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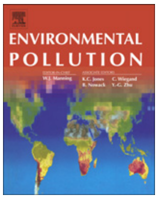

Altered behavior of neonatal northern watersnakes (*Nerodia sipedon*) exposed to maternally transferred mercury

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ABSTRACT

Little is known about effects of maternally transferred contaminants in snakes. The purpose of this study was to evaluate sublethal effects of maternally transferred mercury (Hg) on neonatal northern watersnakes (*Nerodia sipedon*). We captured 31 gravid females along a historically Hg-contaminated river. Following birth, we measured litter Hg concentrations and assessed locomotor performance, foraging ability (i.e., number of prey eaten, latency to first strike, strike efficiency, and handling time), and learning (i.e., change in foraging measures over time) in their offspring ($n = 609$). Mercury concentrations in offspring negatively correlated with motivation to feed and strike efficiency. Over time, strike efficiency and latency to strike decreased for all snakes in the study. However, offspring from contaminated areas maintained consistently lower efficiencies than reference individuals. This study is the first to examine sublethal behavioral effects of maternally transferred contaminants in snakes and suggests that maternally transferred Hg negatively affects offspring behavior.

1. Introduction

Organisms are exposed to mercury (Hg) primarily through their diets, but Hg can also be transferred from females to their offspring (i.e., maternal transfer) (Bergeron et al., 2010; Latif et al., 2001). Maternally transferred Hg can directly affect offspring development and increase embryonic mortality, as well as induce sublethal effects that continue to affect offspring after birth (Bergeron et al., 2011a; Scheuhammer et al., 2007; Wolfe et al., 1998). For example, Bergeron et al. (2011b) found that American toad (*Bufo americanus*) tadpoles from mothers from contaminated areas took 11% longer to swim one meter and needed 34% more stimulation to swim than those from reference mothers. Mercury is also a known neurotoxicant and maternally transferred Hg can damage cells as well as alter neurotransmitter function when the nervous system is most sensitive, thereby impacting coordination and cognition (Weis et al., 2001). For example, a decrease in brain cell density and decreased performance in learning of a spatial task was observed in juvenile zebrafish (*Danio rerio*) exposed to Hg during development (Smith et al., 2010). Additionally, maternally exposed young can exhibit behavioral changes, such as decreased activity and motivation and delayed reaction to stimuli, which may hinder growth and survival (Alvarez et al., 2006; Onishchenko et al., 2007). Effects of maternally derived inorganic contaminants have been examined in a variety of vertebrates, but have seldom been evaluated in reptiles.

Environmental contaminants, such as Hg, are a significant threat to reptiles, but reptiles remain one of the most understudied vertebrate groups in terms of contaminant exposure and effects (Campbell and Campbell, 2002). Among reptiles, snakes are particularly overlooked, and no information exists on environmental contaminants for 6 of the 15 snake families (Campbell and Campbell, 2002). Snakes are strict carnivores and as secondary, tertiary, and apex predators, are susceptible to bioaccumulation of environmental pollutants (Carrasco et al., 2011). Moreover, natural history characteristics such as longevity and small home range size make snakes, especially aquatic or piscivorous species, susceptible to bioaccumulation of contaminants from localized sources (Beaupre and Douglas, 2009; Todd et al., 2010). Despite increasing interest in snake conservation, few studies have evaluated exposure to contaminants in snakes and even fewer have examined sublethal effects in this group (Grillitsch and Schiesari, 2010). The only studies of maternally transferred contaminants in snakes documented transfer of selenium in brown house snakes

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(*Lamprophis fuliginosus*) and Hg in northern watersnakes (*Nerodia sipedon*), but neither study examined sublethal behavioral effects on offspring (Chin et al., in press; Hopkins et al., 2004).

In a companion study, we documented that northern watersnakes maternally transfer high concentrations of Hg to their offspring, but we observed no adverse effects of maternally transferred Hg on reproductive output and embryonic survival (Chin et al., in press). The objective of this study was to evaluate sublethal effects of maternally transferred Hg in neonatal *N. sipedon*. To address this objective, we captured gravid female watersnakes along an Hg contamination gradient at a historically-contaminated river in Virginia, USA, held them until parturition, and evaluated locomotor performance, foraging performance, and learning in their offspring. Specifically, we evaluated the following hypotheses: (1) maternally transferred Hg negatively affects swimming and crawling performance in neonates, (2) maternally transferred Hg negatively affects foraging performance (i.e., number of prey eaten, latency to first strike, strike efficiency, and handling time), and (3) maternally transferred Hg negatively affects learning (i.e., improvement in foraging measures over time).

2. Methods

2.1. Study species

The moderately sized (to 150 cm total length) northern watersnake (*Nerodia sipedon*) is a widely distributed, nonvenomous colubrid that inhabits most freshwater habitats in the eastern United States (Gibbons and Dorcas, 2004). Fish compose the bulk of their diet, especially in lotic habitats, and their extensive list of documented prey includes over 80 fish species (Gibbons and Dorcas, 2004). As a piscivorous species, *N. sipedon* is particularly susceptible to Hg bioaccumulation, and they have been proposed as important indicators of Hg contamination in aquatic ecosystems (Burger et al., 2007; Wolfe et al., 1998).

2.2. Study site

The South River, located in central Virginia, USA, is one of two tributaries of the South Fork of the Shenandoah River, which flows into the Potomac River and the Chesapeake Bay. The South River has a history of Hg contamination from an acetate fiber manufacturing plant located in Waynesboro, VA, that used mercuric sulfate between 1929 and 1950 (Carter, 1977). An Hg contamination gradient now spreads for at least 200 km downstream of the former plant, ranging from low concentrations upstream of the point source to exceedingly high concentrations downstream (Eggleston, 2009).

2.3. Animal collection and husbandry

Gravid female *N. sipedon* ($n = 31$) were captured by hand at sites along the South and Middle Rivers between 14 June and 31 July, 2011. Nine females were captured from reference locations along the Middle River, a river with low Hg concentrations located approximately 37 km northwest of Waynesboro, VA, and from the South River, upstream of the Hg source. The remaining 22 females were collected along the Hg contamination gradient at the South River, providing a wide range of Hg concentrations in watersnake litters.

Watersnakes were located by searching appropriate microhabitats and turning rocks and other cover objects. Upon capture, snakes were sexed by examination of tail morphology and reproductive status of females was assessed by gently palpating the posterior body for presence of developing ova. Gravid females were returned to the laboratory at Virginia Tech and maintained in a walk-in environmental chamber set at 25 °C until parturition. Snakes were housed individually in 75-L aquaria with aspen bedding substrate, two hideboxes, a large water bowl, and a basking lamp, providing a thermal gradient to facilitate thermoregulation. Parturition occurred from 9 August to 4 September, 2011. Neonates were housed communally at 25 °C until locomotor performance trials in aquaria (38–75 L) with paper towel substrate, a hidebox, and a water bowl and were fed during feeding trials (see foraging performance).

2.4. Mercury analysis

To determine total Hg (THg) concentrations of litters, three randomly selected neonates from each litter were euthanized via overdose of buffered tricaine methanesulfonate (MS-222), lyophilized, and homogenized. A single composite sample containing equal portions from each of the three neonate samples was analyzed for THg for each litter. Whole-body neonate samples were analyzed for THg by combustion–amalgamation–cold vapor atomic absorption spectrophotometry (Direct Mercury Analyzer 80, Milestone, Monroe, CT, USA) at the College of William and Mary, Williamsburg, VA, according to U.S. Environmental Protection Agency (U.S. EPA) method 7473 (USEPA, 1998). For quality assurance, control samples included a replicate, blank, and standard reference material (SRM; DOLT-4 dogfish liver, DORM-3 fish protein (National Research Council of Canada (NRCC)), Ottawa, ON). Method detection limits (MDLs; threefold the standard deviation of procedural blanks) for samples were 0.0013 mg/kg (ppm), and all samples had THg concentrations that exceeded that limit. Average relative percent differences (RPD) between replicate sample analyses were 12.11 ± 24.44%. Mean percent recoveries of THg for the DOLT-4 and DORM-3 were 103.85 ± 0.66% ($n = 8$) and 103.44 ± 1.35% ($n = 8$), respectively. All THg concentrations are reported on a dry weight (dwt) basis.

2.5. Experiment 1: locomotor performance

Normal antipredator behavior of young *N. sipedon* consists of diving, swimming away, and dropping into vegetation (Cooper et al., 2008). Because these behaviors involve both terrestrial and aquatic substrates, snake locomotor performance was evaluated in both media. Terrestrial locomotor performance (TLP) and aquatic locomotor performance (ALP) trials were conducted to determine whether neonatal watersnakes with high maternally derived Hg concentrations exhibited reduced sprint velocity in either environmental media compared to young from females collected at reference sites.

Both TLP and ALP trials were conducted in a walk-in environmental chamber set at 25 °C. Snakes were conditioned to both terrestrial and aquatic racetracks on day 2 post-birth by being raced twice in succession on each track. TLP trials were performed on day 3 post-birth, and ALP on day 4 post-birth. In a previous study, snakes achieved their fastest velocity 97% of the time within their first two laps, so neonates were tested for two consecutive laps in each media (Hopkins et al., 2005). Terrestrial locomotor performance followed the methods of Willson and Hopkins (2011) using a 2.3 m linear track lined with photocells emitting infrared beams at 10 cm intervals and interfaced with a computer (Columbus Instruments, Columbus, Ohio, USA). As snakes interrupted the beams, the computer recorded the time at which each gate was passed. Before beginning each trial, a single neonate was placed in a box attached to the starting point and allowed to acclimate for approximately 30 s. A gate separating the box from the track was then lifted and the snake was chased by hand down the track and prodded by lightly touching their tails as frequently as necessary to prompt a flight response. A single observer, blind to snake capture locations and Hg levels, acted as the chaser in all trials. After completion of the TLP trials, each snake was measured (mass, snout-vent length [SVL], tail length), sexed, and individually housed in a 591 ml plastic container with a small amount of water and a moist paper towel.

Aquatic locomotor performance trials occurred on the day following TLP (day 4 post-birth) following the methods of Hopkins et al. (2005). In short, one observer chased a snake by hand down a 3 m long track with walls 4 cm high, filled to a depth of 3 cm with water (25 °C). A second observer filmed the trials using a digital video camera with a frame rate of 30 frames per second (Sony Handycam, JVC Everio HDD). Videos were analyzed using Adobe® Premiere Pro CS5 software. An independent video reviewer, also blind to snake capture locations and Hg levels, recorded the time (nearest 0.03 s) snakes reached each 10 cm interval along the track. For each individual snake, the time taken to cover 30 cm was calculated for each 30 cm segment of each racetrack in both media. Each snake's fastest velocity over a 30 cm segment in each media was used as the measure of maximum locomotor performance in statistical analyses (Hopkins et al., 2005).

2.6. Experiment 2: foraging performance

Foraging performance trials were conducted to determine whether maternally transferred Hg influences a snake's willingness to feed or ability to capture prey. Foraging provides an integrative performance measure incorporating motor function, sensory function, and cognition. Foraging tests were conducted in a walk-in environmental chamber set at 25 °C and were recorded and analyzed via the same equipment and software as ALP. Foraging assessment consisted of two parts: 1) an initial feeding test to determine a snake's inclination to feed on easily-captured prey, and 2) a foraging performance test designed to evaluate the snake's abilities to capture and ingest prey in a more challenging environment. For the initial feeding test, each snake was offered two mosquitofish (*Gambusia affinis*) a week after birth for an hour-long period in a 591 ml plastic container containing a small amount of water (25 °C) and the number of fish they consumed was recorded. Six neonates (3 male, 3 female) that ate during the initial feeding trial were selected per litter for use in foraging performance trials, which occurred one week later. Snakes were tested individually in foraging arenas consisting of a 20 × 20 × 33 cm open-topped plastic bucket lined with heavy gauge plastic fencing to provide a structurally complex environment, and filled with water (25 °C) to a depth of 1 cm. Snakes were given an hour to acclimate to the arena, after which four live *Gambusia*, each weighing 7–10% of the snake's body mass, were introduced from behind a blind. Snakes were then allowed to forage undisturbed for one hour and trials were recorded by a digital video camera suspended above the arena. Foraging performance videos were analyzed by recording the time each snake performed any of a set of predefined behaviors (Table 1). Several endpoints were then calculated and used as dependent variables in statistical analyses of foraging performance (Table 2).

2.7. *Experiment 3: learning*

Young snakes have been shown to strike more readily, improve prey capture efficiency, and decrease prey-handling time following feeding experience (Krause and Burghardt, 2001; Savitzky and Burghardt, 2000). Therefore, additional foraging trials were conducted to determine if snakes with high concentrations of maternally derived Hg exhibited impaired learning or improved less at capturing prey than those from reference locations. Two snakes per litter were randomly selected from those that ate during the initial foraging performance trial to participate in learning trials. These snakes completed three additional foraging trials, each seven days apart, for a total of four trials. Learning trials followed the same procedure as foraging performance trials and used the same endpoints in video analysis. Following foraging and learning trials, females and neonates were released at the female's capture location.

2.8. *Statistical analyses*

All statistical analyses were performed in Microsoft Excel or SAS 9.2 (SAS Institute, Cary, NC, USA) and litter means were used as the statistical unit for all analyses except learning (see below). Statistical significance was assessed at the $\alpha = 0.05$ level and data were examined for normality and homoscedasticity prior to all tests.

The statistical difference between THg concentrations of litter samples from reference and contaminated sites was determined using a one-way analysis of variance (ANOVA). To assess the relationship between Hg concentrations and locomotor performance, maximum velocity achieved over 30 cm was first calculated for each neonate in TLP and ALP. To determine if body size accounted for differences in performance, velocity was then regressed against SVL. Body size was positively correlated with velocity (TLP: $r^2 = 0.167$, $p < 0.001$, ALP: $r^2 = 0.179$, $p < 0.001$), indicating a need to correct for SVL. Mean litter residual values from velocity-SVL correlation were used as body size-corrected measures of locomotor performance and regressed against litter THg concentrations to examine the relationship of exposure to maternally transferred Hg and locomotor performance.

The overall effect of maternally transferred Hg on foraging performance measures (proportion of litter that ate during the initial feeding test, and the litter means for number of prey eaten, latency to first strike, strike efficiency, and handling time) was assessed using a generalized linear model for mixed distributions multivariate analysis of variance (MANOVA; SAS PROC GLIMMIX), a procedure that is capable of modeling non-continuous distributions. Each endpoint was then analyzed separately. The effect of maternally transferred Hg on willingness to feed (expressed as the proportion of individuals that ate in a litter) was analyzed using an ANOVA (SAS PROC GLIMMIX), with a binomial distribution and a logit link function. This ANOVA specifically compared proportional differences among litters with different THg concentrations. The remaining foraging performance endpoints were analyzed by regressing mean litter performance measures against litter THg concentrations. Body size was not included as a covariate in these analyses because prey items were standardized for snake size. Individuals that did not attempt to forage (i.e., never struck at a prey item) were excluded from analyses.

Repeated measures analyses of variance were used to assess differences in learning among individuals from reference and contaminated sites for each foraging performance endpoint over the four weekly feeding trials. Site (reference vs. contaminated), time, and their interaction were included as main effects in the model and litter was included as a random effect.

3. Results

Litter sizes ranged from 5 to 37 neonates, yielding a total of 609 offspring from 31 female *N. sipedon*. Whole-body THg concentrations for litters were strongly and positively correlated with THg concentrations of maternal tail tissue (Chin et al., in press). Litter whole-body THg concentrations from reference sites ranged from 0.06 to 1.09 mg/kg, dwt (mean ± SE = 0.20 ± 0.11 mg/kg) and from 1.08 to 10.10 mg/kg (mean ± SE = 3.42 ± 0.45 mg/kg) for litters from mothers collected along contaminated sections of the South River. Mean litter THg concentrations differed significantly between sites (ANOVA; $F_{1,\ 30} = 20.21$, $p < 0.001$). On average, 95% of the Hg transferred was in the more toxic form of MeHg (Chin et al., in press).

Table 1
Foraging and feeding behaviors of neonatal northern watersnake (*Nerodia sipedon*) exposed to maternal Hg recorded during foraging video analysis.

Measure	Definition
Strike	Faster than normal, directional motion toward a prey item
Unsuccessful strike	Snake strikes but does not catch prey
Successful strike	Snake captures prey in mouth
Positioned	Prey aligned parallel with snake head
Ingested	Prey completely consumed by snake and can no longer be seen

Table 2
Dependent measures of foraging performance of neonatal northern watersnakes (*Nerodia sipedon*) exposed to maternal Hg, calculated from foraging video analysis.

Measure	Definition
No. of prey eaten	Number of prey items consumed by test snake during a one hour foraging trial.
Strike efficiency	Mean number of successful strikes divided by number of total strikes for each prey eaten.
Latency to first strike	Mean time (sec) from time fish are introduced to the arena to time of first strike.
Handling time	Mean time (sec) from successful strike to time ingested for each prey item eaten.

Mean litter maximum locomotor velocities over 30 cm ranged from 27.36 to 52.28 cm/s for TLP and from 55.73 to 74.80 cm/s for ALP. Lack of significant relationships between litter THg concentrations and mean litter velocities corrected for body size (Fig. 1: linear regression; TLP: $p = 0.73$; ALP: $p = 0.79$) suggested that there was no effect of maternally transferred Hg on either terrestrial or aquatic locomotor performance, after accounting for body size.

Multivariate analysis of foraging performance measures yielded a significant interaction between Hg and response variables (MANOVA; Hg × response: $F_{5,\ 117} = 17.24$, $p < 0.001$), indicating that there were differential responses to Hg among foraging performance measures. We found evidence that litters with higher THg values had decreased willingness to feed when offered prey in a confined environment (Fig. 2A: ANOVA; $F_{1,\ 29} = 10.06$, $p = 0.003$); a larger proportion of litters with high THg values did not eat during the initial feeding test compared to litters with lower THg values. Of the three foraging performance measures analyzed, we observed a significant effect of Hg on strike efficiency (Fig. 2B: linear regression; $p = 0.03$), with strike efficiency decreasing from approximately 80%–30% as litter THg concentrations increased. No significant effects of THg on number of prey eaten (linear regression: $p = 0.14$), latency to first strike (linear regression: $p = 0.73$) or handling time (linear regression: $p = 0.98$) were detected.

Consistent with our observations during foraging trials, strike efficiency was the most strongly affected measure over the four-week learning trial. Contrary to our predictions, all snakes exhibited decreasing strike efficiency over time (Fig. 3A: RM-ANOVA; time: $F_{3,\ 174} = 3.87$, $p = 0.01$). However, mean strike efficiency of individuals from mothers captured at reference areas was approximately 10% higher than those from mothers captured from contaminated areas (site: $F_{1,\ 174} = 4.09$, $p = 0.04$) and that difference did not change over time (site × time: $F_{3,\ 174} = 0.04$, $p = 0.98$). Latency to first strike significantly decreased over time (Fig. 3B; time: $F_{3,\ 174} = 6.45$, $p < 0.01$). Neither number of prey eaten (Fig. 3C; site $p = 0.76$, time $p = 0.58$, site × time, $p = 0.65$) nor handling time (Fig. 3D; site $p = 0.88$, time $p = 0.46$, site × time, $p = 0.44$) differed over time or between reference and contaminated sites.

4. Discussion

Despite ample evidence of maternal transfer of contaminants in wildlife, sublethal effects of maternally transferred contaminants

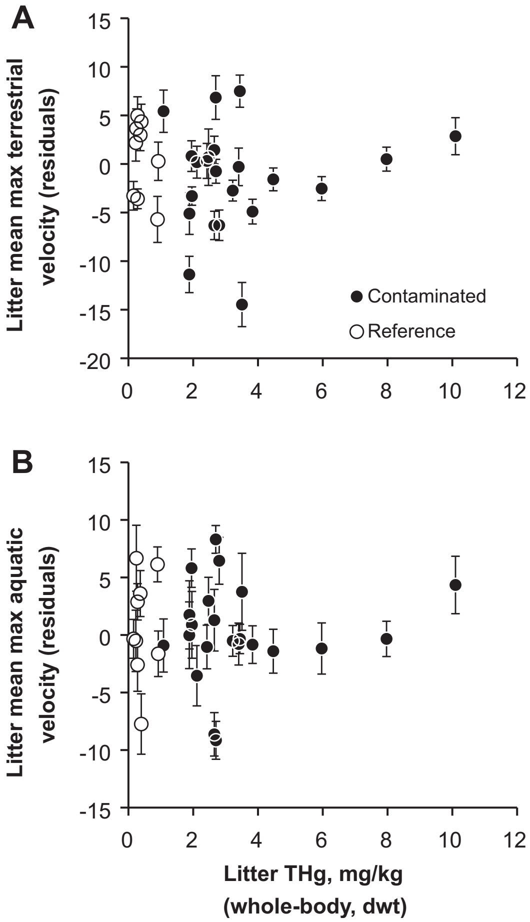

Fig. 1. Relationships between body size-corrected locomotor performance and litter THg concentrations, based on residuals from a regression of SVL and maximum velocities achieved by each individual over 30 cm. Negative values indicate that individuals were slower than expected for their body size and positive values indicated that they were faster. (A) Relationship between mean litter terrestrial locomotor performance residuals and litter THg values; (B) relationship between mean litter aquatic locomotor performance residuals and litter THg values.

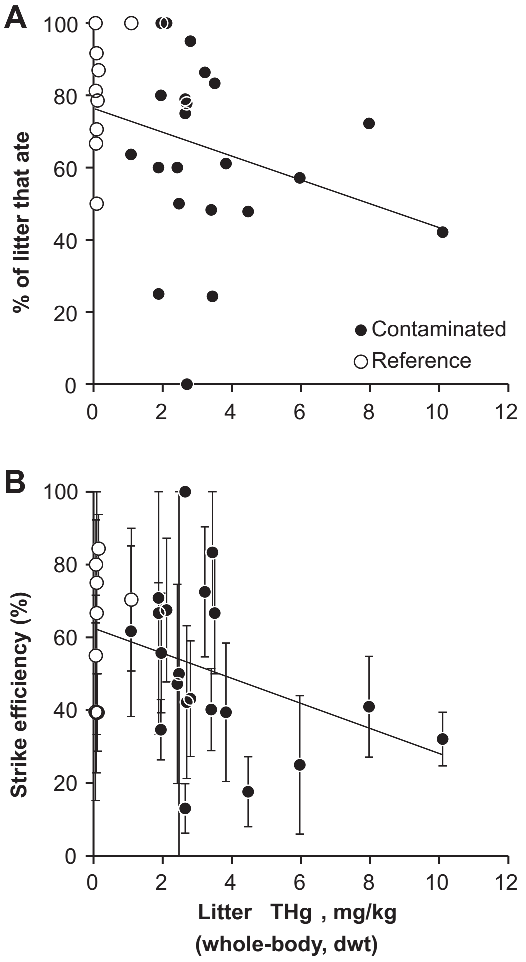

Fig. 2. Relationships between foraging measures and litter THg concentrations in juvenile *N. sipedon*. (A) Percentage of litter that ate during an initial feeding test in a confined environment; (B) litter mean strike efficiency (ratio of successful strikes to total strikes per prey item) during feeding performance trials. Significant interaction in a multivariate analysis supported differential responses among foraging measures to Hg (MANOVA: response × Hg interaction; $F_{1,4} = 23.44$, $p < 0.01$). Note that statistical significance in (A) was determined using a generalized linear model for mixed distributions (SAS PROC GLIMMIX), but a linear trendline is provided for visualization.

remain poorly understood. Offspring exposed to maternally transferred Hg can experience reduced body size and growth, impaired motor function and cognition, and altered behavior, such as reduced response to stimuli representing predator or conspecific interactions (Alvarez et al., 2006; Bergeron et al., 2011b; Heinz, 1975; Heinz and Hoffman, 1998; Onishchenko et al., 2007). Our study examined sublethal effects of maternally transferred Hg on watersnakes and considered a range of endpoints including locomotor performance, foraging ability, and learning. We observed an effect of maternally transferred Hg on foraging, including evidence for decreased motivation to feed with increasing Hg and negative effects on strike efficiency that persisted through successive foraging trials. Our study supports growing evidence that maternally transferred Hg can have subtle sublethal effects that may ultimately compromise the fitness of individuals inhabiting contaminated habitats (Alvarez et al., 2006; Bergeron et al., 2011a, 2011b; Todd et al., 2012).

Although snakes transfer high concentrations of Hg to their offspring (Chin et al., in press), their locomotor abilities may be less affected by maternal Hg than other taxa. Watersnakes from the present study maternally transferred much higher concentrations of Hg (up to 10.10 mg/kg THg [dried neonate body tissue] Chin et al., in press), than amphibians (American toad, pickerel frog [*Rana palustris*], and wood frog [*Rana sylvatica*]) collected at the South river that transferred Hg concentrations ranging from 0.36 to 0.98 mg/kg (dwt) THg to their eggs (Bergeron et al., 2011a; Bergeron and Hopkins, unpublished data). Bergeron et al. (2011b) found that larvae of American toads collected from contaminated sections of the South River floodplain exhibited reduced swimming speed, despite their low egg THg concentrations (0.15 mg/kg) relative to watersnakes. Similarly, Burke et al. (2010) evaluated locomotion of adult two-lined salamanders (*Eurycea bislineata*) exposed to both dietary and maternal Hg from the South River. Salamanders from contaminated sites had a mean THg concentration of 4.52 mg/kg (dwt) and crawled more slowly than individuals from reference areas (Burke et al., 2010). Despite the high concentrations in snake tissues, we observed no effects of maternally transferred Hg on locomotion in watersnakes. Thus, it is possible that snakes have greater tolerance to Hg exposure than amphibians, but further studies of other snake species are needed to support this hypothesis.

Numerous studies have documented negative effects of contaminants on feeding behavior of wildlife (Burke et al., 2010; Little et al., 1990; Reichmuth et al., 2009). Specifically, Hg and other

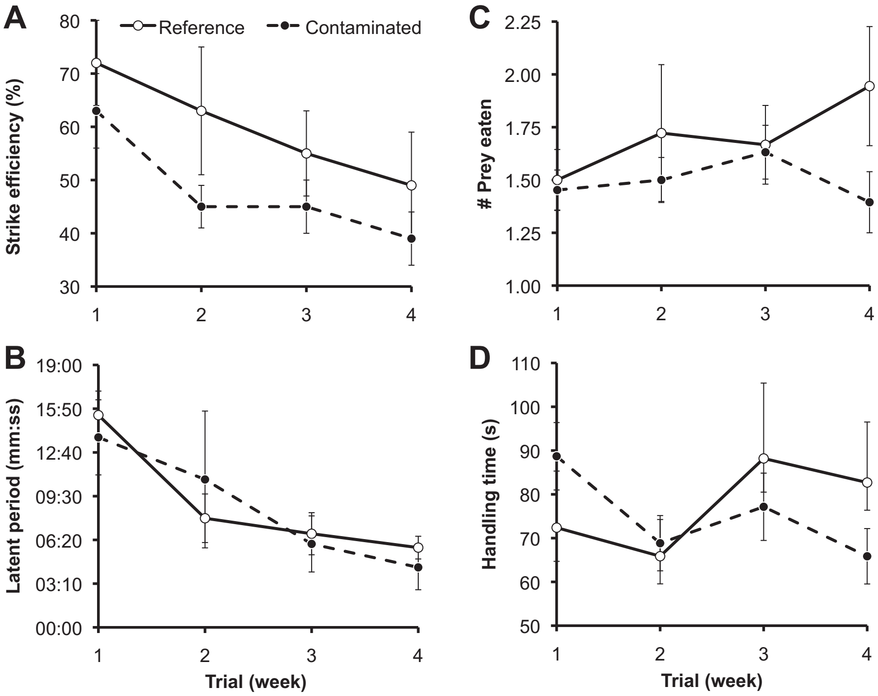

Fig. 3. Effect of maternally transferred Hg on learning in juvenile *N. sipedon*, as indicated by changes in foraging performance measures within individuals over four successive weekly foraging trials. Litters were assigned to reference or contaminated categories based on the female's capture location. (A) Mean strike efficiency per prey item, (B) mean latency to first strike, (C) mean number of prey consumed, and (D) mean handling time per prey item. Strike efficiency significantly decreased over time with differences among sites. Latency to first strike also significantly decreased over time.

contaminants reduce motivation to feed and decrease prey availability, which can impede growth and lengthen the time when young are most susceptible to predation (Madsen and Shine, 2000). We observed a negative relationship between litter THg and the proportion of littermates that ate during the initial feeding test, suggesting that transferred Hg adversely impacts motivation to feed. This result is consistent with several studies documenting reduced appetite in wildlife exposed to dietary or maternally transferred Hg (Burke et al., 2010; Dansereau et al., 1999; Spalding et al., 2000). Mercury has also been shown to affect prey capture in various study systems. For example, blue crabs (*Callinectes sapidus*) and graylings (*Thymallus thymallus*) exposed to Hg exhibited reduced foraging ability and prey capture efficiency (Fjeld et al., 1998; Reichmuth et al., 2009). Striking is an integrated behavior involving motor function, sensory function, and cognition that is essential to prey capture. Strike efficiency was the most affected measure in both the foraging performance test and subsequent learning trials. With increasing maternal Hg, litters had significantly lower mean strike efficiencies; indicating that these individuals were striking frequently, but often missing their mark. However, it is important to note that there was considerable variation in strike efficiency among litters and our sample size at the highest end of Hg concentrations was relatively low. Although the litters with the highest THg concentrations did not exhibit the lowest mean strike efficiencies, all litters with THg concentrations greater than 4 mg/kg averaged less than 50% strike efficiency. More intensive sampling of snakes from highly contaminated sites would be useful in determining the severity of effects at the high end of the Hg contamination spectrum and evaluating whether thresholds exist for the effects we observed. Although it is difficult to tease apart which aspect of striking behavior was most affected by Hg, a lack of significant effect of Hg on locomotion suggests that maternal Hg may more strongly impact cognitive and sensory functions than gross muscular control. Impaired cognition early in life may put individuals from contaminated areas at high risk of mortality and reduce their probability of reaching reproductive maturity.

We also assessed learning by examining changes in watersnake foraging performance over time. Neonatal garter snakes (*Thamnophis sirtalis*) have been shown to improve their prey-handling times over a period of 190 days (three feeding tests, with weekly feedings in-between) (Krause and Burghardt, 2001). Mercury is known to cause nervous system and brain cell death and therefore negatively affect learning and memory (Falluel-Morel et al., 2007; Smith et al., 2010). Thus, we hypothesized that neonatal *N. sipedon* from reference areas would improve in foraging performance over time, because they would learn from prior experience, whereas individuals from contaminated areas would fail to improve. Contrary to our expectations, all litters showed a decrease in strike efficiency over time. This result might be explained by snakes increasing their willingness to strike because of acclimation to the foraging arena, which could increase the total number of strikes, relative to successful strikes. This explanation is supported by a significant decrease in latency to first strike over time, a result also observed in juvenile diamondback watersnakes (Savitzky and Burghardt, 2000). Despite the decrease in strike efficiency over time, the initial negative effect of Hg on strike efficiency persisted, with litters from mothers from contaminated areas continuing to exhibit lower mean strike efficiencies than those from reference areas. This result underscores the idea that effects of maternally derived contaminants on performance may persist well past birth or even be permanent. Indeed, effects of maternally transferred Hg on early growth in American toads have been shown to persist for at least a year in terrestrial juveniles (Todd et al., 2012) and graylings exposed to Hg during development exhibited impaired foraging on live prey at three years of age (Fjeld et al., 1998).

Inhibited prey capture ability in the form of reduced strike efficiency may have implications for snake populations along the

South River and in other contaminated habitats. We observed a reduction in strike efficiency from approximately 80%–30% with increasing Hg concentrations. Moreover, our assessment of this effect is likely conservative because foraging in the wild occurs in a more complex environment than the simple arenas we used in the laboratory. In addition, prey availability and composition may be altered at contaminated sites, which could influence foraging success of generalist predators (Clements and Rees, 1997). The low strike efficiencies we observed could lead to decreased prey-capture rates and subsequent reductions in growth, delays in maturation, and increased vulnerability to predators. Furthermore, maternal Hg exposure may put offspring at a disadvantage at birth and continued exposure to dietary Hg thereafter may exacerbate the effects on foraging we have described. For example, maternal or dietary exposure to Hg resulted in sublethal effects on American toad larvae but combined exposure led to a 125% increase in mortality at metamorphosis (Bergeron et al., 2011b). Effects of combined exposure via multiple pathways may be stronger than either pathway alone, yet few studies have examined interactive effects of dietary and maternal exposure to Hg in wildlife.

Maternal transfer is recognized as an important pathway of exposure to contaminants, but sublethal effects remain poorly understood. As the first study to examine sublethal effects of maternally derived contaminants in snakes, we have provided a strong foundation for future research. Extensions of this study could involve examining effects on other complex behaviors such as defensive strategies, mate finding, and courtship. Finally, snakes play important roles in ecosystems by acting as both abundant predators and prey and any impacts on their survival may in turn affect other species in the food web as well as the overall function of Hg-contaminated ecosystems (Matthews et al., 2002; Reid and Croxall, 2001). Therefore, long-term studies are needed to more fully understand factors influencing Hg accumulation and maternal transfer and whether the effects we observed are sufficient to affect snake populations inhabiting the South River or other contaminated sites.

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