TOXICITY OF MALATHION AND ROUNDUP® TO THE SAN DIEGO FAIRY SHRIMP

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ABSTRACT: The purpose of this study was to measure the toxicity of malathion and glyphosate (the active ingredient in Roundup®, to San Diego fairy shrimp (*Branchinecta sandiegonensis*). The endemic San Diego fairy shrimp has been listed as endangered due to habitat loss and degradation of southern California vernal pools. The risk to fairy shrimp from the potential impact of herbicides and insecticides sprayed near pools has not been assessed. The LC_{50} for malathion was 24.5 mg/L (estimated active ingredient in Dexol Home Insect Killer). Based on possible environmental concentrations of malathion, it is unlikely to pose a threat to individual San Diego fairy shrimp. The LC_{50} for glyphosate was 11.8 µg/L (estimated active ingredient in Monsanto Ready-To-Use Weed & Grass Killer). Environmental concentrations of glyphosate are usually well below concentrations that show acute toxicity to other crustaceans, but assuming a 1/20 safety margin as recommended by the U.S. Environmental Protection Agency, they may be above safe concentrations estimated from toxicity data measured here for *B. sandiegonensis*. However, concentrations in pools are not known, so caution is recommended when making management decisions about these pesticides. We recommend that spraying be avoided in pool watersheds in the rainy season, and that future testing be done on different chemicals, other organisms, and possible sublethal effects on shrimp.

Key words: acute toxicity, *Branchinecta sandiegonensis*, glyphosate, malathion, Southern California vernal pools, San Diego fairy shrimp.

2002-2003 TRANSACTIONS OF THE WESTERN SECTION OF THE WILDLIFE SOCIETY38/39:13-21

Southern California vernal pools are freshwater ephemeral wetlands. The vernal pools on the coastal mesas of San Diego County contain distinctive floral and faunal communities including a diverse crustacean assemblage of anostracans, ostracods, copepods, and cladocerans. In San Diego County, >98% of vernal pools have been destroyed by development (Oberbauer 1990). Several plant and animal species using these pools as habitat have been listed as endangered including the endemic San Diego fairy shrimp (Federal Register 1997). Conservation and restoration activities are currently underway to protect the remaining vernal pools and their inhabitants (U.S. Fish and Wildlife Service 1998) but these efforts are limited by lack of information on the importance of some threats to the pools.

Although obliteration by development is the most pressing problem for pool conservation, degradation also is occurring from near-by construction activity, agriculture, and other human use of the habitat. Proximity to roads exposes pools to motor exhaust and tire wear, which contain heavy metals as well as hydrocarbons. Downwind drift or accidental overspray of insecticides such as malathion that are being used for mosquito or fruit fly control near pools also may contaminate them. Herbicides such as Roundup® are also sprayed to control weeds along the highways in areas close to pools.

Effects on anostracans from contaminants are not as well known as for other crustacean taxa such as cladocerans. Compared to cladocerans, the sensitivity of anostracans to malathion seems to be very low (Calleja et al. 1994, Crisinel et al. 1994, Lahr et al. 2001, Verschueren 2001), while their sensitivity to Roundup® may be higher (Fochtman et al. 2000). Little other information exists concerning the effects of insecticides, herbicides and other pollutants on the flora and fauna of temporary pools (Lahr 1997). There are no toxicity data for any chemical for *B. sandiegonensis*.

The purpose of this study was to measure the toxicities (as 24-hr LC_{50}) of malathion and Roundup® to *B. sandiegonensis*, to estimate the risk to this species from these chemicals, and to provide guidelines for their use around pools. In addition, we wanted to determine if another species could be used as a surrogate for *B. sandiegonensis*, which would eliminate the need to use the endangered species in further toxicity assays.

MATERIALS AND METHODS

Test Chemicals

Malathion (O,O-dimethyl S-(1,2-dicarbethoxyethyl) dithiophosphate) is an organophosphate insecticide that is widely used to control mosquitoes and fruit flies. The mode of action is as an acetylcholinesterase inhibitor. It

is soluble in water up to 145 mg/L at 20°C (Verschueren 2001). However, malathion degrades quickly in water, with an estimated half-life of 36 h at pH of 8.0 at 27°C, and in soil (Verschueren 2001). It may not be detectable in surface waters even after local application (Kuivila and Foe 1995), but is toxic enough in the laboratory that exposure in the environment is a concern.

The active ingredient in Roundup® (Monsanto) is in the isopropylamine salt of glyphosate, N-(phosphonomethyl)glycine. Glyphosate is a broad-spectrum herbicide commonly used to control weeds and may be obtained commercially under several brand names including Rodeo®, Vision®, and Rondo®. Its toxic action is by inhibiting 5-enolpyruvylshikimate-3-phosphate synthase, which prevents synthesis of essential aromatic amino acids necessary for synthesis of proteins. Glyphosate is soluble in water up to 10,000 mg/L at 25°C (Verschueren 2001). No substantial reduction in glyphosate concentration occurred over many days in water-only assays performed by Servizi et al. (1987) and Anton et al. (1993). However, in water-sediment systems, concentrations are reduced rapidly because the chemical sorbs to soil and organic matter (Rueppel et al. 1977). Estimates of the half-life of the chemical in water-sediment systems range from 1.5 days (Goldsborough and Beck 1989) to 28 days (Rueppel et al. 1977). Soil microorganisms degrade the chemical rapidly and completely once it is adsorbed (Rueppel et al. 1977).

We tested the commercial formulations because these are sometimes more toxic than the active ingredients alone (Servizi et al. 1987, Pereira et al. 2000) and it is the commercial formulations that the organisms are exposed to in nature. We tested a commercially available malathion formulation from Dexol® Industries, Home Insect Killer (EPA Registation Number 192-96), which was a 50:50 malathion to "inert" ingredient mixture. We tested a commercial formulation of Roundup® from Monsanto, Ready-To-Use Weed & Grass Killer (EPA Registration Number 71995-18), which was 1:99 glyphosate to "inert" ingredients. LC_{50} values are reported as nominal concentration of the commercial formulation used and as estimated concentration of active ingredient, based on active ingredient concentration in the commercial formulation.

Test Organisms

We tested two potential surrogate species in addition to *B. sandiegonensis*, the cladoceran *Ceriodaphnia dubia* and the anostracan *Thamnocephalus platyurus*. *Ceriodaphnia dubia* co-occurs with *B. sandiegonensis* in San Diego vernal pools (Ebert and Balko 1987, Simovich 1998) and is commonly used in toxicity tests (e.g., Masters et al. 1991, Oris et al. 1991, Janssen and Persoone 1993, Persoone and Janssen 1993). *Thamnocephalus platyurus* is taxonomically closer to *B. sandiegonensis* than the cladoceran and thus is expected to have more similar sensitivity to chemicals (Mayer and Ellersieck 1986). It is also available in a commercial toxicity testing kit which is convenient to use.

Branchinecta sandiegonensis were obtained by hydrating surficial soil taken from a vernal pool in San Diego. Soil was placed in 10-L glass aquaria and covered with dechlorinated tapwater to a depth of 5 cm. Tanks were incubated at a temperature of 10°C under constant light and examined daily for hatchlings. Larval shrimp were removed from the tanks by pipette when they were detected (at 24-48 hr old) and held in plastic cups containing simulated pond water. Simulated pond water was made by rinsing dechlorinated and aerated tap water through soil from the pool where the shrimp were collected. Dechlorination was done using sodium thiosulfate. Larval shrimp were tested within 2 hours of collection.

A *Ceriodaphnia dubia* culture was obtained from Aquatic Biosystems (Fort Collins, Colorado). The culture was maintained in 1-L plastic jars under a 12-hour light/dark cycle at a temperature of approximately 20°C throughout the experiment. We used 8:2 Nanopure water and Perrier® mineral water media that had been aerated for at least 24 hours (U.S. Environmental Protection Agency 1993). *Ceriodaphnia dubia* were fed once daily with 1 ml of yeast/Cerophyl®/trout chow (YCT) solution from Aquatic Biosystems, and 1 ml of the green algae *Selenastrum capricornutum* (Carolina Biological Supply Co., Burlington, NC) per 1-L jar (Cooney et al. 1992, U.S. Environmental Protection Agency 1993). The algal culture was maintained in Evian® spring water and fed once every 2 weeks with nutrient medium (Carolina Biological Supply Co., Burlington, NC). The *C. dubia* culture medium was changed and cultures were thinned by half every other day unless we were preparing to perform an assay. In this case, neonates were isolated from adults daily using a mesh aquarium net that only allowed neonates through. After the second day of separating adults and neonates, we knew that all of the neonates isolated were less than 24 hr old. These were held for another 24 hours before testing so that they would be of the same age as shrimp larvae, 24-48-hr old.

Thamnocephalus platyurus cysts were obtained as part of the Thamnotox F® test kit (Creasel Ltd., Belgium). Cysts were hatched and larvae were tested according to kit directions using the equipment supplied. The "standard freshwater" culture medium included in the kit was reconstituted according to the directions and stored at a temperature of 4°C in amber bottles for the duration of the experiment.

Toxicity Assays

Twenty-four hour acute toxicity assays using *B. sandiegonensis* and *C. dubia* were performed generally according to U. S. Environmental Protection Agency guidelines (U.S. Environmental Protection Agency 1993) with the exception that measurements of water chemistry were not made at the time of the tests and batch cultures were at densities higher than those recommended. However, ephippia were never produced. Assays were performed in a temperature-controlled aquarium facility at a temperature of 20º C. Stock solutions and the series of dilutions for each test concentration were prepared in Nanopure/Perrier® media for *C. dubia* and in simulated pond water for *B. sandiegonensis* immediately prior to the assays. Control media were 100% culture media for cladocerans and 100% simulated pond water for shrimp.

We used 30-ml plastic cups as test vessels and put 15 ml of test solution and 5 organisms in each. Two cups were used (for a total of 10 organisms) per concentration. A replicate consisted of testing 10 organisms for each concentration including the no-chemical control. Three replicates were performed on different days for each species with each chemical. An additional assay was performed for malathion for *C. dubia* with neonates that were <24-hr-old to be able to compare standard assays using 24-hr-old neonates with ones comparable in age to the shrimp used in our assays.

For *C. dubia* assays, complete replicates were performed at one time. For *B. sandiegonensis* assays, a different procedure had to be used because limited numbers of shrimp were available on any day. Hatching in this species is low and irregular because of an incomplete hatch-based bet-hedging strategy (Simovich and Hathaway 1997). The number of hatchlings per day per hydration varied from 20 to 60 individuals. Each test consisted of 10 control shrimp and as many concentriations as we had shrimp to complete. The remainder of the concentrations for a replicate were performed when there were more shrimp available from another hatching (about 1 week later).

Although organisms are usually not fed during toxicity assays, fairy shrimp hatchlings die if they are not fed, so we fed both species to make the methods of the tests comparable. They were fed once at the beginning of the experiments with a drop each of *Selenastrum capricornutum* culture and YTC for daphnids and the algae and a pinch of finely ground Tetrafin® goldfish flakes for shrimp.

After the 24-hour exposure, *C. dubia* were counted and classified as living or dead. They were counted as dead if they were motionless and lying on the bottom of the cup, and did not start swimming if disturbed. For shrimp, the number swimming were counted as live the number missing were assumed dead. Larval shrimp decompose in pond water in less than 12 hours so the carcasses cannot necessarily be found to count (J. Holtz pers. com.).

Thamnocephalus platyurus were hatched and tested according to directions in the package insert (Creasel Ltd., Belgium). Cysts were incubated in dilute standard freshwater medium under constant light at a temperature of 25ºC for 24 hours. Larvae were transferred to fresh, undiluted standard freshwater medium for 2-4 hours before assays were started. Assays were performed using the 24-well test plates with 1-ml wells supplied with the kit. Test chemicals were diluted using standard freshwater medium and each assay consisted of a no-chemical control and 5 test dilutions. To begin the assay, vigorously swimming hatchlings were pipetted into a "rinse" well before being transferred to the test well with the same

Table 1. LC_{50} values for vernal pool invertebrates reported (± 2 95% CI) as the concentration of commercial solution. Concentrations in commercial solutions are 1 part malathion to 1 part other ingredients and 1 part glyphosate to 99 parts other ingredients for Roundup®. LC_{50} values for active ingredient are only estimated from the concentration of the active ingredient in the commercial solution.

Species	Malathion (µg/L)		Roundup® (µg/L)	
	Solution	Active Ingredient	Solution	Active Ingredient
B. sandiegonensis	48,900 ± 10.3	24,450	1,180 ± 0.19	11.8
C. dubia: <24 hr	6.57 ± 0.98	3.28	–	–
24-48 hr	7.17 ± 0.96	3.58	1,940 ± 0.41	19.4
T. platyurus	50,100 ± 11.6	25,050	576,000 ± 17.6	570

concentration of chemical. A replicate consisted of exposing 10 shrimp to each concentration, with all 10 shrimp in 1 well. Shrimp were not fed. They were checked for mortality 24 hours later using the same criteria as for *C. dubia*. Three replicates of the assay were performed on different days for each chemical.

For all 3 species, several range-finding assays were completed before beginning the 3 replicate assays for each chemical. Test series were eliminated from analysis if there was >90% mortality in the control or if there were no concentrations with partial mortality.

Analysis

For B. *sandiegonensis* and *C. dubia* assays, we wanted to combine the data from the 2 cups used in each replicate because counting the cups as separate tests would be pseudoreplication (Hurlburt 1984). Before pooling data, we wanted to make sure that mortality was not significantly different in the 2 cups for each concentration. The comparison was made across all species, chemicals, and series simultaneously (n = 105), using the marginal homogeneity test (MH Program v. 1.0, J. Uebersax, 2000). This is a non-parametric test based on the χ^2 distribution for differences in multinomial paired data (SPSS 1999). Unpaired cups from the shrimp assays were eliminated from the analysis.

We calculated the LC_{50} for each replicate for each species and chemical separately using the trimmed Spearman-Karber method (Hamilton et al. 1977, Salsburg 1986). Analyses was performed using the USEPA TSK program v. 1.5 with automatic trim (U.S. Environmental Protection Agency 1993). Assuming that LC_{50} is a normally-distributed random variable, differences between LC_{50} values for specific species and/or chemical pairs were analyzed by two-tailed independent samples *t*-tests. Levene's test was used to evaluate homogeneity of variances; if it was not significant, we assumed that variances were homogenous and the exact value of *t* was calculated. Analyses were performed using SPSS v.11.0 unless otherwise noted (SPSS, Inc., Chicago, IL).

RESULTS

There was no significant difference (χ^2 = 4.14, 4 df, P = 0.39) between cups across all tests on *C. dubia* and *B. sandiegonensis*, therefore we pooled cups to calculate LC_{50} values (Table 1). LC_{50} values were not significantly different between *C. dubia* neonates <24 hours old and those 24-48 hours old (Table 2).

LC_{50} values for malathion for *B. sandiegonensis* and *T. platyurus* were not significantly different (Table 2) but were 4 orders of magnitude higher than LC_{50} values for *C. dubia*. (A higher LC_{50} value means that the organism is less sensitive to that chemical.)

The LC_{50} for Roundup® for *T. platyurus* was 2 orders of magnitude higher than for the other 2 species. LC_{50} values for *B. sandiegonensis* and *C. dubia* were similar for Roundup®, however the difference was significant at α = 0.05 (Table 2). Although this result is statistically significant at the 5% level, the difference between the LC_{50} values is only about 50%. Typically, differences of at least an order of magnitude between organisms are required to be considered meaningful (Mayer and Ellersieck 1986), so we do not think that the difference is biologically significant.

DISCUSSION

Potential risk from contaminants is assumed if estimated environmental concentration is greater than 1/2 LC_{50}. However, for endangered species, a suggested unacceptable level is concentration greater than 1/20 LC_{50} (Urban and Cook 1986). Therefore, for malathion (measured as active ingredient), an unacceptable concentration for fairy shrimp would be >1.2 mg/L. Although the acute LC_{50} for larvae of the mosquito *Anopheles quadrimaculatus* was measured as 1.0 µg/L, exposure to tens of micrograms may be necessary to control mosqui-

Table 2. Statistical comparisons of LD_{50} values for vernal pool invertebrates by two-sample *t*-test assuming equal variances, because for all comparisons a Levene's test for equality of variances was not significant.

Comparison			Levene's test		*t*-test		
Species	Chemical	Age	*F*	*P*	df	*t*	*P*
C. dubia	malathion	24 hr v. 48 hr	0.007	0.94	4	-0.87	0.433
C. dubia vs. B. sandeigonensis	Roundup®	48 hr	3.65	0.13	4	-3.36	0.028
B. sandeigonensis vs. T. platyurus	malathion	48 hr	0.03	0.87	4	1.31	0.261

toes in the field (Milam et al. 2000). Environmental concentrations of this chemical are not frequently reported, but malathion concentrations of 0.061 µg/L were reported in Sacramento-San Joaquin River Delta waters (Werner et al. 2000). These concentrations are orders of magnitude lower than the estimated unacceptable concentration for *B. sandiegonensis*, so we can conclude that if comparable levels are present in vernal pools, malathion is unlikely to pose a threat to individual San Diego fairy shrimp, especially for adults which will be less sensitive than larvae. Sublethal effects, such as decreased fertility, were not measured in this study but may be important if present (Hallam et al. 1993, Sanchez et al. 1999), and should be investigated further.

For Roundup®, the estimated unacceptable risk concentration for *B. sandiegonensis*, which is 1/20 the LC_{50} estimated for glyphosate, would be about 0.59 µg/L. Environmental measurements of concentrations of glyphosate in ponds receiving overspray ranged from 25 to 141 µg/L within a few hours of application (Goldsborough and Beck 1989). These levels are above the risk level estimated from the LC_{50} measured for *B. sandiegonensis*. Our results contrast with those of other authors who have found, for example, that in experimental ponds, *D. magna* survived exposures up to 100 times the typical field dose with survival of 96% after 8 days (Hildebrand et al. 1980). Perschbacher et al. (1997) concluded that there would be no effect on plankton productivity or zooplankton populations due to overspray of glyphosate settling into aquaculture ponds. Linz et al. (1999) did not find significant differences in abundance of crustaceans in prairie potholes treated with glyphosate and untreated ones. Glyphosate is generally not considered a threat to crustacean communities (Smith and Oehme 1992), but our results show that there may be risk to the endangered San Diego fairy shrimp from the use of commercial formulation of glyphosate products.

This result may be partly due to the difference in toxicity between glyphosate and the other ingredients in these formulations. Extrapolating from the toxicity of the commercial solution to toxicity of glyphosate is probably a gross overestimate of risk because the surfactant used

Table 3. Acute toxicity (24 or 48 hr) of glyphosate products to Anostracan (*T. platyurus*) and Cladoceran (*Daphnia* spp.) species. In cladoceran assays, neonates <24 hr old were used and tests were performed at about 20°C. Species used in this study are in bold. All tests are static.

Species	Test (hrs)	LC_{50} (mg/L)	Chemical Form	Source
T. playturus	24	0.35	Perzocyd®	Foctman et al. 2000
Daphnia spinulata	24	94.87	Rondo®	Alberdi et al. 1996
D. magna	24	95.96	Rondo®	Alberdi et al. 1996
D. magna	48	2.5	Perzocyd®	Fochtman et al. 2000
D. magna	48	1.40[1]	Perzocyd®	Fochtman et a. 2000
D. magna	48	61.72	Rondo®	Alberdi et al. 1996
D. magna	48	218	Rodeo®	[2]Henry et al. 1994
D. magna	48	3.0	glyphosate	LeBlanc 1984
D. magna	48	3.0	glyphosate	Johnson & Finley 1980
D. magna	48	3.0	Roundup®	Folmar et al. 1979
D. magna	48	53.–37	Roundup®	WHO 1994
D. magna	48	42	Sting®	WHO 1994
D. magna	48	930	Roundup® D-max	WHO 1994
D. magna	48	780	glyphosate	Verschueren 2001
D. magna	48	66.18	Rondo®	Alberdi et al. 1996
D. pulex	48[3]	7.9	Roundup®	Hartman & Martin 1984

[1] using the Daphtoxkit™

[2] assays used a non-standard water representative of oligoslaine prairie potholes

[3] using adult animals

in commercial formulations is more toxic than the active ingredient. One of the "inert" ingredients is the surfactant MOMO818, which has been shown to be almost 500 times more toxic to Daphnia pulex than the active ingredient (Servizi et al. 1987). The toxicity of the surfactant and the high concentration of it in the formula we tested may explain why our results are much lower than other reported values (Table 3). It is also possible that feeding the shrimp had an effect on the toxic repsonse, possibly by causing them to ingest the chemical.

Reported LC_{50} values for malathion for anostracans range from 6.5 mg/L to >145 mg/L, which is greater than its solubility in water (Table 4). Our results for *T. platyurus* and *B. sandiegonensis* are within this range, so it is probably safe to conclude that as a group, anostracans are extremely insensitive to malathion. We only found one LC_{50} reported for glyphosate in an anostracan. It was for T. platyurus and was reported as 0.35 mg/L (Fochtman et al. 2000). Assuming this concentration is of active ingredient, their result is about 60% of the value we measured so it is similar to our result for *T. platyurus*, but is much higher that the results for *B. sandiegonensis*. Possible reasons for this were discussed above.

Values measured in this study on *C. dubia* were close to *B. sandiegonensis* for Roundup®, but not for malathion (Table 1). Because age of neonates used did not significantly affect LC_{50}, we can compare values measured on *B. sandiegonensis* larvae to <24 hour-old nauplii of cladocerans reported in the literature for malathion. The toxicity of malathion to *C. dubia* is reported as 3.18 µg/L (Nelson and Roline 1998), which is only 5% different from the value we measured on that species. The toxicity of glyphosate formulations is more difficult to compare because concentrations are reported differently by different authors. Reported toxicities to daphnids ranges from 1.4 to 930 mg/L (Table 3), but for many of these values it is unclear whether the reported value is for active ingredient or for the commercial mixture. Assuming that these values are active ingredient suggests that our results are lower than would be expected.

Although more closely related species tend to have the most similar toxic responses (Mayer and Ellersieck

Table 4. Acute toxicity (24 or 48 hour exposures only) of malathion to Anostraca, Cladocera, and Ostracoda. Tests on cladocerans used neonates <24 hr old and were reported as either EC_{50} (immobilization) or LC_{50}. Species used in this study are in bold. All tests were static and were performed at about 20°C.

Order	Species	Toxicity (µg/L)		Source
		24 hr	48 hr	
Anostraca	*Streptocephalus sudanicus*	>145000	67750	Lahr et al. 2001
	S. proboscidius	6400		Verschueren 2001
	S. proboscidius	81500		Crisinel et al. 1994
	S. proboscidius	644004		Calleja et al. 1994
	S. rubricaudis	73700		Crisinel et al. 1994
	S. texanus	54600		Crisinel et al. 1994
Cladocera	***Ceriodaphnia dubia***	**3.18**	**1.14**	Nelson & Roline 1998
	C. dubia		**2.12**	Ankley et al. 1991
	Daphnia magna	8.04×10^{-15}		Calleja & Persoone 1993
	D. magna	0.9	1.8	Verschueren 2001
	D. magna	2.6×10^{-12}		Verschueren 2001
	D. magna		33	Hermens et al. 1984
	D. magna		1.0	LeBlanc 1984
	D. magna		<9	Bailey & Liu 1980
	D. magna		1.0	Johnson and Finley 1980
	D. pulex		1.8	Sanders & Cope 1966
	Simocephalus serrulatus		0.59-6.2	Mayer & Ellersieck 1986
	S. serrulatus		3.5	Sanders & Cope 1966
Ostracoda	*Cypridopsis vidua*	220		Mayer & Ellersieck 1986
	C. vidua		47	Johnson and Finley 1980

[4]converted from µmol/L assuming M.W. of 330.36 g/mol

1986), we observed that *B. sandiegonensis* had a more similar response to *C. dubia* than the *T. platyurus* when exposed to Roundup®. *Ceriodaphnia dubia* was more sensitive to malathion than the fairy shrimp were, so it would be protective of the endangered species to use its tolerance as a guideline for this chemical. *Thamnocephalus platyurus* was much less sensitive than the other 2 species when exposed to Roundup®, which would make it a poor choice to use for selecting a guideline despite the convenience of using a test kit. Overall, because the toxic responses of *B. sandiegonensis* differed relative to the 2 potential surrogates species depending on the chemical tested, we cannot recommend either as a surrogate.

Our results suggest that cladocerans are more sensitive to malathion than anostracans are (Table 1). Sensitivity of the 5 other cladoceran species that can occur in high abundance in San Diego County vernal pools may be represented by *C. dubia* because it has similar toxic responses to many other cladoceran species (Versteeg et al. 1997). Ostracods can also make up a large portion of the biomass in pools late in the season, and they are much more sensitive to malathion than anostracans are as well (Table 4). If cladocerans and ostracods are heavily impacted by it, the pool community would undergo profound changes after exposure to malathion.

Changes to the ecosystem from toxins can be extensive and difficult to detect immediately (Boudou and Ribeyre 1989, Ramade 1989). Although there seems to be little risk due to malathion for fairy shrimp in pools, there may be negative indirect effects due to impacts on other species in the pool. In laboratory toxicity tests, Lahr (1998) found that the fairy shrimp *Streptocephalus sudanicus* was the crustacean species most sensitive to a variety of insecticides. Populations were the most reduced by the toxins and had the slowest recovery time of all the invertebrate species tested. Lahr (1997) suggested that due to their special life history strategies, organisms living in ephemeral waters might be especially vulnerable to contamination-induced changes in the pools. In further work, Lahr et al. (2000) demonstrated that cladoceran densities are markedly reduced by application of insecticides to ponds but that populations recovered in 3-6 weeks. However, they also showed that if the fairy shrimp *S. sudanicus* was eradicated by a pesticide application, it did not reappear in the pool until the next hydration (Lahr et al. 2000). This shows that impacts of chemicals depend on the pattern of reproductive events of the organisms exposed relative to the pattern of pool filling events.

MANAGEMENT IMPLICATIONS

The most important threats to southern California vernal pools are habitat loss and degradation. The extent of degradation due to pesticides in pools has previously not been known. Our results provide the first measurements of LC_{50} values for *B. sandiegonensis* on malathion and Roundup® that can be used to estimate risk based on concentrations measured in pools where these chemicals are sprayed. Although the fairy shrimp are not particularly sensitive to malathion, *Ceriodaphnia dubia* is much more sensitive, so chemical contaminants may have indirect negative effects on the San Diego fairy shrimp by altering the community composition and food web in pools. Glyphosate also poses a potential risk to shrimp and cladocerans. Because of these unknown impacts, caution is recommended when making management decisions about pesticides entering vernal pools. We recommend that spraying in the watershed of pools be avoided during the rainy season.

ACKNOWLEDGMENTS

We would like to thank Coralie Cobb for supporting this project. C. Owens, J. Holtz, and D. Parsick and many undergraduate workers contributed to this project and we thank them. We also thank Brian Cypher, Douglas Gilkey, and the two anonymous reviewers whose comments improved the manuscript. Funding was provided by the University of San Diego. This research was conducted under U.S. Fish and Wildlife Service permit TE-787037-1.

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