumerated and identified, again to species and life stage.

Statistical analysis

There were three meiofauna data sets for comparison in each experiment: initial meiofaunal densities, densities in final controls, and densities in the final treatment sediments. All data were log-transformed to normality with homogenous variance before statistical analyses. Data for all three experiments were analyzed concurrently by randomized block ANOVA with interaction (experiment \times treatment) tests. Dunnett's *t* test was used to compare initial and final treatment abundances against final control abundances. Tukey's multiple comparison test ($p < 0.05$) was used to compare experiments I, II, and III over time. If experimentwise differences were found, then treatment differences were judged invalid if significant (experiment \times treatment) interactions occurred. Because our experimental design was unbalanced, all statistical analyses were conducted using a generalized linear modeling ANOVA approach (Proc GLM, SAS[®] software, SAS Institute, Cary, NC, USA) that is robust to unbalanced designs.

Chemical analyses

Ten-milligram sediment samples were removed from treatment and control microcosms in experiment I for organic carbon and total nitrogen measurements. Total nitrogen and organic carbon was measured using a Perkin-Elmer model 2400 Elemental Analyzer by the Marine Micropaleontology Laboratory, Department of Geological Sciences, University of South Carolina, Columbia, South Carolina, USA.

Replicate samples were taken and analyzed for initial sediment CHPY concentration in each experiment. Replicate sediment and overlying water samples were taken from each microcosm for final CHPY concentrations. The percentage of water of each sediment sample was determined by weighing, drying, and reweighing triplicate subsamples. At time zero, CHPY was extracted from sediment and overlying water samples through steam distillation [25]. For each sediment anal-

ysis, approx. 5 ml of sediment slurry (0.5 g dry weight) was weighed and transferred to a 125-ml boiling flask containing 30 ml of deionized water and three to four Teflon[®] boiling stones. For the overlying water samples, 20 ml of sample were added to 125-ml boiling flasks containing three to four boiling chips. For both sediment and water samples, the boiling flasks were connected to a steam distillation unit, and 9 ml of 80:20 hexane:isooctane extraction solvent was added to each. Samples were refluxed for 6 to 8 h. Extraction solvent was collected, and each steam distillation apparatus was rinsed three times with extraction solvent. Solvent residues were removed by freezing each extract in a dry ice/acetone bath, then collecting the extraction solvent. The extraction solvent was exchanged with isooctane by adding 2 ml of isooctane and concentrating to 2 ml by evaporation under a dry nitrogen stream. Sulfur was removed by shaking this solution with 1.5 ml of tetrabutylammonium hydrogen sulfate saturated with sodium sulfite [26]. Water was again removed from the extract, and total volume was ensured at 2 ml by adding isooctane or removing solvent by further evaporation under a dry nitrogen stream.

Chlorpyrifos was quantified by injecting 2 μl of the extract into a Hewlett-Packard (Avondale, PA, USA) model 5890 series II gas chromatograph equipped with an electron capture detector and electronic pressure control. The gas chromatograph was equipped with a J&W Scientific (Rancho Cordova, CA, USA) 30-m \times 0.32-mm-inner-diameter column having a film thickness of 0.25 μm . The carrier gas was helium with a flow rate of 1 ml/min. The makeup gas was nitrogen with a flow rate of 50 ml/min. The injection port temperature was 250°C, and the detector was set at 300°C. The oven temperature was initially set to 80°C with a 1-min hold followed by an increase of 5°C/min to 210°C, then a ramp of 30°C/min to 300°C with a 10-min hold. The retention time of CHPY was 30.65 min. Samples were quantified by comparison to an external standard regression using Hewlett-Packard Chemstation[®] software.

RESULTS

Total sediment organic carbon ranged from 3.92 to 4.19% in control microcosms and from 3.51 to 3.90% in treatment microcosms. Total nitrogen ranged from 0.40 to 0.43% in controls and from 0.36 to 0.43% in treatments. The initial and final amounts of measured CHPY for the three experiments are illustrated in Figure 2. Initial overall CHPY mean concentration was 24.1 ng/g. Note the replicability within experiments (lots A and B) in which we duplicated chemical analysis and the general replicability across experiments. The amount of CHPY remaining in sediments at termination of the experiments ranged from 15.6% (experiment III) to 65.7% (experiment II, lot A) (Fig. 2).

Nematodes and harpacticoid copepods made up >97% of the fauna in the initial field samples. We identified nine harpacticoid copepods to species level, but three (*Microarthridion littorale*, *Enhydrosoma propinquum*, and *Paronychocamptus wilsoni*) consistently made up >90% of all the copepods in all experiments; thus, these were the species focused on in the analyses.

Nematodes as a general taxon were enumerated in experiment I and in 14 of 18 samples of experiment II. At the broad taxon level, nematodes were not significantly affected by CHPY (Fig. 3 and Table 1). Nematode species characterizations are difficult and beyond our expertise. Thus, no nematode

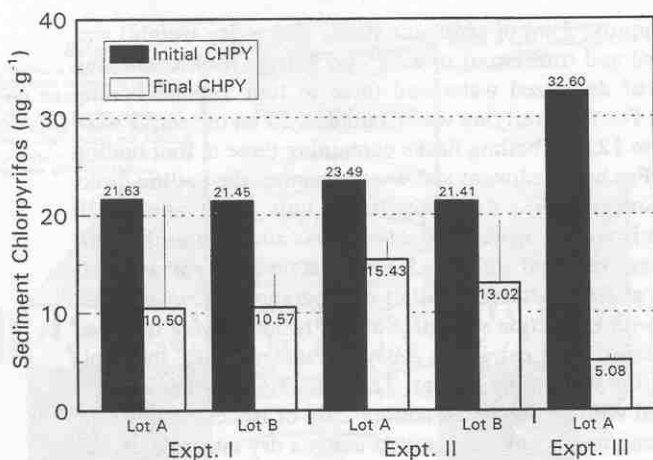


Fig. 2. Initial and final concentrations of chlorpyrifos (CHPY) in replicate microcosm sediments. Units are expressed on a dry-sediment-weight basis.

species data are presented. Total copepods as a general taxonomic grouping were also analyzed by generalized linear modeling ANOVA (Table 2) and found to exhibit no significant density differences across treatment groupings (Fig. 4). Total larval densities were also statistically indistinguishable across treatments (Fig. 4). *Amphiascus* densities were not included in these total density counts and are analyzed and presented separately below.

In Figure 5, we provide abundance data for the three predominant naturally occurring copepod species at time zero (initial) and in the final control and treatment microcosms. The most important experimental result is the comparison of final control densities with final treatment densities. The only significant negative effects (i.e., decreased abundance) occurred for *M. littorale*, and this reduction was due primarily to a reduced number of adults (females and males) in the treatment microcosms (Fig. 5A). Surprisingly, for both *P. wilsoni* (Fig. 5B) and *E. propinquum* (Fig. 5C), copepod densities in the CHPY treatment microcosms increased. For *P. wilsoni* the increase was driven by a significant 57% increase in copepodite juveniles (Fig. 5B), whereas for *E. propinquum* the overall increase is reflective of an increase in adult females (Fig. 5C).

For the cultured copepod *Amphiascus*, which we added to every microcosm at time zero, the microcosm environment

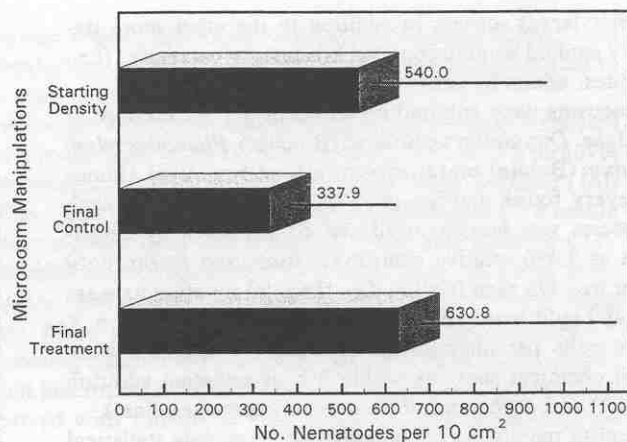


Fig. 3. Total nematode mean densities, averaged over all experiments, at onset of the experiment, after 21 d of exposure to sediment-associated chlorpyrifos (final treatment), and after 21 d of exposure to no chlorpyrifos (final control). Error bars = ± 1 SD.

appeared to be an ideal culture vessel. From an initial starting density of 8.8/10 cm², *A. tenuiremis* increased to 73/10 cm² in control microcosms and to 87/10 cm² in treatment microcosms, a 10-fold increase in 14 to 21 d (Fig. 6). In both microcosm treatments, reproduction was clearly occurring, as indicated by the high copepodite and naupliar densities in the final counts (Fig. 6). Additionally, it appears that in spite of the naupliar LC50 exposure level, CHPY had enhanced *A. tenuiremis* reproduction. Naupliar densities in treatment microcosms were significantly higher (19%) than those in non-treatment (control) microcosms; such was not the case, however, for non-*Amphiascus* nauplii, which exhibited no significant differences among treatments (Fig. 6). Because the initial samples had zero *Amphiascus* (the species we added after initial samples were taken), there was, of course, always a difference between initial *Amphiascus* and any other treatment.

DISCUSSION

It is clear from this and previous meiofaunal studies [7] that the often-used approach of determining whether chemical X affects general meiofaunal densities (e.g., total copepods or nematodes) provides poor resolution of effects of low- to mid-level contaminant exposures. Thus, contaminant risk assess-

Table 1. Summary of the results of two-way generalized linear model ANOVA and Dunnett's *t* test on the mean densities of total nematodes and individual copepod species in microcosms with and without chlorpyrifos^a

Species	Experiment		
	1	2	3
<i>Microarthridion littorale</i>	I < FC* FT = FC	I = FC FT < FC*	I > FC* FT < FC*
<i>Enhydrosoma propinquum</i>	I = FC FT > FC*	I = FC FT = FC*	I = FC FT = FC
<i>Paronychocamptus wilsoni</i>	I < FC* FT = FC	I < FC* FT > FC	I < FC* FT > FC*
<i>Amphiascus tenuiremis</i>	Initial value was 100 ovigerous females		
	FT = FC	FT = FC	FT = FC
Total nematodes	I = FC FT > FC*	I = FC FT = FC ^b	

^a I = initial density; FC = final density in control microcosms; FT = final density in microcosms spiked with chlorpyrifos.

^b Based on 14 samples (only two from the final control).

* Significant difference ($p < 0.05$).

Table 2. Mean densities by experiment and treatment of all copepod species (except *Amphiascus*) combined (general linear model ANOVA results presented by variance source with appropriate *F* table probabilities)

Experiment 1			Experiment 2		
Initial density 27.5 ± 11.6	Final control 69.9 ± 5.5	Final treatment 94.0 ± 20.1	Initial density 173.2 ± 36.3	Final control 139.8 ± 20.8	Final treatment 156.3 ± 57
GLM ANOVA: Total copepods (log ₁₀ [Count + 1]-transformed data)					
Source ^a	df	SS	MS	F	Pr > F
Experiment (time)	2	3.4978	1.7489	101.73	0.001
Treatments	2	0.003	0.0015	0.089	0.916
Experiment × treatment	4	0.5325	0.1331	7.74	0.001
Error	63	1.083			
Total	71				

Experiment 3 result: Initial density, 356.8 ± 80.0; Final control, 338 ± 65.6; Final treatment, 289.0 ± 122.7.

^a df = degrees of freedom; SS = sums of squares; MS = mean squared error; F = F-table statistic; Pr = probability.

ments for meiofauna conducted at gross taxonomic levels may underestimate contaminant effects on individual species, some of which may be key to benthic processes. This is why we discontinued counting total nematodes partway through experiment II. We did not have the expertise to identify nematodes to species and believed that data at the taxon level were not very useful. Similarly, combining copepod species into a total copepods group did not reveal any significant CHPY effects (positive or negative), even though at the species level significant positive and negative effects occurred. Numerous nonmicrocosm studies have shown that different meiofaunal species, and especially life stages within species, possess varying sensitivities to toxicants [7,19,20,27]. Previous meiofauna and pollution microcosm studies [28,29] have found little to no contaminant effects at the general taxon level. Responses, if they occurred, were noted at the species level [29].

Meiofaunal microcosms can allow species-specific toxicant sensitivities to be deciphered conservatively and, at a life-history level, if all or most taxa exhibit either no net loss or net gains in population densities over the course of each chronic bioassay (e.g., approximately one generation in our study). The approach used here met this conservative culturing criterion. That is, most copepod taxa maintained or increased their densities over time in control microcosms. Few, if any, previous meiofaunal microcosm studies have maintained or exceeded starting densities for most taxa over the majority of

their life cycle. We achieved this criterion for every taxon except for our first experiment with *Microarthridion* (Figs. 5 and 6) using these microcosm culturing methods.

The CHPY concentration tested in our study (21–35 ng/g) was approximately 10 times below the highest reported sediment value for U.S. estuaries (245 ng CHPY/g) [16]. The latter concentration was observed in sediments collected near cranberry bog agriculture on Buzzards Bay, Massachusetts, USA, and probably represents a medium to high value for an estuarine system. Chlorpyrifos is used extensively in agriculture, forestry, golf courses, and by homeowners in coastal areas throughout the southeast and Gulf of Mexico regions of the United States. It also has the highest hazard rating for potential pesticide toxicity to estuarine fish and shellfish [16]. We justified testing only a 21- to 35-ng/g concentration in our study because (1) we thought it represented a realistic low-end estimate of CHPY sediment loadings in urbanized and agriculturally affected areas of southeastern U.S. estuaries, (2) we had extensive a priori knowledge of CHPY toxicity to our reference sentinel species *A. tenuiremis*, and (3) we wanted to target an LC_{20–25} adult exposure. We rationalized a priori that a majority life-cycle exposure of the entire meiofaunal community to the acute 96-h LC_{20–25} of a consistently sensitive taxon (*A. tenuiremis*) would produce sufficient toxicity to see toxic effects at the population and community levels in these whole-sediment microcosms. As is often the case in ecological studies, our best rationalizations were almost completely wrong. We also do not know how rapidly the 21-d decline in CHPY sediment concentrations occurred (Fig. 2). If CHPY concentrations dropped rapidly and then stabilized at a lower, nontoxic level, then microcosm meiofauna may not have been exposed to enough CHPY for a sufficiently long duration to produce negative density effects. However, given the CHPY lethality levels observed by Green [41] in 96-h exposures, these 21-d exposures to approx. 30% of Green's test concentrations would be expected to elicit negative effects at least on *A. tenuiremis*.

At the general taxon level (i.e., total copepods and nematodes), CHPY had no effects relative to controls. However, at the copepod species level, the general effect was positive for three of the four predominant species, including our reference, and presumably most sensitive, sentinel species *A. tenuiremis*. Only the harpacticoid copepod *M. littorale* showed a possible CHPY-induced density decline in the meiofauna microcosms (Fig. 5A and Table 1). Clear life-stage-specific differential density changes were observed in CHPY treatments (i.e., adult

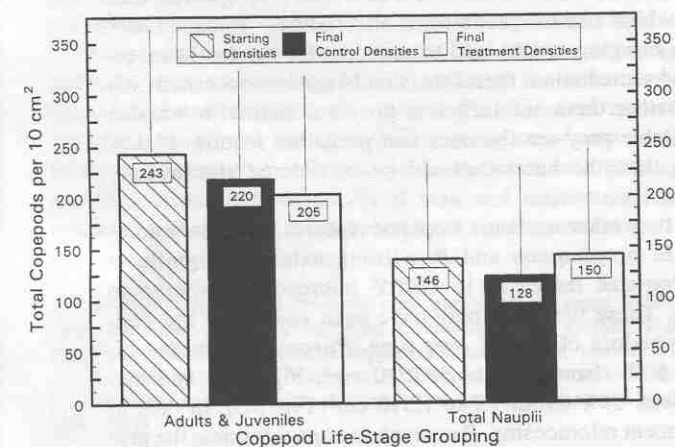


Fig. 4. Total copepod (adult and juvenile and naupliar) mean densities, averaged over all experiments, at onset of the experiment, after 21 d of exposure to sediment-associated chlorpyrifos (final treatment), and after 21 d of exposure to no chlorpyrifos (final control). Error bars = ± 1 SD.

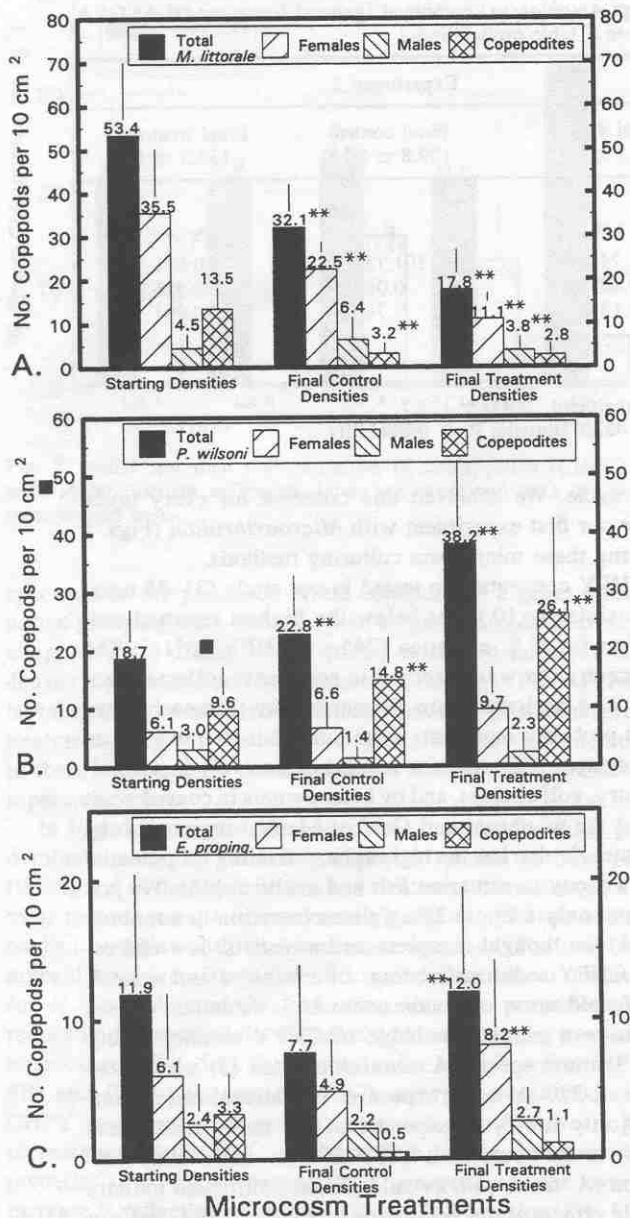


Fig. 5. Mean densities per 10 cm² microcosm area of the copepods *Microarthridion littorale* (A), *Paronychocamptus wilsoni* (B), and *Enhydrosoma propinquum* (C). Densities presented as total adults and copepodites, by sex and by juvenile life stage (copepodites). Larvae (nauplii) could not be identified to species. **Mean is significantly different ($p < 0.02$) from like treatment in preceding histogram group (to the left). Error bars = ± 1 SD.

density declines were greater than those of larvae and juveniles), but we do not yet know the mechanisms through which CHPY may have effected these differences. Naupliar larvae of this and most other harpacticoid copepods are selective sediment grazers on bacteria and microalgae [30,31]. *Microarthridion* adults, however, ingest bulk sediments as well (T. Chandler, unpublished data) and may have received a correspondingly higher exposure through the trophic route. Alternatively, the *M. littorale* response may have been driven by increases in other species densities.

Why *M. littorale* was the only species negatively affected in CHPY treatments is unknown. Previous 96-h sediment bioassays with *M. littorale* and azinphosmethyl (APM), a similar organophosphorous insecticide, showed this species to be 3–

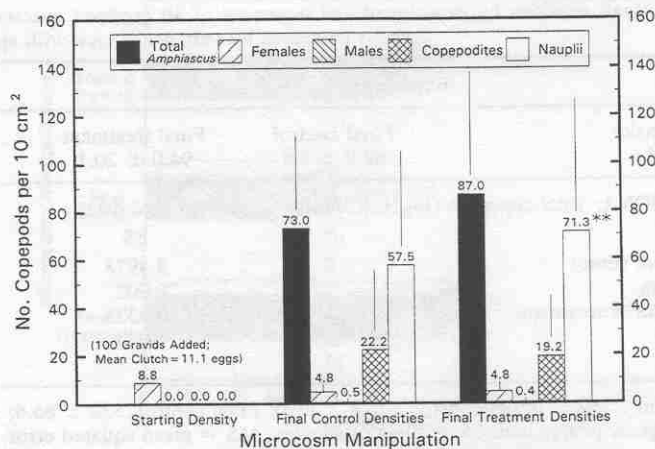


Fig. 6. Total densities of the copepod *Amphiascus tenuiremis* by sex and all life stages at onset of the experiment, after 21 d of exposure to sediment-associated chlorpyrifos (final treatment), and after 21 d of exposure to no chlorpyrifos (final control). **Mean is significantly different ($p < 0.02$) from like treatment in preceding histogram group (to the left). Error bars = ± 1 SD.

4 times less sensitive than our reference copepod, *A. tenuiremis* [32]. Yet, in our study, *A. tenuiremis* exhibited no negative CHPY effect and, to the contrary, exhibited significant net gains in naupliar production under CHPY exposure. Chlorpyrifos has a K_{ow} of 1.8×10^5 , which is more than 500 times higher than APM, and presumably would be much less bioavailable than APM in organic sediments, yet CHPY was significantly toxic to *M. littorale* adults at only 25 ng/g. The sediment lowest-observed-effect concentration reported for APM and *M. littorale* was 500 ng/g [32].

Ecologically, the sensitivity of *M. littorale* to CHPY may be important. *Microarthridion* is a primary and predominant food item for juvenile fishes [33–35], and it is a species known to transfer sediment-bound contaminants to juvenile fish [32,36,37]. Overall, it is the most abundant benthic copepod in South Carolina salt marsh ecosystems [38], and, given its role as a primary food for fish, decreased abundances of *Microarthridion* could have profound effects on an ecosystem. Reducing this critical link in the food chain would certainly reduce the suitability of a habitat as foraging ground. If a chemical such as CHPY reduces a keystone prey species, then usable habitat (for the predator) is also reduced. Reduced habitats for foraging would lead to reduced fish stocks. Management and remediation, therefore, should not be concerned only with whether there are sufficient prey but also with whether the available prey are the ones that predators require. If they are not, then the habitat should be considered significantly degraded.

The two other endemic copepod species in these microcosms, *E. propinquum* and *P. wilsoni*, exhibited significant population-size increases in CHPY microcosms relative to controls. These increases may have been caused by the degradation or loss of CHPY over time. *Paronychocamptus* increased 60% (from 22.8 to 38.2/10 cm², Fig. 5B), and *Enhydrosoma*, 64% (from 7.7 to 12/10 cm², Fig. 5C). In fact, in the treatment microcosms, *Paronychocamptus* became the predominant copepod, supplanting *Microarthridion*. Such a rapid switch in copepod dominance is not known in the natural field populations from which these microcosms came [38]. If such a taxon shift were to occur in field-exposed populations, then the copepods predominating, and therefore most available as

prey to bottom-feeding juvenile fishes and shrimps, may be species that are not preferred by predators. *Paronychocamptus* and *Enhydrosoma* are not common prey of resident juvenile spot (Pisces) in the North Inlet ecosystem (see Fig. 4 of Feller et al. [33] and Fig. 3 of Ellis and Coull [39]). Thus, although CHPY appeared to have little effect overall on total meio-benthos, species-specific CHPY effects may be profound for sustenance of local juvenile fish populations.

The observed net gains in treatment microcosm copepod abundance also may have been an indirect copepod response to a CHPY toxic effect on microcosm microfauna and flora (e.g., ciliates, flagellates, diatoms, bacteria, etc.). Such microfauna and flora serve as food for meiofauna [40]. If CHPY made these organisms more abundant or easier to capture or harvest, then subsequent reproductive gains from enhanced feeding would be expected. On the basis of extensive previous work [19,41], we attempted to estimate the tolerance levels of our cultured copepod, *A. tenuiremis*, and its life-history stages to CHPY. Green's [41] adult 96-h LC₂₀₋₂₅ value was chosen as an appropriate low-end test concentration that should have elicited measurable, but not total, toxicity to the copepod community. Surprisingly, *Amphiascus* (and others) grew in microcosms as if there was no CHPY present. Possibly, organic carbon in the microcosm sediments (and/or pore waters) caused CHPY to be less bioavailable than in Green's studies, or CHPY degradation or loss from sediments was initially quite rapid and then plateaued at the 10- to 15-ng/g concentration. Regarding CHPY bioavailability, Green [41] reported pore-water dissolved oxygen concentration (DOC) in static 96-h bioassays to be 19 to 22 mg/L. Field sediments from our microcosm collection site had values ranging from 60 to 65 mg DOC/L pore water. These elevated DOC levels may have driven available CHPY to nontoxic levels as has been observed with other high K_{ow} toxicants [42,43]. In another study of CHPY bioavailability in the presence of DOC, Brown et al. [44] found a two- to threefold attenuating effect of humic acid on aqueous CHPY toxicity to the cladoceran *Ceriodaphnia dubia*.

Meiofaunal microcosms are a useful tool for assessing population-level effects of sediment-associated contaminants. In testing the entire sediment biocenose, toxicant effects may be attenuated or enhanced and more or less strong than in single-species laboratory bioassays [7,29,45,46]. The associated organic matter, microbial community, and other faunas (meio and macro) no doubt change bioavailability and persistence of contaminants in microcosms compared with single-species bioassays. Studies conducted without the entire assemblage probably overestimate contaminant toxicity for some meiofauna but underestimate it for others (e.g., *Microarthridion* in our study). Sediment risk assessments using meiofauna would be more conservative, informative, and predictive if both approaches were used, especially if taxa and microcosms are collected and cultured from actual field sites of concern.

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