ENVIRONMENTAL Science & Technology

ARTICLE

pubs.acs.org/est

Interactive effects of maternal and dietary mercury exposure have latent and lethal consequences for amphibian larvae

Christine M. Bergeron,[†] William A. Hopkins,[*,†] Brian D. Todd,[†,§] Mark J. Hepner,[†] and Jason M. Unrine[‡]

[†]Wildlife Ecotoxicology and Physiological Ecology Program, Department of Fish and Wildlife Conservation, Virginia Tech, Blacksburg, Virginia 24061, United States

[‡]Department of Plant and Soil Sciences and The Tracy Farmer Institute for Sustainability and the Environment, University of Kentucky, Lexington, Kentucky 40546, United States

Supporting Information

ABSTRACT: Organisms born into the same contaminated environment as their parents can be exposed both maternally and environmentally to contaminants, potentially placing them at greater risk of adverse effects than when exposed via either of the two pathways independently. We examined whether embryonic exposure to maternally derived mercury (Hg) interacts with dietary exposure to negatively influence larval development in American toads (*Bufo americanus*). We collected eggs from breeding pairs at reference and Hg-contaminated sites and monitored performance, development, and survival of larvae fed three experimental Hg diets (total Hg, 0.01, 2.5, and 10 μg/g). The negative sublethal effects of maternal and/or dietary Hg manifested differently, but maternal Hg exposure had a greater overall influence on offspring health than dietary exposure. However, the combination of sublethal effects of the two exposure routes interacted with lethal consequences; larvae exposed to maternal Hg and high dietary Hg experienced 50% greater mortality compared to larvae from reference mothers fed the control diet. This study is the first to demonstrate that the latent effects of maternally transferred contaminants may be exacerbated by further exposure later in ontogeny, findings that may have important implications for both wildlife and human health.

INTRODUCTION

Early development is a critical period in the ontogeny of vertebrates, and adverse effects of maternally derived contaminants during this sensitive stage have been well documented. For example, in many animals, maternal transfer of contaminants reduces reproductive success through embryo mortality and malformations (e.g., ref 1). Due to early disruptions in nervous, endocrine, or immune system development,[2] there is growing evidence that maternally derived contaminants can also have effects that are expressed later in ontogeny. These latent adverse effects may be lethal, as in the case of snapping turtles (*Chelydra serpentina*), where maternal transfer of polychlorinated biphenyls (PCBs) reduced survival over the first 14 months of life.[3] Alternatively, latent effects of maternal transfer of contaminants may be sublethal. The effects of maternally derived contaminants that occur long after hatching or birth are beyond the scope of most studies, but these latent adverse effects may have important implications for both wildlife and human health.

The latent effects of maternal transfer of contaminants may manifest differently depending on the environment in which offspring develop. Offspring of organisms that forage in one location but breed in another, such as migratory birds and many amphibians, may be exposed to maternally transferred contaminants but may or may not be further exposed to contaminants after the embryonic stage. For example, migratory white-faced ibis (*Plegadis chihi*) nesting at Carson Lake, NV, show reduced reproductive success with elevated egg concentrations of dichlorodiphenyldichloroethylene (DDE), but prey on the nesting grounds contained little DDE.[4] However, prey in wintering habitats had elevated DDE levels, suggesting that the main source in offspring was maternal transfer from energy stores gathered at the wintering grounds. Conversely, organisms born into the same contaminated environment as their parents can be exposed both maternally and environmentally to contaminants, potentially placing them at greater risk of adverse effects than when exposed via the two pathways independently.

There has been little research on the combined effects of multiple exposure routes through early development. However, Nye et al.[5] recently investigated the effects of maternal and direct exposure to sediments contaminated with polycyclic aromatic hydrocarbons (PAHs) from Elizabeth River, VA, in larvae of the

Received: December 15, 2010
Accepted: March 9, 2011
Revised: March 8, 2011
Published: March 23, 2011

estuarine fish *Fundulus hereroclitus*. The effect of maternal exposure on growth and body condition in larvae (14 days posthatch) was stronger than direct larval exposure.[5] In another recent study, Eisenreich et al.[3] found a strong negative maternal influence of PCBs on survival of juvenile snapping turtles, but dietary PCB exposure only reduced metabolic rates. Both studies observed latent effects of maternal contaminant transfer, but neither observed interactive effects of the multiple exposure routes. Additional studies are needed to evaluate the relative contributions of maternally and environmentally induced variation in offspring phenotype[6] and to ensure that latent and potential interactive effects are not overlooked.

We investigated the individual and interactive effects of maternally and trophically derived mercury (Hg) contamination on larval development in the American toad (*Bufo americanus*). Mercury is a contaminant of global concern due to its ubiquity, toxicity, and ability to bioaccumulate and biomagnify, especially in the form of (mono)methlymercury (MMHg).[7,8] Early life stages can be particularly sensitive to Hg exposure, and subtle sublethal effects on behavior and reproduction can occur at concentrations much lower than lethal effects due to the neurotoxic and endocrine-disrupting nature of Hg.[9,10] Although there is a paucity of information regarding the effects of Hg on amphibians compared to other vertebrates,[11] amphibian larvae are highly efficient at accumulating elevated concentrations of trace elements, including Hg, due to their feeding ecology and ectothermy.[12] In a previous study, we determined female American toads from a historically Hg-contaminated site maternally transferred Hg to their eggs,[13] disrupting early development of embryos,[14] but the effects of maternal transfer of Hg could also manifest during critical developmental periods weeks to months after hatching. In an additional field study, we found that larvae bioaccumulated very high concentrations of Hg in ephemeral breeding pools,[15] suggesting that significant environmental exposure occurs after maternal transfer. However, adult American toads have the opportunity to oviposit in either contaminated or uncontaminated pools because they are highly mobile for amphibians and can travel up to 1 km from their breeding pool.[16] Thus, their offspring have the potential to experience maternal, environmental (e.g., aqueous or dietary), or both routes of exposure. Here, we used field-collected adults and an experimental feeding study to test the hypothesis that trophic exposure to Hg during the larval period has a negative effect on the development and performance of larvae from Hg-exposed females.

MATERIALS AND METHODS

Field Collection. On April 17 and 18, 2009, we collected 27 reproductive pairs of American toads from breeding pools along the South River (VA) floodplain at three locations [river mile (RM) −1.7, 9, and 20] upstream and downstream from a Hg contamination source (RM 0; see ref 15 for additional information). An analysis of surface water and sediment at the South River confirmed that Hg was the primary contaminant, while organochlorine pesticides, polycyclic aromatic hydrocarbons, and other trace metals were generally low.[17] We transported amplexing pairs into the laboratory and placed them in a shallow bin with dechlorinated tap water to allow them to breed. The next morning, we removed the adult toads from the bins and counted the eggs. We froze a small portion of each egg mass (~500 eggs) for subsequent Hg analysis. Following the methods of Bergeron et al.,[15] we collected ~0.25 mL of whole blood from each anesthetized mother via cardiocentesis and released them at their capture location within 24 h.

Experimental Design. We used a 2 × 3 factorial design to test the individual and interactive effects of maternally and trophically derived Hg on the larval development of American toads. Experimental diets consisted of a dry feed mix spiked with or without Hg [inorganic (HgII) and organic (MMHg); Alfa Aesar] and suspended in an agar−gelatin mixture similar to the diet formulated by Unrine and Jagoe.[18] The resulting diet was in a semisolid matrix, which allowed the larvae to graze naturally while preventing the diet from dissolving. We conducted a preliminary study to determine if Hg was leaching from the food to the water and found that Hg concentrations in the water were below the detection limit of 50 ng/L. The target total Hg (THg) concentration for the low Hg treatment was 2.5 μg/g, dry weight (dw) (2.75% MMHg). This concentration corresponds to approximately twice the highest measured THg concentrations in the guts of larval leopard frogs (*Rana sphenocepala*) from ephemeral wetlands in southeastern US receiving Hg solely from atmospheric deposition.[19] The target THg concentration for the high Hg treatment was 10 μg/g, dw (1.05% MMHg). This corresponds to the upper limits of Hg concentrations found in periphyton at the Hg-contaminated South River (K. R. Tom, Master's thesis, The College of William and Mary, VA, 2008). Percent MMHg in periphyton is generally low (<15%) (e.g., refs 18, 20, and 21). Thus, we used the equations in Unrine et al.[20] to determine the MMHg concentrations in this study. See the Materials and Methods in the Supporting Information for details of the experimental diet formulation.

To determine which eggs to allocate to our experimental groups from the 27 collected clutches, we used the known strong correlation between female blood THg concentrations and those of eggs.[13] The reference and maternally Hg exposed (hereafter: Hg-exposed) groups included hatchlings from females with blood THg concentrations <250 and >1000 ng/g, wet weight (ww), and originated from five and six combined clutches, respectively. On April 28, 2009, at ~4 days posthatch, we mixed free swimming hatchlings with normal morphology across clutches within each maternal Hg group (reference and Hg-exposed) to homogenize genetic variation. We then randomly allocated the hatchlings among three diet treatments (control, low Hg, and high Hg; n = 25/treatment). We placed one larva into each of the 150 4-L polypropylene aquaria containing 3 L of dechlorinated tap water. We individually weighed larvae every 9 days to document growth and increase food rations accordingly. We fed larvae 6% of their body weight per day (wet weight basis), and raised them under a 12 h light/12 h dark photoperiod. At the beginning of the experiment, air temperature was 18 °C and was increased weekly in 0.5 °C increments until 20 °C was reached and maintained for the remainder of the study. Every third day, we replaced 50% of the water in each aquarium. At this time, we siphoned out accumulated feces and uneaten food and provided fresh food.

After the larvae had fed on the experimental diets for 26−28 days [Gosner stages (GS) 26−30], we conducted a swimming performance test to determine if speed or responsiveness to stimuli differed among the six treatment combinations using methods similar to Hopkins et al.[22] We tested 50 larvae per day for three consecutive days (May 23−25, 2009), ensuring a representative daily sample of each treatment combination. See Materials and Methods in the Supporting Information for details of the larval swimming performance experiment.

We inspected larvae daily for developmental stage and mortality. As larvae neared metamorphosis, they were checked at 12 h intervals for front limb emergence (GS 42), completion of tail resorption (GS 46), or mortality. At the time of front limb emergence, larvae were removed from aquaria, weighed, measured, and individually placed in 500 mL cups with ~20 mL water and a clean unbleached paper towel to allow them to climb out of the water following complete tail resorption. The first larva reached metamorphic climax on June 15, 2009, and the last completed metamorphosis on July 22, 2009. In addition to quantifying the proportion of individuals that successfully completed metamorphosis in each treatment, we also measured mass and size at metamorphosis (GS 46) and the amount of time to reach metamorphic climax (GS 42) and complete metamorphosis (GS 46). Toads surviving through metamorphosis were humanely euthanized with buffered tricaine methane sulfonate (MS-222) 24 h after completion of metamorphosis and then frozen for analyses of Hg in tissue.

Mercury Analyses. In a previous study,[13] percent MMHg of the total Hg burden in female toad blood and eggs from the same study site was 71.4 ± 2.8% and 47.8 ± 3.3% (mean ±1 standard error hereafter), respectively. Thus, in the present study, we analyzed only THg for these tissues. We homogenized whole blood from each female toad and report THg concentrations of blood on a wet weight basis. We lyophilized and homogenized eggs and report THg concentrations of eggs on a dry weight basis. Percent moisture of eggs was 95.4 ± 0.2%. We analyzed subsamples (~20 mg) for THg content by combustion−amalgamation−cold vapor atomic absorption spectrophotometry (Direct Mercury Analyzer 80, Milestone, Monroe, CT). See Materials and Methods in the Supporting Information for method details and quality assurance.

We measured both Hg(II) and MMHg in the experimental diet (n = 3 samples/diet level) and metamorphs (n = 3 composite samples/treatment). We lyophilized and homogenized the samples and report Hg concentrations on a dry weight basis. Percent moisture of the diet and metamorphs was 58.6 ± 0.4% and 90.4 ± 0.3%, respectively. Samples were then analyzed by Quicksilver Scientific (Lafayette, CO). See Materials and Methods in the Supporting Information for method details and quality assurance.

Statistical Analyses. We used nonparametric Mann−Whitney U tests to compare blood and egg THg concentrations between reference and Hg-exposed females, because data were non-normally distributed. We used a multivariate analysis of variance (MANOVA) to test for effects of diet, maternal Hg exposure, and their interaction on MMHg and Hg(II) concentrations in metamorphs.

We used a MANOVA to test the effects of diet, maternal Hg exposure, and their interaction on the mass (g) of tadpoles at GS 42 and the time (d) to reach GS 42 (larval duration). We log transformed larval duration to normalize the data to meet assumptions for analyses of variance. Because several animals died during metamorphosis before reaching GS 46, we used a separate MANOVA to test the effects of diet, maternal Hg exposure, and their interaction on the mass of tadpoles at GS 46 and days required to complete tail resorption (GS 42−46). We inverse transformed mass at GS 46 and log transformed days for tail resorption to normalize data and meet statistical assumptions.

Initial iterations of a repeated measures analysis of covariance (ANCOVA) model to test for differences in larval swimming performance among treatments revealed that larvae swam similarly in all three laps and the main effects did not differ over time (swimming speed, $P \geq 0.094$ for all; responsiveness, $P \geq 0.271$ for all). Thus, we used ANCOVA to test for effects of maternal and trophic exposure and their interaction on the average of the three laps for the performance end points of time and responsiveness, using developmental stage and mass as covariates.

We used animals fed control diet from reference mothers to determine expected survivorship under optimal conditions. We subsequently examined both metamorphic success (i.e., survivorship to GS 46) and survivorship during metamorphic climax (GS 42−46) in our five experimental crosses using χ^2 tests of independence to determine whether the observed number of animals surviving in an experimental cross differed from that expected under optimal conditions. Lastly, we used logistic regression to examine the relationship between survival and mass of larvae (GS 42) from Hg-exposed and reference mothers.

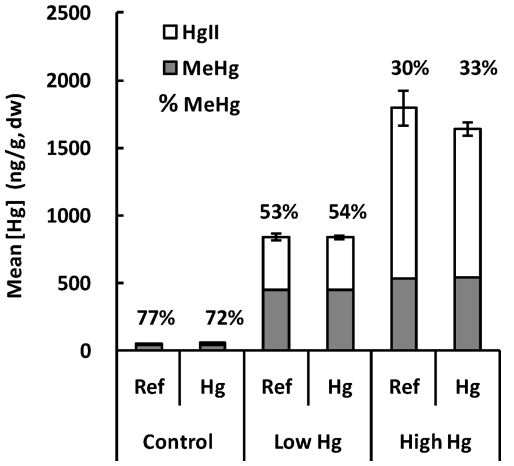

Figure 1. Whole body mercury (Hg) concentrations [ng/g, dry weight; inorganic mercury (HgII) and methylmercury (MMHg)] and the percent of the total Hg that is MMHg (% MMHg) in American toad metamorphs from the 2 × 3 factorial design of maternal Hg exposure [reference (ref) and Hg-exposed (Hg)] and the three diet treatments (control, low Hg, and high Hg). Error bars represent the standard error of the mean.

RESULTS

Mercury Concentrations. We allocated eggs to maternal experimental groups based on the THg concentrations in female blood, and we found a significant difference in the blood THg concentrations of reference females (160 ± 18.6 ng/g, ww) compared to Hg-exposed females (2250 ± 490 ng/g, ww; Z = −2.74, P = 0.006). We later analyzed eggs from each clutch for THg concentrations, and the expected significant difference was confirmed between clutches from reference females (20.6 ± 1.3 ng/g, dw) and Hg-exposed females (149 ± 17.9 ng/g, dw; Z = −2.71, P = 0.006). Total Hg concentrations in diets (dw) were 0.010 ± 0.001 μg/g (56.7 ± 5.5% MMHg), 2.50 ± 0.06 μg/g (3.19 ± 0.03% MMHg), and 10.1 ± 2.27 μg/g (1.05 ± 0.01% MMHg) for the control, low Hg, and high Hg treatments. We found a significant effect of diet on tissue concentrations of Hg in metamorphic toads in our overall MANOVA (Figure 1; diet, $F_{4,24}$ = 184, P < 0.001). However, there was no effect of maternal Hg exposure on Hg tissue concentrations of metamorphs (maternal Hg exposure, $F_{2,11}$ = 1.39, P = 0.29; interaction,

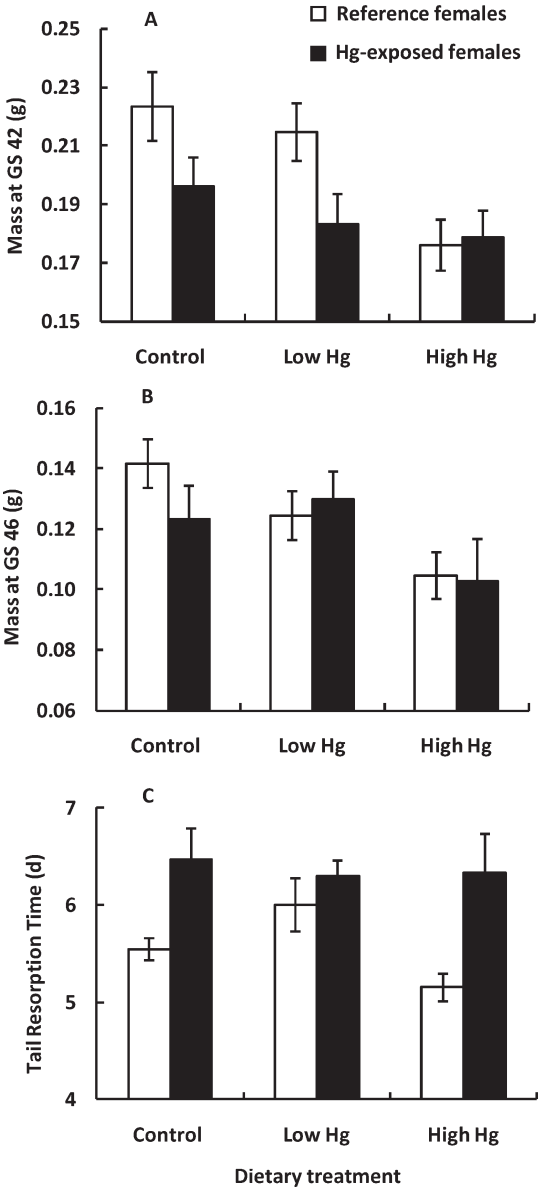

Figure 2. Components of the multivariate analysis of variance for American toad larvae from the 2 × 3 factorial design of maternal mercury (Hg) exposure (reference females and Hg-exposed females) and the three diet treatments (control, low Hg, and high Hg). (A) Mass at Gosner stage (GS) 42, front limb emergence. (B) Mass at GS 46, completion of metamorphosis. (C) Tail resorption time (GS 42−46). Error bars represent the standard error of the mean.

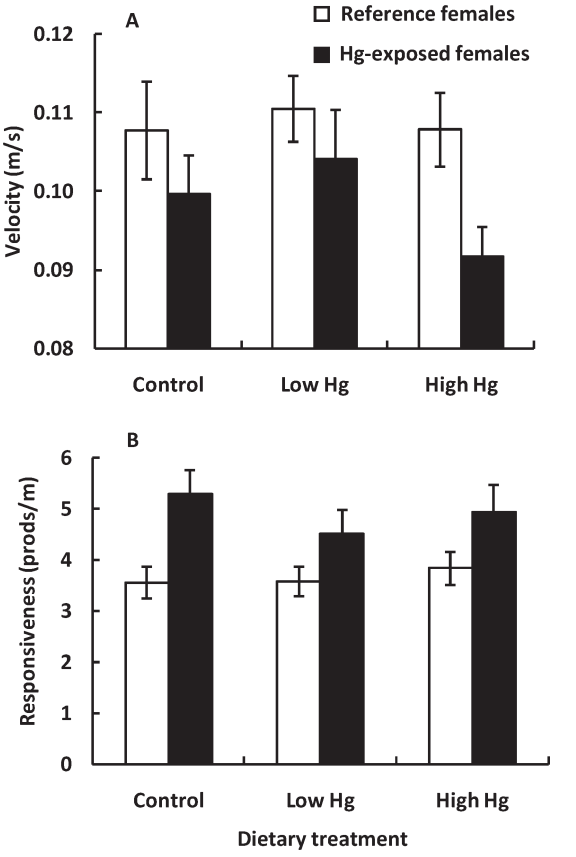

Figure 3. Larval swimming performance of American toads from the 2 × 3 factorial design of maternal mercury (Hg) exposure (reference females and Hg-exposed females) and the three diet treatments (control, low Hg, and high Hg). (A) velocity (m/s) and (B) responsiveness (prods/m). Error bars represent the standard error of the mean.

$F_{4,24}$ = 1.08, P = 0.39). Component ANOVAs revealed that diet had a significant effect on accumulation of both MMHg (Figure 1; $F_{2,12}$ = 494, P < 0.001) and Hg(II) (Figure 1; $F_{2,12}$ = 318, P < 0.001) in metamorphic toads. As expected, mean percent MMHg decreased in metamorphic toads with increasing diet concentration (Figure 1). Posthoc Tukey's tests revealed that Hg tissue concentrations in metamorphs differed among all three diets for both MMHg and Hg(II) (P < 0.001).

Biological Responses to Maternal and Dietary Mercury. Survival was high in larvae until the onset of metamorphic climax (n = 20, 23, 24 for larvae from reference mothers fed control, low Hg, and high Hg diet, respectively, and n = 23, 24, 24 for larvae from Hg-exposed mothers fed control, low Hg, and high Hg diet, respectively). Both dietary and maternal Hg exposure significantly affected growth and development of larvae until the onset of metamorphic climax, but there was no interaction effect in the overall MANOVA (GS 42; diet, $F_{4,264}$ = 2.97, P = 0.02; maternal Hg exposure, $F_{2,131}$ = 3.70, P = 0.027; interaction, $F_{4,264}$ = 1.56, P = 0.19). Component ANOVAs revealed that diet had a significant effect on mass at GS 42 (Figure 2a; $F_{2,132}$ = 5.77, P = 0.004) but not on the duration of larval period (Supporting Information, Figure S1; $F_{2,132}$ = 0.23, P = 0.79). Posthoc Tukey's tests showed that mass at GS 42 differed significantly only between the control diet and high Hg diet (P = 0.004). On average, animals fed high Hg diet were 16% smaller than those fed control diet. Maternal Hg exposure also had a significant effect on mass at GS 42 ($F_{1,132}$ = 5.61, P = 0.019) but not on the duration of the larval period ($F_{1,132}$ = 1.34, P = 0.25). On average, animals from contaminated mothers were 10% smaller at GS 42 than those from reference mothers.

Survival decreased during metamorphic climax (n = 12, 11, 13 metamorphs from reference mothers fed control, low Hg, and high Hg diet, respectively, and n = 14, 10, 6 metamorphs from Hg-exposed mothers fed control, low Hg, and high Hg diet, respectively). Both dietary and maternal Hg affected toads at the completion of metamorphosis, but there was no interaction effect in the overall MANOVA (GS 46; diet, $F_{4,120}$ = 2.56, P = 0.042; maternal Hg exposure, $F_{2,59}$ = 8.28, P = 0.001; interaction, $F_{4,120}$ = 1.21, P = 0.31). Component ANOVAs revealed that

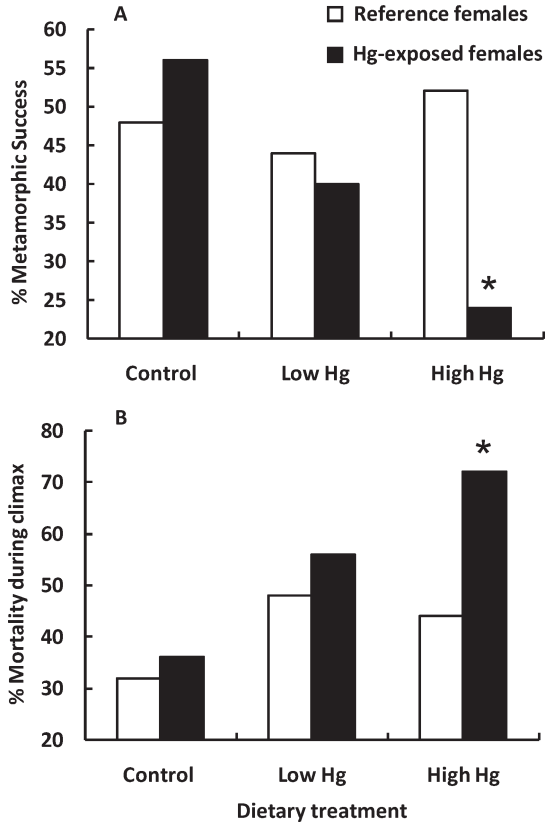

Figure 4. (A) Percent of American toads successfully completing metamorphosis and (B) the percent of mortalities during metamorphic climax from the 2 × 3 factorial design of maternal mercury (Hg) exposure (reference females and Hg-exposed females) and the three diet treatments (control, low Hg, and high Hg). Asterisks (*) denote a significant difference from the reference females fed control diet.

diet had a significant effect on mass at GS 46 (Figure 2b; $F_{2,60}$ = 4.27, P = 0.018) but not on days to complete tail resorption (Figure 2c; $F_{2,60}$ = 1.51, P = 0.23). Posthoc Tukey's tests showed that mass at GS 46 differed significantly only between the control diet and high Hg diet (P = 0.016), with animals fed the high Hg diet being 21% smaller at GS 46 than those fed the control diet. In contrast, maternal Hg exposure had a significant effect on days for tail resorption (Figure 2c; $F_{1,60}$ = 15.7, P < 0.001) but no effect on mass at GS 46 (Figure 2b; $F_{1,60}$ = 0.38, P = 0.54). Larvae from contaminated mothers took 14% longer to fully resorb their tails than did those from reference mothers.

We found maternal Hg exposure, but not dietary exposure, affected average swimming speed of larvae, and we found no evidence of an interaction between these two modes of exposure (Figure 3a; maternal Hg exposure, $F_{1,133}$ = 5.86, P = 0.017; diet, $F_{2,133}$ = 1.61, P = 0.203; interaction, $F_{2,133}$ = 0.769, P = 0.465). Larvae from contaminated mothers took 11% longer to traverse 1 m than did those from reference mothers, even after correcting for size and developmental stage. Likewise, maternal Hg exposure, but not dietary exposure, affected average larval responsiveness, and we found no evidence of an interaction between modes of exposure (Figure 3b; maternal Hg exposure, $F_{1,133}$ = 13.9, P < 0.001; diet, $F_{2,133}$ = 0.432, P = 0.650; interaction, $F_{2,133}$ = 0.835, P = 0.436). Larvae from contaminated mothers had to be prompted to swim 34% more often than those from reference mothers.

Whereas both maternal and dietary Hg exposure independently produced sublethal effects, the combination of the two exposure routes was lethal. The combination of maternal Hg exposure and high Hg diet led to a 50% reduction in metamorphic success (survivorship to GS 46; Figure 4a; χ^2 = 5.77, P = 0.016) compared to larvae from reference mothers that were raised on control diet as an optimal scenario (all other experimental crosses, χ^2 < 0.7, P > 0.4). Importantly, larval survival was high in all treatments (ranging from 80 to 96%) until the onset of metamorphic climax. However, during the critical period of metamorphic climax (from GS 42 to GS 46), the combination of maternal Hg exposure and high Hg diet led to 125% greater mortality (Figure 4b; χ^2 = 12.3, P < 0.001) compared to larvae from reference mothers that were raised on control diet (all other experimental crosses, χ^2 < 0.3, P > 0.06). Lastly, mortality of small individuals was higher than large individuals from Hg-exposed mothers (P = 0.047, β = 6.87) but not from reference mothers (P = 0.20) during metamorphic climax.

DISCUSSION

Depending on their life history, organisms may be at risk of one or more routes of contaminant exposure during early development. Our study is one of the few to investigate the individual and interactive effects of maternally and environmentally derived contaminants on early vertebrate development. Mercury-exposed female American toads transferred ~14 times more Hg to their eggs than reference females, and larvae were efficient at accumulating Hg from their diet regardless of maternal origin. The whole-body THg concentrations in metamorphs at the completion of metamorphosis were ~800 and ~1700 ng/g for the low and high diets, respectively, which is similar to concentrations found in free-ranging American toad larvae from contaminated portions of our study site (~2100 ng/g at GS 28−32).[15] We found both maternal and dietary Hg exposure independently produced negative, but different, sublethal effects on larval development. Most importantly, the latent effects of maternal exposure to Hg combined with high dietary Hg exposure later in ontogeny had a lethal effect in larvae.

The transfer of Hg from mother to offspring and resulting effects have been well documented in several species,[11] but less is known about effects that manifest in offspring later in life. We found maternal exposure negatively affected growth, duration of metamorphic climax, and swimming performance in American toad larvae. Larvae from Hg-exposed mothers were smaller than larvae from reference mothers at the onset of metamorphic climax. However, maternal Hg exposure did not affect mass at the completion of metamorphosis due to the high mortality of small individuals from Hg-exposed mothers, but not from reference mothers, during metamorphic climax. In addition, maternal Hg exposure increased the duration of metamorphic climax, a period of increased vulnerability for immunological,[23] energetic,[24] and ecological[25] reasons, by ~1 day compared to larvae from reference mothers, which may increase mortality risk in natural settings. Unrine et al.[26] also found increased tail resorption time in leopard frog larvae fed Hg diets and suggested that Hg may inhibit the thyroid axis, which is known to play an important role in metamorphic climax.[27] Lastly, swimming performance was negatively affected by maternal Hg exposure, which could impair foraging efficiency and increase predation risk in nature. Mercury is known to affect behavior and performance in fish,[28] but only one study has investigated the effects of

maternal transfer of Hg on behavior.[29] Our study is one of the first to investigate the effects of Hg on performance in amphibians (but see ref 30). Here, metamorphs from Hg-exposed mothers did not have elevated tissue concentrations due to dilution of maternally transferred Hg during growth. In addition, effects of maternal Hg exposure on performance were independent of larval body size and developmental stage. Because Hg is a neurotoxicant and suspected endocrine disruptor,[9,10] the effects we observed were likely caused by physiological or neurological changes during embryonic development but were detectable during later larval development.

Dietary exposure alone negatively affected larval size at the onset and completion of metamorphic climax, but did not affect any other measured traits. Individuals fed the high Hg diet were smaller than those fed the control diet at GS 42 and 46, respectively. Reduced amphibian size at metamorphosis is linked to adverse effects on fitness-related traits, including survival, body size/age at first reproduction, and fecundity (e.g., ref 31). Because of the strong influence of size, an important next step will be to determine whether small body size at metamorphosis in Hg-exposed individuals affects postmetamorphic growth and survival in the terrestrial environment. Interestingly, in a similar study investigating the effects of dietary Hg on leopard frog larvae,[18,26] adverse effects on development and decreased survival were observed at THg concentrations (236 and 412 ng/g) much lower than the concentrations where effects were observed in our study. These differences may be due to differences in sensitivity or length of larval period between species.

We demonstrated that the sublethal, latent effects of maternal Hg exposure interacted with the sublethal effects of high dietary Hg exposure to reduce survival in amphibian larvae. The combination of maternal and dietary exposure to environmentally relevant Hg concentrations had lethal consequences; larvae experienced a 125% increase in mortality during metamorphic climax and a 50% decrease in overall metamorphic success compared to larvae from reference mothers fed the control diet. Until the onset of metamorphic climax, larval survival in all treatments was high, but metamorphic climax is a critical and sensitive stage in the life history of amphibians. Our findings support the hypothesis that metamorphic climax may be a period that is sensitive to the remobilization of Hg from tissues into circulation during tail resorption[26] due to the extensive morphological, physiological, and behavioral changes that occur as animals prepare for the transition to terrestrial life. Further, timing of mortality can have important implications for amphibian population dynamics. In some cases, mortality of eggs or larvae can have slight, or even positive, effects on amphibian populations because they release surviving larvae from the detrimental effects of intraspecific competition (e.g., ref 32). In our study, mortality occurred during metamorphic climax, suggesting larvae in the natural environment may suffer from both density-dependent effects of competition and density-independent effects of contaminant exposure.

Although immediate effects of the maternal transfer of contaminants on early development are well studied, there are fewer investigations into potential latent and long-term effects and even fewer on interactions with environmental exposures through ontogeny. We found the majority of the significant sublethal effects in amphibians resulted from maternal Hg exposure, and the latency of these maternal effects is not surprising due to the effects of transferred contaminants on key organizational events that occur early in ontogeny.[1] However, these negative maternal effects are of great importance to the field of ecotoxicology because studies often examine the effects of environmental contaminants using organisms from reference sites, ignoring maternal contaminant exposure. Our findings emphasize the importance of investigating environmentally relevant routes of contaminant exposure over multiple early life stages because studies based on single routes of exposure or single life stages may underestimate the severity of adverse effects, potentially having widespread implications for both wildlife and human health.[33] Futhermore, future studies investigating a greater number of intermediate concentrations of maternal and dietary Hg exposures in amphibians could help to identify the lowest exposure concentrations causing interactive effects and potentially aid in identifying the route of toxicity. In our study, amphibian larvae were raised individually, but further studies are necessary to determine whether embryonic exposure to maternally derived Hg and/or larval dietary exposure have different effects in more complex environments, such as in the presence of competitors or predators. Additionally, investigating how different contaminant exposure routes impact the terrestrial juvenile stage will ultimately aid in predicting their effects on amphibian population dynamics.

■ ASSOCIATED CONTENT

Supporting Information. Further details are provided of the experimental diet formulation, swimming performance experiment, and methods and quality assurance for THg and MMHg analyses; Figure S1 depicts larval duration of American toads from all treatments. This information is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: 540-231-7292; fax: 540-231-7580; e-mail hopkinsw@vt.edu.

Present Addresses

§Department of Wildlife, Fish, and Conservation Biology, University of California, Davis, One Shields Ave., Davis, CA 95616.

■ ACKNOWLEDGMENT

We thank the landowners along the South River and the Waynesboro Parks and Recreation Department for access to sampling locations; J. Schmerfeld, D. Cristol, K. Carlson-Drexler, A. Condon, M. Howie, C. Ramos, J. Burke, J. Callihan, S. DuRant, M.K. McCaleb, J. McPherson, and H. Wada for their support and field and/or laboratory assistance; and C. Shade of Quicksilver Scientific for MMHg analysis. The manuscript was improved by the comments of J. Willson. Collection of animals was in conformance with appropriate permits, and sample methods were in compliance with Virginia Polytechnic and State University's animal care and use protocols. Financial support was provided by E. I. DuPont de Nemours, the National Science Foundation (NSF # IOB-0615361), and a U.S. EPA STAR Graduate Fellowship (FP-9170040-1) to C.M.B. The EPA has not officially endorsed this publication and the views expressed herein may not reflect the views of the EPA. Research was completed with oversight from the South River Science Team,

which is a collaboration of state and federal agencies, academic institutions, and environmental interests.

REFERENCES

(1) Di Giulio, R. T.; Tillitt, D. E. *Reproductive and Developmental Effects of Contaminants in Oviparous Vertebrates*. SETAC Press: Pensacola, FL, 1999.

(2) Guillette, L. J.; Crain, D. A.; Rooney, A. A.; Pickford, D. B. Organization versus activation—The role of endocrine-disrupting contaminants (EDCS) during embryonic development in wildlife. *Environ. Health Perspect.* **1995**, *103*, 157–164.

(3) Eisenreich, K. M.; Kelly, S. M.; Rowe, C. L. Latent mortality of juvenile snapping turtles from the upper Hudson River, New York, exposed maternally and via the diet to polychlorinated biphenyls (PCBs). *Environ. Sci. Technol.* **2009**, *43* (15), 6052–6057.

(4) Yates, M. A.; Fuller, M. R.; Henny, C. J.; Seegar, W. S.; Garcia, J. Wintering area DDE source to migratory white-faced ibis revealed by satellite telemetry and prey sampling. *Ecotoxicology* **2010**, *19* (1), 153–162.

(5) Nye, J. A.; Davis, D. D.; Miller, T. J. The effect of maternal exposure to contaminated sediment on the growth and condition of larval *Fundulus heteroclitus*. *Aquat. Toxicol.* **2007**, *82* (4), 242–250.

(6) Bernardo, J. Maternal effects in animal ecology. *Am. Zool.* **1996**, *36* (2), 83–105.

(7) Fitzgerald, W. F.; Engstrom, D. R.; Mason, R. P.; Nater, E. A. The case for atmospheric mercury contamination in remote areas. *Environ. Sci. Technol.* **1998**, *32* (1), 1–7.

(8) Mason, R. P.; Reinfelder, J. R.; Morel, F. M. M. Uptake, toxicity, and trophic transfer of mercury in a coastal diatom. *Environ. Sci. Technol.* **1996**, *30* (6), 1835–1845.

(9) Weiner, J. G.; Spry, D. J. Toxicological significance of mercury in freshwater fish. In *Environmental Contaminants in Wildlife: Interpreting Tissue Concentrations*; Beyer, W. N., Heinz, G. H., Redmon-Norwood, A. W., Eds.; Lewis Publishers: Boca Raton, FL, 1996; pp 297−340.

(10) Scheuhammer, A. M. Effects of acidification on the availability of toxic metals and calcium to wild birds and mammals. *Environ. Pollut.* **1991**, *71* (2−4), 329–375.

(11) Scheuhammer, A. M.; Meyer, M. W.; Sandheinrich, M. B.; Murray, M. W. Effects of environmental methylmercury on the health of wild birds, mammals, and fish. *Ambio* **2007**, *36* (1), 12–18.

(12) Unrine, J. M.; Hopkins, W. A.; Romanek, C. S.; Jackson, B. P. Bioaccumulation of trace elements in omnivorous amphibian larvae: Implications for amphibian health and contaminant transport. *Environ. Pollut.* **2007**, *149*, 182–192.

(13) Bergeron, C. M.; Bodinof, C. M.; Unrine, J. M.; Hopkins, W. A. Bioaccumulation and maternal transfer of mercury and selenium in amphibians. *Environ. Toxicol. Chem.* **2010**, *29* (4), 989–997.

(14) Bergeron, C. M.; Hopkins, W. A.; Bodinof, C. M.; Budishack, S. A.; Wada, H.; Unrine, J. M. Counterbalancing effects of maternal mercury exposure during different stages of ontogeny. *Sci Total Environ* Submitted.

(15) Bergeron, C. M.; Bodinof, C. M.; Unrine, J. M.; Hopkins, W. A. Mercury accumulation along a contamination gradient and nondestructive indices of bioaccumulation in amphibians. *Environ. Toxicol. Chem.* **2010**, *29* (4), 980–988.

(16) Forester, D. C.; Snodgrass, J. W.; Marsalek, K.; Lanham, Z. Post-breeding dispersal and summer home range of female American toads (*Bufo americanus*). *Northeast. Nat.* **2006**, *13* (1), 59–72.

(17) URS. *Evaluation for Removal of Organochlorine Pesticides, Polycyclic Aromatic Hydrocarbons, and Trace Metals Analyses in the Phase I System Characterization, South River, VA. Appendix F, Phase I, Year 1 Progress Report: Ecological Study of the South River and a Segment of the South Fork Shenandoah River, Virginia*; Contract No.: 18985057.70030, 2007.

(18) Unrine, J. M.; Jagoe, C. H. Dietary mercury exposure and bioaccumulation in southern leopard frog (*Rana sphenocephala*) larvae. *Environ. Toxicol. Chem.* **2004**, *23* (12), 2956–2963.

(19) Unrine, J. M.; Jagoe, C. H.; Brinton, A. C.; Brant, H. A.; Garvin, N. T. Dietary mercury exposure and bioaccumulation in amphibian larvae inhabiting Carolina Bay wetlands. *Environ. Pollut.* **2005**, *135* (2), 245–253.

(20) Bell, A. H.; Scudder, B. C. Mercury accumulation in periphyton of eight river ecosystems. *J. Am. Water Resour. Assoc.* **2007**, *43* (4), 957–968.

(21) Tom, K. R.; Newman, M. C.; Schmerfeld, J. Modeling mercury biomagnification (South River, Virginia, USA) to inform river management decision making. *Environ. Toxicol. Chem.* **2010**, *29* (4), 1013–1020.

(22) Hopkins, W. A.; Congdon, J.; Ray, J. K. Incidence and impact of axial malformations in larval bullfrogs (*Rana catesbeiana*) developing in sites polluted by a coal-burning power plant. *Environ. Toxicol. Chem.* **2000**, *19* (4), 862–868.

(23) Todd, B. D. Parasites lost? An overlooked hypothesis for the evolution of alternative reproductive strategies in amphibians. *Am. Nat.* **2007**, *170* (5), 793–799.

(24) Beck, C. W.; Congdon, J. D. Energetics of metamorphic climax in the southern toad (*Bufo terrestris*). *Oecologia* **2003**, *137*, 344–351.

(25) Arnold, S. J.; Wassersug, R. J. Differential predation on metamorphic anurans by garter snakes (*Thamnophis*)—Social-behavior as a possible defense. *Ecology* **1978**, *59* (5), 1014–1022.

(26) Unrine, J. M.; Jagoe, C. H.; Hopkins, W. A.; Brant, H. A. Adverse effects of ecologically relevant dietary mercury exposure in southern leopard frog (*Rana sphenocephala*) larvae. *Environ. Toxicol. Chem.* **2004**, *23* (12), 2964–2970.

(27) Denver, R. J.; Boorse, G. C.; Glennemeier, K. A. Endocrinology of complex life cycels: Amphibians. In *Hormones, Brain and Behavior*, Pfaff, D. W., Arnold, A. P., Etgen, A. M., Fahrbach, S. E., Rubin, R. T., Eds.; Academic Press, Inc.: San Diego, CA, 2002; pp 469−513.

(28) Weis, J. S. Reproductive, developmental, and neurobehavioral effects of methylmercury in fishes. *J. Environ. Sci. Health Part C: Environ. Carcinog. Ecotoxicol. Rev.* **2009**, *27* (4), 212–225.

(29) Alvarez, M. D.; Murphy, C. A.; Rose, K. A.; McCarthy, I. D.; Fuiman, L. A. Maternal body burdens of methylmercury impair survival skills of offspring in Atlantic croaker (*Micropogonias undulatus*). *Aquat. Toxicol.* **2006**, *80* (4), 329–337.

(30) Burke, J. N.; Bergeron, C. M.; Todd, B. D.; Hopkins, W. A. Effects of mercury on behavior and performance of nothern two-lined salamanders (*Eurycea bislineata*). *Environ. Pollut.* **2010**, *158*, 3546–3551.

(31) Semlitsch, R. D.; Scott, D. E.; Pechmann, J. H. K. Time and size at metamorphosis related to adult fitness in *Ambystoma talpoideum*. *Ecology* **1988**, *69* (1), 184–192.

(32) Vonesh, J. R.; De la Cruz, O. Complex life cycles and density dependence: Assessing the contribution of egg mortality to amphibian declines. *Oecologia* **2002**, *133* (3), 325–333.

(33) Heindel, J. J. Role of exposure to environmental chemicals in the developmental basis of disease and dysfunction. *Reprod. Toxicol.* **2007**, *23* (3), 257–259.