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Using Non-destructive Techniques to Measure Mercury (Hg) Concentrations in Gravid Blanding's Turtles (*Emydoidea blandingii*) in Northeastern Illinois

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Abstract
Aquatic turtles are suitable biomonitors of wetland ecosystem health because they are long-lived and occupy elevated trophic positions in wetland food webs. This study aimed to determine Hg exposure in adult Blanding's turtles (*Emydoidea blandingii*), an imperiled prairie-wetland species endemic to the northern U.S. and southern Canada. Claw samples were collected from gravid females from four wetland sites in northeast Illinois. Claw Hg concentrations ranged from 654 to 3132 ng/g and we found no effect of body size (carapace length, CL) and some evidence for an effect of wetland site (WS) on mean Hg (i.e. weak effect of site on Hg, detected between WS1 and WS3). Claw Hg concentrations reported in this study were lower than claw concentrations published for other freshwater turtles (e.g. *Chelydra serpentina, Sternotherus oderatus*). This is the first Hg-related study on Blanding's turtles and can serve as a reference for other Hg studies in Illinois wetlands.

Keywords Mercury · Wetlands · *Emydoidea blandingii* · Non-lethal tissue sampling · Biomonitor · Toenail

Wetlands play an important role in the mercury (Hg) cycle by functioning as sinks for inorganic forms of Hg and as a net source of methylmercury (MeHg) production. The anaerobic conditions and high concentrations of dissolved organic carbon and sulfur-containing compounds in wetland sediments support active microbial communities that readily methylate Hg(II) into MeHg (Gilmour et al. 1992; Benoit et al. 2001; Driscoll et al. 2007). The production of MeHg can vary among wetlands as it depends on environmental factors that support these microbial communities, such as the hydroperiod and the frequency of alternating oxic and anoxic conditions in the surface substrate (Hall et al. 2008).

The methylmercury form of Hg is highly toxic and bioavailable to wildlife as it biomagnifies up the trophic ladder, resulting in higher concentrations in long-lived predators (e.g. aquatic turtles; Bergeron et al. 2007; Turnquist et al. 2011; Hopkins et al. 2013c; Châteauvert et al. 2015).

The accumulation of Hg in tissues of freshwater turtles exposed to environmental Hg has been well-documented (Green et al. 2010; Turnquist et al. 2011; Hopkins 2012; Yu et al. 2011; Hopkins et al. 2013a; Meyer et al. 2014; Powell 2014; Zapata et al. 2014; Châteauvert et al. 2015; Schneider et al. 2015; Landler et al. 2017; Slimani et al. 2018). Turtles that inhabit freshwater wetlands can serve as effective biomonitors for these increasingly threatened ecosystems because of their wide distribution, long life expectancies and their higher trophic positions eating fish, amphibians, and invertebrates (Golet and Haines 2001; Guillot et al. 2018). Additionally, the keratinized tissues of turtles (e.g. scutes, claws) have been shown to correlate with Hg concentrations in environmental matrices, particularly in sediments that turtles inhabit, and as such are useful as predictors of ecosystem-level exposure risk (Hopkins et al. 2013a; Smith et al. 2016; Slimani et al. 2018).

Although long-term effects of Hg exposure on freshwater turtle populations are largely unknown (Hopkins et al. 2013c, but see; Yu et al. 2011), elevated tissue Hg

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concentrations have been associated with the disruption of thyroid hormones (Meyer et al. 2014), orientation behavior (Landler et al. 2017), and reproduction via maternal Hg transfer, causing infertility and decreased hatchling success (Hopkins 2012; Hopkins et al. 2013a, c). These Hg-related impairments that limit growth, development, and reproduction in turtles can have deleterious consequences on already imperiled populations of freshwater turtle species.

Blanding's turtles (*Emydoidea blandingii*) are an IUCN Red List endangered wetland-dependent species of North America (van Dijk and Rhodin 2011). Owing to their life history and foraging ecology, they may also be a candidate for monitoring Hg contamination in aquatic ecosystems. These long-lived emydids use core prairie-wetlands and adjacent terrestrial habitats during the active season (Ernst et al. 1994; Hartwig 2004), and wetland sediment and vegetation during winter dormancy (Newton and Herman 2009). Blanding's are primarily carnivorous, foraging at a moderate trophic level on a diet of snails, crayfish, earthworms, insects, vertebrates, and some plant material (Rowe 1992). Blanding's currently exist as discontinuous populations throughout their range (Rubin et al. 2001) and have experienced severe declines due to their vulnerability to anthropogenic change coupled with their life history attributes of low reproductive output, delayed maturity, and expansive habitat requirements (Browne and Hecnar 2007). Blanding's are listed as endangered by Illinois Endangered Species Protection Board (2015) and are now rare in the Greater Chicago Metropolitan Area (GCMA). Consequently, population augmentation programs have been established (e.g. the Blanding's Turtle Recovery Project in 1996; BTRP) to increase juvenile survival via head-starting, in which eggs are collected, incubated, and hatchlings are reared in captivity for 1–2 years before release in the wild.

Blanding's turtles may be at risk of exposure to harmful concentrations of Hg, which is of particular concern given their population status. However, Hg exposure in Blanding's has not been examined. The purpose of the present study was to determine Hg concentrations in female Blanding's turtles breeding in GCMA wetlands. Using non-destructive sampling of claws (also referred to as toenails; e.g. Hopkins et al. 2013a, b), we aimed to provide the first documentation of Hg exposure in Blanding's and to examine site-specific differences in gravid female Hg concentration with body size as a covariate.

Materials and Methods

Claws were used to examine Hg exposure as they accurately reflect cumulative dietary Hg exposure during the months before collection in turtles, including in gravid females (Hopkins et al. 2013a, b). In addition, claws are non-destructive to collect and simple to store and prepare for analysis (Hopkins et al. 2013b). Claws were sampled from female Blanding's turtles inhabiting four GCMA (location previously defined) wetland sites (WS) of northeast IL. Site WS3 (~ 145 ha), contained seasonal ponds that were sparsely vegetated (mostly *Chara* sp.), while the other three sites: WS1, WS2 (both ~ 1400 ha) and WS4 (~ 60 ha) had permanent ponds and were densely vegetated. All sites but WS3 contained multiple freshwater fish species. All sites were in close proximity to one another (~ 15 km), particularly WS1 and WS2 (~ 3.2 km) and thus were likely receiving similar amounts of atmospheric Hg deposition.

Twenty-seven female Blanding's were collected between 17 May and 22 June 2017 from four GCMA wetland sites: WS1 (n = 12), WS2 (n = 11), WS3 (n = 3), and WS4 (n = 1). WS1 consisted of younger, reproductively mature, head-started females (~ 14–20 years of age based on BTRP individual hatching records), whereas WS2, WS3, and WS4 consisted of older females (> 20 years of age; some head-started, some wild). As a part of the BTRP, all females were previously radio-tagged and captured via radio telemetry. Female Blanding's lay a single clutch per season and all were confirmed to be gravid via X-ray. Standard morphometric measurements were taken for each individual, including mass (g), straight carapace and plastron length to the nearest mm. The tips of three claws (2–3 mm) were removed from the left and right hind feet of each turtle using canine nail clippers. Each claw sample was rinsed with reverse osmosis water, cleaned using a soft bristled brush, dried at room temperature for 24 h, and placed in a 2.0 mL screw cap microcentrifuge tube for storage in room temperature prior to Hg analysis.

Three claw samples from each turtle were loaded into a single sample boat and analyzed together as a composite sample to account for potential variation in Hg among claws. The samples were analyzed for total Hg using a Nippon MA-3000 direct mercury analyzer. As the Hg in keratinized tissues such as feathers and claws are predominately in the methylated form of MeHg (72%–98%; Hopkins et al. 2007), total Hg was measured as a proxy for MeHg. Analysis of total Hg concentration was achieved via thermal decomposition, gold amalgamation, and atomic absorption following U.S. EPA method 7473. Standard reference materials (DOLT-5 and TORT-3, from the National Research Council Canada) were analyzed to ensure quality assurance and control. Two samples of DOLT-5 and two samples of TORT-3 were analyzed after every ~ 15 samples. Mean percent recovery for DOLT-5 was 84.0% ± 0.1% (n = 6), relative significant difference among replicates was 1.13%. Mean percent recovery for TORT-3 was 97.1% ± 1.1% (n = 6), relative significant difference among replicates was 1.2%. Method detection limit was 0.001 ng of Hg, no samples fell below the detection limit. All claw Hg concentrations were

recorded in ng/g fresh weight ± standard deviation (SD) and were $\log_{10}$-transformed prior to analysis to improve normality and homoscedasticity of variance.

Because the bioavailability of Hg in wetlands can vary spatially due to site-specific wetland factors (e.g. hydroperiod, food web complexity), we tested for differences in Hg concentration in gravid females (with and without carapace length [CL] as a covariate) among the four sites. Carapace length, rather than mass, best represents body size as it correlates to age, whereas mass of gravid females also represents egg development stage and body condition. Carapace length was not significantly correlated with Hg concentration (Pearson product moment correlation coefficient; $\rho = -0.29$, $df = 25$, $p = 0.14$). However, we included it as a covariate in one of our models because other studies have shown a positive correlation between Hg and body size (with and without a site interaction; e.g. Bergeron et al. 2007; Turnquist et al. 2011; Hopkins et al. 2013a, b, c; Guillot et al. 2018). Prior to settling on the models we report below, we ran an interactions model to test for a significant interaction (non-homogeneity of slopes). Because this interaction model reported no significant interactions, we identified two separate models that would form the basis of our inference for this study.

To test for differences in Hg among sites, we used two general linear models—Model 1 was an analysis of variance with a covariate (i.e. ANCOVA) and Model 2 was an analysis of variance (i.e. ANOVA). Because a preliminary model with an interaction term found no evidence of an interaction, Model 1 was intended to test for the effect of the CL covariate (Table 2). However, Model 1 did not report a significant effect of CL (-0.003, see Table 2). This allowed us to use an intercepts-only model (Model 2) to test for site-specific differences in mean Hg without CL as a covariate (Table 2). Models 1 and 2 were fit using Bayesian estimation, because Bayesian approaches accommodate small samples sizes well and provide more robust inference on model estimations and multiple comparisons (Kruschke 2010). Bayesian methods allowed us not only to calculate parameter estimates, but also to provide probabilistic estimates of uncertainty (credible intervals) around the parameter estimates, where strong effects are determined by non-overlapping 95% credible intervals, moderate effects by non-overlapping 90% credible intervals, and weak effects by non-overlapping 80% credible intervals. Additionally, a frequentist statistical approach would have required error-rate adjustments or post-hoc multiple comparisons corrections that are not required in a Bayesian context. For our analysis, all parameters were given diffuse Normal priors. We ran three concurrent Markov chains for 30,000 iterations, beginning each with a randomly generated value. The first 10,000 iterations of each chain were discarded as burn-in, and the remaining 60,000 were thinned by retaining every

third value, resulting in a sample of 20,000 iterations. Convergence was assessed with the Brooks–Gelman–Rubin statistic, $\hat{R}$, where values < 1.1 indicate convergence; we recorded no values > 1.01. Analyses were run using JAGS (Version 4.1.0) in the *rjags* package (Plummer 2016), run from within *R* (R Core Team 2016).

Results and Discussion

Mean (± SD) claw Hg concentration of gravid female Blanding's turtles at all sites was 1479.8 ± 371 ng/g and was greatest at WS1 (Table 1; Fig. 1). We found no effect of body size (carapace length, CL) and a weak effect of wetland site on mean Hg. Specifically, Model 1 revealed no effect of CL on mean Hg among sites, with all mean Hg among sites being similar (Table 2 Model 1; Fig. 1a). Model 2 found weak evidence for a difference in mean Hg between WS1 and WS3, based on non-overlapping 80% credible intervals (Table 2 Model 2; Fig. 1b). Although several previous turtle Hg studies have documented positive relationships with turtle body size (mass, CL) and Hg concentrations (see above for references), our results are congruent with the various studies that have documented mixed or no effects of size on Hg (e.g. Golet and Haines 2001; Powell 2014; Landler et al. 2017).

Because there are no known point sources of Hg pollution near our sites, atmospheric deposition is the primary source of Hg into these wetlands and is likely uniform across this
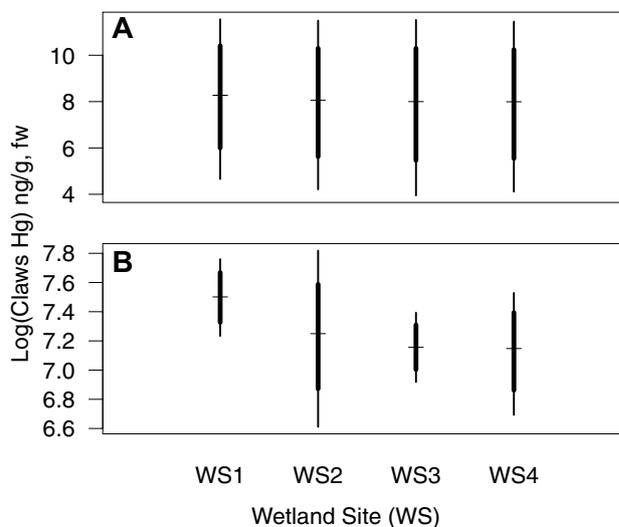
Fig. 1 Mean log claw Hg concentrations (ng/g, fresh weight) of gravid Blanding's turtles at four wetland sites WS1 (n = 11), WS2 (n = 12), WS3 (n = 3), WS4 (n = 1) in the Greater Chicago Metropolitan Area (GCMA). In both panels, site-specific log(Hg) medians (tick mark) are presented along with 95% (thin line) and 80% (thick line) credible intervals. **a** Represents Model 1 (ANCOVA) that included a carapace length (CL) covariate, and **b** represents Model 2, the intercepts-only (ANOVA) model (see Table 2 for model details)

Table 1 Mean (±SD) and range of claw Hg concentrations (ng/g, fresh weight), mean (±SD) carapace length (CL, mm) of gravid female Blanding's turtles at four wetland sites (WS) within the Greater Chicago Metropolitan Area (GCMA) of northeast Illinois (IL)

	WS1	WS2	WS3	WS4	All sites
n	11	12	3	1	27
Hg Mean±SD	2039.9±680.5	1371.9±687.6	1160.3±116.0	1347±0	1479.8±371.0
Hg Range	849–3132	654–3111	1033–1260	na	654–3132
CL Mean±SD	203.5±10.0	216.2±9.3	226.3±10.1	210±0	214.0±7.3

Table 2 Model equations, model estimates with 95% credible intervals (CI), and interpretation for the two models used in this study

Model	Equation	Site (α_j)	Posterior estimate (95% CI)	Interpretation
1	$\log(Hg_i) \sim N(\alpha_{ji} + \beta x_i,\ \sigma_i{}^2)$	WS1	8.18 (4.77, 11.63)	No effect of site and CL on Hg (Fig. 1a)
		WS2	7.89 (4.25, 11.52)	
		WS3	7.88 (4.11, 11.60)	
		WS4	7.96 (4.33, 11.58)	
2	$\log(Hg_i) \sim N(\alpha_{ji},\ \sigma_i{}^2)$	WS1	7.50 (7.23, 7.76)	Weak effect (80% confidence level) of site on Hg, detected only between WS1 and WS3 (see Fig. 1b)
		WS2	7.16 (6.92, 7.40)	
		WS3	7.14 (6.69, 7.53)	
		WS4	7.24 (6.62, 7.83)	

Subscript i indicates the individual (observation) level of the data, and subscript j indicates the group (site) level of the model. Model 1 examined the effect of carapace length (CL), (x_i) on log(Hg), which was found to have no effect [$\beta = -0.003$ (-0.020, 0.013), where β is the slope estimate with 95% CI]. Model 2 was a reduced intercepts-only model without CL, in which the site means were compared. Posterior estimates and 95% CI for site-specific log(Hg) means are reported for both models

relatively small geographic scale. Thus, the lack of strong effect of wetland site on mean Hg may be due to factors such as (1) small sample size (e.g. WS3 and WS4), (2) similar wetland environmental conditions important for Hg methylation, and/or (3) the close proximity of wetland sites coupled with the spatial ecology of Blanding's turtles. Female Blanding's, in particular, move up to 2 km from resident wetlands to nesting sites, use multiple nesting sites across different wetlands (Congdon et al. 2011), and have large home ranges, (mean = 20.3 ± 3.5 ha; Millar and Blouin-Demers 2011). Therefore, Hg concentrations reported here may reflect the availability of Hg across wetlands (e.g. between nearby WS1 and WS2), and the mobility of Blanding's may mask any differences in Hg among sites due to possible wetland-specific methylation rates and trophic structures. Understanding wetland-specific patterns of Hg availability in this wetland complex may require a more sedentary biomonitor to detect.

Overall, claw Hg concentrations detected in Blanding's turtles in northeastern IL were relatively low and matched those of other aquatic turtle species such as spiny softshell turtles (*Apalone spinifera*), snapping turtles (*Chelydra serpentina*), and pond sliders (*Trachemys scripta*) in eastern Oklahoma (mean claw Hg = 700 ng/g fw; Powell 2014). Our results were also similar to turtle species inhabiting uncontaminated portions of the South River, VA (*C. serpentina*, musk turtles: *Sternotherus odoratus*, map turtles: *Graptemys geographica*; mean claw Hg = 2,464 ± 339 ng/g fw; Hopkins et al. 2013b). However, our concentrations

were substantially lower than those same South River turtle species inhabiting superfund sites and who's populations experienced Hg-related reproductive impairment (*C. serpentina;* mean claw Hg = 161,109 ng/g fw, Hopkins et al. 2013c, b; *C. serpentina, S. odoratus, G. geographica*; mean claw Hg = 42,250 ng/g fw). Low claw Hg concentrations in Blanding's suggest minimal health risk to turtles in this wetland complex.

To our knowledge, this is the first study to measure Hg concentrations of reptiles in this geographic region (GCMA), as well as the first documentation of Hg exposure in Blanding's turtles. Results from this study can serve as a reference for other Hg studies on wildlife in northeast IL wetlands and for future studies on Blanding's, ultimately adding to the body of knowledge on this endangered species. Moreover, monitoring Hg concentrations in gravid females of an endangered species is particularly important, as Hg tissue accumulation can have negative consequences for reproductive and population health. Despite declining populations of Blanding's in the GCMA, there is little evidence to suggest that Hg is causing harm to population health; however, continued monitoring is necessary to gauge Hg exposure in other populations, as Hg availability can vary substantially through time and space.

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