Chemosphere 135 (2015) 265–271

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Comparative toxicities of organophosphate and pyrethroid insecticides to aquatic macroarthropods

Neal T. Halstead *, David J. Civitello, Jason R. Rohr

Department of Integrative Biology, University of South Florida, Tampa, FL 33620, United States

HIGHLIGHTS

- We exposed crayfish and water bugs to pyrethroid and organophosphate insecticides.
- Insecticide class was a significant predictor of risk of mortality during the study.
- Pyrethroid insecticides were consistently more toxic than organophosphates.
- Malathion was the only insecticide identified as posing low risk to macroarthropods.
- Identifying low-risk insecticides is critical to minimize adverse ecosystem effects.

ARTICLE INFO

Article history:
Received 16 January 2015
Received in revised form 25 March 2015
Accepted 29 March 2015
Available online 15 May 2015

Keywords:
Malathion
Ecosystem function
Crayfish
Water bug
Risk quotient

ABSTRACT

As agricultural expansion and intensification increase to meet the growing global food demand, so too will insecticide use and thus the risk of non-target effects. Insecticide pollution poses a particular threat to aquatic macroarthropods, which play important functional roles in freshwater ecosystems. Thus, understanding the relative toxicities of insecticides to non-target functional groups is critical for predicting effects on ecosystem functions. We exposed two common macroarthropod predators, the crayfish *Procambarus alleni* and the water bug *Belostoma flumineum*, to three insecticides in each of two insecticide classes (three organophosphates: chlorpyrifos, malathion, and terbufos; and three pyrethroids: esfenvalerate, λ-cyhalothrin, and permethrin) to assess their toxicities. We generated 150 simulated environmental exposures using the US EPA Surface Water Contamination Calculator to determine the proportion of estimated peak environmental concentrations (EECs) that exceeded the US EPA level of concern ($0.5 \times LC_{50}$) for non-endangered aquatic invertebrates. Organophosphate insecticides generated consistently low-risk exposure scenarios (EECs $< 0.5 \times LC_{50}$) for both *P. alleni* and *B. flumineum*. Pyrethroid exposure scenarios presented consistently high risk (EECs $> 0.5 \times LC_{50}$) to *P. alleni*, but not to *B. flumineum*, where only λ-cyhalothrin produced consistently high-risk exposures. Survival analyses demonstrated that insecticide class accounted for 55.7% and 91.1% of explained variance in *P. alleni* and *B. flumineum* survival, respectively. Thus, risk to non-target organisms is well predicted by pesticide class. Identifying insecticides that pose low risk to aquatic macroarthropods might help meet increased demands for food while mitigating against potential negative effects on ecosystem functions.

1. Introduction

Global sales of insecticides have increased over the past several decades (Grube et al., 2011). Insecticide use is positively correlated with cropland (Meehan et al., 2011) and is almost certain to increase with the agricultural expansion necessary to feed the increasing global human population (Tilman et al., 2011; Tilman et al., 2001). Pyrethroid use in particular has increased worldwide, especially to control vector-borne diseases (van den Berg et al., 2012). Additionally, the organophosphate insecticides chlorpyrifos and malathion remain among the most-frequently detected insecticides in surface waters of the United States (Gilliom, 2007), even as agricultural use of organophosphates in the United States has stagnated (Grube et al., 2011; Thelin and Stone, 2013).

Agrochemical pollution from insecticide run-off can have important negative consequences for non-target taxa (Brock et al., 2000; McMahon et al., 2012; Rohr et al., 2013; Rohr et al., 2008b). Insecticides can adversely impact aquatic macroarthropods (Brock et al., 2000; Van Wijngaarden et al., 2006), which play

* Corresponding author at: Department of Integrative Biology, University of South Florida, 4202 E Fowler Ave, SCA 110, Tampa, FL 33620, United States.

E-mail addresses: nhalstea@usf.edu, neal.halstead@gmail.com (N.T. Halstead), civitello@usf.edu (D.J. Civitello), jasonrohr@gmail.com (J.R. Rohr).

http://dx.doi.org/10.1016/j.chemosphere.2015.03.091
0045-6535/

many important functional roles in wetland ecosystems (Wallace and Webster, 1996), including as predators of aquatic herbivores (Kesler and Munns, Jr., 1989; Weber and Lodge, 1990) and as prey for vertebrate predators (Jordan et al., 1996). Because they occupy intermediate trophic levels, macroinvertebrates can mediate the effects of both top-down and bottom-up pressures on ecosystems (Wallace and Webster, 1996). Thus, changes in the abundances of macroarthropod predators can indirectly affect aquatic community composition and ecosystem properties (Halstead et al., 2014; Rohr and Crumrine, 2005).

As new pesticides are developed and approved for use, it is important that risk assessors can predict the risk these chemicals pose to non-target wildlife. Pesticides may vary both in their toxicity to organisms and in their estimated environmental exposures, the latter of which is based on recommended application rates and the physicochemical properties of the pesticide. Insecticides with similar modes of action often have similar safe threshold values in terms of toxic units (concentrations of different pesticides that are standardized by dividing by the geometric mean of reported EC_{50} values of the most sensitive standard test species (typically *Daphnia magna*); Brock et al., 2000). Therefore, pesticides of the same class (i.e., organophosphate vs. pyrethroid insecticides) might be expected to pose similar risk to focal species even though individual pesticides within a class might vary in their relative estimated environmental exposures and toxicities.

The United States Environmental Protection Agency (US EPA) has developed standardized methods for assessing risk to non-target organisms. Environmental exposure scenarios can be generated using the US EPA's Surface Water Contamination Calculator software (SWCC v1.106), which incorporates recommended pesticide application rates for a given crop, local weather and soil characteristics, and the physicochemical properties of the pesticide to generate a 30-year series of peak estimated environmental concentrations (EECs) for a standardized wetland (US EPA, Washington, DC, USA). The ratio of the EEC for a given pesticide relative to its median lethal concentration (LC_{50}) for an organism of concern is then used to determine a risk quotient for that organism ($RQ = EEC/LC_{50}$; US EPA, 2014). The US EPA considers RQ values of 0.5 or greater as representing acute high risk to aquatic organisms (US EPA, 2014a).

Here we compare the relative toxicities of three insecticides in each of two classes of compounds (three organophosphates: chlorpyrifos, malathion, and terbufos; and three pyrethroids: esfenvalerate, λ-cyhalothrin, and permethrin) for two important macroarthropod predators of snails: the crayfish *Procambarus alleni* and the water bug *Belostoma flumineum*. Additionally, as an exploration of relative environmental risk within and between insecticide classes, we compared simulated peak environmental exposures for each insecticide to the US EPA level of concern ($0.5 \times$ LC50) for each species. Both classes of these insecticides affect the nervous systems of target organisms; organophosphates inhibit acetylcholinesterase activity (Newman and Unger, 2002) and pyrethroid insecticides act on voltage-sensitive ion channels in the axonal membranes of neurons to prevent repolarization of action potentials (Soderlund et al., 2002). Our goals were to determine if individual insecticides within a chemical class pose similar threats to these arthropods, and if there are individual chemicals or classes that might pose a lower risk to these taxa if runoff events occur.

2. Methods

2.1. Study organisms

Two common macroarthropod predators were selected for this study. Both *P. alleni* and *B. flumineum* are ubiquitous in freshwater wetlands throughout Florida. *B. flumineum* occur throughout much of North America (Henry and Froeschner, 1988). While *P. alleni* are endemic to Florida (Jordan et al., 1996), the genus is widespread throughout southeastern North America, northern Central America and the northern Caribbean (Hobbs Jr., 1984), and *P. clarkii* have been introduced to every continent except Australia and Antarctica (Hobbs III et al., 1989). Juvenile *P. alleni* (10–43 mm total length) and adult *B. flumineum* (11–20 mm total length) were collected from a pond in Tampa, FL, located at 28°4.172′N, 82°22.665′W. This pond was located far from agricultural land and so was not likely to have been contaminated with agrochemicals with the exception of malathion, which is used ubiquitously throughout Hillsborough County, FL, for adult mosquito control. Individuals in the experiment were maintained separately in the lab in artificial spring water (ASW; Cohen and Neimark, 1980) at 22 °C, on a 14:10 photoperiod, and fed snails (*Physa* spp.) *ad libitum*. Artificial spring water had a pH of 6.8, dissolved oxygen of 6.1 mg/L, and specific conductance of 174.4 μS/cm.

2.2. Insecticides

Three organophosphate (chlorpyrifos, malathion, and terbufos) and three pyrethroid (esfenvalerate, λ-cyhalothrin, and permethrin) insecticides were selected for this study. With the exception of terbufos, all of these chemicals have been used extensively over at least the past two decades in this region (Stone, 2013; Thelin and Stone, 2013). We generated 150 simulated annual peak EEC values in ponds for each pesticide based on the manufacturer's recommended application rate, the physicochemical properties of the pesticide (acquired from the University of Hertfordshire's Pesticide Properties DataBase; 2013), and local abiotic conditions using the US EPA SWCC software (v1.106) and standard EPA scenarios for corn production in five US states (Illinois, Mississippi, North Carolina, Ohio, and Pennsylvania). Corn was used for all exposure scenarios to reduce variation associated with application recommendations for other crops and because applications for corn were included for each insecticide product label. The range of EEC values and the parameters used to calculate them are reported in Table S1.

We selected pesticide concentration ranges that included both the range of EECs and known LC_{50} concentrations for closely-related species and/or similar pesticides where data were available in the US EPA's Ecotox database (US EPA, 2014b). No toxicity data for *P. alleni* were available in the Ecotox database for any of the six insecticides used. However, toxicity data were available for other species of *Procambarus*. Toxicity data for these taxa are summarized in Table S2. No effect of malathion concentrations ranging from 130 to 460 μg/L was observed on *B. flumineum* mortality in mesocosm trials (Relyea and Hoverman, 2008). No other toxicity data were available for *B. flumineum* in the Ecotox database. However, 24-h LC50 concentrations of 15 and 60 μg/L were reported for an unidentified *Belostoma sp*. exposed to chlorpyrifos and parathion, respectively.

Technical-grade insecticides were used for all trials (purity > 98%; Chemservice, West Chester, PA, USA). Actual chemical concentrations applied to the replicates were confirmed using ELISA test kits for detection of organophosphates and pyrethroids (Abraxis, LLC, Warminster, PA, USA). ELISA assays were calibrated by using standards of known concentration for each insecticide. For any nominal concentrations below the limit of detection for the kit, we confirmed the concentration of the stock solution used for serial dilutions.

2.3. Experimental design

We used a static, nonrenewal (no water changes) dose–response design with 5 concentrations of each insecticide

(Table S3), solvent (0.0625 mL/L acetone) and water controls, and 5 replicates of each concentration and control. The *B. flumineum* trials included 10 replicates of each control. Each replicate consisted of one individual *P. alleni* or *B. flumineum* in a 500 mL glass jars filled with 400 mL ASW. Each individual was fed physid snails *ad libitum* (snails were replaced as they were consumed). Observations were made at 3, 12, and 24 h after pesticide application and daily thereafter for a total of 10 days. *P. alleni* trials were conducted from 22 August 2012 to 1 September 2012, and *B. flumineum* trials were conducted from 20 September 2012 to 30 September 2012.

2.4. Data analysis

Dose–response curves and estimation of LC_{50} values were performed using package "drc" (Ritz and Streibig, 2005) in R statistical software (R Core Team, 2013). We used the two parameter logistic model to estimate 96-h and 10-d LC_{50} values and used bootstrapping to determine the 95% confidence intervals around each LC_{50} value. In cases for which the bootstrapping method in package "drc" gave confidence interval estimates that did not include the estimated LC_{50} value, 95% confidence intervals were instead approximated using the variance of the estimate in package "drc" and back-transformed from the log scale used for concentrations (Ritz and Streibig, 2005). We determined the proportion of simulated EEC values that exceeded the US EPA's level of concern ($0.5 \times LC_{50}$) for each LC_{50} estimate in addition to its lower and upper 95% confidence limits.

Survival analysis was performed using the Cox proportional hazard model in package "survival" (Therneau, 2013) in R. To explore differences in survival that could be attributed to either chemical class or the individual chemicals, we ran a mixed-effects Cox model using package "coxme" (Therneau, 2012) in R. To account for variation in absolute toxicity and estimated environmental exposure among chemicals, we converted all concentrations to toxic units (TUs) using SPEAR Calculator software (v0.8.1, Department System Ecotoxicology – Helmholtz Centre for Environmental Research, 2014). Because the range of toxic units for each insecticide did not always overlap (e.g., malathion TUs were up to 5× greater than the highest TU for permethrin) numerical estimation results fitted poorly. Therefore, we added simulated dead individuals at similarly high concentrations for other chemicals. In all such cases, 100% mortality was observed at concentrations orders of magnitude below these simulated higher concentrations. We used standardized chemical concentration as a fixed effect with random intercepts for individual chemicals (esfenvalerate, λ-cyhalothrin, etc.) nested within insecticide class (organophosphate or pyrethroid). Coefficients of the random effects were extracted from the model to assess the contribution of each chemical and class to overall mortality risk.

3. Results

Toxicity data for *Procambarus spp*. from the US EPA's Ecotox database were generally consistent with observed toxicities to *P. alleni* (Table 1, Table S2). Reported 96-h LC_{50} toxicities for *P. clarkii* exposed to λ-cyhalothrin, permethrin, chlorpyrifos, and malathion were all within the 95% confidence intervals for the observed 96-h LC_{50} values for *P. alleni* in this experiment. Terbufos was reported to be more toxic to *P. clarkii* than *P. alleni*, although reported toxicity to an unidentified *Procambarus* species was similar to that of *P. alleni*. Likewise, chlorpyrifos was reported to be more toxic to *P. acutus* and an unidentified *Procambarus* species than to *P. alleni*. Conversely, *P. blandingii* exposed to permethrin in a flow-through experimental design was much more resistant to permethrin exposure than was *P. alleni* in this experiment (Table 1, Table S2). Chlorpyrifos toxicity was greater for an unidentified *Belostoma* spp. in a 24-h trial than was observed for *B. flumineum* over 96-h in this experiment (USEPA, 2014b).

Simulated environmental exposure scenarios indicated generally consistent patterns of environmental risk associated with insecticides of the same class (Table 1). Organophosphate exposures generated consistently few EECs above the US EPA level of concern of half the LC_{50} for both *P. alleni* and *B. flumineum* (Table 1, Figs. 1 and 2), with the exception of 10-d crayfish mortality after exposure to chlorpyrifos (Table 1). Alternatively, the majority of all pyrethroid exposure scenarios were above the level of concern for *P. alleni* (Table 1, Fig. 1). Only λ-cyhalothrin presented consistently high-risk exposure scenarios for *B. flumineum*, while the high degree of uncertainty in the 96-h LC_{50} estimate for permethrin rendered risk assessment impractical (Table 1, Fig. 2).

Cox mixed-effects models indicated that insecticide class accounted for 55.7% and 91.1% of the variance in mortality during

Table 1
96-h and 10-d LC_{50} values (μg/L) for *Procambarus alleni* and *Belostoma flumineum* exposed to multiple concentrations of three pyrethroid (esfenvalerate, λ-cyhalothrin, and permethrin) and three organophosphate (chlorpyrifos, malathion, and terbufos) insecticides. The second column for each endpoint reports the proportion out of 150 annual peak estimated environmental concentrations (EEC) calculated from the US EPA Surface Water Contamination Calculator (v1.106) that exceeded the US EPA risk quotient threshold of one-half the estimated LC_{50}.

Species	Chemical	96-h endpoint		10-d endpoint	
		LC_{50} (95% C.I.)	EECs > 0.5 × LC_{50}	LC_{50} (95% C.I.)	EECs > 0.5 × LC_{50}
P. alleni	Esfenvalerate	0.22 (0.14–0.26)	0.97 (0.93–0.98)	0.22 (0.14–0.26)	0.97 (0.93–0.98)
P. alleni	λ-cyhalothrin	0.21 (0.14–0.28)[b]	1.00 (0.99–1.00)	0.21 (0.14–0.28)[b]	1.00 (0.99–1.00)
P. alleni	Permethrin	0.58 (0.54–1.39)	0.99 (0.83–0.99)	0.58 (0.54–1.39)	0.99 (0.83–0.99)
P. alleni	Chlorpyrifos	29.3 (19.2–32.9)	0.05 (0.01–0.19)	6.26 (5.12–7.66)[c]	0.87 (0.77–0.91)
P. alleni	Malathion[a]	48,936 (14,984–159,822)[c]	0.00 (0.00–0.00)	32,935 (12,738–85,157)[c]	0.00 (0.00–0.00)
P. alleni	Terbufos	8.89 (7.77–12.8)	0.22 (0.13–0.24)	8.89 (7.77–12.8)	0.22 (0.13–0.24)
B. flumineum	Esfenvalerate	1.62 (0.88–3.00)	0.03 (0.00–0.33)	1.31 (0.36–4.71)[c]	0.12 (0.00–0.86)
B. flumineum	λ-cyhalothrin	0.25 (0.14–0.43)[c]	0.99 (0.99–1.00)	0.25 (0.08–0.82)[c]	0.99 (0.87–1.00)
B. flumineum	Permethrin	6.85 (2.64–17.8)[c]	0.09 (0.00–0.57)	3.10 (0.96–10.0)[c]	0.46 (0.01–0.94)
B. flumineum	Chlorpyrifos	37.0 (24.3–56.5)[c]	0.01 (0.00–0.10)	18.9 (9.06–39.3)[c]	0.20 (0.01–0.67)
B. flumineum	Malathion	2695 (1544–7558)	0.00 (0.00–0.00)	2058 (598–7090)[c]	0.00 (0.00–0.00)
B. flumineum	Terbufos	74.3 (18.8–117)	0.00 (0.00–0.09)	39.4 (11.8–132)[c]	0.01 (0.00–0.14)

[a] The logistic model performed poorly because of low mortality, so a probit model was used instead.
[b] All treatments exhibited either 0% or 100% mortality, so the 95% confidence interval is defined by the consecutive concentrations for which 0% and 100% mortality occurred.
[c] The bootstrap method could not be computed in package "drc", so the 95% confidence interval was approximated using the variance estimate and back-transformed from the log scale used to transform concentrations.

the trials for *P. alleni* and *B. flumineum*, respectively (Tables S4 and S5). When accounting for concentration, pyrethroid insecticides were more toxic than organophosphates, accounting for, on average, 342% and 47% increases (relative to organophosphates) in the probability of mortality for *P. alleni* and *B. flumineum*, respectively. Individual insecticides accounted for 44.3% of the variance in mortality during *P. alleni* trials. Examination of the random effects coefficients suggests that the variation accounted for by individual insecticides was largely driven by malathion presenting a very low risk to *P. alleni* (Table S4).

Pyrethroid concentrations increased risk of mortality per unit increase in concentration more rapidly than did organophosphates (Table 2). In addition, time to death after insecticide exposure was longer for *B. flumineum* than for *P. alleni* (Figs. S1 and S2). Mean time to death was less than 96-h for *P. alleni* exposed to concentrations of esfenvalerate ⩾0.26 μg/L, λ-cyhalothrin ⩾0.28 μg/L, permethrin ⩾2.84 μg/L, chlorpyrifos ⩾32 μg/L and terbufos ⩾17 μg/L (Fig. S1). Only one individual *P. alleni* exposed to the highest concentration of malathion died in less than 96-h. Mean time to death was less than 96-h for *B. flumineum* exposed to

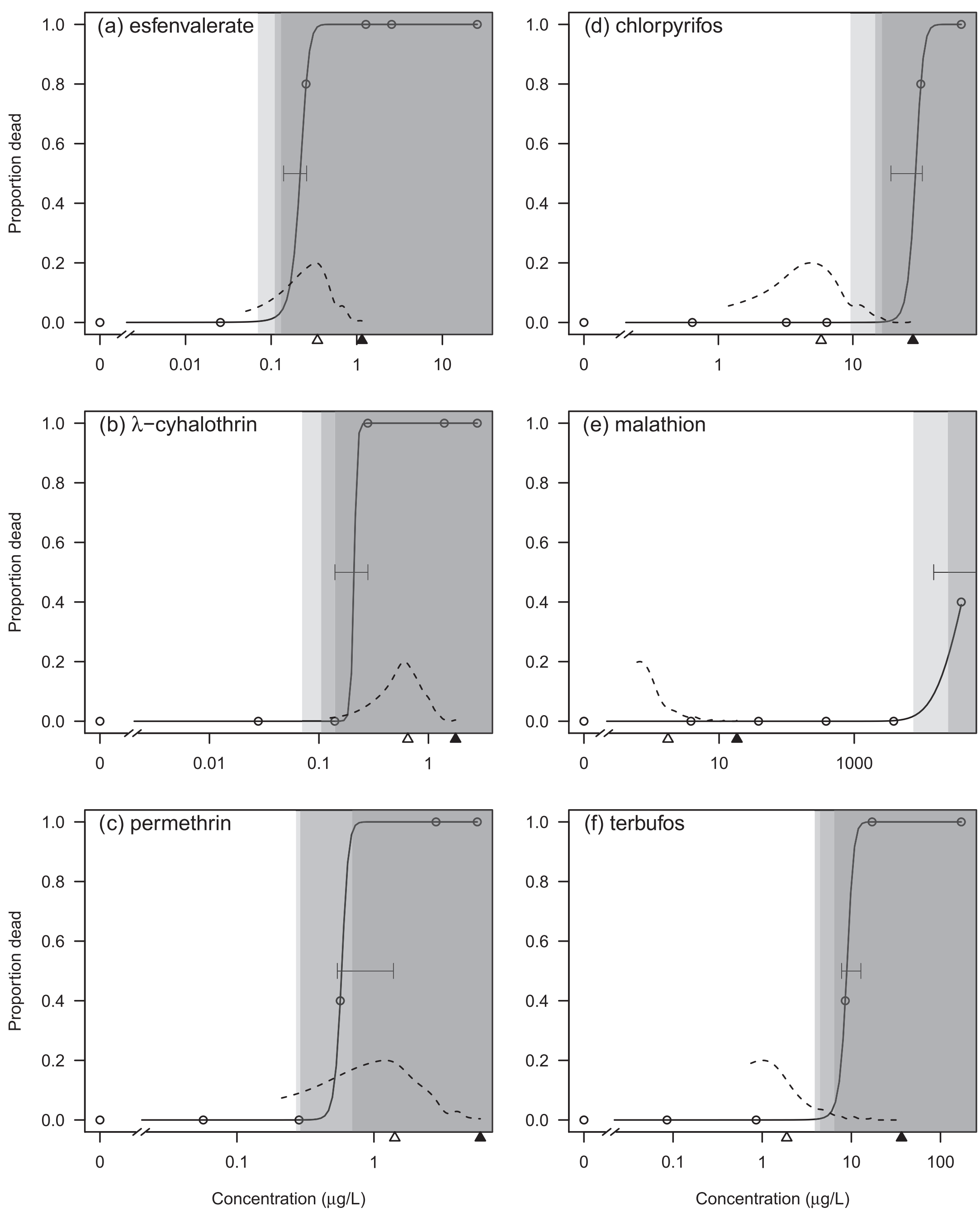

Fig. 1. 96-h dose–response curves for *Procambarus alleni* exposed to multiple concentrations of three pyrethroid (a–c) and three organophosphate (d–f) insecticides. The horizontal bar represents the 95% confidence interval around the LC_{50} estimate with the estimate itself as the point where the confidence interval intersects the curve. The shaded areas represent concentrations above the US EPA's level of concern of $0.5 \times LC_{50}$ (medium gray) for acute high risk to aquatic organisms. The light gray and dark gray regions represent the area of concern calculated from the lower and upper 95% confidence limits of the LC_{50} estimate, respectively. The dashed curves give the kernel density estimates from 150 simulated annual peak environmental concentrations (EECs) in ponds determined from the US EPA Surface Water Contamination Calculator (SWCC) for each insecticide. Thus, those portions of the curve within the shaded areas of each plot indicate simulated peak EECs above the US EPA's level of concern. The open and black triangles along the x-axes indicate the median and maximum EECs, respectively, from the SWCC simulations.

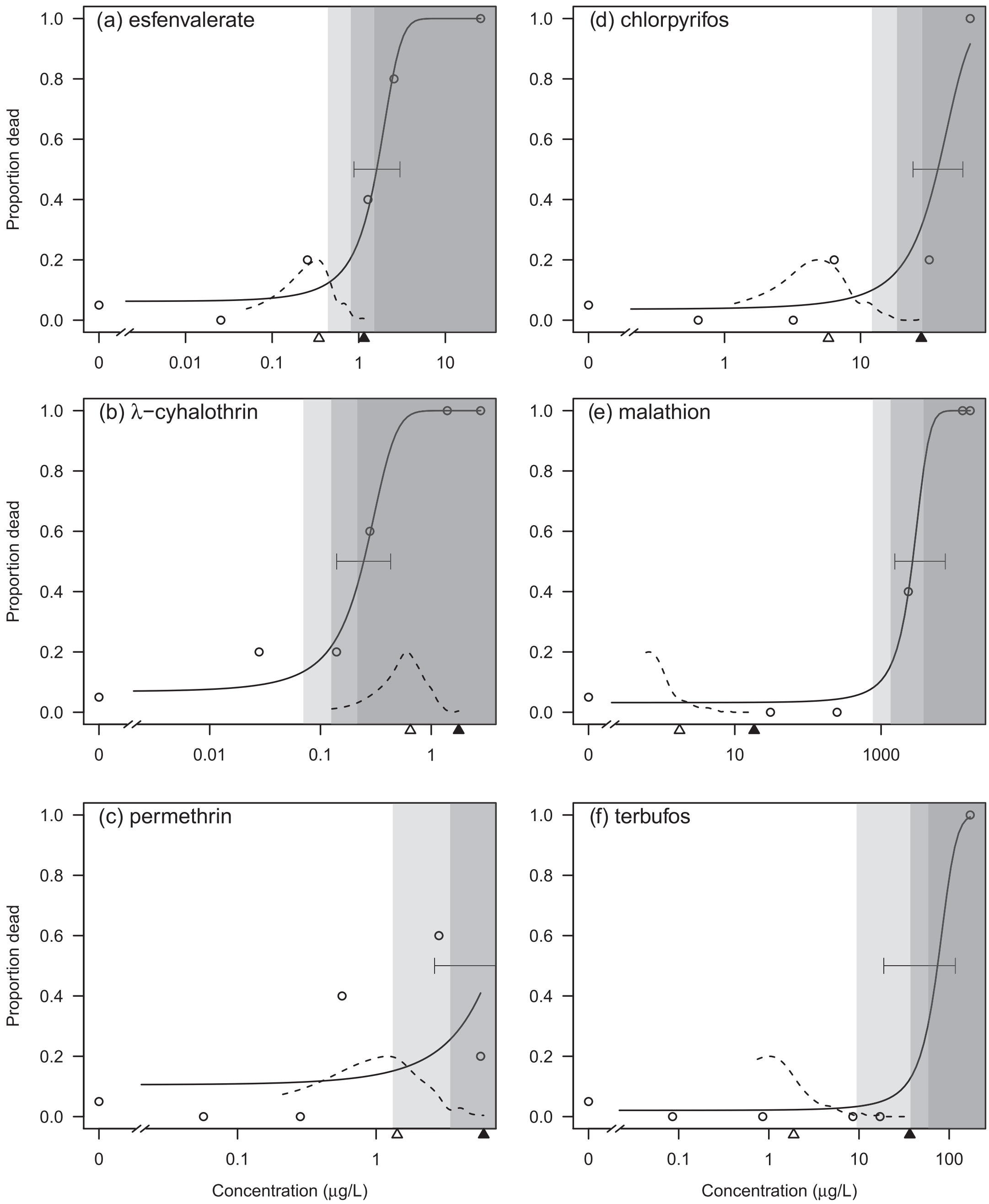

Fig. 2. 96-h dose–response curves for *Belostoma flumineum* exposed to multiple concentrations of three pyrethroid (a–c) and three organophosphate (d–f) insecticides. The horizontal bar represents the 95% confidence interval around the LC_{50} estimate with the estimate itself as the point where the confidence interval intersects the curve. The shaded areas represent concentrations above the US EPA's level of concern of $0.5 \times LC_{50}$ (medium gray) for acute high risk to aquatic organisms. The light gray and dark gray regions represent the area of concern calculated from the lower and upper 95% confidence limits of the LC_{50} estimate, respectively. The dashed curves give the kernel density estimates from 150 simulated annual peak environmental concentrations (EECs) in ponds determined from the US EPA Surface Water Contamination Calculator (SWCC) for each insecticide. Thus, those portions of the curve within the shaded areas of each plot indicate simulated peak EECs above the US EPA's level of concern. The open and black triangles along the *x*-axes indicate the median and maximum EECs, respectively, from the SWCC simulations.

concentrations of esfenvalerate ⩾2.6 µg/L, λ-cyhalothrin ⩾1.4 µg/L, chlorpyrifos ⩾64 µg/L, malathion ⩾13,013 µg/L, and terbufos ⩾171 µg/L (Fig. S2). Mean time to death for *B. flumineum* exposed to 5.68 µg/L permethrin was 192.1-h (±32.1 SE).

4. Discussion

Our results suggest that relative environmental risks among insecticides to aquatic invertebrates can be predicted based on chemical class and/or mode of action. Likely environmental exposures to organophosphates rarely approached levels of concern for either *P. alleni* or *B. flumineum*. In contrast, all three pyrethroid insecticides posed consistently high-risk to *P. alleni*, but not *B. flumineum*. Thus, under similar exposure scenarios, pyrethroid insecticides generally posed a greater risk to these aquatic invertebrates than did organophosphate insecticides. Additionally, toxicity of organophosphates and pyrethroids to *P. alleni* was generally consistent with toxicity levels previously reported for *P. clarkii* and other *Procambarus* species, suggesting that sensitivity to these contaminants is conserved among crayfish species, at least within the genus (Table 1). These results support recent evidence that both phylogeny and the physico-chemical

Table 2
Cox survival analysis for *Procambarus alleni* and *Belostoma flumineum* exposed to multiple concentrations of three pyrethroid (esfenvalerate, λ-cyhalothrin, and permethrin) and three organophosphate (chlorpyrifos, malathion, and terbufos) insecticides for ten days. Positive coefficients (coef) indicate a greater probability of mortality during the study per unit increase in concentration. The hazard ratio is the exponent of the coefficient and indicates the probability of an increase in mortality for every 1 μg/L increase in concentration. For example, the hazard ratio of 1.097 for *P. alleni* exposed to esfenvalerate indicates that every 1 μg/L increase in esfenvalerate increases the probability of mortality during the study increases by 9.7%. The 95% confidence intervals are provided for the hazard ratio.

Species	Chemical	χ^2	*P*	Coef	SE	Hazard ratio	95% CI
P. alleni	Esfenvalerate	12.17	<0.001	0.092	0.024	1.097	(1.046–1.150)
P. alleni	λ-cyhalothrin	24.58	<0.001	1.283	0.251	3.609	(2.206–5.905)
P. alleni	Permethrin	33.93	<0.001	1.007	0.224	2.738	(1.766–4.244)
P. alleni	Chlorpyrifos	30.62	<0.001	0.073	0.015	1.076	(1.045–1.107)
P. alleni	Malathion	8.17	0.004	<0.001	0.683	1.001	(0.263–3.813)
P. alleni	Terbufos	24.58	<0.001	0.026	0.006	1.027	(1.015–1.039)
B. flumineum	Esfenvalerate	21.78	<0.001	0.158	0.036	1.171	(1.091–1.256)
B. flumineum	λ-cyhalothrin	19.60	<0.001	1.035	0.218	2.816	(1.837–4.318)
B. flumineum	Permethrin	2.88	0.090	0.195	0.106	1.215	(0.988–1.495)
B. flumineum	Chlorpyrifos	24.84	<0.001	0.068	0.014	1.070	(1.041–1.100)
B. flumineum	Malathion	39.12	<0.001	<0.001	<0.001	1.000	(1.000–1.000)
B. flumineum	Terbufos	24.12	<0.001	0.028	0.007	1.028	(1.015–1.042)

properties of pesticides can be used to predict the responses of untested species-pesticide pairs (Guénard et al., 2014).

Cox mixed-effects survival analysis indicated that a significant proportion of variation in crayfish survival during our trials could be attributed to differences among individual insecticides. However, examination of the random effects coefficients suggests that this might largely be explained by low mortality associated with exposure to malathion. In particular, because malathion has been heavily used in peninsular Florida for at least the last two decades (Stone, 2013) and is approved for mosquito control with applications directly onto water bodies, it is possible that local populations of *P. alleni* and *B. flumineum* have undergone selection for malathion resistance, as evidenced by the very low toxicity of malathion in this study. While malathion resistance for either species has not been documented, insecticide resistance has been identified in hundreds of arthropod species (Roush and McKenzie, 1987) and mechanisms of resistance to insecticides are becoming increasingly well understood (Taylor and Feyereisen, 1996). However, despite the relative lack of toxicity of malathion to macroarthropods in this study, malathion concentrations of 101 μg/L were sufficient to significantly alter zooplankton community composition and ecosystem properties in mesocosms seeded with organisms from the same area as the current study (Halstead et al., 2014). Likewise, malathion has been observed to have lethal and sublethal effects on a variety of other aquatic organisms (Relyea and Diecks, 2008; Rohr et al., 2008a; USEPA, 2014b; Verbruggen and van den Brink, 2010).

Toxicity of organophosphates or pyrethroids to *B. flumineum* has not been previously reported. In general, *B. flumineum* was less sensitive to insecticides than *P. alleni*. Furthermore, there was less variation in mortality of *B. flumineum* exposed to insecticides within the same class than was observed for *P. alleni*. While the mechanisms behind this pattern remain equivocal, it might be driven by physiological differences in the mechanisms by which these phylogenetically distinct species respond to toxins. Anatomical differences between these species might also mediate exposure to aquatic contaminants. For example, *B. flumineum* acquire oxygen for respiration from trapped air bubbles taken above the surface of the water (Severin and Severin, 1911) whereas crayfish obtain dissolved oxygen directly from the water through their gills (Larimer and Gold, 1961).

The loss of macroarthropods in wetlands has the potential to alter ecosystem functions by removing important functional groups (Wallace and Webster, 1996). As predators, crayfish and giant water bugs have important functions and indirect effects on aquatic communities (Dorn and Wojdak, 2004; Wojdak, 2005). In particular, both crayfish and giant water bugs consume snails, and thus probably regulate snail abundance (Halstead et al., 2014; Hoverman et al., 2014; Sokolow et al., 2014; Wojdak, 2005). Top-down regulation of snails from macroarthropod predators can indirectly mediate the effects that these herbivores have on ecosystem properties in aquatic habitats (Wojdak, 2005) and might even affect disease risk given that increases in snail densities have been demonstrated to increase the prevalence and intensity of trematode infections transmitted by snails to humans and wildlife (Picquet et al., 1996; Rohr et al., 2008b). In fact, native prawns (*Macrobrachium vollenhovenii*) and non-native crayfish (*Procambarus clarkii*) have been identified as promising biocontrol agents to reduce human trematode diseases transmitted by snails (Hofkin and Hofinger, 1992; Mkoji et al., 1999; Sokolow et al., 2014). Identifying insecticides that pose low risk to important functional groups in wetlands, such as macroarthropods, should help to enhance food production without compromising ecosystem function and human health.

Author contributions

NTH and JRR conceived and designed the experiment. NTH conducted the experiment. NTH and DJC performed the statistical analyses. All authors contributed to preparation of the manuscript.

Acknowledgements

This work was supported by grants from the US Environmental Protection Agency (STAR R83-3835 and CAREER 83518801) to JRR. The funding agency had no other involvement in the research or preparation of this manuscript.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chemosphere.2015.03.091.

References

Brock, T.C.M., Van Wijngaarden, R.P.A., Van Geest, G.J., 2000. Ecological risks of pesticides in freshwater ecosystems Part 2: Insecticides. Wageningen.
Cohen, L., Neimark, H., 1980. Schistosoma mansoni: response of cercariae to a thermal gradient. J. Parasitol. 66, 362–364.
Department System Ecotoxicology – Helmholtz Centre for Environmental Research, 2014. SPEAR Calculator.
Dorn, N.J., Wojdak, J.M., 2004. The role of omnivorous crayfish in littoral communities. Oecologia 140, 150–159. http://dx.doi.org/10.1007/s00442-004-1548-9.

Gilliom, R.J., 2007. Pesticides in US streams and groundwater. Environ. Sci. Technol., 3409–3414
Grube, A., Donaldson, D., Kiely, T., Wu, L., 2011. Pesticides Industry Sales and Usage: 2006 and 2007 Market Estimates. Washington, DC.
Guénard, G., Carsten von der Ohe, P., Walker, S.C., Lek, S., Legendre, P., 2014. Using phylogenetic information and chemical properties to predict species tolerances to pesticides. Proc. R. Soc. B-Biol. Sci. 281, 20133239.
Halstead, N.T., McMahon, T.A., Johnson, S.A., Raffel, T.R., Romansic, J.M., Crumrine, P.W., Rohr, J.R., 2014. Community ecology theory predicts the effects of agrochemical mixtures on aquatic biodiversity and ecosystem properties. Ecol. Lett. 17, 932–941. http://dx.doi.org/10.1111/ele.12295.
Henry, T.J., Froeschner, R.C. (Eds.), 1988. Catalog of the Heteroptera, or True Bugs, of Canada and the Continental United States. E.J. Brill, New York, NY.
Hobbs Jr., H.H., 1984. On the distribution of the crayfish genus Procambarus (Decapoda: Cambaridae). J. Crustac. Biol. 4, 12–24.
Hobbs III, H.H., Jass, J.P., Huner, J.V., 1989. A review of global crayfish introductions with particular emphasis on two North American species (Decapoda:Cambaridae). Crustaceana 56, 299–316.
Hofkin, B., Hofinger, D., 1992. Predation of Biomphalaria and non-target molluscs by the crayfish *Procambarus clarkii*: implications for the biological control of schistosomiasis. Ann. Trop. Med. Parasitol. 86, 663–670.
Hoverman, J.T., Cothran, R.D., Relyea, R.A., 2014. Generalist versus specialist strategies of plasticity: snail responses to predators with different foraging modes. Freshw. Biol. 59, 1101–1112. http://dx.doi.org/10.1111/fwb.12332.
Jordan, F., Babbitt, K.J., McIvor, C.C., Miller, S.J., 1996. Spatial ecology of the crayfish *Procambarus alleni* in a Florida wetland mosaic. Wetlands 16, 134–142.
Kesler, D.H., Munns Jr., W.R., 1989. Predation by *Belostoma flumineum* (Hemiptera): an important cause of mortality in freshwater snails. J. North Am. Benthol. Soc. 8, 342–350.
Larimer, J.L., Gold, A.H., 1961. Responses of the crayfish, *Procambarus simulans*, to respiratory stress. Physiol. Zool. 34, 167–176.
McMahon, T.A., Halstead, N.T., Johnson, S.A., Raffel, T.R., Romansic, J.M., Crumrine, P.W., Rohr, J.R., 2012. Fungicide-induced declines of freshwater biodiversity modify ecosystem functions and services. Ecol. Lett. 15, 714–722. http://dx.doi.org/10.1111/j.1461-0248.2012.01790.x.
Meehan, T.D., Werling, B.P., Landis, D.A., Gratton, C., 2011. Agricultural landscape simplification and insecticide use in the Midwestern United States. Proc. Natl. Acad. Sci. U.S.A. 108, 11500–11505. http://dx.doi.org/10.1073/pnas.1100751108.
Mkoji, G.M., Hofkin, B.V., Kuris, A.M., Stewart-Oaten, A., Mungai, B.N., Kihara, J.H., Mungai, F., Yundu, J., Mbui, J., Rashid, J.R., Kariuki, C.H., Ouma, J.H., Koech, D.K., Loker, E.S., 1999. Impact of the crayfish *Procambarus clarkii* on *Schistosoma haematobium* transmission in Kenya. Am. J. Trop. Med. Hyg. 61, 751–759.
Newman, M.C., Unger, M.A., 2002. Fundamentals of Ecotoxicology, second ed. CRC Press, Boca Raton, FL.
Picquet, M., Ernould, J.C., Vercruysse, J., Southgate, V.R., Mbaye, A., Sambou, B., Niang, M., Rollinson, D., 1996. Royal Society of Tropical Medicine and Hygiene meeting at Manson House, London, 18 May 1995. The epidemiology of human schistosomiasis in the Senegal river basin. Trans. R. Soc. Trop. Med. Hyg. 90, 340–346.
R Core Team, 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing.
Relyea, R.A., Diecks, N., 2008. An unforeseen chain of events: lethal effects of pesticides on frogs at sublethal concentrations. Ecol. Appl. 18, 1728–1742.
Relyea, R.A., Hoverman, J.T., 2008. Interactive effects of predators and a pesticide on aquatic communities. Oikos 117, 1647–1658. http://dx.doi.org/10.1111/j.1600-0706.2008.16933.x.
Ritz, C., Streibig, J.C., 2005. Bioassay analysis using R. J. Stat. Softw. 12, 1–22.
Rohr, J.R., Crumrine, P.W., 2005. Effects of an herbicide and an insecticide on pond community structure and processes. Ecol. Appl. 15, 1135–1147. http://dx.doi.org/10.1890/03-5353.
Rohr, J.R., Raffel, T.R., Sessions, S.K., Hudson, P.J., 2008a. Understanding the net effects of pesticides on amphibian trematode infections. Ecol. Appl. 18, 1743–1753.
Rohr, J.R., Schotthoefer, A.M., Raffel, T.R., Carrick, H.J., Halstead, N.T., Hoverman, J.T., Johnson, C.M., Johnson, L.B., Lieske, C., Piwoni, M.D., Schoff, P.K., Beasley, V.R., 2008b. Agrochemicals increase trematode infections in a declining amphibian species. Nature 455, 1235–1239. http://dx.doi.org/10.1038/nature07281.
Rohr, J.R., Raffel, T.R., Halstead, N.T., McMahon, T.A., Johnson, S.A., Boughton, R.K., Martin, L.B., 2013. Early-life exposure to a herbicide has enduring effects on pathogen-induced mortality. Proc. R. Soc. B-Biol. Sci. 280, 20131502.
Roush, R.T., McKenzie, J.A., 1987. Ecological genetics of insecticide and acaricide resistance. Annu. Rev. Entomol. 32, 361–380.
Severin, H., Severin, H., 1911. Habits of Belostoma (= Zaitha) flumineum Say and Nepa apiculata Uhler, with observations on other closely related aquatic hemiptera. J. New York Entomol. Soc. 19, 99–108.
Soderlund, D.M., Clark, J.M., Sheets, L.P., Mullin, L.S., Piccirillo, V.J., Sargent, D., Stevens, J.T., Weiner, M.L., 2002. Mechanisms of pyrethroid neurotoxicity: implications for cumulative risk assessment. Toxicology 171, 3–59.
Sokolow, S.H., Lafferty, K.D., Kuris, A.M., 2014. Regulation of laboratory populations of snails (Biomphalaria and Bulinus spp.) by river prawns, Macrobrachium spp. (Decapoda, Palaemonidae): implications for control of schistosomiasis. Acta Trop. 132, 64–74. http://dx.doi.org/10.1016/j.actatropica.2013.12.013.
Stone, W.W., 2013. Estimated annual agricultural pesticide use for counties of the conterminous United States, 1992–2009: U.S. Geological Survey Data Series 752.
Taylor, M., Feyereisen, R., 1996. Molecular biology and evolution of resistance of toxicants. Mol. Biol. Evol. 13, 719–734. http://dx.doi.org/10.1093/oxfordjournals.molbev.a025633.
Thelin, G.P., Stone, W.W., 2013. Estimation of annual agricultural pesticide use for counties of the conterminous United States, 1992–2009: scientific investigation report 2013–5009. Reston, VA.
Therneau, T.M., 2012. coxme: Mixed Effects Cox Models.
Therneau, T.M., 2013. A package for survival analysis in S.
Tilman, D., Fargione, J., Wolff, B., D'Antonio, C., Dobson, A., Howarth, R., Schindler, D., Schlesinger, W.H., Simberloff, D., Swackhamer, D., 2001. Forecasting agriculturally driven global environmental change. Science 292, 281–284 (80-) http://dx.doi.org/10.1126/science.1057544.
Tilman, D., Balzer, C., Hill, J., Befort, B.L., 2011. Global food demand and the sustainable intensification of agriculture. Proc. Natl. Acad. Sci. U.S.A. 108, 20260–20264. http://dx.doi.org/10.1073/pnas.1116437108.
University of Hertfordshire, 2013. The Pesticide Properties DataBase (PPDB) developed by the Agriculture & Environment Research Unit (AERU), University of Hertfordshire, 2006–2013.
USEPA, 2014a. Technical overview of ecological risk assessment: Risk characterization [WWW Document]. <http://www.epa.gov/oppefed1/ecorisk_ders/toera_risk.htm>.
USEPA, 2014b. ECOTOX Release 4.0 database [WWW Document]. <http://cfpub.epa.gov/ecotox/>.
Van den Berg, H., Zaim, M., Yadav, R.S., Soares, A., Ameneshewa, B., Mnzava, A., Hii, J., Dash, A.P., Ejov, M., 2012. Global trends in the use of insecticides to control vector-borne diseases. Environ. Health Perspect. 120, 577–582. http://dx.doi.org/10.1289/ehp.1104340.
Van Wijngaarden, R.P.A., Brock, T.C.M., van den Brink, P.J., Gylstra, R., Maund, S.J., 2006. Ecological effects of spring and late summer applications of λ-cyhalothrin on freshwater microcosms. Arch. Environ. Contam. Toxicol. 50, 220–239. http://dx.doi.org/10.1007/s00244-004-0249-z.
Verbruggen, E., van den Brink, P.J., 2010. Review of recent literature concerning mixture toxicity of pesticides to aquatic organisms.
Wallace, J.B., Webster, J.R., 1996. The role of macroinvertebrates in stream ecosystem function. Annu. Rev. Entomol. 41, 115–139.
Weber, L., Lodge, D., 1990. Periphytic food and predatory crayfish: relative roles in determining snail distribution. Oecologia 82, 33–39.
Wojdak, J., 2005. Relative strength of top-down, bottom-up, and consumer species richness effects on pond ecosystems. Ecol. Monogr. 75, 489–504.