Effects of River Water and Salinity on the Toxicity of Deltamethrin to Freshwater Shrimp, Cladoceran, and Fish

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Abstract Deltamethrin is a pyrethroid insecticide used extensively to control invertebrate pests on cotton and other crops. It is acutely toxic to nontarget aquatic organisms, but existing toxicity data are mostly from toxicity tests using purified laboratory water that differs greatly from the turbid, high-conductivity rivers in the cotton-growing regions of Australia. The aim of this study was to determine whether the water quality variables conductivity, suspended particles, and dissolved organic matter alter the toxicity of deltamethrin to freshwater crustaceans and a fish. We tested three Australian native species: a cladoceran (Ceriodaphnia cf. dubia), a freshwater shrimp (Paratya australiensis), and larvae of the eastern rainbow fish (Melanotaenia duboulayi). Conductivity of the test solutions ranged from 200 to 750 **1**S/cm, but such changes did not modify the toxicity of deltamethrin to any of the test species. However, the toxicity of deltamethrin to C. cf. dubia and P. australiensis in river water was significantly decreased (1.8-fold to 6.3-fold reduction) compared to that in laboratory water. Variability in the toxicity data limited our ability to detect differences between laboratory and river water for M. duboulayi. Despite reductions in toxicity in natural waters, deltamethrin remained highly toxic [all L(E)C_{50} values ⟍0.26 **1**g/L] to all organisms tested; thus, further investigation of the hazard of deltamethrin is warranted.

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Introduction

Deltamethrin is a pyrethroid insecticide used extensively for the control of invertebrate pests on a variety of crops and turf grass, and for broadscale mosquito control in many countries. In Australia, deltamethrin is an essential component of cotton pest management strategies (Farrell and Johnson 2005), but the proximity of many cotton farms to rivers to facilitate irrigation means that there is a significant contamination risk via spray drift and runoff.

Deltamethrin is acutely toxic to all groups of aquatic organisms (Caquet et al. 2007; Hanson et al. 2007; Solomon et al. 2001; Thomas 2001), but the available toxicity data are mostly derived from studies using purified water and conducted under laboratory conditions that might differ greatly from conditions in natural waters that might be turbid and/or have high conductivity. Because deltamethrin is lipophilic (log K_{ow} = 4.6) and has a very low aqueous solubility, its bioavailability, and hence toxicity, is often reduced by the presence of suspended sediment (Bradbury

and Coats 1989; Kukkonen and Landrum 1998; Muir et al. 1985; Ortego and Benson 1992). In contrast, increasing conductivity can increase the osmotic stress on biota (Hart et al. 1991), leading to enhanced toxicity. Given the strong influence of these variables on toxicity, it is questionable whether toxicity data generated from tests using filtered, low-conductivity laboratory water are an appropriate basis for risk assessment in naturally turbid and saline waters.

The aim of this study was to reveal whether the water quality variables conductivity, suspended particles, and dissolved organic matter alter the toxicity of deltamethrin to freshwater crustaceans and a fish. Specifically, we test the hypotheses that (1) high conductivity will modify the toxicity of deltamethrin and (2) the presence of suspended particles and dissolved organic matter in river water will modify the toxicity of deltamethrin. We tested these hypotheses by exposing three Australian native species to deltamethrin in river water and in laboratory water at varying salinities.

Materials and Methods

Experimental Synopsis

Toxicity tests using all three species were conducted in filtered laboratory culture water, conductivity-adjusted culture water, and Namoi River water. All tests were conducted under laboratory conditions at 23 ± 1°C, with a 16:8 h light–dark regime with a light intensity of 800 lux. Test vessels were covered with plastic film to reduce volatilization and evaporation. Animals were not fed during the tests.

Shrimp and fish tests were run for 96 h, with toxicant solutions renewed after 48 h. Cladoceran tests were run for 48 h without test-solution renewal. The conductivity, pH, turbidity, dissolved oxygen, and temperature of new and old solutions were monitored at the beginning and the end of the test and at each renewal. The ranges of these variables recorded during the tests are given in Table 1.

Each test consisted of seven treatments (i.e., five concentrations of deltamethrin, a control, and a solvent control). The nominal concentrations of deltamethrin used are given in Table 1. The largest volume of solvent used for deltamethrin dosing in each test was also used for the solvent control treatment. Due to difficulties in analyzing low concentrations of deltamethrin in the test solutions, all concentrations are reported as nominal values.

Test Waters and Solutions

Technical grade (C98% a.i.) deltamethrin [(S)-a-cyano-3-phenoxybenzyl (1R,3R)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate; CAS no. 52918-63-5)] was

Table 1 Nominal deltamethrin concentrations and physicochemical variables of test solutions from toxicity tests using P. australiensis, C. cf. dubia, and M. duboulayi

Test water	Nominal test concentrations (μg/L)	pH	Temperature (°C)	Dissolved oxygen (% sat.)	Conductivity (μS/cm)	Turbidity (NTU)
Paratya australiensis						
FW_{200}	C, SC, 0.01, 0.02, 0.06, 0.16, 0.40	7.14–8.28	21.6–22.9	—	178–184	0
FW_{750}	C, SC, 0.01, 0.03, 0.07, 0.19, 0.50	7.94–7.99	23.1–24.6	93–103	710–810	0
RW_{750}	C, SC, 0.01, 0.03, 0.07, 0.19, 0.50	7.78–8.25	23.2–24.2	98–103	694–752	11–20
Ceriodaphnia cf. dubia						
CW_{500}	C, SC, 0.01, 0.02, 0.06, 0.14, 0.36	7.95–8.02	21.6–22.0	—	498–521	0
CW_{500}	C, SC, 0.01, 0.02, 0.06, 0.14, 0.36	7.32–8.61	22.3–23.8	—	510–527	0
CW_{750}	C, SC, 0.01, 0.02, 0.04, 0.10, 0.20	6.76–7.97	22.0–22.4	—	771–797	0
CW_{750}	C, SC, 0.01, 0.02, 0.04, 0.10, 0.20	6.59–7.78	22.5–24.9	—	775–793	0
RW_{750}	C, SC, 0.05, 0.12, 0.28, 0.64, 1.50	7.98–8.33	23.2–23.4	—	816–847	6–28
RW_{750}	C, SC, 0.03, 0.05, 0.10, 0.17, 0.30	7.98–8.50	21.1–23.0	—	794–821	7–29
Melanotaenia duboulayi						
FW_{200}	C, SC, 0.025, 0.05, 0.10, 0.20, 0.40	7.33–7.38	21.1–21.8	—	200–206	0
FW_{200}	C, SC, 0.025, 0.05, 0.10, 0.20, 0.40	7.82–7.88	23.7–24.2	—	208–236	0
FW_{750}	C, SC, 0.025, 0.05, 0.10, 0.20, 0.40	7.90–8.21	21.7–22.7	—	821–903	0
FW_{750}	C, SC, 0.025, 0.05, 0.10, 0.20, 0.40	7.82–7.88	23.7–24.2	—	784–809	0
RW_{750}	C, SC, 0.02, 0.10, 0.20, 0.40, 0.80	7.47–8.10	24.1–25.3	89–111	810–841	25–28
RW_{750}	C, SC, 0.05, 0.11, 0.24, 0.50, 1.20	7.73–7.91	22.5–24.6	—	801–850	11–25

Note: C = control, SC = Solvent Control, — = data not available, equipment malfunction

provided by Rhone-Poulenc. Stock solutions of 40 mg/L and 400 mg/L were prepared in nanograde methanol and stored in the dark at 4°C. Appropriate volumes of the stock solutions of deltamethrin were added to the test solutions by injection below the water surface using a solvent-rinsed borosilicate glass microsyringe.

Shrimp and fish toxicity tests were conducted in fish culture water (FW) that had a conductivity of 200 **1**S/cm (denoted as FW_{200}). Fish culture water was Sydney tap water that had been passed through a mixed-bed filter, activated carbon filter, 5-**1**m filter, a second activated carbon filter, and, finally, an ultraviolet sterilizer. Conductivities were adjusted to 200 **1**S/cm using seawater treated with 1-**1**m filtration and ultraviolet sterilization.

Cladoceran tests were conducted in cladoceran culture water (CW) that had a conductivity of 500 **1**S/cm (denoted as CW_{500}). Cladoceran culture water was filtered Sydney tap water passed through a mixed-bed filter and an activated carbon filter, aged for at least 3 months in holding tanks, and passed through a 5-**1**m filter before use. Conductivities were adjusted to 500 **1**S/cm using the treated seawater described earlier.

Tests on all species were also conducted in Namoi River water (RW) that had a conductivity of *750 **1**S/cm (denoted as RW_{750}) and 25–30 mg total suspended particles/L. The river water used for testing was collected from the Namoi River in July and August 1997 and transported to the laboratory in Sydney. Colloidal aggregates form in river water samples following collection; thus, 1-L aliquots of river water were sonicated (Branson Sonifier 450, at maximum power for 5 min) to break up colloidal aggregates prior to using the water for toxicity testing (Leigh and Hyne 1999). Sonicated aliquots of water were combined and allowed to cool for 1 h before use. Trace level analysis (ppb) by gas chromatography confirmed that the RW_{750} was free from organochlorine and organophosphate pesticides. There were large differences in the conductivity of the laboratory and river waters, so additional toxicity tests were conducted using FW and CW with conductivities adjusted (using treated seawater) to 750 ± 50 **1**S/cm water (FW_{750}, CW_{750}) to match the conductivity of the river water.

Paratya australiensis Toxicity Tests

The glass shrimp, Paratya australiensis, is common throughout southeastern Australia (Walsh and Mitchell 1995), where it inhabits a wide range of ecological conditions (Richardson et al. 2004) but seemingly prefers still littoral habitats (Richardson and Cook 2006). P. australiensis is an omnivorous scavenger-browser that feeds on detrital and particulate material (Richardson et al. 2004). Field-collected P. australiensis are widely used in Australian toxicity tests (e.g., Abdullah et al. 1994; Daly et al. 1992; Hose and Wilson 2005; Olima et al. 1997; Phyu et al. 2005).

Paratya australiensis collected from the Namoi River appeared stressed after the 6-h road trip to the laboratory and so were not used. Instead, P. australiensis were collected from the upper Colo River (33° 26′ S, 150° 51′ E) located approximately 100 km to the north-west of Sydney, Australia. The site of collection is immediately downstream of the Wollemi National Park. This river is a "Protected River" indicating it is one of the least polluted rivers in NSW, Australia (Birch et al. 1998; NSW Department of Environment and Planning 1983). The animals were acclimated to test conditions for 7 days prior to use in toxicity tests. P. australiensis were fed Seramin® tropical flake food during the acclimation period but were not fed for 24 h prior to or during testing. Animals used for the tests were between 1.5 and 2 cm long.

Toxicity tests were conducted in FW_{200}, FW_{750}, and RW_{750} waters. Tests were conducted in 1-L beakers that contained 800 mL of test solution. Three replicates were used per treatment, and five P. australiensis were used per replicate. The exception was the FW_{200} test, in which only two replicates were used per treatment due to a shortage of test animals. Tests were aborted if control mortality exceeded 10%. Due to a shortage of test animals, tests were not repeated.

Ceriodaphnia cf. dubia Toxicity Tests

Ceriodaphnia cf. dubia is morphologically similar but not identical to the US species of the same name (Julli et al. 1990). Cladocerans are important grazers of algae and bacteria and are major prey items for many species of vertebrates and invertebrates. Cladocerans were cultured using the methods set out in Warne (1995) which differ from the US Environmental Protection Agency (EPA (US EPA1994) method by using mass cultures and the amount and type of food.

Toxicity tests were conducted in CW_{500}, CW_{750}, and RW_{750} waters. Duplicate tests were performed in each water type. Tests were conducted using 200 mL of water in 250-mL glass beakers. Each treatment had four replicates and each replicate contained five cladocerans. The test end point was immobilization after 48 h, which was defined as the failure to move within 15 s of the beaker being gently swirled (Day and Maguire 1990).

Melanotaenia duboulayi Toxicity Tests

The eastern rainbowfish Melanotaenia duboulayi (Melanotaeniidae) is an Australian native fish that is common in vegetated natural streams in subtropical eastern

Queensland (McDowall 1996). Rainbow fish are foragers that feed on algae and terrestrial and aquatic insects (King 2005). Consequently, they play an important role in the riverine food chain (Llewellyn 1971) by regulating algal biomass and nuisance insect larvae populations as well as being a source of food for larger fish.

Melanotaenia duboulayi larvae were bred from 4-year-old, fourth-generation stock that were originally captured at Wilsons River near Lismore NSW in 1989. Fifty adult fish were maintained in a 1600-L fiberglass tank (the "brood tank") with a 16:8 h light–dark regime. All fish culturing and toxicity tests used FW, which was prepared as described earlier.

One day prior to spawning, the water in the brood tank was warmed from 23°C to 26°C over a 4-h period and conductivity was increased from 175 to 750 ± 50 μS/cm using clean seawater treated with 1-μm filtration and ultraviolet irradiation. These conditions were maintained for 24 h during which time spawning occurred.

Eggs were laid onto an artificial substrate consisting of nylon mesh strips fixed to an acrylic rope and weighted with bags of aquarium gravel. After spawning, the artificial substrate supporting the eggs was removed and dipped in a malachite green solution (0.13 mL/L brood tank water) for 30 s to minimize fungal infection. The mesh was then rinsed in water from the brood tank and transferred to the hatching tank, which had the same dimensions and water as the brood tank. Hatched larvae (≤1 day old) were collected in a net and gently transferred to a partially submerged glass beaker. The larvae were pooled and then randomly allocated to test replicates.

Toxicity tests were conducted in FW_{200}, FW_{750}, and RW_{750} waters. Duplicate tests were performed in each water type. Tests were conducted in 100-mL beakers containing 80 mL of test solution. Tests using FW had 4 replicates per treatment each containing 10 larvae. Tests using RW_{750} consisted of 3 replicates each containing 10 larvae. Only three replicates were used due to the limited supply and logistics of obtaining river water. The test end point was mortality and was defined as the absence of a heartbeat or blood circulation when examined under a dissection microscope (Holdway et al. 1994).

Statistical Analysis

Concentration response curves were estimated by fitting a nonlinear two-parameter log-logistic regression function with a binomial error structure using the DRC package (Ritz and Streibig 2005) in R version 2.5.0 (R Development Core Team 2007). The model parameters b (slope) and e [inflection point = $L(E)C_{50}$ = median lethal (effective) concentration] were estimated using maximum likelihood, with starter values determined by the programs self-starter function. Lethal (effective) concentration values affecting 10% of the population ($L(E)C_{10}$ values) were extrapolated from the fitted curve.

The DRC package allows multiple curves to be fit simultaneously and makes it possible to compare concentration response curves from independent tests using mixed-model nonlinear regression analysis (Nielsen et al. 2004). The method assumes that any for particular concentration response curve, the parameters (b and e) are fixed but might vary from test to test due to some random effect; that is, the parameters for any test represent the "average" parameters for that curve plus or minus a test-specific error. In this way, including both fixed and random effects in our nonlinear regression model makes it possible to account for variation among repeated tests. Thus, the full nonlinear regression model is one that includes random effects; that is, individual parameters are determined for each individual response curve.

Under a null hypothesis of no difference between two or more response curves, the parameters of the curves can be considered to be drawn from the same population; that is, the random effects that represent the intertest variability should be negligible. Therefore, replacing the individual parameters with a common parameter (thereby removing the random effects) should not affect the residual deviance of the log-logistic model. The test for reduction of a full model (with random effects) to a simpler model (without random effects) can be tested using a likelihood ratio test (Nielsen et al. 2004).

Slope, $L(E)C_{10}$, and $L(E)C_{50}$ values were estimated from the individual and combined curves. These toxicity values and regression parameters were compared using t-tests with Satterthwaite's (1946) method of accounting for nonequal variances. Bonferroni correction was used to adjust the significance level (a) to correct the experimentwise type 1 error rate (Quinn and Keough 2002). Accordingly, significance levels for pairwise comparisons were 0.017, 0.017, and 0.005 for the shrimp, cladoceran, and fish tests, respectively.

Results

Paratya australiensis Tests

There was no difference in the toxicity of deltamethrin in the FW tests (i.e., FW_{200} and FW_{750}) at different conductivities. Concentration response curves were similar among the FW tests, and there was no significant difference ($p > 0.017$) in the slope, 96-h LC_{10} or LC_{50} values (Table 2).

The toxicity of deltamethrin was reduced in the RW_{750} test compared to the tests in FW. The slope of the RW_{750}

Table 2 Concentration response curve parameters and toxicity values for tests of the toxicity of deltamethrin to Australian native biota in laboratory fish water (FW) and laboratory cladoceran water (CW) at varying salinities, and river water (RW)

Test type	Test No.	Duration (h)	Full model				Reduced model		
			Slope	L(E)C_{10} (μg/L)	L(E)C_{50} (μg/L)		Slope	L(E)C_{10} (μg/L)	L(E)C_{50} (μg/L)
Paratya australiensis									
FW_{200}	1	96	−2.396[a]	0.013[a] (0.004)	0.032[a] (0.007)				
FW_{750}	1	96	−2.004[a]	0.012[a] (0.004)	0.037[a] (0.007)				
RW_{750}	1	96	−3.896[a]	0.037[b] (0.008)	0.065[b] (0.008)				
Ceriodaphnia cf. dubia									
CW_{500}	1	48	−2.342	0.011 (0.003)	0.029 (0.004)	⌊	−1.823[a]	0.008[a] (0.002)	0.027[a] (0.003)
CW_{500}	2	48	−1.479	0.006 (0.002)	0.025 (0.005)				
CW_{750}	1	48	−2.061	0.007 (0.002)	0.020 (0.003)	⌊	−2.031[a]	0.007[a] (0.001)	0.020[a] (0.002)
CW_{750}	2	48	−2.002	0.007 (0.002)	0.021 (0.003)				
RW_{750}	1	48	−4.891	0.056 (0.010)	0.088 (0.010)	⌊	−3.413[b]	0.044[b] (0.006)	0.084[b] (0.007)
RW_{750}	2	48	−2.983	0.040 (0.007)	0.083 (0.009)				
Melanotaenia duboulayi									
FW_{200}	1	96	−1.999	0.071 (0.011)	0.212 (0.022)		−1.999[ab]	0.071[ab] (0.011)	0.212 [ab] (0.022)
FW_{200}	2	96	−3.417	0.122 (0.014)	0.232 (0.017)		−3.417[b]	0.122[a] (0.014)	0.232[ab] (0.017)
FW_{750}	1	96	−2.758	0.061 (0.008)	0.135 (0.011)		−2.758[ab]	0.061[b] (0.008)	0.135[ac] (0.011)
FW_{750}	2	96	−2.809	0.094 (0.015)	0.253 (0.026)		−2.809[b]	0.094[a] (0.015)	0.253[b] (0.026)
RW_{750}	1	96	−1.563	0.046 (0.010)	0.186 (0.022)	⌊	−1.240[a]	0.032[b] (0.007)	0.187[c] (0.018)
RW_{750}	2	96	−1.013	0.021 (0.008)	0.187 (0.030)				

Note: Values in parentheses indicate standard errors. Common superscript letters in each column and test denote nonsignificant differences between parameters. " ⌊ " indicates data pooled to create a reduced model for the specific water type and species

concentration response curve was not significantly different ($p \geq 0.017$) from those of the FW tests, but the 96-h LC_{10} and LC_{50} values were both significantly greater ($p < 0.017$) than the corresponding values from the FW tests (Table 2). The ratio of 96-h LC_{50} values from the FW_{750} and RW_{750} tests suggests a 1.8-fold reduction in the toxicity in RW. The same ratio based on 96-h LC_{10} values suggests a 3.1-fold reduction in toxicity.

Ceriodaphnia cf. dubia Tests

Ceriodaphnia cf. dubia was highly sensitive to deltamethrin, with 48-h EC_{50} values B0.09 μg/L across all tests (Table 2). Duplicate CW_{500}, CW_{750}, and RW_{750} tests could each be pooled without a significant change in the residual deviance of the model ($p = 0.50$). The slope, 48-h EC_{10} or EC_{50} values from the reduced model for the CW_{500} and CW_{750} tests were not significantly different ($p \geq 0.017$; Table 2) and indicate that there was no difference in the response of C. cf dubia to deltamethrin at different conductivities. However, the toxicity of deltamethrin in CW_{750} tests was significantly greater ($p < 0.017$) (lower EC_{10} and EC_{50} values) than in RW_{750} that had the same conductivity (Table 2). The RW_{750} curve also had a steeper slope than the CW tests (Table 2). The ratio of 48-h EC_{50} values from the CW_{750} and RW_{750} tests suggests a 4.2-fold reduction in toxicity in river water. The same ratio based on EC_{10} values suggests a 6.3-fold reduction in toxicity.

Melanotaenia duboulayi Tests

Deltamethrin was acutely toxic to M. duboulayi although the concentration response curves and parameters were variable (Table 2). Consequently, replicate FW_{200} and FW_{750} tests could not be collapsed into single curves without causing a significant change to the residual deviance of the model. However, a single curve could be created for the RW_{750} tests with the likelihood ratio test being not significant ($p \geq 0.05$). There were no significant differences ($p \geq 0.005$) among the slope parameters of the FW tests, although there were significant differences ($p < 0.005$) in the 96-h LC_{50} and LC_{10} values within and between the FW_{200} and FW_{750} tests. As a result, we could not conclude whether there was a significant effect of salinity on deltamethrin toxicity to rainbowfish.

The reduced-model RW_{750} curve was less steep than the slopes of the FW curves, but not all comparisons between the slopes of the RW_{750} and the FW curves were significant ($p \geq 0.005$; Table 2). The 96-h LC_{10} and LC_{50} values for the reduced-model RW_{750} curve were generally lower than those of the FW tests but not always significantly so ($p \geq 0.005$). Consequently, it could not be concluded

whether the presence of suspended sediment affected deltamethrin toxicity to M. duboulayi.

Discussion

Changes in the conductivity of river water did not affect the toxicity of deltamethrin to P. australiensis or C. cf. dubia in this study. Similar in situ tests in which the conductivity of river water increased from 200 to 600 **1**S/cm also exhibited no change in deltamethrin toxicity to P. australiensis (Thomas 2001). For M. duboulayi, there was no conclusive evidence that conductivity affected deltamethrin toxicity. The range of salinities tested (i.e., 200 to 800 **1**S/cm) is almost identical (i.e.. 175–750 **1**S/cm) to that used to induce spawning in laboratory M. duboulayi cultures. We therefore expect that such changes are within the range of tolerance for this species. Furthermore, Dyer et al. (1989) reported no significant difference in the toxicity of fenvalerate (a closely related pyrethroid) to bluegill sunfish (Lepomis macrochirus) as conductivity increased from 431 to 735 **1**S/cm.

The range of conductivities we recorded during the tests (178–903 **1**S/cm; Table 1) approximates the range recorded in the Namoi River (146–894 **1**S/cm) during the year of the study (Wood 1997). Conductivity in the Namoi River is decreased by the release of water for irrigation from an upstream reservoir (Gordon 2000). Thus, our results suggest that the use of deltamethrin during periods of low flow when riverine conductivity is high will not affect its toxicity to nontarget organisms.

More importantly, toxicity of deltamethrin to the crustaceans was reduced in RW that contained suspended and dissolved organic matter relative to that in clean laboratory water. Day (1991) showed that the toxicity of deltamethrin to Daphnia magna was reduced with increasing dissolved organic carbon concentration and Karim et al. (1985) also showed a reduction in the toxicity of deltamethrin to a range of invertebrate species in natural waters compared to laboratory water. Despite the protective capacity of natural waters, crustaceans were among the most affected organisms in outdoor pond communities (Caquet et al. 1992).

The reduced toxicity of deltamethrin in RW is likely to have occurred through decreased bioavailability (Yang et al. 2006a, 2006b). The adsorption of hydrophobic organic contaminants, including pyrethroids, to organic matter can significantly reduce their bioavailability without saturating the sorbent (Garbarini and Lion 1986; House and Ou 1992). Between 60% and 80% of the deltamethrin added to test solutions might become bound to dissolved organic carbon (Day 1991). Reductions in deltamethrin toxicity of between 2.5-fold and 13-fold have been reported as a result of sorptive processes (Day 1991; Yang et al. 2006b). Our results were similar, with 1.8-fold to 6.3-fold reductions in deltamethrin toxicity in RW. Interestingly, for P. australiensis and C. dubia, the reductions in toxicity were greater when calculated using $L(E)C_{10}$ than $L(E)C_{50}$ values, emphasizing the greater slopes of the RW response curves compared to those of the clean laboratory waters.

In addition to sorptive processes, the presence of organic matter might increase the degradation rate of deltamethrin via humus-mediated photosensitization, as has been reported for other pyrethroid insecticides (Jensen-Korte et al. 1987). Alternatively, the microorganism populations associated with organic matter in water (Rao et al. 1991) might increase the rate of loss of deltamethrin due to bacterial degradation of the compound (e.g., Das and Mukherjee 1999; Haider 1983; L'Hotellier and Vincent 1986). Irrespective of the mechanisms, this study provides further evidence that the toxicity of deltamethrin to aquatic invertebrates is reduced in RW that contains suspended particulates and dissolved organic matter.

The decreased toxicity of deltamethrin in RW comes despite the likelihood that both P. australiensis and C. cf. dubia will ingest the pesticide-sorbed particles. The reduced toxicity of deltamethrin in RW therefore indicates that the pesticide is strongly sorbed to the suspended particulates and that ingestion is a relatively minor route for toxicity. This supports the work of Adams et al. (1985) who suggest that the dissolved fraction of hydrophobic compounds will be more available to invertebrates than sorbed fractions.

We could not conclude either way whether sensitivity of M. duboulayi differs in river and laboratory waters, although we expected to see a decline in the toxicity of deltamethrin in RW. Ghillebaert et al. (1996a, 1996b) showed that the addition of humic acids (i.e., dissolved organic matter) such as found in natural waters reduced the effects of deltamethrin on the mobility and mortality of Cyprinus carpio (carp) larvae. Karim et al. (1985) also showed a reduction in the toxicity of deltamethrin to fish in natural waters compared to laboratory water. However, apart from reducing bioavailablility, dissolved organic matter, specifically humic acids, might have a protective effect on fish. Varanka et al. (2002) showed that tannic acids had cytoprotective capacity that reduced ultrastructural damage caused by deltamethrin to hepatocytes in C. carpio.

The toxicity of deltamethrin to M. duboulayi was not obviously reduced in RW_{750} as seen for the crustaceans. Given the likely reduction in bioavailability of deltamethrin in the RW, our results suggest an alternate cause of toxicity in the RW_{750} tests. Indeed, our greater than expected toxicity in RW_{750} tests might be an artifact of the suspended particles, which alone can be toxic to fish, particularly larval stages (e.g., Isono et al. 1998;

McFarland and Peddicord 1980). We can discount natural temporal fluctuations in larvae and/or parental animal health as a cause of the greater than expected toxicity in the RW_{750} tests because control mortality rates were consistently low. It is possible, however, that small changes in the age (which might have varied by several hours) of the test organisms between the tests contributed to the variability of the concentration response curves and toxicity estimates. This might also account for the inconsistencies in toxicity estimates in the FW_{750} tests.

Deltamethrin was acutely toxic to all test species, with all L(E)C_{50} values below 0.26 μg/L. Both crustacean species were more sensitive to deltamethrin than the rainbowfish larvae, which is consistent with the outcomes of the risk assessment by Solomon et al. (2001) that identified crustaceans as the most sensitive of the aquatic fauna tested. However, the status of crustaceans as the most sensitive to deltamethrin might be challenged by recent research (Beketov 2004) showing a number of mayfly larvae and a damselfly nymph (96-h LC_{50} < 0.015 μg/L) were more sensitive to deltamethrin than the cladoceran D. magna (96-h LC_{50} = 0.03 μg/L), and the crustaceans tested in this study. Importantly, Beketov (2004) used filtered, particle free pond water. He attributed the high sensitivity of the test species, in part, to the lack of suspended particles in the water.

Compared to other crustaceans, our 96-h LC50 values for P. australiensis in FW (0.032–0.065 μg/L) were close to an order of magnitude lower than the 96-h LC50 for the marine prawn Penaeus duorarum (0.35 μg/L; L'Hotellier and Vincent 1986). For C. cf. dubia, our 48-h LC_{50} values (0.02–0.09 μg/L) were at the low end of the range reported in the literature for other cladocerans (0.03–0.5 μg/L; Barata et al. 2006; Beketov 2004; Day and Macguire 1990; L'Hotellier and Vincent 1986).

The 96-h LC_{50} values (i.e., 0.135–0.253 μg/L) determined for M. duboulayi indicate that this species is among the most sensitive fish species tested to date. Among the more sensitive species are freshwater catfish Clarius gariepinus (96-h LC_{50} = 0.004 μg/L; Datta and Kaviraj 2003), rainbow trout Onchorynchus mykiss (96-h LC_{50} = 0.39 μg/L; L'Hotellier and Vincent 1986), juvenile M. duboulayi (96-h LC_{50} = 0.73–1.86 μg/L; Thomas 2001), eels Anguilla anguilla (96-h LC_{50} = 1 μg/L; Nemcsok et al. 1999), and juvenile C. carpio (96-h LC_{50} = 1.65 μg/L; Calta and Ural 2004). Our 96-h LC_{50} values for M. duboulayi (i.e., 0.135–0.253 μg/L) are clearly lower than many of the LC_{50} values for fish that are reported in the literature. This result is unsurprising because we have used very young (<1 day old) larvae, and the early life stages of M. duboulayi are the most sensitive to environmental stress (Thomas 2001). Our results are similar to those of Köprücü and Aydin (2004), who reported a 48-h LC_{50} for C. carpio larvae of 0.074 μg/L, showing that they too are more sensitive than adult fish (96-h LC_{50} = 4.47 μg/L; Calta and Ural 2004).

The toxicity of deltamethrin reported in this study is probably underestimated given the likely adsorption of deltamethrin onto glassware (Day 1991; Sharom and Solomon 1981) and our use of nominal concentrations. Day and Kaushik (1987) suggested that toxicity of pyrethroids recorded in bioassays in glass beakers are underestimated by up to one-third when concentrations are recorded as nominal rather than measured concentrations. However, the proportion of pyrethroid sorbed to glass decreases with increasing organic carbon concentration in the water (Day and Kaushik 1987). Therefore, the laboratory-water tests are likely to underestimate toxicity (relatively) more than river-water tests, further emphasizing the significant mitigating influence of river water on toxicity.

Our results suggest that increasing the conductivity of test waters does not alter the toxicity of deltamethrin to C. cf. dubia or P. australiensis. However, the reduced toxicity of deltamethrin in RW suggests that the presence of suspended particulates and dissolved organic matter might mitigate the toxicity of this pesticide to these crustaceans. High variability precluded detecting changes in deltamethrin toxicity to M. duboulayi due to either conductivity or suspended particulates and dissolved organic matter in RW. Overall, deltamethrin was highly toxic [L(E)C_{50} values <0.26 μg/L] to all organisms tested and thus further investigation of the hazard of deltamethrin is warranted.

Acknowledgments The authors gratefully acknowledge the financial support of the Land and Water Resources Research and Development Corporation, Australia. We also thank the NSW DECC for use of laboratories.

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