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Heavy Metals Alter the Survival, Growth, Metamorphosis, and Antipredatory Behavior of Columbia Spotted Frog (*Rana luteiventris*) Tadpoles

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Abstract. Amphibian populations appear to be declining around the world. Although there is no single cause, one factor may be pollution from heavy metals. As a result of mining in the Silver Valley of Idaho, heavy metals have been released into habitats containing many species of sensitive organisms, including spotted frogs (*Rana luteiventris*). While the gross extent of pollution has been well documented, the more subtle behavioral effects of heavy metals such as lead, zinc, and cadmium are less well studied. We tested the effects of heavy metals on the short-term survival (LC_{50}) of spotted frog tadpoles. Compared to single metals, metals presented together were toxic at lower doses. We also raised the tadpoles in outdoor mini-ecosystems containing either a single heavy metal or soil from an EPA Superfund site in the Silver Valley known to be composed of numerous heavy metals. Exposure to Silver Valley soil resulted in delayed metamorphosis. We tested the ability of metal-exposed tadpoles to detect and respond to chemical cues emanating from predacious rainbow trout. We found that high levels of Silver Valley soil, medium levels of zinc, and medium and high levels of lead resulted in a decreased fright response. Low levels of cadmium, zinc, and lead did not cause a significant effect, but low levels of soil did result in a decreased fright response. Heavy metals may alter interactions between tadpoles and their predators.

Numerous species of amphibians are declining around the world (Blaustein and Wake 1990). Although many causes have been proposed, *e.g.*, natural fluctuations (Pechmann *et al.* 1991), pathogens (Blaustein *et al.* 1994a), habitat destruction, habitat fragmentation, acid rain, declining ozone layer (Blaustein *et al.* 1994b), and oil pollution (Lefcort *et al.* 1997), most of these causes involve human activities. One cause of this decline in areas of mining, such as the Appalachian or Rocky Mountains, may be heavy metal pollution.

The decline of amphibians from these habitats is not surprising given the extent of heavy metal pollution. Heavy metals such as lead (Pb), zinc (Zn), cadmium (Cd), mercury (Hg), silver (Ag), copper (Cu), arsenic (As), manganese (Mn), molybdenum (Mo), and antimony (Sb) originate from mines, mine tailings, and smelters at the head waters of the South Fork of the Coeur d'Alene River in the Silver Valley of Northern Idaho. The metals' impact on the terrestrial and aquatic environment in this portion of the Northern Rockies is dramatic. For example, the mountains surrounding the towns of Kellogg and Smelterville, located in the Silver Valley of the Coeur d'Alene basin, are denuded of vegetation because of volatile smelter byproducts (Krieger 1990; Neufeld 1987). The point source of contamination is associated with the Bunker Hill Environmental Protection Agency (EPA) Superfund site (Horowitz *et al.* 1993).

During the later part of the 19th and early 20th centuries, it was common practice to deposit metal-rich mine tailings and smelter byproducts directly into and along the banks of rivers. This ecosystem has been contaminated by more than 10^7 tons of metal-rich mine tailings over the past 110 years. Past and present studies (Ridolfi Engineers 1993) indicate that contamination levels are high and extensive in the river and lake system. Heavy metals leaching from tailings, as well as contaminated soil and sediments, continue to enter the ecosystem. For example, a 1986 US EPA NPDES permit allowed up to 5 tons per year of lead to enter the river system (Neufeld 1987). In flood years such as 1996 and 1997 even more sediments are disturbed. Heavy metals in the ecosystem continue to adversely affect the biota of the valley (Hoiland and Rabe 1992). Only remnant, nonrecruiting populations of anurans occur in the upper reaches of the valley near the point source (Lefcort unpublished data).

Because tadpoles feed off both the substrate and attached algae, and continuously process water for respiration, their tissues are potentially exposed to a variety of environmental pollutants, including dissolved toxins, sediment-bound contaminants, and bioconcentrated metals (Hall and Mulhern 1984, 1989; Freda 1991; Horne and Dunson 1995). Therefore, their physiology makes them ideal organisms for monitoring levels of contaminants and investigating long-term patterns of natural and anthropogenically induced changes in aquatic ecosystems (Harfenist *et al.* 1989). Similarly, their relatively easily monitored behavior makes them excellent models for detecting subtle behavioral effects that may occur at lower doses of contamination than those doses that induce gross physiological effects.

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up. One day later a single Gosner stage 22–25 (*i.e.* without leg buds, Gosner 1960) tadpole was added to each container. At 12-h intervals, for the next 96 h, the tadpoles were monitored to see if they were still alive. Tadpoles were not fed after being added. Probit analysis (Finney 1947) was used to determine calculated LC_{50} values.

Experiment Two: Survival, Growth, and Development

In Experiment Two, we tested the effects of heavy metals on the survival, growth, and metamorphosis of tadpoles. Forty-eight 64-L plastic containers (some containing heavy metals, see below) were set up on the roof of a building at Gonzaga University. The containers were filled with dechlorinated tapwater and a measured inoculate of leaf litter, algae, and zooplankton. One liter of sand was added as a substrate. These mini-ecosystems were self-sustaining and no external sources of food were added. Distilled water was added each week to compensate for evaporation. The different treatments were: *Control* (no metals), *Lead* from lead nitrate (50 ppm, high treatment, 5 ppm, medium treatment, and 0.01 ppm, low treatment), *Zinc* from zinc nitrate hexahydrate (50 ppm, 15 ppm, and 0.05 ppm), *Cadmium* from cadmium nitrate tetrahydrate (20 ppm, 5 ppm, and 0.1 ppm), and *Soil* from the Superfund site in the Silver Valley of Idaho (1,000 ml and 100 ml). The metal concentrations matched those found in ponds in the Silver Valley, with high levels near the point source and lower levels downstream (Lefcort and Ettinger unpublished data). Three days after adding the metals, 50 2-week-old tadpoles were added to each mini-ecosystem container.

One week after setting up the high- and medium-level metal treatments we noticed that some of the high single-metal-level tadpoles were dying. It was at this point that the low-metal-level treatments were set up using tadpoles from the original clutches that remained in the laboratory. Because of the difference in starting dates the high and medium treatments were analyzed separately from the low level of metals (see Results).

Each treatment was replicated four times for a total design of 48 mini-ecosystems. Once each week five tadpoles from each container were measured to determine their length, weight, and stage of development. Metal levels in tadpoles and the container water were measured partway through the experiment when the tadpoles were 5 weeks of age. To test for treatment effects, Student's *t* tests were used with alpha set to 0.05. One-way ANOVA with subsequent Student-Newman-Keuls multiple comparison of means tests were also used. Because four response variables were analyzed with one-way ANOVA tests, alpha was conservatively Bonferroni-adjusted to 0.0125 (Sokal and Rohlf 1995).

Experiment Three: Fright Response

We tested the behavioral response of Experiment Two tadpoles to chemical cues present in water that had been in contact with predacious rainbow trout. The apparatus was similar to that used in previous studies (Petranka *et al.* 1987; Lefcort and Eiger 1993; Lefcort 1996). Tadpoles were tested in two gravitational flow-through systems (Figure 1) each composed of three 25-L plastic tubs (51 cm × 37 cm × 21 cm). In each system, the tubs were arrayed at different heights so that water flowed from one to another at 0.5 L/min. Freshwater from the uppermost tub flowed into the middle tub and picked up any chemical cues that might be present. It then flowed into the lower tub, which contained four tadpoles. The lower (tadpole) tub had an output opening and never contained more than 10 L of water.

The uppermost tub (water storage) was filled with 23 L of water. The middle tub (chemical-cue treatment) was either unoccupied or contained one of three randomly chosen trout. From the middle tub, water flowed to the lowest tub, which was divided across its length by a faint pencil line to delineate the sides such that half the tub contained plants (*Limnophila*), as a potential refuge, and half did not. Since the tub was divided lengthwise, plant placement was not confounded with proximity to incoming water. The middle and lower tubs were wrapped in black plastic to prevent visual cues. The side of the tub without plants was further divided along its width by a faint pencil line to measure activity within that half.

Data were recorded while looking through a slit in a 2-m tall black curtain. The lowest tub was rinsed between replicates so that the tadpoles were always added to a tub containing untreated water. The tadpoles were given 30 min to acclimate to the tub before the flow was started. Ten minutes after flow was initiated, we recorded the number of tadpoles on the side of the tub without plants. These data were recorded for an additional 10 min at 1-min intervals. Throughout this second period of 10 min we monitored movement by recording the number of times any tadpole crossed between the half of the tub that contained plants to the half that did not, and the number of times any tadpole crossed between the upper and lower halves of the side of the tub without plants. Individual tadpoles were not followed, therefore the number of moves may be due to only a few of the four tadpoles.

The experiment was conducted when the tadpoles were between 3 and 5 weeks of age (Gosner stages 22–25). Tadpoles exposed to medium metal levels were tested first, and 1 week later tadpoles exposed to low metal levels were tested. Because of a possible ontogenetic trend in behavior, control (no metal) tadpoles were tested during both the medium- and low-metal-level trials. To test for significant treatment effects, two-way ANOVA with subsequent Student-Newman-Keuls multiple comparison of means tests were used with alpha set to 0.05.

Results

Experiment One: Short-Term Survival in High Concentrations of Metals (LC_{50})

The calculated metal concentration that killed half of a group of tadpoles at 24, 48, 72, and 96 h are presented in Table 1. None of the animals in the control (no metal) treatment died. Interestingly, the interactions between zinc and cadmium caused the two metals to be much more toxic in combination than either was alone. Unfortunately, lead would not stay in solution. All chemicals that we used to keep the lead in solution had mildly toxic effects and when presented alone caused the death of some tadpoles. For this reason the LC_{50} of lead was not determined.

Experiment Two: Survival, Growth, and Development

We examined how high, medium, and low concentrations of lead, zinc, cadmium, and soil from the Silver Valley Superfund site would affect the growth and development of tadpoles. The concentrations of metals in the various treatments are presented in Table 2 (concentrations below 0.1 ppm are near the limits of detectibility of the ICP metal analyzer). Tadpoles exposed to high levels of metals did not survive to metamorphosis.

The number of tadpoles that survived to metamorphosis was dependent on the metal treatment the tadpoles were exposed to (one-way ANOVA, $F_{6,27} = 28.7$, $p < 0.001$, Table 3). Survival of the control and the low-lead-treatment animals was higher

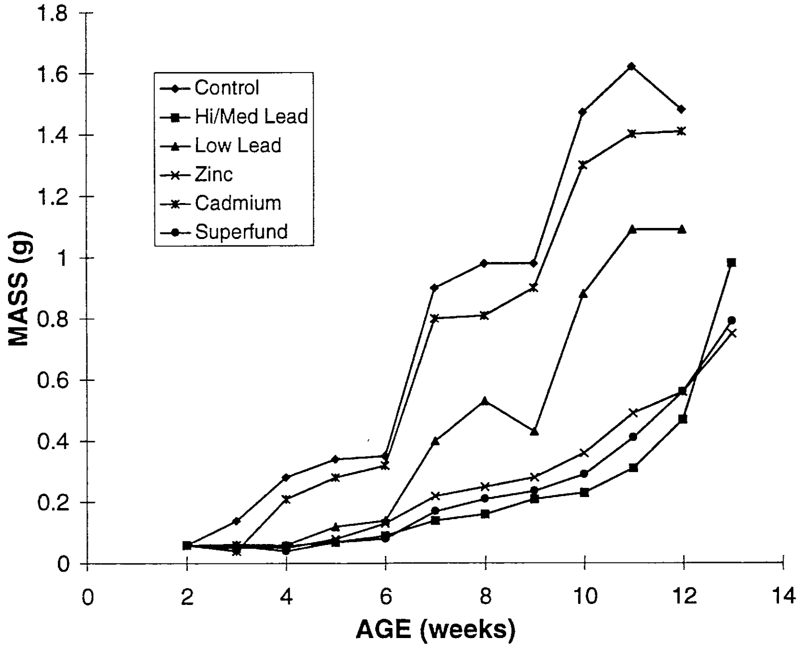

Fig. 2. Mean mass of tadpoles prior to metamorphosis. Due to low numbers of survivors the Superfund treatment is composed of both high and low Superfund animals

phose than the control, low cadmium, and low lead tadpoles (Student-Newman-Keuls multiple comparisons of treatment means).

Tadpoles that undergo metamorphosis at an older age tended to be heavier. The mean weight at metamorphosis was dependent on which treatment the tadpoles were exposed to (one-way ANOVA, $F_{6,27} = 20.9$, $p < 0.001$, Table 3), with the high and low Superfund tadpoles and the high/medium lead combination tadpoles weighing significantly more than the low lead, low zinc, low cadmium, and control tadpoles (Student-Newman-Keuls multiple comparisons of treatment means). Considering just mass at metamorphosis is deceptive because the high and low Superfund tadpoles, and the high/medium lead combination tadpoles were older than the control water tadpoles and many of the exposed tadpoles did not undergo metamorphosis. At all ages after week two, the high and low Superfund tadpoles and the high/medium lead combination tadpoles actually weighed less than the control water tadpoles (Student's t test, $p < 0.01$, Figure 2). The control water tadpoles weighed significantly more than low lead and low zinc tadpoles at metamorphosis.

The mean snout to urostyle length at metamorphosis was not dependent on which treatment the tadpoles were exposed to (one-way ANOVA, $F_{6,27} = 2.26$, $p = 0.073$, Figure 3).

Experiment Three: Fright Response

High/Medium Lead, Medium Zinc, and High Superfund: Although the medium zinc animals died before metamorphosis, we completed Experiment Three before they died.

Metals had a significant effect on refuge use. The mean number of moves (total number of times tadpoles crossed from one half of the tub to the other half) was dependent on which metal treatment the tadpoles were exposed to (Table 4 and Figure 4), with the unexposed tadpoles moving significantly more than the lead-, zinc-, and soil-exposed tadpoles both in the presence and the absence of the fish (Student-Newman-Keuls test). There was a significant effect for fish overall, but when each individual metal treatment was compared, only the unexposed tadpoles altered (decreased) their activity levels in the presence of the fish (Student-Newman-Keuls multiple comparisons of treatment means). However, there was no statistically significant interaction between the presence of the predator and the metal treatment.

The presence of the fish had a significant effect on decreasing the mean number of animals on the side of the tub away from the pond plants (Table 4 and Figure 5). This was due to the zinc and soil treatment animals being in the open more than the lead and the unexposed treatments (Student-Newman-Keuls test). However, this resulted in a significant interaction effect between the treatment (no metal, lead, or zinc) and the presence of the predator since only unexposed animals increased refuge use in the presence of the predator (Student-Newman-Keuls multiple comparisons of treatment means). Looking at Figure 5, the lead treatment animals appear to differentiate between the presence and absence of the predator but this behavioral difference is not statistically significant (Student-Newman-Keuls multiple comparisons of treatment means).

Low Lead, Low Cadmium, Low Zinc, and Low Superfund: The mean number of moves (total number of times tadpoles crossed from one part of the open side of the tub to the other open part) was not dependent on which metal treatment the tadpoles were exposed to (Table 4 and Figure 6). There was a significant effect for the presence of the fish, with all treatments except "soil" (Student-Newman-Keuls multiple comparisons of treatment means) decreasing their movements when the fish was present. Their was no interaction between the presence of the predator and the metal treatment.

Significant treatment effects were also found for the effect of the predator on the mean number of animals on the open side of

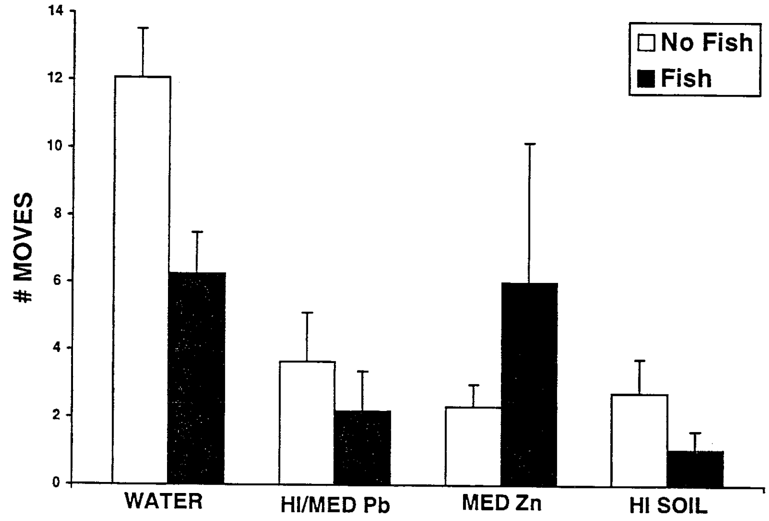

Fig. 4. Mean (+SE) of moves made by groups of five tadpoles when exposed to water with and without fish chemical cues

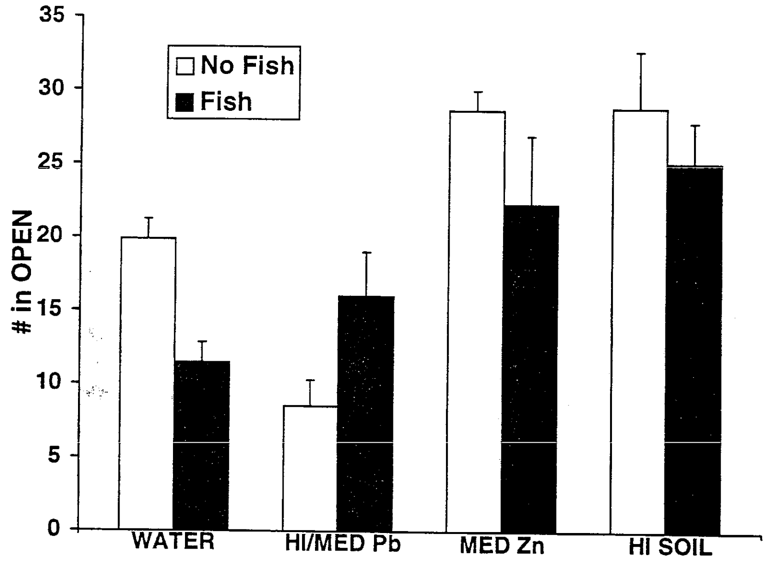

Fig. 5. Mean (+SE) number of animals in the open (outside of refuge) made by groups of five tadpoles when exposed to water with and without fish chemical cues

mass at metamorphosis (Dilling and Healey 1926). Although metamorphosis at a large mass is positively correlated with adult survival and future fitness (Smith 1987), it is a risky life-history trait in environments such as temporary ponds that might dry up. However, tadpoles used in our study were gathered from a permanent lake, so increased time to metamorphosis may be adaptive. Interestingly, regardless of the time to metamorphosis, tadpoles in all treatments underwent metamorphosis at similar body lengths.

Third, the metals reduce the normal antipredatory behavior of the tadpoles. When control tadpoles were exposed to the odor of a fish that had fed on tadpoles they fled to plant refugia and then reduced their activity levels. However, tadpoles exposed to medium levels of lead and zinc, or high levels of soil from the Superfund site did not flee to refugia or reduce their already low level of activity. At lower levels of lead, zinc, or cadmium, tadpoles exhibited normal antipredatory behaviors. However, at low levels of contaminated soil they failed to take evasive action when presented with chemical cues from fish. Heavy metals can interfere with olfaction in fishes (Hara 1982) and the fright response of bullfrog *R. catesbeiana* has also been found to be reduced by heavy metals from coal ash (Raimondo *et al.* 1998). Reduction in the fright response increases susceptibility to predation of southern leopard frog (*Rana utricularia*) tadpoles by sunfish (Lefcort 1996). However, it is difficult to predict the ecological ramifications of heavy metal–induced altered tadpole fright responses. If metal-exposed fishes also suffer from altered foraging abilities then tadpole "carelessness" may not affect tadpole mortality.

We also found that the metals had a greater effect when presented together. Acute survival times were lower when zinc and cadmium were presented together than when presented singly. The effects were twice as great as one might predict from a simple additive effect. Mine wastes in the Silver Valley area are composed of a mixture of over a dozen heavy metals. Although some metals are present in low concentrations, their cumulative effect may be great. The low level of the behaviorally altering Superfund soil treatment had lower concentrations of cadmium and zinc than the treatments composed solely of low levels of cadmium or low levels of zinc, which did not alter high levels of metals accumulating in plants and algae represent a greater threat to primary consumers than to higher trophic levels (Henney *et al.* 1991). Mercury is the only element that becomes appreciably biologically magnified and only then in its methylated or alkylated forms (Boudou and Ribeyre 1981).

In conclusion, heavy metals exerted detrimental effects on tadpole growth, development, and survival to metamorphosis. The metals also reduced predator avoidance behavior. If metal-exposed tadpoles are more susceptible to predation, then predators may indirectly include a higher percentage of metal-exposed tadpoles in their diets than would be expected from the percentage of metal-exposed tadpoles in an area.

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