



ELSEVIER

Journal of Experimental Marine Biology and Ecology 321 (2005) 43–57

**Journal of
EXPERIMENTAL
MARINE BIOLOGY
AND ECOLOGY**

www.elsevier.com/locate/jembe

Influence of natural dissolved organic matter (DOM) on acute and chronic toxicity of the pesticides chlorothalonil, chlorpyrifos and fipronil on the meiobenthic estuarine copepod *Amphiascus tenuiremis*

Adriana C. Bejarano*, G. Thomas Chandler, Alan W. Decho

Department of Environmental Health Sciences, Arnold School of Public Health, University of South Carolina, Columbia, SC 29208, USA

Received 16 August 2004; received in revised form 10 October 2004; accepted 4 January 2005

Abstract

Dissolved organic matter (DOM) represents an important carbon phase in coastal environments and influences the partitioning of organic contaminants. In this study, we evaluated the role of salt-marsh sediment-derived DOM vs. DOM-free seawater on the acute and chronic toxicity of three pesticides (chlorothalonil—CHTH, chlorpyrifos—CHPY and fipronil—FIP) to the meiobenthic copepod *Amphiascus tenuiremis*. Acute toxicity was evaluated via standard 96-h median lethal concentration (LC_{50}), while chronic toxicity was evaluated for 16 days using a 96-well microplate life-cycle bioassay. DOM significantly reduced ($p < 0.05$) acute toxicity of CHTH and CHPY to male and female copepods relative to copepods exposed in DOM-free seawater. In contrast, DOM elevated the acute toxicity of FIP to male copepods. In chronic exposures with/without DOM, CHTH and CHPY did not significantly affect copepodite-to-adult development. In these treatments, plus controls, the majority (95%) of copepodites developed into adults by 8 days. Individuals exposed to FIP in the presence of DOM showed a slower development rate than FIP individuals in DOM-free seawater. Overall, FIP exposed copepodites developed into adult copepods 4 days later than controls. CHTH, CHPY and FIP significantly reduced reproductive success by 33%, 31% and 89%, respectively. DOM, however, mitigated 30% ($p = 0.006$) and 20% ($p = 0.05$) of the reproductive failure attributed to CHTH and CHPY. FIP-induced reproductive failure was high (~80%) in all exposures regardless of DOM presence/absence. An exponential growth model predicted that exposure to CHTH, CHPY and FIP in the absence of DOM significantly reduced ($\geq 38\%$) projected naupliar production relative to DOM-free controls. In the presence of DOM, naupliar production under CHTH and CHPY exposures was not significantly different ($p > 0.05$) from control projections. These results indicate that DOM generally reduced the acute and chronic toxicity of CHTH and CHPY to *A. tenuiremis*, while certain compound-specific pesticide (FIP): organic associations may enhance acute toxicity of FIP, particularly to *male* copepods.

© 2005 Elsevier B.V. All rights reserved.

Keywords: Dissolved organic matter; Estuarine copepods; Pesticide toxicity

* Corresponding author. Tel.: +1 803 7776452; fax: +1 803 7773391.

E-mail address: ACBejara@mailbox.sc.edu (A.C. Bejarano).

1. Introduction

Estuarine salt marshes are of particular ecological and biological importance because they provide feeding and nursery grounds for economically valuable fish and shellfish, as well as for a wide variety of macro- and meiofauna (Mitsch and Gosselink, 2000). Estuarine salt marshes are among the most productive ecosystems on earth and mediate a dynamic balance of nutrients and organic matter (Mitsch and Gosselink, 2000). These ecosystems are rich in dissolved organic matter (DOM) (Dafner and Wangersky, 2002), which may serve as a trap for anthropogenic non-polar organics such as pesticides.

The nature of DOM in aquatic environments plays an important role in determining the partitioning of organic contaminants. DOM mitigates the effects of hydrophobic organic contaminants (Landrum et al., 1987; Servos et al., 1989) by reducing their free-concentration in solution, potentially reducing contaminant uptake and bioavailability to aquatic organisms. For instance, DOM reduced the bioavailability of the hydrophobic contaminants benzo[*a*]pyrene (BaP) and 3,3',4,4'-tetrachlorobiphenyl (TCB; Akkanen and Kukkonen, 2003) to the water flea *Daphnia magna*. In DOM-free artificial freshwater, the 24-h bioconcentration factors (BCF) of BaP and TCB in *D. magna* were 18 and 3 times higher, respectively, than in undiluted natural freshwater containing 20 mg DOC/L (Akkanen and Kukkonen, 2003). Similarly, the presence of natural pore-water DOM filtrates and fulvic acids reduced the bioconcentration of chlorpyrifos to the juvenile bivalve *Mercenaria mercenaria*, relative to DOM-free seawater (Bejarano et al., 2005). Consequently, the presence of DOM likely reduces the bioavailability and bioconcentration of hydrophobic contaminants such as pesticides, resulting in a potential attenuation of toxicological effects.

DOM effects on bioavailability and toxicity may depend in part on compound-specific physicochemical properties such as chemical hydrophobicity. Properties such as the *n*-octanol–water partition coefficient (K_{OW}) are important in predicting the environmental fate of organic compounds (Wania and Mackay, 1999). The K_{OW} measures the tendency of a chemical to partition between an environmental/physiological non-polar organic phase (i.e., *n*-octanol used as a surrogate for lipids) and an aqueous phase

(Schwarzenbach et al., 1993). Chemicals with high K_{OW} values (>1000) tend to have low water solubility, thus high bioconcentration potential in aquatic organisms (Schwarzenbach et al., 1993; Wania and Mackay, 1999). This environmentally relevant partition coefficient has been used in models predicting the distribution of organic compounds in environmental compartments (Wania and Mackay, 1999; Mackay, 1991). Further, the K_{OW} is used as one of the molecular descriptors of the toxic effects of chemicals in quantitative Structure Activity Relationships (QSAR) models (Niculescu et al., 1998; Van Leeuwen et al., 1992). Fisher et al. (1993) found a significant correlation between $\log K_{OW}$ and acute sediment toxicity of 12 insecticides (i.e., organophosphorous and carbamate insecticides) to the midge *Chironomus riparius*. Similarly, Saito et al. (1993) found a significant correlation between the $\log K_{OW}$ of 101 chemicals (i.e., alcohols, aromatics, phenols and pesticides) and the acute midpoint cytotoxicity to goldfish scale cells. Thus, pesticides with increasing K_{OW} values will be expected to be more acutely toxic to exposed aquatic organisms.

In the present study, we focused on the toxicological effects of three pesticides (chlorothalonil—CHTH, chlorpyrifos—CHPY and fipronil—FIP) with varying modes of actions, physicochemical properties (i.e., K_{OW}) and affinities for carbon/lipid on an estuarine meiobenthic copepod *Amphiascus tenuiremis* cf. Mielke. Chlorothalonil (2,4,5,6-tetrachloroisophalonitrile, CHTH) is a widely applied organochlorine non-systemic fungicide. CHTH inhibits important cellular enzymes (i.e., NADPH oxidase) by binding to the sulfhydryl groups altering metabolic functions in cellular respiration (Caux et al., 1996). This pesticide also depletes glutathione (GSH), a non-enzymatic reducing agent involved in cell detoxification (Tillman et al., 1973). Studies with estuarine and marine organisms showed a high acute toxicity to sheep head minnow (96-h LC_{50} =32 μ g/L), pink shrimp (96-h LC_{50} =154 μ g/L) and eastern oyster (96-h ED_{50} =3.6 μ g/L) (USEPA, 1999). CHTH is highly toxic to the copepod *A. tenuiremis*, with increasing toxicity to females (96-h LC_{50} =86.05 μ g/L, 95% CI=74.45–100.17 μ g/L), males (96-h LC_{50} =38.13 μ g/L, 95% CI=28.75–47.66 μ g/L) and stage-I copepodites (96-h LC_{50} =24.34 μ g/L, 95% CI=20.99–27.39 μ g/L) (Bejarano, A.C., unpublished). CHTH has an estimated \log

K_{OW} ranging from 3.05 (Krawchuk and Webster, 1987) to 3.66 (Estimation Program Interface Suite™, EPASUIT, version 3.11, USEPA, Washington, DC, USA).

Chlorpyrifos (*O,O*-diethyl-*O*-[3,5,6-trichloro-2-pyridyl]-phosphorothioate, CHPY) is an organophosphorus insecticide that inhibits acetylcholinesterase (AChE), an enzyme responsible for ACh catalysis at nerve synapses. CHPY is known to pose acute and chronic risks to many non-target aquatic wildlife (Odenkirchen and Eisler, 1988), especially estuarine species. CHPY use for household insect control is currently being eliminated (USEPA, 2000) in favor of FIP. CHPY is highly toxic to the copepod *A. tenuiremis* (96-h LC_{50} =66 µg CHPY/kg sediment) with a greater sensitivity in early (i.e., nauplius) life stages (96-h LC_{50} =40 µg CHPY/kg sediment) (Green et al., 1996). CHPY has an estimated log K_{OW} ranging from 4.66 (Estimation Program Interface Suite™, EPASUIT, version 3.11, USEPA, Washington, DC, USA) to 4.96 (PhysPro Database, Syracuse Research Corp., Syracuse, NJ, USA).

The new phenylpyrazole insecticide fipronil (5-amino-[2,6-dichloro-4-(trifluoromethyl) phenyl]-4-[(1*R,S*)-(trifluoromethyl) sulfinyl]-1*H*-pyrazole-3-carbonitrile, FIP) is a potent insecticide that blocks the flow of chloride ions in neurons by acting at the γ -aminobutyric acid (GABA)-gated chloride channel (Hainzl et al., 1998). Although FIP is effective against a variety of target species, there are concerns regarding its potential effects on non-target species (USEPA, 1996). FIP is acutely toxic to a wide range of estuarine and marine invertebrates such as oysters (96-h EC_{50} =770 µg/L), mysids (96-h EC_{50} =0.14 µg/L) and sheep head minnow (96-h EC_{50} =130 µg/L) (USEPA, 1996). A recent study found that FIP is acutely toxic to male (96-h LC_{50} =3.5 µg/L) and female (96-h LC_{50} =13.0 µg/L) *A. tenuiremis* (Chandler et al., 2004). Furthermore, FIP at low concentrations (0.22 µg/L) caused significant developmental delays to sexual maturity and reproductive failure (Chandler et al., 2004). FIP has an estimated log K_{OW} ranging from 4.0 (The Merck Index, 2001) to 6.63 (Estimation Program Interface Suite™, EPASUIT, version 3.11, USEPA, Washington, DC, USA).

Hence, the purpose of this study was to determine how the presence of relatively low salt-marsh sediment-derived DOM concentrations influence acute

(i.e., survival) and chronic (i.e., developmental and reproductive effects) toxicities, and potential population level effects of these three common pesticides on *A. tenuiremis*.

2. Materials and methods

2.1. Test organisms

The marine meiobenthic copepod *A. tenuiremis* inhabits muddy inter- and subtidal estuarine sediments and is distributed from the Baltic Sea to the southern Gulf of Mexico (Lang, 1948). *A. tenuiremis* has a generation time of 21 days (egg-to-egg) at 20 °C in sediment (Chandler and Green, 1996), and a life cycle consisting of clearly distinguishable naupliar and copepodite stages, and sexually dimorphic adults. In microplate cultures, gravid females produce multiple clutches (5–6) extruded as dual egg sacs each with up to 12 embryos distributed in a planar arrangement (Bejarano and Chandler, 2003). This copepod has been mono-specifically cultured in contaminant-free muddy flow-through microcosms at salinity $30 \pm 0.5\text{‰}$, pH 8 ± 0.3 , 23 ± 2 °C, and has been used in acute and chronic toxicity testing of a wide variety of pesticides (Bejarano and Chandler, 2003; Green et al., 1996; Klosterhaus et al., 2003; Strawbridge et al., 1992).

2.2. Dissolved organic matter preparation

Estuarine sediments were collected from a pristine *Spartina* salt-marsh estuarine reserve in North Inlet, SC, USA and washed/sorted to a <63-µm particle size (Chandler and Green, 1996). Salt-marsh sediment derived DOM was extracted by homogenizing these sediments in a blender with artificial seawater (30‰; Instant Ocean®) and placing the resulting sediment slurry in a refrigerator (4 °C) for 2 h. Overlying water was decanted without disturbing the sediments, and sediments were then centrifuged for 5 min at 4700 rpm. The DOM-rich supernatant was collected into a clean beaker, filtered (0.45 µm), gently aerated overnight and filtered again (0.45 µm) prior to use. Water aliquots were analyzed in triplicates for total organic carbon (TOC) concentration by combustion method using a TOC analyzer (TOC-5000A; Shimadzu

Scientific Instruments, Norcross, GA, USA). The measured TOC concentration of final salt-marsh sediment DOM was 11.57 ± 0.18 mg/L, with little variation across extractions (<5%).

2.3. Target pesticides

In this study, we used three pesticides (chlorothalonil—CHTH, chlorpyrifos—CHPY and fipronil—FIP) with distinctive physical and toxicological properties covering a wide range of K_{OW} values. Pesticides were purchased from Chem Service ($98 \pm 0.5\%$ purity; West Chester, PA, USA), with concentrated stocks made in 100% grade-acetone and kept in the dark at -20 °C. Test solutions were made by adding μ L amounts of pesticide stocks to 100 mL of filtered (0.45 μ m) and aerated (>90% O_2 saturation) seawater (30‰), or seawater containing salt-marsh sediment DOM. Control treatments (pesticide-free seawater) received the highest acetone volume added to any pesticide containing solution (0.03% by volume). Spiked solutions were homogenized in the dark for 1 h at room temperature (20 °C) and under constant stirring using Teflon®-coated magnetic stirring bars.

2.4. Acute and chronic pesticide toxicity

All acute and chronic experiments were performed using the same source of DOM-free seawater and seawater containing salt-marsh sediment DOM (11.57 ± 0.18 mg/L, TOC). Standard aqueous 96-h median lethal concentration (LC_{50}) toxicity tests using cultured adult female and male *A. tenuiremis* were performed following general ASTM guidelines (ASTM, 1988). Briefly, 30 mL of well-mixed pesticide-spiked or acetone-carrier control solutions were added in quadruplicate to acetone-rinsed test chambers (50-mL crystallizing dishes). To assess the DOM effects on pesticide acute toxicity, exactly 20 male and 20 non-gravid female copepods were transferred into each chamber, and incubated static at 20 ± 1 °C for 96-h under 12:12 light/dark conditions. At the end of the 96-h exposure period, surviving male and female copepods were counted in each chamber. Water chemistry (i.e., DO, salinity and pH) was recorded prior and post-exposure. Experimental design for tests with each of these pesticides included spiked DOM-

free seawater or seawater containing salt-marsh sediment DOM solutions with 5 concentrations and a carrier control per pesticide, with 4 replicates each.

Upon aqueous 96-h LC_{50} determination and to assess DOM effect on chronic pesticide toxicity, 96-well microplate life cycle bioassays were performed (after ASTM, 2004; Bejarano and Chandler, 2003). Briefly, stage-I juvenile copepodites (C-I) were gently collected from stock cultures, sorted out and placed individually into microwells (i.e., ultra-low attachment polystyrene 96-well microplates; Corning Costar, NY, USA) containing 200 μ L of test solution. C-Is were reared to sexual maturity, and virgin male and female copepods removed from wells and mated pair-wise in new wells containing original treatments. Test solutions were replaced (>90% water replacement) every third day with fresh test solutions (>90% DO), and individuals fed every 6 days with 3 μ L of a fresh 1:1 concentrated (10^7 cells/mL) phytoplankton mixture of *Isochrysis galbana* and *Dunaliella tertiolecta*. Covered microplates were held in humidified chambers in an incubator (Cryo-fridge™, Baxter, Thousand Oaks, CA, USA) at 25 ± 1 °C and 12:12 light/dark conditions. The chronic exposure lasted 16 days with individuals followed from C-I juvenile copepodite stage (1–2 days post-nauplius molting into copepodites) to sexual reproduction through female extrusion of the second brood. Each C-I was monitored daily through copepodite development via inverted stereomicroscopes. Developmental endpoints included C-I survival to mature adult, days to successful sexual differentiation and sex ratios. Likewise, each mating pair was monitored during the mating period lasting up to 7 days post-mating to accommodate potential delays in reproduction. Reproductive endpoints included reproductive success/failure, first and second brood sizes and total viable offspring production. Reproductive success was defined as those mating pairs able to extrude viable embryos over the entire mating period (7 days). Experimental design for each of the pesticides included spiked DOM-free seawater or seawater containing salt-marsh sediment DOM solutions with one concentration and 64 starting C-I copepodites per pesticide distributed over 4 microplates. A single DOM-free seawater and seawater containing DOM solution was used as a carrier control for all pesticides, using the highest acetone volume used in chronic exposures (0.024% by volume). Target

pesticide concentration in microplate bioassays for each pesticide was the mean of DOM-free seawater and seawater containing salt-marsh sediment DOM male 96-h LC₁₅'s values. Male 96-h LC₁₅'s were used to ensure C-I survival to sexual maturity as previous observations have shown that C-I and adult male copepods have similar pesticide sensitivities (Bejarano, A.C., unpublished).

2.5. Stage-structured population growth model modeling

Multi-generational population-level effects of CHTH, CHPY and FIP in the presence and absence of DOM were estimated using empirical microplate life-cycle data fitted to a matriarchal stage-structured Lefkovich matrix model (RAMAS[®] EcoLab 2.0, Applied Biomathematics, Setauket, NY, USA) (Akçaya et al., 1999; Caswell, 2001; Lefkovich, 1965). A 5-stage (embryo to nauplius to copepodite to virgin-female to gravid-female) matrix model was used to project offspring production (e.g., nauplius) through 3 generations based on: (1) proportions of stage-specific survival, (2) proportion of copepodites developing into virgin females (i.e., female sex ratio), (3) proportion of females able to produce at least two viable broods and (4) fecundity (i.e., viable offspring production per female) through two broods.

Offspring production for each pesticide treatment and DOM condition was projected using the Lefkovich exponential growth model starting with 60 C-I individuals. This model assumes that fecundity and stage-specific survival rates are density independent and uses only the stage matrix for population size predictions. For deterministic purposes, stage-specific survival rates and fecundities are assumed to remain constant through time. Final projected offspring production from this matriarchal model was considered relative to controls rather than as absolute values. A total of 10 simulations were run for each microplate in each pesticide treatment and DOM condition.

2.6. Water chemistry

Sub-samples of test solutions and controls per pesticide were collected in triplicate (1.5 mL) prior to and after each acute 96-h exposure. Likewise, triplicate water samples (1.5 mL) were collected

during chronic exposures from fresh test solutions and controls prior to each water change ($n=7$ total water changes) and prior to solution addition to microplate wells. Also, water samples per treatment and controls collected during every water change were pooled over 8 wells (total volume ~1.6 mL) to quantify change in the pesticide concentration over each 3-day exposure period. Water sample aliquots were processed by liquid 2:1 extraction by collecting water sample aliquots into clean 20-mL amber vials containing 3.0 mL of methyl tertiary-butyl ether (MTBE HPLC-grade, Fisher Scientific, Pittsburgh, PA, USA). All samples were tested with 0.25 μ L 4-bromanisole/L of MTBE as an internal standard. Samples were vigorously vortexed for 1.5 min and sonicated at 25 °C for 5 min, following by transfer of MTBE-containing extractable analytes into 15-mm i.d. GC-amber vials. Vials were immediately crimped and stored at –70 °C for further analysis. The pesticide extracts were analyzed using a Hewlett Packard 5890 Series II gas chromatograph (GC) coupled to a ⁶³Ni electron capturer detector (GC-ECD) (Hewlett Packard, Palo Alto, CA, USA), operating in splitless flow mode. Injector and detector temperatures were 230 and 310 °C, respectively. The GC column oven was programmed with initial temperature of 100 °C (1-min hold) ramped to 270 °C at 10 °C/min and a 10-min hold. A fused silica column (30 m (L)×0.254 mm (i.d.)) coated with 0.25 μ m DB-5 MS (Agilent Technologies, Folsom, CA, USA) was used to separate target pesticide analytes. Under these conditions, retention time for CHTH, CHPY and FIP were 13.11 min, 15.03 min and 12.9, respectively.

2.7. Statistical analyses

For each pesticide (i.e., using measured concentrations), analysis and comparisons of sex-specific mortality dose response curves between DOM-free seawater and seawater containing salt-marsh sediment DOM solutions were performed using Generalized Linear Interactive Modeling, GLiM (Piegorisch and Bailer, 1997) fit via PROC GENMOD (SAS[®] Institute, Cary, NC, USA). GLiM uses generalizations of the normal-based linear models (i.e., ANOVA and linear regression) to account for non-normal responses (Piegorisch and Bailer, 1997). Simultaneously, Probit Analysis (PROC PROBIT, SAS) was used to estimate

sex-specific 96-h LC₅₀ values (Piegorsch and Bailer, 1997).

Within each pesticide treatment, copepodite-to-adult developmental curves between the DOM-free and salt-marsh sediment DOM solutions were compared as above using the GENMOD procedure (GENMOD, SAS). Additionally, contrasts were performed to determine differences in developmental curves between individuals exposed to pesticides and controls. All endpoints obtained from the microplate life cycle bioassay were tested for normality and homogeneity of variance using the Shapiro–Wilk “goodness of fit test” and Levene test, respectively. Variables failing normality were transformed accordingly. Within pesticides, differences in total viable offspring production (two consecutive broods) between DOM-free seawater and seawater containing salt-marsh sediment DOM solutions were determined by a one-way analysis of variance (PROC GLM, SAS) using the Tukey test for pair-wise comparisons. The two endpoints following a binomial distribution, sex ratios and reproductive success/failure, were analyzed using Fisher’s exact test (i.e., 2×2 contingency tables) and a Pearson’s chi-square goodness-

of-fit test (row by column (R×C) contingency tables) (Piegorsch and Bailer, 1997). Population-level effects of CHTH, CHPY and FIP in the presence and absence of DOM were analyzed using population projections from individual microplates per pesticide and DOM condition. All variance estimates were computed at the level of individual microplates. Data were logarithmically transformed (i.e., log₁₀(x+1)), and within DOM condition differences in projections across pesticide treatments and controls were determined by a one-way analysis of variance (PROC ANOVA, SAS). Within DOM condition, multiple comparisons between pesticide and control exposures were done by Dunnett’s procedure. All tests for significance were performed using an alpha level of 0.05 ($\alpha=0.05$).

3. Results

3.1. Acute pesticide exposures

Water chemistry conditions after the 96-h acute exposure were consistent across all pesticides and

Table 1

Nominal and measured pesticide concentrations ($\mu\text{g/L}$) in acute (96-h) and chronic (16-day) copepod toxicity tests in the presence and absence of salt-marsh sediment dissolved organic matter (DOM). Measured pesticide concentrations were obtained from triplicate water samples. CHTH=chlorothalonil, CHPY=chlorpyrifos and FIP=flupyrifluor. ND=non-detectable or below instrument detection limit

Pesticide	Acute (96-h LC ₅₀)			Chronic (microplate bioassay)		
	Nominal ($\mu\text{g/L}$)	Measured ($\mu\text{g/L}$)—seawater	Measured ($\mu\text{g/L}$)—DOM	Nominal ($\mu\text{g/L}$)	Measured ($\mu\text{g/L}$)—seawater	Measured ($\mu\text{g/L}$)—DOM
CHTH	Control	ND	ND	Control	ND	ND
	20	16.2±6.2	15.20±1.9	24	23.5±2.4	24.5±1.8
	40	40.51±5.89	32.60±2.89			
	70	63.51±4.63	58.47±5.46			
	120	115.16±5.58	93.59±5.6			
	200	204.62±15.65	188.29±9.47			
CHPY	Control	ND	ND	Control	ND	ND
	1	ND	ND	0.18	ND	ND
	2	ND	ND			
	5	5.31±2.8	5.79±0.72			
	10	10.97±0.81	8.64±1.39			
	20	20.45±1.72	18.96±1.62			
FIP	Control	ND	ND	Control	ND	ND
	4	2.11±0.12	2.71±0.28	1.4	1.41±0.4	1.42±0.22
	6	3.91±0.22	4.27±0.36			
	10	6.24±0.74	6.06±0.67			
	18	17.69±1.44	16.58±2.32			
	30	28.63±3.6	28.34±3.82			

DOM treatments (salinity= $30.14 \pm 0.35\%$, pH= 8.16 ± 0.07 , dissolved oxygen saturation (%) DO= 85.64 ± 9.08). Measured concentrations for chlorothalonil (CHTH) and chlorpyrifos (CHPY) were between 22% below and 10% above nominal concentrations, while for fipronil (FIP) between 2% and 47% below nominal (Table 1). The two lowest CHPY concen-

trations (1 and 2 $\mu\text{g/L}$, nominal) were below instrument detection limits (1 $\mu\text{g/L}$). Pesticide loss over the 96-h acute exposure was $\leq 15\%$ of initial concentrations.

Sex-specific mortality dose response curves for all pesticides and DOM treatments were analyzed using measured pesticide concentrations, except for CHPY. Nominal concentrations of CHPY were used when

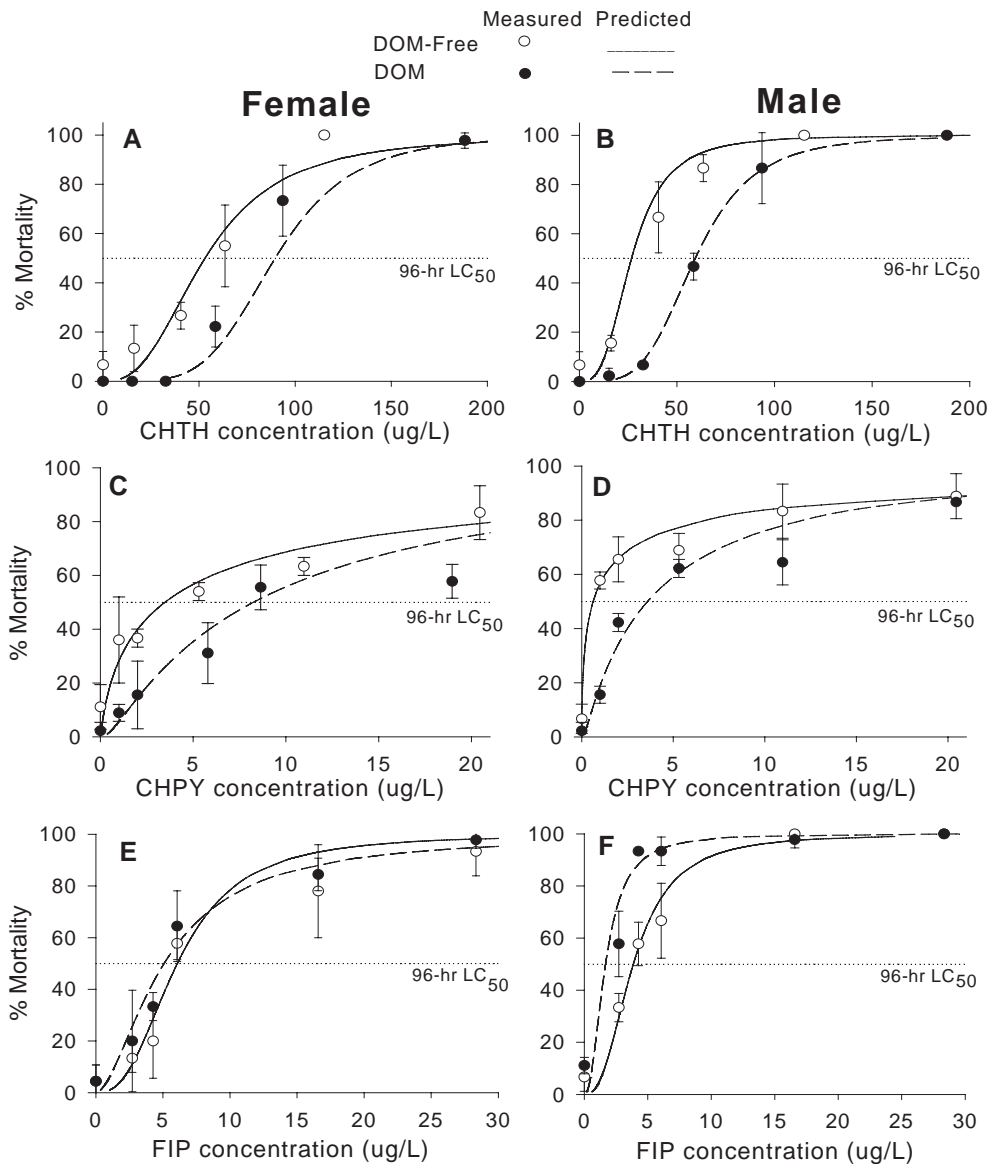


Fig. 1. Sex-specific mortality dose response curves for *Amphiascus tenuiremis* exposed to chlorothalonil (CHTH; A and B), chlorpyrifos (CHPY; C and D) and fipronil (FIP; E and F) in the presence and absence of salt-marsh sediment dissolved organic matter (DOM). Measured and predicted copepod mortality are represented by symbols and lines, respectively.

measured concentrations were below detection limits. All three pesticides were acutely toxic to *A. tenuiremis*. Male *A. tenuiremis* copepods were in all cases (i.e., pesticides and DOM treatments) more sensitive than females. Dose–mortality curves of copepods exposed to all three pesticides in the absence of DOM (DOM-free seawater) showed a very similar shape (i.e., slope and intercept) as copepods exposed to these pesticides in the presence of DOM ($p>0.05$). However, mortality responses significantly changed in the presence of DOM ($p<0.05$; Fig. 1). Both female and male copepods exposed to CHTH and CHPY in the absence of DOM showed a significant increase in acute pesticide toxicity (i.e., lower 96-h LC₅₀ values) compared to individuals exposed to these pesticides in the presence of salt-marsh sediment DOM (Fig. 1). Female and male copepods exposed to CHTH in the DOM-free seawater treatment showed 96-h LC₅₀ values 41% and 55% lower, respectively, than female and male copepods exposed to CHTH in the presence of salt-marsh sediment DOM (Table 2, Fig. 1A and B). Similarly, female and male copepods exposed to CHPY in the DOM-free seawater treatment showed 96-h LC₅₀ values 58% and 83% lower, respectively, than female and male copepods exposed to CHPY in the presence of DOM (Table 2, Fig. 1C and D). In FIP exposures, the presence of DOM increased FIP toxicity to females by 16%; yet, dose–mortality curves responses in the presence and absence of DOM were not significantly different from each other ($p>0.05$; Table 2, Fig. 1E). In contrast, the presence of DOM significantly increased ($p<0.0001$) by 43% the acute toxicity of FIP to male copepods (Table 2, Fig. 1F).

To compare DOM effects on acute toxicity across pesticides and between sexes within pesticides, we calculated the relative pesticide toxicity index (mean 96-h LC_{50(DOM)}/mean 96-h LC_{50(DOM-free)}). Relative pesticide toxicity index to male and female copepods}}

varied independently of pesticide log K_{OW} 's. The pesticide with the highest estimated log K_{OW} (CHPY) had the largest index indicating much lower toxicity of CHPY in the presence of DOM. However, the pesticide with the lowest estimated log K_{OW} (FIP) had a <1 relative pesticide toxicity, indicating much higher acute toxicity of FIP in the presence of DOM. In the presence of DOM, CHTH was 2.22 and 1.69 times less toxic to male and female copepods than CHTH alone, while CHPY was 5.9 and 2.35 times less toxic to male and female copepods than CHPY alone. This indicates that the presence of DOM was more advantageous for male copepods in reducing CHTH and CHPY acute toxicity. In contrast, relative FIP toxicity in the presence of DOM resulted in greater toxicity to males (0.43) than to female copepods (0.84).

3.2. Chronic pesticide exposures

Water quality conditions were consistent across all pesticides and DOM treatments. Water conditions prior to microwell water change were salinity=30‰, pH=8.1±0.2, dissolved oxygen saturation DO>95%. Likewise water quality conditions in microwells after each 3-day chronic exposure period were salinity=30±1‰, pH=7.9±0.3, dissolved oxygen saturation DO>75%. The presence of DOM did not compromise water quality during chronic exposures, as DO in the microwells containing DOM was similar to that of DOM-free seawater.

CHTH and FIP measured concentrations throughout the length of the chronic exposure (16 days) were 10% below and 1.4% above nominal concentrations (24 µg/L and 1.4 µg/L), respectively, with little pesticide loss (<15%) between consecutive water changes. Target chronic CHPY concentration (0.18 µg/L) was below instrument detection limits (1 µg/L; Table 1).

Table 2

Estimated sex specific 96-h median lethal concentration (96-h LC₅₀) toxicity in *Amphiascus tenuiremis* exposed to chlorothalonil (CHTH), chlorpyrifos (CHPY) and fipronil (FIP) with and without salt-marsh sediment dissolved organic matter (DOM). Values represent Probit estimated 96-h LC₅₀'s (µg/L) and 95% confidence intervals

Pesticide	DOM-free seawater		DOM	
	Female	Male	Female	Male
CHTH	53.12 (45.66–61.11)	26.72 (22.67–30.81)	89.68 (80.52–100.11)	59.4 (53.01–66.33)
CHPY	3.56 (2.23–5.16)	0.61 (0.12–1.27)	8.37 (6.58–10.79)	3.6 (2.70–4.64)
FIP	6.07 (5.23–7.5)	3.86 (3.25–4.46)	5.09 (4.13–6.24)	1.68 (0.99–2.17)

Control copepodite survival to adult copepods between presence and absence of DOM was not statistically significant ($p>0.05$) indicating that salt-marsh sediment DOM did not increase copepodite mortality during microplate chronic exposures. Survival of copepodites exposed to CHTH and CHPY in the presence and absence of DOM was not statistically different from controls ($p>0.05$) with $99\pm 3\%$ of the initial copepodites surviving to the adult copepod stage. However, survival in FIP exposed individuals in the presence and absence of DOM was significantly lower than controls ($p<0.0001$, $81\pm 12\%$).

The cumulative copepodite-to-adult developmental curves for individuals reared in clean DOM-free seawater were not significantly different ($p=0.142$) from individuals reared in estuarine salt-marsh sedi-

ment DOM. Developmental rates under DOM-free and DOM conditions were 2.11/day and 3.68/day, respectively ($p=0.11$). Similarly, the cumulative copepodite-to-adult developmental curves for individuals exposed to CHTH and CHPY in the presence and absence of DOM were not significantly different ($p>0.05$) from control developmental curves. Fifty percent of the surviving copepodites exposed to CHTH, CHPY and controls in the presence and absence of DOM developed into sexually mature adult copepod after 7 days of microplate initiation, with 95% developing to adult by day 8.

In contrast, individuals exposed to FIP showed significantly different developmental curves than controls ($p<0.05$; Fig. 2). In FIP exposures with DOM-free seawater, copepodite developmental rate

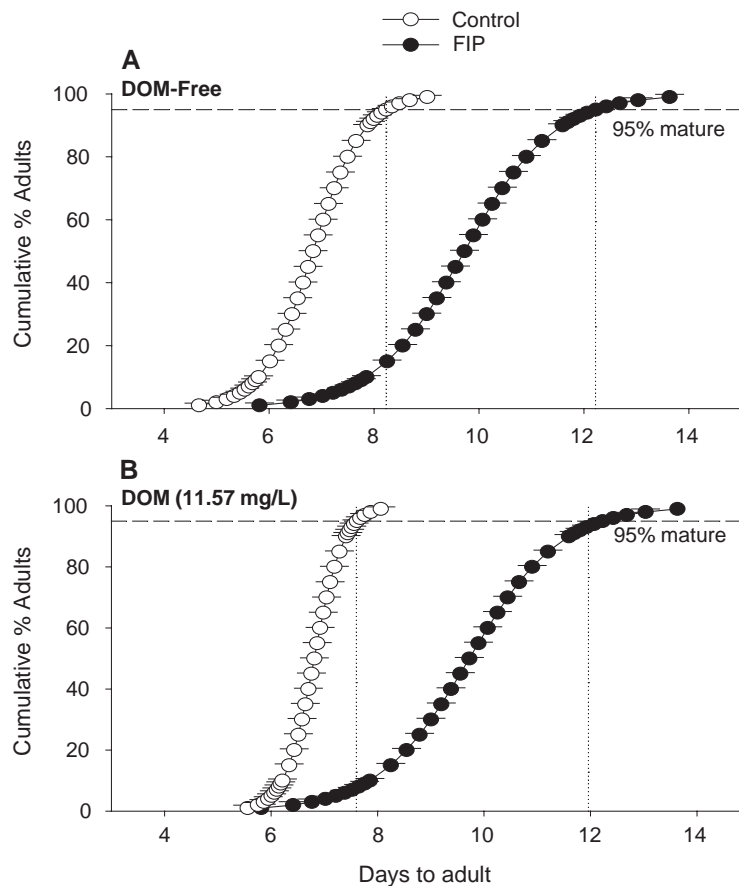


Fig. 2. *Amphiascus tenuiremis* copepodite developmental times to mature adult in control and fipronil (FIP) exposed individuals in the presence and absence of salt-marsh sediment dissolved organic matter (DOM). $n=51-64$ copepodites per treatment and DOM condition. Values represent mean predicted values and ± 1 standard deviation.

was significantly slower ($p=0.0027$, 1.18/day) than controls (2.11/day). While 50% of the control copepodites developed into adult male and female copepods after 7 days of exposure, individuals exposed to FIP in DOM-free seawater showed a 3- and 2-day delay in development into female and male copepods, respectively. Overall 95% of the Copepodites exposed to FIP developed into adults 4 days later than control copepodites (Fig. 2A). FIP exposed copepodites in the presence of DOM showed developmental rates significantly slower ($p=0.002$, 0.8/day) than DOM controls (3.68/day). The majority of copepodites (95%) developed into adults 4 days later than control copepodites (Fig. 2B). Although the initial copepodite development into adult copepods in FIP exposed individuals was accelerated by 2 days in the presence of DOM, overall developmental rates were slower for individuals exposed to FIP in DOM-free seawater. DOM did not attenuate the developmental delays caused by FIP.

Adult male to female sex ratios in copepods exposed to the three pesticides in the presence or absence of DOM were not statistically significant ($p>0.05$) from controls. Male to female sex ratios across all pesticides and DOM conditions were 1.12 ± 0.51 .

During a 7-day mating period control females produced on average 36 ± 6 viable embryos over 3 to 4

consecutive broods. Mating pairs with females extruding at least 2 broods over the 7-day mating period were considered reproductive successes. Reproductive success of mating pairs exposed to control DOM-free seawater was 93% (Fig. 3). In mating pairs exposed to CHTH, CHPY and FIP with DOM-free seawater, reproductive success was significantly reduced by 26% ($p=0.01$), 24% ($p=0.02$) and 83% ($p<0.0001$) relative to controls. Similarly, reproductive success in mating pairs exposed to control seawater in the presence of DOM was 85% but not different from that of DOM-free seawater controls ($p>0.05$). Reproductive success of mating pairs exposed to CHTH and CHPY in the presence of DOM showed a reproductive success similar to that of controls (96% and 89%, respectively, $p>0.05$). In contrast, reproductive failure in FIP-exposed mating pairs was significantly higher (80%, $p<0.0001$) compared to controls. Comparing reproductive success within pesticides, the presence of DOM mitigated 30% ($p=0.006$) and 20% ($p=0.05$), respectively, of the reproductive failure attributed to CHTH and CHPY. DOM did not mitigate FIP-induced reproductive failure.

Brood sizes and total viable offspring production were analyzed only for control females and females exposed to CHTH and CHPY. Low reproductive success in FIP exposures did not allow for statistical

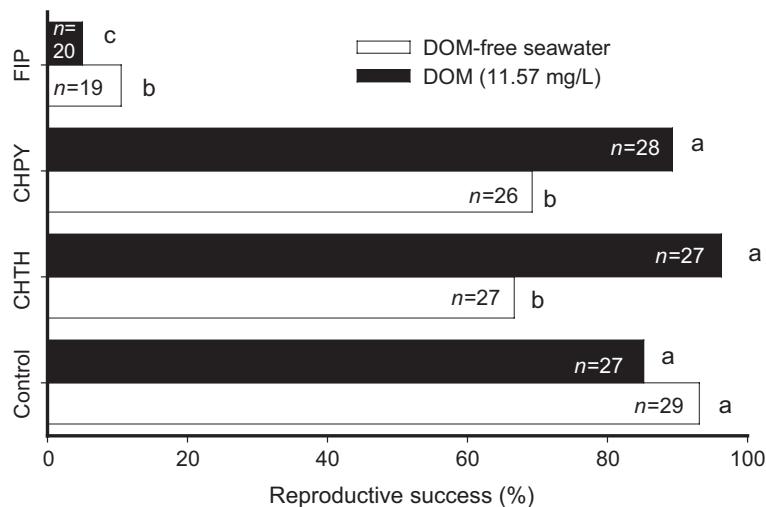


Fig. 3. Reproductive success of *Amphiascus tenuiremis* mating pairs chronically exposed to chlorothalonil (CHTH), chlorpyrifos (CHPY) and fipronil (FIP) in the presence and absence of salt-marsh sediment dissolved organic matter (DOM). n =total number of mating pairs per treatment. Within bars of same color, bars with the same letter are not significantly different from controls. For pesticides relative to controls, bars with the same letter are not significantly different from each other.

Table 3

Mean first, second and total viable offspring production (i.e., nauplius) (± 1 standard deviation) of female *Amphiascus tenuiremis* exposed to chlorothalonil (CHTH) and chlorpyrifos (CHPY) with and without salt-marsh sediment dissolved organic matter (DOM)

Pesticide	Brood	DOM-free seawater	DOM
Control	Number of viable females	26	23
	First brood	8.69 \pm 2.48	7.61 \pm 2.84
	Second brood	12.42 \pm 2.17	8.57 \pm 2.67
	Total production	21.12 \pm 3.75	16.17 \pm 5.13
CHTH	Number of viable females	17	26
	First brood	7.18 \pm 2.04	5.56 \pm 3.05
	Second brood	12.29 \pm 2.86	9.96 \pm 3.7
	Total production	19.47 \pm 3.43	15.52 \pm 5.21
CHPY	Number of viable females	18	26
	First brood	9 \pm 2.89	6.68 \pm 2.96
	Second brood	13.72 \pm 2.05	11.24 \pm 2.34
	Total production	22.72 \pm 4.16	17.92 \pm 3.59

analysis of the above endpoints. First and second brood sizes, and total viable offspring production in females exposed to CHTH and CHPY in the presence and absence of DOM were not significantly different from controls ($p > 0.05$; Table 3). Control females and females exposed to pesticides in DOM-free seawater generally produced on average 4 more nauplius ($p = 0.0004$) than females reared in same treatments with DOM.

3.3. Stage-structured population growth modeling

As mentioned previously, neither CHTH nor CHPY affected stage-specific survival in the presence or absence of DOM. In the absence of DOM, all three pesticides significantly reduced reproductive success of exposed mating pairs resulting in reduced projected production (e.g., nauplius; Fig. 4). The exponential growth model predicted a naupliar production through three generations 37% lower than DOM-free controls for individuals exposed to CHTH and CHPY and 98% lower than controls for individuals exposed to FIP ($p < 0.0001$; Fig. 4a). In contrast, the mitigating reproductive effects attributed to CHTH and CHPY in the presence of DOM resulted in a projected naupliar production similar to that of control-DOM individuals ($p > 0.05$; Fig. 4b). In the presence of DOM, projected naupliar production relative to controls was elevated on average by 20% in individuals exposed to CHTH and reduced by only 3% in individuals exposed to CHPY. Projected naupliar production in individuals exposed to FIP in the presence of DOM was 99% lower than DOM-controls. A difference of 41% in projected naupliar production was predicted for control DOM-free seawater and control DOM resulting from reduced fecundity of control-DOM females.

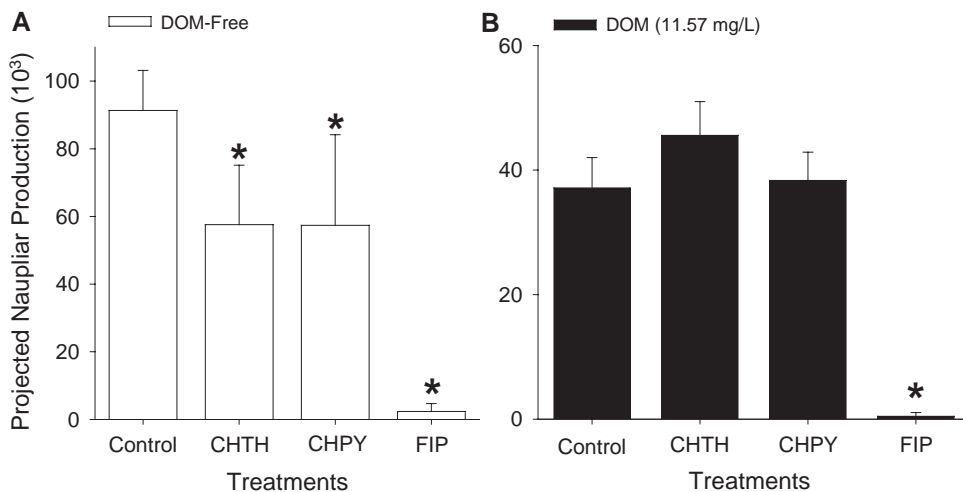


Fig. 4. Three-generation Lefkovich matrix projections of *Amphiascus tenuiremis* naupliar production by individuals exposed to control seawater, chlorothalonil (CHTH), chlorpyrifos (CHPY) and fipronil (FIP) in the absence (A) and presence (B) of salt-marsh sediment dissolved organic matter (DOM).

4. Discussion

In this study, we evaluated the role of salt-marsh sediment DOM on the acute and chronic toxicity of three pesticides (chlorothalonil—CHTH, chlorpyrifos—CHPY and fipronil—FIP) that have been detected at low levels in estuarine and coastal waters (Pait et al., 1992; USGS, unpublished data). For chronic exposures, our study used pesticide concentrations (CHTH=24 µg/L, CHPY=0.18 µg/L, FIP=1.4 µg/L) within the same order of magnitude and near the worse case scenario concentrations (CHTH=16 µg/L, CHPY=0.08 µg/L, FIP=1.5 µg/L) in estuarine waters (Demcheck and Skrobialowski, 2003; Lee, pers. comm.; Pait et al., 1992; USGS, unpublished data).

Dissolved organic matter (DOM) represents an important source of carbon in marine environments (Dafner and Wangersky, 2002). In coastal areas, carbon content as DOM is often >100 µM, with the highest concentrations found in estuarine ecosystems (Dafner and Wangersky, 2002). Carbon content in estuarine DOM is frequently >145 µM, with concentration depending on source inputs (Guo and Santschi, 1997). The sorptive material contained in salt-marsh sediment DOM may influence the partitioning of organic contaminants, as DOM provides an environment allowing the binding of hydrophobic compounds (Schwarzenbach et al., 1993). A previous study with natural forms of DOM at environmentally realistic concentrations showed a reduction in CHPY uptake and bioconcentration to the bivalve *M. mercenaria* (Bejarano et al., 2005). DOM generally decreases the tendency of pesticides to bioconcentrate in aquatic organisms (Schwarzenbach et al., 1993) resulting in a reduction of free-pesticide binding to cell membranes and other structures, thus reducing acute pesticide toxicity. Our results showed that salt-marsh sediment DOM with relatively low dissolved organic carbon content (11.57 ± 0.18 mg/L) reduced the acute toxicity of CHTH and CHPY to male and female copepods as evident by elevated 96-h LC₅₀ estimates. Other studies have also suggested the attenuation of toxic effects of pesticides by organic carbon (Chandler et al., 1997; Mézin and Hale, 2004). For instance, Day (1991) found that the acute toxicities of three pyrethroid insecticides to *D. magna* decreased inversely proportional to DOC concentration. DOC at a concentration of 15.5 mg/L reduced the acute toxicity

of fenvalerate to *D. magna* by a factor of 17 (Day, 1991). Also, a study with naturally occurring meiofauna communities and the laboratory-cultured copepod, *A. tenuiremis*, reared in whole-sediment microcosms containing CHPY spiked-sediments, found no CHPY effects on copepod densities (Chandler et al., 1997). These results were attributed to high organic content, which may have driven the bioavailable pesticide fraction to non-toxic levels.

In this study, we evaluated three pesticides with distinct *n*-octanol–water partition coefficients (log K_{OW}) and modes of action. The log K_{OW} value of hydrophobic non-polar organic compounds has been traditionally used to predict chemical toxicity (Van Leeuwen et al., 1992; Di Toro et al., 2000). However, studies have shown that chemical log K_{OW} values do not sufficiently describe toxicity (Fisher et al., 1993; Hodson et al., 1988). We found that the presence of DOM had a more profound effect on the acute and chronic toxicity of CHPY, which had the highest log K_{OW} . However, the pesticide with the intermediate log K_{OW} , FIP, showed an apparent enhancement of acute toxicity to male copepods when DOM was present. A similar study showed that the addition of sediment to toxicity tests resulted in a significant reduction in the toxicity (i.e., 24-h EC₅₀) of cholinergic insecticides (organophosphorous and carbamates) to the midge *C. riparius* (Fisher et al., 1993). In that study, however, chemical log K_{OW} did not fully describe insecticide toxicity. In these and other species, toxicity likely depends on chemical descriptors such as the log K_{OW} and also on particular molecular functional groups that dictate pesticide modes of action.

As mentioned previously, we found that the presence of DOM increased the acute toxicity of FIP to male copepods relative to its toxicity in seawater-only exposures. A possible explanation for the apparent increased toxicity of FIP is that the presence of DOM could have reduced the light-dependent formation of the less or equally toxic metabolite fipronil-desulfinyl. In fact, Ajajoud et al. (2003) found that the presence of organic matter protected FIP from photodegradation favoring the formation of fipronil-sulfone and fipronil-sulfide. These two metabolites were more acutely toxic to fourth-instar larvae of *Aedes aegypti* than the parent compound FIP (Ajajoud et al., 2003). This may also explain the slower developmental rates of copepodites chronically

exposed to FIP in the presence of DOM; however, we did not quantify any FIP metabolites.

Our data also shows greater acute toxicity of CHTH, CHPY and FIP to male copepods compared to female *A. tenuiremis*. Consistently, we also found that the presence of DOM was more advantageous for male copepods exposed to CHPY and CHTH than for female copepods. DOM likely provided the additional contaminant storage lacking in males, serving as a surrogate for lower body lipid concentration. Females across copepod species have larger lipid storage than males (i.e., Klosterhaus et al., 2003) providing not only an important role in reproduction but also facilitating higher contaminant body burdens. For instance, percent total lipid content (dry wt.) in adult non-gravid female copepod *Microarthridion littorale* was 1.8 times higher than in males, resulting in higher non-gravid female fluoranthene and benzo[*a*]anthracene body burdens (Klosterhaus et al., 2002). Similarly, higher body burdens in female *A. tenuiremis* may explain their reduced sensitivity to CHTH, CHPY and FIP. A study with the calanoid copepod *Acartia tonsa* (Medina et al., 2002) also showed sex-specific differences during the first 24 h of exposure to the pyrethroid insecticide cypermethrin. In that study, female copepods were less sensitive to acute pesticide toxicity, possibly due to the elimination of cypermethrin via egg production (McManus et al., 1983). Higher male sensitivity to contaminants could also be explained by energy budget differences between male and female copepods, such as faster respiration rates in male and higher food ingestion rates in female copepods (Irigoien et al., 2000). Sex-specific differences in pesticide toxicity, particularly greater male sensitivity, could result in lower female fertilization, skewed sex ratios and reduction in population growth. However, relatively low levels of DOM could attenuate these ecological consequences.

The presence DOM not only reduced the acute toxicity of CHTH and CHPY but also reduced reproductive failure in mating pairs chronically exposed to these pesticides. DOM could have absorbed the pesticide fraction that directly or indirectly caused reproductive failure in seawater exposures only. In order to ensure individual survival under seawater-only pesticide exposures, copepods may have increased their metabolic requirements for

maintaining homeostasis by diverting energy traditionally channeled into processes such as reproduction. (Maund et al., 1992). Nonetheless, the mechanistic process by which DOM reduces the reproductive failure attributed to CHTH and CHPY is still not clear. The benefit of such reproductive failure mitigation was further demonstrated in naupliar production projections, where exposures to CHTH and CHPY in the presence of DOM resulted in similar naupliar projections as DOM control projections. The ecological consequences of this pesticide-effect mitigation could result in optimum population maintenance and fitness. Notice, however, that the exponential population growth model employed in the present study oversimplifies copepod population dynamics by ignoring competition, predation and environmental carrying capacities. Interestingly, we found that DOM reduced reproductive output in females reared in the presence or absence of pesticide. Höss et al. (2001) found that, at ecologically relevant DOM concentrations, the origin of DOM influenced reproduction by the nematode *Caenorhabditis elegans*. Substances such as fulvic acids, isolated from soil leachate, stimulated *C. elegans* reproduction, while fulvic acids from a humified lake inhibited nematode reproduction.

In conclusion, the presence of salt-marsh sediment DOM mitigated the acute and chronic effects of CHTH and CHPY in *A. tenuiremis*, while enhancing the acute toxicity of FIP particularly to male copepods. Therefore, seawater-only exposures likely overestimate acute and chronic pesticide effects under environmentally realistic conditions; however, DOM likely has non-generic, compound (pesticide)-specific chemical influences on toxicity to meiobenthos. Further studies are needed to elucidate specific pesticide–DOM interactions and the mechanisms by which DOM decreases/increases pesticide toxicity.

Acknowledgement

The authors would like to thank J. Pender for technical assistance and the anonymous reviewer whose suggestions greatly improved this manuscript. This research was made possible by the National Oceanic and Atmospheric Administration (NOAA)—the South Atlantic Bight Land Use–Coastal Ecosys-

tem Study (LU-CES) and the US Environmental Protection Agency (US EPA)–Science to Achieve Results (STAR) Program, Award No. R827397 (GT Chandler, PI). This research has not been subject to either agencies' peer or policy review and no endorsement should be inferred. [SS]

References

- Aajoud, A., Ravel, P., Tissut, M., 2003. Fipronil metabolism and dissipation in a simplified aquatic ecosystem. *J. Agric. Food Chem.* 51, 1347–1352.
- Akçakaya, H.R., Burman, M.A., Ginzburg, L.R., 1999. Applied Population Ecology: Principles and Computer Exercises Using RAMAS EcoLab 2.0, 2nd edition. Applied Biomathematics, Setauket, NY.
- Akkanen, J., Kukkonen, J., 2003. Measuring the bioavailability of two hydrophobic organic compounds in the presence of dissolved organic matter. *Environ. Toxicol. Chem.* 22, 518–524.
- American Society for Testing Materials (ASTM), 1988. Standard Guide for Conducting Acute Toxicity Tests with Fishes, Microinvertebrates and Amphibians. ASTM Standard No. E 1192-88. ASTM, Philadelphia, PA, pp. 102–121.
- American Society for Testing Materials (ASTM), 2004. Standard Guide for Conducting Renewal Microplate-based Life-cycle Toxicity Tests with a Marine Meiobenthic Copepod. ASTM Standard No. E2317-04. ASTM, Philadelphia, pp. 1–16.
- Bejarano, A.C., Chandler, G.T., 2003. Reproductive and developmental effects of atrazine on the estuarine meiobenthic copepod *Amphiascus tenuiremis*. *Environ. Toxicol. Chem.* 22, 3009–3016.
- Bejarano, A.C., Decho, A.W., Chandler, G.T., 2005. The role of various dissolved organic matter (DOM) forms on chlorpyrifos bioavailability to the estuarine bivalve *Mercenaria mercenaria*. *Mar. Environ. Res.* 60, 111–130.
- Caswell, H., 2001. Matrix Population Models—Construction, Analysis, and Interpretation, 2nd edition. Sinauer Associates, Sunderland, MA.
- Caux, P.Y., Kent, R.A., Fan, G.T., Stephenson, G.L., 1996. Environmental fate and effects of chlorothalonil: a Canadian perspective. *Crit. Rev. Environ. Sci. Technol.* 26, 45–93.
- Chandler, G.T., Green, A.S., 1996. A 14-day harpacticoid copepod reproduction bioassay for laboratory and field contaminated muddy sediments. In: Ostrander, G.K. (Ed.), *Tech. Aquat. Toxicol.*, pp. 23–39.
- Chandler, G.T., Coull, B.C., Schizas, N.V., Donelan, T.L., 1997. A culture-based assessment of the effects of chlorpyrifos on multiple meiobenthic copepods using microcosms of intact estuarine sediments. *Environ. Toxicol. Chem.* 16, 2339–2346.
- Chandler, G.T., Cary, T.L., Volz, D.C., Walse, S.S., Ferry, J.L., Klosterhaus, S.L., 2004. Fipronil effects on copepod development, fertility and reproduction: a rapid life-cycle assay in 96-well microplate format. *Environ. Toxicol. Chem.* 23, 117–124.
- Dafner, E.V., Wangersky, P.J., 2002. A brief overview of modern directions in marine DOC studies: Part II. Recent progress in marine DOC studies. *J. Environ. Monit.* 4, 55–69.
- Day, K.E., 1991. Effects of dissolved organic carbon on accumulation and acute toxicity of fenvalerate, deltamethrin and cyhalothrin to *Daphnia magna* (Straus). *Environ. Toxicol. Chem.* 10, 99–101.
- Demcheck, D.K., Skrobialowski, S.C., 2003. Fipronil and degradation products in the rice-producing areas of the Mermentau River Basin, Louisiana, February–September 2000. U.S. Geological Survey Fact Sheet, FS-010-03. U.S. Government Printing Office, Washington, DC.
- Di Toro, D.M., McGrath, J.A., Hansen, D.J., 2000. Technical basis for narcotic chemicals and polycyclic aromatic hydrocarbon criteria: I. Water and tissue. *Environ. Toxicol. Chem.* 19, 1951–1970.
- Fisher, S.W., Lydy, M.J., Barger, J., Landrum, P.F., 1993. Quantitative structure–activity–relationships for predicting the toxicity of pesticides in aquatic systems with sediment. *Environ. Toxicol. Chem.* 12, 1307–1318.
- Green, A.S., Chandler, G.T., Piegorsch, W.W., 1996. Life-stage-specific toxicity of sediment-associated chlorpyrifos to a marine infaunal copepod. *Environ. Toxicol. Chem.* 15, 1182–1188.
- Guo, L., Santschi, P.H., 1997. Isotopic and elemental characterization of colloidal organic matter from the Chesapeake Bay and Galveston Bay. *Mar. Chem.* 59, 1–15.
- Hainzl, D., Cole, L.M., Casida, J.E., 1998. Mechanisms for selective toxicity of fipronil insecticide and its sulfone metabolite and desulfinyl photoproduct. *Chem. Res. Toxicol.* 11, 1529–1535.
- Hodson, P.V., Dixon, D.G., Kaiser, K.L.E., 1988. Estimating the acute toxicity of waterborne chemicals in trout from measurements of median lethal dose and the octanol–water partition coefficient. *Environ. Toxicol. Chem.* 7, 443–454.
- Höss, S., Bergtold, M., Haitzer, M., Traunspurger, W., Steinberg, C.E.W., 2001. Refractory dissolved organic matter can influence the reproduction of *Caenorhabditis elegans* (Nematoda). *Freshw. Biol.* 46, 1–10.
- Irgoien, X., Obermüller, B., Head, R.N., Harris, R.P., Rey, C., Hansen, B.W., Hygum, B.H., Heath, M.R., Durbin, E.G., 2000. The effect of food on the determination of sex ratio in *Calanus* spp.: evidence from experimental studies and field data. *ICES J. Mar. Sci.* 57 (6), 1752–1763.
- Klosterhaus, S.L., Ferguson, P.L., Chandler, G.T., 2002. Polycyclic aromatic hydrocarbon bioaccumulation by meiobenthic copepods inhabiting a superfund site: techniques for micromass body burden and total lipid analysis. *Environ. Toxicol. Chem.* 21, 2331–2337.
- Klosterhaus, S.L., DiPinto, L.M., Chandler, G.T., 2003. A comparative assessment of azinphosmethyl bioaccumulation and toxicity in two estuarine meiobenthic harpacticoid copepods. *Environ. Toxicol. Chem.* 22, 2960–2968.
- Krawchuk, B.P., Webster, G.R.B., 1987. Movement of pesticides to groundwater in an irrigated soil. *Water Pollut. Res. J. Can.* 22, 129–145.
- Landrum, P.F., Nihart, S.R., Eadie, B.J., Herche, L.H., 1987. Reduction in bioavailability of organic contaminants to the

- amphipod *Pontoporeia hoyi* by dissolved organic matter of sediment interstitial water. *Environ. Toxicol. Chem.* 6, 11–20.
- Lang, K., 1948. Monographie der Harpacticiden. Nordiska-Bokhändeln, Stockholm, Sweden.
- Lefkovich, L.P., 1965. The study of population growth in organisms grouped by stages. *Biometrics* 21, 1–18.
- Mackay, D., 1991. Multimedia Environmental Models. The Fugacity Approach. Lewis Publications, Chelsea, MI, USA.
- McManus, G.B., Wyman, K.T., Peterson, W.T., Wurster, C.F., 1983. Factors affecting the elimination of PCBs in 310 the marine copepod *Acartia tonsa*. *Estuar. Coast. Shelf Sci.* 17, 421–430.
- Medina, M., Barata, C., Telfer, T., Baird, D., 2002. Age-and sex related variation in sensitivity to the pyrethroid cypermethrin in the marine copepod *Acartia tonsa* Dana. *Arch. Environ. Contam. Toxicol.* 42 (1), 17–22.
- Mézin, L.C., Hale, R.C., 2004. Effect of humic acids on toxicity of DDT and chlorpyrifos to freshwater and estuarine invertebrates. *Environ. Toxicol. Chem.* 23, 583–590.
- Mitsch, W.J., Gosselink, J.G., 2000. Wetlands, 3rd edition. J. Wiley.
- Maund, S.J., Taylor, E.J., Pascoe, D., 1992. Population responses of the freshwater amphipod crustacean *Gammarus-pulex* (L) to copper. *Freshw. Biol.* 28, 29–36.
- Niculescu, S.P., Kaiser, K.L.E., Schüürmann, G., 1998. Influence of data preprocessing and kernel selection on probabilistic neural network modeling of the acute toxicity of chemicals to the fathead minnow and *Vibrio fischeri* bacteria. *Water Qual. Res. J. Can.* 33, 153–165.
- Odenkirchen, E.W., Eisler, R., 1988. Chlorpyrifos hazard to fish, wildlife and invertebrates: a synoptic review. U.S. Fish and Wildlife Service. Resources Report, 85.
- Pait, A.S., DeSouza, A., Farrow, D.R.G., 1992. Agricultural Pesticide Use in Coastal Areas: a National Summary. National Oceanic and Atmospheric Administration, Rockville, MD.
- Piegorsch, W.W., Bailer, A.J., 1997. Statistics for Environmental Biology and Toxicology. Chapman & Hall, London, UK.
- Saito, H., Koyasu, J., Yoshida, K., Shigeoka, T., Koike, S., 1993. Cytotoxicity of 109 chemicals to goldfish GFS cells and relationships with 1-octanol/water partition coefficients. *Chemosphere* 26, 1015–1028.
- Schwarzenbach, R.P., Gschwend, P.M., Imboden, D.M., 1993. Environmental Organic Chemistry. John Wiley, New York, USA.
- Servos, M.R., Muir, D.C.G., Webster, G.R.B., 1989. The effect of dissolved organic matter on the bioavailability of polychlorinated dibenzo-*p*-dioxins. *Aquat. Toxicol.* 14, 169–184.
- Strawbridge, S., Coull, B.C., Chandler, G.T., 1992. Reproductive output of a meiobenthic copepod exposed to sediment-associated fenvalerate. *Arch. Environ. Contam. Toxicol.* 23, 295–300.
- The Merck Index, 2001. In: O’Neal, M.J., Smith, A., Heckelman, P.A., Obenchain, J.R., D’Arecca (Eds.), An encyclopedia of chemicals, drugs and biologicals, 13th ed. Merck and Co., INC, Whitehouse Station, NJ, USA.
- Tillman, R.W., Siegel, M.R., Long, J.W., 1973. Mechanism of action and fate of the fungicide chlorothalonil (2,4,5,6-tetrachloroisophalonitrile) in biological systems: 1. Reactions with cells and subcellular components of *Saccharomyces pastorianus*. *Pestic. Biochem. Physiol.* 3, 160–167.
- USEPA. New Pesticide Fact Sheet, 1996. US EPA, Office of Prevention, Pesticides and Toxic Substances, Washington, DC. EPA-737-F-96-005.
- USEPA. Registration Eligibility Decision (RED) Chlorothalonil, 1999. US EPA, Office of Prevention, Pesticides and Toxic Substances, Washington, DC. EPA-738-R-99-004.
- USEPA. Chlorpyrifos Revised Risk Assessment and Agreement with Registrants, 2000. US EPA, Office of Prevention, Pesticides and Toxic Substances, Washington, DC.
- Van Leeuwen, C.J., Vanderzandt, P.T.J., Aldenberg, T., Verhaar, H.J.M., Hermens, J.L.M., 1992. Application of QSARS, extrapolation and equilibrium partitioning in aquatic effects assessment: 1. Narcotic industrial pollutants. *Environ. Toxicol. Chem.* 11, 267–282.
- Wania, F., Mackay, D., 1999. The evolution of mass balance models of persistent organic pollutant fate in the environment. *Environ. Pollut.* 100, 223–240.