# Individual and Interactive Effects of Maternally- and Trophically-Derived Mercury on Development of Amphibian Larvae

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***Introduction:***

The transfer of contaminants from a female to her offspring may be an important mechanism of impaired reproductive success in amphibians. While maternal transfer of contaminants has been studied in many fish and wildlife species, only two studies have documented maternal transfer of contaminants in wild populations of amphibians (Hopkins et al. 2006; Kadokami et al. 2004). In 2007, we discovered that American toads (*Bufo americanus*) inhabiting the floodplain of the South River maternally transfer Hg to their eggs. We also determined that the toad larvae developing in ephemeral breeding pools along the South River accumulate extremely high concentrations of Hg in their tissues (up to 3,923 ng/g, dry wt). High accumulation of Hg in larvae is consistent with their feeding habits (benthic omnivores that ingest detritus and sediment) and the likelihood that their breeding sites are subject to high methylation rates. Thus, toads developing in wetlands in the South River floodplain may encounter both maternally-derived Hg as embryos (depending on the exposure history of the mother) and prolonged dietary exposure to Hg as larvae (depending on breeding sites selected by breeding adults).

***2008 Research Highlights***

In 2008 we tested the following hypotheses in order to clarify the ultimate individual and interactive impacts of Hg from maternal and dietary sources on embryonic and larval development, as well as female reproductive success:

- **H1.** Maternal transfer of Hg negatively impacts reproductive success by affecting hatching success and morphological development of embryos.
- **H2.** Embryos that successfully hatch from Hg-exposed females experience latent effects of maternal transfer later in larval development that decrease the number and size of individuals recruited to the local population.
- **H3.** Embryonic exposure to 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.

**H1.** Based on two years of data, we found that female toads transferred approximately 5% of their total Hg [THg] body burden to their eggs in both the reference and contaminated site. THg concentrations (dry wt) in eggs ranged from 10.6 to 46.2 ng/g in the reference site and from 9.2

to 359.0 ng/g in the contaminated site. In 2007 and 2008, there were significant negative relationships between THg concentrations in eggs and hatching success, female whole body THg concentration and hatching success, and THg concentrations in female blood and hatching success. Contrary to our predictions, malformation frequencies decreased with increasing Hg concentration in the eggs. Combined with the hatching success results, this finding suggests that if there were any teratogenic effects due to Hg, the afflicted embryos were incapable of completing early development and failed to hatch. Thus, fewer hatchlings were produced at high Hg concentrations, but successful hatchlings were less likely to be malformed than those exposed to low Hg concentrations. Most importantly, we calculated an overall index of hatchling viability for each clutch by combining hatching success with malformation frequency according to the methods of Hopkins et al. (2006). We found a significant negative relationship between Hg concentration in eggs and the percentage of eggs in a clutch that produced viable hatchlings. Although correlative, this is the most compelling evidence in the amphibian literature that a female can maternally transfer a contaminant and influence the hatching success of her embryos.

**H2.** Early embryonic exposure to contaminants can have latent effects that are not detectable for weeks-to-months after hatching. Such latent effects have been observed with pesticides (Budischak, 2007; Budischak et al., 2008; Rohr and Palmer, 2005), but have not been examined with Hg or other metals. Hatchlings with normal morphology from 6 females from the reference site (wet wt; reference < 200 ng/g in female blood) and 3 females from the contaminated site (1,000-3000 ng/g) were transferred to 12 outdoor pond mesocosms (2 Hg contamination levels x 6 replicates x 100 hatchlings per replicate = 1200 hatchlings) within 4 days of hatching. Mesocosms were constructed out of 1000L polyethylene cattle watering tanks containing water, leaf litter, and plankton communities. Because we were only interested in latent effects of maternal exposure, no Hg was added to any mesocosms. Mesocosms were monitored daily and metamorphosing individuals were moved into the lab. This approach allowed determination of latent effects of maternal transfer on larval growth and survival to metamorphosis, which can influence the number and quality of individuals recruited to the adult population (Beebee et al., 1996; Berven, 1990). Overall, maternal exposure to Hg had no significant latent effects on the larval development of individuals that hatched with normal morphology. The percentage of individuals that successfully metamorphosed was high in both of our treatment groups (reference: 45.3 ± 3.8%, Hg: 54.5 ± 2.9%), suggesting that the most severe effects of maternally-derived Hg are confined to early embryonic development and do not have detectable effects on larvae thereafter. The fact that body size, body condition, time to metamorphosis, and a variety of other larval and metamorphic traits did not differ between treatments further supports this conclusion. In addition, THg concentrations (dry wt) in metamorphs did not differ between treatments ($p = 0.776$; reference: 42.1 ± 2.2 ng/g; Hg: 43.2 ± 2.4 ng/g).

**H3.** Based on our field results from 2007, it is clear that developing toads can face the interactive insults of embryonic (maternally-derived) and larval (trophically-derived) exposure to Hg. We used a 2X2 factorial feeding study to determine if trophic exposure to Hg during the larval period has an interactive effect on development of tadpoles from Hg-exposed females. Egg masses were collected from breeding pairs from 2 sites representing the full range of Hg concentrations in female blood (wet wt; reference < 200 ng/g, high = 1,000-3000 ng/g). After hatching, hatchlings with normal morphology from each clutch were mixed to homogenize genetic variation within each Hg level. Individual tadpoles were then randomly selected and allocated among four treatments containing 36 tadpoles per treatment:

**1)** reference tadpoles ? control diet,
**2)** reference tadpoles? Hg diet,
**3)** High Hg tadpoles ? control diet,
**4)** High Hg tadpoles ? Hg diet.

Husbandry and experimental Hg diet provisioning were similar to methods we have already developed (Unrine et al., 2004). The control diet contained 4.96 ng/g Hg dry wt, and the Hg-spiked diet contained 2,571 ng/g Hg dry wt. The latter concentration is well within the range of concentrations observed in periphyton in the South River (up to 26,000 ng/g, dry wt; Newman, unpublished). Ratios of MeHg:Hg(II) were calculated using the regression equations in Unrine and Jagoe (2004). The Hg-spiked diet was only 4% MeHg which is consistent with Mike Newman's data for periphyton from the South River. Tadpoles were fed ~ 6% of their body weight daily and growth rates and survival were monitored closely throughout the study. Tadpoles in all treatment groups exhibited positive growth rates, on average increasing in body size by 30-fold over the first 10 weeks of the study. Survival was high in all treatments to Gosner stage 42 (front limb emergence), ranging from 69 – 89 % among treatments. The first tadpoles began metamorphosing during the $11^{th}$ week of the experiment. Metamorphs resulting from the Hg dietary treatments accumulated 1,072 ± 30.8 ng/g THg dry wt compared to only 59.0 ± 3.1 ng/g in tadpoles fed the control diet. Maternal exposure to Hg did not appear to influence accumulation of Hg during the larval period. Unfortunately, we observed unacceptably high mortality during metamorphic climax (from front limb emergence to tail resorption), ranging from 61 – 78%. These values are approximately 3X higher than what we expect to see in healthy tadpoles undergoing metamorphosis, and therefore prevented us from interpreting the remaining results of the study. To address these issues, we ran three additional feeding experiments this summer. These side projects suggested that the problems during metamorphic climax were related to the composition of the diet, rather than other husbandry issues. This winter, we will reformulate our diet to include a mix of algal resources (the one notable component missing from our current diet) working with an expert in aquaculture nutrition. By successfully completing this experiment in 2009, we will determine the relative importance of maternally- and trophically-derived Hg on larval development that may influence juvenile recruitment from aquatic habitats. In addition, the study will provide information regarding potential source-sink dynamics that occur in the South River for a wide variety of animals (e.g., amphibians and birds) that move in and out of the site during the reproductive season.

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