SUBLETHAL EFFECTS OF LEAD ON NORTHERN LEOPARD FROG (RANA PIPIENS) TADPOLES

TE-HAO CHEN,*† JACKSON A. GROSS,†‡ and WILLIAM H. KARASOV†
†Department of Wildlife Ecology, ‡Department of Animal Sciences, University of Wisconsin–Madison, Madison, Wisconsin 53706, USA

Abstract—Northern leopard frogs (Rana pipiens) were exposed to environmentally relevant concentrations of lead in water (3, 10, and 100 μg/L, as lead nitrate) from the embryonic stage to metamorphosis. Survival, growth, deformity, swimming ability, metamorphosis, and lead tissue concentrations were evaluated. Tadpole growth was significantly slower in the early stages (Gosner stages 25–30) in 100 μg/L treatment. More than 90% of tadpoles in the 100 μg/L treatment developed lateral spinal curvature, whereas almost all the tadpoles in the other groups were morphologically normal. Spinal deformity was associated with abnormal swimming behavior. Maximum swimming speed of tadpoles in the 100 μg/L treatment was significantly lower than that in the other groups. No significant effect of lead exposure was found on percentage metamorphosis, snout–vent length, mortality, and sex ratio of metamorphs. Time to metamorphosis was delayed in 100 μg/L treatment. Lead tissue concentrations in the tadpoles ranged from 0.1 to 224.5 mg/kg dry mass, were positively related to lead concentrations in the water, and fell within the range of tissue concentrations of wild tadpoles found in previous studies. According to our results, the current U.S. Environmental Protection Agency freshwater-quality chronic criterion for lead (2.5 μg/L) is appropriate to protect northern leopard frog larvae.

Keywords—Sublethal Lead Amphibian Tadpoles Development

INTRODUCTION

Lead (Pb) occurs naturally in the earth’s crust, but the environmental distribution of Pb is most influenced by human activities [1]. Although the use of Pb by humans has thousands of years of history, demand has increased considerably since the Industrial Revolution of the 18th and 19th centuries [1]. Sources of Pb inputs into surface water include deposition of atmospheric Pb and point sources of emissions, such as discharges from sewage treatment plants, industries, and mine works. In addition to the point sources, Pb also may enter waterways from highway runoff or leaching from mine tailings [1]. Large amounts of spent lead shot as a result of hunting may elevate Pb concentrations in surface water [2]. In the United States, levels of Pb measured in the surface water typically range between 5 and 30 μg/L, with averages of 3.9 μg/L, but levels as high as 890 μg/L have been reported [3]. Lead exposure may cause deleterious effects on aquatic wildlife, including amphibians [1].

Lead is a nonessential heavy metal and is toxic to organisms. The most widely studied effects in animals are those on survival, development, reproduction, behavior, nervous system, and hematological system [1]. Toxicity of Pb has been studied extensively in fish, birds, and mammals, but information related to amphibians is relatively sparse [4]. A few field studies have related Pb levels in amphibians to those in their ambient environment (see, e.g., [5]). Although without correlating Pb levels in amphibians with negative effects on amphibians, these studies did suggest that amphibians living at contaminated sites might face the risk of Pb toxicity. However, field studies alone may not be able to establish causes of effects because of the potential confounding influences of other contaminants or environmental stressors [4]. Thus, laboratory-based dose–response toxicity tests are essential to confirm the effects caused by a single metal, which is Pb in our case.

Most studies have focused on survival after acute exposure [4], but some sublethal endpoints have been reported in the literature as well. For example, Perez-Coll et al. [6] found that toad (Bufo arenarum) embryos exposed to Pb experienced arrested development, underdeveloped gills, curved tails, and increased mortality. Sobota and Rahwan [7] showed that Pb exposure resulted in neural tube defects and spinal deformities in Xenopus laevis tadpoles. Other researchers found more subtle effects, including inhibited learning performances and hypoxia-like responses in bullfrog (Rana catesbeiana) tadpoles exposed to Pb [8]. However, research has been done only with a few native North American species [9–11]. Concerning application in ecological risk assessment and amphibian conservation, it is important to conduct toxicity tests on native species, because sensitivity between species may be quite different. Chronic-exposure studies also are necessary, because sublethal effects, such as those on growth, behavior, and metamorphosis, usually cannot be detected in acute-exposure studies [4].

The objective of the present study was to determine the effects of chronic Pb exposure at environmentally relevant concentrations on survival, growth, development, behavior, and metamorphosis of larval northern leopard frogs (Rana pipiens). We chose northern leopard frogs because they have a widespread distribution across most of northern North America. To the best of our knowledge, the present study would be the first to address the effects of chronic Pb exposure in this species.

MATERIALS AND METHODS

Study organisms and animal husbandry

Six clutches of fertilized eggs were obtained from captive adult frogs collected from the Sensiba Wildlife Area (Suamico, WI, USA) according to procedures of artificial insemination.
described previously [12]. Briefly, six females were dosed with luteinizing hormone–releasing hormone agonist to induce ovulation. Eggs were stripped from the females by abdominal squeeze and then mixed with solution of minced testes from six killed males. Gosner stage (GS [13]) 17 embryos were transferred to 150-ml Petri dishes containing 100 ml of one of four treatment solutions (described below) until reaching GS 25. Tadpoles were then transferred to 5-gal I-Chem Certified® low-density polyethylene (LDPE) tanks (Fisher Scientific, Hampton, NH, USA) containing the respective treatment solution through metamorphosis. The temperature was maintained at 21 to 22°C and a constant 14:10-h light:dark photoperiod. Total solution changes occurred every day while larvae were in the Petri dishes and every other day while in the LDPE tanks. Tadpoles were fed with ground rabbit chow (Harland-Teklad, Madison, WI, USA) ad libitum every other day following solution changes from GS 25 to metamorphosis.

On forelimb emergence (GS 42), metamorphs were transferred to plastic boxes with small compartments. Every metamorph was individually placed in a single compartment (115 × 51 × 45 mm) with an appropriate amount of original treatment solution and a piece of paper towel to prevent desiccation. The solution in each compartment was changed every other day. These plastic boxes were placed on a slant to provide a bank for developing juveniles to climb out of the water. Food was not provided at this time, because during the metamorphosis process, the animal did not eat and all nutrition was provided by the resorption of the tail [14]. On complete tail resorption, juveniles were killed with 0.5 g/L of MS-222 (tricaine methane sulfonate; Western Chemical, Ferndale, WA, USA).

*Treatment solutions*

The stock solutions (72.58, 241.92, and 2,419.2 mg/L as Pb(NO₃)₂ for the low, medium, and high treatments, respectively) were prepared by dissolving Pb(NO₃)₂ powder (Sigma-Aldrich, St. Louis, MO, USA) in ultrapure water (Millipore, Billerica, MA, USA). To reach the nominal Pb concentrations (3, 10, or 100 µg/L) in each tank, 1 ml of the respective stock solution was added to 4 gal of dechlorinated, ultraviolet radiation-sterilized water (pH 7.9; hardness, 170 mg/L as CaCO₃) in the tanks following water changes. For the controls, nothing was added to the tanks after water changing.

*Experimental design*

Ten embryos (GS 17) from each of the six clutches were randomly assigned to 1 of 20 Petri dishes, so 60 larvae were in each Petri dish. Each Petri dish was randomly assigned to one of the control, low, medium, or high (0, 3, 10, or 100 µg/L, respectively) treatments, with five replicates in each treatment. When tadpoles reached GS 25, they were transferred to 5-gal LDPE tanks containing 4 gal of dosed or control water and allowed to develop through metamorphosis. On days 34, 52, and 66, ten tadpoles from each tank were randomly selected for snout–vent length (SVL) measurement. The length from the tip of the snout to the posterior margin of the vent of each tadpole was measured; any deformities also were recorded. Tadpoles were then returned to their original tanks. When larvae reached metamorphic climax (i.e., emergence of forelimbs at GS 42), they were transferred to the plastic boxes described above through the metamorphosis process. On complete tail resorption, juveniles were killed in MS-222 solution. Their SVLs were measured, and the gender of each juvenile was determined by examination of the relative size of its gonads under a dissecting microscope. The gonad/kidney complexes of juveniles were dissected out and preserved in 10% formalin for future histological study. Some metamorphs died before completing tail resorption. We performed the same procedures on those animals except for measuring their SVL. The experiment was terminated on day 154 post-hatch. All tadpoles that failed to metamorphose were killed and frozen at −20°C for metal analysis.

*Swimming ability test*

Swimming ability of tadpoles was evaluated on day 68 (methods modified from those described by Jung and Jagoe [15]). Ten tadpoles (GS 26–38) were randomly selected from each tank. Each tadpole was placed at one end of a Plexiglas (Rohm and Haas, Philadelphia, PA, USA) testing channel (60 × 5 × 4 cm) marked at 1-cm intervals. The channel was filled to a 2-cm depth with clean, dechlorinated water at the same temperature as that in the tadpole tanks. Tadpoles were chased at apparent maximum speed using a piece of blunt plastic stick (without being touched) along the channel and were videotaped over a 20-cm distance in the middle of the channel by a TR-140 camcorder (Sony, Tokyo, Japan). A stopwatch was placed next to the channel and recorded in the video. The distance each tadpole swam in 1 s was determined from the recording using slow-motion analysis. The fastest sprint speeds of each tadpole were used for statistical analysis. This measurement is to simulate the maximum sprint speed that could be used by the tadpoles to escape predators.

*Tissue Pb analysis*

Unfortunately, tadpole tissues designated for metal analysis in this experiment were contaminated with metals during the homogenizing process. Therefore, we later repeated a similar exposure experiment for collection of tissue for Pb concentrations analysis. In this experiment, approximately 900 fertilized *R. pipsiens* eggs from three clutches were purchased from Nasco (Fort Atkinson, WI, USA). Embryos were pooled, and 60 embryos (GS 20) were randomly sampled and distributed to each treatment. Two replicates were used in each treatment, and 30 animals were in each replicate. Tadpoles were raised in water dosed with or without Pb following the same protocol used in the main experiment. When the first tadpole reached forelimb emergence, all tadpoles were killed with MS-222, pooled within treatment groups, and freeze-dried. From each pool, two subsamples of 1 g of dried tadpoles were randomly selected and sent to the Soil and Plant Analysis Lab of the University of Wisconsin (Madison, WI, USA) for analysis. Tissue Pb concentrations were determined by induced coupled plasma–mass spectroscopy after an open-vessel HNO₃ plus H₂O₂ hot-plate digestion. The minimum detection level was 0.04 mg/kg dry mass.

*Statistical analyses*

Statistical analyses were performed with SYSTAT® (Ver 11; Systat Software, Richmond, CA, USA). Shapiro-Wilk’s test was used to test the assumption of normality. Proportion data for mortality, deformity, metamorphosis, and sex ratio were adjusted with an arcsine square-root transformation. Differences between treatments were compared by analyses of variance (ANOVA). Tadpole swimming speed was analyzed with an analysis of covariance (ANCOVA), with larval total length as a covariate. In these and other ANOVAs, F values were presented with their respective degrees of freedom as sub-
RESULTS

Survival, growth, and deformities

Tadpole mortality (mortality from GS 17 to GS 42) was dependent on the Pb treatments ($F_{3,16} = 3.68$, $p = 0.03$). Mortalities were not significantly different between the control and any of the Pb treatments. Significantly higher mortalities were found, however, in the high-Pb treatment when compared to the medium-Pb treatment (Tukey’s test, $p = 0.02$) (Fig. 1).

Tadpole SVL in high-Pb treatment was significantly lower than in all the other treatments on day 34 post-hatch and was significantly lower than that in the medium-Pb treatment on day 52 post-hatch (Tukey’s test, $p < 0.05$). On day 66 post-hatch, although tadpole SVL in the high-Pb treatment was still the lowest, no significant difference was found between treatment groups ($F_{3,16} = 0.59$, $p = 0.63$) (Fig. 2).

Tadpole spinal deformity was observed in the present study. Examined on day 66, scoliosis (lateral spinal curvature) was found in 92% of tadpoles sampled in the high-Pb treatment but in only 6% of those in the control; no tadpoles in the other two treatments were observed to have scoliosis. The prevalence of scoliosis was dependent on Pb treatments ($F_{3,16} = 157; p < 0.001$). Varying degrees of severity were found, but all consisted of a lateral curvature of the tail originating at some point between the tail base and the tail end. Some deformed tadpoles developed an only slightly curved spine, but some seriously deformed ones had a nearly 90° spinal curvature. The spinal malformation usually persisted after metamorphosis if the scoliosis originated in the urostyle region, but the malformation often was not observed after metamorphosis if the scoliosis originated in the lower-tail region.

Swimming performance

Maximum swimming speed was positively related to tadpole total body length (ANCOVA, $F_{1,97} = 18.6$, $p < 0.001$) and was dependent on Pb treatments ($F_{3,93} = 11.8$, $p < 0.001$) (Fig. 3). Swimming speed of high-Pb tadpoles was significantly slower than that in all other treatments (Tukey’s test, $p < 0.02$). Tadpoles in the medium-Pb treatment swam slower, but not significantly so (Tukey’s test, $p > 0.2$), compared with those in the control and low-Pb treatment. We also observed that tadpoles with severe spinal curvature were unable to swim straight forward but swam in a circular pattern.
Metamorphosis

The first tadpole to reach GS 42 (forelimb emergence) was observed on day 63. An increased time to initial metamorphosis was observed in the high-Pb treatment (Fig. 4). The first tadpole in a tank to reach GS 42 from that group was significantly delayed compared to all the other groups (Tukey’s test, $p < 0.05$). Time required for 50% of tadpoles in a tank to reach GS 42 was longest in the high-Pb treatment, but this was not statistically significant ($F_{3,16} = 1.68$, $p = 0.21$) (Fig. 4). No significant effect of Pb concentrations was found on percentage metamorphosis. In addition, mortality during tail resorption (GS 42–46) as well as SVL and sex ratio of metamorphs were not significantly different between treatments (Table 1).

Tissue Pb analysis

In the second tadpole exposure study, the first animal to reach GS 42 was observed on day 60, and all tadpoles were killed at that time. Whole-body Pb concentrations in tadpoles were positively associated with nominal Pb concentrations in water ($r = 0.995$) (Fig. 5). Tadpole Pb body concentrations in the control, low-, medium-, and high-Pb treatments were 0.1, 8.0, 25.5, and 224.5 mg/kg dry mass, respectively. Tadpole Pb body concentrations (log transformed) were significantly different between treatments ($F_{3,4} = 2.614, p < 0.001$) and were significantly different from each other (Tukey’s test, $p < 0.001$).

**DISCUSSION**

Survival and growth

Studies of short-term acute exposure to Pb showed that the 48-h median lethal concentration (LC50) of *B. arenarum* was 470 µg/L [6], and the 96-h LC50 of *Rana hexadactyla* was 33,280 µg/L [16]. Rice et al. [11] showed that exposure of 1,000 µg Pb/L for 14 weeks did not increase mortality in bullfrog (*R. catesbeiana*) tadpoles. Stansley et al. [9] observed that exposure to surface water (291–1,934 µg Pb/L) from a Pb-contaminated trap and siete range for 10 days caused high tadpole mortality in *Rana palustris* but not in *R. catesbeiana*. In the present study, tadpole mortality was significantly different between treatments and was highest at the 100 µg/L treatment; however, the dosed treatments were not significantly different from the control. It is noticeable that tadpole mortality...

---

**Table 1. Percentage metamorphosis and snout–vent length (SVL), mortality (by Gosner stages 42–46), and percentage male of *Rana pipiens* metamorphs exposed to lead via culture water** *(mean ± standard error; n = 5 tanks in each treatment)*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Metamorphosis (%)</th>
<th>SVL of metamorphs (mm)</th>
<th>Mortality of metamorphs (%)</th>
<th>Male metamorphs (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>39.2 ± 4.6</td>
<td>20.4 ± 0.5</td>
<td>55.7 ± 5.7</td>
<td>47.4 ± 2.0</td>
</tr>
<tr>
<td>3 µg/L</td>
<td>36.5 ± 4.8</td>
<td>19.5 ± 0.4</td>
<td>46.1 ± 2.3</td>
<td>49.2 ± 8.8</td>
</tr>
<tr>
<td>10 µg/L</td>
<td>37.1 ± 4.2</td>
<td>20.2 ± 0.4</td>
<td>49.2 ± 8.1</td>
<td>30.0 ± 8.0</td>
</tr>
<tr>
<td>100 µg/L</td>
<td>26.4 ± 2.9</td>
<td>20.9 ± 0.2</td>
<td>60.4 ± 2.3</td>
<td>60.4 ± 8.9</td>
</tr>
<tr>
<td>ANOVA</td>
<td>$F_{3,16} = 1.89$, $p = 0.17$</td>
<td>$F_{3,16} = 2.01$, $p = 0.15$</td>
<td>$F_{3,16} = 1.47$, $p = 0.26$</td>
<td>$F_{3,16} = 2.86$, $p = 0.07$</td>
</tr>
</tbody>
</table>

*Values are presented as the mean ± standard error (n = 5 tanks in each treatment).*

*Respective F-ratio statistics and their corresponding $p$ values from the analysis of variance (ANOVA).*
was relatively high (~30%) in the controls. We observed very low mortality (<5%) in the Petri dishes (i.e., GS 17–25), suggesting that the majority of the tadpole mortality occurred between GS 25 and GS 41. Throughout the entire exposure period, we did not observe any sign of hypoxia (e.g., tadpoles floating near the water surface), lethargy, or a major die-off of tadpoles, so we do not feel that the exposure system was the cause of excessive tadpole mortality. One possibility may be the quality of eggs. The adult female and male frogs used for artificial breeding were collected at Sensiba Wildlife Area from a wild population with relatively low exposure to heavy metals, polychlorinated biphenyls, or chlorinated pesticides [17]. They were maintained at room temperature for two months, then hibernated in our facility for eight weeks before breeding. We were not able to make the water cold enough (water should have been at 4°C but was ~9°C in our hibernation tank) during hibernation. We did observe that the frogs still swam occasionally in the hibernation tank. Inadequate nutrition before hibernation (frogs were provided with crickets feeding on vitamin-fortified food) and undesirable water temperature during hibernation could have affected egg development in females. Because of the elevated mortality in the controls, we suggest that further study is necessary to verify the effect of Pb on R. pippens tadpole mortality at these exposure levels.

Researchers have observed decreased SVL in R. catesbeiana tadpoles exposed to 1,000 μg Pb/L for 10 weeks [11]. In another study, R. catesbeiana tadpoles exposed to 780 μg/L for 7 days ate less food and lost mass compared with controls [18]. In the present study, a significant effect of Pb was found on SVL in the high-Pb treatment during early tadpole stages (by day 34 post-hatch), but the effect disappeared during later stages. The reason for this observation may be tadpole density. A "crowding effect," which reduces tadpole growth, has been reported in R. pippens [14]. Tadpole density was lower in the high-Pb treatment tanks because of elevated, but not statistically significant, mortality; lower density may allow surviving tadpoles to grow faster. Although the effect on growth was only significant during early tadpole stages, it still has ecological relevance, because smaller tadpoles are more vulnerable to predation by some gape-limited predators in the field [19]. In addition, our swim test showed that smaller tadpoles swim slower and, thus, are less able to evade predators [20,21].

Swimming performance

Researchers have been using tests of swimming performance parameters to address behavioral toxicology in amphibians [15]. We chose the maximum sprint swim speed as the parameter to test in the present study, because from the perspective of hydromechanics, tadpoles do not appear to be designed for sustained swimming at high speed [24]. Maximum sprint speed is crucial for tadpoles to escape from predator attacks, and slower tadpoles are more susceptible to predation [20,21]. Decreased maximum sprint speed was observed in our Pb-exposed tadpoles, suggesting that Pb-exposed tadpoles may face higher risk of predation in the field. In our high-Pb treatment, significant decrease in swimming performance was associated with high prevalence of spinal deformities, an association also observed in bullfrog tadpoles collected from coal ash–polluted sites [23].

A previous study has shown that Pb can affect amphibian neuromuscular transmission [25], which is important to behavioral performance. Lead has been found to inhibit acquisition and retention of learning in bullfrog tadpoles [8] and to result in decreased fright response in Columbia spotted frog (Rana luteoventris) tadpoles [10]. Thus, it is possible that slower tadpole swimming speed also was caused by neural or neuromuscular damage resulting from chronic Pb exposure. In addition, Pb also is known to decrease hemoglobin levels, damage erythrocytes, and alter respiratory surfaces, which may cause decreased oxygen uptake in Pb-exposed animals. Rice et al. [18] observed hypoxia-like responses and decreased activity in bullfrog tadpoles exposed to sublethal levels of Pb. This may be another factor that resulted in slower swimming speed in our tadpoles.

Metamorphosis

Age and size at metamorphosis are crucial parameters of life history in amphibians [26]. In the natural environment, various combinations of biotic and abiotic factors can influence development and growth of amphibians. Biotic factors include availability and quality of food, competition, crowding, and predation; abiotic factors include environmental temperature, photoperiod, and the hydric environment [27]. It is optimal to have a shorter larval period and larger size at metamorphosis, but it often is not possible because of a trade-off between size and age at metamorphosis [28]. Some trace elements have been suggested to affect age or size of amphibians at metamorphosis [29]. In the present study, Pb exposure delayed the onset of metamorphosis in northern leopard frogs without affecting the size at metamorphosis. Metamorphosing later, without increasing body size, generally is a disadvantage. Beck and Congdon [30] found that locomotion performance, measured by sprint speed and endurance, of Bufo terrestris metamorphs was negatively related to age at metamorphosis, and the authors suggested that the poor performance might affect their ability to disperse from natal pond, to forage, or to avoid predators. Delayed metamorphosis has been found to correlate with delayed maturity and to reduce recruitment to the breeding pop-
ulation in the chorus frogs (Pseudacris triseriata) [31]. Delayed metamorphosis also may increase the risk of desiccation in temporary water bodies (e.g., ponds or wetlands), which are major habitats of R. pipoens tadpoles.

It is noteworthy that mortality of metamorphs (GS 42–46) was high in the controls (~56%). Frogs are relatively vulnerable to numerous factors during metamorphosis, because the immune and other physiological systems are restructured [14]. We speculate that the paper towel put in the compartments of the plastic boxes caused the high mortality. In other experiments conducted later in our laboratory, we used the same plastic boxes and the same animal care regime, but without providing the paper towel, and the mortality of metamorphs dropped to 10% (unpublished observations). It is possible that different hydric conditions, or even leaching of some chemical(s) from the paper towel, caused mortality in metamorphs. Because of the high mortality of metamorphs, we suggest that further study is necessary to examine the effect of Pb on survival of metamorphs at these levels of exposure.

**Lead levels in tadpole tissues**

Tadpoles had been exposed to Pb for 153 d in the main experiment but for 60 d in the second exposure experiment. We recognized that it would be most desirable if we could have done the second experiment for the same length of time and with the same number of tadpoles. However, because of limitations in our laboratory space, we started the second exposure study with fewer animals (fewer tanks to save space), and we decided to end the exposure (to kill all the tadpoles) when the first tadpole reached GS 42 (forelimb emergence). This way, we also were able to be confident that we would have sufficient tadpole tissues and that all tadpoles had the same length of exposure period. The first tadpole reached GS 42 on day 60 after exposure began, which was quite close to when the first metamorph was observed in the main experiment (day 63). A previous study showed that R. catesbiana larvae exposed to 1.000 μg Pb/L for 48 h, 7 d, and eight weeks all had similar tissue Pb concentrations [32]. If this also were true in R. pipoens larvae, one could assume that Pb concentrations in tadpoles exposed to Pb for 60 d would have similar Pb levels to those exposed for 150 d. However, further exposure studies are needed to verify this assumption.

Bioaccumulation of Pb in tadpoles has been reported in previous laboratory or field-exposure studies [11,17]. In the present study, we also observed bioaccumulation of Pb in the tadpoles. The tadpole Pb body concentrations were positively related to nominal Pb concentrations in water. Previous field studies have shown elevated Pb concentrations in amphibians in some contaminated sites in the United States. For example, in the bullfrog and green frog tadpoles living in highway drainages in Maryland and Virginia, Pb concentrations ranged from 0.07 to 270 mg/kg dry mass [5]. Lead concentrations in bullfrogs of two lead mining districts of southeastern Missouri, USA, had been reported to be as high as 545 mg/kg dry mass [33] (corrected to a dry-mass basis assuming 5 g wet/g dry mass [34]). Lead concentrations in unspecified tadpoles collected in the "New Lead Belt" area of southeastern Missouri ranged from 28 to 1,560 mg/kg dry mass [35]. Mean Pb concentrations in our tadpoles ranged from 0.1 to 224.5 mg/kg dry mass, which are values comparable to or lower than the levels observed in those studies. No direct evidence shows the effects of consumption of Pb-contaminated tadpoles in wildlife, but diets with Pb levels comparable to those observed in our tadpoles have been shown to cause negative physiological and reproductive effects in some avian and mammalian species [36]. Thus, it is possible that Pb in tadpoles may negatively affect the physiology and reproduction in their predators, although Pb is not considered to be biomagnified through the food chain [37].

**CONCLUSION**

The present study regarding northern leopard frogs is, to our knowledge, the first to address the effects of chronic exposure of Pb from early life stage through metamorphosis in this species. The adverse effects observed in the present study include decreased tadpole SVL in early stages, spinal deformity, slower sprint speed, delayed metamorphosis, and accumulation of Pb in the body. We did not observe any apparent effects in tadpoles exposed to 3 μg Pb/L, which is very close to the U.S. Environmental Protection Agency freshwater-quality chronic criterion for Pb (2.5 μg/L, but 4.5 μg/L at the hardness of 170 mg/L [38]). Therefore, according to our results, northern leopard frog exposed only as larvae would be protected by this water-quality standard. However, as mentioned previously, tadpoles of other species with tissue Pb levels as high as or even much higher than those observed in the present study have been reported in the field. The adverse effects observed in our study might be found in tadpoles inhabiting those field sites contaminated with Pb. Furthermore, because stressors acting together can have impacts that are stronger than the sum of their individual effects [39], the influence of the sublethal effects resulting from Pb exposure in amphibians may be worsened while interacting with other abiotic or biotic stressors, such as other contaminants, habitat degradation, pathogens, and predators.

Acknowledgement—We thank Bruce Darken and Kathleen LeVering for their assistance. This study was funded by the University of Wisconsin Sea Grant Institute under grants from the National Sea Grant College Program, National Oceanic and Atmospheric Administration, U.S. Department of Commerce, and from the State of Wisconsin (Grant NA46RG0481, Project R/MW-84). T.-H. Chen also was supported by the Government Scholarship provided by Ministry of Education, Republic of China (Taiwan).

**REFERENCES**


8. Strickler SS, Taylor DH. 1991. Lead inhibits acquisition and re-


