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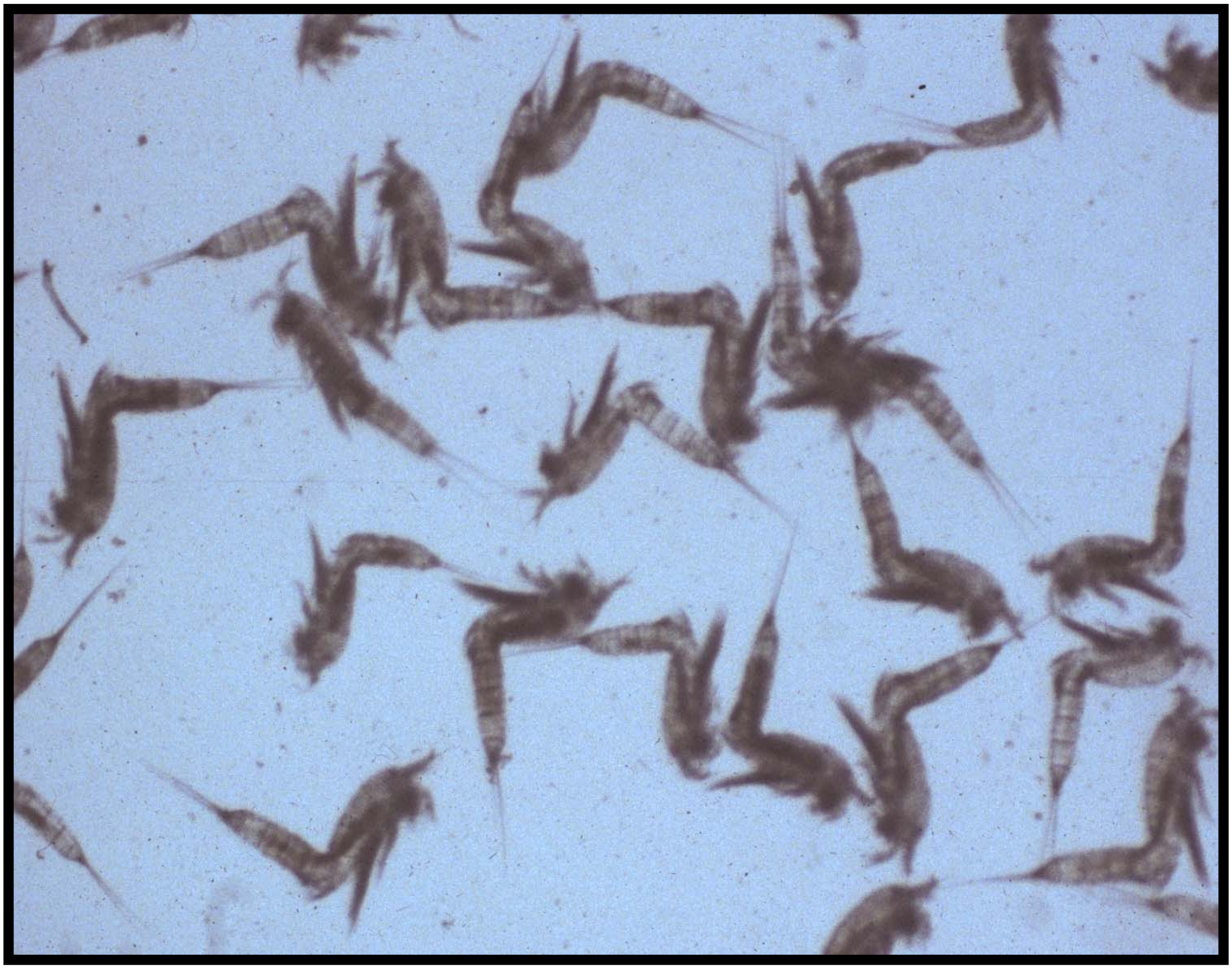
Mixtures of Metals and Polynuclear Aromatic Hydrocarbons May Elicit Complex, Nonadditive Toxicological Interactions



U.S. Department of the Interior
Minerals Management Service
Gulf of Mexico OCS Region

Cooperative Agreement
Coastal Marine Institute
Louisiana State University

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ABOUT THE COVER

Amphiascoides atopus collected from a mass laboratory culture. Body lengths of adult females average approximately 1 mm. *A. atopus* was one of two meiobenthic copepod species used in experiments to determine toxicological interactions of metals and polynuclear aromatic hydrocarbons.

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ABSTRACT

Metals and polynuclear aromatic hydrocarbons (PAH) may be elevated around oil and gas production platforms, including those in the Gulf of Mexico (Kennicutt et al. 1996; Peterson et al. 1996). The exposure of sediment-dwelling organisms to metal and PAH mixtures may result in toxic endpoints that differ from exposure to individual contaminants. The purpose of this study was to identify acute and sublethal metal-PAH nonadditive interactions in selected benthic organisms.

Cadmium, Hg, Pb, and Zn were elevated in close proximity to platforms, and of these, Cd, Hg, and Pb occurred at concentrations that may be expected to cause lethal or sublethal toxic responses in marine biota based on single-compound exposures in laboratory tests (Kennicutt et al. 1996). Total PAH concentrations in sediments were found to be elevated at a spatial scale similar to the distribution of metals. PAH near platforms were observed at concentrations typically just below minimum effects criteria established for PAH.

Acute and sublethal mixture toxicology of contaminants (i.e., Cd, Hg, Pb, fluoranthene and phenanthrene) associated with sediments around offshore oil production platforms was investigated using two species of meiobenthic harpacticoid copepod. *Schizopera knabeni* was exposed to sediment amended with single contaminants and contaminant mixtures in 96-h LC_{50} lethality and grazing-rate bioassays. Contaminant effects in mixtures were delineated using toxic unit methodology and factorial experiments. Adult *S. knabeni* was shown to be highly tolerant of single-contaminant exposures to phenanthrene, Cd, Hg, and Pb, as well as a mixture of Cd, Hg, and Pb. However, when the mixture of Cd, Hg, and Pb was combined with phenanthrene, a greater than additive response was demonstrated; the mixture was 1.5 x more lethal than predicted by separate exposures. Binary experiments revealed that although phenanthrene was individually synergistic with Cd, Hg, and Pb, the phenanthrene-Cd synergism was particularly strong (2.8 x more lethal than predicted). A Cd-phenanthrene synergism in *S. knabeni* was also observed in aqueous exposures suggesting the interaction was related to a pharmacological insult rather than a sediment-related exposure effect. Cadmium did not influence phenanthrene uptake kinetics suggesting that Cd had no effect on phenanthrene biodynamics. An antagonism between Cd, Hg, and Pb was also indicated, and this antagonism may have moderated an observed Cd-phenanthrene synergism in metal-phenanthrene mixtures. Grazing-rate bioassays suggest a response-additive sublethal toxicology between metals and phenanthrene. Experiments with *Amphiascoides atopus* revealed that phenanthrene and fluoranthene are both synergistic with Cd. Overall, our studies suggest that metal-PAH interactions may be common among benthic copepods (and perhaps other benthic taxa) and that strong nonadditive effects observed in binary mixtures may be moderated in more diverse contaminant mixtures. The strength of observed synergisms suggests that established sediment quality criteria may not be protective for joint exposures of PAH and metals, especially Cd-PAH mixtures.

INTRODUCTION

Waste discharges and spills that may occur during offshore oil and gas exploration and production can alter the benthic environment through the addition of metals and polynuclear aromatic hydrocarbons (PAH) to sediments. The drilling muds which are discharged and settle on the sea floor contain trace amounts of metals, including mercury and cadmium. Barite with greater amounts of impurities was in use prior to 1993. Other metals such as lead and zinc may originate from corrosion of galvanized pipe or welding operations. The PAHs may be present in sediment as result of pipeline spills of crude oil or oil-based drilling fluids, spills of oil from machinery or supply boats, or improperly treated produced water and deck drainage discharges.

Through the Outer Continental Shelf Lands Act (OCSLA), the Department of the Interior (DOI) is directed to responsibly manage the Outer Continental Shelf oil and natural gas resources while maintaining the protection of the human, marine, and coastal environment. The Minerals Management Service (MMS) with the DOI is tasked to conduct studies to monitor the health of the offshore environmental impacted by oil and gas exploration and production. MMS works with the US Environmental Protection Agency (USEPA) which regulates all discharges to waters through the Clean Water Act, National Pollutant Discharge Elimination System (NPDES). This study addressed the toxic interactions that may occur from metal and PAH contaminant mixtures associated with offshore activity.

Hazard assessment of contaminated sediments is based primarily on laboratory testing that quantifies the lethal responses of model organisms to sediments contaminated with single compounds (Long et al. 2000; USEPA 2000a; 2000b). However, benthic organisms often experience persistent exposure to mixtures of pollutants in diverse chemical classes (Kennicutt et al. 1996). Because few laboratory tests examine the toxicity of contaminant mixtures (Cassee et al. 1998; Steevens and Benson 1999), hazard assessment protocols incorporate assumptions about the toxicity of mixtures. If contaminants have a similar mode of toxic action, dose (or concentration)-additive toxicity is typically hypothesized (Cassee et al. 1998). This assumption appears to be well met within classes of organic contaminants, including polynuclear aromatic hydrocarbons (PAH) (Broderius 1991). Joint-toxicity of chemicals with dissimilar toxic action is usually hypothesized to be independent and elicit response-addition toxicity (Broderius 1991; Faust et al. 2000; Price et al. 2002). However, biota may respond to contaminant mixtures in unexpected ways because individual contaminants sometimes interact modifying the overall magnitude or nature of toxicity (Cassee et al. 1998). These nonadditive toxicant interactions, expressed as synergisms (greater than additive toxicity) or antagonisms (less than additive toxicity), pose a significant challenge to hazard assessment, but have been considered rare (Pape-Lindstrom and Lydy 1997; de Zwart and Posthuma 2005).

Recent research, however, suggests that interactive effects may be more common than previously considered, at least within and between certain chemical classes with different modes of toxic action. For example, synergisms between insecticides and herbicides appear to be frequent (Pape-Lindstrom and Lydy 1997). In a comprehensive review of studies of mixtures of heavy metals, Norwood et al. (2003) concluded that synergisms and antagonisms are more common than response-addition toxicity. Metals and PAH are frequent co-contaminants in sediments (Sanger et al. 1999a; 1999b; Callender and Rice 2000; Van Metre et al. 2000) and have dissimilar toxicology, but several studies suggest co-occurrence may elicit complex, interactive effects (Gust 2005a and citations therein). For example, Moreau et al. (1999) characterized an antagonistic interaction between phenanthrene and Zn that moderated lethality in sheepshead minnow (*Cyprinodon variegates*), although indications of a synergistic interaction at low toxicant levels and at specific phenanthrene:Zn ratios were also noted. The frequency and biological impact of these interactions will remain uncertain until more organisms are tested with a wider range of types and combinations of contaminant mixtures.

Toxicological interactions (i.e., synergisms and antagonisms) between contaminants are usually conceptualized as pharmacological in nature, related to the dose experienced at the site of toxic action after bioaccumulation into an organism's tissues (Cassee et al. 1998; Broderius 1991). However, when contaminants in mixtures interact with the environment (including sediments), "apparent" interactions associated with effects on exposure/bioavailability may occur (Norwood et al. 2003; Gust and Fleeger 2006). Deposit-feeding infauna may be particularly susceptible to exposure-mediated effects because the route of uptake for many contaminants is sediment ingestion (Wang and Fisher 1999; Lu et al. 2004; Kukkonen et al. 2005; Luoma and Rainbow 2005), and deposit feeders are frequently highly selective in the size and type of particles ingested (Self and Jumars 1988). Contaminants have also been shown to influence the size of particles ingested by deposit feeders (Millward et al. 2001b). By way of example, PAH partition strongly with the organic carbon fraction of particulate matter in sediments (Reible et al. 1996), while metals associate with fulvic and humic acids, metal oxyhydroxides and particulate organic carbon in oxidized and acid-volatile sulfides in reduced sediments (Decho and Luoma 1994; Chapman et al. 1998). Because these particles differ in size and type, contaminants in different chemical classes may be differentially available among sediments to selectively feeding invertebrates. Furthermore, sediment-associated contaminants may interact by biogeochemical processes to influence desorption kinetics of co-occurring contaminants. For example, hydrocarbons may influence ligand-binding sites for metals, altering metal free-ion concentration in pore water, and thus exposure (Millward et al. 2001a). Alternatively, a contaminant may influence the bioavailability of co-occurring contaminants indirectly by its effects on sediment pH, redox potential or the ratio of acid volatile sulfides and simultaneously extracted metals. Finally, joint exposure to metal and organic contaminants may influence digestive processes in deposit-feeding invertebrates (Chen et al. 2000; Mayer et al. 2001). One possibility is that organic pollutants may alter metal interactions with other organic compounds, such as amino acids, during gut passage and modify metal absorption efficiency. Little research has been conducted on these questions, however it is clear that interactions between co-occurring contaminants associated with sediments may be a function of exposure, mediated by several possible mechanisms.

Two recent studies with deposit-feeding invertebrates suggest that interactions between Cd and phenanthrene are related to sediment exposure but by different mechanisms. In the bulk deposit-feeding oligochaete *Ilyodrilus templetoni*, phenanthrene reduced exposure to the co-pollutant Cd by slowing the rate of sediment ingestion (presumably by a narcotizing effect) which resulted in reduced Cd toxicity (Gust and Fleeger 2006). Alternatively, Cd bioaccumulation (and thus toxicity) in the amphipod *Hyalella azteca* increased in the presence of a sublethal concentration of phenanthrene in sediment but not in water-only exposures, indicating an exposure-related effect on uptake from sediment (Gust 2005b; Gust and Fleeger 2005). Because sediment toxicity bioassays form the basis for assessing sediment quality guidelines (Long et al. 2000), information regarding joint-toxic effects of contaminants among various taxa (especially model species frequently used in toxicity bioassays) will prove necessary for accurate assessment of environments where contaminant mixtures persist. In addition, knowledge of the frequency with which interactive mixture effects occur (and if they result from pharmacological interactions or contaminant-related alterations in chemical exposure) is required to fully interpret mixture effects and thereby improve hazard assessment.

Metals and PAH may be elevated around oil and gas production platforms, including those in the Gulf of Mexico (Kennicutt et al. 1996; Peterson et al. 1996). Benthic amphipods and harpacticoid copepods were found to decrease in abundance, while more tolerant deposit-feeding, infaunal annelids increased in abundance near platforms (Peterson et al. 1996). Cadmium, Hg, Pb, and Zn were elevated in close proximity to platforms, and of these, Cd, Hg, and Pb occurred at concentrations that may be expected to cause lethal or sublethal toxic responses in marine biota based on single-compound exposures in laboratory tests (Kennicutt et al. 1996). Total PAH concentrations in sediments were found to be elevated at a spatial scale similar to the distribution of metals. PAH near platforms were observed at concentrations typically just below minimum effects criteria established for PAH. These observations suggest that elevated metals may be responsible for changes in biotic communities in close proximity to platforms. However without experiments designed to identify toxicant interactions, the hypothesis that metal-PAH interactions are responsible for the observed changes in community structure cannot be excluded. As illustrated by the studies mentioned above, PAH may interact synergistically with metals in amphipods reducing amphipod abundance by a direct toxic effect. On the other hand, deposit-feeding annelids may respond to joint contamination in an independent or antagonistic fashion, perhaps leading to increases in abundance by hormesis (Calabrese and Baldwin, 2003) or by indirect ecological effects (Fleeger et al. 2003). Other areas associated with oil production and transportation in the Gulf of Mexico (*e.g.*, produced-water outfall sites) also exhibit high concentrations of metals and PAH (Rabalais et al. 1991), and, if common, nonadditive interactions may broadly impact benthic communities in many affected areas.

The purpose of the study was to identify the existence, frequency of occurrence and (potentially) the cause of acute and sublethal metal-PAH nonadditive interactions in selected benthic organisms. Initially, an amphipod, an annelid and a harpacticoid copepod were examined because earlier studies (Peterson et al. 1996) found members of these taxa either increase or decease in abundance near platforms with elevated metal and PAH contamination. Experiments with the macrofaunal *Leptocheirus plumulosus* (an amphipod) and *Neanthes arenaceodentata* (a polychaete) were conducted; however, several problems with test sediment and culturing precluded successful, timely outcomes. Experiments with the meiobenthic copepod *Schizopera knabeni* were successful and lethal and sublethal endpoints (related to ingestion rate) associated with Cd-phenanthrene exposure were examined. In addition, a second species of meiobenthic copepod (*Amphiascoides atopus)* was examined in a limited number of experiments.

METHODOLOGY

Acute Tests

Laboratory Culture and Test Organisms

The laboratory-reared meiobenthic harpacticoid copepods *Schizopera knabeni* Lang and *Amphiascoides atopus* Lotufo and Fleeger were used as test organisms. Cultures of *S. knabeni* were initiated in 1993 from collections made in a salt marsh near Port Fourchon, Louisiana (Lotufo and Fleeger 1997) and have been continuously maintained since in sediment-free, 1-L Erlenmeyer flasks at room temperature with 20‰ artificial seawater (ASW). Cultures of *A.*

atopus were established in 1992 (see Lotufo and Fleeger 1995; Sun and Fleeger 1995) and were maintained during the course of the experiments in conditions identical to *S. knabeni* except for the use of 30‰ ASW. Copepods were fed weekly with T-*Isochrysis* paste (Brine Shrimp Direct), and continuous reproduction in both species was apparent. Copepods were harvested by sieving culture medium through a 125-µm aperture screen, and retained copepods were sorted under a stereo-dissection microscope. *S. knabeni* is a benthic copepod; however, adults can swim although nauplii are restricted to substrates (Fleeger 2005). Adults also readily associate with muddy sediments when present (Lotufo and Fleeger 1997). *A. atopus* adults (but not nauplii) are also capable swimmers. Members of this genus typically live in high-energy beaches with course sediment and do not associate with muddy environments. Therefore, experiments with *A. atopus* were conducted in a sediment-free environment.

Sediment Preparation

Sediment used in all acute and sublethal toxicity bioassays with *Schizopera knabeni* was collected from the upper 2 cm of a mudflat in a *Spartina alterniflora* salt marsh near Cocodrie, Louisiana. This sediment was processed following Chandler (1986) to reduce the organic matter content and to generate a more uniform particle size distribution. Sediment was autoclaved after processing and a liquid slurry (ratio = 1.31) was created by homogenizing sediment with an appropriate volume of 20‰ ASW. The final wet:dry ratio was 1.31:1. Total organic carbon (TOC) content of the processed sediment was measured using a Perkin Elmer 2400 CHN Series II elemental analyzer (Norwalk, CT, USA) and found to be 3.7% ± 0.34%. Samples were refluxed for 6 h in concentrated HCl to eliminate inorganic carbonate and oven dried at 70°C prior to TOC analysis. Prepared sediments were stored in sealed containers in the dark at 4^{o}C until used in bioassays.

Phenanthrene (98% purity, Aldrich Chemical Co. Milwaukee, WI, USA) was amended to processed sediment by dissolving the chemical in HPLC-grade hexane and then volatilizing the solvent in an ultra-high-purity nitrogen gas stream to coat the inside walls of glass loading jars (Reible et al. 1996). The appropriate mass of wet sediment to achieve a targeted concentration was then added to each jar and tumbled on a roller mill at room temperature (~22^{o}C) for 21 d to homogenize and age the phenanthrene-sediment complex. Amended sediment was stored at 4°C for no more than 7 d prior to bioassay initiation. Day-0 sediment phenanthrene concentrations were measured using high-performance liquid chromatography (HPLC) following Gust (2005b). Two replicates of each phenanthrene-amended sediment treatment were frozen, freeze-dried, homogenized, and pre-weighed quantities were transferred to a glass extraction vessel. Sixty mL of a 1:1 mixture of HPLC grade acetone and hexane was added to the dried sediments. The solvent-sediment combination was sonicated for 20 min and then allowed 24 h for solvent extraction of phenanthrene. After extraction, the solution was reduced in volume by 90% via volatilization in an ultra-high-purity nitrogen gas stream, and then brought up to the initial volume with acetonitrile. Samples were analyzed using a Hewlett-Packard 1100 series HPLC. Phenanthrene concentrations were determined by reverse phase HPLC employing an HC-ODS Sil-X, 5 µm particle diameter packed column. Phenanthrene was monitored by fluorescence detection. Five minutes of isocratic elution with acetonitrile/water (4:6) (v/v) were followed with linear gradient elution to 100% acetonitrile over 25 min at a flow rate of 0.5 mL min^{-1}.

Lead (Pb), cadmium (Cd), and mercury (Hg), as chloride salts; 98% purity, Sigma Chemical Co. St. Louis, MO, USA, were amended to processed sediment for bioassays. When testing mixture effects, Pb, Cd, and Hg were always amended in a ratio of 5:3:2 respectively (on average, the ratio of measured final concentrations in mixtures was 4.9: 3.3: 2.1, see below). The required amount of each compound was dissolved in deionized water, and then added to a specific mass of wet sediment to achieve a targeted sediment concentration. To ensure homogenization, the metal-rich solution was added drop wise via a gravity-fed apparatus to 39 g of wet sediment in glass jars undergoing vigorous mixing (Millward et al. 2004). Amended sediment was stored at 4°C for no more than 7 d prior to initiation of toxicity bioassays. For joint-toxicity bioassays, phenanthrene was amended to sediment (as above) prior to addition of metals. Two replicates of each metal treatment were freeze-dried, milled, and weighed. Metals were extracted into 10 mL of trace-metal-grade HNO_3 using a Perkin Elmer/Anton Paar, Multiwave 3000, microwave sample preparation system. Day-0 sediment metal concentrations were analyzed by inductively-coupled argon plasma mass spectrometry (ICP-MS) using a PerkinElmer Sciex, Elan 9000 ICP-MS following Gust (2005b).

96-h Acute Sediment Toxicity Tests

All glassware used to conduct static sediment bioassays was acid cleaned prior to use. Test chambers (28 x 45 mm glass crystallizing dishes) were filled with 25 mL of 20‰ ASW. Sediment from each treatment was dispensed into a test chamber to create a sediment layer 3-4 mm thick. Sediment was allowed to settle for 4 h before copepods were added. Five replicates were used for each treatment and control (without amended contaminants) sediment. Background levels of metals and phenanthrene are low at our sediment collection site (Carman et al. 1997). Typically, five target levels (concentrations) plus a control were used in acute tests. Crystallizing dishes were placed in a loosely covered plastic container lined with wet paper towels to minimize evaporation. Fifteen adult female *S. knabeni* were introduced to each crystallizing dish using a mouth pipette and dishes were placed in an environmental chamber and maintained at 25^{o}C without light. After 96 h, the contents of each dish was poured through a 125-µm mesh sieve and copepods retained were enumerated as live or dead. Missing copepods were presumed dead, and percent mortality was calculated. Copepods immobilized by phenanthrene were considered alive if they displayed body motion when touched with a probe. Range-finding tests (results not shown) were conducted for individual contaminants and the contaminant mixture to help determine appropriate treatment concentrations for the definitive LC_{50} tests described herein.

An LC_{50} and 95% confidence interval for each individual contaminant and an LC_{50} for each contaminant when exposed in a mixture (designated LC_{50}m, Hagopian-Schlekat et al. 2001) were estimated from mortality data using probit analysis (SAS Version 8.0). All LC_{50} estimates from sediment toxicity tests were based on measured contaminant concentrations. These data from tests with mixtures allowed the generation of a compound-specific LC_{50} (LC_{50}m) for each compound within a mixture. The LC_{50}m is, therefore, the LC_{50} for a particular compound in the presence of the other compounds in the mixture when amended in equi-toxic proportions. Concentration-response curves of compounds of interest were compared by examination of LC_{50} (values were considered different if 95% C.I.s did not overlap) and by ANOVA.

Log-normalized concentration-response curves were examined for Cd, phenanthrene and the metal mixture. If the slopes of the response curves are similar (Berenbaum 1989; de Zwart and Posthuma 2005), the sum toxic unit (TU) approach was used to assess the effects of binary mixtures (Marking 1977). Mixture effects between metals (either in a mixture or Cd alone) and phenanthrene were hypothesized to follow a dose-additive model. The TU of a mixture was calculated by summing the ratios of the concentrations of each compound in the mixture divided by its individual LC_{50} value. Thus in a binary mixture (e.g., phenanthrene and Cd), the concentration of each at one half of its LC_{50} yielded a sum TU=1. Sediments in each test were amended using a 70% dilution series projected above and below 1 TU. If a mixture composed of a sum TU of 1 produced 50% mortality (determined as TU_{50} including 1 within the 95% C.I.), we concluded that the binary mixture was concentration additive. A toxic unit of less than 1.0 was considered to be greater-than-additive (synergistic) and greater than 1.0 was considered less-than-additive (antagonistic).

Acute Toxicity in Aqueous Exposures

All containers and apparatus used to conduct water-only exposures of Cd, phenanthrene, fluoranthene or mixtures were acid cleaned prior to use. For single-contaminant exposures with copepods, an appropriate amount of Cd was dissolved into 10 mL of ASW and spiked into beakers containing 250 mL of ASW to create a dilution series of contaminant concentrations. Phenanthrene or fluoranthene were dissolved in 2 mL of acetone and spiked into ASW. Similar spiking methods were used for experiments with *Schizopera knabeni* and *Amphiascoides atopus*, except that 20‰ ASW was used for *S. knabeni* and 30‰ ASW for *A. atopus*. Forty mL of contaminant-amended ASW from each treatment concentration was dispensed into test chambers (100 mL beakers). Five replicates were used in each treatment category, including a control. Beakers were placed in a loosely covered plastic container lined with wet paper towels to reduce evaporation. Fifteen adult female *S. knabeni* or *A. atopus* were introduced to each beaker, and beakers were held in an incubator at $25^{o}C$ without light. Water was replaced twice daily with freshly prepared contaminant-amended ASW. After 96 h, contents were rinsed through a 63-µm aperture sieve and the copepods retained were enumerated as live or dead as described above.

Binary-mixture effects between Cd and phenanthrene or Cd and fluoranthene were examined using factorial experiments. A range of nominal phenanthrene concentrations were tested with and without a nominal sublethal concentration (140 µg L^{-1}) Cd for *Schizopera knabeni* to test the hypothesis that Cd does not alter the toxicity of phenanthrene. Two types of controls were used. The first had no amended contaminants and the second contained the sublethal concentration of Cd without phenanthrene. For *Amphiascoides atopus*, a range of nominal Cd concentrations were tested with and without a nominal sublethal concentration (250 µg L^{-1}) of phenanthrene or fluoranthene to determine if either PAH altered the toxicity of Cd. A control without amended contaminants was used, as well as a control containing only phenanthrene or fluoranthene. The experiments were conducted as 96-h bioassays and nominal LC_{50} values were calculated. Factorial experiments were analyzed by comparison of LC_{50} values (nonoverlapping 95% C.I. were used as criteria for determining differences among treatments) and two-way ANOVA to determine concentration effects and interactive effects among treatments.

Cd Effects on Phenanthrene Bioaccumulation Kinetics

Phenanthrene bioaccumulation kinetics were measured in sediment exposures using equivalent sediment bioassays as described above to test the hypothesis that Cd had no effect on phenanthrene bioaccumulation. Radiolabeled phenanthrene (^{14}C, 15 µCi) was supplemented to the phenanthrene stock solution and amended to sediments as described above. *Schizopera knabeni* was exposed to 52.5 mg kg^{-1} phenanthrene with and without 22 mg kg^{-1} Cd (all values are nominal) for 4, 24, 48, and 72 h. After exposure, copepods were recovered and enumerated using the methods described above, allowed to depurate gut contents for 6 h then transferred to

8-mL scintillation vials containing 0.5 mL of TS-2 tissue solublizer. Samples were heated to 50°C and allowed to digest for 12 h. After digestion, 0.5 mL of 1 N HCl solution was added to neutralize the tissue solublizer and 6 mL of Biosafe II liquid scintillation cocktail was added to each sample. After a 24-h holding period to reduce chemical-induced scintillation, samples were analyzed using a Packard model Tri-carb 2900TR liquid scintillation counter. An empirically derived quench curve incorporating quench stemming from tissue digestion reagents was used to assess counting efficiency and to determine disintegrations per minute (dpm) from counts per minute (cpm) data. A ratio of dpm to analytically measured phenanthrene concentration was used to determine *S. knabeni* tissue concentrations. Two-way ANOVA was used to determine the effects of Cd, exposure duration and their interaction on phenanthrene bioaccumulation kinetics.

Sublethal Tests

Microalgae Labeling

An inoculum of *Isochrysis galbana* in log-phase growth was added to 600 mL of a nutrient culture media (f/2) at 20‰. After 3 days, 250 µCi of $NaH^{14}CO_3$ was added (specific activity 50mCi mmol^{-1}, American Radiolabeled Chemicals). Cultures were sealed to prevent the loss of label as $^{14}CO_2$, and maintained at 21°C with a 14/10 h light/dark cycle. Cultures were monitored every 48 h for cell density and label incorporation, and grown until ^{14}C in the cells became constant (6 or 7 days). Unincorporated ^{14}C was removed by repeated centrifugation and decantation of supernatant and rinsing with 20‰ ASW. The cell density was determined by direct count using a Neubauer haemocytometer, and ^{14}C in algal cells was determined using a liquid scintillation counter (Packard Tri-carb 2900 TR).

Grazing Experiment

To determine the effect of sediment contaminated with phenanthrene and metals (Cd, Pb, and Hg) (alone and in combination) on the feeding rate of *Schizopera knabeni*, copepods were fed ^{14}C-labeled *I. galbana*. In one experiment, adult female *S. knabeni* were exposed to sediment contaminated with phenanthrene and the metal mixture (Pb, Cd and Hg in a nominal 5:3:2 ratio). In another experiment, *S. knabeni* was exposed to Cd alone and a mixture of phenanthrene and Cd. We selected Cd for this experiment because results of lethality experiments suggest it is more toxic than Pb or Hg. Experimental units consisted of crystallizing dishes filled with 25 mL ASW at 20‰ and 3 mL of contaminated sediment. Four replicates were used per treatment. Phenanthrene concentration was 110 mg kg^{-1} dry sediment and total metals concentrations ranged between 100 and 400 mg kg^{-1} dry sediment (all nominal). Cd concentrations ranged from 50 to 200 mg kg^{-1} dry sediment.

Dishes were placed in loosely covered plastic containers underlined with water-soaked paper towels to retard evaporation from the experimental units. Ten adult females were added to each crystallizing dish. Four test units were used as controls (no amended contaminants) and 3 formaldehyde-killed units were used to determine the copepod incorporation of label by means other than feeding (poison control). After an incubation period of 48 h, each dish was inoculated with 330 µL of radiolabeled cells (2.27 x 10^7cell mL^{-1}, 0.19 dpm cell^{-1}). After 4 h, copepods were killed in formaldehyde, concentrated on a 125-µm sieve and sorted under a stereo microscope.

Copepods were placed in scintillation vials and solubilized with 200 μL TS-2 tissue solubilizer. After 24 h, 200 μL 1N HCl was added to neutralize the tissue solubilizer, 6 mL of Biosafe II liquid scintillation cocktail were added and radioactivity was determined by liquid scintillation counting. Radioactivity was converted to the amount of algal cells consumed by dividing the mean radioactivity of copepods by the mean radioactivity per algal cell.

Statistical Analysis

Data from grazing experiments were analyzed using a one-way analysis of variance (ANOVA). *A posteriori* comparisons were performed using the Tukey test (alpha = 0.05). All analyses were carried out using SAS 9.1 software.

RESULTS

S. knabeni Sediment Toxicity Tests with Single Contaminants

Mortality in *Schizopera knabeni* ranged from 0-13% in control sediments for all experiments. *S. knabeni* mortality and sediment concentration did not produce a monotonically increasing order of mortality in Hg-only or Pb-only bioassays, and toxicity was observed only after exceeding sediment saturation (estimated to be 4000 mg Pb kg^{-1} dry sediment and 2500 mg Hg kg^{-1} dry sediment). Therefore, data (not shown) were not fit to concentration-response curves. However, *S. knabeni* mortality increased with increasing phenanthrene and Cd concentration in Cd-only and phenanthrene-only bioassays (Figure 1). Estimates of the LC_{50} for single-compound exposures to phenanthrene and cadmium for *S. knabeni* were 426 ± 70 (error terms are expressed as 95% confidence intervals throughout the text) mg kg^{-1} dry sediment and 230 ± 26 mg kg^{-1} dry sediment, respectively (Table 1). Slopes of concentration-response curves for phenanthrene, Cd, and the metal mixture differed by less than 10% (Figure 2), and following de Zwart and Posthuma (2005), toxic unit methods were deemed appropriate to examine interactive toxicity (see below).

Metals-Mixture Sediment Toxicity Tests with *S. knabeni*

Lead, Cd, and Hg were amended in sediments using a 5:3:2 ratio respectively over a range of concentrations. Measured ratios averaged 4.9 Pb : 3.3 Cd : 2.1 Hg across all treatment levels used (n = 6), suggesting metal ratios were consistent within and among experiments. There was a direct positive relationship between mortality and sediment metals concentration when *Schizopera knabeni* was exposed to the metal mixture (Figure 3). The estimated 96-h LC_{50} for the summed metal concentration in *S. knabeni* was 1462 ± 107 mg kg^{-1} dry sediment. Values for individual metals in the mixture (i.e., an LC_{50}m for Pb, Cd, and Hg) were 773 ± 58.8, 442 ± 33.6, and 309 ± 23.5 mg kg^{-1} dry sediment, respectively (Table 1). Slopes of concentration-response curves for phenanthrene and the metals mixture differed by more than 10% (Figure 2), however their general similarity suggested that TU methods were appropriate to examine interactive toxicity (see below). The 96-h LC_{50} for Cd alone was 230 ± 26 mg kg^{-1} dry sediment compared to the LC_{50}m of Cd in combination with Pb and Hg of 442 ± 33.6 mg kg^{-1} dry sediment (Table 1). This large decrease in Cd toxicity for *S. knabeni* in the presence of Hg and Pb suggests an antagonism between Cd and the other metals, although specific bioassays to test for interactions between Cd and the other metals were not conducted.

Joint-Toxicity Tests in Sediment

Lead and Hg (nominal concentrations, 2000 and 1000 mg kg^{-1} dry sediment respectively) were added to phenanthrene-amended sediment in binary mixtures in a factorial design to determine if either metal influenced the acute toxicity of phenanthrene. Nominal phenanthrene concentrations ranged from 0 to 1000 mg kg^{-1} dry sediment in these tests (Figure 4). Mortality increased significantly with increasing concentration of phenanthrene ($p < 0.001$) in both bioassays and the addition of Pb ($p < 0.001$) and Hg ($p < 0.001$) both significantly increased toxicity (based on two-way ANOVA). The interaction between phenanthrene and Pb ($p = 0.090$) was not significant, however the interaction between phenanthrene and Hg was significant ($p = 0.012$). Least square means tests suggest that Hg amendment caused changes in the observed phenanthrene dose-response curve. Increasing phenanthrene caused significant increases in mortality such that mortality at all phenanthrene exposure concentrations without Hg differed from each other. With Hg amendment, one adjacent phenanthrene treatment combination did not differ from each other.

Interactive effects between phenanthrene and the metals mixture and between phenanthrene and Cd in *Schizopera knabeni* were each tested using toxic unit methods (Figure 5). Measured sediment concentrations (with 96-h TU_{50} values in parenthesis) for phenanthrene and the metals mixture (Table 1), and phenanthrene and Cd (Figure 5) in equi-toxic exposures

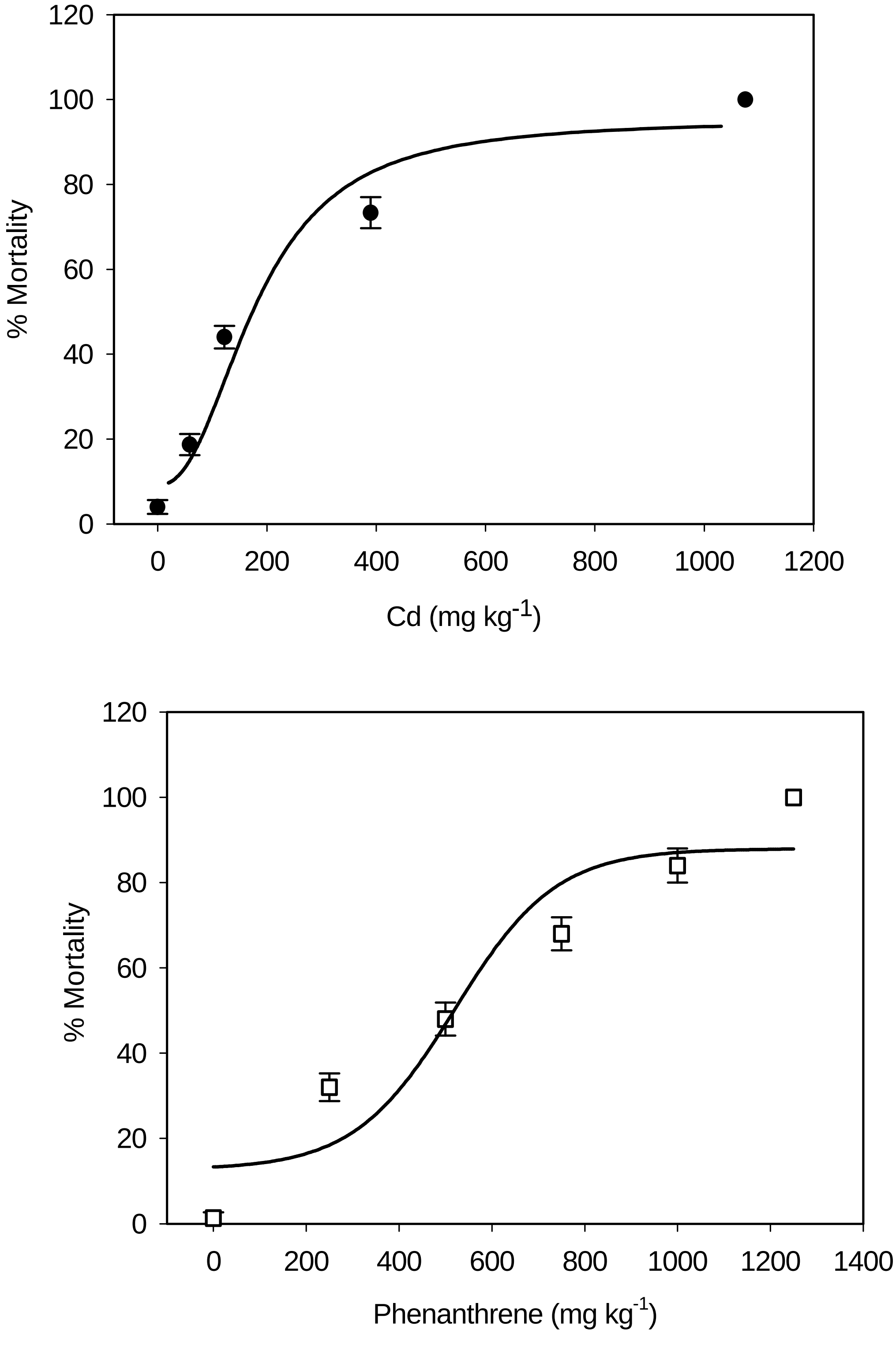


Figure 1. Concentration response of *Schizopera knabeni* to individual contaminant exposures in sediment. Upper figure, Cd alone and lower figure, phenanthrene alone.

Table 1.

Summary of results of sediment lethality tests (expressed as LC_{50} and 95% confidence limits) and toxic unit tests with *Schizopera knabeni* for individual compounds and metal mixtures. Individual values for Pb, Cd, and Hg in the metals mixture experiment represent an $LC_{50}m$ (95% confidence limits) for each metal. LC_{50} and TU_{50} equivalent concentrations are expressed in mg kg^{-1} dry sediment.

	Single-Compound Exposures	
Cd	230 (26)	
Phenanthrene	426 (70)	
	Exposure to a Mixture of Pb, Cd, and Hg	
Metals mixture	1462 (110)	
Pb	773 (59)	
Cd	442 (34)	
Hg	309 (23)	
Toxic Unit Experiments	TU_{50} (95% confidence limits)	Concentration equivalent
Phenanthrene + Cd	0.42 (0.04)	162 (20)
Phenanthrene + metals mixture	0.65 (0.08)	610 (70)

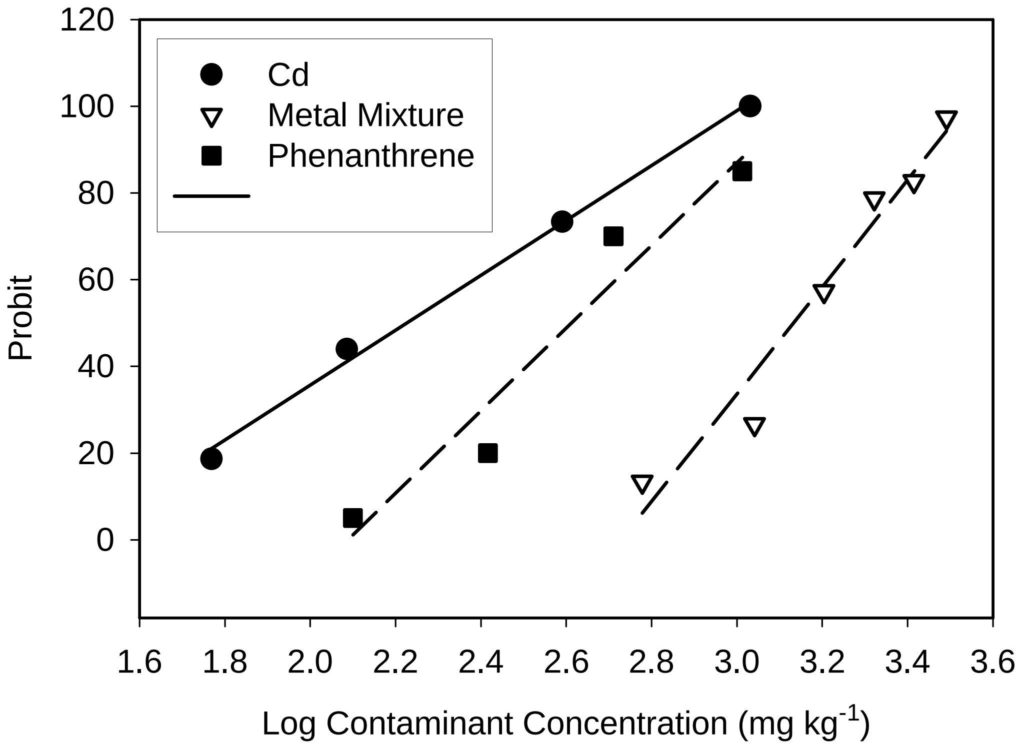


Figure 2. Comparison of log-normalized dose-response curves for Cd, a 5:3:2 mixture of Pb, Cd, Hg ("metal mixture") and phenanthrene. Values represent measured Cd concentrations (mg kg^{-1} dry sediment) and mean percentage mortality (n = 5).

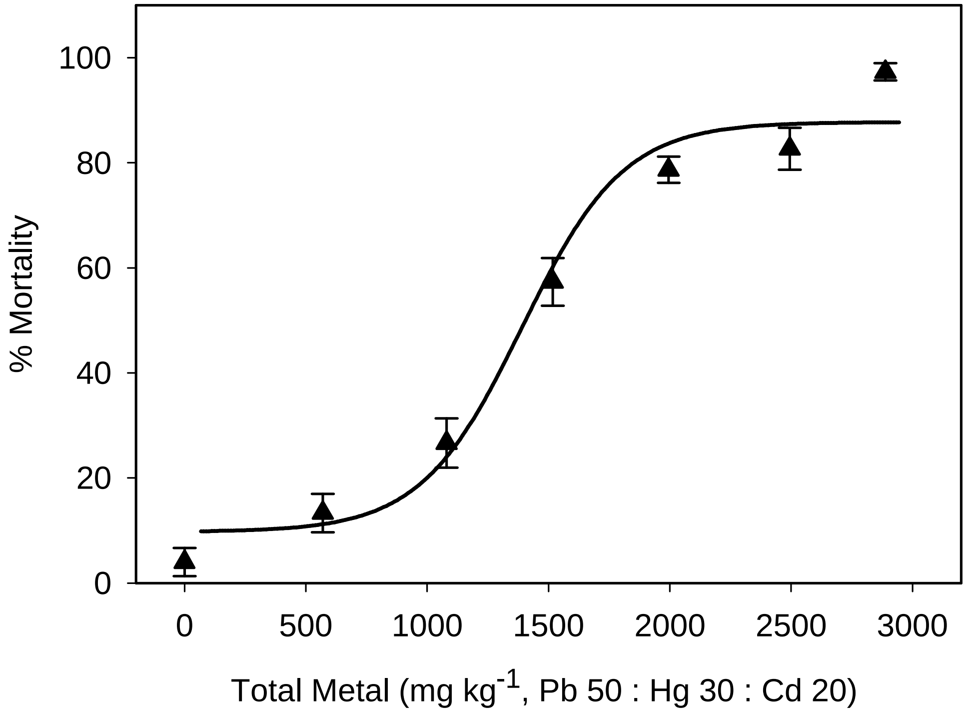


Figure 3. Concentration response of *Schizopera knabeni* to metal mixture in sediment when presented in a ratio of 5Pb:3Cd:2Hg. The metal mixture LC_{50} equals 1462 mg kg^{-1}.

that caused 50% mortality were 610 (TU = 0.65 ± 0.08) and 162 mg kg^{-1} dry sediment (TU = 0.42 ± 0.04), respectively. The confidence interval around TU did not include unity for either test, suggesting a greater-than-additive effect (synergy) in phenanthrene-metal mixtures. The combination of phenanthrene with the metals mixture was 1.5 x more lethal than each separately, and joint phenanthrene and Cd exposures were more strongly synergistic, 2.8 x more lethal as a mixture than either contaminant alone.

Aqueous Toxicity Tests with *S. knabeni*

Based on nominal contaminant concentrations, the 96-h LC_{50} estimates for Cd alone and phenanthrene alone were 276 ± 32 and 580 ± 102 µg L^{-1}, respectively (Figure 6). The Cd control did not increase mortality relative to true control, indicating the Cd concentration used (nominal 140 µg Cd L^{-1}) was sublethal for *Schizopera knabeni*. The Cd-phenanthrene mixture yielded a nominal 96-h LC_{50} for phenanthrene of 280 ± 28 µg L^{-1} (Figure 6). Based on nonoverlapping confidence intervals for phenanthrene alone and Cd-phenanthrene mixture treatments, we conclude that the increase in phenanthrene toxicity in the presence of a sublethal concentration of Cd in aqueous exposure is the expression of a synergism between phenanthrene and Cd.

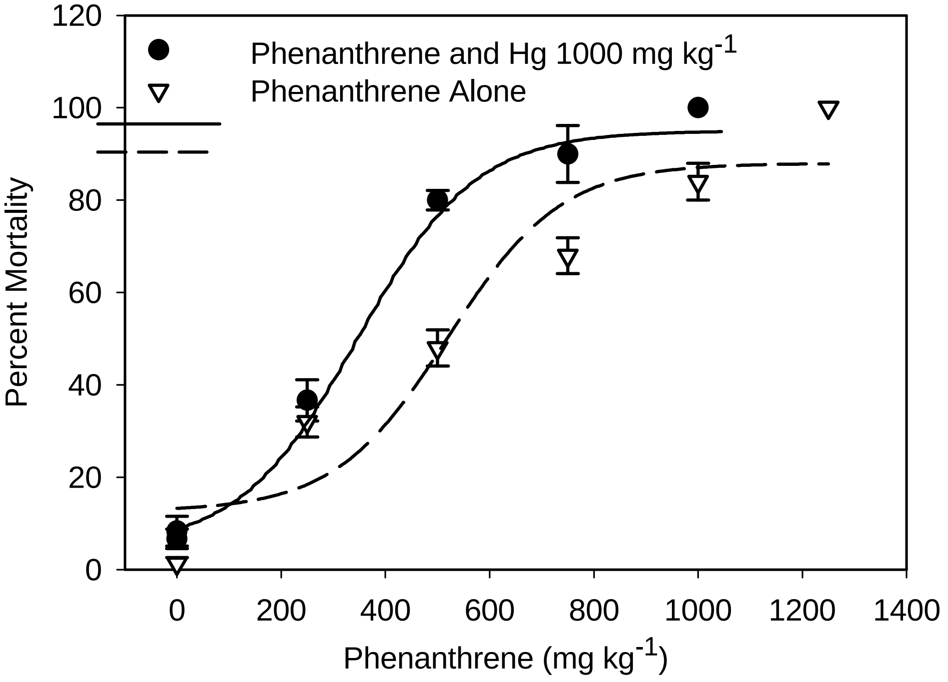
Bioaccumulation Kinetics in *S. knabeni*

Uptake of phenanthrene (based on ^{14}C-labeled phenanthrene) was rapid in *Schizopera knabeni*, reaching an apparent equilibrium in less than 4 h and with no change in tissue values over 72 h (Figure 7). A similarly rapid uptake for phenanthrene in *S. knabeni* was found by Lotufo (1998). The addition of 22 mg kg^{-1} Cd did not influence the uptake of phenanthrene. The experiment was conducted over 72 h to determine if Cd had an effect on phenanthrene tissue concentration that might be associated with an induction of metabolic pathways that breakdown PAH. However, tissue values of phenanthrene at 72 h with and without Cd were almost identical.

Aqueous Toxicity Tests with *A. atopus*

Amphiascoides atopus was highly tolerant of both phenanthrene and fluoranthene in single-compound, water-only exposures. Increasing concentration did not produce a monotonically increasing order of mortality for either phenanthrene or fluoranthene, and mortality did not increase relative to controls even at concentrations equivalent to aqueous saturation (data not shown). Mortality in Cd-only exposures, however, increased with increasing concentration, and a nominal LC_{50} of 549 ± 84 µg Cd L^{-1} was estimated (Figure 8).

Amphiascoides atopus was exposed to a range of Cd concentrations in combination with 250 µg L^{-1} (nominal) phenanthrene or fluoranthene. Controls with only PAH amendment displayed no added mortality relative to controls without amended contaminants. The LC_{50} for Cd with phenanthrene was 388 ± 44 µg L^{-1} (Figure 9). The non-overlapping confidence intervals for the Cd-only and Cd-phenanthrene mixture treatments indicates a synergistic interaction between Cd and phenanthrene. Similarly, when exposed to a range of Cd concentrations with the addition of 250 µg L^{-1} (nominal) fluoranthene (Figure 9), Cd LC_{50} (193 ± 30 µg L^{-1}) was significantly reduced (based on non-overlapping confidence intervals) relative to Cd-only exposures. The large change in Cd LC_{50} with fluoranthene suggests a very strong Cd-fluoranthene synergism.



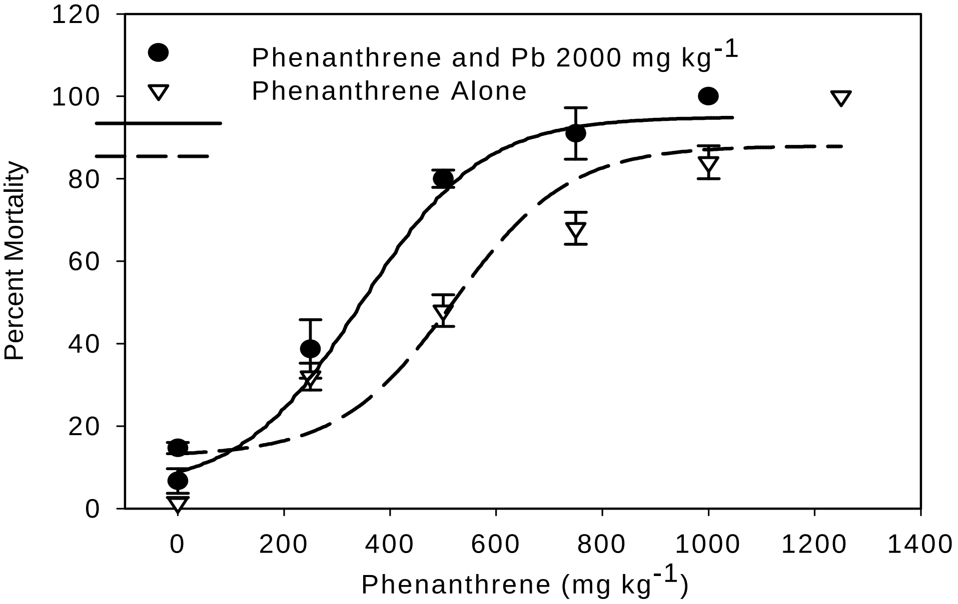
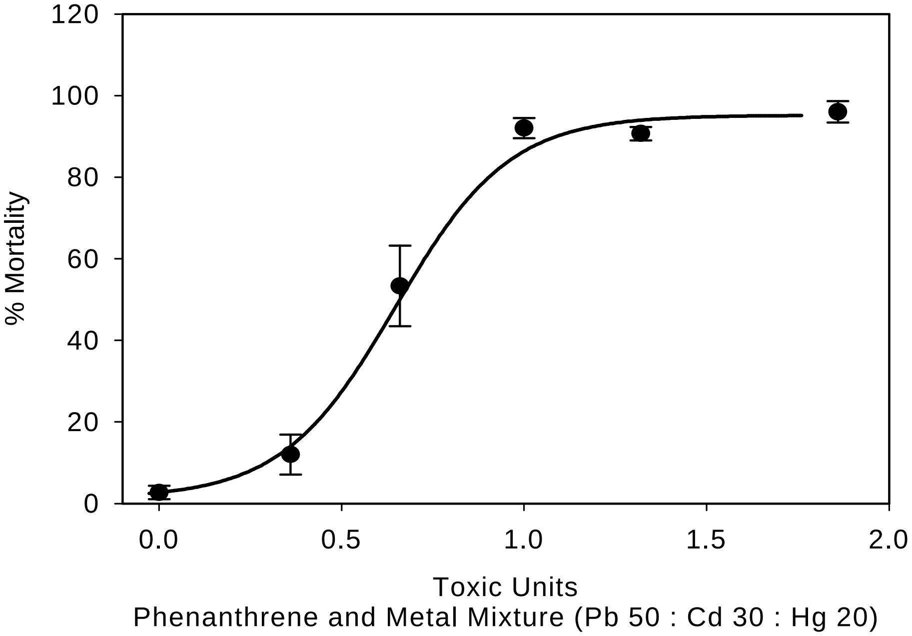


Figure 4. Metal-phenanthrene mixture experiments with *Schizopera knabeni* in sediments. Upper figure is Cd in binary exposures with Hg using a factorial design. Lower figure is Cd in binary exposures with Pb using a factorial design



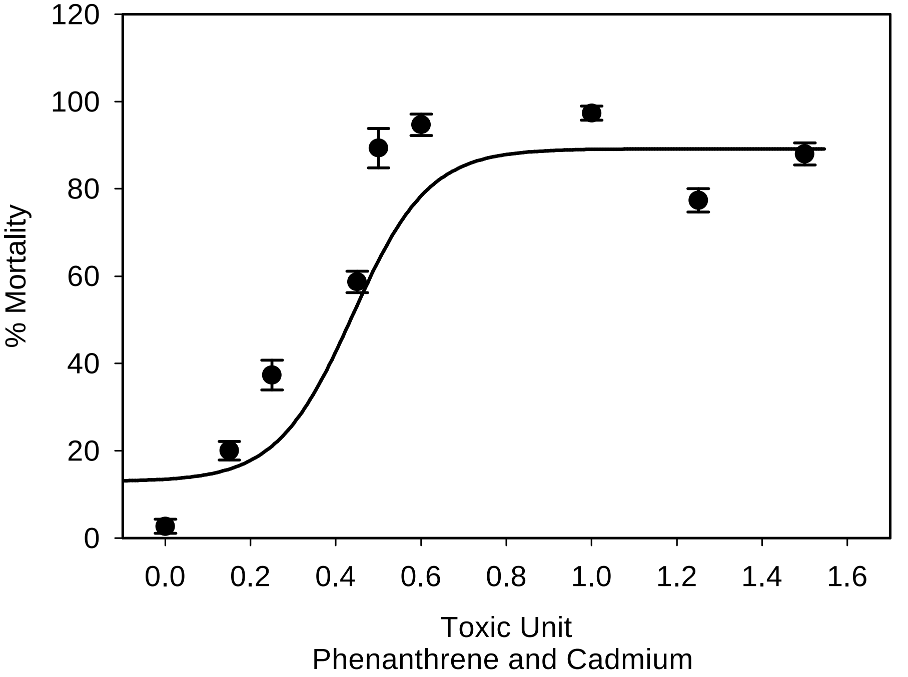


Figure 5. Metal-phenanthrene mixture experiments with *Schizopera knabeni* in sediments. Upper figure is phenanthrene and a metal mixture in a ratio of 5Pb:3Cd:2Hg and lower figure is phenanthrene and Cd, using toxic unit methodology.

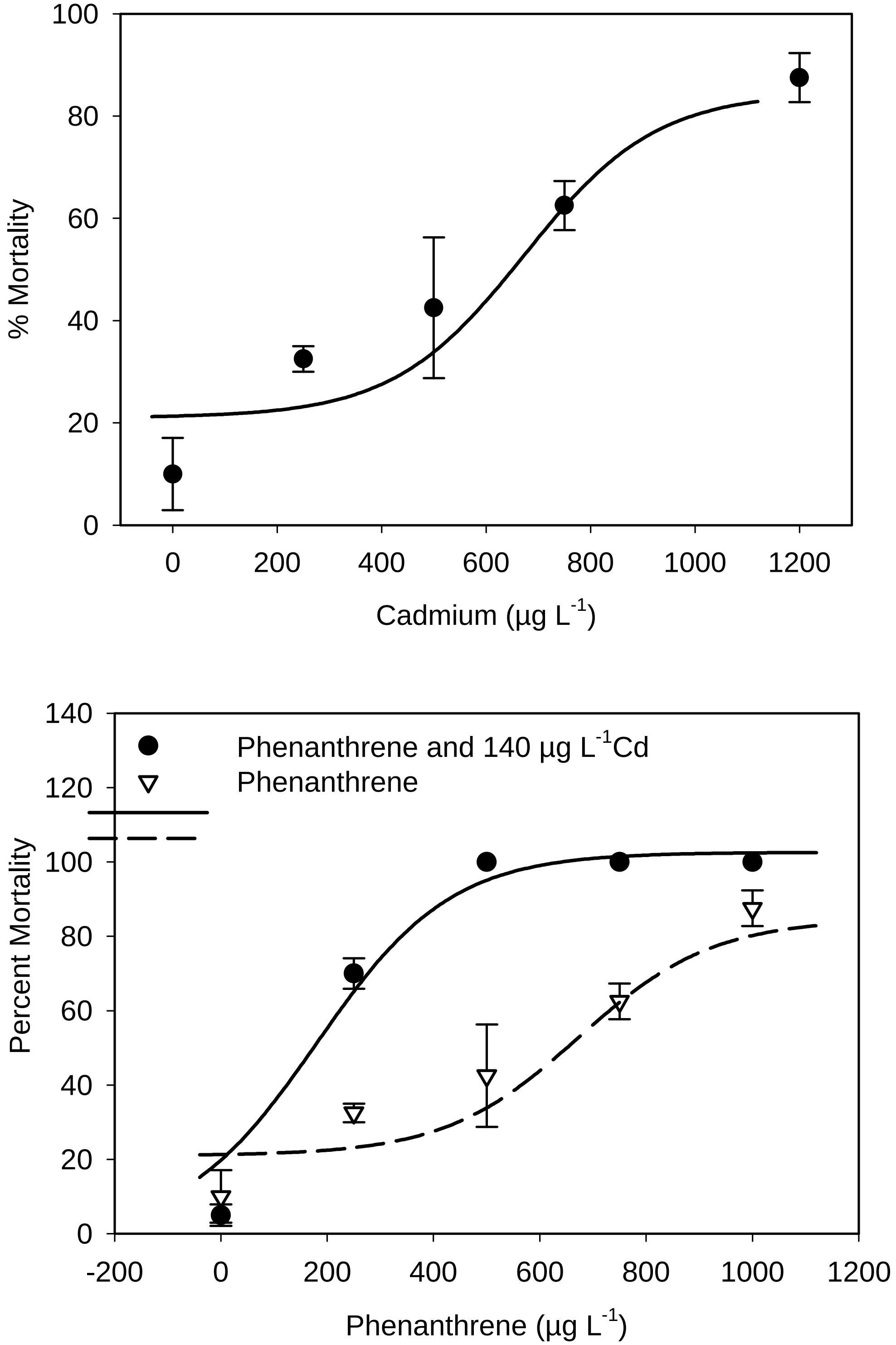


Figure 6. Concentration responses of *Schizopera knabeni* to Cd alone (upper figure) and phenanthrene alone and phenanthrene with Cd (nominal 140 µg L^{-1}Cd) in water-only experiments.

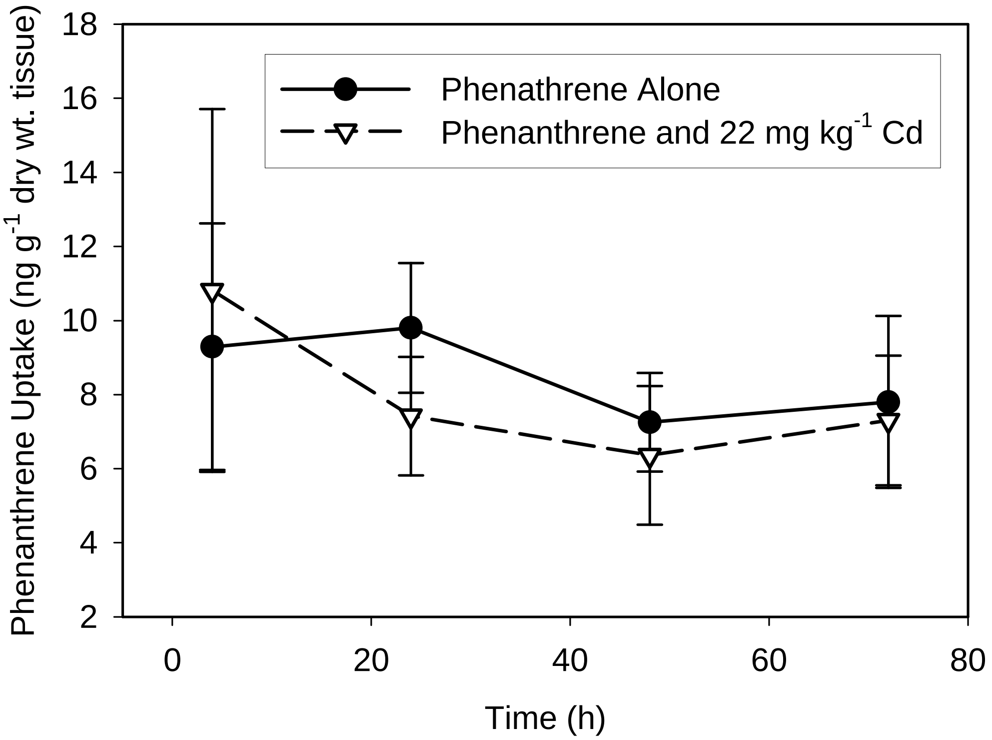


Figure 7. Effects of Cd on phenanthrene bioaccumulation rate in *Schizopera knabeni*. *S. knabeni* were exposed to 52.5 mg phenanthrene kg^{-1} dry sediment (measured concentration). One treatment included phenanthrene alone and the other contained both phenanthrene and 22 mg Cd kg^{-1} dry sediment (measured concentration). Symbols represent treatment means and error bars represent standard deviation (n = 4).

Sublethal, Grazing Rate Experiment

Grazing rates expressed as the number of algal cells ingested per individual copepod during 4 h, were significantly decreased by sediment-associated metals and phenanthrene (Figure 10). The mixture of metals at concentrations higher than 100 mg kg^{-1} dry sediment significantly decreased grazing rates compared to controls ($p < 0.05$). The grazing rate at the lowest metals concentration (100 mg kg^{-1} dry sediment) was not significantly different from controls ($p = 0.74$). Grazing at highest concentrations of metals (300 and 400 mg kg^{-1} dry sediment) was totally suppressed. Phenanthrene alone (110 mg kg^{-1} dry sediment) also caused a significant decrease in grazing rate ($p < 0.05$). The mixture of phenanthrene and metals caused a significant decrease in grazing rate compared to controls ($p < 0.0001$), especially at the highest concentrations of metals (200-400 mg kg^{-1} dry sediment). Grazing rate of *Schizopera knabeni* exposed to phenanthrene combined with the metals mixture did not differ from grazing rate of copepods exposed to phenanthrene alone ($p > 0.05$).

Cd alone and Cd combined with phenanthrene significantly decreased grazing rates of *Schizopera knabeni* ($p < 0.05$) at concentrations above 50 mg kg^{-1} dry sediment (Figure 10). Grazing rate at 50 mg Cd kg^{-1} dry sediment was not significantly different from control ($p = 0.09$), but the mixture of 50 mg Cd kg^{-1} dry sediment with phenanthrene was significantly different from control ($p < 0.0003$). Phenanthrene alone also caused a significant decrease in grazing rate relative to control ($p < 0.0002$). Grazing rates in phenanthrene alone did not differ from those in phenanthrene combined with Cd.

DISCUSSION

Our results suggest that benthic copepods respond synergistically to lethal mixtures of metals and PAH. For example in *Schizopera knabeni*, a mixture of Cd, Hg and Pb was synergistic when combined with phenanthrene in sediment; the combination was 1.5 x more lethal than separate exposures. Overall, synergisms were identified in both copepod species tested with various combinations of two PAH and three metals. Synergistic lethal effects among metals and between metals and organic contaminants have been identified previously in research with harpacticoid copepods (Barnes and Stanburry 1948; Forget et al. 1999; Hagopian-Schlekat et al. 2001), suggesting that synergisms may be common in this taxon. Harpacticoids are known to be sensitive to contamination in single-contaminant exposures in the laboratory (Coull and Chandler 1992) and from field studies of contaminant mixtures (Kovatch et al. 2000; Bejarano et al. 2004; Millward et al. 2004).

To better understand the nature of the metals mixture-phenanthrene synergism in *Schizopera knabeni*, the lethal effects of phenanthrene were measured separately in binary combination with Cd, Pb, and Hg to test the null hypothesis that the strength of the interaction between each metal and phenanthrene is equivalent. The addition of a sublethal concentration of Pb, Hg, and Cd all significantly increased the toxicity of phenanthrene. However, Cd-phenanthrene mixtures were the most strongly synergistic (2.8 x more synergistic together than in individual exposures). Also, both phenanthrene and fluoranthene elicited a synergistic interaction with Cd in *Amphiascoides atopus* suggesting that Cd-PAH interactions may occur with many individual PAH. Gust (2005a) reviewed about 30 studies that examined metal-PAH interactions in aquatic biota. The majority provided evidence for synergistic toxicology, although antagonisms were also common. Response addition and concentration addition

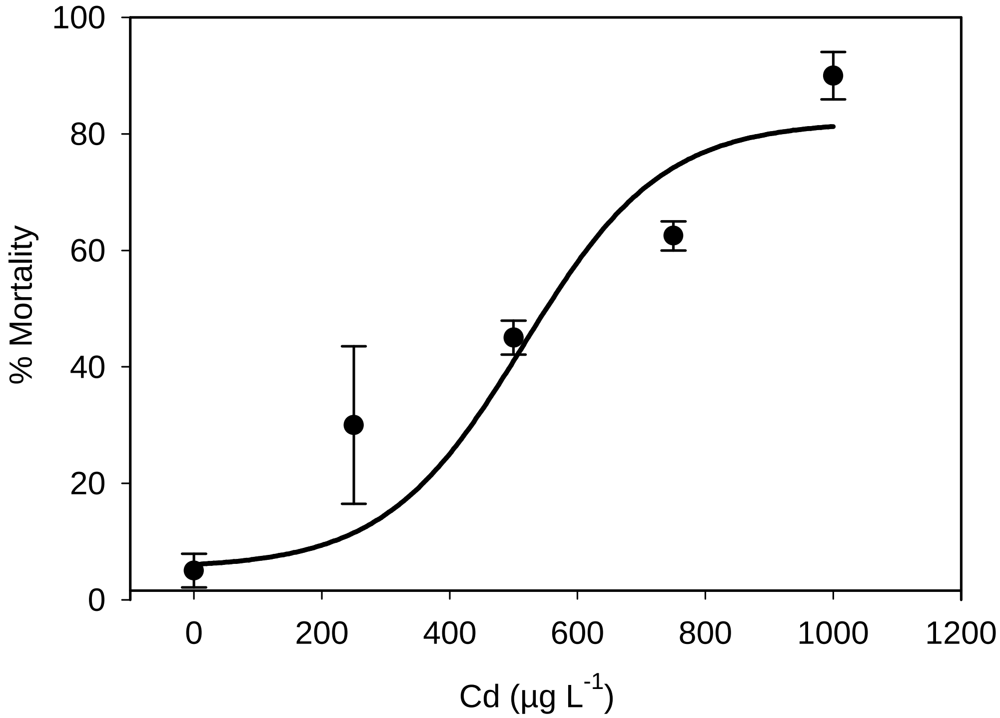


Figure 8. Concentration response of *Amphiascoides atopus* to Cd in water only experiments.

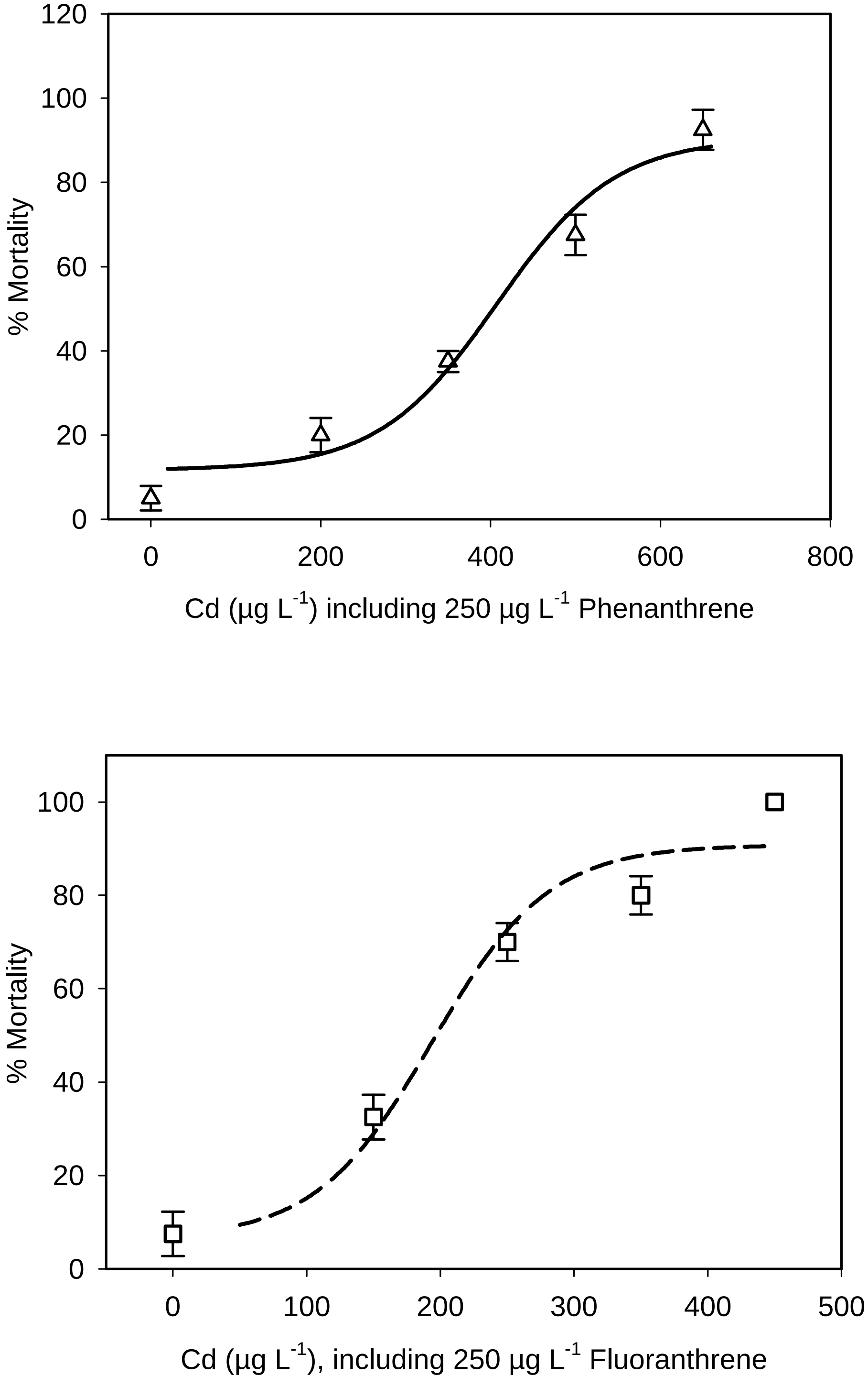


Figure 9. Metal-phenanthrene mixture experiments with *Amphiascoides atopus* in water-only experiments. Upper figure is exposures with 250 µg L^{-1} phenanthrene; lower figure, 250 µg L^{-1} fluoranthene.

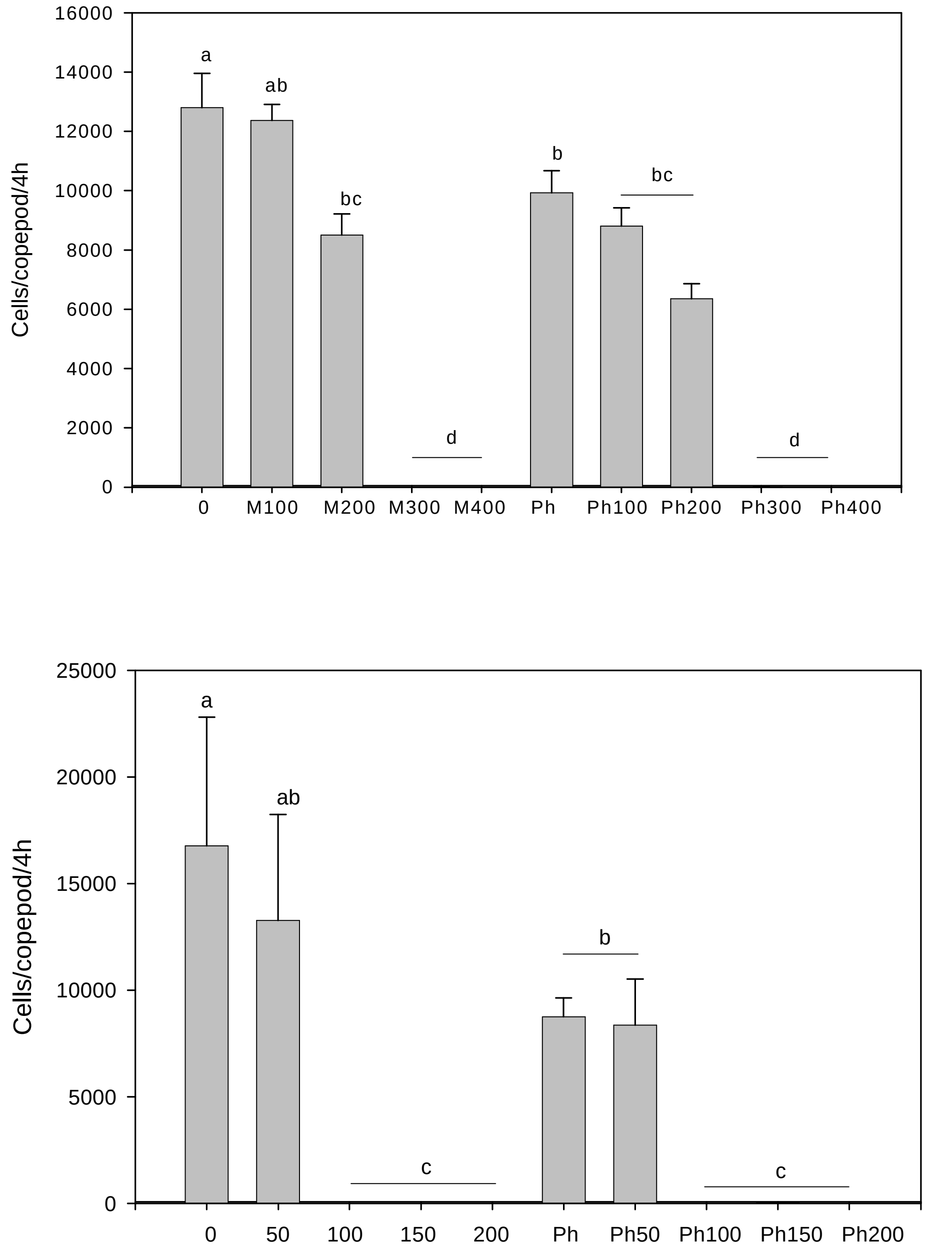


Figure 10. Grazing rates of *Schizopera knabeni* in phenanthrene- and metals-contaminated sediment. Error bars are ± 1 SD (n = 4). Different letters indicates significant difference between treatments (alpha = 0.05). Upper figure is phenanthrene and a metals mixture (0 = Control, M100 through M400 indicates concentration of metals, Ph = phenanthrene alone, Ph 00 through Ph400 indicates phenanthrene combined with metals). Lower Figure is phenanthrene with Cd (0 = Control, 50 through 200 indicates concentration of Cd (ppm), Ph = phenanthrene alone, Ph50 through Ph200 indicates phenanthrene combined with Cd)

were relatively rare. These observations suggest that nonadditive interactions between a variety of metals and PAH occur frequently in aquatic animals (at least in the few studies conducted in binary tests), and that the combination of Cd with PAH may be especially prone to synergistic interactions.

Cadmium proved to be much less toxic to *Schizopera knabeni* in the presence of Hg and Pb. The cause of an antagonism between Cd, Pb, and Hg in *S. knabeni* was not investigated in our studies but could be due to competition for biotic ligands among metal ions. Our concentration-response data suggest that Pb and Hg are less toxic than Cd to *S. knabeni.* The toxic effect of Cd may, therefore, be reduced if Pb and Hg compete with Cd for active sites during or after bioaccumulation. Furthermore, the strength of the Cd-phenanthrene synergism was reduced in mixtures with Pb and Hg, and this appears to be the first report of an antagonism among metals that reduces the strength of a metal-PAH synergism. Although complex mixtures with many contaminants (e.g., >10) in diverse chemical classes have rarely been examined in aquatic organisms, results provide little support for synergistic or antagonistic behavior (Broderius and Kahl 1985; Hermens et al. 1985; Deneer et al. 1988; Altenburger et al. 2004; de Zwart and Posthuma 2005). Studies of binary interactions among contaminants are also infrequent (Pape-Lindstrom and Lydy 1997), but, on the other hand, commonly report strong nonadditive interactions (Pape-Lindstrom and Lydy 1997; Steevens and Benson 1999; Norwood et al. 2003; Jonker et al. 2004; Gust 2005b; Lydy and Austin 2005). The general observation that increasing contaminant complexity reduces the strength of contaminant nonadditive interactions has been noted on several occasions (McCarty and Mackay 1993; Warne and Hawker 1995; de Zwart and Posthuma 2005), although few studies have been conducted with toxicants with different modes of toxic action. Moreover, mechanistic explanations of this phenomenon usually require that toxicants have the same mode of toxic action (Warne and Hawker 1995). However, de Zwart and Postuma (2005) recently proposed that mixtures of toxicants with different modes may express a greater incidence of concentration addition if toxicants have baseline toxic effects (non-specific components) in addition to effects associated with a specific mode of toxic action. Additive effects will be more likely to occur in mixtures in which contaminants are in low concentration (below the threshold concentration at which specific toxic action may occur) such as in complex mixtures at equi-toxic concentrations because they may contribute to the non-specific mode of toxicity. This idea has merit, however the paucity of tests of mixtures of metals and PAH make this theory difficult to test.

A strong Cd-phenanthrene synergism in *Schizopera knabeni* was observed in both water-only and sediment exposures. Cadmium-phenanthrene and Cd-fluoranthene synergisms were also found during aqueous exposures in *Amphiascoides atopus*. Furthermore, our toxicokinetic measurements suggest that the presence of a sublethal concentration of Cd in sediment had no effect on the uptake rate of phenanthrene in *S. knabeni*. Other investigations (i.e., George and Young 1986; Brüschweiler et al. 1996) have found that metals interfere with PAH breakdown and that PAH may cause toxicity as body burdens increase in vertebrates. Of course it is possible that phenanthrene affects the biodynamics, i.e., uptake, excretion or sequestration rate, of Cd in *S. knabeni*, however this effect could not be studied because of the small mass of copepods. Taken together, our results suggest that the observed Cd-phenanthrene synergism in benthic copepods is not due to exposure associated with contaminant uptake from sediment, but is associated with a pharmacological interaction expressed after bioaccumulation. Little is known about the cellular basis of joint Cd-PAH toxicity, however effects on enzyme systems associated with respiration in animals and plants and photosynthesis in plants have been implicated in work with Cu-PAH interactions (Babu et al. 2001).

Gust and Fleeger (2005), however, concluded that an observed synergism between Cd and phenanthrene in the freshwater amphipod *Hyalella azteca* was related to sediment exposure; experiments at similar concentrations and contaminant ratios indicated response-additive toxicity in aqueous conditions but synergistic toxicity in sediments. Benthic copepods are much smaller in body mass than amphipods, and tissue burdens reach equilibrium in hours (Lotufo 1998). Furthermore, a significant fraction of contaminant body burden in harpacticoids is probably accumulated from overlying or pore water because harpacticoids are not bulk deposit feeders (Green et al. 1993). Thus, copepods may be less sensitive to exposure-related effects than the larger amphipods that may ingest whole-sediment particles and take days for body burdens to reach equilibrium (Gust and Fleeger 2005). Only additional research will determine if "apparent" (exposure-related) interactions occur frequently in sediment exposures and if ecological or taxonomic patterns can be discerned (e.g., apparent interactions may be most common in bulk deposit feeders).

A mixture of Cd, Hg, and Pb, as well as Cd alone, significantly reduced grazing rates of *Schizopera knabeni* feeding on labeled microalgae. Feeding ceased above 300 mg kg^{-1} dry sediment of the metal mixture, well below the estimated LC$_{50}$ of 1462 mg kg^{-1} dry sediment. Feeding strategies in meiofauna have been related to different toxicological responses to metal-contaminated sediment (Millward et al. 2001a). The mode of feeding of *S. knabeni* probably consists of selective deposit feeding (Lotufo 1997), as has been found in similar species. Selective deposit feeders select fine, organically enriched particles that adsorb a major fraction of available metals due to a high surface area (Selck et al. 1999). Phenanthrene alone also caused a decrease in *S. knabeni* grazing rate. It has been shown that PAH cause decreases in feeding rate in many aquatic animals irrespective of feeding mode (Fleeger et al. 2003). However, the joint effects of metals (either in a mixture or as Cd alone) and phenanthrene were not found to be synergistic but are probably best described as independent (response-addition) in *S. knabeni*. Similarly, Gust and Fleeger (2006) found that joint exposures to Cd and phenanthrene had independent effects on ingestion rate even though a strong antagonistic lethal interaction occurred in the tubificid oligochaete *Ilyodrilus templetoni*. The absence of interactive effects on feeding suggests that metal-PAH interactive effects on lethality have a different underlying mechanism and that reductions in grazing probably did not directly contribute to the lethality effect in *S. knabeni*. However, the severe reduction in grazing in the presence of metals or phenanthrene suggests this sublethal effect may strongly impact populations in field settings.

Hazard assessment of contaminated sediments is based on estimates such as ERM (effects range mid, Hyland et al. 2003) that predict the likelihood of adverse effects to populations at specific levels of contamination. The ERM for Cd (9.6 mg kg^{-1} dry sediment) is well below our estimated LC$_{50}$ value of 230 mg kg^{-1} dry sediment, suggesting this sediment quality criterion is protective of adult *Schizopera knabeni*. Similarly, the ERM for phenanthrene (1.5 mg kg^{-1} dry sediment) is also well below the LC$_{50}$ of 426 mg kg^{-1} dry sediment and would certainly be expected to be protective of adult *S. knabeni*. However, there are no guidelines for protection in Cd-phenanthrene mixtures when a synergism is indicated. In the present study, the TU$_{50}$ of phenanthrene and Cd mixture was 162 mg kg^{-1} dry sediment; equi-toxic equivalents are 101 mg kg^{-1} dry sediment phenanthrene and 61 mg kg^{-1} dry sediment for Cd given the exposure ratio used. Assuming that 10% of an TU$_{50}$ could serve as a protective standard, equi-toxic sediment concentrations as low as 10 mg kg^{-1} dry sediment for phenanthrene and 6 mg kg^{-1} dry sediment for Cd would be expected to cause lethal or sublethal effects in *S. knabeni*. Therefore, the established ERM for phenanthrene would be protective in a equi-toxic mixture with Cd, but the ERM for Cd may not be protective for adult *S. knabeni* in an equi-toxic mixture with phenanthrene. However, adult *S. knabeni* are very tolerant to phenanthrene compared to its other life history stages; significant effects of phenanthrene on reproductive output were detected at concentrations as low as 22 mg kg^{-1} dry sediment (Lotufo and Fleeger, 1997). Therefore, if Cd-phenanthrene combinations exert a strong synergism on reproduction in *S. knabeni*, effects would be expected to occur at much lower concentrations than for adult lethality. Additional research is needed to determine the interactive effects of Cd and phenanthrene on reproduction in *S. knabeni*.

CONCLUSIONS AND RECOMMENDATIONS

Due to human activity, marine and freshwater sediments are sometimes contaminated with complex mixtures of many individual chemical compounds, in the same or different chemical classes, that threaten environmental health by causing toxicity to sediment-dwelling organisms. Although relatively rare based on present knowledge, the effects of individual compounds on biota sometimes differ in mixtures with other compounds. Very few studies have examined the joint effects of metals and polynuclear aromatic hydrocarbons in toxicity tests, but recent results suggest that interactions among these chemicals may be more common than previously thought. Such concentration-response tests are important because standards for sediment quality are based on their results. Tests allow investigators to estimate risk associated with contaminated sediments, to identify chemicals responsible for effects, and to make appropriate decisions for actions such as remediation. In the absence of specific tests on model organisms, methods that estimate risk make assumptions of the toxicity of classes of compounds when found in mixtures. Our studies suggest that the lethal effects of metals and aromatic hydrocarbons, compounds found jointly around oil platforms, differ from their effects in isolation in such a way that they are more toxic in mixtures. These results differ from the assumptions made in risk analysis. The pharmacological basis of this toxic interaction for lethality in our sediment-dwelling test organisms is unknown; however, sublethal tests suggest that a mixture of metals and aromatic hydrocarbons does not interact to cause effects on food ingestion. The joint effects of the metal cadmium and the hydrocarbon phenanthrene seem particularly toxic, and standards of sediment quality may not be protective when these compounds co-occur.

Researchers are actively exploring the use of concentration-response curves to improve models that predict contaminant low- or no-effects concentrations in sediments (*e.g.*, Scholze et al. 2001). Research that establishes theoretical techniques by which nonadditive toxicological behavior may be incorporated into the estimation of effects criteria is in its infancy, and is greatly hampered by a lack of critical information regarding effects in mixtures (de Zwart and Posthuma 2005). Even if advanced methods were established, they could not be applied to metal-PAH interactions because few studies estimate sediment concentrations at which interactions occur. Data from laboratory experiments are needed to anticipate the frequency of interactions at contaminated sites and, if they are common, to improve predictions based on minimum-effects criteria and resulting hazard assessment. Therefore, we feel that additional research is justified to determine the extent, cause and significance of metal-PAH interactions in benthic populations and communities. de Zwart and Posthuma (2005) suggest methods by multispecies responses to toxicant mixtures may be investigated, and that this research is necessary to improve sediment-quality criteria.

Our research suggests that binary metal-PAH synergisms are common in meiobenthic copepods, and a literature search suggests that synergisms and antagonisms may be generally common in aquatic invertebrates. The cause of metal-PAH synergisms among benthic invertebrates may be diverse; some species express apparent contaminant interactions as a function of exposure associated with uptake from sediment while others express true pharmacological interactions. Furthermore, the strength of an observed Cd-phenanthrene synergism in a species studied intensely (*Schizopera knabeni*) suggests that established sediment quality criteria may not be protective in binary contaminant mixtures. The contaminants used in our studies (PAH, Cd, Pb and Hg) co-occur in sediments at oil production sites at concentrations that sometimes exceed those that may cause effects in our test species. Therefore, synergisms (if as strong as found in *S. knabeni*) could contribute to reductions in abundance of selected taxa observed near oil platforms.

LITERATURE CITED

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The Department of the Interior Mission

As the Nation's principal conservation agency, the Department of the Interior has responsibility for most of our nationally owned public lands and natural resources. This includes fostering sound use of our land and water resources; protecting our fish, wildlife, and biological diversity; preserving the environmental and cultural values of our national parks and historical places; and providing for the enjoyment of life through outdoor recreation. The Department assesses our energy and mineral resources and works to ensure that their development is in the best interests of all our people by encouraging stewardship and citizen participation in their care. The Department also has a major responsibility for American Indian reservation communities and for people who live in island territories under U.S. administration.

The Minerals Management Service Mission

As a bureau of the Department of the Interior, the Minerals Management Service's (MMS) primary responsibilities are to manage the mineral resources located on the Nation's Outer Continental Shelf (OCS), collect revenue from the Federal OCS and onshore Federal and Indian lands, and distribute those revenues.

Moreover, in working to meet its responsibilities, the **Offshore Minerals Management Program** administers the OCS competitive leasing program and oversees the safe and environmentally sound exploration and production of our Nation's offshore natural gas, oil and other mineral resources. The MMS **Minerals Revenue Management** meets its responsibilities by ensuring the efficient, timely and accurate collection and disbursement of revenue from mineral leasing and production due to Indian tribes and allottees, States and the U.S. Treasury.

The MMS strives to fulfill its responsibilities through the general guiding principles of: (1) being responsive to the public's concerns and interests by maintaining a dialogue with all potentially affected parties and (2) carrying out its programs with an emphasis on working to enhance the quality of life for all Americans by lending MMS assistance and expertise to economic development and environmental protection.