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Do effects of mercury in larval amphibians persist after metamorphosis?

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Abstract Despite widespread concern about the role of environmental contaminants in global amphibian declines, and evidence that post-metamorphic life stages contribute disproportionately to amphibian population dynamics, most studies in amphibian ecotoxicology focus on larval life stages. Studies that focus solely on early life stages may miss important effects of contaminant exposure, such as latent effects that manifest some time after previous exposure. Moreover, it is often assumed that effects observed in amphibian larvae will persist to affect survival or reproduction later in life. We used terrestrial enclosures to determine whether exposure to mercury (Hg) through maternal transfer and/or larval diet had any adverse effects in post-metamorphic American toads (*Bufo americanus*). We found a 5% difference in size at metamorphosis that was attributed to maternal Hg exposure persisted for 1 year in the terrestrial environment, resulting in a 7% difference at the conclusion of the study. Although patterns of survival differed among treatments through time, we found no overall difference in survival after 1 year. We also found no evidence of emergent latent effects in the terrestrial toads that could be attributed to earlier exposure. Our results indicate that adverse effects of maternal Hg exposure that were observed in larval amphibians may persist to affect later terrestrial life stages but that no novel adverse effects developed when animals were raised in a semi-natural environment. Moreover, we found no evidence of persistent effects of dietary Hg exposure in larvae, highlighting a need for greater focus on maternal effects in amphibian ecotoxicology. Finally, we suggest an increase in the use of longitudinal studies to better understand contaminant impacts to amphibian populations via effects in both aquatic and terrestrial life stages.

Keywords Amphibian declines · Carryover effects · Latent effects · Maternal effects · Population models

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Introduction

The need to understand functional mechanisms underlying ecotoxicological processes frequently leads to studies of species or systems that can be feasibly controlled and replicated. Even within species, some life stages can receive disproportionate attention because they may lend themselves more readily to experimental research. For example, the vast majority of toxicological research on amphibians has focused on aquatic larval stages that are more easily manipulated in the laboratory or sampled in the field compared to terrestrial stages (Carey and Bryant 1995; Biek et al. 2002; Boone and James 2005). Despite the widespread decline of amphibian populations (Stuart et al. 2004; Hoffmann et al. 2010), and theoretical and empirical evidence that post-metamorphic life stages have a disproportionately high impact on amphibian population dynamics (Biek et al. 2002; Vonesh and De la Cruz 2002; Schmidt et al. 2005), we know comparatively little about the ecotoxicology of terrestrial amphibian life stages or whether effects on larval stages persist to affect post-metamorphic vital rates.

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Concern that environmental contaminants contribute to global amphibian declines has prompted much amphibian toxicological research (Alford and Richards 1999; Houlahan et al. 2000; Wake and Vredenburg 2008; Sparling et al. 2010). Historically, amphibian toxicology has focused on dose-response relationships to determine lethal concentrations of a variety of contaminants (e.g., pesticides, herbicides, metals, or persistent organic pollutants) on embryos or larvae (Boone and James 2005; Palenske et al. 2010). These studies are useful for determining the acute toxicity of many substances and for understanding the sensitivity of diverse species to a wide range of potentially harmful environmental contaminants (Relyea and Jones 2009; Kerby et al. 2010), but they frequently lack ecological realism. Recently, there has been greater interest in understanding the broader ecological and sublethal consequences of environmental contaminants in more relevant contexts.

Contemporary ecotoxicological studies focus more widely on how sublethal concentrations of contaminants interact with natural stressors, including inter- and intraspecific competition (e.g., Relyea et al. 2005; Jones et al. 2011), predation (Relyea and Edwards 2010; Todd et al. 2011a), environmental stochasticity (e.g., Boone and Semlitsch 2002), and parasites (e.g., Budischak et al. 2008), as well as environmentally realistic contaminant mixtures (e.g., Snodgrass et al. 2004; Boone et al. 2005; Relyea 2009). These studies have revealed that environmental contaminants can have a wide array of adverse sublethal effects on larval amphibians (Rowe et al. 2001; Budischak et al. 2008). However, the broader implications of sublethal effects are often discussed under the assumption that they persist throughout ontogeny, presumably reducing survival or reproduction in post-metamorphic life stages, but this assumption is rarely tested. Additionally, studies that focus only on larval life stages may miss important latent effects of contaminant exposure that appear later in ontogeny after previous exposure (e.g., Rohr and Palmer 2005; Budischak et al. 2008).

To test the hypothesis that larval effects of contaminant exposure persist after metamorphosis, we examined the post-metamorphic growth and survival of American toads (*Bufo americanus*) for 1 year after exposure to mercury (Hg) via maternal transfer or larval diet. Previously, we have shown that female American toads inhabiting a contaminated river floodplain accumulate Hg and maternally transfer it to their offspring (Bergeron et al. 2010b). Exposure to maternal Hg results in delayed metamorphosis, smaller body size, and increased prevalence of spinal malformations in newly metamorphosed juveniles (Bergeron et al. 2011; Todd et al. 2011a, b). Additionally, dietary Hg can reduce body size of developing larvae and can interact with maternal Hg to reduce larval survival (Bergeron et al. 2011). Mercury bioaccumulates in tissues, is a known neurotoxicant, and can affect the endocrine and reproductive systems of vertebrates (Eisler 2006; Crump and Trudeau 2009; Tan et al. 2009). Thus, we predicted that negative effects observed at metamorphosis would persist through ontogeny to negatively affect growth and survival of terrestrial juvenile American toads. Lastly, we tested for the onset of any adverse latent effects undetected in the larval stage yet resulting from earlier larval exposure.

Methods

Experimental design

Recently metamorphosed American toads used in the present study were obtained from an earlier factorial experiment that examined the individual and interactive effects of maternal and dietary Hg on larval traits. Detailed methods and results of the earlier study on larvae are reported in Todd et al. (2011b). Briefly, we acquired larvae from clutches of eggs from toads collected in either contaminated or uncontaminated portions of the South River floodplain near Waynesboro, VA, USA (Todd et al. 2011b). Reference and contaminated mothers had total Hg (THg) concentrations of 159.5 ± 18.6 (mean ± SE hereafter) and 2,250 ± 489.8 ng/g ww in their blood, respectively (Todd et al. 2011b). Reference and contaminated eggs had THg concentrations of 20.6 ± 1.3 and 149.1 ± 17.9 ng/g dw, respectively (Todd et al. 2011b). Soon after hatching, larvae were allotted to dietary treatments and fed either control diet (0.01 ± 0.001 μg/g dw THg) or a diet spiked with environmentally relevant concentrations and proportions of inorganic and methyl-Hg (10.1 ± 2.3 μg/g THg dw, 1.05% monomethyl-Hg), creating four experimental crosses: (1) reference mother—control larval diet, (2) reference mother—Hg larval diet, (3) Hg mother—control larval diet, (4) Hg mother—Hg larval diet. For more information on larval diet, see Bergeron et al. (2011) or Todd et al. (2011b). We drew animals haphazardly during the peak of metamorphosis for placement into terrestrial enclosures ("pens"). We created eight replicates for each possible 2 × 2 experimental cross and each replicate consisted of a group of nine individuals drawn from the same larval treatment. A different subset of animals, drawn at the same time, was used to determine initial Hg-tissue concentrations at the beginning of the experiment.

Each group of nine recently metamorphosed toads was placed into a terrestrial enclosure in a stratified block design such that a block of four pens contained each of the four experimental treatments. The terrestrial pens measured 1.75 × 1.75 m and were located in a deciduous forest on

the campus of Virginia Tech, Blacksburg, VA, USA. The pens were constructed of smooth aluminum flashing that stood 60 cm high and was buried 30 cm into the ground. Each pen also featured a 20-cm deep hole with a diameter of 20-cm that was located centrally in the pen and was packed with leaf litter. Each hole was covered with a 61 × 61 cm piece of ¼″ untreated plywood so that toads could use the areas as refugia. We individually marked each animal by clipping one front toe and one hind toe prior to release. We released the animals within 48 h of completing metamorphosis in late June 2009. Total Hg concentrations of the recently metamorphosed animals at the time they were added to the terrestrial pens are shown in Table 1. Because amphibians lose mass at metamorphosis (Bergeron et al. 2011; Todd et al. 2011b), THg-tissue concentrations of metamorphosed animals are likely greater than those of younger, untransformed larvae. Thus, the THg-tissue concentrations of metamorphosed animals fed dietary Hg were higher than those of younger tadpoles collected at contaminated field sites, which averaged approximately 2,100 ng/g dw at Gosner stages 28–32 (Bergeron et al. 2010a), but were environmentally relevant. No additional dietary Hg was provided to the animals at any point after metamorphosis.

We censused pens throughout 2009 and again in May 2010 after the animals had overwintered. Beginning 3 weeks after release, we visually searched each pen for 3 min at daybreak on two consecutive days. During searches we gently wet the interior of the pens by spraying well water from a hose for 1 min to stimulate toad activity. Upon each capture, we recorded the identity, body size (snout-vent-length, SVL), and mass of each animal and returned them to their pens within 2 h. Searches were conducted on two consecutive days every 3 weeks until 6 October 2009 (15 weeks later) when temperatures began to drop and surface activity ceased. On 10–11 May 2010 we conducted a final census during which we removed all captured animals. On 12–14 May, we carefully removed all leaf litter by hand from each pen and searched for any animals that may have been missed during the prior surveys.

In general, animals had to rely on the leaf-litter insect communities within pens to provide forage during the experiment. However, we added 2 kg of leaf litter collected from the surrounding forest to each pen in September 2009 to provide additional forage. On 13 April 2010, as temperatures warmed and toads resumed activity after overwintering, we added 0.5 g of small domestic crickets to each pen and on 20 April 2010 we added 2.7 g of small domestic crickets to each pen to replenish prey populations that likely perished over the winter.

Mercury analyses

For juvenile toads, we created composite samples for each replicate and we lyophilized and homogenized each composite sample. Our composite samples were composed of 1–3 toads from each of the four replicate pens for each experimental cross and the percent moisture of the juvenile toads was 86.96 ± 0.17%. We analyzed subsamples of the homogenized tissues (approximately 20 mg) for THg content by combustion amalgamation-cold vapor atomic absorption spectrophotometry (Direct Mercury Analyzer 80, Milestone, Monroe, CT USA) according to U.S. Environmental Protection Agency method 7473. For quality assurance, each group of 10 to 15 samples included a replicate, blank, and standard reference material (SRM; DOLT-4 dogfish liver or DORM-3 fish protein [National Research Council of Canada (NRCC), Ottawa, ON]). We calibrated the instrument using solid SRMs (DOLT-4 and DORM-3). Method detection minimum limits (3 times the standard deviation of procedural blanks) for samples were 0.33 ng, and all samples had THg concentrations that exceeded the limit. Average relative percent differences between replicate sample analyses were 6.08 ± 1.91% ($n = 25$). Mean percent recoveries of THg for the SRMs, DOLT-4 and DORM-3, were 99.50 ± 0.20% ($n = 54$) and 98.67 ± 0.42% ($n = 54$), respectively.

Table 1 Total mercury concentrations in recently metamorphosed American toads, *Bufo americanus*, (originally reported in Todd et al. 2011b) and juvenile toads after 1 year in terrestrial enclosures

Maternal group	Dietary treatment	Mean total Hg concentration (±1 SE) at metamorphosis (ng/g dry wt)	Mean total Hg concentration (±1 SE) at 1 year (ng/g dry wt)	Fold change
Reference	Control diet	21.5 ± 1.4	56.3 ± 3.2	2.6 fold increase
Reference	Hg diet	3250.6 ± 127.8	238.8 ± 24.2	13.6 fold decrease
Hg contaminated	Control diet	33.7 ± 2.0	71.9 ± 13.4	2.1 fold increase
Hg contaminated	Hg diet	4122.2 ± 154.8	334.3 ± 37.4	12.3 fold decrease

Statistical analyses

All statistical analyses were run in SAS 9.2 (SAS Institute Inc., Cary, NC, USA) and statistical significance was recognized at $\alpha < 0.05$. All statistical assumptions were examined prior to analyses; unless otherwise noted, raw data values were used in statistical models. Pen means were used as statistical units in all analyses. Due to problems with normality and heteroscedasticity, we used the conservative Scheirer-Ray-Hare test as a non-parametric equivalent of a two-way analysis of variance (ANOVA) to determine whether maternal group, dietary treatment, or their interaction had a significant effect on THg concentrations in juvenile toads after 1 year of growth in the terrestrial environment (Dytham 2003). We investigated the latent effects of exposure to Hg via maternal transfer, larval diet, and their interaction on mean body size (SVL) of juvenile American toads using repeated measures ANOVA (SAS Proc Mixed), using each block of four pens as a blocking factor. Change in mean body size over time may not accurately represent growth due to possible size-specific mortality or variation in detection of differently sized animals over time. Thus, we assessed individual growth, corrected for body size, by calculating the proportional change in body size of individuals captured in any two successive intervals: $(SVL_{t+1}-SVL_t)/SVL_t$. We used repeated measures ANOVA to test for effects of time, maternal Hg exposure, dietary Hg exposure, and their interactions on mean proportional change in SVL, using each block of four pens as a blocking factor. Mass was highly correlated with SVL but was prone to fluctuation depending on whether recent rain allowed animals to hydrate and whether animals urinated during handling. Thus, we did not analyze mass but instead used SVL as a measure of body size.

We also used repeated measures ANOVA to test for effects of time, maternal Hg exposure, dietary Hg exposure, and their interactions on minimum number of juvenile toads known alive (survival). Retrospective examination of capture histories demonstrated that within each sampling session, >90% of the individuals known to be alive were captured, suggesting that minimum number known alive was an appropriate proxy for survival (Todd and Rothermel 2006). Time, maternal Hg exposure, dietary Hg exposure, and their interactions were included as factors and each block of four pens was included as a blocking factor in the model. We analyzed survival separately at the final time point (May 2010) using two-factor ANOVA with each block of four pens as a blocking factor. Finally, we used non-parametric bootstrap resampling (Lunneborg 2000) to test whether, within treatments, animals that survived for 1 year represented a non-random sample of all animals with respect to their initial body size (SVL). In the bootstrap resampling, individuals were treated as the statistical units.

Results

Mercury tissue concentrations

THg concentrations in the toads did not differ significantly between the two maternal groups 1 year after metamorphosis ($F_{1,26} = 0.26$, $P = 0.61$; Table 1). However, those fed dietary Hg as larvae still had THg concentrations 3–6 times greater 1 year after metamorphosis than did those fed control diet ($F_{1,26} = 5.3$, $P = 0.02$; Table 1). There was no significant interaction between maternal group or dietary treatment on THg concentrations in the one-year-old animals ($F_{1,26} = 0.0$, $P = 0.97$). The mean dry weight of toads assayed for THg increased from 0.021 g at metamorphosis to 0.197 g 1 year later, representing a 9.3-fold average increase in body mass. Toads fed dietary Hg as larvae exhibited a 12.3–13.6-fold decrease in THg concentrations after 1 year of growth in the terrestrial environment, whereas those fed control diets experienced a 2.1–2.6-fold increase in THg concentrations over the same time period (Table 1).

Biological endpoints

Juvenile American toads raised in the terrestrial enclosures grew rapidly, more than doubling their initial length after 1 year (Fig. 1). At metamorphosis, juvenile toads from Hg-exposed mothers were 5% smaller than those from reference mothers. This initial difference in body size persisted through 1 year of terrestrial growth (Maternal: $F_{1,21} = 86.31$, $P < 0.001$), but the lack of a maternal-by-time interaction (Maternal × Time: $F_{6,166} = 0.50$, $P = 0.811$) indicated that no latent effects of maternal Hg on growth manifested in the terrestrial stage. Rather, the result is more accurately viewed as the persistence of effects seen at metamorphosis; 1 year after metamorphosis, toads from Hg-exposed mothers were 7% smaller than those from reference mothers. We also found no evidence of latent effects of dietary Hg (Diet: $F_{1,21} < 0.01$, $P = 0.950$; Diet × Time: $F_{6,166} = 0.24$, $P = 0.961$) or an interaction between maternal and dietary Hg exposure (Maternal × Diet × Time: $F_{6,166} = 0.32$, $P = 0.923$) on juvenile body size, but we found a significant difference between blocks of pens ($F_{7,21} = 5.36$, $P = 0.001$). We found no effects of Hg on size-corrected growth (Maternal, Diet, Maternal × Time, Diet × Time, Maternal × Diet × Time, all $P \geq 0.13$). Juvenile toads grew most rapidly in the first 9 weeks of the study (Time: $F_{5,98.2} = 0.89.38$, $P < 0.0001$), increasing their body size by 15–20% during

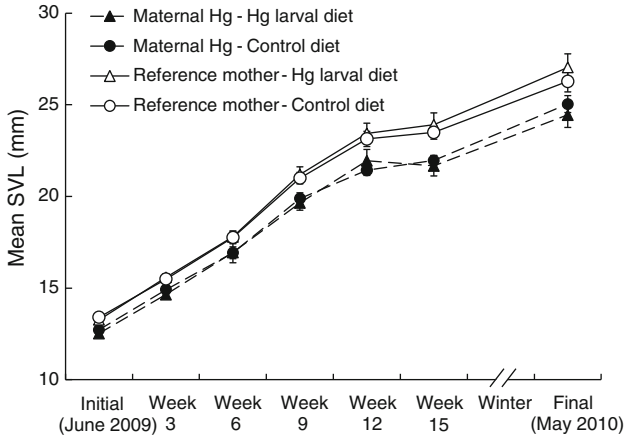

Fig. 1 Body size (*SVL*, snout-vent-length) of juvenile *Bufo americanus* exposed to Hg through maternal transfer and/or larval diet and raised in terrestrial enclosures from metamorphosis to 1 year of age. Values represent means (±1 SE) across pens ($n = 8$ per treatment) initially stocked with nine newly metamorphosed *B. americanus*. Note the disproportionately long time interval (broken axis) during overwintering

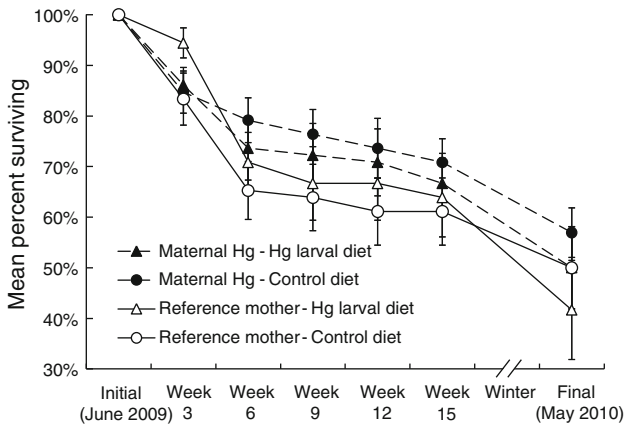

Fig. 2 Survival of juvenile *Bufo americanus* exposed to Hg through maternal transfer and/or larval diet and raised in terrestrial enclosures from metamorphosis to 1 year of age. Values represent means (±1 SE) across pens ($n = 8$ per treatment) initially stocked with nine newly metamorphosed *B. americanus*

each 3 week interval. Growth slowed during the fall and winter but increased again in the spring. We found no effect of block on size-corrected growth ($F_{7,56} = 0.94$, $P = 0.484$).

Survival of toads decreased to approximately 70% during the first 6 weeks of the study, but plateaued thereafter (Fig. 2). An additional 15% drop in survival occurred over winter, yielding an overall mean survival of approximately 50% at the end of 1 year. Although we detected significant effects of maternal Hg on juvenile survival when survival data were analyzed in time-series (Maternal × Time: $F_{5,140} = 0.2.34$, $P = 0.044$), the mean survival rate after 1 year did not differ among treatments (Diet, Maternal, Diet × Maternal, all $P \geq 0.23$). Exposure to dietary Hg in the larval stage did not affect terrestrial survival (Diet × Time: $F_{5,140} = 1.68$, $P = 0.144$) or interact with effects of maternal Hg exposure (Maternal × Diet × Time: $F_{5,140} = 0.39$, $P = 0.857$). Survival also did not differ among blocks of pens ($F_{7,21} = 1.64$, $P = 0.180$). Results of non-parametric bootstrap resampling suggested that survival was not related to initial body size; in all cases, the mean initial body size of surviving individuals did not differ significantly from a randomly drawn subset of the pool of initial body sizes within each treatment (Table 2).

Discussion

Most ecotoxicological studies of amphibians measure effects on larval life stages and assume that sublethal effects observed in larvae persist to affect survival and reproduction later in life. We used terrestrial enclosures to determine whether maternal or dietary Hg exposure in larval American toads had any adverse effects in the terrestrial environment following metamorphosis, including the possible persistence of effects seen at metamorphosis or the onset of any latent effects not seen previously. We found no evidence for the onset of latent effects on growth or survival of terrestrial juveniles during the first year following metamorphosis. However, size differences that were present at metamorphosis as a result of maternal Hg exposure persisted for at least 1 year in the terrestrial environment. Our results demonstrate that sublethal larval effects attributed to maternal transfer of Hg may have lasting consequences in the terrestrial environment following metamorphosis.

Studies of maternal effects in amphibians have typically examined the effects of maternal contributions to egg size on larval development and size at metamorphosis (e.g., Kaplan 1985; Semlitsch and Gibbons 1990; Parichy and Kaplan 1992; Laugen et al. 2002). These studies have found that, depending on resource availability in the aquatic environment, maternal effects on egg size can persist to metamorphosis. However, few studies have examined the persistence of maternal effects after metamorphosis, and fewer still have investigated the ecological consequences of maternal contaminant exposure in amphibians. One recent study demonstrated that maternal effects of contaminant exposure can include reduced hatching success and increased developmental abnormalities in recently hatched offspring of narrowmouth toads, *Gastrophryne carolinensis* (Hopkins et al. 2006). Previous research on larval American toads has shown that maternal Hg can delay metamorphosis, lead to smaller body size, and increase prevalence of spinal malformations (Bergeron et al. 2011; Todd et al. 2011a). The present study demonstrates that maternal effects of contaminant exposure on size at metamorphosis can persist

Table 2 Mean initial body size of juvenile American toads (*Bufo americanus*) surviving for 1 year in terrestrial enclosures and confidence intervals of randomly drawn survivors from within treatments

Maternal group	Dietary treatment	*n* surviving	Mean initial snout-to-vent length (mm) of survivors	90% CI
Reference	Control diet	37	13.47	13.08–13.71
Reference	Hg diet	30	13.27	12.97–13.53
Hg contaminated	Control diet	40	12.78	12.45–12.99
Hg contaminated	Hg diet	36	12.36	12.20–12.84

Confidence intervals were derived from 1,000 bootstrap resampled subsets (size = *n* surviving) drawn from the overall pool of initial body sizes within each treatment ($n = 72$ per treatment)

after metamorphosis in terrestrial life stages. Despite dietary Hg constituting the vast majority of accumulated Hg in animal tissues, the results of the present study suggest that timing of contaminant exposure (early in ontogeny via maternal transfer) may be more important than the degree of contaminant accumulation. However, at least one study has demonstrated that the combination of maternal Hg and high dietary Hg can cause significant mortality at metamorphosis (see Bergeron et al. 2011 for additional discussion).

Sublethal effects on amphibian larvae are often assumed to affect post-metamorphic survival or reproduction, but this assumption is seldom tested. One of the most frequently observed sublethal effects of contaminants on amphibians is a reduction in body size of larvae or newly metamorphosed juveniles (Boone and Bridges 2003). For example, reductions in larval growth rates or size at metamorphosis can result from exposure to pesticides (Bridges 2000; Boone 2005), fungicides, (Fioramonti et al. 1997), and coal combustion wastes (Snodgrass et al. 2004). In previous studies of Hg in larval American toads, reduced body size was one of the most prevalent sublethal effects of Hg exposure (Bergeron et al. 2011; Todd et al. 2011a). The broader implications of body size differences are often argued under the assumption that they persist to affect fecundity of terrestrial adults. For example, because maturity in female amphibians is generally size-dependent (Berven 1990; Scott 1994), and because smaller females produce smaller clutches (Berven 1988; Semlitsch and Gibbons 1990), it is often suggested that smaller body size at metamorphosis can reduce lifetime reproductive fitness (Boone and Bridges 2003). However, this assumption is seldom tested and studies of natural stressors such as competition have suggested that size differences at metamorphosis can be overcome in toads by compensatory growth in the terrestrial environment (Beck and Congdon 1999; Boone 2005; Sams and Boone 2010). Our study provides evidence that effects of contaminants on larval body size can in fact persist in post-metamorphic amphibians.

Our results suggest that sublethal effects of Hg on amphibians may be more persistent than those of short-lived environmental contaminants such as pesticides. The few studies that have examined latent or persistent effects of larval contaminant exposure in post-metamorphic amphibians have focused on pesticides (Rohr and Palmer 2005; Distel and Boone 2009; Distel and Boone 2010; Webber et al. 2010). Moreover, some studies have indicated that juvenile toads can compensate for pesticide-induced reductions in size at metamorphosis via increased growth in the terrestrial habitat (Boone 2005; Distel and Boone 2009; Distel and Boone 2010), calling into question the lasting consequences of larval effects. In contrast, we found that a 5% reduction in body size at metamorphosis due to maternal Hg exposure persisted in the terrestrial environment and resulted in a 7% difference in body size after nearly a year of growth in the terrestrial environment. The discrepancy between our results and those of previous studies might be explained by differences between Hg and the contaminants other studies have evaluated. For example, many currently used pesticides are designed to degrade rapidly in the environment, whereas Hg and other metals are persistent and can bioaccumulate in tissues (Eisler 2006). Thus, metals might be more likely to result in persistent long-term effects than some other environmental contaminants. Alternatively, the failure of terrestrial toads to show compensatory terrestrial growth in the present study may indicate that feeding ability was impaired in affected animals or that there were metabolic costs associated with Hg accumulation, either via increased metabolic maintenance costs or via reduced metabolism and growth. Metals are known to cause increased malformation frequencies (Hopkins et al. 2000; Todd et al. 2011a), which may negatively affect feeding abilities in malformed individuals. Also, metal contamination in vertebrates can alter metabolic function and reduce somatic growth (Sherwood et al. 2000).

Our study was also designed to test for the onset of any latent adverse effects of larval of maternal Hg exposure. Laboratory studies have shown that sublethal contaminant exposure can affect behavior (Semlitsch et al. 1995; Bridges 1997, 1999; Burke et al. 2010) and physiology (Rowe et al. 1998; Palenske et al. 2010) of larval amphibians. Often these

sublethal effects are suspected to reduce survival under natural conditions, resulting in latent lethal effects in the terrestrial stage (Boone and Bridges 2003). For example, Rohr and Palmer (2005) found that terrestrial juvenile streamside salamanders (*Ambystoma barbouri*) exhibited altered behavior several months after larval exposure to the herbicide Atrazine, increasing their susceptibility to desiccation. When reared in terrestrial enclosures with no predators or heterospecific competitors, we found little evidence that prior Hg exposure affected survival up to 1 year of age. Although we found a significant maternal effect when survival data were analyzed in time series, survival rates at the end of 1 year did not differ among treatments. One possible explanation for the slight difference in survival trajectories is that mortality was size-dependent during some seasons and thus, differences among treatments were driven by initial differences in body size. However our non-parametric bootstrap resampling analysis suggested that survival was independent of initial body size.

Although our results suggest that exposure to Hg through maternal transfer or larval diet has limited adverse effects on post-metamorphic survival, they should be interpreted with caution given the simplified conditions of the terrestrial enclosures in the present study. Under more challenging natural conditions, larger body size may reduce risk of predation by gape-limited predators (Willson and Hopkins 2011), or facilitate dispersal or predator evasion through enhanced locomotor performance and stamina (Arnold and Wassersug 1978; John-Adler and Morin 1990). Additionally, the present study does not address terrestrial Hg accumulation and possible effects that such accumulation may have on post-metamorphic animals. A valuable next step in longitudinal studies of contaminant effects in amphibians would be the addition of terrestrial sources of contaminant exposure, either via dietary or direct exposure, in concert with larval and/or maternal exposures.

Despite the lack of added dietary Hg in the terrestrial enclosures after metamorphosis, the animals displayed interesting patterns of Hg accumulation. For example, toads fed control diet as larvae accumulated low levels of Hg in the terrestrial environment, although their concentrations were still below those of adult American toads and aquatic and terrestrial salamanders that inhabit uncontaminated reference sites in Virginia (Bergeron et al. 2010a). The Hg accumulation in toads fed uncontaminated larval diet likely reflects the ingestion in the terrestrial environment of background levels of Hg from sources such as atmospheric deposition and the trophic shift from a primarily herbivorous larval diet to a carnivorous diet in terrestrial life stages. In contrast, juvenile toads fed dietary Hg in the larval environment exhibited a more than 12-fold decrease in THg concentrations after 1 year of growth in terrestrial enclosures. Given their nearly 10-fold increase in dry weight over this time, the decrease in THg concentrations can be explained primarily by dilution as juveniles gained body mass. However, terrestrial animals with high THg burdens at metamorphosis may have also eliminated some Hg from their tissues in the year following metamorphosis. Nevertheless, even after a full year in an uncontaminated terrestrial environment, toads that were fed dietary Hg as larvae still exhibited significantly elevated THg tissue concentrations compared to those raised on the control larval diet. The persistence of elevated Hg tissue concentrations after metamorphosis highlights the possibility that toads metamorphosing and leaving contaminated larval habitats may be important vectors for Hg dispersal into surrounding terrestrial food webs.

When scaling from individual-based toxicological studies to population-level effects, it is important to understand the degree to which contaminant effects translate across life stages. Specifically, post-metamorphic life stages are thought to be the primary drivers of amphibian population dynamics (Biek et al. 2002; Vonesh and De la Cruz 2002; Schmidt et al. 2005). Thus, it is critical to know whether sublethal effects observed in larvae persist in post-metamorphic life stages and whether latent effects emerge after initial exposure to contaminants. Our results indicate that adverse effects of maternally transferred Hg on larval amphibians can persist to affect subsequent terrestrial life stages. The reduction in body size at metamorphosis in the present study persisted for at least 1 year in terrestrial juveniles. The degree to which a 5–7% reduction in body size may influence population dynamics is unclear. However, in a study of marbled salamanders (*Ambystoma opacum*), Scott (1994) found that a reduction of approximately 10% in body size at metamorphosis equated to a 20% reduction in the return rate of breeding animals within 2 years and an 8% difference in body size of reproductive females equated to a 33% reduction in clutch size. Thus, a 5–7% reduction in body size of American toads may be linked to a reduction in lifetime reproductive output through delayed maturity, lower survival, and/or smaller clutches. Further, by examining growth and survival of post-metamorphic toads, we were able to demonstrate a lack of significant latent effects from earlier maternal or dietary exposure in the larval environment. Longitudinal studies like ours are increasingly needed to comprehensively assess the diverse effects of environmental contaminants throughout ontogeny when the ultimate goal is translating individual effects to the viability of natural populations.

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