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Differential Toxicity to Cd, Pb, and Cu in Dragonfly Larvae (Insecta: Odonata)

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Abstract Odonate larvae are important organisms in aquatic ecosystems but have been rarely studied in laboratory toxicity tests. Only a few previous studies have been conducted on odonates and their responses to heavy metals. We exposed two species of libellulid larvae (Anisoptera: Libellulidae) to equimolar concentrations of cadmium, lead, or copper in 7-day survival tests. Larvae were tolerant of high concentrations of cadmium and lead, as no significant decrease in survival was observed at exposures as high as 0.893 and 2.232 mM, respectively. In contrast, larvae were more sensitive to copper exposure, demonstrating significantly decreased survival to exposures as low as 2.360 μM. In whole animal samples, larvae accumulated very high concentrations (>1000 μg/g dry weight) of all three metals in an exposure-related manner. Much of this accumulation could probably be attributed to adsorption or accumulation of metal within the exoskeleton, because odonate larvae are known to sequester metals into this material. Our results were generally consistent with previous observations indicating that odonates are tolerant to metal exposures, even in comparison with other aquatic invertebrates. However, there are few studies that have used odonates in toxicity tests and compared these organisms to other aquatic life. Based on their abundance and their simple requirements in the laboratory, we believe that odonate larvae can be useful toxicological model organisms.

Odonates (Insecta: Odonata; dragonflies and damselflies) are abundant and important members of a variety of freshwater ecosystems (Corbet 1999). The aquatic larvae are predators of invertebrates as well as vertebrates such as fish and amphibian larvae. Odonate larvae, in turn, serve as an important prey base for fish and other aquatic predators. Upon metamorphosis and emergence, adult odonates become important predators on insects and continue to act as a food source for terrestrial predators such as amphibians and birds.

Because they have such an important role in freshwater systems, odonate larvae are included in many environmental assessments (Rutherford and Mellow 1994; Karouna-Renier and Sparling 2001; Scher and Thièry 2005). However, few studies have documented the responses of odonates to environmental contaminants. As might be expected, the effects of insecticides have been frequently studied (Anadu et al. 1996; Beketov 2004; Bhardwaj and Tyagi 1993; Giddings et al. 1996; Hardersen and Wratten 2000; Rohr and Crumrine 2005; Schroer et al. 2004). However, less information has been collected on the responses of odonates to metal contaminants. None of these studies are very recent, and some are not very thorough. Sloof (1983) exposed a variety of invertebrate larvae, including the odonate *Ischnura elegans*, to toxicants, including mercury and cadmium. *I. elegans* larvae had considerably higher tolerance of metals, based on 48-h median lethal concentrations (LC_{50}s), compared to most other species tested. Jones (1985) made casual observations of odonate larvae development in settling tanks within a former tin mine. Some malformations were observed, but no empirical data were provided. Correa (1985) exposed *Somatochlora cingulata* larvae to aluminum and low pH levels. Adverse effects such as decreased oxygen consumption were the result of exposure to low pH rather than aluminum. Meyer et al. (1986) exposed *Libellula depressa*, *Libellula quadrimaculata*, and *Aeshna cyanea* larvae to

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lead and measured organ bioaccumulation and oxidative enzyme activity. Most of the accumulated lead was found in the cuticle, and enzyme activity was suppressed. Mackie (1989) determine 96-h LC_{50}s for *Enallagma* sp. larvae exposed to cadmium, lead, and aluminum. Cadmium was found to be the most toxic of the three metals**.** Rockwood and colleagues (1990, 1991) observed decreases in weight and oxygen uptake and changes in hemolymph chemistry in *Libellula julia* larvae exposed to aluminum. The most recent study was conducted by Tennessen (1993), who documented hatching success and development of *Libellula lydia* and *Pachydiplax longipennis* larvae exposed to iron.

Because there are so few laboratory studies regarding effects of metals on odonate larvae, we conducted a series of basic exposures with cadmium, copper, and lead to determine the effects on survivability. Equimolar exposures were used so that we could directly compare the relative toxicity of these three metals. The study species were larvae of *P. longipennis* and *Erythemis simplicicollis* (Anisoptera: Libellulidae). To our knowledge, this work is the first study of copper toxicity in odonate larvae**.** In addition to toxicity tests, tissue levels of metals were measured in *P. longipennis* so that we could compare toxicity and bioaccumulation. This topic has also not been adequately addressed in odonates. Our ultimate goal was to provide recent information regarding the toxicity of metals to odonate larvae.

Materials and Methods

Collection of Larvae

Odonate larvae were collected from 2004 to 2006 from a small pond on the campus of the University of South Alabama, Mobile, Alabama, USA. The most common species included *P. longipennis, E. simplicicollis*, and *Ladona deplanata* (Anisoptera: Libellulidae). Specimens were identified through dichotomous keys (Richardson 2003). The most consistently abundant species in all years was *P. longipennis*; therefore, these larvae were used in the majority of the experiments described in this article. Larvae were collected with a D-frame net within leaf litter and aquatic vegetation. Specimens as small as 5 mm and as large as 40 mm (late instar *P. longipennis*) were collected; most individuals were between 10 and 25 mm. Larvae were brought back to the laboratory room and allowed to acclimate to room temperature (23°C) in the collection water. Water from the pond contained only trace amounts of metals (Pb and Cu <5 μg/L; Cd below detection limit).

Maintenance of Odonate Larvae

The maintenance and housing design has been described previously (Rice 2008). Briefly, after adjusting to laboratory temperature, larvae were placed into housing chambers made from 480-mL (16-oz) plastic drinking cups. Four 3 × 5-cm windows were cut into the sides of each cup; each window was covered with nylon window screen (mesh size: 0.84 mm). Typically, one to three larvae, depending on size, were placed in each chamber. A maximum of 10 housing chambers were placed in a 38 × 38 × 16.5-cm translucent polyethylene box. Each plastic box was filled to a depth of 11 cm with reconstituted hard water (FETAX solution; ASTM 2000). The water did not to need to be changed because it was filtered and recirculated. The entire system was held in a laboratory room under a 12-h light:12-h dark photoperiod regime at 23°C. Larvae were collected for all trials within 2 weeks or less prior to use in experiments. The size of larvae used for experiments ranged between 10 and 15 mm. They were fed two to three *Daphnia magna*, two to three times per week, prior to use in experiments. *Daphnia magna* were purchased from Carolina Biological Supply (Burlington, NC, USA). They were maintained in 1-L polypropylene tricorner beakers containing reconstituted hard water and were fed a yeast/fish flake/cereal grass mix (YTC) according to standard methods (Landis et al. 2005).

General Experimental Design

All experiments were conducted over 7 days in the laboratory room at 23°C and a 12-h light:12-h dark photoperiod. Exposure chambers consisted of 400-mL polyethylene beakers filled to a test volume of 360 mL of FETAX solution at the designated treatment concentration, with one larva per beaker. Water quality parameters generally measured in the exposure chambers were as follows: pH = 6.24, hardness = 120 mg $CaCO_3$/L, and temperature = 23°C. Stock solutions of 10 g/L Pb, Cu, and Cd were made from metal salts (lead nitrate: $Pb(NO_3)_4$; copper sulfate pentahydrate: $CuSO_4 \cdot 5\ H_2O$; cadmium chloride hemipentahydrate: $CdCl_2 \cdot 2½\ H_2O$), which were dissolved in ultrapure water. Larvae were not fed during the exposure. Four to five replicate beakers (i.e., four to five larvae) per treatment were used, depending on availability of larvae. Beakers were checked once per day for dead larvae, as determined by lack of response to prodding. A complete water change was conducted on day 3 of each trial.

Exposure to Cd, Pb, and Cu

The main series of experiments used *P. longipennis* larvae that were exposed to nominal concentrations of 0, 0.045,

0.357, 0.893, and 2.232 mM of Cd, Pb, or Cu. One trial was conducted with all three metals during the same 7-day exposure period, with four replicate beakers per treatment for each metal. Additionally, a second Cd trial (five replicates per beaker) and two trials each of Cu or Pb (four replicates per beaker) were conducted during single 7-day periods. Statistical analysis (below) was conducted on composite data for each metal.

Additional experiments were also performed. Because of low survivability in the initial Cu treatments described earlier, a single 7-day trial with *P. longipennis* was conducted using low levels of Cu, at exposures of 0, 0.295, 0.590, 1.180, and 2.360 μM, with four replicates per treatment. In 2004, *E. simplicicollis* were abundant in the collection pond. Therefore, we exposed larvae (similar in size to *P. longipennis*) to Cd at 0, 0.022, 0.045, 0.134, 0.357, and 0.893 mM. Two separate trials, with five replicates per exposure, were conducted.

Metal Levels in Whole Animal Samples

Using *P. longipennis*, four larvae were selected each from 0-, 0.045-, or 2.232-mM exposures of Cd, Pb, or Cu from various trials to determine metal levels in whole animal samples. Tissue processing methods were modified from US EPA Method 3051 (US EPA 1994). Specimens were frozen after removal from the particular exposure experiment. Individual larvae were thawed, rinsed with ultrapure water, and placed in a 45-mL Teflon digestion vial with 2 mL ultrapure nitric acid. These vials were placed into Parr® microwave digestion bombs (Parr Instrumental Company, Moline, IL, USA), which were then placed into a microwave and heated at 750 W for 3 min. Bombs were cooled, vented, and then microwaved a second time at 600 W for 2 min. The resulting digestate was quantitatively transferred to an acid-washed 50-mL centrifuge tube for analysis and diluted to 20 mL with ultrapure water. To report the data on a dry weight basis, a subset of larvae were weighed wet, dried for 48 h at 65°C, and weighed again. The average dry:wet ratio was 10%; this value was used to convert the wet weights of digested larvae to dry weight.

Standard reference materials were digested with batches of larvae samples to monitor extraction efficiency. These reference materials consisted of 0.25 g of NIST 1566b (National Institute of Standards and Technology: oyster tissue) during Pb and Cd extraction and NIST 2976 (mussel tissue) during Cu extraction. Recovery efficiency from these reference materials was 95–100%. Metal levels were analyzed on a Varian® SpectrAA220 graphite furnace atomic absorption spectrophotometer (Varian, Inc., Palo Alto, CA, USA). The instrumental detection limit was approximately 0.01 μg metal/g dry weight.

Statistical Analysis

Statistical analysis consisted of analysis of variance (ANOVA) to compare survival time among exposure concentrations within each metal. Separate analyses were conducted on the main series with Cd, Pb, and Cu (composite of all separate metal trials), on the low Cu trial, and on a composite of the two Cd trials with *E. simplicicollis*. Tukey's multiple comparisons were used to separate significant differences among all metal treatments within a particular metal. Student's *t*-tests were used to compare survival time between *P. longipennis* and *E. simplicicollis* within 0.045-, 0.357-, or 0.893-mM Cd exposures. ANOVA was also used to compare metal levels in larvae among 0-, 0.045-, and 2.232-mM treatments within a metal. For this analysis, data were $\log_{10}$-transformed due to extreme heterogeneity of variances among the treatments.

Results

Analysis of composite trials with Pb revealed no significant difference in survival time for *P. longipennis* exposed to any level of Pb ($F_{4,\,55} = 0.64$, $p = 0.639$; Fig. 1). For composite Cd trials, survival time was significantly lower in the 2.232-mM treatment compared to 0.357- and 0.045-mM treatments ($F_{4,\,40} = 3.18$, $p = 0.023$; Fig. 1). In contrast to results from Cd or Pb exposure, *P. longipennis* larvae exposed from 0.045 to 2.232 mM Cu showed significant decreases in survival time compared to unexposed larvae ($F_{4,\,55} = 24.29$, $p = 0.0001$; Fig 1). No other significant

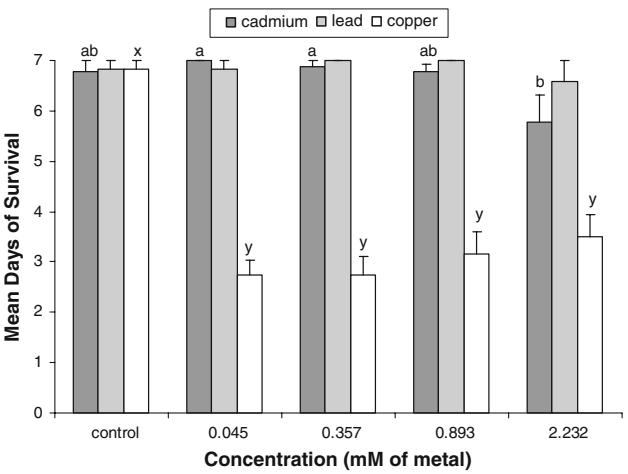

Fig. 1 Mean survival (±1 SE) for *P. longipennis* larvae over 7-day exposures to equimolar concentrations of cadmium, lead, or copper. There were two trials with cadmium ($N = 9$) and three trials each with lead and copper ($N = 12$). Each trial used four to five larvae per treatment. Within Cd or Cu treatments, different letters indicate significant differences in survival time. There were no significant differences among any Pb treatments

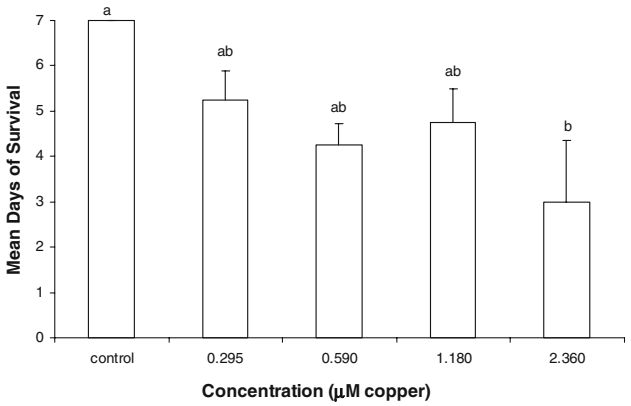

Fig. 2 Mean survival (±1 SE) for *P. longipennis* larvae over a 7-day exposure to copper. Data consists of a single 7-day trial with four larvae per treatment. Different letters indicate significant differences in survival time

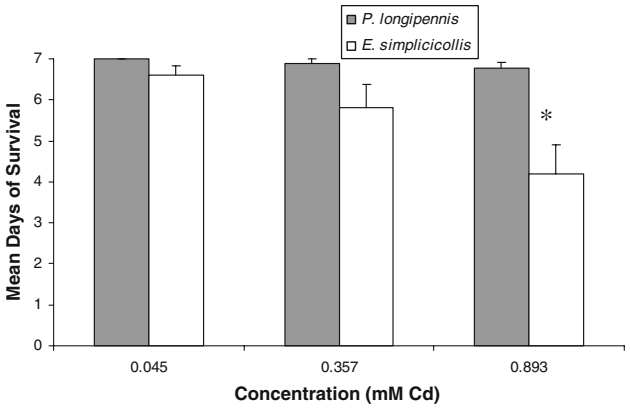

Fig. 4 Mean survival (±1 SE) for *P. longipennis* and *E. simplicicollis* larvae over 7-day exposures to cadmium. Data for each species consisted of a composite of two trials. Each trial used four to five larvae per treatment. Asterisks indicate significant differences in survival time between species within a treatment. See Figure 1 (*P. longipennis*) and Figure 3. (*E. simplicicollis*) for other details

differences were observed among any other concentrations. Cu levels as low as 2.360 µM significantly decreased survival time compared to unexposed larvae ($F_{4,\ 15} = 3.55$, $p = 0.031$; Fig. 2).

A significant treatment effect was detected in survival time for *E. simplicicollis* larvae exposed to Cd ($F_{4,\ 54} = 2.92$, $p = 0.021$; Fig. 3). However, the only significant pairwise comparison was between the 0.893- and 0.045-mM treatments. *E. simplicicollis* larvae exposed to 0.045 or 0.357 mM Cd had a similar survival time compared to that of *P. longipennis* in the same treatments ($df = 17$, $t < 1.71$, $p > 0.05$; Fig. 4) but a significantly lower survival time in the 0.893-mM treatment ($df = 17$, $t = 3.36$, $p = 0.001$; Fig. 4).

Pachydiplax longipennis larvae exposed to 0.045 or 2.232 mM Cd, Pb, or Cu accumulated high levels of these metals (Table 1). There was considerable variability within each treatment, but, in general, the level of metals was consistent with exposure level. For both Cd and Cu, there were significant differences in metal concentrations among all three treatments ($F_{2,\ 9} > 62.74, p < 0.001$, based on $\log_{10}$–transformed data; Table 1). For Pb, unexposed larvae had significantly lower concentrations than both 0.045 and 2.232 mM, but there were no differences between the two Pb exposure treatments ($F_{2,\ 9} = 9.19$, $p < 0.001$, based on $\log_{10}$-transformed data; Table 1).

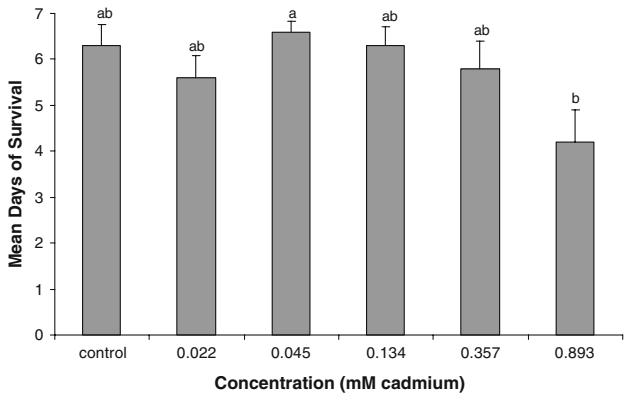

Fig. 3 Mean survival (±1 SE) for *E. simplicicollis* larvae over 7-day exposures to cadmium. Data consists of a composite of two trials. Each trial used five larvae per treatment; total sample sizes = 10 for each treatment. Different letters indicate significant differences in survival time

Discussion

Both *P. longipennis* and *E. simplicicollis* larvae exhibited high tolerance to Pb and Cd, at least in terms of survivability. No appreciable mortality was observed in either species at concentrations below 0.893 mM (100 mg Cd/L, 185 mg Pb/L). *Pachydiplax longipennis* larvae were able to tolerate up to 2.232 mM Cd and Pb (250 mg Cd/L, 462 mg Pb/L). Only exposures to Cu demonstrated any effect on mortality at concentrations above 2.360 µM (150 µg Cu/L). All of the concentrations of Pb, Cu, or Cd that caused mortality were well above any concentration to which odonate larvae would be exposed in the field, except under extreme contamination scenarios. These concentrations also greatly exceed the US EPA-recommended Criterion Continuous Concentration to protect aquatic life [CCC at water hardness of 100 mg/L $CaCO_3$: Pb < 2.50 µg/L (0.012 µM); Cu 9.00 µg/L (0.142 µM); Cd < 0.25 µg/L (0.002 µM); US EPA 2005].

The mortality from exposure to Cu but not to Cd or Pb might be due to the ability of aquatic insects such as odonates to more readily bioaccumulate Cu. Metal-binding metallothionein proteins have been found in some species

Table 1 Mean concentrations (±1 SE) of cadmium, lead, or copper in whole-body samples of *P. longipennis* larvae ($N = 4$) exposed to equimolar concentrations over 7 days

Metal	0 mM	0.045 mM	2.232 mM
Cadmium	10.91 ± 8.64 a	1,085.52 ± 200.83 b	21,423.80 ± 3,330.18 c
Lead	336.39 ± 139.76 a	90,066.66 ± 16,730.46 b	189,320.60 ± 47,302.76 b
Copper	33.95 ± 10.14 a	3,190.68 ± 625.07 b	20,783.31 ± 8,612.65 c

Note: Metal levels are in micrograms per gram dry weight; the mean dry:wet weight ratio of *P. longipennis* was 10%. Different letters indicate significant differences among treatments within a metal, based on $\log_{10}$-transformed data.

of insect larvae. Cu is preferentially bound more readily by these proteins than Cd, whereas Pb is minimally bound (Maroni and Watson 1985, Suzuki et al. 1988, 1989). If odonate larvae have metal-binding proteins, then they might bioaccumulate Cu more readily that Cd or Pb and then show toxic effects. However, the presence of these proteins in odonates remains unexplored.

The concentrations in the present study were also considerably higher than levels used in the few previous experiments on odonates exposed to Cd or Pb; no investigators have examined the toxicity of Cu in odonates. Meyer et al. (1986) exposed *Libellula depressa*, *L. quadrimaculata*, and *A. cyanea* larvae to 20 μg/L (0.097 μM) of Pb for 6 weeks. No mortality was observed, but activity of oxidative enzymes was decreased. Meyer et al. (1986) also observed that food-catching behaviors were markedly decreased after 2 weeks of exposure. In contrast, we observed no changes in appetite for *E. simplicicollis* exposed to 0.357 mM (73.97 mg/L) during a single 14-day trial (data not shown). Mackie (1989) conducted 96-h exposures of Cd, Pb, and other metals with *Enallagma* sp. larvae. Median lethal concentrations (LC_{50}) ranged from 7.05 to 10.66 mg Cd/L (0.063 to 0.095 mM), well below the highest concentration used in our experiments where no mortality was observed. The mortality observed by Mackie (1989) might be explained by differences in species or in general experimental design. In contrast to exposures to Cd, Mackie (1989) observed no mortality in *Enallagma* sp. larvae exposed to concentrations of Pb above 60 mg/L (0.290 mM). These results were consistent with our observations of little appreciable mortality in Pb-exposed libellulids. Sloof (1983) exposed *I. elegans* larvae to Cd and determined the LC_{50} to be >56 mg/L (0.500 mM). Chessman and McEvoy (1998) did not conduct exposures, but, instead, they calculated indexes of sensitivity to various environmental insults in Australian watersheds. The authors determined that lestid and libellulid larvae appeared to be relatively insensitive to metal pollution compared to other insults such as sewage or dams. The results of the above studies all suggest that odonate larvae, at least libellulids, are tolerant of water-borne heavy metals. Even when effects were observed, the exposure concentrations were typically in the milligram per liter (millimolar) range, well above any levels that would be expected in the field.

The above-cited studies represent the only investigations of odonate responses to Cd or Pb. Furthermore, because no experiments have been conducted with Cu, we cannot be confident that the high mortality we observed compared to Cd or Pb exposure would be expected or instead is a unique phenomenon among aquatic insects. Considering that odonates include two distinct suborders (Zygoptera and Anisoptera) and a variety of natural histories within these groups, more controlled studies need to be conducted with a variety of species to more fully examine metal bioaccumulation and toxicity in these organisms.

It would be useful to compare the sensitivity of metals between odonates and other aquatic invertebrates, but only two studies have made such direct comparisons. Sloof (1983) determined that *I. elegans* were more tolerant to Cd compared to other noninsect aquatic invertebrates but similar in tolerance to other aquatic insect larvae. Mackie (1989) observed that *Enallagma* sp. were more tolerant to Cd or Pb compared to molluscs or ephemeropteran larvae.

Because of the lack of any other direct comparisons to odonates, an indirect alternative would be to examine how other aquatic invertebrates respond differentially to Cd, Pb, and Cu. Warnick and Bell (1969) exposed a variety of aquatic insect larvae to Cd, Cu, and Pb and measured LC_{50}s. All species were most sensitive to Cu and least sensitive to Pb. Nehring (1976) observed that plecopteran and ephemeropteran larvae were both more sensitive to Cu compared to Pb (Cd not tested). Anderson et al. (1980) determined that the chironomid *Tanytarsus dissimulis* was most sensitive to Cd compared to Cu and least sensitive to Pb. Rayms-Keller et al. (1998) observed that mosquito larvae (*Aedes aegypti*) were less sensitive to Cu compared to Cd (Pb not examined). Milani et al. (2003) exposed four species of aquatic invertebrates (not odonates) to Cd and Cu (Pb not examined). Based on 96-h LC_{50}s during water-only exposures, *Hyalella* sp. and *Chironomus* sp. were more sensitive to Cd, whereas *Hexagenia* sp. and *Tubifex* sp. were more sensitive to Cu. These studies generally indicate that aquatic insect larvae, much like the odonate larvae in the present study, are tolerant of Pb compared to Cd or Cu. In contrast, sensitivity to Cu versus Cd varies

among insect species. It should be noted that the above-cited studies observed toxicity at concentrations far below those used in the present study. Therefore, in general, odonates do appear to be more tolerant to metals compared to other aquatic invertebrates.

We measured considerable amounts of Cd, Pb, and Cu in *P. longipennis* larvae collected during various trials. These whole-body concentrations appear to be extraordinarily high, especially considering that little toxicity was observed for Cd or Pb. We do not believe that these high levels are the result of sample contamination. Certainly, there was a great deal of insoluble metal precipitate that formed in the exposure chambers and the larvae would be covered with this material. However, we thoroughly rinsed the specimens prior to both freezing and processing. Furthermore, recovery from standard reference materials processed with the larvae samples was never higher than 100%, indicating no contamination of reagents. Therefore, we are confident that the levels presented here represent the actual levels in the specimens. However, much of this metal might not be bioavailable but adhered onto or sequestered into the exoskeleton. In this event, rinsing the specimens prior to digestion would not remove these bound metal forms.

There is considerable documentation that odonates and other aquatic insect larvae sequester high levels of metals, particularly Pb, in the cuticle (for a review, see Hare 1992). For example, Giesy et al. (1981) observed that the exuviae of *Pantala hymenaea* exposed in artificial microcosms contained 68% of the Cd in whole specimens. Meyer et al. (1986) exposed three species of anisopteran larvae to Pb and measured levels in multiple organs and exoskeleton. Composite samples of these species demonstrated that most accumulated Pb was sequestered in the exoskeleton compared to the brain, fat bodies, midgut, or rectum. Gupta (1995) collected *Crocothemis servilia* from lakes in India and measured Cd, Pb, and Cu levels. The greatest proportion of whole-body metal levels was sequestered into the exoskeleton (100%, 75%, and 68% for Cd, Pb, and Cu, respectively). Based on these previous observations, it is possible that *P. longipennis* larvae in the present study had high levels of metals adsorbed onto or accumulated into their exoskeletons. These metal species were unlikely to be very bioavailable, given the lack of any obvious toxicity even at high exposure concentrations.

We measured relatively high levels of Pb even in our unexposed control larvae, and we have no good explanation for these high levels. Analysis of water from the collection area indicated only trace amounts of all three metals (Pb and Cu <5 μg/L; Cd below detection limit). The levels in the unexposed treatments were higher than any Pb amounts reported in whole-larvae samples from field sites. In most cases, the Pb concentrations in odonates collected from these areas were <20 μg/g dry weight (Anderson 1977; Barak and Mason 1989; Karouna-Renier and Sparling, 2001; Mathis et al., 1979; Nummelin et al., 2007; Scheuhammer et al., 1997). Gupta (1995) did measure up to 50 μg/g dry weight in *C. servilia* from lakes in India, but there was no indication that these lakes were contaminated.

It is possible that odonate larvae are capable of bioaccumulating high amounts of Pb even under low-level exposures, perhaps from the collection area or the exposure water. As described previously, Meyer et al. (1986) exposed three species of Anisoptera to Pb and found that the exoskeleton had the highest levels of bioaccumulation. Furthermore, the levels in the exoskeleton of Pb-exposed and unexposed larvae were nearly equivalent. This observation, much like that in the present study, indicated that unexposed larvae were carrying relatively high levels of Pb (Meyer et al. 1986). However, the concentrations of Pb reported by Meyer et al. (<10 μg/g dry weight using our dry:wet weight ratios) were considerably lower than observed in the present study. These investigators used a lower exposure concentrations (20 μg/L = 0.097 μM) for 6 weeks. Meyer et al. (1986) hypothesized that most of the Pb in the odonate exoskeleton was held in the mesocuticle, which becomes reincorporated into the new cuticle during subsequent molting periods. Therefore, previously accumulated Pb remains and so there would be potential, even under low Pb-exposure scenarios, for odonate larvae to accumulate a relatively high Pb burden with repeated molts. We did observe molting by several larvae during various experiments; therefore, the extremely high levels of metals we measured in unexposed *P. longipennis* could be consistent with these observations from Meyer et al. (1986). Unfortunately, Meyer et al. (1986) are the only investigators who observed Pb uptake within multiple organs between exposed and unexposed odonate larvae. Therefore, more studies are required that examine the sites of uptake of Pb and other metals in odonates and other aquatic insects.

Unlike the relatively high Pb levels in unexposed *P. longipennis* in the present study, the levels of Cd and Cu in unexposed larvae were comparatively low. These levels probably represent low background levels; they were within values reported in previous studies for odonate larvae collected from various field sites. These field levels were generally <25 μg Cu/g and <2 dry μg Cd/g dry weight (Anderson 1977; Barak and Mason 1989; Currie et al. 1997; Karouna-Renier and Sparling 2001; Nummelin et al. 2007; Scheuhammer et al. 1997). The values measured in the present study bracket these previously reported concentrations. Some investigators have reported higher levels, in some cases from contaminated areas. Brown (1977) measured Cu ranging from 48 to 768 μg/g dry

weight from *Libellula* sp. and *Agrion* sp. larvae collected in a mine drainage area in Cornwall, United Kingdom. Mathis et al. (1979) measured Cd levels of ~35.4 μg Cd/g wet weight (354 μg/g dry weight based on our dry:wet ratio) from power-plant ponds in Illinois, United States. Gupta (1995) measured up to 45 μg Cu/g dry weight, but only 6 μg Cd/g dry weight, in *C. servilia* from lakes in India. Based on these studies, we believe that the Cd and Cu levels in unexposed *P. longipennis* larvae in the present study could be considered within normal background values.

In conclusion, *P. longipennis* and *E. simplicicollis* larvae appear to be tolerant of heavy metals and capable of accumulating high body burdens with little effect on mortality. We suggest that future studies examine other sublethal end points, such as predator-avoidance, development, or changes in appetite. In field situations, odonate larvae might not be at risk of toxic effects from metal exposure even when high levels have been bioaccumulated. However, predators of odonate larvae might be at risk from ingestion of metal-laden larvae. Unfortunately, no studies have examined the transfer of metals from odonate larvae to predators.

Similar properties of metal tolerance have been observed for frog larvae, which also do not demonstrate toxic effects until high levels of metals have been bioaccumulated (Ferreira et al. 2004; Hopkins et al. 2000; Rice et al. 1999, 2002). Odonate larvae share some similar properties to frog larvae. The larval stage of both types are abundant members of a variety of freshwater ecosystems and serve as food base for high-level predators such as fish. Furthermore, both odonate larvae and frog larvae undergo extensive metamorphosis into adults that are important insect predators while still serving as an important food base. Much support in recent years has been presented for frog larvae as important organisms for environmental toxicology in both field and lab studies (e.g., Hopkins et al. 2000; Rice et al. 1999, 2002; Sparling et al. 2006). We propose that the same recognition be given to odonate larvae.

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