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Effects of Cadmium and Body Mass on Two Anti-Predator Behaviors of Five Species of Crayfish

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Abstract: Five crayfish species (*Orconectes placidus*, *O. virilis*, *Procambarus acutus*, *P. alleni* and *P. clarkii*) were subjected to Cd exposure in 96 h acute toxicity tests to assess changes in two anti-predator behaviors, the tail-flip response and the claw-raise response. The tail-flip response was significantly affected by Cd exposure in three of five cases (ANOVA $p<0.05$). In three species, planned comparisons with Duncan's Test revealed that at least one exposure concentration of Cd decreased the frequency of the tail-flip behavior significantly compared to controls ($p<0.05$). The lowest level of Cd to significantly impair the tail flip response was 0.306 mg Cd L^{-1} for *O. virilis*. Regression analysis detected significant decreasing trends in the tail-flip behavior as cadmium concentrations increased in four species of crayfish ($p<0.05$, $r>0.42$). In two cases, planned comparisons with Duncan's test revealed that at least one exposure concentration of Cd increased the frequency of the claw-raise response significantly compared to controls ($p<0.05$). The lowest level of Cd to significantly increase the claw raise response was 3.50 mg Cd L^{-1} for *P. clarkii*. Regression analysis indicated that the claw-raise behavior was related to Cd concentration in two species ($p<0.05$, $r>0.50$). When control groups were compared across species, a significant correlation was measured between body mass and both the tail flip and claw raise behaviors. Across the five species, as body mass increased, the tail flip response decreased in frequency ($r = -0.72$; $p<0.001$) and the claw raise response increased in frequency ($r = 0.70$; $p = 0.001$). Interference with either behavior, but especially the tail flip response, could have important survival consequences, especially for juvenile crayfish which are typically more sensitive to cadmium exposure.

Key words: Crayfish, cadmium, metal toxicity, behavior, body mass

INTRODUCTION

North America has 363 species of freshwater crayfish, about 61% of the worldwide total of more than 520 recognized species. It has been estimated that approximately 48 to 51% of crayfish species in North America are imperiled or vulnerable to extinction (Taylor *et al.*, 2007; Stein *et al.*, 2000). Some species, such as *Orconectes virilis*, have a very wide distribution encompassing many states while others have very limited ranges, sometimes as small as a single cave or stream (Stein *at al.*, 2000; Hobbs, 1976).

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Crayfish can constitute a large fraction of the animal biomass in an aquatic ecosystem, sometimes exceeding 50% of the macroinvertebrate biomass in a lake or stream (Momot, 1995). Crayfish are also important food sources to a variety of aquatic and terrestrial predators (Holdich, 2002). Their central location in many aquatic food webs makes them a species of special interest, possibly allowing them to transfer toxicants between trophic levels. Additionally, crayfish are important model organisms for neurophysiological study, including details relating to nerve conduction, control of heart and ventilatory rate, innervation of various organs and the neuromuscular junction and behavior (Shuranova *et al*., 2006).

Cadmium is a common, widespread toxicant and can be found in approximately half of hazardous waste sites on past and present U.S. EPA National Priorities Lists (Taylor *et al*., 1999; U.S. EPA National Priorities List web site: http://www.epa.gov/superfund/sites/npl /index.htm accessed March 1, 2010). It enters the environment primarily through mining activities and fossil fuel combustion (Taylor *et al*., 1999). The Cd^{2+} ion is the most bioavailable form and the most toxic to aquatic organisms. It is thought to be taken up through the gills by the same mechanism as calcium and zinc. It is a nonessential metal and can cause a wide range of effects in aquatic organisms, including slowed growth, reduced reproductive output, impaired Ca metabolism, gill damage and altered behavior, in addition to mortality (Jiraungkoorskul *et al*., 2007; Rainbow and Black, 2005; Albert *et al*., 1992). These factors, especially the impact of Cd on Ca metabolism, make Cd a toxicant of special interest to crustaceans because of its implications to the molting process, a time of greatly increased Ca metabolism (Wheatly and Ayers, 1995).

The behavioral effects of heavy metals on fish include a wide variety of effects including avoidance and/or attraction to heavy metals, altered swimming behavior and decreased competitiveness and social dominance in various species. There is considerable interspecific variation in response (Black and Birge, 1980; Scarfe *et al*., 1982; Yorulmazlar and Gul, 2003; Sloman *et al*., 2003a, b). Invertebrates experience a similar variety of effects including scallops closing their shells, disruptions to feeding behaviors, avoidance of low metal concentrations and difficulty with prey capture (Inda and Cuturrufo, 1999; Heinis *et al*., 1990; Lefcort *et al*., 2004; Wallace *et al*., 2000). Few studies have been done on the effects of heavy metal exposure on crayfish behavior, despite this group's wide use in behavioral research. Maciorowski *et al*. (1980) found that the crayfish *Cambarus acuminatus* was attracted to Cd at low and moderate levels, 0.0058 to 0.0185 mg L^{-1}, but was repelled at 0.1483 mg L^{-1}. Exposure to Cu at levels as low as 0.02 mg L^{-1} caused the crayfish *Cambarus bartoni* to stop moving towards food sources and sometimes actively avoid food sources (Sherba *et al*., 2000). These results are similar to those for Steele *et al*. (1992), who tested the crayfishes *Procambarus clarkii*, *C. bartoni* and *Orconectes rusticus*. They found that a sublethal mixture of Cu, Cr, As and Se in proportions that simulated their proportions in coal fly ash slurry significantly reduced all three species' preference to move into areas with a feeding stimulant fluid relative to control areas with attractant. Juvenile rusty crayfish (*O. rusticus*) showed hyperactivity resulting in reduced shelter use relative to controls when exposed to sublethal concentrations of Cd (Alberstadt *et al*., 1999). Juvenile red swamp crayfish (*P. clarkii*) showed similar although, weaker, trends (Misra *et al*., 1996).

While, avoidance/attraction, feeding and shelter seeking behaviors have been investigated under heavy metal exposure, there has been no work assessing the potential effects of heavy metal pollution on anti-predator behaviors. This research begins to fill this gap by investigating the effects of cadmium on two basic anti-predator behaviors. One is the typical escape response involving backwards swimming using the uropod (also called the

tail-flip behavior or simply escape behavior) (Wine and Krasne, 1982). The other is the predator response or defense behavior (also called the claw-raise behavior or meral spread) (Hayes, 1975, 1977; Bruski and Dunham, 1987). Any impairment to the performance of these two behaviors could make crayfish more vulnerable to predation. Additionally, further information on the relationship between crayfish body mass and these two behaviors is presented.

MATERIALS AND METHODS

Five toxicity tests were conducted with adult crayfish between August 2002 and November 2003 in the Department of Biology at the University of Kentucky, Lexington, KY, USA. The species included *Procambarus acutus* (Girard 1852), *P. alleni* (Faxon, 1884), *P. clarkii* (Girard 1852), *Orconectes placidus* (Hagen 1870) and *O. virilis* (Hagen 1870). Individuals of the species *P. acutus* (Average mass = 15.5 g) were purchased from Blue Spruce Biological Supply Company, Boulder CO; *P. alleni* (5.14 g) from Fish2U.com, Gibsonton, FL and *P. clarkii* (18.5 g) from Louisiana Seafood Exchange, New Orleans LA. *O. placidus* (7.06 g) was collected from Blackburn Fork near Cookeville, TN. *O. virilis* (12.8 g) was obtained from Clear Creek Fisheries, Martinsville, IN. Water samples were taken at the time of collection and analyzed to determine if there were any environmental contaminants that might complicate toxicity testing. Organisms were either purchased or collected in advance to allow a minimum of three days to acclimate to laboratory conditions.

Toxicity test and water quality analysis methods were based on standard methods (U.S. EPA, 1993; Eaton, 1995). Test procedures and toxicological results are presented in detail by Wigginton and Birge (2007). All toxicity tests were conducted for 96 h in a temperature controlled environmental room at 25±1°C and a 16 h light/8 h dark photoperiod. Individuals were housed in 11.3 L polypropylene tanks. Control and exposure water consisted of carbon filtered tap water diluted with >17.6 Mohm high purity water to a hardness of 42.5-52.9 mg $CaCO_3$ L^{-1}. The salt $CdCl_2$ was used to make all exposure concentrations. Concentrations of Cd chosen for *O. placidus*, the first test in this series, were based on results from a related species (Wigginton and Birge, 2007). *Procambarus clarkii* was the second of this series of toxicity tests conducted. A broad range of Cd concentrations was used to ensure the LC_{50} value was encompassed within the range of concentrations, based upon additional literature review (Ramo *et al*., 1987; U.S. EPA, 2001). Subsequent tests used a more narrow range of Cd concentrations based on experience from the *P. clarkii* test. Aeration was provided to each tank to ensure that dissolved oxygen levels remained above 4.0 mg L^{-1}, as stipulated by U.S. EPA (1993). For each test, five crayfish were housed in each tank; four replicates were used. In all tests, 800 mL polypropylene beakers were used to isolate each crayfish within the tank to prevent injury or mortality from fighting. Each beaker was well perforated to allow water flow throughout the test chamber. Toxicant exposure levels, expressed as mg of Cd L^{-1}, are given in Fig. 1 and 2. In all tests, 4.0 L of each test or control solution was used per treatment. All solutions were renewed daily. In tests with *P. acutus*, *P. clarkii* and *O. placidus*, checks were conducted every 6 h. In tests with *P. alleni* and *O. virilis*, the animals were checked every 24 h. At each check, animals were examined for behavioral response by tapping them lightly on the abdomen five times with a glass rod. The escape or tail flip response was defined as the animal sweeping its tail fully downward at least one time (Hayes, 1975). The predator response pattern or claw raise response was defined as the chelae being raised above parallel to the abdomen (Hayes, 1975, 1977). A lack of either of these behavioral

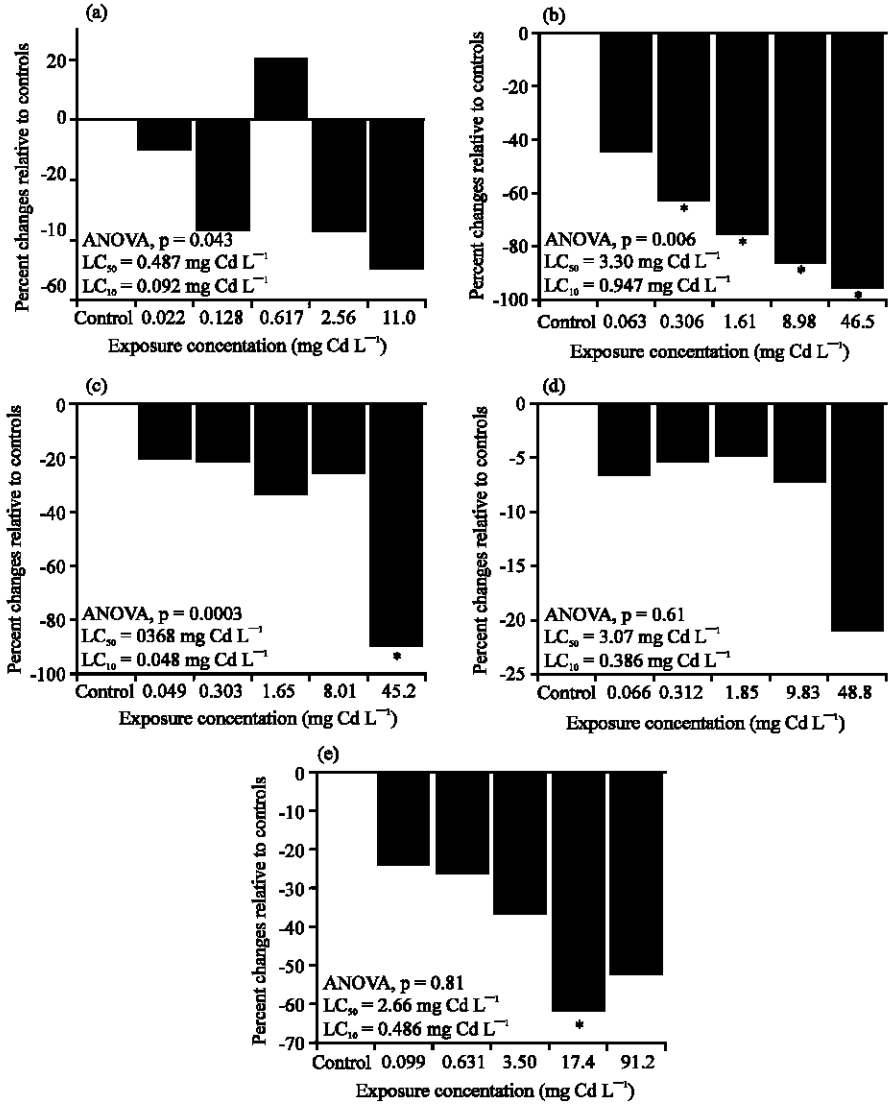

Fig. 1: Percent change in tail-flip behavior at each exposure concentration for 5 species of crayfish relative to controls. (a) *Orconectes placidus*, (b) *Orconectes virilis*, (c) *Procambarus acutus*, (d) *Procambarus alleni* and (e) *Procambarus clarkii*. An asterisk (*) indicates a significant difference from control responses ($p \leq 0.05$, Duncan's test). ANOVA, LC_{50} and LC_{10} values are also given (LC_{50} and LC_{10} values from Wigginton and Birge, 2007)

responses was scored as a zero. Complete lack of response to the five taps on the abdomen (e.g., no movement of the legs, tail, scaphognathite, antennae, etc.) was counted as mortality.

For statistical analysis of these toxicity tests, the average response of each crayfish was obtained by dividing the number of performances of each behavior by the number of measurement opportunities for that behavior. Because the five crayfish in each tank were not fully separated, the response of all crayfish per replicate were averaged. These proportions,

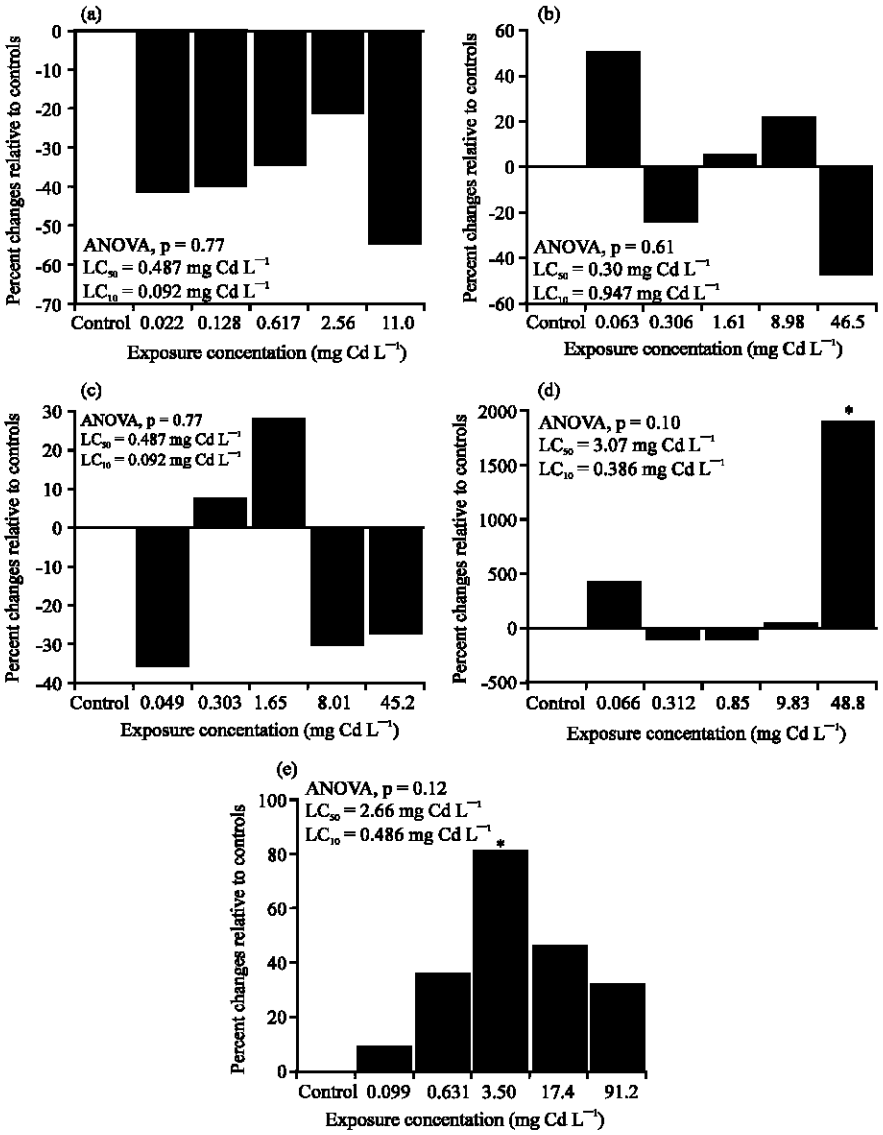

Fig. 2: Percent change in claw-raise behavior at each exposure concentration for 5 species of crayfish relative to controls. (a) *Orconectes placidus*, (b) *Orconectes virilis*, (c) *Procambarus acutus*, (d) *Procambarus alleni* and (e) *Procambarus clarkii*. An asterisk (*) indicates a significant difference from control responses ($p \leq 0.05$, Duncan's test). ANOVA, LC_{50} and LC_{10} values are also given (LC_{50} and LC_{10} values from Wigginton and Birge, 2007)

grouped by treatment, were then analyzed by ANOVA and pair-wise comparison with Duncan's Test. These proportions were also tested with regression analysis to determine if increasing or decreasing trends could be detected. In most cases, simple linear regression was used. However, for *P. clarkii*, residual analysis indicated that concentration should be transformed; a cube root transformation was selected empirically because it corrected the

problematic distribution of residuals observed initially. Additionally, the unusual shape of the claw raise response data for *P. clarkii* indicated that a quadratic model was need. The proportions derived for control animals were tested with simple linear regression to determine if body mass might have an effect upon the likelihood of performing either behavior. This analysis was performed within individual species and across all species. When done within a species, each crayfish in each control replicate was considered individually. Analyzed across four or five species of crayfish, the responses of individuals in each control replicate were averaged, as done previously. Statisitca (Student Version 5.5 for MS Windows, StatSoft Inc.) was used for all ANOVA and Duncan's Tests while SPSS (Student Version 12 for MS Windows, SPSS Inc.) was used for all regression analysis.

In each test, water samples were collected from at least two replicates of each exposure concentration daily. The sampling scheme was designed so that some samples were taken immediately before and after a water change to determine whether Cd levels remained constant. These samples were analyzed using either inductively-coupled plasma optical emission spectrometry (ICP OES-Varian Vista MPX) or graphite furnace atomic absorption spectrometry (GFAAS-Varian SpectrAA-20 with GTA-96 Graphite Tube Analyzer) to determine actual Cd exposure concentrations. Values reported on Fig. 1 and 2 are averages of measured Cd concentration from multiple water samples and so some variation occurs between tests for similar levels of Cd exposure.

RESULTS

Cadmium had a significant effect on three species of crayfish for the tail flip response. One-way ANOVA was performed and indicated Cd had a significant effect on the tail-flip response in *O. placidus, O. virilis* and *P. acutus* (Fig. 1a-e). Duncan's Test used in planned comparisons of the effects of Cd levels versus the control indicated that at least one concentration of Cd significantly decreased the tail-flip response in *O. virilis*, *P. clarkii* and *P. acutus* ($p<0.05$). A general trend was seen in each test whereby this reduction increased as Cd concentrations increased. This trend was significant in all species except *P. alleni* ($r = 0.395$, $p = 0.069$; Table 1). Between the five tests, the lowest test concentration to have a response significantly different from controls was 0.306 mg Cd L^{-1} (Fig. 1).

The effect of Cd on the claw raise response was less clear. Cadmium had a significant effect on *P. alleni* and *P. clarkii* when tested with one-way ANOVA (Fig. 2a-e). For *P. alleni* only the highest concentrations of Cd (48.8 mg Cd L^{-1}) resulted in a significant increase in the claw raise response (Fig. 2). *Procambarus clarkii*, on the other hand, showed a significant increase at the middle concentration (3.50 mg Cd L^{-1}), but not at the higher concentrations.

Table 1: Regression models for the effect of cadmium on five species of crayfish including pearson correlation coefficient (r), significance (p) and model equation

Species	Behavior	r-value	p-value	Equation
O. placidus	Tail-flip	0.420	0.041	$y = -0.21x+0.490$
	Claw-raise	0.362	0.195	$y = -0.003x+0.102$
O. virilis	Tail-flip	0.446	0.029	$y = -0.004x+0.201$
	Claw-raise	0.397	0.055	$y = -0.003x+0.310$
P. acutus	Tail-flip	0.790	<0.001	$y = -0.11x+0.557$
	Claw-raise	0.172	0.421	$y = -0.001x+0.167$
P. alleni	Tail-flip	0.395	0.069	$y = -0.003x+0.913$
	Claw-raise	0.579	0.005	$y = 0.005x+0.009$
P. clarkii	Tail-flip	0.474	0.019	$y = -0.025x+0.191$[a]
	Claw-raise	0.502	0.047	$y = 0.193x^2-0.038x+0.363$[a]

[a]Examination of residuals indicated data transformation was necessary for y values in these regressions. A cube root transformation was used

Table 2: Regression models for the effect of mass on either the tail flip response or claw-raise response for five individual species and across five or four species of crayfish including Pearson correlation coefficient (r), significance (p) and model equation

Grouping	Behavior	r-value	p-value	Equation
O. placidus [a]	Tail-flip	-0.848	<0.001	y = -0.066x+0.989
	Claw-raise	0.454	0.045	y = 0.017x+0.026
O. virilis [a]	Tail-flip	-0.644	0.002	y = -0.074x+1.315
	Claw-raise	0.591	0.006	y = 0.075x-0.646
P. acutus [a]	Tail-flip	-0.608	0.004	y = -0.050x+1.435
	Claw-raise	0.549	0.012	y = 0.035x-0.357
P. alleni [a]	Tail-flip	-0.861	<0.001	y = -0.049x+1.206
	Claw-raise	0.259	0.271	y = 0.006x-0.020
P. clarkii [a]	Tail-flip	-0.449	0.047	y = -0.022x+0.649
	Claw-raise	0.259	0.276	y = 0.016x-0.056
All species [b]	Tail-flip	-0.724	<0.001	y = -0.036x+0.988
	Claw-raise	0.702	0.001	y = 0.020x-0.045
All species except	Tail-flip	-0.860	<0.001	y = -0.045x+1.020
P. acutus [b,c]	Claw-raise	0.774	<0.001	y = 0.023x-0.056

[a]Each organism in each control replicate was treated individually in this analysis. [b]Responses of all organisms in a replicate were averaged for analysis. [c]*P.acutus* was excluded from this analysis because the high level of molting that occurred in this species' test probably altered their behavioral response dramatically

O. placidus showed a general decrease in response at all concentrations, although, the trend was not significant; *O. virilis* did not show any clear pattern. Regression analysis indicated a correlation between Cd concentration and claw-raise response in *P. alleni* and *P. clarkii* (Table 1).

Significant correlations existed between body mass and the tail flip behavior for all species and between mass and the claw raise behavior for three of five species. When individual control organism behavioral responses were compared, as body mass increased, the tail flip behavior became less common ($r = -0.449$ to -0.861, $p < 0.05$) while the claw raise behavior often became more common ($r = 0.454$ to 0.591, $p<0.05$ for *O. placidus*, *O. virilis* and *P. acutus*; Table 2). These trends also existed between species for all control organisms pooled. As body mass increased, the frequency of the tail flip response decreased ($r = -0.724$; $p<0.001$), while the frequency of the claw raise behavior increased ($r = 0.702$; $p = 0.001$) (Table 2). The assay with *P. acutus* had an unusually high frequency of tail flips based on the size of these crayfish. It was significantly higher than assays with all other crayfish except *P. alleni* ($p<0.01$ Duncan's Test). Given that the data from *P. acutus* were somewhat anomalous because of an unusually high molting rate in the test (Wigginton and Birge, 2007), these inter-species regressions were recalculated omitting data from that assay. The closeness of fit increased for both the tail flip response ($r = -0.860$; $p<0.001$) and the claw raise response ($r = 0.774$; $p<0.001$) (Table 2).

DISCUSSION

The effect of Cd exposure on the tail flip behavior was more consistent than on the claw raise response. Additionally, the tail flip response was typically more sensitive to Cd insult. *O. virilis* was the species with the most sensitive tail-flip response to Cd exposure with a significant effect at 0.306 mg Cd L^{-1} (Fig. 1). This value is ten times lower than the concentration lethal to 50% of the population (LC_{50}) and three times lower than the 10% lethality concentration (LC_{10}; Fig. 1). This level of Cd can be found in the environment in certain circumstances (Albert *et al*., 1992; Concas *et al*., 2006; Canovas *et al*., 2007). One unusual case was the response of *O. placidus* at 0.617 mg Cd L^{-1}. It exhibited a 20.1% higher incidence of the tail flip behavior compared to controls. While, the average mass for this

group was not significantly different from control organisms (t-test, p = 0.184), it started at a higher rate of the tail flip behavior with a daily average response of 70.3% compared to 51.3% for control crayfish. From the 24 h check to the 96 h check, the daily average tail flip response for the 0.617 mg Cd L^{-1} exposure concentration decreased in frequency from 70.3 to 41.7%. The next higher exposure concentration showed a similar 31.8% decrease between the 24 and the 96 h daily average. While most species exhibited a significant negative correlation between Cd and tail-flip behavior, *P. alleni* did not (Table 1), perhaps because members of this species were, on average, smaller than individuals in any other test and so the tendency for them to tail flip was likely strongest, as has been noted by Lang *et al.* (1977), Kellie *et al.* (2001) and Keller and Moore (2000). A significantly greater percentage of this species' control group tail flipped compared to any other test's control group (Duncan's test $p<0.01$). Thus, while Cd has clear effects upon crayfish behavior, especially the tail flip response, the variability of that response complicates the use of this behavior as an indicator of Cd exposure. However, it does seem that Cd, in some cases, can have significant effects upon the tail flip behavior at levels below acutely lethal exposure concentrations. Further studies could be done to more fully establish whether these effects can be found at more environmentally relevant concentrations, especially on a chronic timescale.

The claw raise response, when comparing control groups across all tests, was a generally less common occurrence than the tail flip response, except in the assay with *P. clarkii*. Individuals in this test were on average larger than those in any other test and actually exhibited a greater proportion of claw-raises than tail-flips. In the test with *P. clarkii*, it is likely that a significant response for the highest exposure groups was not seen because of the extreme lethality of these concentrations of Cd. All members of the highest and all but two of the second highest groups died by the end of the assay. Only tests with *Procambarus* species contained treatments that were significantly different from controls. The most sensitive significant response was in the assay with *P. clarkii* at an exposure level of 3.50 mg Cd L^{-1}, 32% greater than the LC_{50} value. The only other significant responses came from the highest exposure concentration in the *P. alleni* assays which was many times higher than that species' LC_{50} value. Additionally, the relationship in *P. alleni* is suspect because very few observations were made at the highest concentration before all organisms died. This indicates that the claw raise behavior is much less susceptible to Cd perturbation than the tail flip response and much less useful as a potential behavioral indicator of Cd stress. The fact that the claw raise was increased significantly in *P. clarkii* perhaps is explicable because it was on average the largest and larger crayfish tend to use the claw-raise response more frequently than the tail-flip response (Table 2). Thus, there were a sufficient number of observations of the claw raise response to allow for reliable measurement. In addition, *P. clarkii* is known to be an aggressive species that can exclude other species from their native habitats (Holdich, 2002), perhaps increasing its likelihood of performing the claw-raise response compared to other species.

When conducting the toxicity tests listed in this study, it became apparent that the *P. acutus* assay was different from the others. It experienced an unusually high molt frequency (41.7%) compared to the other tests (0.556% for *O. juvenilis* to 13.3% for *P. clarkii*.) (Wigginton and Birge, 2007). It has been shown that molting lobsters have altered threat responses compared to non-molting individuals (Cromarty *et al.*, 2000). Because of this, *P. acutus* was excluded from the analysis of the interspecific effects of size on tail flip and claw raise behavior. Periods of very high molting rates are known to occur in nature

(Taylor and Schuster, 2004) and given that they are times of considerable additional stress could represent times of unusually high sensitivity to toxicant insult (Wigginton and Birge, 2007).

The crayfish in this series of tests tended to exhibit the tail-flip response less often as average crayfish mass increased. Conversely, they evinced an increase in the frequency of the claw-raise behavior as average body mass increased. American lobsters (*Homarus americanus*) increase the frequency of their more aggressive defensive responses, including the claw-raise response, as they grow in size (Lang *et al*., 1977; Wahle, 1992). Also, as observed in this study, *H. americanus* and *P. clarkii* are less likely to exhibit a tail flip response as body mass increases (Lang *et al*., 1977; Kellie *et al*., 2001). In agreement with present findings, Keller and Moore (2000) found that smaller crayfish (*O. virilis*) tended to tail flip more often and larger crayfish to raise their claws more often when encountering fish, an important predatory threat to crayfish. In this series of tests, because the response was clear in each species' test, it would seem that size was a more important determinant of crayfish behavioral response to stimuli than interspecific variation meaning that comparison between species would be most meaningful if individuals were of the same size. The relative level of aggressiveness between crayfish species has been assessed in dominance contests between individuals of different crayfish species. The interspecific level of aggression does seem to be significantly different in some cases, which may be a factor that has aided invasive crayfish species, such as *O. rusticus*, in displacing native species such as *O. immunis* (Tierney *et al*., 2000). However, no pattern was found between body mass and the sensitivity of the behaviors to Cd exposure. Thus, while overall trends in behavioral response may be governed by body mass, the individual species seem to have intrinsic differences in how they respond to Cd. Further studies within and between groups would be needed to control for and assess both interspecific variability in behavioral response and the effect of body mass on response.

The assay with *P. acutus*, the second heaviest species on average, had a significantly higher frequency of tail flips than assays with smaller crayfish ($p<0.05$, Duncan's Test), except *O. placidus* which showed no statistical difference. However, as previously mentioned, this assay experienced an unusually high number of molts which probably explains the altered response (Cromarty *et al*., 2000).

The condition of an individual's chela (i.e., both present, one missing, two missing, one or more being a product of regeneration) was not controlled in this study which is a confounding factor since loss of one or both chela can increase the likelihood of the tail-flip behavior compared to the claw-raise response (Kellie *et al*., 2001; Page *et al*., 2007). Additionally, this may explain why *P. clarkii*, despite being generally larger than other crayfish in this series of tests, did not have a significant relationship between mass and behavioral response. Some crayfish were missing one chela in this test. The lack of a significant increasing pattern for the claw raise behavior in *P. alleni* was probably because the individuals were so small. Only one claw raise behavior was observed in the control organisms in the entire *P. alleni* test. The complete separation of individuals would also be beneficial, since in the current study, to ensure that Cd was equally available to all individuals, crayfish were housed in perforated beakers in common tanks of exposure medium. This allowed one individual to potentially hear or smell the reaction of another crayfish when tapped, which may have altered its reaction as crayfish are known make extensive use of both tactile and chemical cues in communication and the assessment of their environment (Schneider and Moore, 2000; Gherardi *et al*., 2002). This is a potential weakness of the analysis of crayfish behavior versus body mass within each species. In other analysis,

the responses of crayfish within a replicate were averaged to avoid the possibility of pseudo-replication since organisms with a replicate were imperfectly separated. However, for body mass versus behavior assessment within a given species, the averaging of responses within replicates left too few data points for effective analysis (n = 4). In addition, crayfish in a particular test were randomly assigned to replicates which resulted in relatively similar average mass values between control replicates for a species. Given that results for individual species generally matched the interspecies trends and results from other researchers, the pseudo replication in this part of the analysis did not seem to affect intraspecies analysis.

Overall, given that the tail flip response is more commonly used by smaller crayfish and it is more strongly and consistently affected by cadmium exposure, disruption of the tail flip response may be an important sublethal toxicological effect because juvenile crayfish can be much more susceptible to cadmium exposure than adults. The LC_{50} value for juvenile *O. juvenalis* and *O. placidus* are 0.060 and 0.037 mg Cd L^{-1}, respectively (Wigginton and Birge 2007). If the tail flip behavior were disrupted at a fraction of the, respective LC_{50} value, as in adult *O. virilis*, then survival could be adversely affected at low and environmentally relevant concentrations.

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