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Evidence and patterns of maternal transfer of metals and trace elements in Western tiger snakes (*Notechis scutatus occidentalis*) – a pilot study

Damian Christopher Lettoof, James Urban Van Dyke, Marthe Monique Gagnon

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Abstract:

Urban wildlife are regularly exposed to a variety of anthropogenic contaminants that have the potential to bioaccumulate in body tissues. As a consequence, developing embryos and offspring can be at risk from exposure to maternally-accumulated contaminants, yet this has rarely been reported in reptiles. We opportunistically collected one pregnant Western tiger snake (*Notechis scutatus occidentalis*) from each of three wetlands with differing sediment metal contamination around Perth, and analysed maternal snake livers and three foetuses per litter for a suite of 17 elements representing either alkaline earth metals, transition metals or metalloids. We detected 14 elements, and compared their concentrations in maternal livers to foetus whole-bodies to determine preliminary patterns of maternal transfer. Our results suggest antimony, arsenic, manganese, mercury, molybdenum and zinc are maternally transferred in Western tiger snakes. We urge further research to further quantify patterns of contaminant maternal transfer in viviparous snakes, and determine their impacts on the development and health of contaminated offspring.

Keywords:

ecotoxicology, bioaccumulation, embryonic development, urbanisation, pollution

Introduction:

Urban wildlife are often chronically exposed to anthropogenic contaminants, such as metals and pesticides (Murray et al. 2019). For terrestrial vertebrates, diet is usually the most significant route of contaminant exposure; however, developing embryos can be exposed to maternally transferred contaminants. Currently, research on maternal transfer of contaminants in reptiles is mostly limited to the oviparous taxa: turtles (Ehsanpour et al. 2014) and crocodilians (Rauschenberger et al. 2004); whereas squamates, particularly viviparous species, have received little attention. In addition, most of this research has focused on organic contaminants due to their lipophilic properties (Rowe 2008), despite many metals being shown to transfer to offspring during development (Ehsanpour et al. 2014). The knowledge of maternal transfer of elements in snakes specifically, is limited to mercury in the viviparous wetland species *Nerodia sipedon* (Chin et al. 2013a; Cusaac et al. 2016) and selenium in the oviparous terrestrial species *Lamprophis fuliginosus* (Hopkins et al. 2004).

During an ecotoxological study in Perth, Western Australia (Lettoof et al. 2020), Western tiger snakes (*Notechis scutatus occidentalis*) were collected and euthanised to analyse element concentration and potential bioaccumulation in their livers. Of these snakes, one pregnant female from each of three wetlands was collected. We took this opportunity to screen three foetuses from each litter to compare concentrations of 14 elements (five non-essential metals and nine trace elements) to element concentrations in their mothers' livers and explore patterns of maternal transfer. Herein we present a preliminary study on the first evidence of maternal transfer in a viviparous snake. Our results justify further research on this system and enrich the global knowledge of maternal transfer of metals and trace elements in snakes.

Methods:

Between March and April 2019, one pregnant tiger snake was hand collected from each of three wetlands (Herdsman Lake and Bibra Lake within urban Perth, and Loch McNess within Yanchep National Park) that differed in their degree of urbanisation and contamination with metals and other trace elements (Lettoof et al. 2020). Specifically, Herdsman Lake generally had the highest sediment concentration of all screened elements including arsenic, copper, lead and zinc concentrations exceeding Australian government guidelines. Bibra Lake and Loch McNess differed in element concentrations; however, selenium exceeded Australian government guidelines in both wetlands (Loch McNess four times higher than Bibra Lake). Snakes were humanely euthanised by blunt force trauma, and we removed their livers, foetuses, and infertile ova. After removing the foetuses and ova, we measured the snout-vent length (SVL) and body mass of females and foetuses. We randomly selected three foetuses per litter to analyse for whole-body concentrations of metals. Maternal livers and foetuses were screened for 17 elements (i.e. metals and trace elements) by ChemCentre (Perth, Western Australia); these elements were chosen based on the lack of historical data from these wetlands and regular screening of this suite for environmental monitoring (Leif Cooper, ChemCenter, personal communication). We screened livers only as an index of the snakes' accumulation of the elements that could be compared across sites. Whole livers and foetuses were homogenised, extracted with nitric and hydrochloric acid, and analysed for metals using a combination of inductively coupled plasma-atomic emission spectroscopy (ICP-AES) and inductively coupled plasma-mass spectrometry (ICP-MS). For more detailed descriptions on sites and chemical analysis see Lettoof et al. (2020). For statistical analyses, samples that were recorded below detectable limits were entered as half the detection limit (Zeghnoun et al. 2007). We compared the relationships between maternal liver concentrations (independent variable) and foetus total body concentrations (dependent variable) using a separate mixed-effects regression model for each metal with maternal ID as a random effect, which grouped foetuses by litter to avoid pseudoreplication. All data were log-transformed. Due to small sample sizes we also assessed the relationships visually using scatter plots. Snakes were collected under Western Australia's Department of Biodiversity, Conservation and Attractions Permit No. 08-002624-1. Curtin University's Animal Research Ethics Committee approved the use and handling of snakes for this research under Approval No. ARE2018-23.

Results:

Sixteen elements were detected in foetuses (Table 1; see supplementary material for limits of detection (LODs)). Beryllium was not found above detection limits in any maternal liver or foetus, tin and lead were not detected in most samples hence these metals are excluded from further discussion. Foetuses from the Bibra Lake and Yanchep snakes were smaller, and had less pigmentation, indicating they were a slightly earlier stage of development. Due to small tissue samples, elements could only be quantified as wet weight.

There were significant positive relationships between Western tiger snake maternal liver and foetus concentrations of manganese ($r^2 = 0.61$, $F_{1,6} = 14.19$, $p = 0.009$) and molybdenum ($r^2 = 0.57$, $F_{1,6} = 9.28$, $p = 0.023$), while barium showed a near-significant negative correlation ($r^2 = 0.75$, $F_{1,6} = 4.78$, $p = 0.07$). Relative to maternal liver concentrations, foetus concentrations of antimony, arsenic, mercury, and zinc appeared to increase, while foetus concentrations of cadmium and silver appeared to decrease, but all of these relationships were non-significant, possibly due to small sample sizes (Fig. 1). Foetus concentrations of cobalt, copper, chromium, nickel and selenium remained relatively constant as maternal liver concentrations increased.

Discussion:

Our data indicate statistical evidence of maternal transfer of manganese and molybdenum between contaminated female Western tiger snakes and their foetuses, and potential maternal transfer of antimony, arsenic, mercury and zinc. Evidence for maternal transfer of arsenic, mercury and zinc exists in other reptiles, however this is limited to turtles and one snake (Chin et al. 2013a; Ehsanpour et al. 2014; Van Dyke et al. 2014). We could not find any published literature on maternal transfer of antimony, manganese or molybdenum in reptiles. Although these elements have been shown to maternally transfer in humans (Krachler et al. 1999), we present here the first evidence of maternal transfer in a reptile. We acknowledge that the element concentrations lacking statistical relationships or correlative patterns are not indicative of a lack of maternal transfer, and may suggest partial maternal elimination. We also acknowledge that these patterns could change or be clarified with more data; and thus, our preliminary results indicate that further studies exploring maternal transfer of metals and trace elements in snake populations likely to be exposed to pollution are warranted.

Interpreting patterns of maternal transfer from small data sets and single points in time is difficult in viviparous squamates, as they potentially rely on two periods of nutritional allocation: vitellogenesis prior to ovulation and placental transport during pregnancy (Van Dyke and Griffith 2018). These two potential periods of reproductive allocation could transfer the same maternal metals to developing foetuses differently, depending on the stage of development when the mother is exposed to the elements. For example, if a mother accumulates a contaminant during or before vitellogenesis, it should be incorporated in the yolk during vitellogenesis (Van Dyke et al. 2012), from where it would expose developing embryos. As vitellogenesis takes place in the liver, the element concentrations of foetuses should reflect that of their mother's liver if they were incorporated into yolk during vitellogenesis. A similar pattern would be present if the same mother was exposed to the element post-ovulation and it was transported via the placenta. However, most viviparous squamates are not highly placentotrophic (Van Dyke and Griffith 2018), so the placenta may be incapable of transporting metals that were accumulated post-ovulation. Alternatively, the placenta may exclude elements bound to metallothionein as is shown in placental mammals (Yoshida et al. 2002). If no placental transport occurred, the data would show higher maternal liver concentration and lower foetus body concentration for elements the mother accumulated during gestation. This would suggest maternal transfer does not occur for said element across a placenta, which could be reflected by the absent or negative correlations that our data show for barium, cadmium and silver (Fig. 1). Our small sample size and single-point in time testing (during late development) do not allow us to confidently deduce whether these elements are transferred during vitellogenesis or placentation, but provide some indication that either or both mechanisms might be possible in viviparous squamates like tiger snakes.

Regardless of the mechanism, manganese and molybdenum's significant positive correlations between maternal livers and foetuses suggest these metals were maternally transferred to the developing foetuses, while antimony, arsenic, mercury, and zinc all exhibit patterns supporting the potential for maternal transfer. Arsenic, manganese, molybdenum and zinc are trace elements essential for biological functions, and therefore some degree of maternal transfer of these is expected. Maternal liver and foetus manganese concentrations are similar and potentially reflect essential developmental requirements, whereas molybdenum concentrations are much lower in foetuses and therefore this element may not be as important during this stage of tiger snakes lifecycle. The non-essential metals, i.e. antimony, cadmium, mercury etc. are known to maternally transfer due to their lipophilic properties and affinity for metalloproteins (Guirlet et al. 2008).

Although trace elements are usually regulated by the body, over exposure can lead to accumulation and toxicity, and non-essential metals usually bioaccumulate more easily and are toxic at much lower concentrations. Some of the sampled wetlands have sediment contaminated with arsenic, zinc and mercury concentrations exceeding Australian and New Zealand Environment and Conservation Council (ANZECC) sediment quality guidelines; and antimony, arsenic, mercury, molybdenum and zinc bioaccumulate in tiger snakes at these wetlands (Lettoof et al. 2020). The potential for these elements to maternally transfer in tiger snakes suggests they are not only cycling throughout the food-web of these wetlands, but have potential to impact vertebrates during embryogenesis, when they may be most at risk of biological effects of contamination (Wolfe et al. 1998).

Exposure to contaminants via maternal transfer may be more harmful to the foetus than exposure from diet in adults, as the organism is exposed during sensitive stages of development (Russell et al. 1999). The developmental consequences of maternally transferred contaminants in snakes are virtually unknown, as previous research is limited to three studies of a single non-essential metal and snake species. Maternally acquired mercury was shown to not have any effect on *Nerodia sipendon* reproductive output or offspring survival (Chin et al. 2013b), nor stress levels (Cusaac et al. 2016); however, it did reduce offspring strike efficiency and motivation to feed (Chin et al. 2013a). In other taxa, maternal transfer of selenium and strontium resulted in lower hatchling success, developmental abnormalities and abnormal swimming in *Gastrophryne carolinensis* toad larvae (Hopkins et al. 2006), while *Esox lucis* fry suffer higher frequency of deformities and edema when maternally exposed to selenium (Muscatello et al. 2006). Offspring starting life with such handicaps may experience lower survival, higher predation and ultimately reduce population replenishment. Eventually, exposure to contaminants during embryonic and foetal development can have detrimental impacts on both the individual organism and population.

Data from this study, although limited, offer preliminary patterns of maternal transfer of a suite of metals and trace elements in Western tiger snakes. Tiger snakes in Perth may be particularly sensitive to population impacts from maternal transfer as they are restricted to wetlands both enclosed and chronically contaminated with various metals, metalloids and other organic compounds from urbanisation. We suspect tiger snakes maternally transfer antimony and mercury that they have accumulated in urban Perth wetlands, and arsenic, manganese, molybdenum and zinc as part of natural offspring development but may be susceptible to higher loads from over exposure and bioaccumulation. Thus, we urge further research into the mechanism of transfer and impacts on the development and health of offspring.

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Table 1. Morphometrics and metal concentrations (wet weight) of wild-caught pregnant *Notechis scutatus occidentalis* livers and whole body concentrations of three randomly chosen foetuses (mean ± SD) per litter. Mothers mass was measured after extraction of foetuses and infertile ova. SVL = snout-vent length, N = no. of foetus/infertile ova, < = below detection limit, $^{\#}$ likely process acting between mother and foetus, * indicates statistical positive relationship between maternal liver and foetuses concentrations.

Figure 1. Relationships between Western tiger snake (*Notechis scutatus occidentalis*) maternal liver and foetus whole-body concentrations (wet weight) of a) non-essential metals, and b) trace elements. Regression line indicate a statistical significant (p = <0.05) relationship. Concentration units are µg/Kg for non-essential metals (a), and mg/Kg for trace elements (b). Axis ranges are unique for each metal.