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Environmental causes and reproductive correlates of mercury contamination in European pond turtles (*Emys orbicularis*)

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ABSTRACT

Assessing Hg contamination in aquatic ecosystems is difficult because wetlands are part of large and complex networks, and potential sources of Hg contamination are highly diverse. To investigate environmental determinants of Hg contamination, we studied one of the largest continental French wetlands structured as a dense network of artificial ponds. Such context allows to investigate the influence of pond characteristics on Hg contamination in an area relatively disconnected from direct sources of pollution. We relied on a bioindicator organism, the European pond turtle (*Emys orbicularis*) to assess Hg contamination in a relatively large number of sites ($N > 255$ turtles from 15 ponds sampled in 2016 and 2017). Non-invasive sampling in the claws of turtles show that Hg concentrations were not related to their sex or size, but we found an effect of age (1.62 ± 0.20 in juveniles and $2.21 \pm 0.06\ \mu g\ g^{-1}$ dw in adults), suggesting that turtles do bioaccumulate Hg through their life. Turtle Hg was different between ponds, and we found that pond age and pond usage (draining events linked to pond maintenance) were the main environmental determinants of Hg concentrations in turtles. Finally, and more importantly, our dataset allowed us to highlight potential negative effect of Hg concentrations on the proportion of reproductive females, suggesting an influence of Hg on reproductive mechanisms in this species. This result indicates that Hg contamination, even in absence of direct and strong sources of pollution, may have a critical impact on reproduction and thus the persistence of a long-lived vertebrate.

1. Introduction

Mercury (Hg) is one of the most (in)famous environmental contaminants (Wiener et al., 2003). Through various transport processes, Hg tends to concentrate in aquatic environments, which are considered as sinks of Hg (Mason et al., 2012; Driscoll et al., 2013). Assessing the sources of Hg contamination in aquatic ecosystems is difficult because wetlands are part of large and complex networks composed of both surface and underground waters. Contaminants can enter aquatic environments through various pathways such as atmospheric deposition, erosion or anthropogenic sources including agricultural drainage, mining, or industrial effluents (Förstner and Wittman, 1981; Wiener et al., 2003; Wang et al., 2004). Cryptic sources can further complicate our understanding of the extent of Hg contamination. For instance, it has recently been shown that fish farming is responsible for the transfer of marine Hg to areas that superficially appear unaffected from other sources of pollution (Hansson et al., 2017; see also Lemaire et al., 2018; Guillot et al., 2018).

Aquatic environments are important sources of methyl-Hg (MeHg) (Ackerman et al., 2016; Eagles-Smith et al., 2018; Gilmour et al., 1992; Hsu-Kim et al., 2013), a form of Hg responsible for toxic effects on humans and wildlife (e.g. Tan et al., 2009; Scheuhammer et al., 2008; Crump and Trudeau, 2009; Green et al., 2010; Rutkiewicz et al., 2011; Wolfe et al., 1998; Tartu et al., 2013; Schneider et al., 2013). Several processes, relatively specific to wetlands, are known to influence the rates at which inorganic Hg can be biotransformed to MeHg (Eagles-Smith et al., 2018; Hsu-Kim et al., 2018). Such factors involve sedimentation and biomethylation by microorganisms, which depends on physical (e.g., anoxia in slow moving water bodies) and biological (e.g., development of periphytic biofilm on algae and macrophytes) processes (see Hsu-Kim et al., 2018 for a review, Gochfeld, 2003). Once methylated, Hg becomes readily bioavailable, is bioaccumulated within organisms and biomagnified through the food chain (Lavoie et al., 2013). In turn, these processes can also be influenced by several ecological factors such as primary productivity, habitat structure, bioenergetics, and food web structure (Eagles-Smith et al., 2018).

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In complex aquatic networks, monitoring Hg contamination is logistically complicated. To circumvent these difficulties, the use of biological indicators is often required and allows spatiotemporal biomonitoring of the contaminant. Traditionally, freshwater fish have been intensively investigated for Hg contamination (e.g., Depew et al., 2012; Åkerblom et al., 2014; Scheuhammer et al., 2015; Eagles-Smith et al., 2016), because fish are widely used as a food source by many human populations (Futsaeter and Wilson, 2013; Dong et al., 2015; Lepak et al., 2016; Fliedner et al., 2016). More recently, aquatic reptiles (mainly turtles and snakes) have been shown to be particularly useful to monitor Hg in freshwater habitats (Bergeron et al., 2007; Burger et al., 2009; Schneider et al., 2009, 2010, 2013; Turnquist et al., 2011; Hopkins et al., 2013a; Eggins et al., 2015; Adel et al., 2017). Because of their relatively limited dispersal capacity and their high degree of philopatry, Hg concentrations found in their tissues should be closely linked to those of their small home-ranges, thereby allowing a spatially explicit sampling. In addition, reptiles are considered as meso- or apex-predators thereby allowing to integrate information from the underlying levels of the trophic web. Contrarily to endothermic vertebrates, their relatively lower metabolic rates and relatively higher tissue conversion rates allow a greater capacity to integrate long-term Hg contamination in their organism (Slimani et al., 2018; Lemaire et al., 2018). Finally, recent developments of non-invasive techniques to assess individual Hg contamination allow sampling of relatively large sample sizes while respecting important ethical considerations. For instance, easily accessible tissues such as scales or claws in which Hg bioaccumulates due to its binding to keratins (Schneider et al., 2011; Bezerra et al., 2013) allow the use of such a non-invasive technique to quantify Hg contamination in these models (Hopkins et al., 2013a; Lemaire et al., 2018; Slimani et al., 2018).

Using the European pond turtle *Emys orbicularis* as a bioindicator of Hg, we took advantage of a natural, semi-experimental context in one of the largest continental French wetlands: the Brenne region. In this area, each pond harbours a faithful turtle population and is characterized by a suite of features (age, size, usage; Benarrous, 2017). Based on a relatively large sample size (255 turtles from 15 ponds), the goals of our study were three-fold. First, we investigated the individual determinants of Hg concentrations (i.e., sex, age and body size). Second, we aimed at assessing the environmental determinants of Hg contamination based on each site's unique combination of physical features (i.e., age of the pond, its size and usage). Finally, we investigated the potential deleterious consequences of such contamination in our study species by examining relationships between Hg concentrations and body-condition and reproduction.

2. Material and methods

2.1. Study species and study sites

The European pond turtle *Emys orbicularis* is a small European freshwater turtle species. Our study took place in 2016 and 2017 in the Brenne Natural Park (hereafter "Brenne"). Brenne spreads over 1700 km^2 and is characterized by a matrix of ~4000 large artificial ponds created since the Middle-Age in order to raise fish (Benarrous, 2017).

Sampling (see below) took place on 15 ponds, mainly located in the vicinity of the Réserve Naturelle Nationale de Chérine (46°47′25.23″N, 1°12′3.54″E, Fig. 1). Sites were sampled either in 2016 (May to September, N = 9 sites and N = 148 turtles) or in 2017 (April to June, N = 6 sites and N = 107 turtles). Because each pond is characterized by a unique suite of features, those were assessed as follow. We measured the size of each pond (ha) using QGIS (QGIS Development Team (2017). QGIS Geographic Information System. Open Source Geospatial Foundation Project. http://qgis.osgeo.org). We recorded the age of each pond from local archives (Benarrous, 2017). Because local usage is characterized by a full draining of each pond during a whole year (for pond maintenance) at a variable frequency (typically once every 7–25 years), the elapsed time since the last draining event was also recorded (hereafter latency since last draining "LSLD"). Finally, we created two categories of local management: ponds located in protected areas (or managed by the Natural Reserve staff) were classified as "reserves", while ponds not submitted to any regulations were classified as "not protected". The combination of this detailed information for various sites distributed across a relatively similar environmental context and over a relatively small spatial scale represents a powerful opportunity to investigate the environmental determinants of Hg contamination in a typical continental wetland.

2.2. Field procedures and sampling

Turtles were captured with baited funnel traps from April to July usually during three 4 days-long capture sessions per site. Funnel traps were visited every day in order to avoid long-term disturbance for captured individuals.

Upon capture, each turtle was individually and permanently marked with notches in the marginal dorsal and ventral scutes. The straight carapace length (SCL) was measured with a calliper (± 0.01 mm) and body mass was measured with a digital scale (± 1 g). Most individuals were sexed based on the morphology of the plastron (concave in males, Zuffi and Gariboldi, 1995). Sexing was not possible in a few juvenile individuals and these individuals were kept as "unsexed" in our analyses. Individuals were classified as juveniles when a large growth ring was visible on the ventral scutes, while individuals lacking this growth mark were classified as adults (Olivier, 2002). Gravidity of adult females was systematically assessed using manual pelvic palpation (Duguy and Baron, 1998; Olivier, 2002).

Finally, the distal extremities of 2 claws from each leg per individual were collected with a nail clipper for further Hg measurements. Such non-invasive technique has been successfully used in several freshwater species (e.g., Hopkins et al., 2013a; Slimani et al., 2018) and validated in *Emys orbicularis* from the Brenne area (Guillot et al., 2018). Importantly, each turtle is relatively faithful to its home-pond (Owen-Jones, 2015) thereby allowing for a precise determination of the Hg contamination level for each site. When possible, we aimed at collecting claws from 10 males and 10 females per site. Overall, we collected claw samples from 255 individuals (232 adults and 23 juveniles), representing 126 females, 122 males and 7 unsexed individuals.

Each individual was released at the location of capture, usually within an hour after capture.

2.3. Mercury analysis

Prior to chemical analysis, claws were cleaned three times with a mixture of 2:1 chloroform/methanol solution in an ultrasonic cleaner, rinsed in milli-Q quality water, and dried for 48 h (Guillot et al., 2018). Total Hg concentrations were measured using an atomic absorption spectrophotometer (Advanced Mercury Analyser-254, Altec) on dried tissue aliquots (ranging from 0.5 to 3 mg) as described by Chouvelon et al. (2009). The analytical quality (i.e. accuracy and reproducibility) of the Hg measurements was assessed by the analyses of blanks and certified reference material (CRM) TORT-2 Lobster Hepatopancreas (NRC, Canada; certified Hg concentration: 0.27 ± 0.06 μg g^{-1} dw). The CRM were analysed at the beginning and at the end of the analytical cycle, and by running controls for every 10 samples (Bustamante et al., 2006). Mass of the CRM was adjusted to represent an amount of Hg similar to that in turtle samples. Our measured values for the CRM were 0.255 ± 0.014 μg g^{-1} dw (N = 38) showing a recovery of 94%. Blanks were analysed at the beginning of each set of samples and the quantification limit of the method was 0.05 ng. Data for Hg concentrations are presented as μg.g^{-1} relative to the dry weight (dw).

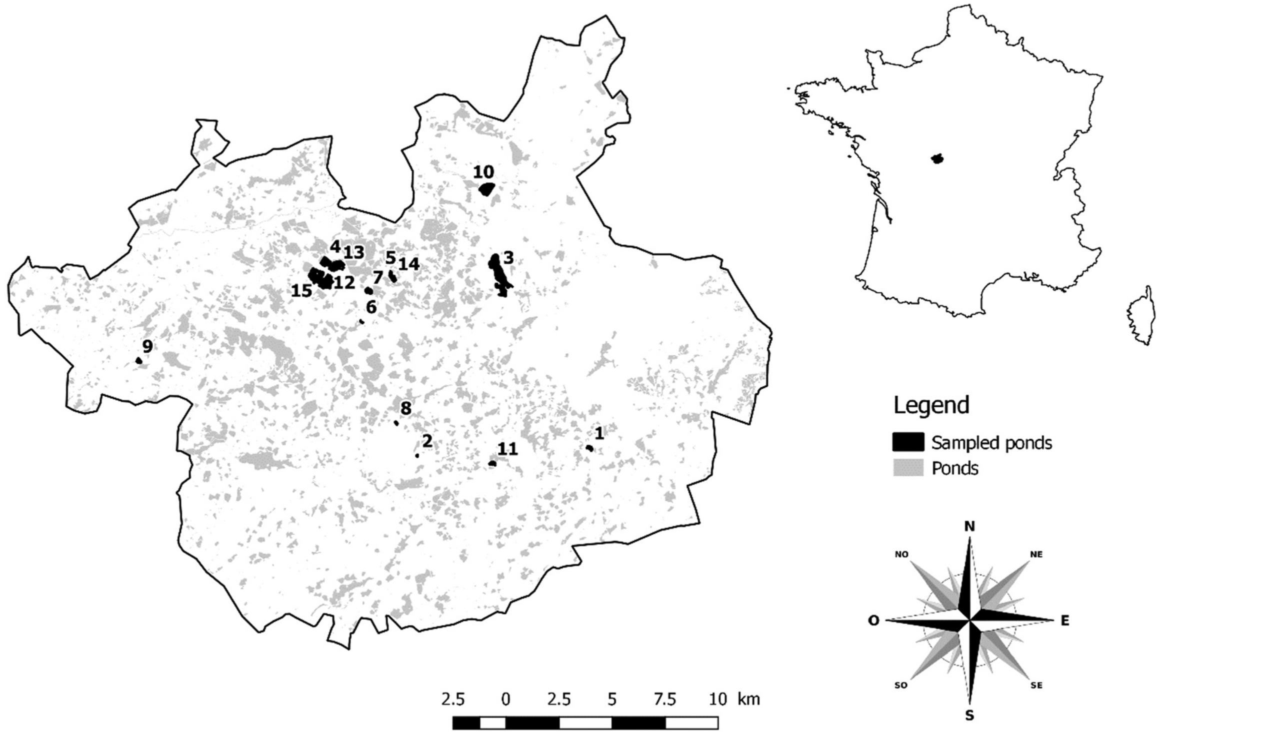

Fig. 1. Map of the study sites and general situation of the Brenne area in France. Grey areas represent all the ponds of the area, while black areas represent the ponds sampled for the present study.

2.4. Statistical analyses

We used general linear models to investigate individual determinants of Hg concentration, with Hg concentration as the dependent variable and individual characteristics as the factors (sex [unsexed, female, male], age class [juvenile, adult] and size [SCL]).

We also used general linear models to investigate environmental determinants of Hg concentration, with Hg concentration as the dependent variable, and pond characteristics as the factors (pond size, pond age, LSLD, and management category).

To explore the potential correlates of Hg concentration, we relied on two proxies of turtle state. First, we computed a body-condition index (calculated as the residuals of the regression of log-transformed body mass against SCL) separately for males and females. The relationship between body-condition index and Hg concentration assessed with linear regressions.

Second, for each site, we assessed the proportion of gravid females. It was calculated as the number of gravid females divided by the total number of females restricted to those captured during the reproductive period solely (defined as period between the date of capture of the first gravid females and the date of capture of the last gravid female). Gravidity can be influenced by environmental conditions (e.g., temperature, precipitation, food availability). Indeed, both years show strong variation of the proportion of gravid females (i.e., 0–18% for the sites sampled in 2016 versus 0–53% for the sites sampled in 2017, see results). As a consequence, both years were analysed separately. Because there was only one value per site (and per year) for the proportion of gravid females (see above for our calculations of this proportion), relationships between this metric and mean Hg concentration (restricted to adult females with SCL > 133 mm)) were assessed with Spearman rank correlations. Importantly, both estimates should be relatively independent as the proportion of gravid females was calculated for each site's captured individuals (i.e., including individuals not sampled for Hg) while the mean Hg concentration for adult females was calculated on a sub-sample of the captured population.

All analyses were performed with Statistica 12.

3. Results

Mean Hg concentrations (± SD) for each site and sex are presented in Table 1.

Hg concentration was not linked to turtle size ($F_{1250} = 2.35$, $p = 0.13$) nor to turtle sex ($F_{2250} = 1.79$, $p = 0.17$) but was influenced by turtle age classes ($F_{1250} = 10.10$, $p = 0.001$) with juveniles having lower Hg concentration than adults (mean Hg 1.62 ± 0.20 for juveniles and 2.21 ± 0.06 $\mu g\, g^{-1}$ dw for adults, Fig. 2).

Hg concentrations diverged strongly between sites ($F_{14,240} = 7.27$, $p < 0.0001$; range 1.44 ± 0.18 – 3.17 ± 0.25 $\mu g\, g^{-1}$ dw, Table 1). Hg concentrations was not linked to management category ($F_{1127} = 0.02$, $p = 0.87$) or pond size ($F_{1127} = 0.06$, $p = 0.81$) but it was positively related to pond age ($F_{1127} = 7.17$, $p = 0.008$) and negatively related to LSLD ($F_{1127} = 4.43$, $p = 0.03$, Fig. 3).

We did not find a significant influence of Hg concentrations on the body condition of males ($F_{1117} = 1.31$, $p = 0.25$) or females ($F_{1123} = 0.007$, $p = 0.93$). Similar results were found when the analyses were restricted to adult individuals (all $p > 0.32$).

We found significant negative relationships between the proportion of gravid female per pond and the Hg concentrations of adult females for both 2016 (Spearman rank correlation, $r_s = -0.50$, $p < 0.0001$, Fig. 4) and 2017 (Spearman rank correlation, $r_s = -0.39$, $p = 0.004$, Fig. 4.).

4. Discussion

Overall, the results we found on the individual determinants of Hg concentrations in *E. orbicularis* support the use of freshwater turtles as bioindicators of environmental contamination, as already demonstrated in other studies (Bergeron et al., 2007; Burger et al., 2009; Schneider et al., 2009, 2010; Turnquist et al., 2011; Hopkins et al., 2013b; Slimani et al., 2018). Importantly, the semi-experimental context of our field sites allowed to highlight several environmental variables (other than direct anthropogenic contamination) that appear to influence Hg bioavailability to turtles. Finally, the concentrations of Hg we found in

Table 1
Summary of the characteristics of the study sites (15 ponds) and associated sampled turtles. "Number" refers to the number displayed in Fig. 1. "Management" indicates management type (R: reserves, NP: not-protected). "Age" indicates the age of the pond in years. "LSLD" stands for "latency since last draining" in years. "Sex" indicates the sex of the sampled turtles (U: unsexed individuals, F: females, M: males). "SCL" stands for straight shell length (mean ± SD, mm). Hg concentrations in turtle claws are given as the mean ± SD (μg g^{-1} dw). See text for details.

Number	Name	Management	Age (years)	LSLD (years)	Sex	SCL	Hg	N
1	Beaugu	R	NA	3	U	111.53	1.22	1
					F	158.94 ± 12.15	1.69 ± 0.35	11
					M	145.68 ± 8.97	1.99 ± 0.52	9
2	Beauju	NP	NA	NA	U	96.49	2.50	1
					F	157.42 ± 8.42	3.39 ± 2.23	4
					M	135.60 ± 3.82	2.13 ± 0.56	4
3	Bellebouche	NP	618	NA	F	144.15 ± 14.13	3.12 ± 1.39	11
					M	137.19 ± 7.49	2.55 ± 0.71	11
4	Cistude	R	37	4	U	126.66	0.94	1
					F	166.21 ± 6.13	2.10 ± 0.99	8
					M	146.68 ± 8.91	1.64 ± 0.41	7
5	Courlis	NP	NA	NA	F	159.89 ± 4.67	1.67 ± 0.48	10
					M	151.46 ± 4.77	1.80 ± 0.62	7
6	Gorgeat	R	578	10	F	161.09 ± 10.93	2.04 ± 0.37	11
					M	149.88 ± 10.53	2.06 ± 0.44	8
7	Hautes-Rondières	R	800	15	F	157.16 ± 7.71	1.81 ± 0.37	10
					M	145.34 ± 7.12	1.62 ± 0.33	10
8	La Cure	NP	NA	NA	U	98.78	2.60	1
					F	160.6 ± 3.82	2.46 ± 1.00	5
					M	142.65 ± 11.23	3.21 ± 0.66	6
9	La Touche	R	NA	3	F	143.98 ± 18.93	2.52 ± 0.82	4
					M	148.32 ± 9.84	3.53 ± 0.94	7
10	Lion	R	337	6	F	153.83 ± 12.94	1.33 ± 0.29	11
					M	151.28 ± 7.67	1.55 ± 0.99	10
11	Ménettrie	NP	NA	NA	U	124.61 ± 10.04	1.13 ± 0.23	3
					F	154.47 ± 12.66	2.37 ± 0.54	7
					M	148.59 ± 8.56	2.07 ± 0.82	11
12	Monmelier	NP	562	5	F	155.36 ± 9.09	2.83 ± 0.54	8
					M	138.93 ± 2.31	2.11 ± 1.27	3
13	Nuret	NP	549	8	F	160.80 ± 6.87	1.82 ± 0.48	10
					M	152.86 ± 9.80	1.59 ± 0.25	10
14	Petit Ajonc	NP	NA	NA	F	167.70 ± 7.80	1.90 ± 0.56	2
					M	146.97 ± 6.37	1.64 ± 0.41	8
15	Ricot	R	500	1	F	151.81 ± 7.21	3.19 ± 1.04	14
					M	144.25 ± 6.10	2.32 ± 1.09	11

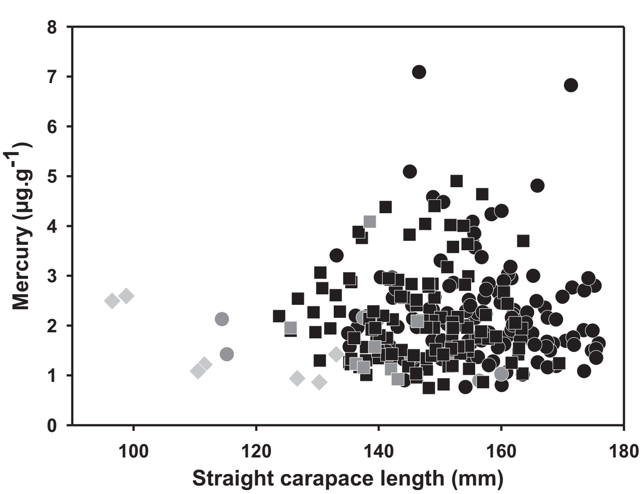

Fig. 2. Relationship between turtle straight shell length (SCL) and Hg concentration (μg g^{-1} dw) in turtle claws. Symbols stand as follow: circles represent females, squares represent males, diamond represent unsexed individuals. Black symbols represent adults, while dark grey symbols stand for juveniles.

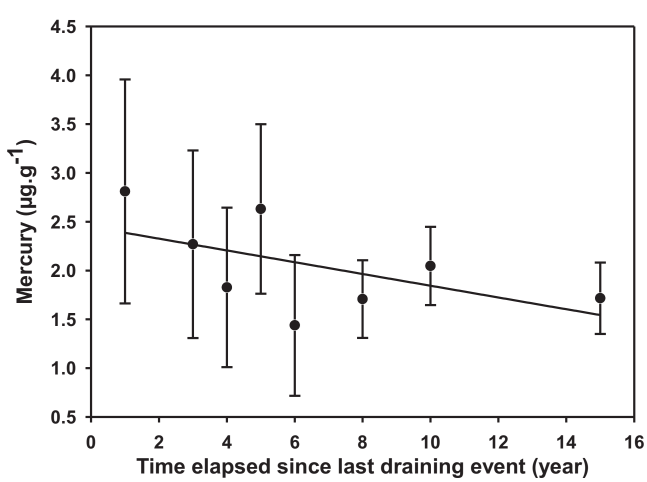

Fig. 3. Relationship between the time since last draining event and Hg concentration ($r_s = -0.28$). For each site, Hg levels are given as the mean ± SE (μg g^{-1} dw) in turtle claws.

E. orbicularis from our study site appear related to their propensity to reproduce and thus may bear consequences for the persistence of this population. All of these findings are discussed sequentially below.

4.1. Individual determinants of Hg concentrations

The usefulness of freshwater turtles as bioindicators of Hg contamination has already been demonstrated in this species (Guillot et al., 2018) and others (Bergeron et al., 2007; Burger et al., 2009; Schneider et al., 2009, 2010; Turnquist et al., 2011; Hopkins et al., 2013b; Slimani et al., 2018). Consistent with these studies, our investigations also

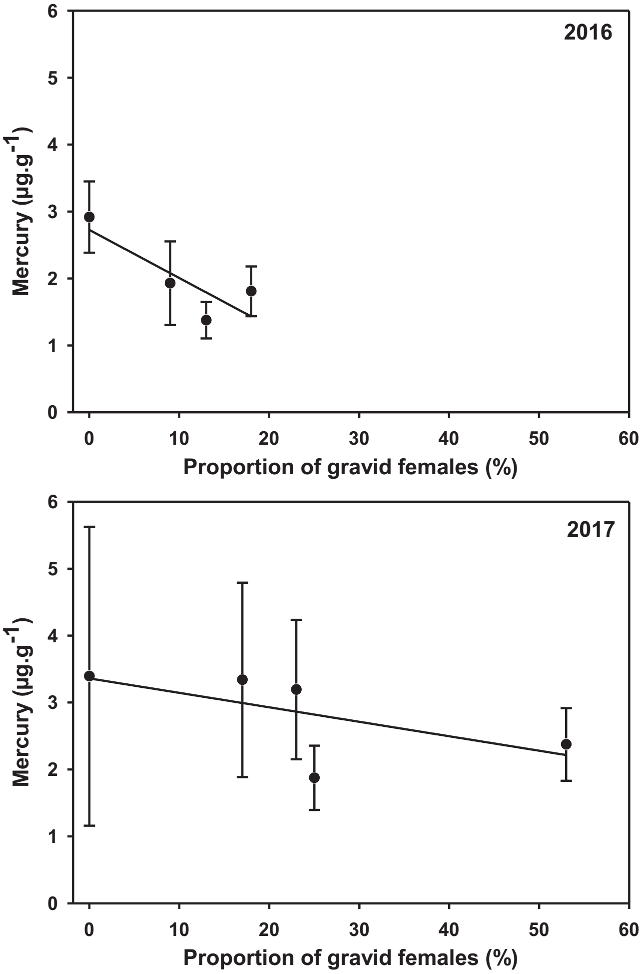

Fig. 4. Relationship between the proportion of gravid females and Hg concentrations in the claws of adult females (year 2016 upper panel, $r_s = -0.50$, year 2017 lower panel, $r_s = -0.39$). For each site, Hg concentrations are given as the mean ± SE (µg g^{-1} dw).

indicate that analysis of the claws of *E. orbicularis* is a useful non-invasive technique to monitor Hg contamination in a large number of individuals. We found that juvenile individuals had lower Hg concentrations in their claws than older adult turtles indicating that Hg bioaccumulates during the life of *E. orbicularis* (Guillot et al., 2018). Despite this difference between age classes (juveniles versus adults), we did not find any relationship between turtle size and Hg concentration, while such correlation has been previously recorded (Guillot et al., 2018; Hopkins et al., 2013a). We suggest two complementary, non-mutually exclusive hypotheses to explain this result. First, the range of size available in our study is restricted to relatively large individuals (e.g. all SCL > 96.5 mm corresponding to large juveniles and adults), while a previous study included neonates and very small individuals (see Guillot et al., 2018). Such restricted range of size inevitably obscured any relationship between Hg concentration and turtle size. Second, *E. orbicularis* has a relatively determinate growth. Indeed, although young adults can continue to grow, most older individuals have nearly ceased growing. As a result, individuals having a similar SCL can differ in age (i.e., mixing young and very old adults). Because Hg bioaccumulates with age, individuals having a similar size can display highly different Hg concentrations. Again, such phenomena would obscure any relationship between Hg concentrations and size among adult individuals. Future studies should investigate such hypotheses, notably by sampling individuals of known age from long-term mark-recapture studies.

As already suggested for this species (Guillot et al., 2018), and for other freshwater turtles (Adel et al., 2005; Bergeron et al., 2007; Schneider et al., 2009; Turnquist et al., 2011; Hopkins et al., 2013a; Slimani et al., 2018) sex did not influence Hg concentrations. This suggests that feeding, metabolism and/or growth rates are presumably fairly similar for both sexes (Allender et al., 2015; Yu et al., 2011), contrarily to what has been found in another species of freshwater turtle (Nagle et al., 2001) and in seaturtles (Guirlet et al., 2008). In addition, this further suggests that reproduction (egg production and laying) does not represent a major excretion pathway in female *E. orbicularis* (but see Burger and Gibbons, 1998; Hopkins et al., 2013b). Yet, mechanisms through which female *E. orbicularis* would limit Hg deposition in their eggs remain puzzling, and future studies should usefully investigate this question.

4.2. Environmental determinants of Hg concentrations

Our study highlights the role of several environmental factors affecting the concentrations of Hg in the claws of *E. orbicularis*. Importantly, these determinants are not related to direct sources of pollution due to anthropogenic activities (e.g., industrial effluents, Wang et al., 2004), but instead indicate subtler sources of Hg bioavailability in the wild (Driscoll et al., 2013). First, the management category of each pond does not seem to significantly influence Hg concentrations in turtles. This suggests that, in Brenne, intensive fish farming (in unprotected ponds) may not be responsible for the transfer of marine mercury through fish food as shown in trout farming systems (Hansson et al., 2017; Lemaire et al., 2018). Indeed, in our study area, fish farming is mainly dedicated to omnivorous species (i.e., carps) for which food supplementation is represented by corn, rather than commercial pellets based on proteins from fishery products of marine origin (Hansson et al., 2017). Second, our results show that *E. orbicularis* inhabiting older ponds display relatively higher Hg concentrations. This result suggests that Hg concentrations, and thus its bioavailability, increase with the age of the water body. Because natural sources of Hg include both hydrologic and atmospheric deposition (e.g., Mason et al., 2012), older water body have been subjected to these natural processes for a longer time period, and may thus display naturally higher Hg concentrations (Eagles-Smith et al., 2018). Although it may be complicated to assess such parameter in other geographic areas, we believe that this natural source of variation of Hg concentration is a major issue to take into account. Finally, we found an influence of the latency since the last draining event of the ponds on the Hg concentrations of *E. orbicularis*. Periodic year-long draining of each pond has been ancestrally used in Brenne to maintain pond and associated structures (e.g., pond drain). During such a year-long draining event, the organic matter contained in the mud is mineralized. As a consequence, the years following such draining episode are usually characterized by a significant bloom of aquatic vegetation both in terms of density and species richness (Richier and Broyer, 2014). Such process can influence Hg bioavailability (i.e., the methylation of inorganic Hg) by promoting growth of bacterial biofilm on algae and macrophytes, all of which play a role in Hg methylation, and thus in the increase of its bioavailability (Gentès et al., 2013, 2017). Furthermore, the macrophytic rhizosphere is involved in Hg methylation, and may therefore increase Hg bioavailability to local fauna (Gentès et al., 2013). Such process is likely to explain the negative and significant relationship we found between the latency since the last draining event and the Hg concentrations in *E. orbicularis*. Although draining events are no doubt favourable to promote aquatic vegetation blooming (Richier and Broyer, 2014), our results suggest that if they are too frequent, they may provoke an increase of Hg bioavailability. Future management policies should take this result into account, and should aim at a finely tuned balance between the required periodical blooms of aquatic vegetation and the associated increased bioavailability of Hg.

4.3. Reproductive correlates of Hg concentrations

Finally, our study allowed to highlight potential negative effects of Hg concentrations on the reproduction in *E. orbicularis*. Specifically, we found negative relationships between mean Hg concentrations of adult females and the proportion of reproductive females for a given site. Importantly, this negative trend was repeatedly detectable during the two consecutive years of our study. Although Hg has been shown to negatively influence hatching success in another species of freshwater turtle (Hopkins et al., 2013b), to our knowledge our study is the first to document a negative relationship between Hg concentrations and the propensity of adult females to reproduce. Such negative relationship may be attributable to endocrine disruptive properties of Hg. Indeed, Hg has been shown to affect thyroid hormones in freshwater turtles (Meyer et al., 2014). Our results on body condition (i.e. no effect of Hg on turtles' body condition) do not support an influence of Hg on feeding, growth or indeed, metabolism. Our results rather suggest an influence of Hg on reproductive mechanisms. For instance, Hg and especially MeHg is known to disrupt reproductive hormones (Frederick and Jayasena, 2011; Tan et al., 2009; Zhu et al., 2000) and have been shown to influence reproductive probability in other long-lived vertebrates (Tartu et al., 2013; Goutte et al., 2014). Our dataset does not allow teasing apart causation and our conclusion needs to be taken with caution. Despite the lack of evidences of direct causation, Hg concentrations found in *E. orbicularis* (up to 7 $\mu g\ g^{-1}$ dw) are coherent with those known to provoke reproductive failure in other study systems (e.g., 1 $\mu g\ g^{-1}$ in blood and from 2.4 to 40 $\mu g\ g^{-1}$ in feathers in birds for instance, Burger and Gochfeld, 1997, Evers et al., 2008), suggesting the relationships we found may not be trivial for *E. orbicularis*. Future studies should investigate reproductive hormone levels and Hg concentrations in female *E. orbicularis* in order to test for this hypothesis. Interestingly, although the Brenne population of *E. orbicularis* is thought to be one of the largest in France, the proportion of neonates is among the lowest (Beau, 2015). Clearly the negative relationship between Hg concentrations and the proportion of reproductive female we detected suggest that Hg contamination, even in absence of direct and strong sources of pollution, may have a critical impact on reproduction and thus the persistence of population in a long-lived vertebrate.

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