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Counterbalancing effects of maternal mercury exposure during different stages of early ontogeny in American toads

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ABSTRACT

Maternal transfer of environmental contaminants is a disadvantageous parental effect which can have long-lasting implications for offspring fitness. We investigated the effects of mercury (Hg) on the reproductive success of female amphibians and the subsequent effects of maternal transfer on the development of their offspring. American toads (*Bufo americanus*) maternally transferred Hg to their eggs, and there was a negative relationship between Hg concentrations and the percentage of viable hatchlings produced in clutches. However, when we continued to monitor larvae that successfully hatched, we found 21% greater metamorphic success in larvae from Hg-exposed mothers compared to reference larvae. The negative effect in the embryonic stage and positive effect in the larval stage counterbalanced one another, ultimately resulting in no difference in predicted terrestrial recruitment, regardless of maternal Hg exposure. Our findings demonstrate that maternal effects on survival manifesting at different stages in ontogeny have the potential to produce complicated outcomes.

1. Introduction

The condition or physiological state of parents can be affected by the environment, and these effects can be transmitted to offspring (usually by the mother) in the form of nutrition, hormones, and antibodies (Bernardo, 1996). Parental effects on offspring fitness are highly context-dependent and can be positive or negative based on different environmental circumstances (Rossiter, 1996). However, exposure to environmental contaminants is one of the most obvious disadvantageous parental effects. Contaminants may directly affect parental health, fertility, or fecundity, but females may also maternally transfer bioaccumulated contaminants to developing embryos. In the majority of circumstances that have been investigated, maternal transfer of contaminants is deleterious to offspring due to the effects of transferred contaminants on key organizational events that occur early in ontogeny (Russell et al., 1999). For example, rapid declines of North American raptor and piscivorous bird populations in the mid-20th century were largely attributed to the pesticide dichlorodiphenyltrichloroethane (DDT) and its metabolites which caused eggshell thinning and subsequently reduced hatching success (Blus, 1996). In addition to immediate effects, parental effects can have long-term consequences (Lindstrom, 1999). For instance, alligators maternally exposed to endocrine-disrupting chemicals from Lake Apopka, FL experienced low reproductive success as a result of reduced hatching success and increased juvenile mortality, along with abnormal development of the endocrine and reproductive systems (Guillette et al., 2000). In humans, prenatal exposure in females to the synthetic estrogen diethylstilbestrol (DES) increased the risk of vaginal clear cell adenocarcinoma, abnormalities of the reproductive tract, and infertility (Swan, 2000). However, unlike the effects of maternal exposure of DDT on hatching success in birds, the effects of DES were latent and manifested only at the onset of offspring adolescence, another critical period in ontogeny (Swan, 2000). These examples highlight the range in timing of the expression of deleterious effects that maternal exposure to chemicals can have on offspring.

Compared to other vertebrate classes, few studies have investigated the effects of contaminants on reproduction in amphibians, even though environmental contamination is one of several factors suspected of contributing to worldwide amphibian population declines (Corn, 2000). In particular, maternal exposure and transfer of contaminants may be important mechanisms of impaired reproductive success in amphibians, but most of the information regarding the effects of contaminants on amphibian development is from aqueous exposure of embryos (Linder and Grillitsch, 2000). In amphibians, developmental processes dominate the embryonic stage, however, considerable growth and development continues during the larval stage, culminating in metamorphosis. Only three studies have examined the effects of maternal transfer of

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contaminants on amphibian offspring (Bergeron et al., 2011; Hopkins et al., 2006; Kotyzova and Sundeman, 1998), and there are few studies of the potential for latent effects of maternal exposure to contaminants beyond embryonic development. Much like DES, it is possible that the effects of maternal transfer of contaminants could manifest during critical developmental periods weeks to months after hatching (Budischak et al., 2008; Rohr and Palmer, 2005).

The current study sought to investigate the effects of maternal exposure to mercury (Hg) on the reproductive success of female amphibians across a large Hg-contamination gradient and the subsequent development of their offspring through metamorphosis at the extremes of this gradient. Mercury is an environmental contaminant of global concern due to its ubiquity, toxicity, and ability to bioaccumulate in animals, especially as (mono)methylmercury (MMHg) (Fitzgerald et al., 1998; Mason et al., 1996). Due to the neurotoxic, teratogenic, and endocrine-disrupting nature of Hg, subtle effects on behavior and reproduction may occur at concentrations well below levels associated with overt toxicity and death (Scheuhammer, 1991; Weiner and Spry, 1996). Indeed, reproductive success is the demographic parameter expected to be most affected by exposure to Hg in fish and birds (Crump and Trudeau, 2009; Scheuhammer et al., 2007; Weiner and Spry, 1996). Although there is little information about the effects of Hg in amphibians, larval development and metamorphic climax are both stages during ontogeny when amphibians are known to be sensitive to Hg exposure (Unrine et al., 2004). In a previous study, we determined that *Bufo americanus* (American toads), one of the most common amphibians inhabiting the floodplain of the historically Hg-contaminated South River, VA, USA (Carter, 1977), maternally transferred Hg to their eggs (Bergeron et al., 2010a). Here, we predicted that offspring from female *B. americanus* collected at Hg-contaminated sites would be negatively affected by the maternal transfer of Hg through both decreased embryonic viability (i.e., decreased hatching success and increased frequency of morphological abnormalities) and decreased metamorphic success due to the latent effects of Hg during the larval stage, thus decreasing the females' overall reproductive success.

2. Material and methods

2.1. Breeding and egg collection

We captured amplexing pairs of *B. americanus* in March and April of 2007 ($n=53$) and 2008 ($n=30$) from breeding pools located within 180 m of the South River, along a broad contamination gradient upstream (river mile [RM] −1.7 and −5; $n=24$) and downstream (RM 2, 5, 9, 16, and 20; $n=59$) of a Hg contamination source (RM 0; see Bergeron et al., 2010b for additional information). The South River was historically contaminated with mercuric sulfate used by a manufacturing plant in Waynesboro, VA (Carter, 1977) and an analysis of surface water and sediment at the South River and the reference sites confirmed that Hg was the primary contaminant while organochlorine pesticides, polycyclic aromatic hydrocarbons, and other trace metals, such as cadmium, copper, chromium, lead, selenium, and zinc, were generally low (URS, 2007).

To determine whether Hg maternally transferred to eggs in *B. americanus* has any effect on reproductive success and development, we followed the methods of Hopkins et al. (2006) and Bergeron et al. (2010a). Briefly, amplexing pairs of *B. americanus* were transported to the laboratory where they were allowed to breed in dechlorinated tap water. We recorded mass and snout–vent length (SVL) of females after oviposition. In 2007, we held most females for an additional 48 h to void gut contents, then collected blood and sacrificed them with an overdose of buffered tricaine methane sulfonate (MS-222) to examine relationships between Hg in female carcasses and blood/eggs (see Bergeron et al., 2010a, 2010b). In a parallel study we determined that the percentage of Hg that was methylated (MMHg) in female carcass, blood, and eggs was 53.3 ± 2.3%, 71.4 ± 2.8%, and 47.8 ± 3.3% (mean ± 1 standard error hereafter), respectively (Bergeron et al., 2010a). Thus, in the present study, we report only total Hg (THg) concentrations for these tissues. By establishing mathematical relationships between Hg in female blood and carcass in this supporting study (Bergeron et al., 2010a), we were also able to avoid sacrificing females in 2008 and instead analyzed blood for Hg analysis. Females and males were individually marked by toe clipping and released at their point of capture.

2.2. Embryonic developmental assessment

After determining the clutch size of each female, we allocated subsets from each clutch for Hg analyses, hatching and morphological assessments, and mesocosm experiments (2008 only). To assess hatching and morphological development, subsets of 500 eggs from 52 females were allowed to develop to hatching (~Gosner stage [GS] 20) at 17–20 °C in ~3 L of dechlorinated tap water. Hatchlings from each subset were counted to quantify hatching success, and then fixed in formalin and stored in 70% ethanol. We classified each hatchling as either morphologically "normal" or "abnormal" according to the methods of Bantle et al. (1991) using a dissecting microscope. Morphological abnormality classifications included edema or swelling, craniofacial malformations, and/or four types of axial malformations (dorsal flexure, lateral flexure, wavy tail, and axial shortening). All morphological assessments were performed blind to female identity. Finally, we calculated the overall viability of embryos in each clutch by combining hatching success and the frequency of abnormalities (assuming abnormal hatchlings were not viable) (Hopkins et al., 2006).

2.3. Latent effects on larvae

To examine latent effects of maternally-derived Hg on larval traits and recruitment (i.e., successful metamorphosis) at the extremes of the Hg-contamination gradient, we established replicated ($n=12$) outdoor aquatic mesocosms in 1500 L polyethylene stock tanks at Virginia Tech in Blacksburg, VA. On February 29, 2008, we filled the mesocosms with approximately 475 L of well water and 475 L of dechlorinated city water. No Hg was added to any of the mesocosms. To provide nutrients, each mesocosm received 1 kg of air-dried deciduous leaf litter (50:50 poplar and oak mix) and 17 g of finely ground Purina Rabbit Chow® (St. Louis, MO, USA). To initiate algal and periphyton growth, we added 2 L of filtered water to each mesocosm from two ponds within Montgomery County, VA on three separate dates before March 14, 2008. To decrease the variability in initial phytoplankton communities, portions of water were repeatedly exchanged among mesocosms prior to the addition of hatchlings. We covered mesocosms with black mesh lids to provide shade and exclude predators and competitors.

After embryos hatched, "normal" hatchlings were added to the mesocosms where they remained until the initiation of metamorphic climax. Because female blood and egg THg concentrations are closely correlated (Bergeron et al., 2010a), we used female blood THg to initially characterize clutches as either reference (female blood THg concentrations<250 ng/g, wet weight) or maternally Hg-exposed (hereafter, Hg-exposed; female blood concentrations>1000 ng/g, wet weight). These threshold concentrations represented the extremes of the Hg-contamination gradient as females from the reference sites did not exceed 250 ng/g THg in their blood and the upper quartile of blood from females at the South River exceeded 1000 ng/g THg. On April 9, 2008 clutches (reference $n=6$, Hg-exposed $n=3$) within each group were combined to homogenize genetic variation to avoid confounding mesocosm effects with clutch effects (Boone and James, 2005). Next, equal densities of 100 hatchlings were added to each randomly chosen mesocosm ($n=6$ mesocosms/group). We monitored mesocosms daily and moved metamorphosing individuals (≥GS 42) into

the lab to complete metamorphosis. At the time of front limb emergence, we placed individuals in separate 500 mL cups with ~20 mL mesocosm water and a dry area to climb onto during tail resorption. We checked each metamorphosing tadpole once a day for mortality or completion of tail resorption (GS 46). All surviving metamorphosed toads were weighed and measured, euthanized with buffered MS-222, and then frozen for subsequent Hg analyses.

2.4. Estimating terrestrial recruitment

We estimated overall recruitment to the terrestrial environment as the percent of a clutch to metamorphose for the reference and Hg-exposed clutches by using a simple algorithm:

$$\text{Estimated recruitment } (\%) = \frac{\text{clutch size} * \% \text{ viable} * \% \text{ metamorphic success}}{\text{clutch size}}.$$

The model incorporates clutch size and associated viability for each female sampled and relies on the mean metamorphic success from the two mesocosm groups (Hg-exposed or reference) to estimate terrestrial recruitment. We made the following assumptions: 1) hatchlings with morphological abnormalities were not viable; 2) the percent viability for the 500 egg subset is representative of the entire clutch; 3) the metamorphic success of pooled clutches from reference and Hg-exposed mesocosms at the extremes of the Hg-contamination gradient (45.3% and 54.5%, respectively) is indicative of metamorphic success of individual clutches from reference and Hg contaminated sites. While we fully recognize that this estimate is overly simplistic and does not account for individual clutch effects on larval development or important ecological factors such as density-dependence and competition, it is a useful first order approximation of metamorph production for each female.

2.5. Sample preparation and mercury analyses

We lyophilized and homogenized adult carcasses (2007), eggs (2007 and 2008), and metamorphs from the mesocosm experiment (2008) and we report THg concentrations on a dry wt basis. Whole blood from each adult *B. americanus* was homogenized using a vortex mixer and we report THg concentrations in blood on a wet wt basis. Percent moisture was 77.8 ± 0.4% (mean ± 1 standard error of the mean hereafter) for female carcasses, 96.3 ± 0.2% for eggs, and 87.1 ± 0.1% for metamorphs. We analyzed subsamples (20–150 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 (U.S. EPA) method 7473 (USEPA, 1998). For quality assurance, each group of 10 to 15 samples included a replicate, blank, and standard reference material (SRM; TORT-2 lobster hepatopancreas, DOLT-2 dogfish liver, DOLT-3 dogfish liver, DORM-3 fish protein [National Research Council of Canada (NRCC), Ottawa, ON] or SRM 966 Toxic Metals in Bovine Blood Level 2 [National Institute of Standards and Technology, Gaithersburg, MD, USA]). We calibrated the instrument using solid SRMs (TORT-2 and DORM-2 dogfish muscle [NRCC], or DOLT-3 and DORM-3). Method detection limits (MDLs; 3 times the standard deviation of procedural blanks) for samples were 0.95 ng, and all samples had THg concentrations that exceeded the limit. Average relative percent differences (RPD) between replicate sample analyses were 5.98 ± 0.75% ($n = 75$). Mean percent recoveries of THg for the SRMs ranged from 89.66 ± 0.01% to 106.87 ± 0.51%.

2.6. Statistical analyses

We examined the relationship between female, blood, or egg THg concentrations and female body size on clutch size using multiple regression analyses. We used linear regression to examine the relationship between female body size and THg concentrations in tissues. We used analysis of variance (ANOVA) to test for differences in female body size and clutch size by year.

To assess the effects of Hg on embryonic development, we used linear regression to describe the relationships between female body THg concentrations (log-transformed) in 2007 (the only year we sacrificed females) and hatching success, the frequency of abnormalities, or viability (all angular-transformed). We used analysis of covariance (ANCOVA) to compare relationships between blood or egg THg concentrations (log-transformed) and hatching success, the frequency of abnormalities, or viability (all angular-transformed) between years (2007 and 2008). Although female body size (SVL) did not influence hatching success or viability, there was a weak tendency for it to influence abnormalities ($r = 0.243$, $p = 0.083$), where smaller females produced more abnormal individuals. Thus, to correct for body size, we used the residuals of SVL and the frequency of abnormalities in the linear regression and ANCOVA analyses.

To compare development of larvae in mesocosms from reference and Hg-exposed females, we used multivariate analysis of variance (MANOVA) with several parameters describing traits of recruits to the terrestrial environment. These endpoints included: SVL and condition ($\text{mass}/\text{SVL}^3 * 10^5$) at the completion of metamorphosis (GS 46), days until the beginning of metamorphic climax (GS 42), days to complete metamorphic climax (from GS 42 to 46), and the percentage of individuals to complete metamorphosis. Mean responses of each mesocosm were used in all statistical analyses. Lastly, we used linear regression to examine the relationship between THg concentrations in eggs and blood and estimated recruitment.

We performed all analyses with SAS 9.1 (SAS Institute, Cary, NC, USA) and used $\alpha = 0.05$ to determine statistical significance.

3. Results

3.1. Effects of mercury on clutch characteristics

There were no significant effects of female, egg, or blood THg concentrations on clutch size ($p > 0.244$, for all), but female mass and SVL were both positively correlated with clutch size ($p < 0.0001$, for all). Females were larger in 2008 (70.5 ± 2.8 g, 87.17 ± 1.58 mm) than in 2007 (56.1 ± 2.1 g, 80.38 ± 0.74 mm; $F_{1,80} > 16.68$, $p < 0.0001$, for both), resulting in larger clutch sizes in 2008 (mean, SE: 7495 ± 495) compared to 2007 (6078 ± 366; $F_{1,80} = 5.28$, $p = 0.024$). In 2007, there was no relationship between female body THg concentrations and SVL ($r^2 = 0.022$, $p = 0.319$, $n = 48$). However, with both years combined, female SVL was positively correlated with blood and egg THg concentrations ($r^2 = 0.073$, $p = 0.013$, $n = 83$ and $r^2 = 0.089$, $p = 0.006$, $n = 83$, respectively), suggesting a trend toward larger females accumulating and transferring more Hg to their eggs.

3.2. Effects of mercury on embryonic development

The percentage of embryos that successfully hatched (clutch range: 0.6–100%) decreased with increasing THg concentrations. This relationship was best explained by the correlation with female body THg concentrations in 2007 (Fig. 1A; $r^2 = 0.472$, $p < 0.001$, $n = 23$). A strong negative relationship was also found between maternal blood THg and hatching success (Fig. 1B; $F_{1,53} = 13.11$, $p < 0.001$) and between egg THg and hatching success (Fig. 1C; $F_{1,53} = 19.27$, $p < 0.001$). For blood and eggs, the effect of THg on hatching success was consistent across years ($F < 0.93$, $p > 0.339$, for all year and THg by year combinations).

We assessed the morphology of newly hatched embryos from 52 clutches ($n = 20{,}551$ hatchlings). Contrary to our predictions, the frequency of abnormalities (clutch range: 0.2–56%) decreased as THg concentrations increased in female body (Fig. 2A; $r^2 = 0.297$, $p = 0.013$), blood (Fig. 2B; $F_{1,48} = 7.35$, $p = 0.009$), and eggs (Fig. 2C;

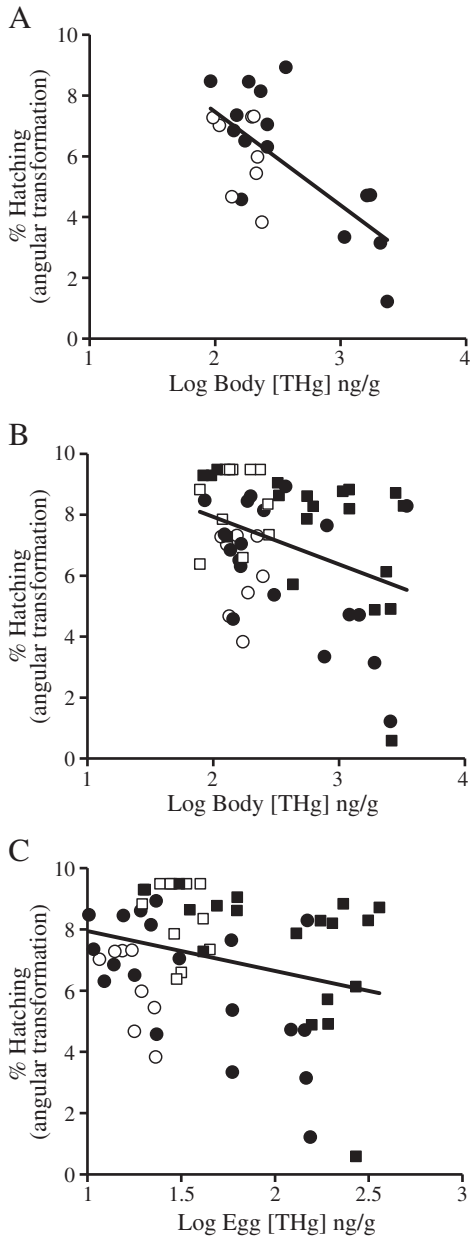

Fig. 1. Relationship between log total mercury (THg; ng/g) concentration and the percentage of embryos that successfully hatched (angular transformation) in A) female body (dry weight; 2007 only), B) female blood (wet weight) and C) eggs (dry weight) from the reference (open symbols) and contaminated (closed symbols) portion of the South River (VA, USA) in 2007 (circle symbols) and 2008 (square symbols). Note that the y-axis is expressed as an angular transformation of the percent hatching.

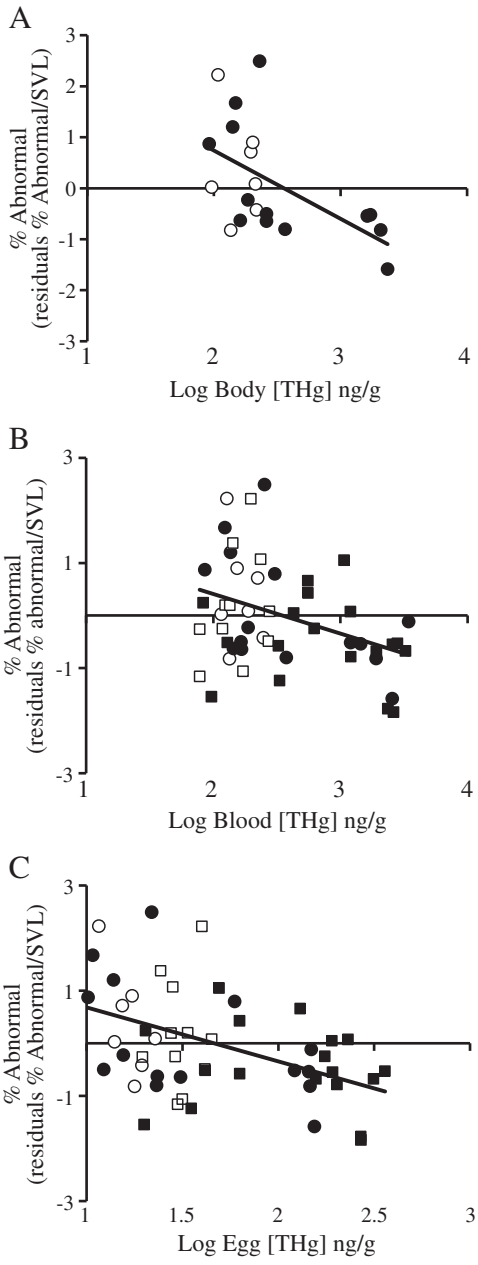

Fig. 2. Relationship between log total mercury (THg; ng/g) concentration and the percentage of abnormal hatchlings (angular transformation) corrected for body size (snout–vent length; SVL) in A) female body (dry weight; 2007 only), B) female blood (wet weight) and C) eggs (dry weight) from the reference (open symbols) and contaminated (closed symbols) portion of the South River (VA, USA) in 2007 (circle symbols) and 2008 (square symbols).

$F_{1,48} = 9.15$, $p = 0.004$). For blood and eggs, the effect of THg on the frequency of abnormalities was consistent across years ($F < 2.10$, $p > 0.154$, for all year and THg by year combinations). Of the total abnormalities observed, 83% were axial, 12% were craniofacial, 3.2% were edema, and 4.4% were considered "other" which included abnormal gut coiling, reduced tail margins, and no head.

The decrease in the frequency of abnormalities with increasing THg concentrations was not sufficient to offset the effect of decreased hatching success on overall hatchling viability (clutch range: 0.4–95%), which decreased with increasing THg concentrations in female body (Fig. 3A: $r^2 = 0.292$, $p = 0.014$, $n = 20$), blood (Fig. 3B: $F_{1,48} = 5.55$, $p = 0.023$), and eggs (Fig. 3C: $F_{1,48} = 6.88$, $p = 0.012$). Again, for blood and eggs, the effect of THg on viability was consistent across years ($F < 0.37$, $p > 0.544$, for all year and THg by year combinations).

3.3. Effects of maternal mercury on larval development

The mean THg concentrations in the blood of females from the reference and contaminated site used in the mesocosm experiment were 181.6 ± 28.0 and 2122.0 ± 480.4 ng/g, respectively. The corresponding mean THg concentrations in the eggs were 29.8 ± 3.5 and 286.1 ± 38.3 ng/g for the reference and contaminated clutches, respectively. The overall MANOVA model describing metamorphic responses to maternal Hg exposure extremes at the South River revealed a significant difference between the Hg-exposed and reference groups (Pillai's Trace = 0.80, $F_{5,6} = 4.76$, $p = 0.042$). Component ANOVAs revealed no difference ($p > 0.133$, for all) between the reference and Hg-exposed groups in SVL (mm: 13.8 ± 0.2 and 13.0 ± 0.5), body condition (9.0 ± 0.2 and 9.4 ± 0.3), days to the start of metamorphic

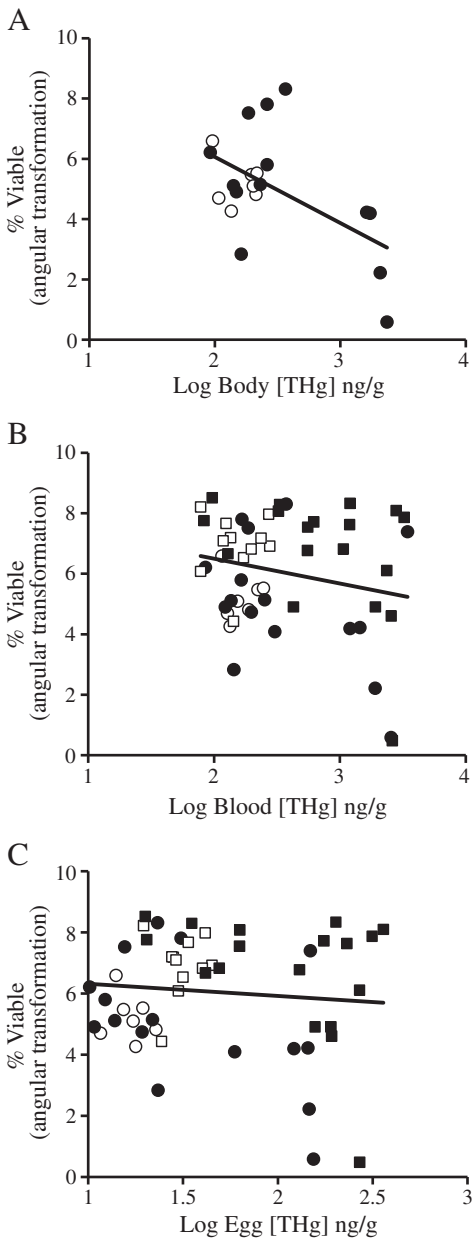

Fig. 3. Relationship between log total mercury (THg; ng/g) concentration and the percentage of viable hatchlings (angular transformation) in A) female body (dry weight; 2007 only), B) female blood (wet weight) and C) eggs (dry weight) from the reference (open symbols) and contaminated (closed symbols) portion of the South River (VA, USA) in 2007 (circle symbols) and 2008 (square symbols). Overall viability of embryos in each clutch was estimated by combining hatching success and the frequency of abnormalities (assuming abnormal hatchlings were not viable). Note that the y-axis is expressed as an angular transformation of the percent viable.

climax (47.8 ± 0.5 and 49.0 ± 0.6), and days to complete metamorphic climax (5.2 ± 0.1 and 5.3 ± 0.1), respectively. Although the significance of the MANOVA may be attributable to the variance properties of the combined endpoints rather than a single strong effect on any single endpoint, the most notable difference was a 21% decrease in metamorphic success in the reference group ($45.3 \pm 3.8\%$) compared to the Hg-exposed group ($54.5 \pm 2.9\%$; $p = 0.084$). Because individuals were not subjected to additional exposure of excessive Hg during larval development, THg concentrations (dry weight) in metamorphs were low and did not differ between individuals exposed as embryos to reference and high maternal Hg (42.1 ± 2.2 ng/g versus 43.1 ± 2.4 ng/g; $F_{1,10} = 0.09$, $p = 0.776$).

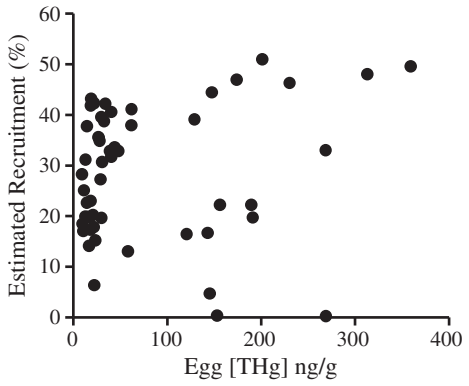

Fig. 4. Relationship between total mercury (THg; ng/g, dry weight) concentrations in eggs and estimated recruitment (%) ($r^2 = 0.024$, $p = 0.273$).

We found no significant relationship between the THg in the eggs or maternal blood and estimated recruitment (Fig. 4; $r^2 = 0.024$, $p = 0.273$ and $r^2 = 2.8 \times 10^{-5}$, $p = 0.970$, respectively), suggesting that the negative effect of maternal Hg exposure on the embryonic stage was offset by enhanced survival during the larval stage, resulting in no effect on the number of juveniles entering the terrestrial environment.

4. Discussion

Individuals can be directly affected by their environment, but also indirectly affected by the environment in which their parents and even their grandparents inhabited (Bernardo, 1996; Mousseau and Fox, 1998; Rossiter, 1996). Because these environmental factors can influence offspring phenotypes, there are widespread implications for both wildlife and human health. For instance, parental exposure to environmental contaminants or poor nutritional state can have negative, transgenerational effects on adult offspring in terms of disease susceptibility and reproductive success (Anway and Skinner, 2006; Bateson et al., 2004). In some cases, there are compensatory mechanisms within an individual to offset the effects of the maternal environment either during the embryonic stage or later in development. For example, in mice, developmental exposure to the endocrine-active chemical, bisphenol A (BPA) modified the epigenome via DNA methylation. However, maternal nutrient supplementation negated the hypomethylating effect of BPA (Dolinoy et al., 2007). Alternatively, individuals that experience nutritional deficits during early development can compensate by accelerating growth in a later stage if conditions improve, however this compensatory growth can be associated with long-term costs in the adult stage (Metcalfe and Monaghan, 2001). Interestingly, here we demonstrate a counterbalancing effect within an amphibian population. Although we found a negative effect of maternal Hg exposure on amphibian reproduction through reduced embryonic viability, the effect was counterbalanced by relatively high metamorphic success in surviving larvae from Hg-exposed females at the extreme of the Hg-contamination gradient. Based on a simple model that combines survival from these two stages of ontogeny, we observed no net effect of maternal Hg exposure on estimated terrestrial recruitment, suggesting that the counterbalancing effect could have important ecological consequences for the population.

Maternal exposure to environmental contaminants can negatively affect reproduction by influencing a variety of factors including egg production, embryo viability, and successful offspring development. Maternal Hg exposure did not affect female clutch size in *B. americanus*, even at the elevated concentrations of Hg documented in this study. This stands in contrast to other studies that have found impaired ovary development, egg production, and spawning success in females of oviparous species exposed to Hg, particularly in fish (reviewed in Crump and Trudeau, 2009; Tan et al., 2009). Alternatively, even though female *B. americanus* only transfer a small

proportion (~5%) of their pre-ovipositional Hg body burden to their eggs (Bergeron et al., 2010a), the resulting Hg concentrations in eggs appear to be sufficient to decrease hatching success. Although correlative, this relationship provides compelling evidence that female amphibians can maternally transfer a contaminant and influence the survival of offspring in the early stages of development.

Offspring that successfully hatch may be at a functional disadvantage due to the effect of maternal exposure to contaminants on embryonic neurodevelopment or morphology. Contrary to the prevailing body of literature on Hg and many other contaminants (e.g., Weis and Weis, 1991) as well as our predictions, we found that the frequency of abnormalities decreased with increasing Hg concentrations. Our findings suggest that Hg induces mortality rather than a teratogenic effect in amphibian embryos from high Hg clutches. Consequently, we hypothesize that a greater proportion of lower quality embryos hatched successfully in clutches from reference mothers, resulting in a greater proportion of abnormal hatchlings. However, the decrease in the frequency of abnormalities in high Hg clutches was not sufficient to offset the effect of decreased hatching success, and ultimately, overall hatchling viability decreased with increasing Hg concentrations. Interestingly, the strongest correlation between Hg concentrations in all the tissues sampled (maternal carcass, maternal blood, and eggs) and the embryonic developmental endpoints (hatching success, frequency of abnormalities, and viability) were with maternal carcass. We expected the strongest correlations to be with Hg concentrations in eggs, assuming that the direct effect of transferred Hg causes the reduction in viability. The stronger relationship between female Hg concentrations and embryonic development suggests that the effects of Hg on the female's reproductive axis or other aspects of her physiology might contribute to the observed embryonic effects. As a result, exposure to Hg could lead to suboptimal egg quality or maternal deposition of other hormones (e.g., stress hormones) into the egg. For example, Verboven et al. (2009) found that egg quality in Glaucous Gulls (*Larus hyperboreus*) exposed to persistent organic pollutants may be affected by the direct maternal transfer of pollutants to eggs and indirectly through changes in egg size and composition (lipid and water content).

Few studies have investigated the long-term effects of maternal transfer of contaminants on offspring development despite the fact that important organizational events often occur early in development. This is one of the first studies in amphibians to examine potential long-term or latent effects of maternal contaminant exposure during the larval period. We found no difference in body size or condition, time to metamorphosis, or days to complete tail resorption between the metamorphs from reference or Hg-exposed groups. However, contrary to our predictions, we found that larvae from the Hg-exposed group actually had greater metamorphic success than larvae from the reference group. Based on these observations, we hypothesize that the poorest quality embryos in the clutch were eliminated due to elevated maternal Hg exposure and the surviving larvae were more robust than larvae from the reference clutches, which were not subjected to similar selective pressures. In our simplified developmental environments (i.e., excluding predators and heterospecific competitors), the "selection" by Hg for more robust larvae appears to be advantageous. However, Hg is a neurotoxicant with known effects on behavior and performance, even when maternal exposure is the only exposure to Hg. For example, larval Atlantic croaker (*Micropogonias undulates*) from parents fed MMHg-contaminated diets showed reduced performance including altered swimming behavior and startle response (Alvarez et al., 2006). Thus, it remains unknown how amphibian larvae maternally-exposed to Hg would respond to predators or intense competition.

To assess the overall impact of maternal Hg exposure in *B. americanus* on the number of surviving metamorphs, we used a simple model to estimate recruitment to the terrestrial environment. We found that the negative effect of maternal Hg exposure on viability in the embryonic stage was counterbalanced by the selection for more robust larvae in the Hg-exposed group, resulting in no differences in recruitment across a wide range of Hg concentrations. This counterbalancing effect is a significant finding because it suggests that, assuming all else is equal, maternal Hg exposure ultimately has little effect on amphibian juvenile recruitment, which can later influence the future reproducing population (Beebee et al., 1996; Berven, 1990). However, while juvenile recruitment may be unaffected, we did not investigate the potential impact of maternal Hg exposure on the population through changes in genetic variability. For example, the selection for robust larvae in the Hg-exposed group may drive evolution in ways that are not adaptive in terms of resistance to natural stressors (e.g., predation or disease) (Medina et al., 2007). In addition, it is possible that the counterbalancing effect may produce variable results under natural conditions because we began the larval experiment with equal densities of hatchlings in each of the simulated ponds (mesocosms) and did not account for known density-dependent interactions in amphibian larvae. For example, in natural conditions, if hatching success was reduced in Hg-contaminated ponds, the density of surviving larvae would be reduced, releasing them from competitive pressure and potentially increasing their metamorphic success beyond that observed in this experiment compared to larvae from reference ponds. Because competition is an important factor influencing metamorphic success of amphibians (e.g., Semlitsch and Caldwell, 1982; Wilbur, 1977), demographic population modeling (e.g., Vonesh and De la Cruz, 2002) will ultimately be a more informative method to estimate recruitment provided that juvenile and adult parameters can be obtained or estimated.

The maternal environment can greatly influence reproductive conditions and both the immediate and long-term development of offspring. Our findings shed further insight into the effects of maternal contaminant exposure on reproductive success and are among the first to correlate contaminant concentrations in the field with deleterious effects (reduced hatchling viability) on amphibian reproduction. In addition, comparatively few studies investigate the long-term consequences of maternal transfer of contaminants. Our work demonstrates that maternal effects which manifest at different stages in ontogeny have the potential to offset one another. This is of broad importance because it suggests that advantageous or disadvantageous parental effects on survival during early life stages may be counterbalanced in a later stage, resulting in no net effect on recruitment to the adult population. However, because maternal effects are highly context-dependent, future studies should account for differing environmental circumstances. For example, an important next step will be to incorporate greater environmental complexity by determining whether the latent effects of Hg are altered under more environmentally realistic situations where individuals are required to compete for resources with heterospecifics or avoid predators. In addition, larval amphibians are highly efficient at accumulating Hg compared to other life stages (Bergeron et al., 2010b), and exposure to dietary Hg alone may have negative effects on larval recruitment (Unrine et al., 2004). Thus, it is important to determine whether maternally-derived Hg interacts additively or synergistically with larval dietary exposure to negatively impact the number and size of individuals recruited to the local population.

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