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ADVERSE EFFECTS OF ECOLOGICALLY RELEVANT DIETARY MERCURY EXPOSURE IN SOUTHERN LEOPARD FROG (*RANA SPHENOCEPHALA*) LARVAE

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Abstract—Southern leopard frog (*Rana sphenocephala*) larvae were exposed to experimental diets supplemented with *aufwuchs* from control and mercury-enriched mesocosms combined in proportions intended to mimic mercury concentrations and speciation in *aufwuchs* observed from aquatic systems contaminated by atmospheric deposition. Observations on rates of mortality, malformation, and larval growth and development were made for 254 d. Increased incidence of mortality, malformation, and changes in growth and development were observed at concentrations that reflect the highest concentrations expected in the amphibian diet from atmospheric deposition (1,500–3,300 ng Hg/g dry wt). The results of this study are probably more ecologically realistic than results obtained from previous studies of aqueous mercury toxicity and suggest that dietary mercury exposure in habitats contaminated primarily by atmospheric deposition has the potential to cause adverse effects in amphibian larvae.

Keywords—Mercury Amphibian Trophic transfer Development Growth

INTRODUCTION

Declines in amphibian populations over the past 50 years have been documented worldwide, and pollutants, such as mercury, are hypothesized to be among many possible contributing factors in some of these declines [1]. Mercury from atmospheric deposition tends to accumulate in biota inhabiting wetlands that are critical amphibian breeding sites [2], yet very little is known about the effects of mercury exposure in amphibians [3]. A recent review of metal ecotoxicology in amphibians identified numerous studies of acute (2–8 d) exposures to aqueous mercuric chloride and methylmercuric chloride in embryos and larvae and two subchronic studies (30–60 d) of reproductive effects in adults [4]. These studies have shown that aqueous mercury exposure in the μg/L range can cause lethality, malformations, and reproductive changes; however, these exposure conditions are not representative of conditions in natural ecosystems. Aqueous total mercury (THg) concentrations in the field range from 0.3 to 8.0 ng/L in sites free of significant local sources of mercury and 10 to 40 ng/L in sites directly contaminated by industrial mercury emissions [5], both of which are orders of magnitude less than concentrations used in laboratory toxicity studies.

Diet is probably the most significant route of Hg exposure for amphibians. Concentrations of mercury in the diet of anuran amphibians may be as high as 1.6 μg/g dry-weight THg in habitats free of local mercury sources [6] and as high as 50 μg/g dry-weight THg in contaminated sites [7]. Thus, THg concentrations in the amphibian diet can be higher than aqueous concentrations by a factor of approximately 10^6. Effects of toxicants often differ by route of exposure because differences in uptake, metabolism, distribution, and elimination can result in different target tissue concentrations. For example, while short-term exposure (100 μg/L for 48 h) to aqueous inorganic mercury (Hg [II]) in eastern mosquitofish (*Gambusia holbroki*) increased standard metabolic rate [8], transgenerational exposure to predominantly dietary Hg (up to 60 μg/g dry wt THg) did not [9].

The purpose of this study was to determine if dietary mercury concentrations relevant to sites contaminated primarily by atmospheric deposition have the potential to cause adverse effects in amphibian larvae. We chose to focus on life history traits and responses that have demonstrated or theoretical implications for population dynamics, including timing and size at metamorphosis, metamorphic success rate, larval growth, and morphological development [10]. Based on previous short-term pilot studies, we hypothesized that dietary mercury exposure at concentrations far exceeding aqueous lethal concentrations would not cause lethality or malformations but would have other sublethal effects. We also hypothesized that dietary mercury exposure would decrease growth rate and size at metamorphosis in larvae while increasing the length of the larval period. We tested our hypotheses by exposing larvae of southern leopard frog, *Rana sphenocephala*, to diets containing mercury-enriched aufwuchs in the laboratory over the entire larval period (60–254 d). Aufwuchs is defined here as the accumulation of periphyton and associated organisms as well as dead and abiotic material on submerged surfaces, which is an important component in the diet of anuran amphibian larvae. We chose southern leopard frogs because they are part of a complex of closely related species (*Rana pipiens* complex) with a range that covers most of North America [11]; furthermore, they are locally abundant and breed year-round in South Carolina, USA [12].

METHODS

Preparation of experimental diets

Experimental diets were formulated with *aufwuchs* from control and Hg-enriched mesocosms combined with ground, vitamin-enriched rabbit pellets (Classic Blend Rabbit Food, L/M Animal Farms, Pleasant Plain, OH, USA) and trout pellets (Aquamax Grower 600, PMI Nutrition International, Brentwood, MO, USA) and embedded them in a matrix of agar and

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gelatin. The mesocosms that served as a source of *aufwuchs* were polyethylene-lined 7,250-L wading pools filled with water and sediments from local, uncontaminated sources. They were spiked with mercuric chloride ($HgCl_2$) in 1992 and have been open to inputs of rainwater, evaporation, and colonization by biota since construction. The construction of the Hg-enriched mesocosms is described in greater detail elsewhere [11]. Control and Hg-enriched mesocosm aufwuchs were combined in proportions intended to yield ecologically relevant THg concentrations of 100, 500, and 1,000 ng/g THg fresh weight as well as a control diet. Preparation of diets is described in greater detail in Unrine and Jagoe [12]. The control, 100-, and 500-ng/g fresh-weight diets encompass the range of THg concentrations found in aufwuchs from environments with no significant local anthropogenic or geologic sources of Hg, while the 1,000-ng/g diet is twice the highest concentration yet measured in aufwuchs from environments with no local Hg sources [6]; however, sites with local sources of Hg may have Hg concentrations in aufwuchs concentrations up to 20 times our highest dietary concentration [7]. Percent of THg present as methylmercury (MMHg) was also consistent with what has been observed in aufwuchs in natural systems [12].

Nutrient analysis of diet

Complete proximate analysis of the diets was performed in triplicate. Crude fat content was determined as the loss in mass after hot petroleum ether extraction with a semiautomated extraction system and ash content as the mass of the residue remaining after burning a sample at 550°C. Energy, crude protein, and crude fiber determinations were made by The University of Georgia, Department of Poultry Science Analytical Research Services Laboratory (Athens, GA, USA). Energy content was determined by bomb calorimetry, crude protein content with the Dumas combustion method (combustion, gas chromatography/thermal conductivity), and crude fiber by calculating the loss of mass on ignition for dry samples rendered free of fat by ether extraction and digested in weak acid followed by weak base.

Egg collection and hatching

Three southern leopard frog egg masses were collected from Peat Bay (14.3 Ha; pH = 4.48; alkalinity = 0 mg/L; dissolved organic carbon = 51 mg/L; conductivity = 37 μS/cm at 25°C), a wetland in Barnwell County (SC, USA) on the U.S. Department of Energy, Savannah River Site, and transported in natal water to the laboratory. The embryos hatched within 48 h of collection and reached Gosner stage (GS) 25 [13], the end of embryonic development and onset of feeding, within 96 h. Fifty percent of their natal water was replaced with frog embryo teratogenesis assay–*Xenopus* (FETAX) medium [14] daily from hatching until larvae reached stage 25.

One hundred normal GS 25 larvae from each egg mass were mixed in 25 L of FETAX medium to homogenize genetic variation. One individual was assigned from this pool of 300 larvae to each of 72 4-L polypropylene aquaria containing 2 L of FETAX medium. The four Hg treatments were randomly assigned to each of the aquaria (18 replicates per treatment). Fifty percent of the FETAX medium was replaced in each aquarium three times per week. Air temperature in the building was regulated by a thermostat at 20 to 25°C. Light cycle may play an important role in timing of metamorphosis [15], so the natural light cycle at the time of the experiment was mimicked by providing tadpoles with a combination of natural light and full-spectrum fluorescent light. Timing of the artificial lights was adjusted weekly to reflect the natural light cycle at the time of the experiment (September–June). Tadpoles were fed ad libitum, but each individual was provided with the same size ration at each feeding. If any tadpoles had consumed their entire ration between feedings (every 1–2 d), the ration size was increased for all individuals so that food was not limited. On forelimb emergence, each tadpole was provided with a floating sponge to allow them to leave the medium during metamorphosis. When metamorphs completed two-thirds of tail resorption, FETAX medium was replaced with moistened paper towels.

The FETAX medium was brought to nearly 100% oxygen saturation prior to each water change by aerating for approximately 10 min. Conductivity and pH of the medium were measured, prior to adding it to the aquaria, each time it was prepared (two to three times per week). Temperature was determined in a randomly selected aquarium just prior to renewing medium. Total ammonia concentrations were determined once per week in random samples from aquaria (just prior to renewing the medium) with a commercially available semiquantitative test kit (Tetra, Blacksburg, VA, USA) based on the phenol-hypochlorite method. Random samples of medium from the aquaria were also analyzed for Hg (II) and methylmercury (MMHg) six times during the experiment in order to verify the diet as the primary exposure route (see the section *Mercury analysis*).

Biological responses

These methods generally followed those of Snodgrass et al. [16]. Tadpoles were inspected every 1 to 2 d for survival, food consumption, and developmental abnormalities. External malformations were determined and classified according to Bantle et al. [17] in tadpoles and according to Meteyer [18] in metamorphs. The number of days elapsed posthatching (DPH) at which tadpoles reached three developmental stages—complete hind-limb development (HL; GS 39), forelimb emergence (FL; GS 42), and complete tail resorption (TR; GS 46)—was also recorded. Complete tail resorption was considered to be completion of metamorphosis. Individual mass was determined at each of these stages with an analytical balance to the nearest 0.1 mg. Mass at 55 DPH was also determined to quantify initial larval growth prior to major developmental changes; at this time, the majority of tadpoles (97%) were in the early stages of hind-limb development (GS 25–35).

Mercury analysis

Up to 50 mg of experimental diet or 100 mg of tadpole tissue were weighed up into closed, heavy-walled, acid-washed polytetrafluoroethylene vials containing 2.5 to 10 ml (depending on sample size) of 25% weight/volume (w/v) KOH in methanol, and samples were allowed to digest at 70°C overnight. The digestates were then diluted to twice the initial volume with methanol. Small aliquots of the digestates were directly analyzed for inorganic mercury (Hg II) and methylmercury (MMHg) by aqueous phase ethylation, room temperature precollection, gas chromatography, and cold vapor atomic fluorescence spectrophotometry with the methods of Liang et al. [19]. The FETAX medium (50-ml samples) was filtered with 0.45-μm polypropylene syringe filters and polypropylene syringes prior to analysis and analyzed by gas chromatography and cold vapor atomic fluorescence spectrophotometry according to the methods of Liang et al. [20]. Details of quality control parameters and detection limits are discussed in Unrine and Jagoe [12].

Statistical analysis

All statistical analyses were performed with SAS® 8.01 (SAS Institute, Cary, NC, USA). Assumptions of normality and homoscedasticity were tested with Shapiro–Wilk's test and Bartlett's test, respectively. Nonparametric statistics were used if data could not be transformed to meet the assumptions for analysis of variance (ANOVA). Frequency distributions were also examined by constructing normal probability plots as described by Sokal and Rohlf [21].

Repeated-measures ANOVA was performed for mass at 55 DPH, HL, FL, and TR for animals that completed metamorphosis to compare larval growth among treatments. A Greenhouse–Geisser correction was applied to F statistics for within-subject effects after we rejected the null hypothesis of a type H covariance structure (Huynh–Feldt condition) with a sphericity test. Significant effects were then analyzed by performing individual ANOVAs comparing treatments to controls with Dunnett's tests.

Differences in time required to reach developmental stages (HL, FL, and TR) were compared with survival time analysis (SAS Lifetest Procedure). Survival time analysis, also known as failure time analysis or lifetime data analysis, is commonly used to analyze differences in time to occurrence of a particular endpoint, usually death or malfunction [22]. Here we use survival time analysis in examining time to reach developmental stages instead of time to failure or death. Nonparametric log-rank tests described by Peto et al. [23] were used because data for time to stage in some treatments had no apparent underlying distribution.

Log-likelihood ratio tests (G tests) of independence were used to examine the effect of treatments on survival and metamorphic success. Exact p values for G statistics were estimated by Monte Carlo simulation because we had insufficient cell counts to assume that asymptotic estimates of p values were good approximations of the true p values.

The relationship between Hg concentration and malformation rate data was assessed with a log-logistic concentration response model. The model was fit by performing linear regression of logit-transformed response frequencies versus ln [THg] for each of the Hg-enriched diets.

Differences in tail resorption time (time between FL and TR) were compared with ANOVA as described previously. Analysis of covariance was used to test for differences in weight loss during tail resorption with tail resorption time as a covariate.

RESULTS

Analysis of diet

No significant differences in energy, protein, fiber, ash, or fat content were observed ($\lambda = 0.0896$, $F_{15, 11.444} = 1.07$, $p = 0.4654$) in the diets used in our experiment. Energy, crude protein, crude fiber, ash, and fat content averaged across all treatments was 433.5 kJ/g, 32, 8.5, 13, and 4.2%, respectively. The actual concentration and speciation of mercury in the diets is provided in the section *Mercury analysis.*

Quality of FETAX medium

Physicochemical parameters measured for FETAX medium were as follows (mean ± 1 standard error of the mean): pH = 7.86 ± 0.02 ($n = 97$), conductivity = 1,676 ± 81 μS/cm at 25°C ($n = 81$), temperature = 22 ± 0.2°C ($n = 97$). Semiquantitative determinations of ammonia never exceeded 0.25 mg/L. No evidence of fouling of the medium, such as bacterial scum present on the medium surface, was observed.

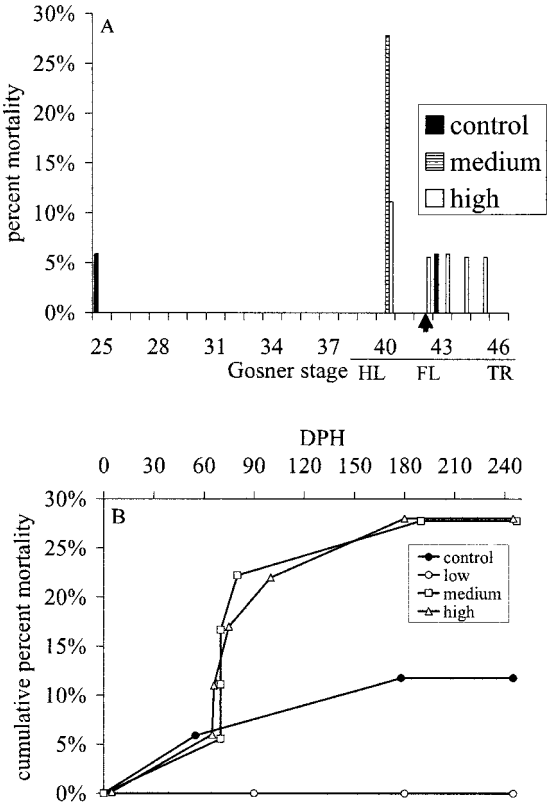

Fig. 1. Plots of percent mortality in *Rana sphenocephala* tadpoles fed diets containing mercury-enriched aufwuchs in the laboratory by Gosner stage (**A**) and cumulative percent mortality by days post hatching (DPH) (**B**) (HL = complete hind-limb development; FL = complete forelimb development; TR = complete tail resorption). Arrow indicates stage corresponding to metamorphic climax (FL; Gosner [13] stage 42) in panel **A**. No mortality occurred in the low mercury dietary treatment. Some points are slightly offset in panel **B** to aid in visualization.

Survival and metamorphic success

Survival of larvae was dependent on mercury treatment ($G = 9.6576$, $p = 0.0406$, $df = 3$). Control tadpoles had a survival rate of 88.2%. Only 17 of the initial 18 control tadpoles were included in the experiment because one control tadpole was injured during replacement of the medium and was eliminated from the experiment. The low mercury group had a survival rate of 100%, whereas the medium and high mercury groups each had survival rates of 72.2%. Note that for controls, one animal died early in development before HL, and one died after FL (Fig. 1A). All the animals that died in the medium and high mercury treatments did so at stage 40 after HL but during the first 180 d of the experiment, primarily in the first 60 to 90 d (Fig. 1B).

Metamorphic success rate was also dependent on mercury exposure ($G = 10.4703$, $p = 0.0293$, $df = 3$). Metamorphic success rates were 82.4, 100, 66.7, and 72.2% for control, low, medium, and high mercury diets, respectively. Only two very small tadpoles (one from the control and one from the high Hg treatment) remained that had not yet developed hind limbs or died after 254 DPH. It became apparent that these animals would not metamorphose, and we concluded that their development may have been arrested.

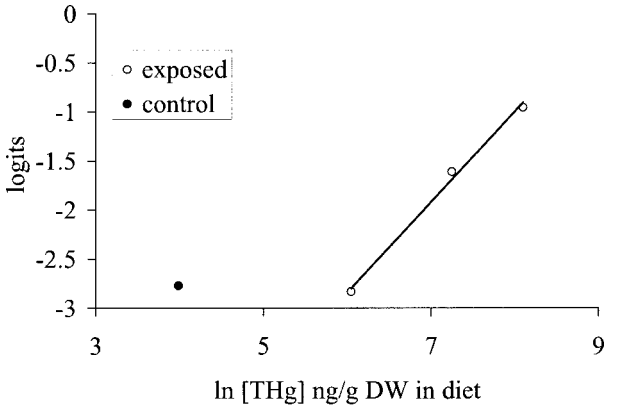

Fig. 2. Log-logit plot of malformation frequency for *Rana sphenocephala* tadpoles fed diets containing mercury-enriched aufwuchs. Values are plotted as ln of total mercury concentration in diet versus logit of frequency malformed (n = 17 for controls, n = 18 for low, medium, and high; DW = dry weight, THg = total mercury).

Malformation

Malformation rate of the exposed tadpoles increased monotonically with treatment and was well explained (r^2 = 0.9945) by a log-logistic concentration response model (Fig. 2; $F_{1,2}$ = 179.29, p = 0.0475). Malformation rates were 5.9% (1/17), 5.6% (1/18), 11.1% (2/18), and 27.8% (5/18) in control, low, medium, and high treatments, respectively. The control and low concentration treatments each had one tadpole with very mild scoliosis. Two of the medium concentration and five of the high concentration tadpoles displayed pronounced scoliosis, with a concentration-dependent increase in severity. We observed the appearance of microphtalmia and micromelia in one tadpole fed the medium mercury diet and ectromelia of the tibiale and fibulare in both hind limbs of a tadpole fed the high mercury diet. Curvature in the tadpoles' notochord persisted as curvature of the spine in metamorphs for tadpoles with severe scoliosis.

Malformation rate and mortality were independent (G = 0.0766, p = 1.000, df = 1). The control and low mercury groups had 0 to 11.1% mortality, and neither of the control tadpoles that died was malformed. The medium and high mercury groups both had 27.8% mortality, but each treatment only had one tadpole that was malformed that also died (5.6%).

Growth

Repeated-measures ANOVA of mass yielded a significant treatment-by-stage interaction ($F_{9,147}$ = 2.56, p = 0.0238) for tadpoles that completed metamorphosis (Fig. 3A). Univariate ANOVA of mass measurements at each stage indicated that treatment effect of mass at 55 DPH ($F_{3,49}$ = 3.20, p = 0.0345) was the source of this interaction. The quadratic component of treatment for mass at 55 DPH was significant ($F_{1,49}$ = 4.49, p = 0.0392). The difference became statistically significant in the high mercury tadpoles, which were 39% larger than control tadpoles at 55 DPH. We found no significant differences among treatments for mass at HL ($F_{3,49}$ = 0.16, p = 0.5222), FL ($F_{3,49}$ = 1.00, p = 0.4015), or TR ($F_{3,49}$ = 0.52, p = 0.6677).

Development

Concentration-dependent decreases in mean DPH to reach each developmental landmark existed with the exception of HL in the low treatment group (Fig. 3B). Log-rank tests yielded statistically significant differences for HL (X^2 = 9.0094, p = 0.0292, df = 3) and FL (X^2 = 8.6365, p = 0.0345, df = 3) but not TR (X^2 = 3.7859, p = 0.2855, df = 3). Comparisons of each treatment against control yielded a significant difference only for the high Hg treatment versus control for HL (X^2 = 4.7294, p = 0.0297, df = 1) and FL (X^2 = 4.2336, p = 0.0396, df = 1).

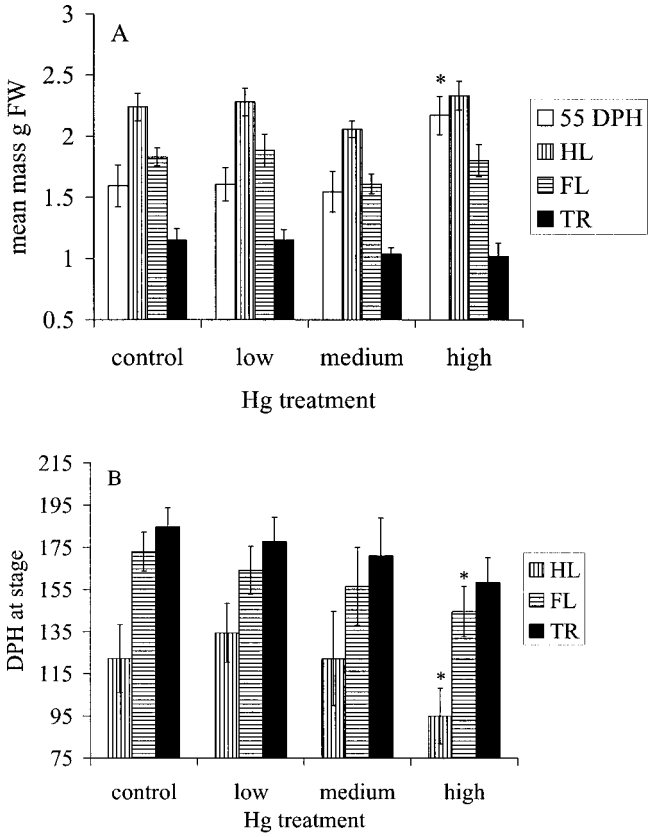

Fig. 3. Mean mass of *Rana sphenocephala* tadpoles fed diets containing mercury-enriched aufwuchs at various developmental stages for tadpoles that successfully completed metamorphosis by treatment (**A**) and mean days posthatching (DPH) at developmental stages (**B**) by Hg treatment. HL = complete hind-limb development; FL = complete forelimb development; TR = complete tail resorption; 55 DPH = 55 d posthatching (n = 14, 18, 11, and 12 for control, low, medium, and high, respectively, in panel **A**; n = 15, 18, 17, and 18 for control, low, medium, and high, respectively, for HL in panel **B**; n = 15, 18, 11, and 16 for control, low, medium, and high, respectively, for FL in panel **B**; n = 14, 18, 11, and 12 for control, low, medium, and high, respectively, for TR in panel **B**). Asterisk indicates difference from controls statistically significant at α = 0.05. Error bars correspond to ±1 standard error of the mean. FW = fresh weight.

The shape of the cumulative frequency distributions for the number of metamorphs reaching TR across time varied by treatment (Fig. 4), although differences were not statistically significant with a log-rank test (X^2 = 3.7859, p = 0.2855, df = 3). While only one individual from the control group (7.1%) reached TR prior to 160 DPH (March 1), the number of individuals reaching TR prior to 160 DPH had a concentration-dependent increase in the low (27.8%), medium (41.7%), and high (46.2%) feeding treatments. As an alternative method for comparing the shape of the cumulative frequency distributions, we tested the hypothesis that they followed a normal distribution. Visual inspection of normal probability plots gave the impression that while the control group had an approximately normal distribution, the low, medium, and high groups had more platykurtotic or even bimodal dis-

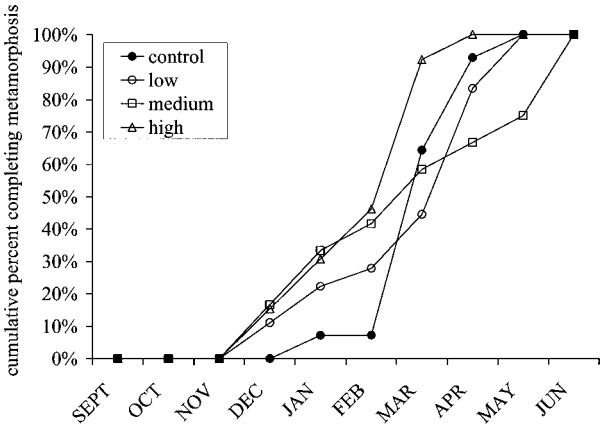

Fig. 4. Cumulative percent of *Rana sphenocephala* tadpoles fed diets containing mercury-enriched aufwuchs attaining complete tail resorption by month and treatment (n = 14, 18, 11, and 12 for control, low, medium, and high, respectively).

tributions. Shapiro–Wilk's test generally confirmed this observation. The p values regarding the null hypothesis of normality were 0.3869, 0.0302, 0.1082, and 0.035 for control, low, medium, and high treatments, respectively.

Differences among treatments for tail resorption time were significant ($F_{3,52} = 3.41$, $p = 0.0240$; Fig. 5). The linear contrast was statistically significant ($F_{1,52} = 6.84$, $p = 0.0117$). Both medium and high Hg treatments had tail resorption times that were significantly longer than for controls ($\alpha = 0.05$). Through analysis of covariance, no significant differences ($F_{3,51} = 1.75$, $p = 0.1691$) occurred in time-corrected loss of mass during tail resorption among treatments. Least-squares means (corrected for tail resorption time) for loss of mass were 0.729, 0.727, 0.534, and 0.769 g for control, low, medium, and high mercury diets, respectively.

Mercury analysis

Aqueous Hg was not detected in any samples of FETAX medium from the control, low, or medium Hg aquaria. Detection limits (mean blank + 3σ) were 0.3 and 0.07 ng/L for MMHg and Hg (II), respectively. However, we detected MMHg in one sample from a high Hg aquarium (5.39 ng/L) and Hg (II) in one sample from high Hg aquarium (1.52 ng/L) at 109 DPH but not in other samples.

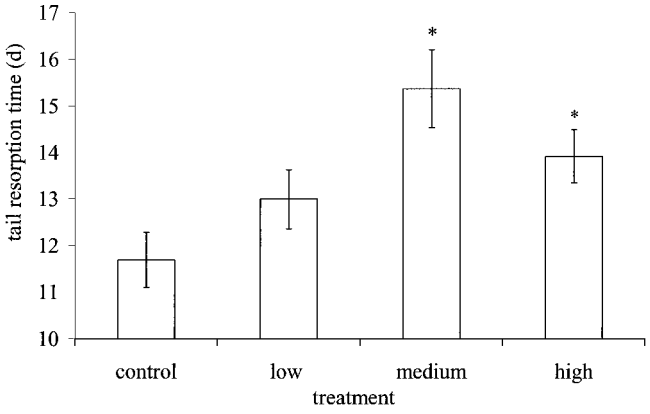

Fig. 5. Tail resorption time (time between forelimb emergence and complete tail resorption) in days by treatment for *Rana sphenocephala* tadpoles fed diets containing mercury-enriched aufwuchs (n = 15, 18, 11, and 12 for control, low, medium, and high, respectively). Asterisk indicates difference from controls statistically significant at $\alpha = 0.05$. Error bars correspond to ±1 standard error of the mean.

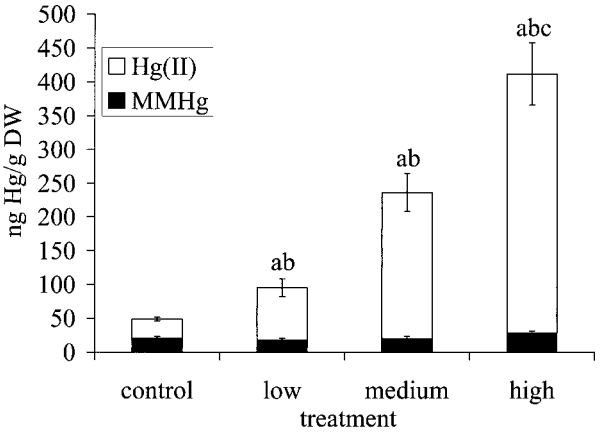

Fig. 6. Plot of body burden of mercury (ng/g dry wt [DW]) in *Rana sphenocephala* tadpoles fed diets containing mercury-enriched aufwuchs (n = 17 for controls and n = 18 for low, medium, and high treatments). Letter indicates that difference from control is statistically significant at $\alpha = 0.05$ (''a'' for inorganic mercury (Hg [II]), ''b'' for total mercury, ''c'' for methylmercury (MMHg)). Error bars correspond to ±1 standard error of the mean.

Actual THg concentrations in the diets were 54, 423, 1,409, and 3,298 ng/g dry weight or 14, 110, 366, and 858 ng/g fresh weight (74% moisture) with 22.0, 3.4, 1.9, and 1.5% present as MMHg for control, low, medium, and high treatments, respectively. Percent present as MMHg was a realistic reflection of what is observed in nature in the tadpole diet [12]. Methylmercury concentration in the diet differed significantly among treatments ($F_{3,16} = 3.62$, $p = 0.0364$) but was higher than controls only in the high Hg treatment ($\alpha = 0.05$). Inorganic mercury concentration differed significantly among treatments ($F_{3,16} = 46.50$, $p < 0.0001$) and was higher in the medium and high Hg diets ($\alpha = 0.05$). Total mercury concentration ($F_{3,16} = 215.39$, $p < 0.0001$) differed significantly among treatments and was higher than controls in all treatments ($\alpha = 0.05$). Tadpole MMHg body concentration (Fig. 6) was significantly different among treatments ($F_{3,68} = 3.76$, $p = 0.0148$) but was higher than controls only in the high Hg treatment ($\alpha = 0.05$). Inorganic mercury concentration ($F_{3,68} = 36.59$, $p < 0.0001$) and THg concentration ($F_{3,68} = 50.04$, $p < 0.0001$) were significantly different among treatments and were significantly higher than controls in all treatments ($\alpha = 0.05$; Fig. 6).

DISCUSSION

This is the first study to demonstrate effects of environmentally realistic dietary Hg exposure in amphibian larvae. Increased rates of mortality, malformation, growth, and development as well as decreased rate of tail resorption occurred as a result of mercury absorbed from the diet. Occurrence of mortality and malformations increased and tail resorption rate decreased at dietary exposure concentrations that reflect the highest Hg concentrations observed in aufwuchs in lakes contaminated solely by atmospheric deposition [6]. The highest dietary Hg concentration, which was more than an order of magnitude lower than Hg concentrations observed in aufwuchs from a site contaminated by local Hg sources [7], resulted in the most frequent and severe malformations as well as changes in growth and development rates. Taken together, these results suggest that dietary Hg concentrations in sites with little or

no local sources of contamination may be sufficient to disrupt normal development and key life history characteristics of amphibians.

Survival and metamorphic success

Increased mortality compared to controls occurred at the two highest dietary Hg concentrations we tested, but mortality decreased in the low Hg group compared to controls. Decreased mortality over controls at low Hg concentrations has also been observed in FETAX assays [24] and might indicate that mercury is capable of causing hormetic effects; however, this study provides no evidence of hormesis according to quantitative criteria for classifying effects as hormetic [25].

Metamorphosis could be a sensitive period for Hg-exposed individuals because tadpoles are required to make a number of behavioral, morphological, physiological, and biochemical changes in order to transition to terrestrial life. Tadpoles that died in the medium high Hg treatments generally did so immediately before or after metamorphic climax (GS 42; FL). Increased sensitivity during major developmental changes has been observed in tadpoles exposed to aqueous Hg (II) [26]. Mercury in fish is largely bound to sulfhydryl-rich proteins in muscle [5], so it is possible that mercury in the tadpoles was mobilized from muscle tissue undergoing regression, such as the tail muscle, during major developmental changes of metamorphosis. This may have increased the tissue dose of mercury in critical organ systems such as the liver and kidneys and may explain the association between metamorphic climax and mortality.

Malformations

This was the first experiment to demonstrate that dietary Hg may cause a concentration-dependent increase in the frequency of malformations. We observed flexure of the notochord, which is often observed in embryos exposed to aqueous Hg [24,27,28]. We also observed hind-limb malformations, which have not been detected in previous studies restricted to the embryonic period. Edema, evident as blisters on the body due to Hg (II) exposure [27], and swelling and distention of the body and hind limbs in tadpoles exposed to MMHg [29] have been observed in studies of acute aqueous Hg toxicity. No evidence of edema was observed in this study. Such differences in observed malformations are probably due to differences in route of exposure. It is possible that edema observed in aqueous exposure is caused by impairment of osmoregulatory function or altered integrity of the integument. Increased incidence of malformations in this experiment occurred at 1,409 and 3,298 ng/g dry-weight Hg and increased by 28% at the highest concentration.

Growth

Compared to controls, we observed an increase in initial larval growth rate in the high Hg group, which probably enabled Hg-exposed larvae to reach developmental stages in a shorter time but at a similar mass. Increased initial growth in high Hg tadpoles was likely due to increased appetite. We noted that when ration sizes were increased to maintain an ad libitum feeding regime, it was because the tadpoles in the high Hg group were consuming their entire rations. While severe stress generally decreases appetite, mild stress can actually increase the appetite of an animal [30]. For example, increase in appetite due to metal exposure has been shown in rainbow trout (*Oncorhynchus mykiss*). Exposure to sublethal Cd (3 μg/L) decreased appetite, while exposure to Cu (75 μg/L) increased appetite [31]. Mercury may have had a similar effect on the tadpoles in our study, although this behavior has not previously been demonstrated in amphibians.

Alternative explanations for enhanced early larval growth in the high Hg group may relate to metabolic energy expenditures or activity level. If Hg-exposed tadpoles had lower metabolic rate and/or activity level than controls, then similar disparities in growth might emerge. Comparison of differences in weight loss during tail resorption did not support the hypothesis of decreased metabolic rate because tadpoles lost the same mass when corrected for tail resorption time. However, one cannot eliminate these alternatives because we did not make systematic observations on activity or measure metabolic rate.

Development

Mercury exposure resulted in increased developmental rate through completion of limb development, but it decreased tail resorption rate. A concentration-dependent decrease was observed in time to HL, no treatment effect on time between HL and FL, and a concentration dependent increase in time between FL and TR (tail resorption time). Increased developmental rate through HL and FL may have been influenced by increased food consumption and growth rate, but rate of development after FL is dependent on hormonal activity because tadpoles cease feeding after FL. Although tail resorption rate was decreased by mercury exposure, many larvae in the Hg-exposed treatments completed metamorphosis precociously. Precocious metamorphosis has also been observed in green frog (*Rana clamitans*) larvae exposed to carbaryl [32]. Precocious metamorphosis in overwintering larvae may be disadvantageous because ecological conditions in the terrestrial environment during the winter may not be as favorable for growth and survival of metamorphs relative to conditions in the spring; therefore, postmetamorphic survivorship could be decreased as a result of Hg exposure. Increased tail resorption time increases the time for which tadpoles have both limbs and a tail. Tadpoles that have both limbs and a tail neither swim nor hop effectively and thus have an increased risk of predation relative to premetamorphic or postmetamorphic individuals [33]. According to Fort et al. [34], increased tail resorption time due to toxicant exposure is frequently mediated through inhibition of the thyroid axis. Changes in thyroid function due to Hg exposure have been demonstrated in fish [35], rodents [36,37], and humans [38]. Decreases in tail resorption rate observed in this experiment suggest that Hg may also inhibit the amphibian thyroid axis.

CONCLUSION

Dietary exposure in sites receiving Hg only through atmospheric deposition may be sufficient to cause adverse effects on amphibian development and decrease survival through metamorphosis. While approximately 10 to 15% of tadpoles were either mildly malformed or died in the control and low mercury treatments, approximately 50 to 60% of tadpoles were either severely malformed or died in the medium and high mercury treatments. Furthermore, many Hg-exposed individuals metamorphosed precociously and had increased tail resorption times. These combined effects have the potential to decrease the number and quality of offspring recruited to the terrestrial environment, ultimately decreasing the number of individuals with future potential for reproduction. Effects of

dietary Hg exposure at low levels could also be exacerbated by other factors, such as habitat destruction, disease, and global climate change. That malformations, decreased survival, and changes in normal growth and developmental rates occurred at dietary Hg concentrations relevant to habitats contaminated solely by atmospheric Hg suggests that Hg pollution from atmospheric deposition has the potential to adversely impact amphibian populations. Further study of the effects of mercury pollution on amphibians is warranted and should focus on dietary Hg exposure.

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