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Trace Element Concentrations in European Pond Turtles (*Emys orbicularis*) from Brenne Natural Park, France

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Abstract

We assessed trace elements concentration in European pond turtle (*Emys orbicularis*) from Brenne Natural Park (France). We sampled road-killed turtles (N = 46) to measure the concentrations of 4 non-essential (Ag, Cd, Hg, and Pb) and 10 essential (As, Co, Cr, Cu, Fe, Mn, Ni, Se, V, and Zn) elements in muscle, skin, liver and claws. Body size or sex did not influence the concentrations of most elements; except for Hg (liver, skin and claws) and Zn (muscle) which increased with body size. We found relatively high concentrations of Hg and Zn, possibly linked to fish farming. This result deserves future investigations to evaluate possible ecotoxicological effects on *E. orbicularis*.

Keywords Trace elements · Contamination · Wetlands · *Emys orbicularis*

Wetlands are important habitats for biodiversity, yet they are among the most endangered ecosystems in the world, suffering from a drastic reduction of their surface and from a degradation of water quality (Schneider et al. 2017). Monitoring environmental contaminations in wetlands is difficult because they are connected to complex and large hydric networks composed by both surface and underground waters. As a consequence, the connectivity of aquatic systems can induce contamination in areas that otherwise appear unscathed from direct sources of pollution (Baker 1992). Among environmental contaminants, trace elements are well-known for their ability to enter and move across aquatic environments (Agarwal 2009). These elements comprise both non-essential elements (that exhibit high toxicity at low concentrations) and essential elements (that may become toxic when they exceed normal levels) (Förstner and Wittman 1981). Overall, trace elements represent a threat to aquatic ecosystems because of their high toxicity, persistence, bioaccumulation in organisms and biomagnification across trophic levels (Agarwal 2009).

Freshwater turtles are suitable organisms to survey contamination levels in complex aquatic ecosystems (Overmann and Krajicek 1995; Ayub et al. 2001; Nagle et al. 2001; Bergeron et al. 2007; Yu et al. 2011; Malik et al. 2013; Yadollahvand et al. 2014; Allender et al. 2015; Slimani et al. 2018). First, they are widely distributed and occupy a variety of habitats. Second, they have a long life expectancy, which allows studying bioaccumulation processes and long-term trends of contaminants. Finally, most species are sedentary and provide information on the contamination at a precise location.

In this study, we used opportunistic sampling of road-killed European pond turtles (*Emys orbicularis*) to study trace element contamination of one of the largest French wetlands: the Brenne Natural Park. The goals of our descriptive study were to document the concentrations of 4 non-essential trace elements (Ag, Cd, Hg, and Pb) and 10 essential trace elements (As, Co, Cr, Cu, Fe, Mn, Ni, Se, V, and Zn) in the muscle, skin, liver and claws of *E. orbicularis*; and to investigate the relationship between the concentrations of these elements and the sex and the size of the turtles.

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Materials and Methods

Emys orbicularis is a small European freshwater turtle species (Priol et al. 2008). Carcasses of accidentally killed pond turtles were found on roads situated in vicinity of the Réserve Naturelle Nationale de Chérine (46°47′25.23″N, 1°12′3.54″E) between 2008 and 2014. A total of 46 turtles were collected including 38 adults (17 females, 16 males, 5 unsexed individuals) and 8 juveniles. We used plastron length as an index of body size.

All individuals were dissected to take samples of the posterior right leg muscles and skin, liver and claws (Table 1). Because of the limiting mass of the claw samples, only Hg concentrations were measured in this tissue. The muscles, skin and liver samples were lyophilized and hand-ground with a porcelain mortar and pestle. Claws were washed three times with a mixture of 2:1 chloroform/methanol solution in an ultrasonic cleaner and rinsed in milli-Q quality water. Claws were then dried for 48 h at 50°C. The average weights of the tissue samples used for trace element analysis were 200 mg for muscles samples, 215 mg for skin samples, 119 mg for liver samples and 12 mg for claws.

Total Hg concentrations were measured using and atomic absorption spectrophotometer (Advanced Mercury Analyser-254, Altec) on dried tissue aliquots (ranging from 2 to 10 mg) as described by Chouvelon et al. (2009). Accuracy was checked using TORT-2 Lobster Hepatopancreas (NRC, Canada; certified Hg concentration: 0.27 ± 0.06 µg g^{-1} dw) as certified reference material (CRM). Our measured values were 0.245 ± 0.003 µg g^{-1} dw (N = 4) showing a recovery of 91%. Blanks were analysed at the beginning of each set of samples and the detection limit of the method was 0.05 ng.

Other elements (Table 1) were analysed using a Varian Vista-Pro ICP-OES and a Thermo Fisher Scientific X Series 2 ICP-MS (following Kojadinovic et al. 2011). CRM (DOLT-4 dogfish liver and TORT-3 Lobster Hepatopancreas, NRC, Canada) and blanks treated and analysed in the same way as the samples were included in each analytical batch. The recovery ratio were in good agreement with DOLT-4 [94% (Ag); 99% (As); 105% (Cd); 104% (Cu); 90% (Fe); 99% (Ni); 87% (Pb); 101% (Se) and 101% (Zn)] and TORT-3 [109% (As); 101% (Cd); 101% (Co); 95% (Cr); 103% (Cu); 86% (Fe); 96% (Mn); 97% (Ni); 93% (Pb); 101% (Se); 99% (V) and 96% (Zn)]. The limits of quantification (µg g^{-1}) were 0.01 (Ag, Co, Cd, Mn, Pb); 0.12 (As, Cr); 0.02 (Cu); 5.03 (Fe, Zn); 0.05(Ni); 0.5 (Se); and 1.26 (V). Trace element concentrations are expressed in µg g^{-1} dw.

When trace elements concentrations were lower than the limit of detection (LoD) in more than 30% of the samples (Table 1), these samples were excluded from statistical analyses (EPA 2000). When trace elements concentrations were lower than LoD in less than 30% of the samples, values below LoD were replaced by (LoD) × 0.5 for statistical analyses (EPA 2000). To investigate the influence of sex on trace element concentrations, possible sexual size dimorphism was considered: plastron length was compared between sexes with a Kruskal–Wallis test (data were not normally distributed). Because females were larger than males (see results), we used ANCOVAs to test for sex effects in

Table 1 Trace elements concentrations (µg g^{-1} dw) in skin, muscle, liver and claws (Hg only) of *E. orbicularis*

Elements	Muscle		Skin		Liver		Claws	
	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD
Essential								
As	35/39	0.64 ± 0.39	42/44	1.17 ± 1.02	15/16	0.56 ± 0.57	–	–
Co	39/39	0.14 ± 0.09	44/44	0.18 ± 0.19	16/16	0.48 ± 0.91	–	–
Cr	39/39	7.36 ± 4.55	42/43	4.00 ± 2.04	8/16	3.87 ± 12.68	–	–
Cu	39/39	5.00 ± 0.82	44/44	3.02 ± 0.92	16/16	11.98 ± 4.54	–	–
Fe	39/39	279 ± 115	42/42	316 ± 173	11/11	464 ± 164	–	–
Mn	34/34	6.79 ± 4.72	40/40	14.32 ± 13.68	16/16	10.44 ± 13.41	–	–
Ni	39/39	2.72 ± 2.13	43/43	1.61 ± 0.82	15/16	0.63 ± 1.42	–	–
Se	38/38	3.17 ± 1.03	44/44	2.25 ± 0.83	16/16	7.56 ± 7.89	–	–
V	0/39	<LoD	2/44	0.99 ± 0.12	5/17	9.64 ± 4.51	–	–
Zn	39/39	161 ± 30	44/44	64 ± 15	16/16	139 ± 36	–	–
Non-essential								
Ag	15/39	0.019 ± 0.010	25/44	0.026 ± 0.021	11/17	0.027 ± 0.016	–	–
Cd	26/39	0.015 ± 0.012	24/44	0.013 ± 0.012	15/15	0.129 ± 0.123	–	–
Hg	39/39	0.662 ± 0.375	44/44	0.484 ± 0.367	17/17	1.128 ± 1.077	43/43	1.346 ± 0.939
Pb	39/39	0.21 ± 0.19	44/44	0.36 ± 0.31	16/18	0.74 ± 2.51	–	–

N = sample with concentration above the limit of detection (LoD)/total sample size trace element concentrations, using plastron length as the covariate. To investigate the influence of body size (hence presumably age) on trace element concentrations, and thus the bioaccumulation of these elements through time, we used Spearman rank's correlation between trace elements and plastron length.

Results and Discussion

The mean concentrations of each trace element are presented in Table 1. Only four elements (Cd in muscle and skin; Cr in liver; Ag in muscle, skin and liver and V in muscle, skin and liver) were below the LoD in > 30% of individuals (Table 1).

Although females were larger than males (Kruskal–Wallis χ^2=19.7449, df = 1, $p < 0.01$), sex did not influence the concentrations of trace elements (all $p > 0.12$). This suggests that feeding, metabolism or growth rates were broadly similar for both genders (Allender et al. 2015; Yu et al. 2011) and that contrarily to what has been shown in another species (Nagle et al. 2001) or in sea turtles (Guirlet et al. 2008), eggs may not represent a major excretion pathway in female *E. orbicularis*.

Most trace elements concentrations did not correlate with body size suggesting that *E. orbicularis* does not bioaccumulate these contaminants with age as showed in other turtle species (Allender et al. 2015; Yadollahvand et al. 2014). Yet, and similarly to other studies (Overmann and Krajicek 1995; Nagle et al. 2001; Bergeron et al. 2007; Yu et al. 2011), we found two notable exceptions to this trend: Zn concentrations (in muscle, $r_s = 0.49$, $p < 0.05$; Fig. 1) and Hg concentrations (in liver, skin and claws) were correlated with body size (respectively $r_s = 0.56$, $p < 0.05$; $r_s = 0.44$, $p < 0.05$; $r_s = 0.40$, $p < 0.05$; Fig. 2). These results suggest that these elements bioaccumulate over the life of the turtle. Interestingly, these two elements were found in relatively high concentrations (up to 208 µg g^{-1} dw for Zn and 4.451 µg g^{-1} dw for Hg), suggesting that excretion rates do not compensate for Zn and Hg exposure.

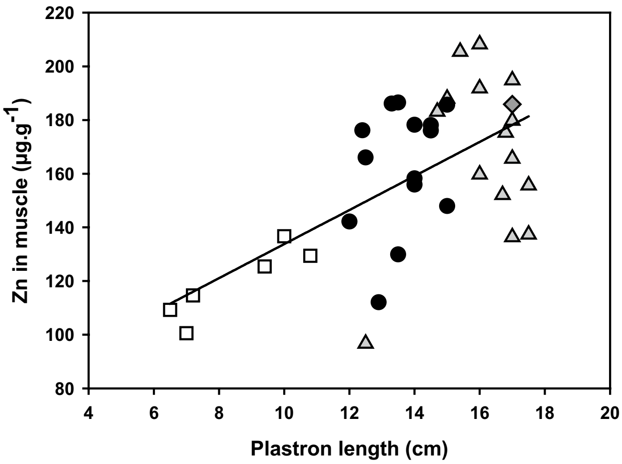

Fig. 1 Relationship between turtle size (plastron length) and concentrations of Zn in muscles. Symbols stand as follow: white squares for juveniles, black circles for adult males, light grey triangles for adult females and dark grey diamonds for unsexed adults

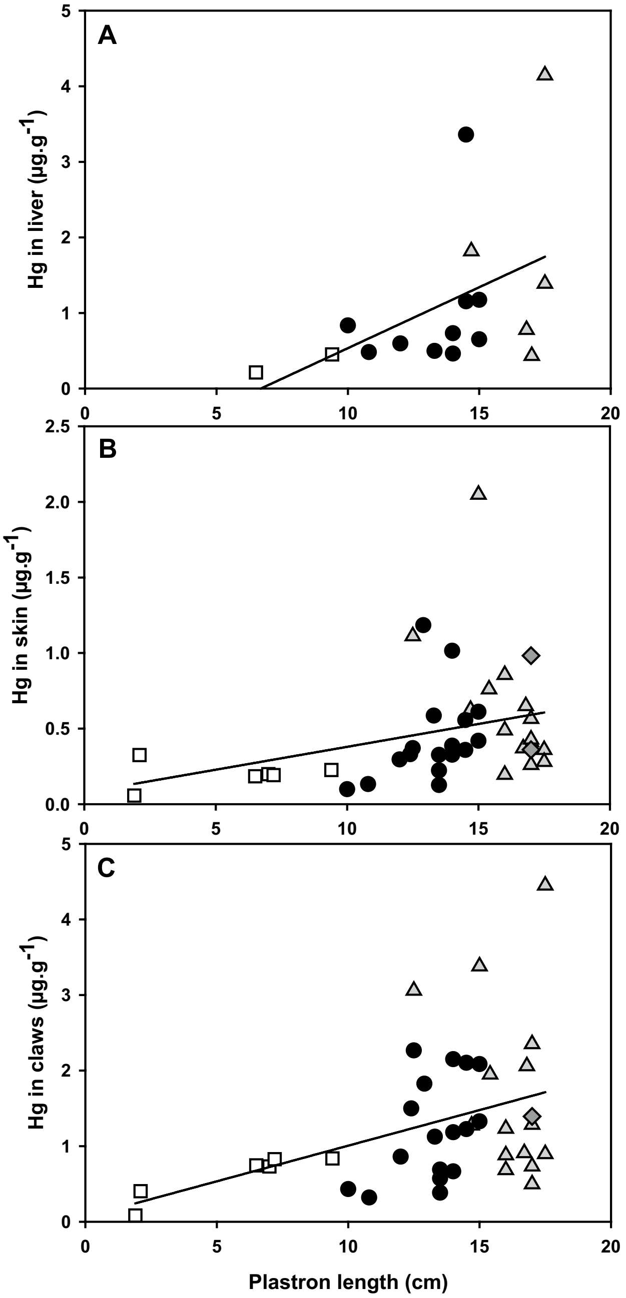

Fig. 2 Relationship between turtle size (plastron length) and concentrations of Hg in **A** liver, **B** skin and **C** claws. Symbols stand as follow: white squares for juveniles, black circles for adult males, light grey triangles for adult females and dark grey diamonds for unsexed adults

Bioaccumulation of Hg in its methylated form is a well-known process in vertebrates as this non-essential metal is not regulated and bound to proteins potentially leading to adverse effects (Eagles-Smith et al. 2018). It is noteworthy that the value of Hg we report in various tissues of *E. orbicularis* are among the highest reported for freshwater turtles (Yu et al. 2011; Zapata et al. 2014; Slimani et al. 2018), and the consequences of such high values should be investigated.

Zn is an essential metal subjected to homeostatic regulation as it included in the functional groups of various enzymes, play a structural role in respiratory pigments and metalloenzymes, and can act as activating co-factor for various proteins (see e.g. Simkiss 1979; Williams 1981). Accordingly, Zn concentrations measured here could be physiological and thus not a problem on the health status of *E. orbicularis*. Nonetheless, the increase of Zn concentrations with age is likely due to its accumulation on specific metalloproteins such as metallothioneins which serve as intracellular protein in metal homeostasis (Vallee 1991). Future investigations on metallothioneins in *E. orbicularis* would be needed to validate this hypothesis. In addition, the values we report are substantially higher than those reported for other species of turtles (Malik et al. 2013; Yadollahvand et al. 2014), and the potential effects of such high values of Zn should be investigated.

Although the sources of Hg and Zn contamination remain unknown, several hypotheses can be proposed. For instance, the concentrations of Hg we detected may be related to a natural contamination due to the methylating activity of microorganisms in anoxic sediments rather than a direct anthropogenic source of contamination (Morel et al. 1998). Alternatively, relatively high concentrations of both Hg and Zn could be linked to the composition of fish pellets used for fish farming that occur in many ponds of Brenne. Indeed, commercial fish pellets exhibit high Hg concentrations of marine origin (Hansson et al. 2017; see also Lemaire et al. 2018) and are enriched with Zn oxide (EFSA 2012). Future studies should usefully investigate both the origin of Hg and Zn as well as the physiological and toxicological consequences of elevated levels of these elements for *E. orbicularis* (Meyer et al. 2014).

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