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Blood levels of metallic trace elements are influenced by sex, age and habitat in the European pond turtle (*Emys orbicularis*)

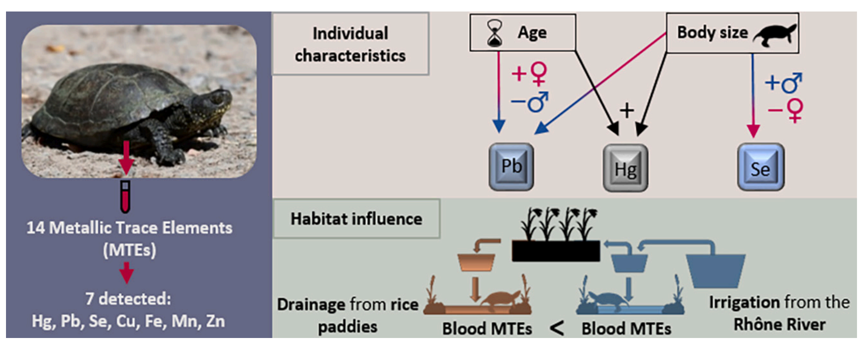
Leslie-Anne Merleau [a,b,c,*], Aurélie Goutte [a], Anthony Olivier [c], Marion Vittecoq [c], Paco Bustamante [d], Carole Leray [c], Olivier Lourdais [b,e]

[a] *École Pratique des Hautes Études, PSL Research University, UMR 7619 METIS, Sorbonne University, Paris, France*
[b] *Centre d'études biologiques de Chizé, CNRS, UMR 7372, Villiers en Bois, France*
[c] *La Tour du Valat, Research Institute for the Conservation of Mediterranean Wetlands, Arles, France*
[d] *Littoral Environnement et Sociétés (LIENSs), UMR 7266 CNRS-La Rochelle Université, 2 rue Olympe de Gouges, 17000 La Rochelle, France*
[e] *School of Life Sciences, Arizona State University, Tempe, AZ, USA*

HIGHLIGHTS

- 7 metallic trace elements among 14 were detected in all individuals.
- Individual traits and study site were the main factors explaining MTE levels.
- Levels of Pb increased with body size and age in females but not in males.
- The intra-individual repeatability was high and significant for Hg, Pb and for Se.

GRAPHICAL ABSTRACT



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ABSTRACT

Metallic trace elements (MTEs) constitute a major source of chemical pollution and represent a threat to aquatic ecosystems and organisms. Important variation in contamination may exist at a local scale in relation to the environment (hydrosystem, trophic ressources) and individual traits (age, sex). Heretofore, the factors influencing MTEs exposure of freshwater reptiles in temperate regions are not fully understood. Freshwater turtles have a relatively high trophic position and a long lifespan, thus being potentially highly exposed due to bioaccumulation and bioamplification processes. We investigated MTE blood concentrations from two populations of the European pond turtle *(Emys orbicularis)* in the Camargue wetland (France). These populations, monitored since 1997, differ in their habitat and exposure (irrigation versus drainage canal). In this study, we detected 7 MTEs (Cu, Fe, Hg, Mn, Pb, Se, and Zn) which levels depended on site and individual characteristics. Hg was positively related to body size and age, indicating an increase of exposure in older individuals. We found differences between males and females with the interaction with body size for Pb and Se and with age for Pb. Nitrogen and carbon stable isotopes varied only marginally between individuals and were poorly associated with MTEs concentrations, showing that trophic position might not explain MTEs contamination for these populations. At the individual level, Hg, Pb, and Se blood values were repeatable over years. Further studies should

* Corresponding author at: EPHE-UMR 7619 - Case Courrier 105, Sorbonne Université, 4 Place Jussieu, 75252 Paris Cedex 05, France.
E-mail address: leslie-anne.merleau@ephe.psl.eu (L.-A. Merleau).

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concentrate on potential ecophysiological effects to such exposure, especially since we recently evidenced that these populations of *E. orbicularis* are highly exposed to organic contaminants, which can lead to synergistic effects.

1. Introduction

Metallic trace elements (MTEs) constitute a significant part of environmental pollution at a global scale (Ullrich et al., 2001; Pacyna et al., 2007; Ali and Khan, 2019). MTEs can originate from natural sources, mainly volcanism and soil and rock erosion, but most of the environmental contamination originates from anthropogenic sources, especially from industry, agriculture and gas combustion (Ullrich et al., 2001; Pacyna et al., 2007; Ali and Khan, 2019). In addition to their production from various and widespread human activities, MTEs can be transported by atmosphere and water, leading to an expansion of the contamination by leaching, transportation by soil particles and deposition of airborne emissions (Pacyna et al., 2007; Ullrich et al., 2001; Vareda et al., 2019). Therefore aquatic environments constitute sinks for MTEs, where the local physicochemical characteristics of the water and the sediments (pH, redox potential, salinity, organic matters) can influence their bioavailability (Deb and Fukushima, 1999; Zhang et al., 2014; Väänänen et al., 2018). In addition, in these ecosystems, the methylation of mercury (Hg) by Fe- and sulfate-reducing bacteria enhances the uptake and toxicity of Hg for organisms (Driscoll et al., 2013).

All the MTEs have a potential for toxicity at certain concentrations, still, some of them play essential roles in the physiological functions of organisms, such as copper (Cu), iron (Fe), manganese (Mn), selenium (Se) and zinc (Zn) (Grillitsch and Schiesari, 2010). In turn, cadmium (Cd), mercury (Hg), lead (Pb) do not participate in molecular or physiological processes and have detrimental effects on organisms (Grillitsch and Schiesari, 2010; Ali and Khan, 2019). These non-essential elements, Cd, Hg, Pb can bioaccumulate with increasing age in regularly polluted environments (Burger, 2008; Lavoie et al., 2013; Pain et al., 2019). Furthermore, Hg has the specificity to biomagnify across the trophic chain, leading to higher levels of concentrations for species in top trophic positions (Lavoie et al., 2013). Exposure to these non-essential elements potentially increases oxidative stress (Ortiz-Santaliestra et al., 2018; Soldatini et al., 2020), endocrine system disruption (Burger, 2008; Tan et al., 2009; Grillitsch and Schiesari, 2010; Tartu et al., 2013; Meyer et al., 2013; Pain et al., 2019) and immune dysfunctions (Grillitsch and Schiesari, 2010). These effects could also be transgenerational as maternal transfer of MTEs to the eggs has been shown in different species such as birds and reptiles (Yu et al., 2011; Hopkins et al., 2013b; Van Dyke et al., 2014; Ackerman et al., 2016; Nilsen et al., 2020).

Stable isotopes can be useful to assess with MTE body burden (Le Croizier et al., 2016) as they can inform on foraging habitat and position in the trophic chain (Post, 2002). Nitrogen stable isotope values ($\delta^{15}N$) increase with trophic position because of the enrichment in ^{15}N with the diet in animals (Peterson and Fry, 1987; Post, 2002). In the case of carbon stable isotopes, the ratio between ^{13}C and ^{12}C ($\delta^{13}C$) is used to determine the origin of carbon by the primary producers in trophic chains (Peterson and Fry, 1987; Post, 2002). Thus, increased levels of Hg are usually related to higher levels of $\delta^{15}N$, illustrating the biomagnification of Hg, as shown in seabirds and freshwater fishes (Lucia et al., 2016; Liu et al., 2018; Mills et al., 2020; Jouanneau et al., 2022), as well as in reptiles (Bergeron et al., 2007; Hopkins et al., 2013a; Lemaire et al., 2021a).

Reptiles are particularly vulnerable to habitat degradation (Cox et al., 2022) and are considered as relevant bioindicators of pollution (Sparling et al., 2010; Weir et al., 2010; Ortiz-Santaliestra et al., 2018; Silva et al., 2020). While they are still largely understudied in ecotoxicological studies, MTEs are among the most monitored contaminants for this group. Long lifespan, spatial fidelity and low metabolism are typical characteristics of chelonian (turtles and tortoises) that make them relevant to address long-term and local contamination of the environment. Mercury levels in different tissues exhibit strong correlation in turtle species, allowing the use of red blood cells from minimal invasive blood samples (Hopkins et al., 2013a). In addition, analysis of MTEs in red blood cells reflects the levels that circulate in the organism and that can thus impact organs and physiological functions (Hopkins et al., 2013b). It also allows us to assess recent contamination when compared to scales that represent long-term and chronic exposure (Turnquist et al., 2011).

Freshwater turtles are particularly relevant models to grasp local contamination of aquatic ecosystems. Previous assessments on MTEs levels in red-eared sliders, common snapping turtles, Iberian pond turtles and European pond turtles have shown that local sources of contamination or environmental characteristics were important factors explaining MTEs levels in these species (Turnquist et al., 2011; Yu et al., 2011; Hopkins et al., 2013b; Beau et al., 2019; Ortiz-Santaliestra et al., 2019). However, assessing the bioaccumulation through the lifespan of individuals was generally restricted to comparison between age classes (juveniles vs. adults) in most of these studies. Long-term population monitoring provides a unique opportunity to address determinants of exposure. We studied MTEs in two neighboring populations of *E. orbicularis* in the Camargue wetland, monitored by capture-mark-recapture since 1976 (Olivier, 2002; Olivier et al., 2010). Only limited exchanges exist between these populations. The area includes several potential sources of heavy metal pollution: irrigation by the Rhône River (Dendievel et al., 2020; Ferrand et al., 2012) and the vicinity of the industrial zone of Fos-sur-Mer on the other side of the Rhône River (Austruy et al., 2019). Our long-term monitoring allows us to examine the influence of specific traits such as age and sex in two contrasted sites (irrigation vs drainage areas). Recent studies have pointed that this species appears to constitute a good bioindicator for chemical pollution (Burkart et al., 2021; Merleau et al., 2024) with local variation between irrigation and drainage areas. However little is known about exposure levels of MTEs for this vulnerable species (Beau et al., 2019). We determined the blood concentrations of 14 MTEs (Ag, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, V, and Zn) over three years (2018 to 2020) in 257 individuals, allowing us to investigate inter- and intra-individual variations in MTE levels. We also measured two isotopes ($\delta^{13}C$ and $\delta^{15}N$) to test for trophic differences during the same period. We examined the following questions:

1) How the population, year and individual traits (sex, body size, age) shape MTE contamination level? We expect significant variation among habitats with higher MTE values in the drainage area. We also expect higher MTE concentrations in older individuals, especially for Hg and Pb.
2) Do stable isotopes ($\delta^{15}N$ and $\delta^{13}C$) correlate with MTE? Notably, are the trophic position or the primary producers responsible for inter-individual differences in Hg and Pb levels?
3) Are MTE and stable isotope values in the blood similar among years at the individual level? We expect MTE levels to fluctuate within the individuals across the years due to the variation of environmental contamination.

2. Materials and methods

2.1. Population monitoring and captures

The study area is situated in the Camargue wetland, in Southern France in the Natural Reserve of the Tour du Valat (43°30′N, 4°40′E).

The two populations are located in an area with multiple sources of anthropogenic release of MTEs: the Rhône River, the industrial site of Fos-sur-Mer and rice paddies (Ribeiro et al., 2005; Ferrand et al., 2012; Austruy et al., 2019; Dendievel et al., 2020) but mainly differ by their hydrology (Olivier et al., 2010; Burkart et al., 2021; Merleau et al., 2024). The Esquineau wetlands are made up irrigation canals (whose water is pumped from the Rhône) and associated marshes irrigated by this canal system (Burkart et al., 2021; Merleau et al., 2024). The second site, the Faïsses, is crossed by drainage canals of the Fumemorte basin, which irrigate the marshes in this area (Burkart et al., 2021; Merleau et al., 2024). Using funnel traps or by hand, *E. orbicularis* were captured in canals and marshes from the end of April to the end of July in 2018 and 2019 and from the beginning of May to the beginning of August in 2020. As part of a long-term capture-mark-recapture program (1976–2024), turtles are identified individually by notching the marginal and nuchal scales (Ficheux et al., 2014; Olivier, 2002). We checked the traps daily and after being sampled in the laboratory of the Tour du Valat, individuals were released on their exact site of capture (Fay et al., 2023; Olivier et al., 2010). Captures of this protected species were authorized by French Departmental Authorities (Permits: DREAL Cerfa 13616-01; N°13-2020-03-27-007).

2.2. Blood sampling

From 2018 to 2020, we collected 410 blood samples from 257 individuals (mass > 300 g). We collected 1.5 mL (< 1 % of the individual body mass) from the dorsal coccygeal vein using a previously heparinized Terumo syringe and a 25G needle. We centrifuged the samples to retrieve red blood cells separately from plasma and the samples were then frozen at −18 °C until processing at the LIENSs laboratory of La Rochelle University (France). 143 individuals were sampled once, 77 over two years and 37 over the three years of monitoring. Two individuals were sampled twice in the same year in 2018. These samples were included in the general analyses, but these two individuals were not included in intra-individual variation analysis. Following the EU Directive 2010/63/EU requirements for animal experiments, the blood sampling procedure was assessed by an Ethic Committee (Permit: APAFIS #17899-201812022345423 v2).

2.3. Variables collected

2.3.1. Morphology

Using a precision scale (Mettler Toledo PB3001-S), turtles were weighed to the nearest g in the laboratory and dorsal shell length (hereafter "carapace length") was measured with a caliper (cm) (Olivier et al., 2010). We estimated the body condition index with the residuals of a linear regression of the log-transformed mass against the log-transformed carapace length with the addition of sex as a control variable. As described by Castanet (1988) and Olivier (2002), we counted the number of growth rings on the plastron scutes to determine the age in individuals <5 years old. The age was known for 128 individuals including 78 females (age range: 5–43 years) and 50 males (age range: 4–25 years). The gravidity of the females was assessed by pelvic palpation (Olivier, 2002; Beau et al., 2019). We recorded the presence of the eggs and the number of palpations performed on a female by capture season.

2.3.2. Metallic trace elements analyses

Red blood cell aliquots were freeze-dried for 48 h and homogenised. As described in Lemaire et al. (2022), total Hg analyses were performed on aliquots weighing between 0.83 and 2.10 mg using an atomic absorption spectrometer AMA-254 (Advanced Mercury Analyser-254; Altec) with a limit of detection (LoD) of 0.1 ng. At least two replicates were analysed for each sample until the relative standard deviation was <10 %. Validation of the method was obtained by the analysis of certified reference material (CRM) TORT-3 (Lobster hepatopancreas from the National Research Council of Canada (NRCC) with certified Hg value: 0.292 ± 0.022 $\mu g.g^{-1}$ dw) at the beginning of the analyses and CRM DOLT-5 (Dogfish liver from the NRCC, with certified Hg value: 0.44 ± 0.18 $\mu g.g^{-1}$ dw). Following the protocol described in Bustamante et al. (2008), all the other trace elements (Ag, As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Se, V, and Zn) were analysed with Inductively Coupled Plasma (ICP) 5800 VDV - Agilent Technologies and Mass Spectrometry (Thermo Fisher Scientific Series II ICP-MS). Recoveries for Hg were 102.0±1.8 % and 96.6±1.2 % for TORT-3 and DOLT-5, respectively. Recoveries for the other elements varied between 94 % and 109 % for TORT-3 and DOLT-5. The LoD for the different elements analysed by ICP were 0.01 (Ag, Cd, Co, Cr, and Pb), 0.02 (Ni), 0.05 (Cu, Mn and Se), 0.1 (As), 0.2 (V) and 2 (Fe and Zn) $\mu g.L^{-1}$. Trace element concentrations were further expressed in $\mu g.g^{-1}$ dry weight (dw). Hematocrit was not collected in this study but the average values for *E. orbicularis* are usually 22–23 % (Yilmaz and Tosunolu, 2010).

2.3.3. Stable isotope analyses

Carbon and nitrogen stable isotopes were quantified in aliquots of 0.3 to 0.5 mg of freeze-dried red blood cells. The aliquots were processed with an elemental analyser (Flash 2000 or EA Isolink, Thermo Scientific) coupled to a mass spectrometer (Delta V Plus with Conflo IV Interface, Thermo Scientific). Results were expressed in the δ unit notation as deviations from standards (Vienna Pee Dee Belemnite for $\delta^{13}C$ and N_2 in air for $\delta^{15}N$) following the formula: $\delta^{13}C$ or $\delta^{15}N$ = [(Rsample/Rstandard) - 1] × 1000, where R is $^{13}C/^{12}C$ or $^{15}N/^{14}N$, respectively. Two-point calibration was provided by standard of caffeine (USGS-61 & USGS-63). The analytical precision was < 0.10 ‰ for $\delta^{13}C$ and < 0.15 ‰ for $\delta^{15}N$. We were able to determine stable isotope values of carbon and nitrogen in all samples but two.

2.4. Statistical analyses

We conducted all statistical analyses with R software (4.2.2) and the following packages lme4 (Bates et al., 2015), emmeans (Lenth et al., 2023), DHARMa (Hartig, 2016) and rptR (Stoffel et al., 2017). Models were progressively reduced through stepwise elimination of non-significant variables ($p > 0.05$). Statistical values provided for non-significant variables are the one obtained in the last step before their removal from the models. Pairwise comparisons were conducted using Tukey post hoc tests in the emmeans package (Lenth et al., 2023).

2.4.1. Variation in ETM

We used LMMs (Linear Mixed Models) with individual identity as a random effect to control for repeated sampling of individuals. We tested the effects of year, site, sex, carapace length, body condition, and the interaction site × year on the concentration of Cu, Fe, Hg, Mn, Pb, Se and Zn. Se and Zn concentrations were log-transformed to best fit the model assumptions. Because age and body condition were strongly correlated, we also conducted the same LMMs by testing the effect of age on the subset of known-aged individuals ($n = 211$). We also tested the effect of the 7 MTE abovementioned on the body condition, used as the response variable. The lme4 package (Bates et al., 2015) was used and model assumptions were checked by examining residual plots.

2.4.2. Variation in isotopic levels

We used LMMs (Linear Mixed Models) with individual identity as a random effect to control for repeated sampling of individuals. We tested the effects of year, site, sex, carapace length, body condition, and the interaction site × year on the concentration of $\delta^{13}C$ and $\delta^{15}N$. Model assumptions were checked by examining residual plots.

2.4.3. Intra-individual variation analysis

To assess variations in individuals sampled multiple years, we compared groups of individuals sampled by pair of years using paired *t*-test and one-way repeated measures ANOVA for the individuals sampled during three years. The "repeatability" is thereafter defined by the stability of the intra-individual measured parameters over time. We performed individual repeatability tests to assess the intra-individual variation between years, using the package rptR (Stoffel et al., 2017).

3. Results

3.1. Determinants of MTE concentrations

Among the 14 metallic trace elements examined, 7 were above the limit of detection: Cu, Fe, Hg, Mn, Pb, Se, and Zn, and included in further analyses. Ag, As, Cd, Co, Cr, Ni, and V were below the limit of detection in all or >80 % of the samples analysed in this study. We found an effect of population on the concentrations of Hg, Pb, and Zn with higher contamination in Esquineau for all three elements (Table 1, Fig. 1). However the effect for Pb concentrations were mainly due to a few individuals in Esquineau, notably one individual having the maximum concentration of 1 μg.g^{-1} dw. The concentrations of Se were higher in Esquineau in 2018 but not in 2019 and 2020 (Table 1, Fig. 1). We did not observe differences between the two sites for the other MTEs (Cu, Fe, and Mn) (Table 1). We found an effect of the year of capture for each MTE, but the differences between years depended on the MTE. Post-hoc tests showed that Cu, Fe, Hg, Se, and Zn concentrations were the highest in 2018 compared to the two other years. Fe, Se, and Zn levels were also higher in 2019 compared to 2020. Mn and Pb levels were significantly lower in 2018 than in 2019 and 2020. Cu levels were significantly higher in 2019 compared to the two other years. Mean concentrations depending on the year and the site are presented in Supplementary Material.

We found effects of body size on Hg concentrations that were positively correlated with carapace length whereas those of Mn were negatively correlated (Table 1, Fig. 2A, B). We found an interaction between body size and sex only for Pb; while Pb concentrations significantly increased with size for females they decreased for males (Fig. 2C). Se levels increased with body size in males but not in females (Fig. 2D). Males had higher Cu levels than females (Table 1). When considering known-aged individuals, Fe and Hg concentrations increased in older individuals (Fig. 3A, B). Older females had higher Pb concentrations whereas older males tended to have lower Pb ones (Fig. 3C). This effect was still observed when retrieving older females and considering the ages of the two sexes. We did not find an effect of age on Cu, Mn, Se, and Zn concentrations. No MTE was related to the palpation of eggs in females. Body condition as response variable was not correlated with MTE concentrations (all *p* value >0.09).

3.2. Relation with stable isotopes

We were able to determine stable isotope values of carbon and nitrogen in all samples but two. We observed very few variations in stable isotope values: $\delta^{13}C$ ranged from −29.19 to −23.45 ‰ (mean ± SE = −27.27 ± 0.99 ‰) and $\delta^{15}N$ ranged from 6.23 to 12.59 ‰ (mean ± SE = 8.59 ± 0.68 ‰).

We found significant but very slight differences for the $\delta^{13}C$ values between the two populations (Fig. 4A, LMER model: $\chi^2 = 9.417$, df = 1, $p = 0.002$; mean ± SE: −27.45 ± 0.81 ‰ and −26.97 ± 1.18 ‰ for Esquineau and Faïsses, respectively) and between females and males (LMER model: $\chi^2 = 7.858$, df = 1, $p = 0.005$; mean values: −27.41 ± 0.94 ‰ and −27.05 ± 1.02 ‰, respectively). Individuals' age did not influence stable isotope values. No difference in $\delta^{15}N$ values was found between the sites of capture (Fig. 4B, LMER model: $\chi^2 = 2.73$, df = 1, $p = 0.098$). $\delta^{15}N$ values tended to be higher in males (mean ± SE = 8.68 ± 0.69 ‰) than females (mean ± SE = 8.55 ± 0.68 ‰) but the difference was marginal (LMER model: $\chi^2 = 3.7$, df = 1, $p = 0.054$). We found a correlation between Hg and $\delta^{13}C$ (Pearson correlation: $r = -0.49$, $p = 0.004$). Any other correlation between MTEs and stable isotopes was nonsignificant.

Table. 1
Effects of environmental and individual variables on the levels of metallic trace elements in the red blood cells of *Emys orbicularis* in two populations (site) of the Camargue wetland (France). Models were selected using a top-to-bottom approach, based on a general model: MTE ∼ site*year + sex*scale(CL)+ body condition or MTE ∼ site*year + sex*age for the subset of known-aged individuals. For each effect tested we present (1) if selected in the final model: the observed significant effect (in bold) or (2) if removed: the level before exclusion.

	All (410 samples)			Known-aged (211 samples)		
	chi^2	df	p value	chi^2	df	p value
A. Hg						
Year	**9.123**	**2**	**0.01**	**8.943**	**2**	**0.011**
Site	**68.246**	**1**	**< 0.001**	**12.863**	**1**	**< 0.001**
Year × site	1.5587	2	0.458	4.929	2	0.085
Body condition	2.138	1	0.144	–	–	–
CL (scaled)	**7.306**	**1**	**0.007**	–	–	–
Sex	0.454	1	0.501	0.821	1	0.365
Sex × CL(scaled)	0.81	1	0.368	–	–	–
Age	–	–	–	**6.395**	**1**	**0.011**
Sex × age	–	–	–	0.353	1	0.552
B. Pb						
Year	**13.058**	**2**	**0.001**	**6.47**	**2**	**0.04**
Site	**4.255**	**1**	**0.04**	0.297	1	0.585
Year × site	0.936	2	0.626	4.35	2	0.114
Body condition	2.296	1	0.13	–	–	–
CL(scaled)	**15.451**	**1**	**< 0.001**	–	–	–
Sex	**49.892**	**1**	**< 0.001**	0.671	1	0.413
Sex × CL(scaled)	**8.979**	**1**	**0.003**	–	–	–
Age	–	–	–	**16.807**	1	**< 0.001**
Sex × age	–	–	–	**10.67**	**1**	**0.001**
C. Se (log)						
Year	**50.084**	**2**	**< 0.001**	**34.166**	**2**	**< 0.001**
Site	**9.708**	**1**	**0.002**	**4.591**	**1**	**0.032**
Year × site	**17.959**	**2**	**< 0.001**	4.485	2	0.106
Body condition	1.013	1	0.314	–	–	–
CL(scaled)	2.379	1	0.123	–	–	–
Sex	**25.756**	**1**	**< 0.001**	**13.389**	**1**	**< 0.001**
Sex × CL(scaled)	**7.491**	**1**	**0.006**	–	–	–
Age	–	–	–	1.325	1	0.25
Sex × age	–	–	–	2.004	1	0.157
D. Cu						
Year	**11.674**	**2**	**0.003**	**10.371**	**2**	**0.006**
Site	2.078	2	0.15	0.4566	1	0.499
Year × site	2.377	2	0.305	1.062	2	0.588
Body condition	1.64	1	0.2	–	–	–
CL (scaled)	0.051	1	0.821	–	–	–
Sex	**19.187**	**1**	**< 0.001**	**13.846**	**1**	**< 0.001**
Sex × CL(scaled)	0.293	1	0.588	–	–	–
Age	–	–	–	1.418	1	0.234
Sex × age	–	–	–	0.736	1	0.391
E. Fe						
Year	**95.08**	**2**	**< 0.001**	**57.792**	**2**	**< 0.001**
Site	0.162	1	0.687	1.15	1	0.284
Year × site	5.387	2	0.068	0.153	2	0.926
Body condition	< 0.001	1	0.981	–	–	–
CL(scaled)	2.624	1	0.11	–	–	–
Sex	0.209	1	0.647	0.932	1	0.334
Sex × CL(scaled)	0.007	1	0.932	–	–	–
Age	–	–	–	**4.748**	**1**	**0.029**
Sex × age	–	–	–	0.584	1	0.445
G. Mn						
Year	**27.46**	**2**	**< 0.001**	**11.946**	**2**	**0.003**
Site	1.902	1	0.168	0.013	1	0.908
Year × site	0.021	2	0.989	4.776	2	0.092
Body condition	0.323	1	0.569	–	–	–
CL (scaled)	**7.294**	**1**	**0.007**	–	–	–
Sex	1.613	1	0.204	2.567	1	0.109
Sex × CL(scaled)	1.442	1	0.229	–	–	–

(continued on next page)

Table. 1 (*continued*)

	All (410 samples)			Known-aged (211 samples)		
	chi^2	**df**	**p value**	**chi^2**	**df**	**p value**
Age	–	–	–	0.386	1	0.535
Sex × age	–	–	–	3.279	1	0.07
H. Zinc (log)						
Year	**27.058**	**2**	**< 0.001**	**22.458**	**2**	**< 0.001**
Site	**12.576**	**1**	**< 0.001**	3.531	1	0.06
Year × site	1.466	2	0.851	0.898	2	0.638
Body condition	0.146	1	0.702	–	–	–
CL(scaled)	0.517	1	0.472	–	–	–
Sex	2.099	1	0.147	0.124	1	0.634
Sex × CL(scaled)	3.276	1	0.07	–	–	–
Age	–	–	–	0.018	1	0.892
Sex × age	–	–	–	0.134	1	0.714

Footnotes: Results of linear mixed models (LMM) for every MTE, with log-transformed data for Se and Zn. Known-aged: subset of individuals of known age. CL: carapace length. The significant variables retained in the final model are in bold font.

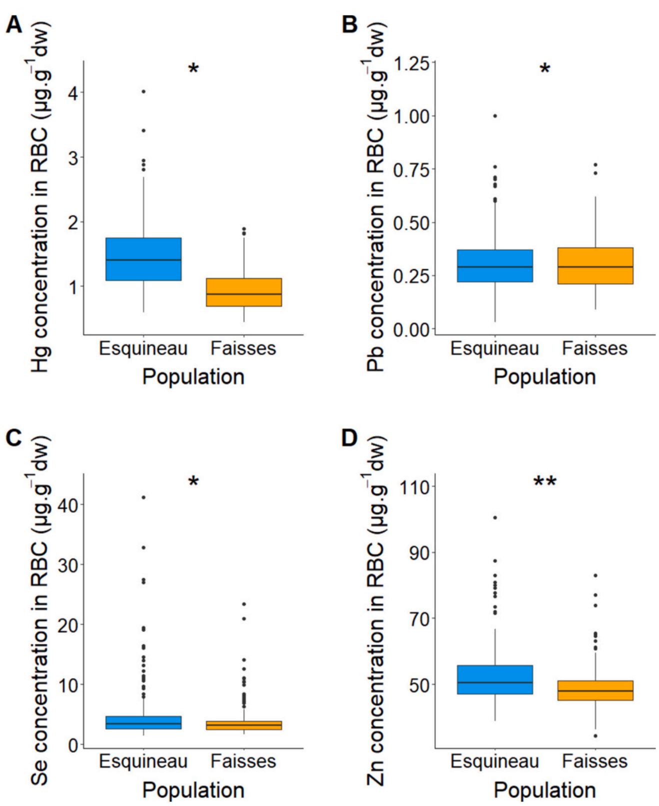

Fig. 1. Variation in Hg (A), Pb (B), Se (C), and Zn (D) concentrations (µg.g^{-1} dw) in red blood cells (RBC) of the individuals sampled in the Camargue wetland, France, among the two populations: Esquineau and Faïsses, $n = 410$ (* and **: significance obtained from the LMM models).

3.3. Repeated measures and individual variation

Individuals sampled during the three years (n = 37) exhibited significant variations in their Fe and Mn levels ($Fe_{2018\text{-}2019\text{-}2020}$: ANOVA, $F_{(2,108)} = 11.242$, $p < 0.001$; $Mn_{2018\text{-}2019\text{-}2020}$: ANOVA, $F_{(2,108)} = 4.771$, $p = 0.01$). Individuals sampled in 2018 and 2019 (n = 20) exhibited higher concentrations of Pb in 2019 (paired *t*-test: $t = -3.02$, df = 19, $p = 0.007$). Individuals sampled in 2019 and 2020 (n = 29), and in 2018 and 2020 (n = 28) exhibited higher Fe and Se concentrations in 2019 and 2018 than in 2020 (paired *t*-tests: $Se_{2019\text{-}2020}$, $t = 3.018$, df = 28, $p <$ 0.01; $Se_{2018\text{-}2020}$, $t = 2.86$, df = 27, p < 0.01; $Fe_{2019\text{-}2020}$, $t = 2.951$, df = 28, p < 0.01; $Fe_{2018\text{-}2020}$, $t = 4.853$, df = 27, p < 0.001). Three MTEs remained statistically constant over the years: Cu, Hg, and Zn.

We found high intra-individual repeatability across time for Hg, Pb, and Se for each pair of years or for all three years (Fig. 5). The repeatability coefficient was respectively between 0.6 and 0.9; 0.6 and 0.8; 0.57 and 0.84 depending on the pairs of years (see Supplementary Material). In turn, the intra-individual repeatability was low and nonsignificant for Cu and Zn, as well as for Fe, except in 2018–2019, and Mn except for 2018–2020 and 2018–2019-2020.

4. Discussion

Despite the ubiquity and toxicity of MTEs, factors influencing their levels in wild populations are still insufficiently understood, notably freshwater turtle species (but see Turnquist et al., 2011; Yu et al., 2011; Hopkins et al., 2013a; Guillot et al., 2018; Ortiz-Santaliestra et al., 2019). Our study is the first to characterize a large set of MTEs, including both essential and non-essential elements, in the blood of *E. orbicularis* and to investigate the environmental and individual factors governing circulating levels. We were able to detect 7 MTEs out of the 14 that were analysed. Most of them were essential trace elements (Cu, Fe, Mn, Se, and Zn). Variation in MTEs were primarily driven by individual characteristics (age, sex) and population.

4.1. Occurrence of trace elements

Two trace elements of major concern were detected in all the samples: Hg and Pb. Blood levels of Hg in our study were in the range of the concentrations found in other freshwater reptiles. In several freshwater turtle species, studies found average blood Hg levels ranging from 0.02 to 4.35 µg.g^{-1} dw (Hopkins et al., 2013a; Meyer et al., 2014; Slimani et al., 2018; Van Dyke et al., 2017). Similar concentrations were found in whole blood of wild caimans, from 0.07 to 2.19 µg.g^{-1} dw (Lemaire et al., 2021a, 2022). However, our results were below the blood concentrations of populations of *Mauremys leprosa* (3.37 to 8.83 µg.g^{-1} dw) living in a region with former mine activities (Ortiz-Santaliestra et al., 2019) and *Chelydra serpentina* (0.05 to 24.95 µg.g^{-1} dw) living downstream a former industrial Hg releasing (Hopkins et al., 2013a). Previous studies in Brenne, France showed two or three fold higher levels in claws *of E. orbicularis* compared to our results (Beau et al., 2019; Guillot et al., 2018). This difference, also observed in other species (Schneider et al., 2011) could be explained by the high bioaccumulation of Hg in keratinized tissues due to the affinity of their protein to metalloid compounds (Grillitsch and Schiesari, 2010). Pb contamination is an issue in the Camargue wetlands due to the use of lead shots for hunting for decades (Hoffmann, 1960; Pain et al., 2019). However, little oxidation of shots seems to occur (Pain, 1991) and Pb was rarely detected in water analyses done by the National Reserve of Camargue (Cheiron, 2019; Cheiron and Bricault, 2020, 2021) probably explaining the similar levels to the ones of freshwater reptiles living in non-contaminated areas (Ortiz-Santaliestra et al., 2019; Lemaire et al., 2022). Interestingly, As, Co, Ni, and V in the blood of *E. orbicularis* were below the detection limit of the method although these MTEs were detected in water samples from the main drainage canal of the area in which the canals of the site of Faisses flow (Cheiron, 2019; Cheiron and Bricault, 2020, 2021). Considering organotropism, levels of certain MTEs could be higher in internal organs in *E. orbicularis* (e.g., Cd in the kidneys) than was what found in this study, especially for trace elements non-detected here.

4.2. Variation among site and year of capture

We found that Hg, Pb, Se, and Zn varied among the two populations with Esquineau showing the highest levels for each of these MTEs, except in 2020 for Se. A difference in trophic position between these two populations is unlikely to explain the difference. Using $\delta^{15}N$ to assess the

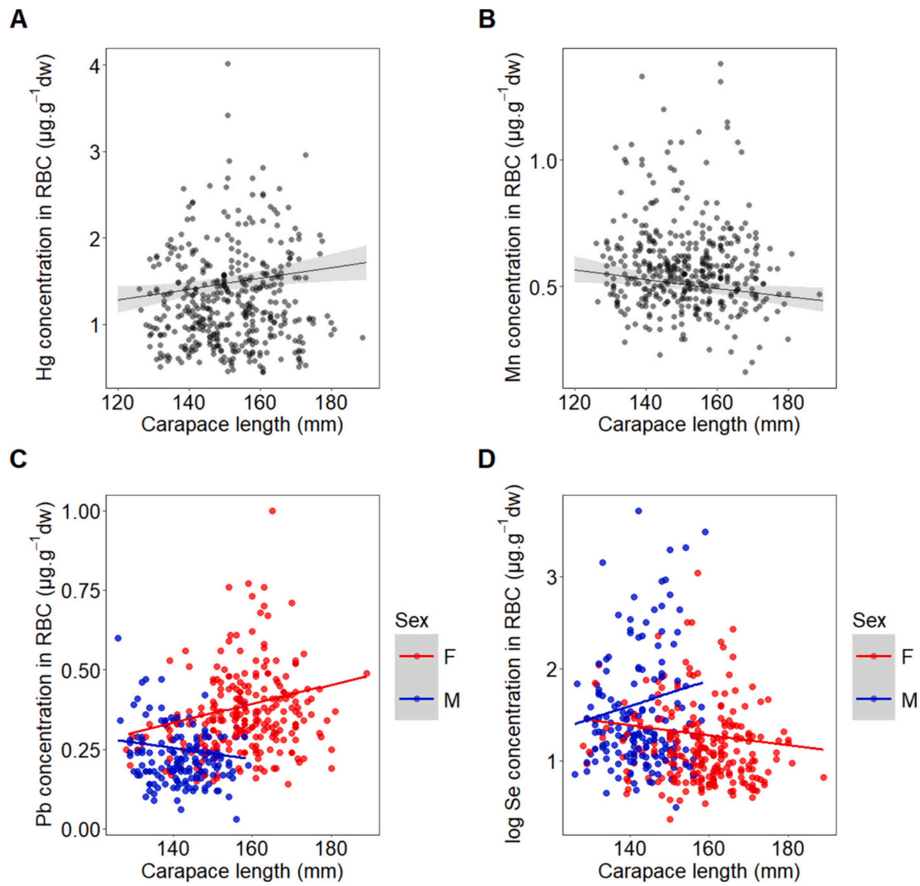

Fig. 2. Relationship between Hg (A), Mn (B), Pb (C), and Se (D) concentrations (μg.g^{-1} dw) in red blood cells (RBC) and carapace length (mm, A, B) or the carapace length in interaction with the sex (C, D) of the individuals sampled in the Camargue wetland, France (dots: data, lines: predictions of the mixed-effect models including (A) population, the sampling year and the CL, R^2 conditional = 0.86, n = 410; (B) the sampling year and the CL, R^2 conditional = 0.44, n_{Female} = 248, n_{Male} = 162).

trophic level we found little variation (coefficient of variation: 8 %) and individuals sampled were distributed in one to two trophic levels only (Post, 2002). Although environmental levels were not measured in our study, several hypotheses can be made. Esquineau site is made up of irrigation canals and marshes irrigated by them. Thus, the water irrigating the wetlands of this site is directly pumped from the Rhône River, 8 km upstream. The difference in MTE levels is probably due to the exposure and the contamination of the site ecosystem by the Rhône River which flows through a valley with many industries upstream of the pumping point (Cheiron, 2019; Cheiron and Bricault, 2020, 2021). Regarding Hg levels, another hypothesis could explain that turtles from Esquineau exhibited higher levels: in this site, marshes have more dry-out periods, which can increase Hg methylation in marshes (Feng et al., 2014). *E. orbicularis* exhibited higher Hg levels in their claws in sites that had been year-long drained the latest (Beau et al., 2019). Overall these results contrast with previous findings on legacy organic pollutants and pesticides in the same populations where the drainage site is the more exposed (Burkart et al., 2021; Merleau et al., 2024). The higher Pb levels at the Esquineau site could also be due to its proximity to a communal hunting ground where hunters do not comply with regulations on the use of non-toxic ammunition (Mondain-Monval et al., 2017, 2020).

We found an effect of the year for every trace element quantified. Differences between years were generally limited (coefficients of variation ranged from 2.2 % for Fe to 17.6 % for Se). These variations were supported when considering individuals with multiple measures across years. Therefore, they illustrate temporal variations in individual exposure, and could be due for instance to potential variations in environmental levels to the primary producers of the trophic chain, as explained by the inter-annual variations of δ^{13}C. However, considering the low turn-over of red blood cells in reptiles, measured trace element concentrations probably reflect exposure over several months (Stacy et al., 2011).

4.3. Influence of sex, body size and age

Several MTEs (Hg, Mn, Pb, and Se) were positively correlated with body size, likely because of a higher exposition through food, with larger individuals expected to consume more (Shine et al., 1998). The correlation between carapace length and levels of these MTEs may reflect bioaccumulation although blood levels result of recent integration rather than accumulation over several years (Grillitsch and Schiesari, 2010). We found no relation between ETM and individual body condition. Previous studies found an adverse effect of heavy metals, and Hg in particular, in reptiles (Finger et al., 2017; Nilsen et al., 2017). In *Mauremys leprosa,* populations with average Hg blood concentrations similar to ours showed a greater body condition compared to those with higher levels of Hg (8.83 ± 1.88 μg.g^{-1} dw) (Ortiz-Santaliestra et al., 2019). In birds, a recent meta-analysis revealed that Hg burden and body condition are essentially not related (Carravieri et al., 2022). However, our results are consistent with a previous study that did not find a relationship between Hg burden in claws and body condition in *E. orbicularis* (Beau et al., 2019).

We found a strong effect of age on Hg and Pb concentrations. Our results rely on red blood cells, a dynamic tissue and thus give complementary information on the exposure to trace elements during the life of

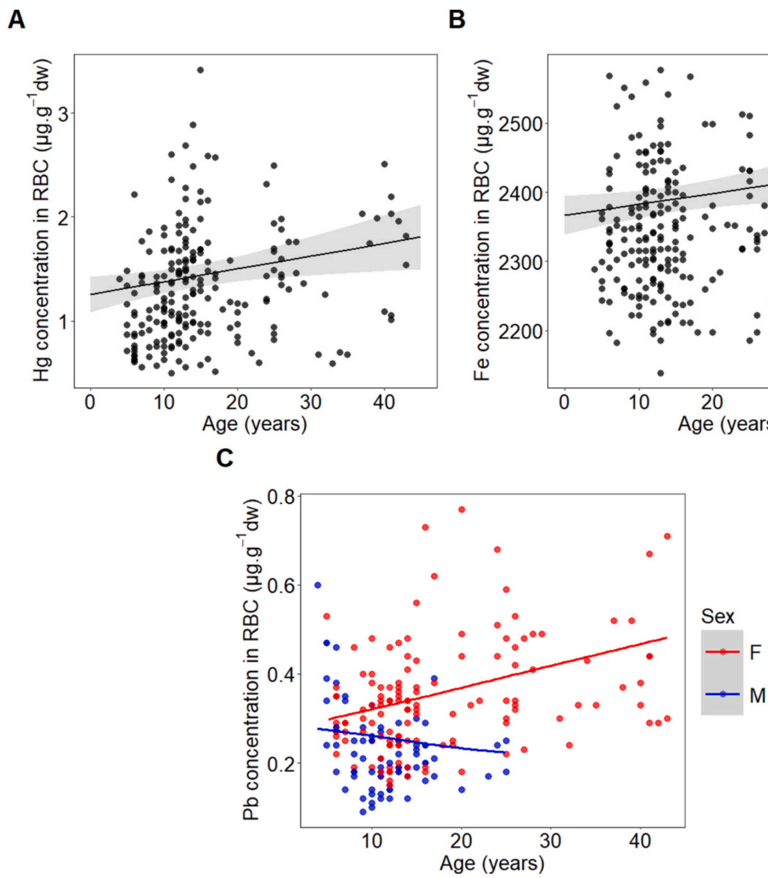

Fig. 3. Relationship between Hg (A), Fe (B), and Pb (C) concentrations (μg.g^{-1} dw) in red blood cells (RBC) and the age (year, A, B) or the age in interaction with the sex (C) of the individuals of known-age sampled in the Camargue wetland between 2018 and 2020 (dots: data, lines: predictions of the mixed-effect models including (A) population, the sampling year and the age, R^2 conditional = 0.83, n = 211; (B) the sampling year and the age, R^2 conditional = 0.45, n = 211; (C) the sampling year and the interaction of the sex and the age, R^2 conditional = 0.71, n_{Female} = 135, n_{Male} = 76).

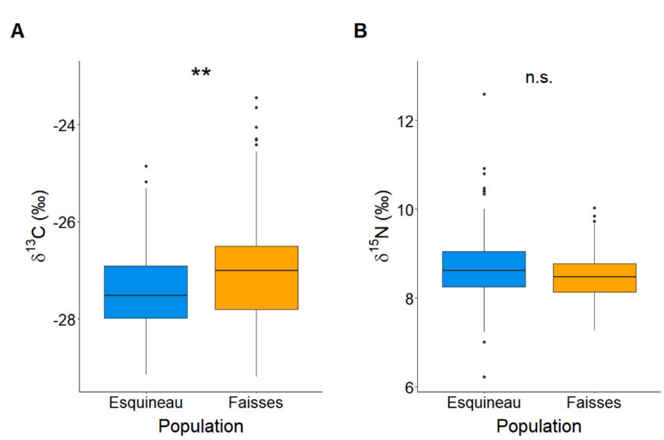

Fig. 4. Variation in $\delta^{13}C$ (A) and $\delta^{15}N$ (B) in red blood cells (RBC) of the individuals sampled in the Camargue wetland among the two populations: Esquineau and Faïsses, n = 409 (**: significance obtained from the LMM models).

the individuals. When blood levels of Hg increase, this element is likely deposited in developing keratinized tissues such as scute layers and claws (Beau et al., 2019; Villa et al., 2019) and thus lower the blood levels. Older individuals having higher circulating levels of Hg and Pb suggest that these individuals keep being more exposed, presumably through feeding. Our long-term study systems allowed to show for the first time that older females are more contaminated than older males, and even though our dataset is limited for old males (maximum age for males: 25 years), this effect remained significant when considering females under 25 years. The effect of age was also detected for Fe and could be explained by a higher number of red blood cells in older individuals. This hypothesis should be tested in further studies using hematocrit values. Increasing concentrations in larger and older females compared to males could be explained by differential absorption between sex, or linked to the reproduction.

We found higher Pb concentrations in females than males and this difference increased with the individual ages. This result was counterintuitive and although not known for Pb in freshwater turtles, has been already shown in other reptiles (Burger et al., 2004; Ciliberti et al., 2011). No differences in feeding habits between males and females were found in some populations of *E. orbicularis* (Çiçek and Ayaz, 2011; Ziane et al., 2020), including in the Camargue (Ottonello et al., 2005) but (Ducotterd et al., 2020) showed that males and females consumed different prey species. Even if little is known about the toxicokinetics of trace elements in reptiles, the higher concentration of Pb in females could also result from a difference in physiology, notably in the absorption of the trace elements. We also observed a significant difference between males and females for Cu, however the means for each sex were extremely close. In turn, we did not observe sexual differences in the concentration of Hg, as previously reported in Brenne for this species (Guillot et al., 2018; Beau et al., 2019). Maternal transfer of trace elements in the eggs has been shown in reptiles (Nilsen et al., 2020; Lemaire et al., 2021b) including freshwater turtles (Guirlet et al., 2008; Yu et al., 2011; Hopkins et al., 2013a,b). However, this potential maternal transfer is not always a sufficient excretion pathway to observe differences in trace element concentrations between males and females

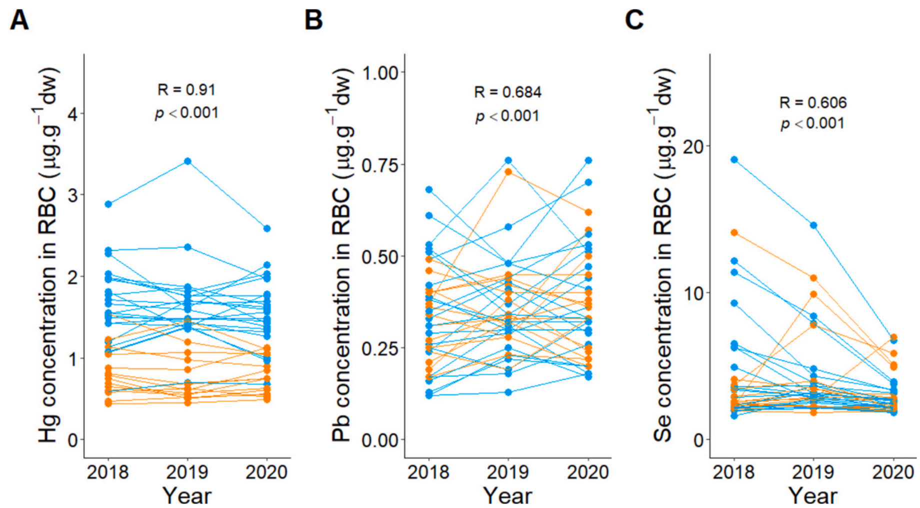

Fig. 5. Hg (A), Pb (B), and Se (C) concentrations (μg.g^{-1} dw) detected in red blood cells (RBC) of *E. orbicularis* of two populations in the Camargue wetland, France, during three years, n = 36 (blue: samples from Esquineau, orange: samples from Faïsses, R: coefficient of individual repeatability, p: significance).

(Schneider et al., 2011; Yu et al., 2011; Lemaire et al., 2021a).

Essential MTEs (Cu, Fe, Mn, and Zn) were less impacted by individual factors than Hg and Pb, which can be explained by a highly controlled regulation of these MTEs. Since they are involved in biological mechanisms, there are pathways of regulation, involving different components like metallothioneins to help maintain optimal concentrations of these MTEs for cell physiology (Deb and Fukushima, 1999). Fe was the MTE with the lower variation coefficient (4.4 %), which probably reflects a high regulation due to its crucial role in the production of hemoglobin. Se exhibited the highest coefficient of variation among the samples (92 %), which raised some concern on its potential impacts. In several Vertebrate species, including reptiles, seabirds, and mammals, Se has been shown to mitigate the toxic effects of Hg (Cuvin-Aralar and Furness, 1991; Manceau et al., 2021). However, high dietary exposure to Se (15 to 30 μg. g^{-1}) in the yellow-bellied slider *Trachemys scripta scripta* is correlated with detrimental effects on several parameters such as red blood cells, immunity (Haskins et al., 2017). In the American alligator *Alligator mississipiensis*, dietary exposure to 1000 and 2000 μg.g^{-1} lead to glucocorticoid release and decreased body condition (Finger et al., 2017, 2018).

4.4. Patterns of covariation and individual repeatability

No significant correlations were found between MTEs except for Hg and δ^{13}C. This correlation is not common but was also found in fish species (Ofukany et al., 2014) or seabirds (e.g. (Binkowski et al., 2021; Carravieri et al., 2014). It might be explained by a slight difference in Hg levels in primary producers and differences in foraging strategies, as *E. orbicularis* exhibit a very varied diet (Ducotterd et al., 2020). In large aquatics ecosystems, differences in carbon stable isotopes have been found between littoral and pelagic food webs (France, 1995). δ^{13}C is a more sensitive measure than δ^{15}N, only a change of 1 ‰ being necessary to change the level of primary producers. We would have expected a correlation between Hg and δ^{15}N considering the biomagnification property of Hg, but the variation was limited in δ^{15}N (6.23–12.59 ‰).

Our study allowed us to access the intra-individual variations of the levels of MTEs along time. No variation between years was found for Cu, Hg, and Zn in the subset of individuals that were sampled multiple times. We also found high and significant repeatability across years for Hg showing that these individuals tended to be exposed consistently to Hg over time. The intra-individual repeatability was also high and significant for Pb and for Se. At the group scale, MTEs values could be different enough to be statistically detectable; yet the concentrations were similar throughout the years for most individuals, exposing them to potential chronic effects. Even if concentrations were rather low, being exposed to such levels of Hg and Pb for several decades could be detrimental to the individuals. Fe levels seemed affected by external factors that impact individuals globally, illustrating the highly conserved nature of the regulation of this element (Kaplan and Ward, 2013). Cu and Zn, on the other side, seemed to be less highly regulated than Fe, as we found no difference in their levels in groups sampled for a pair of years but low and non-significant repeatability at the individual scale.

5. Conclusion

We demonstrate that habitat plays a role in exposure to the most toxic MTEs (Hg and Pb) with the irrigation site exhibiting the highest levels. Our results also highlight the effect of individual characteristics (size, sex, age) on MTE blood burden, however MTE contamination were poorly related to stable isotope values. Our work is also the first to detect a differential effect of the sex depending on the size and the age on the circulating levels of Pb in a freshwater turtle species. Remarkably, blood levels of Hg, Pb, and Se are highly reproducible at the intra-individual level over sampled years. Our study unequivocally supports the need of long-term population monitoring to better understand i) the temporal intra-individual variation of MTE burden and ii) the influence of age on different MTEs. Future work should focus on the toxicokinetics of MTEs in *E. orbicularis*, and on the effects of such exposure, particularly on physiology, reproduction and population dynamics.

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CRediT authorship contribution statement

Leslie-Anne Merleau: Writing – review & editing, Writing – original draft, Visualization, Formal analysis. **Aurélie Goutte:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Anthony Olivier:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Marion Vittecoq:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Paco Bustamante:** Writing – review & editing, Validation, Resources, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **Carole Leray:** Writing – review & editing, Resources, Investigation. **Olivier Lourdais:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Marion Vittecoq reports financial support was provided by the Water Agency Rhône Méditérranée Corse. Olivier Lourdais reports financial support was provided by the French Biodiversity Office. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.177487.

Data availability

Data will be made available on request.

References

Ackerman, J.T., Eagles-Smith, C.A., Herzog, M.P., Hartman, C.A., 2016. Maternal transfer of contaminants in birds: mercury and selenium concentrations in parents and their eggs. Environ. Pollut. 210, 145–154. https://doi.org/10.1016/j.envpol.2015.12.016.

Ali, H., Khan, E., 2019. Trophic transfer, bioaccumulation, and biomagnification of non-essential hazardous heavy metals and metalloids in food chains/webs—concepts and implications for wildlife and human health. Hum. Ecol. Risk Assess. Int. J. 25, 1353–1376. https://doi.org/10.1080/10807039.2018.1469398.

Austruy, A., Yung, L., Ambrosi, J.P., Girardclos, O., Keller, C., Angeletti, B., Dron, J., Chamaret, P., Chalot, M., 2019. Evaluation of historical atmospheric pollution in an industrial area by dendrochemical approaches. Chemosphere 220, 116–126. https://doi.org/10.1016/j.chemosphere.2018.12.072.

Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. J. Stat. Soft. 67 (1), 1–48. https://doi.org/10.18637/jss.v067.i01.

Beau, F., Bustamante, P., Michaud, B., Brischoux, F., 2019. Environmental causes and reproductive correlates of mercury contamination in European pond turtles (Emys orbicularis). Environ. Res. 172, 338–344. https://doi.org/10.1016/j.envres.2019.01.043.

Bergeron, C.M., Husak, J.F., Unrine, J.M., Romanek, C.S., Hopkins, W.A., 2007. Influence of feeding ecology on blood mercury concentrations in four species of turtles. Environ. Toxicol. Chem. 26, 1733–1741. https://doi.org/10.1897/06-594R.1.

Binkowski, L.J., Fort, J., Brault-Favrou, M., Gallien, F., Le Guillou, G., Chastel, O., Bustamante, P., 2021. Foraging ecology drives mercury contamination in chick gulls from the English Channel. Chemosphere 267, 128622. https://doi.org/10.1016/j.chemosphere.2020.128622.

Burger, J., 2008. Assessment and management of risk to wildlife from cadmium. Sci. Total Environ. 389, 37–45. https://doi.org/10.1016/j.scitotenv.2007.08.037.

Burger, J., Campbell, K.R., Campbell, T.S., 2004. Gender and spatial patterns in metal concentrations in brown anoles (*Anolis sagrei*) in Southern Florida, USA. Environ. Toxicol. Chem. 23, 712–718. https://doi.org/10.1897/02-647.

Burkart, L., Olivier, A., Lourdais, O., Vittecoq, M., Blouin-Demers, G., Alliot, F., Le Gac, C., Martin, N., Goutte, A., 2021. Determinants of legacy persistent organic pollutant levels in the European pond turtle (*Emys orbicularis*) in the Camargue wetland, France. Environ. Toxicol. Chem. 40, 2261–2268. https://doi.org/10.1002/etc.5077.

Bustamante, P., González, A.F., Rocha, F., Miramand, P., Guerra, A., 2008. Metal and metalloid concentrations in the giant squid *Architeuthis dux* from Iberian waters. Mar. Environ. Res. 66, 278–287. https://doi.org/10.1016/j.marenvres.2008.04.003.

Carravieri, A., Bustamante, P., Tartu, S., Meillère, A., Labadie, P., Budzinski, H., Peluhet, L., Barbraud, C., Weimerskirch, H., Chastel, O., Cherel, Y., 2014. Wandering albatrosses document latitudinal variations in the transfer of persistent organic pollutants and mercury to southern ocean predators. Environ. Sci. Technol. 48, 14746–14755. https://doi.org/10.1021/es504601m.

Carravieri, A., Vincze, O., Bustamante, P., Ackerman, J.T., Adams, E.M., Angelier, F., Chastel, O., Cherel, Y., Gilg, O., Golubova, E., Kitaysky, A., Luff, K., Seewagen, C.L., Strøm, H., Will, A.P., Yannic, G., Giraudeau, M., Fort, J., 2022. Quantitative meta-analysis reveals no association between mercury contamination and body condition in birds. Biol. Rev. 97, 1253–1271. https://doi.org/10.1111/brv.12840.

Castanet, J., 1988. Les méthodes d'estimation de l'âge chez les chéloniens. Mésogée 48, 21–28.

Cheiron, A., 2019. Rapport d'activité 2018 de la Réserve naturelle nationale de Camargue (Société nationale de protection de la nature – Réserve Naturelle Nationale de Camargue).

Cheiron, A., Bricault, B., 2020. Rapport d'activité 2019 de la Réserve naturelle nationale de Camargue (Société nationale de protection de la nature – Réserve naturelle nationale de Camargue).

Cheiron, A., Bricault, B., 2021. Rapport d'activité 2020 de la Réserve Naturelle Nationale de Camargue (Société Nationale de Protection de la Nature).

Çiçek, K., Ayaz, D., 2011. Food composition of the European pond turtle (*Emys orbicularis*) in Lake Sülüklü (Western Anatolia, Turkey). J. Freshw. Ecol. 1–8. https://doi.org/10.1080/02705060.2011.580536.

Ciliberti, A., Berny, P., Delignette-Muller, M.-L., De Buffrénil, V., 2011. The Nile monitor (*Varanus niloticus*; Squamata: Varanidae) as a sentinel species for lead and cadmium contamination in sub-Saharan wetlands. Sci. Total Environ. 409, 4735–4745. https://doi.org/10.1016/j.scitotenv.2011.07.028.

Cox, N., Young, B.E., Bowles, P., Fernandez, M., Marin, J., Rapacciuolo, G., Böhm, M., Brooks, T.M., Hedges, S.B., Hilton-Taylor, C., Hoffmann, M., Jenkins, R.K.B., Tognelli, M.F., Alexander, G.J., Allison, A., Ananjeva, N.B., Auliya, M., Avila, L.J., Chapple, D.G., Cisneros-Heredia, D.F., Cogger, H.G., Colli, G.R., de Silva, A., Eisemberg, C.C., Els, J., Fong G., A., Grant, T.D., Hitchmough, R.A., Iskandar, D.T., Kidera, N., Martins, M., Meiri, S., Mitchell, N.J., Molur, S., Nogueira, C. de C., Ortiz, J.C., Penner, J., Rhodin, A.G.J., Rivas, G.A., Rödel, M.-O., Roll, U., Sanders, K.L., Santos-Barrera, G., Shea, G.M., Spawls, S., Stuart, B.L., Tolley, K.A., Trape, J.-F., Vidal, M.A., Wagner, P., Wallace, B.P., Xie, Y., 2022. A global reptile assessment highlights shared conservation needs of tetrapods. Nature 605, 285–290. doi:https://doi.org/10.1038/s41586-022-04664-7.

Cuvin-Aralar, Ma.L.A., Furness, R.W., 1991. Mercury and selenium interaction: a review. Ecotoxicol. Environ. Saf. 21, 348–364. https://doi.org/10.1016/0147-6513(91)90074-Y.

Deb, S.C., Fukushima, T., 1999. Metals in aquatic ecosystems: mechanisms of uptake, accumulation and release-Ecotoxicological perspectives. Int. J. Environ. Stud. 56, 385–417. https://doi.org/10.1080/00207239908711212.

Dendievel, A.-M., Mourier, B., Dabrin, A., Delile, H., Coynel, A., Gosset, A., Liber, Y., Berger, J.-F., Bedell, J.-P., 2020. Metal pollution trajectories and mixture risk assessed by combining dated cores and subsurface sediments along a major European river (Rhône River, France). Environ. Int. 144, 106032. https://doi.org/10.1016/j.envint.2020.106032.

Driscoll, C.T., Mason, R.P., Chan, H.M., Jacob, D.J., Pirrone, N., 2013. Mercury as a global pollutant: sources, pathways, and effects. Environ. Sci. Technol. 47, 4967–4983. https://doi.org/10.1021/es305071v.

Ducotterd, C., Crovadore, J., Lefort, F., Guisan, A., Ursenbacher, S., Rubin, J.-F., 2020. The feeding behaviour of the European pond turtle (*Emys orbicularis*, L. 1758) is not a threat for other endangered species. Glob. Ecol. Conserv. 23, e01133. https://doi.org/10.1016/j.gecco.2020.e01133.

Fay, R., Ficheux, S., Béchet, A., Besnard, A., Crochet, P., Leblois, R., Crivelli, A., Wattier, R., Olivier, A., 2023. Direct and indirect estimates of dispersal support strong juvenile philopatry and male-biased dispersal in a freshwater turtle species (*Emys orbicularis*). Freshw. Biol. https://doi.org/10.1111/fwb.14171 fwb.14171.

Feng, S., Ai, Z., Zheng, S., Gu, B., Li, Y., 2014. Effects of dryout and inflow water quality on mercury methylation in a constructed wetland. Water Air Soil Pollut. 225, 1929. https://doi.org/10.1007/s11270-014-1929-6.

Ferrand, E., Eyrolle, F., Radakovitch, O., Provansal, M., Dufour, S., Vella, C., Raccasi, G., Gurriaran, R., 2012. Historical levels of heavy metals and artificial radionuclides reconstructed from overbank sediment records in lower Rhône River (South-East France). Geochim. Cosmochim. Acta 82, 163–182. https://doi.org/10.1016/j.gca.2011.11.023.

Ficheux, S., Olivier, A., Fay, R., Crivelli, A., Besnard, A., Béchet, A., 2014. Rapid response of a long-lived species to improved water and grazing management: the case of the European pond turtle (*Emys orbicularis*) in the Camargue, France. J. Nat. Conserv. 22, 342–348. https://doi.org/10.1016/j.jnc.2014.03.001.

Finger, J.W., Hamilton, M.T., Glenn, T.C., Tuberville, T.D., 2017. Dietary selenomethionine administration in the American alligator (*Alligator mississippiensis*): hepatic and renal se accumulation and its effects on growth and body condition.

Arch. Environ. Contam. Toxicol. 72, 439–448. https://doi.org/10.1007/s00244-017-0370-4.
Finger, J.W., Hamilton, M.T., Kelley, M.D., Zhang, Y., Kavazis, A.N., Glenn, T.C., Tuberville, T.D., 2018. Dietary selenomethionine administration and its effects on the American alligator (*Alligator mississippiensis*): oxidative status and corticosterone levels. Arch. Environ. Contam. Toxicol. 75, 37–44. https://doi.org/10.1007/s00244-018-0530-1.
France, R.L., 1995. Differentiation between littoral and pelagic food webs in lakes using stable carbon isotopes. Limnol. Oceanogr. 40, 1310–1313. https://doi.org/10.4319/lo.1995.40.7.1310.
Grillitsch, B., Schiesari, L., 2010. The ecotoxicology of metals in reptiles. In: Sparling, D., Linder, G., Bishop, C., Krest, S. (Eds.), Ecotoxicology of Amphibians and Reptiles, Second edition. CRC Press, pp. 337–448. https://doi.org/10.1201/EBK1420064162-c12.
Guillot, H., Bonnet, X., Bustamante, P., Churlaud, C., Trotignon, J., Brischoux, F., 2018. Trace element concentrations in European pond turtles (*Emys orbicularis*) from Brenne Natural Park, France. Bull. Environ. Contam. Toxicol. 101, 300–304. https://doi.org/10.1007/s00128-018-2376-7.
Guirlet, E., Das, K., Girondot, M., 2008. Maternal transfer of trace elements in leatherback turtles (*Dermochelys coriacea*) of French Guiana. Aquat. Toxicol. 88, 267–276. https://doi.org/10.1016/j.aquatox.2008.05.004.
Hartig, F., 2016. DHARMa: Residual Diagnostics for Hierarchical (Multi-Level/Mixed) Regression Models. https://doi.org/10.32614/CRAN.package.DHARMa.
Haskins, D.L., Hamilton, M.T., Stacy, N.I., Finger, J.W., Tuberville, T.D., 2017. Effects of selenium exposure on the hematology, innate immunity, and metabolic rate of yellow-bellied sliders (*Trachemys scripta scripta*). Ecotoxicology 26, 1134–1146. https://doi.org/10.1007/s10646-017-1839-7.
Hoffmann, L., 1960. Le saturnisme, fléau de la sauvagine en Camargue. Terre Vie Rev. Hist. Nat. 14, 120–131. https://doi.org/10.3406/revec.1960.6070.
Hopkins, W.A., Bodinof, C., Budischak, S., Perkins, C., 2013a. Nondestructive indices of mercury exposure in three species of turtles occupying different trophic niches downstream from a former chloralkali facility. Ecotoxicology 22, 22–32. https://doi.org/10.1007/s10646-012-0999-8.
Hopkins, B.C., Hepner, M.J., Hopkins, W.A., 2013b. Non-destructive techniques for biomonitoring of spatial, temporal, and demographic patterns of mercury bioaccumulation and maternal transfer in turtles. Environ. Pollut. 177, 164–170. https://doi.org/10.1016/j.envpol.2013.02.018.
Jouanneau, W., Sebastiano, M., Rozen-Rechels, D., Harris, S.M., Blévin, P., Angelier, F., Brischoux, F., Gernigon, J., Lemesle, J.-C., Robin, F., Cherel, Y., Bustamante, P., Chastel, O., 2022. Blood mercury concentrations in four sympatric gull species from South Western France: insights from stable isotopes and biologging. Environ. Pollut. 308, 119619. https://doi.org/10.1016/j.envpol.2022.119619.
Kaplan, J., Ward, D.M., 2013. The essential nature of iron usage and regulation. Curr. Biol. 23, R642–R646. https://doi.org/10.1016/j.cub.2013.05.033.
Lavoie, R.A., Jardine, T.D., Chumchal, M.M., Kidd, K.A., Campbell, L.M., 2013. Biomagnification of mercury in aquatic food webs: a worldwide meta-analysis. Environ. Sci. Technol. 47, 13385–13394. https://doi.org/10.1021/es403103t.
Le Croizier, G., Schaal, G., Gallon, R., Fall, M., Le Grand, F., Munaron, J.-M., Rouget, M.-L., Machu, E., Le Loc'h, F., Laë, R., De Morais, L.T., 2016. Trophic ecology influence on metal bioaccumulation in marine fish: inference from stable isotope and fatty acid analyses. Sci. Total Environ. 573, 83–95. https://doi.org/10.1016/j.scitotenv.2016.08.035.
Lemaire, J., Bustamante, P., Marquis, O., Caut, S., Brischoux, F., 2021a. Influence of sex, size and trophic level on blood Hg concentrations in Black caiman, *Melanosuchus niger* (Spix, 1825) in French Guiana. Chemosphere 262, 127819. https://doi.org/10.1016/j.chemosphere.2020.127819.
Lemaire, J., Marquis, O., Bustamante, P., Mangione, R., Brischoux, F., 2021b. I got it from my mother: inter-nest variation of mercury concentration in neonate Smooth-fronted Caiman (*Paleosuchus trigonatus*) suggests maternal transfer and possible phenotypical effects. Environ. Res. 194, 110494. https://doi.org/10.1016/j.envres.2020.110494.
Lemaire, J., Brischoux, F., Marquis, O., Mangione, R., Caut, S., Brault-Favrou, M., Churlaud, C., Bustamante, P., 2022. Relationships between stable isotopes and trace element concentrations in the crocodilian community of French Guiana. Sci. Total Environ. 837, 155846. https://doi.org/10.1016/j.scitotenv.2022.155846.
Lenth, R.V., Buerkner, P., Giné-Vázquez, I., Herve, M., Jung, M., Love, J., Miguez, F., Riebl, H., Singmann, H., 2023. emmeans: Estimated Marginal Means, aka Least-Squares Means (R Packages).
Liu, Y., Liu, G., Yuan, Z., Liu, H., Lam, P.K.S., 2018. Heavy metals (As, Hg and V) and stable isotope ratios (δ13C and δ15N) in fish from Yellow River Estuary, China. Sci. Total Environ. 613–614, 462–471. https://doi.org/10.1016/j.scitotenv.2017.09.088.
Lucia, M., Strøm, H., Bustamante, P., Gabrielsen, G.W., 2016. Trace element concentrations in relation to the trophic behaviour of endangered ivory gulls (*Pagophila eburnea*) during their stay at a breeding site in Svalbard. Arch. Environ. Contam. Toxicol. 71, 518–529. https://doi.org/10.1007/s00244-016-0320-6.
Manceau, A., Bourdineaud, J.-P., Oliveira, R.B., Sarrazin, S.L.F., Krabbenhoft, D.P., Eagles-Smith, C.A., Ackerman, J.T., Stewart, A.R., Ward-Deitrich, C., Del Castillo Busto, M.E., Goenaga-Infante, H., Wack, A., Retegan, M., Detlefs, B., Glatzel, P., Bustamante, P., Nagy, K.L., Poulin, B.A., 2021. Demethylation of methylmercury in bird, fish, and earthworm. Environ. Sci. Technol. 55, 1527–1534. https://doi.org/10.1021/acs.est.0c04948.
Merleau, L.-A., Lourdais, O., Olivier, A., Vittecoq, M., Blouin-Demers, G., Alliot, F., Burkart, L., Foucault, Y., Leray, C., Migne, E., Goutte, A., 2024. Pesticide concentrations in a threatened freshwater turtle (*Emys orbicularis*): seasonal and annual variation in the Camargue wetland, France. Environ. Pollut. 341, 122903. https://doi.org/10.1016/j.envpol.2023.122903.
Meyer, E., Sparling, D., Blumenshine, S., 2013. Regional inhibition of cholinesterase in free-ranging western pond turtles (*Emys marmorata*) occupying California mountain streams. Environ. Toxicol. Chem. 32, 692–698. https://doi.org/10.1002/etc.2097.
Meyer, E., Eagles-Smith, C.A., Sparling, D., Blumenshine, S., 2014. Mercury exposure associated with altered plasma thyroid hormones in the declining Western pond turtle (*Emys marmorata*) from California Mountain streams. Environ. Sci. Technol. 48, 2989–2996. https://doi.org/10.1021/es4050538.
Mills, W.F., Bustamante, P., McGill, R.A.R., Anderson, O.R.J., Bearhop, S., Cherel, Y., Votier, S.C., Phillips, R.A., 2020. Mercury exposure in an endangered seabird: long-term changes and relationships with trophic ecology and breeding success. Proc. R. Soc. B Biol. Sci. 287, 20202683. https://doi.org/10.1098/rspb.2020.2683.
Mondain-Monval, J.-Y., Rau, P.D.D., Guillemain, M., Olivier, A., 2017. Retour sur 10 ans d'utilisation des munitions non toxiques en Camargue: efficacité des chasseurs et effets sur la contamination des oiseaux par le plomb. Faune Sauvage 314, 10–15.
Mondain-Monval, J.-Y., Olivier, A., Cardoso, O., 2020. Interdiction du plomb de chasse, une difficile mise en oeuvre. Espaces Naturels 71, 36–37.
Nilsen, F.M., Dorsey, J.E., Lowers, R.H., Guillette, L.J., Long, S.E., Bowden, J.A., Schock, T.B., 2017. Evaluating mercury concentrations and body condition in American alligators (*Alligator mississippiensis*) at Merritt Island National Wildlife Refuge (MINWR), Florida. Sci. Total Environ. 607–608, 1056–1064. https://doi.org/10.1016/j.scitotenv.2017.07.073.
Nilsen, F.M., Rainwater, T.R., Wilkinson, P.M., Brunell, A.M., Lowers, R.H., Bowden, J.A., Guillette, L.J., Long, S.E., Schock, T.B., 2020. Examining maternal and environmental transfer of mercury into American alligator eggs. Ecotoxicol. Environ. Saf. 189, 110057. https://doi.org/10.1016/j.ecoenv.2019.110057.
Ofukany, A.F.A., Wassenaar, L.I., Bond, A.L., Hobson, K.A., 2014. Defining fish community structure in Lake Winnipeg using stable isotopes (δ13C, δ15N, δ34S): implications for monitoring ecological responses and trophodynamics of mercury & other trace elements. Sci. Total Environ. 497–498, 239–249. https://doi.org/10.1016/j.scitotenv.2014.07.125.
Olivier, A., 2002. Ecologie, traits d'histoire de vie et conservation d'une population de Cistude d'Europe (*Emys orbicularis*) en Camargue (EPHE diploma dissertation). Ecole Pratique des Hautes Etudes, Paris, France.
Olivier, A., Barbraud, C., Rosecchi, E., Germain, C., Cheylan, M., 2010. Assessing spatial and temporal population dynamics of cryptic species: an example with the European pond turtle. Ecol. Appl. 20, 993–1004. https://doi.org/10.1890/09-0801.1.
Ortiz-Santaliestra, M.E., Maia, J.P., Egea-Serrano, A., Lopes, I., 2018. Validity of fish, birds and mammals as surrogates for amphibians and reptiles in pesticide toxicity assessment. Ecotoxicology 27, 819–833. https://doi.org/10.1007/s10646-018-1911-y.
Ortiz-Santaliestra, M.E., Rodríguez, A., Pareja-Carrera, J., Mateo, R., Martinez-Haro, M., 2019. Tools for non-invasive sampling of metal accumulation and its effects in Mediterranean pond turtle populations inhabiting mining areas. Chemosphere 231, 194–206. https://doi.org/10.1016/j.chemosphere.2019.05.082.
Ottonello, D., Salvidio, S., Rosecchi, E., 2005. Feeding habits of the European pond terrapin *Emys orbicularis* in Camargue (Rhône delta, Southern France). Amphib.-Reptil. 26, 562–565. https://doi.org/10.1163/156853805774806241.
Pacyna, E.G., Pacyna, J.M., Fudala, J., Strzelecka-Jastrzab, E., Hlawiczka, S., Panasiuk, D., Nitter, S., Pregger, T., Pfeiffer, H., Friedrich, R., 2007. Current and future emissions of selected heavy metals to the atmosphere from anthropogenic sources in Europe. Atmos. Environ. 41, 8557–8566. https://doi.org/10.1016/j.atmosenv.2007.07.040.
Pain, D.J., 1991. Lead shot densities and settlement rates in Camargue marshes, France. Biol. Conserv. 57, 273–286. https://doi.org/10.1016/0006-3207(91)90073-I.
Pain, D.J., Mateo, R., Green, R.E., 2019. Effects of lead from ammunition on birds and other wildlife: a review and update. Ambio 48, 935–953. https://doi.org/10.1007/s13280-019-01159-0.
Peterson, B.J., Fry, B., 1987. Stable isotopes in ecosystem studies. Annu. Rev. Ecol. Syst. 18, 293–320. https://doi.org/10.1146/annurev.es.18.110187.001453.
Post, D.M., 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology 83, 703–718. https://doi.org/10.1890/0012-9658(2002)083[0703:USITET]2.0.CO;2.
Ribeiro, C.A.O., Vollaire, Y., Sanchez-Chardi, A., Roche, H., 2005. Bioaccumulation and the effects of organochlorine pesticides, PAH and heavy metals in the eel (*Anguilla anguilla*) at the Camargue Nature Reserve, France. Aquat. Toxicol. 74, 53–69. https://doi.org/10.1016/j.aquatox.2005.04.008.
Schneider, L., Belger, L., Burger, J., Vogt, R.C., Jeitner, C., Peleja, J.R.P., 2011. Assessment of non-invasive techniques for monitoring mercury concentrations in species of Amazon turtles. Toxicol. Environ. Chem. 93, 238–250. https://doi.org/10.1080/02772248.2010.517627.
Shine, R., Harlow, P.S., Keogh, J.S., Boeadi, 1998. The influence of sex and body size on food habits of a giant tropical snake, *Python reticulatus*. Funct. Ecol. 12, 248–258. https://doi.org/10.1046/j.1365-2435.1998.00179.x.
Silva, J.M., Navoni, J.A., Freire, E.M.X., 2020. Lizards as model organisms to evaluate environmental contamination and biomonitoring. Environ. Monit. Assess. 192, 454. https://doi.org/10.1007/s10661-020-08435-7.
Slimani, T., El Hassani, M.S., El Mouden, E.H., Bonnet, M., Bustamante, P., Brischoux, F., Brault-Favrou, M., Bonnet, X., 2018. Large-scale geographic patterns of mercury contamination in Morocco revealed by freshwater turtles. Environ. Sci. Pollut. Res. 25, 2350–2360. https://doi.org/10.1007/s11356-017-0643-5.
Soldatini, C., Sebastiano, M., Albores-Barajas, Y.V., Abdelgawad, H., Bustamante, P., Costantini, D., 2020. Mercury exposure in relation to foraging ecology and its impact on the oxidative status of an endangered seabird. Sci. Total Environ. 724, 138131. https://doi.org/10.1016/j.scitotenv.2020.138131.

Sparling, D.W., Linder, G., Bishop, C.A., Krest, S.K., 2010. Recent advancements in amphibian and reptile ecotoxicology. In: Ecotoxicology of Amphibians and Reptiles. CRC Press.

Stacy, N.I., Alleman, A.R., Sayler, K.A., 2011. Diagnostic hematology of reptiles. Clin. Lab. Med. 31, 87–108. https://doi.org/10.1016/j.cll.2010.10.006.

Stoffel, M.A., Nakagawa, S., Schielzeth, H., 2017. rptR: repeatability estimation and variance decomposition by generalized linear mixed-effects models. Methods Ecol. Evol. 8, 1639–1644. https://doi.org/10.1111/2041-210X.12797.

Tan, S.W., Meiller, J.C., Mahaffey, K.R., 2009. The endocrine effects of mercury in humans and wildlife. Crit. Rev. Toxicol. 39, 228–269. https://doi.org/10.1080/10408440802233259.

Tartu, S., Goutte, A., Bustamante, P., Angelier, F., Moe, B., Clément-Chastel, C., Bech, C., Gabrielsen, G.W., Bustnes, J.O., Chastel, O., 2013. To breed or not to breed: endocrine response to mercury contamination by an Arctic seabird. Biol. Lett. 9, 20130317. https://doi.org/10.1098/rsbl.2013.0317.

Turnquist, M.A., Driscoll, C.T., Schulz, K.L., Schlaepfer, M.A., 2011. Mercury concentrations in snapping turtles (*Chelydra serpentina*) correlate with environmental and landscape characteristics. Ecotoxicology 20, 1599–1608. https://doi.org/10.1007/s10646-011-0718-x.

Ullrich, S.M., Tanton, T.W., Abdrashitova, S.A., 2001. Mercury in the Aquatic Environment: A Review of Factors Affecting Methylation, 3, pp. 241–293.

Väänänen, K., Leppänen, M.T., Chen, X., Akkanen, J., 2018. Metal bioavailability in ecological risk assessment of freshwater ecosystems: from science to environmental management. Ecotoxicol. Environ. Saf. 147, 430–446. https://doi.org/10.1016/j.ecoenv.2017.08.064.

Van Dyke, J.U., Steen, D.A., Jackson, B.P., Hopkins, W.A., 2014. Maternal transfer and embryonic assimilation of trace elements in freshwater turtles after remediation of a coal fly-ash spill. Environ. Pollut. 194, 38–49. https://doi.org/10.1016/j.envpol.2014.07.005.

Van Dyke, J.U., Jachowski, C.M.B., Steen, D.A., Jackson, B.P., Hopkins, W.A., 2017. Spatial differences in trace element bioaccumulation in turtles exposed to a partially remediated coal fly ash spill. Environ. Toxicol. Chem. 36, 201–211. https://doi.org/10.1002/etc.3524.

Vareda, J.P., Valente, A.J.M., Durães, L., 2019. Assessment of heavy metal pollution from anthropogenic activities and remediation strategies: a review. J. Environ. Manag. 246, 101–118. https://doi.org/10.1016/j.jenvman.2019.05.126.

Villa, C.A., Bell, I., Madden Hof, C., Limpus, C.J., Gaus, C., 2019. Elucidating temporal trends in trace element exposure of green turtles (*Chelonia mydas*) using the toxicokinetic differences of blood and scute samples. Sci. Total Environ. 651, 2450–2459. https://doi.org/10.1016/j.scitotenv.2018.10.092.

Weir, S.M., Suski, J.G., Salice, C.J., 2010. Ecological risk of anthropogenic pollutants to reptiles: evaluating assumptions of sensitivity and exposure. Environ. Pollut. 158, 3596–3606. https://doi.org/10.1016/j.envpol.2010.08.011.

Yilmaz, N., Tosunolu, M., 2010. Hematology and some plasma biochemistry values of free-living freshwater turtles (*Emys orbicularis* and *Mauremys rivulata*) from Turkey. North-Western Journal of Zoology 6, 109–117.

Yu, S., Halbrook, R.S., Sparling, D.W., Colombo, R., 2011. Metal accumulation and evaluation of effects in a freshwater turtle. Ecotoxicology 20, 1801–1812. https://doi.org/10.1007/s10646-011-0716-z.

Zhang, C., Yu, Z., Zeng, G., Jiang, M., Yang, Z., Cui, F., Zhu, M., Shen, L., Hu, L., 2014. Effects of sediment geochemical properties on heavy metal bioavailability. Environ. Int. 73, 270–281. https://doi.org/10.1016/j.envint.2014.08.010.

Ziane, N., Fediras, S., Rouag, R., Olivier, A., Benyacoub, S., 2020. Feeding Habits of the European pond turtle *Emys orbicularis* (Linnaeus, 1758) in the Lake Tonga, Algeria. Herpetology Notes 13, 875–881.