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Determinants of mercury contamination in viperine snakes, *Natrix maura*, in Western Europe

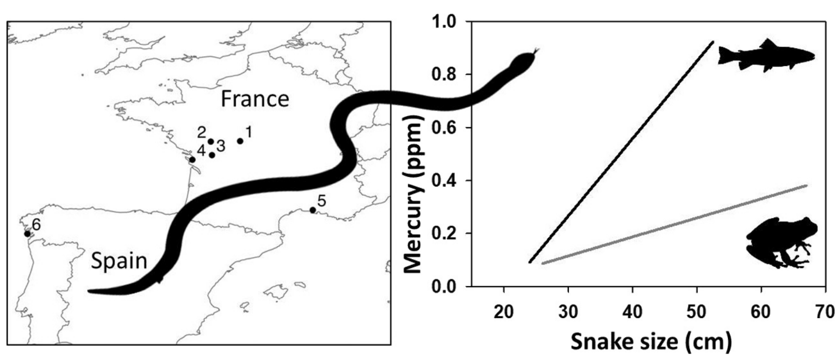
Jérémy Lemaire [a,b], Paco Bustamante [c], Anthony Olivier [d], Olivier Lourdais [a], Bruno Michaud [a], Alexandre Boissinot [a], Pedro Galán [e], François Brischoux [a,*]

[a] *Centre d'Etudes Biologiques de Chizé, CEBC UMR 7372 CNRS-Université de La Rochelle, 79360 Villiers en Bois, France*
[b] *Réserve Naturelle Nationale de Chérine, Maison de la Nature et de la Réserve, 36290 Saint-Michel-en-Brenne, France*
[c] *Littoral Environnement et Sociétés (LIENSs), UMR 7266 CNRS-Université de La Rochelle, 2 rue Olympe de Gouges, 17000 La Rochelle, France*
[d] *Institut de recherche de la Tour du Valat, Le Sambuc, 13200 Arles, France*
[e] *Grupo de Investigación en Bioloxía Evolutiva (GIBE), Departamento de Bioloxía, Facultad de Ciencias, Universidad de A Coruña, Campus da Zapateira, s/n., 15071 A Coruña, Spain*

HIGHLIGHTS

- Hg contamination in freshwater meso-predators has been largely overlooked.
- Hg concentrations were measured in scales of viperine snakes in France and Spain.
- Viperine snakes do accumulate Hg in their scales.
- Diet (fish versus amphibians) influenced accumulation rates of Hg.
- Highest values of Hg were found in individuals from a fish farm.

GRAPHICAL ABSTRACT



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ABSTRACT

The effects of Hg contamination are presumably widespread across the components of aquatic ecosystems, but investigations have been mainly focused on freshwater fish, because this biota represents a major source of Hg for human populations. Yet, the possible bioaccumulation of Hg on other freshwater meso- and apex-predators (e.g., amphibians, reptiles) has been largely overlooked, especially in Western Europe. In this study, the determinants of Hg concentrations were assessed for the viperine snake (*Natrix maura*) across 6 populations (>130 individuals sampled in 2016 and 2017) in France and Spain. Specifically, body size, sex, and diet were compared with Hg concentrations measured in ventral scales. Overall, *N. maura* accumulated Hg in their scales. Sex did not seem to influence Hg concentrations in this species. Significant differences in Hg concentrations were observed between study sites, and these differences were likely to be mediated by site-specific diet. Frog-eating individuals were characterized not only by lower mean values of Hg (0.194 ± 0.018 $\mu g \cdot g^{-1}$ versus 0.386 ± 0.032 $\mu g \cdot g^{-1}$ for piscivorous individuals), but also by weaker slopes of the body size-Hg relationship as compared to fish-eating snakes, suggesting strong differences in accumulation rates due to food resources. Importantly, the highest slope of the body size-Hg relationship and the highest values of Hg were found in individuals foraging on trout raised by a fish farm, suggesting that fish farming may contribute to Hg contamination in inland

* Corresponding author.
E-mail address: francois.brischoux@gmail.com. (F. Brischoux).

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freshwater systems. Finally, our results are compared with data on Hg concentrations in other species of aquatic snakes, in order to provide a comparative point for future studies.

1. Introduction

Mercury (Hg) is a well-known environmental contaminant which can originate from both natural and anthropogenic sources (Fitzgerald et al., 2007; Selin, 2009). Atmospheric and water circulation patterns tend to concentrate Hg in aquatic environments (Mason et al., 2012). In addition, anoxic conditions found in slow-moving water bodies promote the transformation of inorganic Hg in methyl-Hg by micro-organisms (Compeau and Bartha, 1985; Jensen and Jernelöv, 1969), which is known for its toxic effects on humans and wildlife (e.g. Tan et al., 2009; Scheuhammer et al., 2008). Finally, Hg can be bioaccumulated within organisms and biomagnified through the food chain, and concentrated in apex predators as fishes, birds or mammals (Mason et al., 1995; Atwell et al., 1998; Power et al., 2002).

Over the last decades, the potential deleterious effects of Hg have been investigated from both an ecological and public health perspective (Driscoll et al., 2013; Lavoie et al., 2013; Eley, 1997; Wolfe et al., 1998). Effects of Hg are multiple and cover a large spectrum of syndromes such as neurological dysfunction (Steuerwald et al., 2000; Basu et al., 2005; Clarkson and Magos, 2006; Scheuhammer and Sandheinrich, 2007; Depew et al., 2012), endocrine disorders (Wada et al., 2009; Meyer et al., 2014) or altered reproduction and offspring quality (Klaper et al., 2006; Burgess and Meyer, 2008; Bergeron et al., 2011; Hopkins et al., 2013a; Tartu et al., 2013). Heretofore, the comprehensive assessments of Hg contamination across complex environments and trophic webs are challenging. Indeed, although the toxic effects of Hg are presumably widespread across the components of aquatic ecosystems, investigations of Hg contamination have been mainly focused on freshwater fish (e.g., Depew et al., 2013; Åkerblom et al., 2014; Scheuhammer et al., 2014; Eagles-Smith et al., 2016), probably because this biota represents both a major source of protein for many human populations (Futsaeter and Wilson, 2013; Dong et al., 2015; Lepak et al., 2016; Fliedner et al., 2016). Aquatic birds have also attracted considerable scientific attention in this respect (Ackerman et al., 2016; Jackson et al., 2016; Blukacz-Richards et al., 2017; Sullivan and Kopec, 2018; Żarski et al., 2017; see also Whitney and Cristol, 2017 for a review). Yet, other aquatic vertebrates than fishes, and especially meso- and apex-predators, may well suffer from Hg contamination (e.g. Driscoll et al., 2007). Such overlooked organisms include amphibians (e.g., Bergeron et al., 2010; Todd et al., 2011), aquatic snakes (e.g., Burger et al., 2005; Drewett et al., 2013), and turtles (e.g., Meyer et al., 2014; Slimani et al., 2017). This is especially true in Western Europe where investigations of Hg contamination in aquatic tetrapods are very scarce as compared with other geographic areas such as Northern America. Nevertheless, inclusion of these organisms is of crucial importance if we are to globally assess Hg contamination worldwide, and in turn to monitor its effects on biodiversity and human health (Gustin et al., 2016).

In addition, some lineages of these overlooked meso- and apex predators also provide a unique set of features that make them useful biological tools to monitor Hg contamination in the wild (e.g., Burger et al., 2005; Slimani et al., 2017). For instance, as compared with highly mobile fish and birds, aquatic reptiles and amphibians are characterized by high levels of philopatry associated with relatively low capacities for large scale movements (Hillman et al., 2014). As a consequence, Hg concentrations in their tissues should strongly reveal those of their relatively small home ranges while highly mobile organisms such as fish and birds may provide information that integrates Hg contamination over large distances, and thus, different environments (Burger et al., 2007; Drewett et al., 2013; Slimani et al., 2017). Additionally, aquatic reptiles are situated relatively high in the trophic web, and as ectotherms, they display relatively low metabolic rates and relatively high tissue conversion rates of their food resources which should enhance their capacity to integrate long-term Hg contamination in their tissues. Characterized by an indeterminate growth, many aquatic reptile species display very wide size range between minute neonates and large adult individuals which allow access to bioaccumulation processes within a population. Finally, easily accessible tissues such as claws in turtles or scales in snakes, in which Hg tends to accumulate and bind to keratins (Hopkins et al., 2013b), provide a powerful opportunity to adopt a non-invasive technique in order to assess Hg contamination in these organisms (Schneider et al., 2015).

In this study, Hg concentrations were investigated in a widely distributed European semi-aquatic Natricinae, the viperine snake (*Natrix maura*). More than 130 individuals distributed across 6 populations situated in France and Spain were sampled. These populations were associated with contrasted ecological contexts and dominant trophic resources (mainly amphibians in 3 sites and fishes in 3 other sites, Table 1). A non-invasive technique (scale-clipping) was used in order to assay Hg concentrations across this wide geographic area. In addition, on a sub-sample of individuals, scale clipping was combined blood sampling to assess the relationship between Hg concentrations in the blood (reflecting short term Hg exposure, i.e., weeks) and in the scales (integrating Hg exposure over a longer time scale, i.e., months) in this species (as shown in other semi-aquatic snake species, Burger et al., 2005). Specifically, the aims of this study were:

1) to assess Hg contamination in this species across a large ecological context,
2) to investigate bioaccumulation rates across a wide range of body size,
3) and to examine the influence of sex and diet on Hg contamination and bioaccumulation rates.

2. Material and methods

2.1. Study species and study sites

The viperine snake (*Natrix maura*) is a semi-aquatic freshwater natricine widely distributed across Western Europe and Northern Africa, broadly from France to Morocco (Miras et al., 2015). This relatively small-sized species (up to ~80 cm total length) typically forages for fish and amphibians in aquatic environments such as streams, rivers, marshes, and lakes (Miras et al., 2015; Santos and Llorente, 2009). A skin-shedding occurs in *N. maura* at the onset of the activity period in early spring, while another shedding cycle is later associated with ovulation shortly before oviposition in females (June–July). Our sampling occurred in late spring between these two periods.

The six study sites were distributed in France and Spain and cover the different habitat types in which *N. maura* typically occurs (Table 1, see also graphical abstract). From north to south, the study sites were 1 - Réserve Naturelle de Chérine within the Brenne Natural Park, France (hereafter “Brenne”), 2 - Lac du Cébron, France (hereafter “Cébron”), 3 - The Boutonne river at Fontenille-Saint Martin d'Entraigues, France (hereafter “Fontenille”), 4 - The Réserve Naturelle de Moëze-Oléron, France (hereafter “Moëze”), 5 - The Réserve Naturelle de la Tour du Valat, France (hereafter “Tour du Valat”), and 6 - Ons island, Spain (hereafter “Ons”).