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High levels of maternally transferred mercury do not affect reproductive output or embryonic survival of northern watersnakes (Nerodia sipedon)

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HIGH LEVELS OF MATERNALLY TRANSFERRED MERCURY DO NOT AFFECT REPRODUCTIVE OUTPUT OR EMBRYONIC SURVIVAL OF NORTHERN WATERSNAKES (*NERODIA SIPEDON*)

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Abstract—Maternal transfer is an important exposure pathway for contaminants because it can directly influence offspring development. Few studies have examined maternal transfer of contaminants, such as mercury (Hg), in snakes, despite their abundance and high trophic position in many ecosystems where Hg is prevalent. The objectives of the present study were to determine if Hg is maternally transferred in northern watersnakes (*Nerodia sipedon*) and to evaluate the effects of maternal Hg on reproduction. The authors captured gravid female watersnakes ($n = 31$) along the South River in Waynesboro, Virginia, USA, where an extensive Hg-contamination gradient exists. The authors measured maternal tissue and litter Hg concentrations and, following birth, assessed (1) reproductive parameters (i.e., litter size and mass, neonate mass); (2) rates of infertility, death during development, stillbirths, malformations, and runts; and (3) the overall viability of offspring. Mercury concentrations in females were strongly and positively correlated with concentrations in litters, suggesting that *N. sipedon* maternally transfer Hg in proportion to their tissue residues. Maternal transfer resulted in high concentrations (up to 10.10 mg/kg dry wt total Hg) of Hg in offspring. The authors found little evidence of adverse effects of Hg on these measures of reproductive output and embryonic survival, suggesting that *N. sipedon* may be more tolerant of Hg than other vertebrate species. Given that this is the first study to examine the effects of maternally transferred contaminants in snakes and that the authors did not measure all reproductive endpoints, further research is needed to better understand factors that influence maternal transfer and associated sublethal effects on offspring. Environ. Toxicol. Chem. 2013;32:619–626. © 2012 SETAC

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INTRODUCTION

Maternal transfer of contaminants, such as mercury (Hg), is a notable pathway of exposure for wildlife [1]. Mercury is a contaminant of particular concern due to its prevalence in aquatic systems, known toxicity, and ability to be transferred from a female to her offspring [1–3]. The highly toxic organic form, methylmercury (MeHg), readily bioaccumulates and generally comprises a high percentage of the Hg transferred from female to offspring [4–6]. Moreover, exposure to maternally transferred Hg may be more detrimental than dietary or environmental exposure because offspring are subjected to contaminants during sensitive developmental stages [7]. Mercury has been shown to negatively affect reproduction in several vertebrate species. Reduced clutch size and number of fledglings, increased embryonic mortality, and thinned eggshells are among the documented negative effects of Hg on avian reproduction [3,8]. In fish, maternally derived Hg can reduce hatching success and embryonic survival as well as increase rate of infertility [9]. Similar decreases in hatching success as well as overall reductions in viability have been documented in American toad (*Bufo americanus*) embryos maternally exposed to Hg [1]. Although maternal transfer has gained recognition as an important pathway for contaminant exposure, it has seldom been studied in reptiles.

Contaminants are among the six suspected contributors to global reptile declines, but few studies have examined contaminant exposure, maternal transfer, or associated effects in this class of vertebrates [10,11]. Among reptiles, snakes are especially overlooked, with relatively scant contaminant information available for only six of the 15 families [11]. Snakes feed at high trophic levels and exhibit several ecological and life-history traits that increase their susceptibility to bioaccumulation of Hg and other contaminants [12]. For example, longevity and small home range size may subject snakes to prolonged exposure and localized sources of contaminants [13]. In addition, aquatic snake species may be particularly at risk of Hg bioaccumulation. A recent study found that northern watersnakes (*Nerodia sipedon*) from an Hg-contaminated river had among the highest mean Hg concentrations among vertebrates inhabiting the site [14]. Within *N. sipedon*, large females had the highest overall Hg concentrations [14]. Presumably, females that accumulate high Hg concentrations subsequently transfer high concentrations to their offspring. However, little is known about maternal transfer of contaminants in snakes. The only study of which we are aware reported selenium transfer in captive brown house snakes (*Lamprophis fuliginosus*) but did not examine associated effects on offspring [15].

The objectives of the present study were to determine whether Hg is maternally transferred in northern watersnakes (*N. sipedon*) collected along an Hg-contaminated river and to assess any associated effects on reproduction. Specifically, we measured total Hg and the percentage of Hg that was methylated in 31 gravid females (tail tissue and blood) and their offspring (whole bodies). We assessed several measures of reproductive performance (litter size, total litter mass, and mean neonate mass) and litter characteristics (sex ratio, frequency of infertility, stillbirths, embryonic mortality, malformations, runts,

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and overall viability). We hypothesized that Hg is transferred from female tissues to offspring in a residue-dependent manner and that females with high Hg concentrations would exhibit reduced size and mass of litters as well as increased rates of infertility, malformations, and stillbirths.

METHODS

Study species

The northern watersnake (*Nerodia sipedon*) is a mid-sized (to 150 cm total length) nonvenomous colubrid snake that is found in most freshwater habitats in the eastern United States [16]. They are primarily piscivorous and are documented to consume over 80 fish species [16]. Watersnakes have been considered as indicators of Hg contamination due to their aquatic nature and piscivorous diet, which puts them at high risk of Hg bioaccumulation [17].

Mating in *N. sipedon* occurs from May to June, and females give birth to live young in August to September. Gestation lasts from 9 to 12 weeks, during which time gravid females continue feeding [18,19]. Litter sizes range from 4 to 99 but typically average between 20 and 40 young [16]. *Nerodia sipedon* are primarily lecithotrophic (embryos nourished by yolk), but there is recent evidence that the placenta present in *N. sipedon* can transfer nutrients to developing offspring as well as perform respiratory functions [20,21].

Study site

The South River, located in central Virginia, USA, was historically contaminated by Hg from an acetate fiber manufacturing plant located in Waynesboro, Virginia, that used mercuric sulfate from 1929 to 1950 [22]. An extensive Hg gradient now spreads for 200 km downstream of the Hg source, ranging from low concentrations upstream of the plant to extremely high concentrations downstream [23]. Advisories for fish consumption have been in place since 1977, and Hg levels in fish and other wildlife inhabiting the South River remain high [23]. Snakes were collected along the contamination gradient and from two nearby reference sites: a portion of the South River upstream of the Hg point source and the Middle River, a river with low Hg concentrations located 37 km northwest of Waynesboro.

Animal collection and husbandry

Gravid female *N. sipedon* ($n = 31$) were captured by hand at sites along the Middle and South Rivers between June 14 and July 31, 2011 (see Drewett et al. [14] for map). Nine females were captured from reference locations; the remaining 22 females were collected along the contamination gradient (river miles 1–22 downstream of the Hg source) at the South River. Sampling at different locations along the gradient provided a wide range of Hg values in females and their offspring.

On capture, each snake's snout–vent length (SVL), tail length (TL), and mass were measured. Snakes were sexed by examination of tail morphology, and the reproductive status of females was assessed by gently palpating the abdomen for the presence of developing ova. A small tail clip (<1 cm, <0.5 g) and a 0.5-ml blood sample were taken from each gravid female for Hg analysis. After clipping tails, wounds were lightly cauterized with a medical cautery unit [24] to stop bleeding and prevent infection. Blood and tail clip samples have been shown to be reliable nonlethal indicators of heavy-metal concentrations in internal tissues of snakes, including *N. sipedon* [17]. Due to differential turnover rates, the blood and tail are thought to represent shorter-term and longer-term Hg exposure, respectively [17]. One female was not sampled for blood and tail at capture because she had sustained recent injuries. After collection, tissue samples were frozen at -80°C prior to analysis.

Gravid female *N. sipedon* were transported to the laboratory at Virginia Tech and kept in a walk-in environmental chamber set at 25°C. Snakes were housed individually in 75-L aquaria with aspen bedding substrate, two hide boxes, a basking lamp (14 light:10 dark), and a large water bowl. The basking lamp provided a thermal gradient from approximately 25 to 35°C during the day, allowing females to thermoregulate within their preferred body temperature range of 20.8 to 34.7°C [18]. Females were weighed weekly and at parturition. Once per week throughout gestation, females were offered frozen/thawed fish (various minnows, *Lepomis* spp., and *Micropterus* spp.) equal to 15% of their body mass, collected from within one river mile of their capture location.

Females gave birth between August 9 and September 4, 2011. At birth, offspring were separated into the following categories: infertile ova (no embryo present), died during development (embryo present but not fully developed), stillborn (fully developed but dead at birth), and alive at birth. Full-term neonates were measured (mass, SVL, TL), sexed by manual eversion of hemipenes, and examined for gross malformations. Additionally, any neonates (live or stillborn) weighing less than 2.0 g or measuring less than 150 mm SVL were classified as runts (mean of all neonates: 4.24 ± 0.02 g, 177 ± 0.46 mm). Finally, the total number of inviable offspring (defined as all infertile ova, stillborns, offspring that died during development, and those that were born alive but had major spinal malformations or died within 3 d of birth) was tabulated for each litter. Litter mass was calculated for each female by subtracting her mass immediately following parturition from her prepartum mass. Relative litter mass was calculated as litter mass divided by the female's postpartum mass [25]. Females and healthy neonates were subsequently released at the female's capture location.

Mercury analysis

Tail and a subset ($n = 11$) of blood samples taken from females at the time of capture were analyzed for total mercury (THg). Tail samples were cleaned to remove any possible superficial contamination by rinsing with Millipore water and gently scrubbing with a thin-fibered plastic brush and then lyophilized. Three randomly selected neonates from each litter were killed via an overdose of buffered tricaine methanesulfonate (MS-222), lyophilized, and homogenized. A composite sample containing equal portions from each of the three neonates was analyzed for THg for each litter. Tail, blood, and whole-body neonate samples were analyzed for THg by combustion-amalgamation-cold vapor atomic absorption spectrophotometry (Direct Mercury Analyzer 80; Milestone) at the College of William and Mary, Williamsburg, Virginia, according to U.S. Environmental Protection Agency method 7473 [26]. For quality assurance, samples were run with a replicate, blank, and standard reference materials (DOLT-4 dogfish liver and DORM-3 fish protein; National Research Council of Canada) in every batch of approximately 10 samples. Method detection limits (threefold the standard deviation of procedural blanks) for samples ranged from 0.0013 to 0.0054 mg/kg (ppm), and all samples had THg concentrations that exceeded that limit. Average relative percentage of differences between replicate sample analyses were $13.35 \pm 10.86\%$ for tail tissue/blood and

$12.11 \pm 24.44\%$ for homogenized neonates. Mean percentage of recoveries of THg for DOLT-4 and DORM-3 were $101.8 \pm 0.16\%$ ($n = 36$) and $101.9 \pm 0.46\%$ ($n = 36$), respectively, for tail tissue/blood and $103.9 \pm 0.66\%$ ($n = 8$) and $103.4 \pm 1.35\%$ ($n = 8$) for homogenized neonates. All THg concentrations are reported on a dry-weight basis unless otherwise noted.

To determine how much of the THg found in females and litters was comprised of the more bioavailable form, MeHg, 12 female tail samples and 12 homogenized litter samples were individually analyzed using high-pressure liquid chromatography (Quicksilver Scientific; method QS–LC/CVAF–001). A combination of two blanks, standard reference materials (1:TORT-2 and DOLT-4), and matrix spikes was used for quality control. The limit of detection was $5.0\,E^{-5}$ mg/kg, and all samples had Hg concentrations that exceeded these limits. Percentage of recovery for HgII/MeHg for TORT-2 and DOLT-4 were 105.0/104.7 and 91.3/85.1%, respectively. Matrix spike recovery of HgII and MeHg was 89.7 ± 1.1 and $88.8 \pm 0.5\%$, respectively.

Statistical analyses

All statistical analyses were performed in Microsoft Excel or SAS 9.2 (SAS Institute). Statistical significance was assessed at the $\alpha = 0.05$ level. Where appropriate, data were $\log_{10}$-transformed to improve normality and homoscedasticity. Litters were treated as the statistical unit, and all factors were treated as fixed effects.

Maternal transfer of Hg in *N. sipedon* was assessed by regressing whole-body THg concentrations of litters against THg concentrations of maternal tail tissue and blood. Statistical differences between THg concentrations from reference and contaminated sites for both maternal and litter samples were determined using a one-way analysis of variance (ANOVA). To assess the relationship between %MeHg and THg concentrations, litter THg concentrations were regressed against litter %MeHg.

An analysis of covariance (ANCOVA) was used to assess the influence of relative litter mass (mass lost at parturition, divided by postpartum mass of females) on the total amount (mg) of Hg transferred from females to their offspring. In this analysis, relative litter mass was treated as the main effect and THg concentration (mg/kg) in maternal tail tissue as the covariate. The total amount of Hg transferred was calculated for each female by multiplying litter THg concentration by the total mass of fully developed neonates for each litter. Although infertile ova and other material expelled at birth undoubtedly also contained some Hg, we did not know THg concentrations of these tissues and, thus, conservatively excluded them from the calculation of THg transferred. We also used ANCOVAs to assess the influence of THg concentration in maternal tail tissue (main effect), female body size (SVL, covariate), and their interaction on female reproductive characteristics (litter size, mean neonate mass, and total litter mass). Effects of maternally transferred Hg and female body size on litter characteristics expressed as proportions (counts of infertile ova, deaths during development, stillbirths, spinal malformations, runts, sex ratio [number of females], and overall inviability divided by total litter size) were evaluated using generalized linear models for mixed distributions (SAS PROC GLIMMIX), a procedure capable of modeling noncontinuous distributions. All GLIMMIX models included litter THg concentration as the main effect, maternal SVL as a covariate, and their interaction and used a logit link function to compare proportional differences among litters.

RESULTS

Litter sizes, including live neonates, stillborns, and infertile ova, ranged from 9 to 49, yielding a total of 609 viable offspring and 100 inviable offspring from the 31 females. Mercury concentrations were consistently higher, for both maternal tissue and litters, in samples from the contaminated region of the river compared to reference sites (ANOVA: maternal tail $F_{1,29} = 38.54$, $p < 0.01$; maternal blood $F_{1,10} = 26.07$, $p < 0.01$; litter whole body $F_{1,30} = 20.21$, $p < 0.01$). Maternal THg concentrations in tail tissue of gravid females from reference sites ranged from 0.16 to 0.92 mg/kg dry weight (mean $= 0.42 \pm 0.09$ mg/kg) and from 2.83 to 13.84 mg/kg (mean $= 5.78 \pm 0.55$ mg/kg) for females captured along the contamination gradient. Total Hg concentrations in blood of reference females ranged from 0.03 to 0.30 mg/kg wet weight and from 1.72 to 5.32 mg/kg for females captured along the contamination gradient. Whole-body THg concentrations for litters from reference sites ranged from 0.06 to 1.09 mg/kg dry weight (mean $= 0.20 \pm 0.11$ mg/kg) compared to 1.08 to 10.10 mg/kg (mean $= 3.42 \pm 0.45$ mg/kg) for litters from mothers collected along contaminated sections of the South River. Litter whole-body THg concentrations were strongly and positively correlated with THg concentrations in maternal tail tissue (Fig. 1A; linear

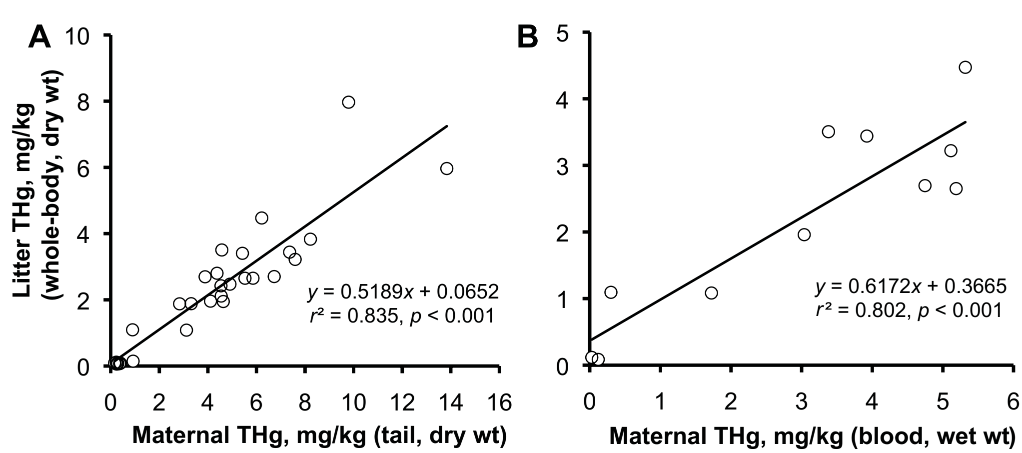

Fig. 1. Relationship between maternal and litter total mercury (THg) concentrations in *Nerodia sipedon* from the South and Middle Rivers, Virginia, USA. Litter values represent whole-body homogenized samples from three randomly selected neonates per litter, whereas maternal values represent (**A**) tail tissues and (**B**) blood.

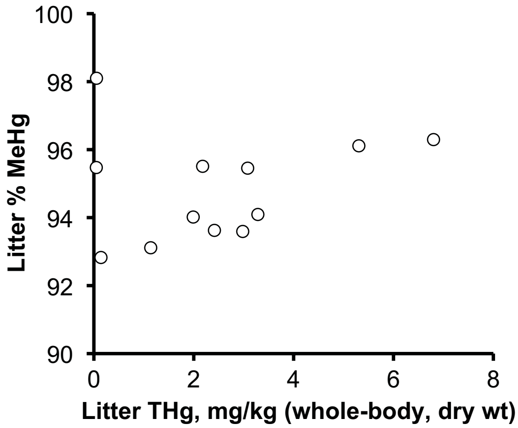

Fig. 2. Relationship between percentage of methylmercury (MeHg) and total mercury (THg) concentrations in litters of *Nerodia sipedon* from the South and Middle Rivers, Virginia, USA.

regression $r^2 = 0.84$, $p < 0.01$) and blood (Fig. 1B; $r^2 = 0.80$, $p < 0.01$). Methylmercury constituted 92.8 to 98.1% (mean 95.0 ± 0.45%) of litter THg and did not correlate with litter THg concentrations (Fig. 2; $r^2 = 0.03$, $p = 0.55$).

The total amount of Hg transferred from females to their offspring ranged from 0.003 to 1.24 mg (mean = 0.27 ± 0.06 mg). As expected, total Hg transferred was strongly influenced by THg concentration in maternal tail tissue (Fig. 3A; ANCOVA $F_{1,27} = 80.32$, $p < 0.01$). However, we also detected a significant effect of relative litter mass on total Hg transferred ($F_{1,27} = 5.98$, $p = 0.02$), indicating that females putting more effort into reproduction excreted higher quantities of Hg than females with lower relative litter masses.

Reproductive data for the 31 litters of *N. sipedon* are summarized in Table 1, as well as the relationships between reproductive characteristics, maternal body size (SVL), and maternal and litter THg concentrations. Maternal SVL was positively correlated with litter size (Fig. 4A, Table 1) and total litter mass (Fig. 4B) but not neonate mass (Fig. 4C). None of these characteristics were significantly influenced by THg concentration in maternal tail tissue (Table 1). Mean relative litter mass (mass lost at parturition divided by postpartum mass) was 45 ± 3% (range 16–76%). No trade-off between offspring size and number was detected; litter size was not correlated with mean offspring mass (linear regression $r^2 = 0.06$, $p = 0.16$). Rates of infertility (mean = 7.58%) and stillbirths (mean = 3.58%) were moderate compared to

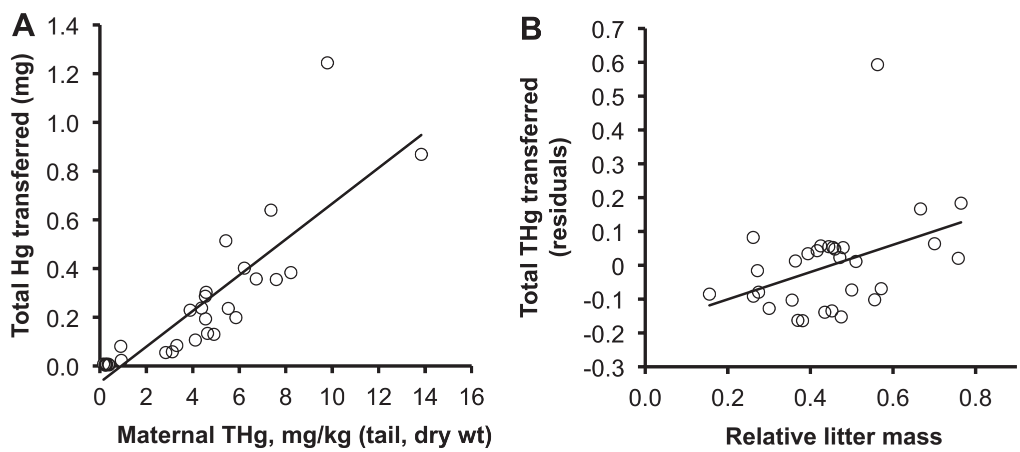

Fig. 3. (**A**) Relationship between total mercury (THg) concentrations in tail tissue of female *Nerodia sipedon* and the THg transferred to the litter. (**B**) Relationship between residuals from regression in (**A**) (THg transferred, correcting for effect of maternal THg concentrations) and relative litter mass (mass lost at parturition/postpartum female mass). Note that residuals are displayed for visualization, but the effects were evaluated statistically using analysis of covariance (ANCOVA).

Table 1. Influence of female body size (snout–vent length [SVL]) and Hg on litter characteristics in *Nerodia sipedon* ($n = 31$ litters, 609 neonates) from the South and Middle Rivers, Virginia, USA[a]

Characteristic	Mean ± SE (%)	Range (%)	SVL effect	Hg effect
Female SVL (mm)	745 ± 15.11	620–942		
Litter size [b,c]	22.87 ± 1.47	9–49	$F_{1,26} = 18.49$, $p < 0.01$[f]	$F_{1,26} = 0.01$, $P = 0.91$
Neonate mass (g)[c]	4.14 ± 0.10	2.60–5.29	$F_{1,26} = 1.09$, $p = 0.31$	$F_{1,26} = 0.62$, $P = 0.44$
Total litter mass[c]	139.10 ± 11.62	35–300	$F_{1,26} = 98.13$, $p < 0.01$[f]	$F_{1,26} = 2.21$, $P = 0.15$
Sex ratio (female)[d]	10.61 ± 0.76 (50.04 ± 1.58)	2–20 (33–71)	$F_{1,27} = 0.21$, $p = 0.65$	$F_{1,27} = 0.18$, $P = 0.67$
No. infertile ova[d]	1.77 ± 0.69 (7.58 ± 2.40)	0–17 (0–48)	$F_{1,27} = 23.18$, $p < 0.01$[f]	$F_{1,27} = 7.75$, $P = 0.01$[f]
No. stillborn[d]	0.90 ± 0.44 (3.58 ± 1.95)	0–11 (0–55)	$F_{1,27} = 14.90$, $p < 0.01$[f]	$F_{1,27} = 0.53$, $P = 0.47$
No. died during development[d]	0.13 ± 0.06 (0.44 ± 0.22)	0–1 (0–4)	$F_{1,27} = 1.47$, $p = 0.24$	$F_{1,27} = 0.06$, $P = 0.80$
No. spinal malformations[d]	0.39 ± 0.20 (1.94 ± 0.97)	0–6 (0–27)	$F_{1,27} = 0.23$, $p = 0.64$	$F_{1,27} = 2.13$, $P = 0.16$
No. runts[d]	0.26 ± 0.11 (0.86 ± 0.37)	0–2 (0–7)	$F_{1,27} = 4.49$, $p = 0.04$[f]	$F_{1,27} = 0.61$, $P = 0.44$
No. inviable offspring[d]	3.23 ± 1.08 (13.70 ± 3.89)	0–24 (0–78)	$F_{1,27} = 35.91$, $p < 0.01$[f]	$F_{1,27} = 2.39$, $P = 0.13$
No. viable offspring[d,e]	19.65 ± 1.44 (86.30 ± 3.89)	5–37 (22–100)		

[a] Characteristics describing litter composition are presented as counts, followed by the value expressed as a percentage of the litter in parentheses. Total litter mass was calculated by subtracting each female's mass immediately following parturition from her prepartum mass.
[b] Includes live and stillborn neonates and infertile ova.
[c] Statistical analyses (ANCOVA) used maternal THg concentrations as the main effect and SVL as a covariate.
[d] Statistical analyses (GLIMMIX) used litter THg concentrations as the main effect and SVL as a covariate.
[e] Not tested statistically because it is the inverse of inviable offspring.
[f] All significant effects of SVL were positive, and the significant effect of Hg was negative.

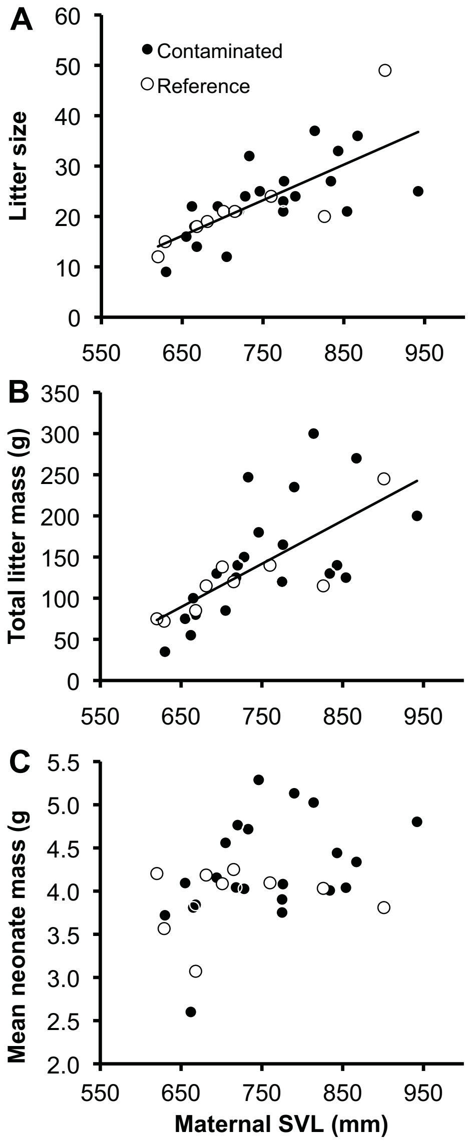

Fig. 4. Influence of body size (snout–vent length [SVL]) on reproductive characteristics of female *Nerodia sipedon* from the South River, Virginia, USA, including (**A**) litter size, (**B**) total litter mass, and (**C**) mean neonate mass. Litters from reference and contaminated sites are indicated, but no relationships between total mercury concentrations in maternal tail tissue and reproductive characteristics were statistically significant (see Table 1).

low frequencies of malformations (mean = 1.94%), runts (mean = 0.86%), and embryos that died during development (mean = 0.44%); however, all of these characteristics exhibited marked variance. Spinal malformations were the only terata observed, consisting of kinks or bends in the spine, sometimes accompanied by abnormal fusion of the epithelium. Maternal SVL was positively correlated with the frequency of infertile ova, stillbirths, and runts (Table 1). We detected a significant, negative influence of litter THg on frequency of infertile ova (Table 1). However, this effect was driven by high rates of infertility in several small, low-Hg females from the contaminated site and one large female from a reference site, as indicated by a significant Hg by SVL interaction (GLIMMIX, SVL by Hg interaction, $F_{1,27} = 8.83$, $p = 0.01$). No other characteristics were significantly influenced by litter THg, either as a main effect or as an interaction with maternal body size (Table 1).

The overall viability (inverse of inviability) of litters was high (mean = 87%) but variable (22–100%). Maternal SVL was negatively correlated with viability, with larger females having lower proportions of viable offspring than smaller females (Table 1, Fig. 5A). As with the other litter characteristics, neither litter nor maternal THg concentrations were significantly related to overall viability of offspring (Fig. 5B).

DISCUSSION

Evidence for maternal transfer of contaminants exists for several taxonomic groups, but in many cases effects remain poorly understood. The present study is the first to examine maternal transfer of Hg and its effects in snakes. We found that THg levels of whole-body tissue of neonates were highly correlated with THg levels found in maternal tail tissue and blood, demonstrating that Hg is transferred from mother to offspring in *N. sipedon*. Although females transferred high amounts of Hg to their offspring, we found no evidence of adverse effects on size, number, or short-term viability of offspring. The present study suggests that *N. sipedon* may be more tolerant of high levels of Hg than other vertebrate taxa and provides a foundation for future research on maternal transfer of Hg and evaluation of effects in snakes. In particular, more research is needed on whether maternally transferred mercury affects behavior and long-term viability of offspring.

Female watersnakes transferred the highest concentrations of THg (up to 10.10 mg/kg of dried neonate body tissue) and the

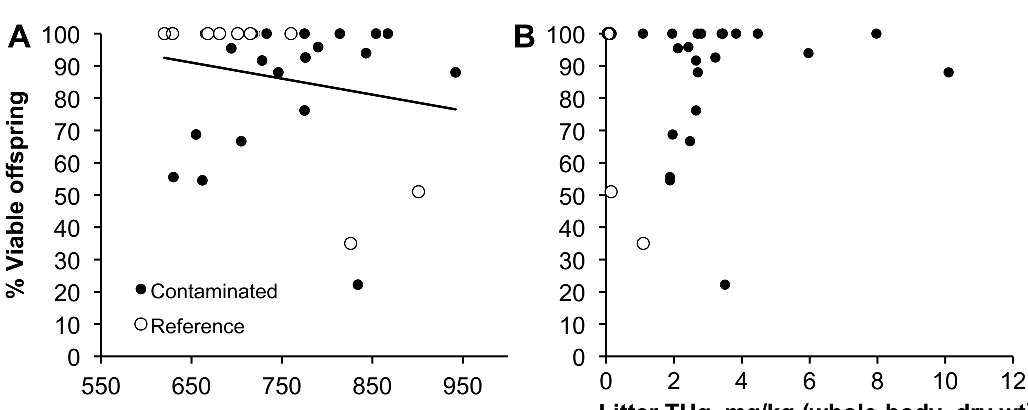

Fig. 5. Relationships between overall percentage of each litter that was viable and (**A**) maternal snout–vent length (SVL) and (**B**) litter total mercury (THg) concentrations of whole-body tissue. Inviable offspring included all infertile ova, stillbirths, offspring that died during development, and those that were born alive but had major spinal malformations or died within three days of birth. Note that the statistical significance of the relationships was assessed using generalized linear models for mixed distributions (GLIMMIX, Table 1), but a linear trend line is provided here for visualization.

highest %MeHg (up to 98%) to their offspring among all vertebrates studied thus far at the South River, one of the most heavily studied Hg-contaminated sites in the U.S. For comparison, peak THg concentrations in American toad eggs ranged from 0.01 to 0.36 mg/kg, and common snapping turtle (*Chelydra serpentina*) eggs had THg concentrations up to 6.61 mg/kg (B. Hopkins, 2012, Master's thesis, Virginia Tech, Blacksburg, VA, USA) [1]. A recent study suggests that the primary reason for higher litter THg concentrations in *N. sipedon* is that they have higher exposure to Hg as indicated by high maternal blood THg concentrations when compared to other species [14]. Studies of other vertebrate species at the South River report mean %MeHg values up to 91% as well as positive relationships between THg concentrations and %MeHg in eggs [4,27,28]. *Nerodia sipedon* neonates also exhibited high %MeHg ($95 \pm 0.45\%$) but, unlike other species, showed a constant relationship between %MeHg and THg concentrations in litters. The lack of a positive relationship between THg and %MeHg might be related to the fact that *N. sipedon* are viviparous and exhibit some degree of placentotrophy [20], which could provide different mechanisms of maternal transfer of Hg compared to oviparous species. Snakes provide excellent opportunities for exploring factors that influence maternal transfer of contaminants because they exhibit a diversity of reproductive strategies (i.e., oviparity, viviparity, income, and capital breeding) and life-history traits. Furthermore, several snake species can often be found in a single habitat, allowing for tractable interspecific comparisons with minimal influence of confounding environmental factors.

Maternal transfer has been proposed to reduce a female's body burden of Hg [29], but this possibility remains poorly understood. Studies of fish and amphibians have suggested that a relatively low percentage (<10%) of female Hg body burden is transferred to offspring [4,9]. We found that female *N. sipedon* transferred up to 1.24 mg of THg to their offspring, but our desire to avoid lethal sampling of adults prohibited us from calculating the percentage of body burden that this amount represents. Field sampling of adult *N. sipedon* at our site has revealed that similarly sized males and females do not differ significantly in THg concentrations of tail tissue [14]. Thus, although females transfer a relatively large absolute amount of Hg to their offspring, this appears to be a relatively small proportion of their overall body burden. This pattern may indicate that the maternal diet is a major source of Hg transfer, as has been observed in fish [5]. Alternatively, if the Hg maternally transferred by females originated from tissues such as internal organs, the study of tail tissue previously mentioned [14] may not have been sufficient to detect sex differences in organs. However, Wylie et al. [30] also found no sex differences in liver Hg concentrations of giant garter snakes (*Thamnophis gigas*). Although we found no evidence that maternal transfer reduces the body burden of Hg in females, we did find that the total amount of Hg excreted may be influenced by the amount of resources allocated to reproduction. After correcting for maternal THg concentration, the total amount of Hg transferred to litters was positively correlated with relative litter mass. This positive relationship indicates that mothers that invest more in reproduction excreted larger quantities of Hg via maternal transfer. This is expected, as females with higher relative litter masses put more resources into reproduction and, thus, transfer greater total amounts of Hg. Contrary to our expectations, we did not detect a positive correlation between maternal SVL and investment, indicating that body size and possibly age do not play a strong role in a female's rate of Hg excretion.

Trophic interactions are often considered to be the primary pathway for movement of Hg through food webs [31], but our results suggest that maternal transfer may also play an important role. The majority of the Hg bioaccumulated by female watersnakes, and subsequently transferred to their offspring, originated from prey acquired in the aquatic environment, particularly fish [16]. Survival of young watersnakes is generally low [18], with known predators including snapping turtles, alligators, predatory fish (bass, catfish, and pickerel), mammals (raccoons, otters, mink, and skunks), predatory birds (herons, egrets, bitterns, rails, and hawks), and other snakes [18]. In addition, juveniles are abundant along streams, with density estimates of 0.4 individuals per linear meter [32]. The high total amounts of Hg transferred from females to their offspring, in conjunction with high densities and frequent predation rates on juveniles, suggest that maternal transfer may play an important role in the movement of Hg in the South River food web. Moreover, because many of the predators known to consume watersnakes are primarily terrestrial, predation on young watersnakes may be an important mechanism for transport of Hg from aquatic to terrestrial habitats.

Mercury has been shown to negatively affect reproductive parameters in several vertebrate species at the South River. Tree swallows (*Tachycineta bicolor*) from contaminated sites produced fewer fledglings than those from reference sites [33]. Bergeron et al. [1] observed decreased hatching success and viability with increasing egg THg concentrations in American toads. Hopkins (2012, Master's thesis) documented adverse effects on reproduction of snapping turtles inhabiting the South River. Specifically, clutches from contaminated areas averaged 11 to 12% lower hatching success, 153 to 425% higher rates of embryonic mortality, and 49 to 174% higher rates of infertility compared to reference locations. In the present study, the only reproductive parameter that was significantly influenced by litter THg concentrations was rate of infertility, but this effect was likely due to a coincidence of site (habitat) effects on rates of infertility and Hg levels in females, rather than a biologically meaningful effect of Hg. Lack of adverse effects of Hg in the present study suggests that *N. sipedon* at this site may be relatively tolerant of Hg compared to other taxa and supports the idea that species differ substantially in their responses to Hg. However, the population that we sampled has been under continuous exposure to Hg for over a half-century; therefore, we cannot rule out the possibility that the tolerance we observed for high Hg body burdens is particular to this population and could be the result of rapid evolution of detoxification mechanisms. Watersnakes at the South River might provide an ideal opportunity to evaluate evolutionary responses to persistent environmental contaminants.

Although we found little evidence of effects of maternally derived Hg on reproductive parameters in *N. sipedon*, our study provides data that will be useful for evaluating factors that affect reproduction in this relatively well-studied species. Our results agree well with other studies in terms of litter size (8–37, Weatherhead et al. [34]; 10–34, Weatherhead et al. [35]), offspring size (1.5–6.1 g, Weatherhead et al. [35]; 1.5–4.8 g, Ernst and Ernst [18]), and sex ratio (51% female, Weatherhead et al. [34]). In addition, our study confirmed expected relationships between female body size, litter size, and relative litter mass [35,36]. However, contrary to Weatherhead et al. [35], we did not observe a trade-off between litter size and offspring size. An additional difference between our study and previous investigations of reproduction in *N. sipedon* is that we found higher rates of infertility and stillbirths (7.7 and 3.9% of all births) and that both of these rates increased with increasing female body size. This relationship could be interpreted as evidence of senescence as larger females are likely older than smaller females. However, we believe that it is more likely a reflection of some snakes being collected from suboptimal habitat. Not all reaches of the South River contained optimal habitat for watersnakes, and sampling along the Hg contamination gradient necessitated focusing on some of these areas that did not provide ideal conditions (e.g., forested areas with deep water and shady banks). Watersnakes were noticeably less abundant in these areas, which may have limited mating opportunities and contributed to increased rates of infertility. Furthermore, suboptimal habitats might have impeded resource acquisition and proper thermoregulation prior to capture.

Maternal transfer is gaining recognition as an important pathway for exposure to contaminants, but the effects of maternally derived contaminants remain poorly understood in reptiles. We have established that female *N. sipedon* transfer high levels of Hg to their offspring and that the majority of this Hg is in the more bioavailable form, MeHg. We also provide the first data on effects of maternally derived contaminants in snakes. Although we found no clear effects of Hg on reproductive output and embryonic survival, future studies are needed to more fully explore this subject. As a potent neurotoxicant, maternally transferred Hg has the potential to adversely impact nervous system development, thereby causing sublethal effects on behavior, coordination, and cognition that might not be detectable until later in life. Thus, an obvious extension of this research would be to explore sublethal and/or latent effects of maternally transferred Hg on the physiology or behavior of young watersnakes and to follow these snakes through development. Furthermore, subsequent dietary exposure could interact with sublethal effects of maternally derived Hg, potentially producing unanticipated consequences. For example, Bergeron et al. [37] found that individually maternal and dietary Hg exposure produced sublethal effects on American toad larvae but combined, these exposures increased mortality at metamorphic climax by 125% in comparison to larvae from reference mothers that were fed control diets. These findings underscore the need to conduct further studies on the effects of maternally derived contaminants in snakes.

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