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Low levels of POPs in *Emys orbicularis* in the Camargue

Determinants of Legacy POPs Levels in the European Pond Turtle (*Emys orbicularis*) in the Camargue Wetland, France

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Abstract: Many banned persistent organic pollutants (POPs) remain for decades in the aquatic environment and can have harmful effects on long-lived predators because of their high bioaccumulation and biomagnification potentials. We investigated the occurrence and levels of 18 polychlorinated biphenyls (PCBs) and 16 organochlorine pesticides (OCPs) in European pond turtles (n = 174) from April to July 2018 in the Camargue wetland, France. Although the Camargue was highly contaminated in previous decades, plasma occurrence and levels of POPs were very low: we were able to quantify only three of the 34 compounds we analyzed in more than 10% of the turtles. POP burdens did not differ between males and females and were uncorrelated with sampling date and body mass. We observed differences in POP burdens between turtles from the two sampling sites. One possible explanation is that the sampling sites were in different agricultural hydraulic system: plasma occurrence and levels were higher for PCB-52 and HCB in turtles captured in drainage channels, and for PCB-153 at the site that receives irrigation. Finally, the occurrence and levels of PCB 153 in turtles increased with age, likely because of bioaccumulation and much higher exposure 20-30 years ago than now.

Keywords: reptiles, PCB, organochlorine pesticides, delta wetlands

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1. Introduction

In Europe, the production and use of persistent organic pollutants (POPs) have been prohibited or severely restricted since 2004 through the Stockholm Convention (http://www.pops.int/), but POPs still cause several environmental concerns today. Polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) remain in the environment for decades because of their slow biodegradation and because of improper handling of contaminated wastes. Levels of POPs in animals increase with age (Vives et al., 2005; Binnington & Wania, 2014) and trophic level (Goutte *et al.*, 2020). Exposure to POPs causes a wide range of adverse effects, including neurotoxicity, endocrine disruption, immune dysfunction, reproductive impairment, and developmental abnormalities, which may ultimately compromise survival and reproductive output and lead to population declines of wild vertebrates (Goutte *et al.*, 2014, 2015; Salice *et al.*, 2014).

Pollution, especially from industrial and agricultural discharges, is one of the major threats to freshwater ecosystems (Holt, 2000; Dudgeon *et al.*, 2006). Wetlands support an extremely rich biodiversity, but are among the most transformed and threatened ecosystems of the world (Revenga *et al.*, 2005), experiencing rates of population declines and species extinctions far higher than those in forests, grasslands, and coastal ecosystems (Dudgeon *et al.*, 2006). Deltas are often exposed to high water pollution from intensive agriculture (Kuenzer & Renaud, 2012) and from discharge of contaminants in upstream waters. The Camargue, in the Rhône River delta, is the largest wetland in France and is of international importance under the Ramsar Convention (https://www.ramsar.org/), but it is heavily impacted by human activities (Cheiron *et al.*, 2018). The contamination of water bodies by agricultural, industrial, and urban discharges has been well studied over the past decades (Comoretto *et al.*, 2007). In particular, levels of PCBs in sediments of the Rhône River increase from upstream to downstream, reaching 417 μg/kg dw (dry weight, for the sum of 7 PCB congeners (28, 52, 101, 118, 138, 153, and 180; Mourier et al. 2014). Mean flux over the 2011–2016 period was 14 kg/year for PCB 180 in suspended particulate matter at the outlet of the Rhône River (Poulier *et al.*, 2019). Previous studies in the Camargue confirmed that both birds (Berny et al., 2002) and fish (Roche et al., 2002, 2003, 2009a) are exposed to OCPs and PCBs.

Freshwater turtles remain poorly studied in ecotoxicology although they can provide useful information on local contamination because of their longevity (Campbell & Campbell, 2002; EL Hassani *et al.*, 2019; Gaus *et al.*, 2019), high trophic-level, and low dispersal capacity (Châteauvert *et al.*, 2015; Ming-ch'eng Adams *et al.*, 2016). Moreover, as ectothermic vertebrates, turtles have a lower ability to metabolize pollutants than endothermic mammals and birds (de Solla, 2015). The European pond turtle, *Emys orbicularis*, a long-lived (> 40-80 years) opportunistic predator (feeding on fish, amphibians, aquatic insect, gastropods, and crayfish) and scavenger (Ottonello et al. 2005; Ficetola & De Bernardi, 2006; Ziane et al. 2020), is facing significant population declines due to multiple environmental alterations, including water pollution (Cheylan 1998). The European pond turtle is listed as "near threatened" (NT) on the UICN Red List of threatened species (Tortoise and

Freshwater Specialist group, 1996). Pollution by trace metal elements has been studied in *E. orbicularis* (Namroodi *et al.*, 2017; Guillot *et al.*, 2018; Beau *et al.*, 2019), but data are lacking on burdens of POPs.

We studied contamination levels in two populations of *E. orbicularis* in the Natural Reserve of the Tour du Valat in the Camargue, France. Several individuals were of known-age owing to a capture-mark-recapture (CMR) program initiated more than 20 years ago (Olivier *et al.*, 2010; Ficheux *et al.*, 2014; Arsovski *et al.*, 2018). The hydraulic system consists of irrigation canals originating from the Rhône river and of drainage canals, which receive various environmental contaminants from the Rhône river and agricultural plots, especially rice fields (Chauvelon, 1996). In the present study, we assessed recent (2018) levels of OCPs and PCBs in plasma of the European pond turtle (n = 174). We also tested the effects of individual traits (mass, sex, and age) on POPs burdens. We expected that older and larger individuals should have higher levels of POPs because of bioaccumulation and length of exposure.

Moreover, we compared POPs burdens of turtles from two populations in different locations in the agricultural hydraulic system (irrigation vs drainage). We expected high levels of OCPs in turtles from the drainage site, as a possible consequence of the remobilization of pesticides from agricultural soils, and high levels of PCBs in turtles from the irrigation channel, as a possible consequence of the historical contamination. We acknowledge the limitations to our inferences about the possible causes of site differences in POPs burdens because we had a single site in irrigation canals and a single site in drainage canals. Therefore, we are unable to disentangle site effects from hydrologic effects. Consequently, we only cautiously offer some plausible explanations for site differences.

2. Material and methods

2.1. Sampling sites and capture

We conducted the study in the Natural Reserve of the Tour du Valat (43°30' N, 4°40' E, Fig. 1) in France. We captured European pond turtles (n = 174) in canals and marshes from 24 April to 26 July 2018 by hand or with funnel traps (Olivier *et al.*, 2010; Ficheux *et al.*, 2014). We captured turtles at two sites: (1) irrigation canals and their associated marshland (site of Esquineau, n = 126) and (2) drainage canals of the Fumemorte basin and marshes filled with water by these canals (site of Faïsses, n = 48). The sex ratio was the same between the two sites (58.3% females at Faïsses and 60.3% females at Esquineau, Pearson's Chi-squared test, p = 0.95).

Individual turtles were identified as part of a long-term CMR program with unique combinations of shallow notches on the marginal and nuchal scales (Olivier, 2002; Olivier *et al.*, 2010). Individuals were weighed and sexed by visual observation of the secondary sexual characters. The year of birth can be determined if the first capture occurred within the first 5 years of life by counting the number of growth streaks (Castanet, 1988). The long-term CMR program started in 1997, but a few dozens of individuals were marked as adults in 1976. In our data set, 66 of the 174 individuals were of known-age (5 to 26 years old, corresponding to a first capture between 1997 and 2018) and some were first captured as adults in 1976, thus being considered as more than 44 years old. When turtles were first captured after their first 5 years of life (n = 108), we determined stages of growth based on growth streaks on the plastron (Olivier, 2002). By combining known ages and stages of growth, we assigned an age class to each turtle: 4-8 years old (n = 20), 9-13 years old (n = 46), 14-

22 years old (n = 54), and 23-44+ years old (n = 54). Turtles were released on the same day at their capture site.

2.2. Blood sampling

Plasma is a good matrix to determine individual burdens of POPs because blood sampling is minimally invasive and because circulating levels of POPs in blood are significantly correlated to concentrations of POPs stored in fat (Keller *et al.*, 2004; Dabrowska *et al.*, 2006), liver, kidney, and muscle tissues (Van de Merwe *et al.*, 2010). We collected blood samples (2 ml, always less than 1% of the turtle body mass) from the dorsal coccygeal vein (Innis & Knotek, 2020; Keller *et al.*, 2004) with a Terumo Company® syringe (Somerset, USA) pre-impregnated with heparin to prevent blood clotting during collection and equipped with a 25G needle (Franklin Lakes, USA). We then centrifuged the samples to separate the plasma from the red blood cells. Samples were stored at −18 °C until analysis in the UMR 7619 METIS of Sorbonne Université, France.

2.3. Chemical analyses

We determined the levels of 17 OCPs (p,p′-DDT and metabolites (p,p′-DDE and p,p′-DDD); pentachlorobenzene (PeCB); hexachlorobenzene (HCB); pentachloronitrobenzene (Quintozene); four isomers of hexachlorocyclohexane (α-, β-, γ- (lindane), δ-HCH); aldrin; endrin; isodrin; telodrin (Isonbenzan); heptachlor; heptachlor epoxide; heptachlor endo-epoxide), 7 marker PCBs (M-PCBs, IUPAC numbers # 28, 52, 101, 118, 138, 153 and 180), and 12 dioxin-like PCBs (DL-PCBs, IUPAC numbers # 77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169 and 189) in the 174 plasma samples.

Samples were processed through Solid Phase Extraction (SPE), using hexane: dichloromethane, 9:1, using a validated protocol (Tapie et al. 2011) that was adapted for plasma samples (see Supplementary Material for a detailed description of the procedure and method validations). PCBs and OCPs were analyzed using an Agilent 7890 A gas chromatograph (GC) coupled to a 7000 B triple quadrupole mass spectrometer system (MS/MS) (Agilent Technologies, Les Ulis, France). Recovery rates (% RR) of compounds were assessed on replicate plasma samples of European pond turtles (n = 4) with spiked solutions (100 ng of each compound). The repeatability of the method was assessed in terms of relative standard deviation (% RSD) of the recovery (Table S2). Recovery rates were not satisfactory (< 75% or > 125%) for isodrin, endrin, heptachlor epoxid, heptachlor-endo-epoxide, p,p'-DDD, PCB-77, PCB-189 (Table S2) and these chemicals were thus not considered further.

2.4. Statistical analyses

We performed statistical analyses with R software version 3.3.2 (R Core Team, 2016). Only quantifiable POPs (i.e., values higher than the limit of quantification (LQ)) in at least 10% of the samples were included in the statistical analyses. Since age and mass were highly positively correlated ($t = 3.65$, $df = 64$, $p = 0.0005$) and since females were significantly heavier than males ($W = 474$, $p < 0.0001$), effects of body mass, sex, and age were evaluated in separate analyses.

We used generalized linear models (GLM) with a binomial distribution and a logit link function to test for the effects of sex, site, the interaction sex * site, sampling date, age, and body mass on contaminant occurrence. For each model, we used a backward elimination to progressively remove non-significant terms ($p > 0.05$). For all analyses, model specification and validation were based on residual analysis.

The effects of sex, sampling site, date, age and body mass on pollutant concentrations were tested by using all data and applying statistical methods for left-censored data to handle values below the LQ (Helsel, 2005). To do so, group comparisons and linear regressions were performed using Peto-Prentice tests and tobit models, respectively, with the function cendiff of the NADA package and the function tobit of the AER R-package (Shoari and Dubé 2018).

3. Results

3.1. POPs levels and occurrence

In 37 of the 174 samples, which represented 10.4% of turtles sampled in Faïsses and 24.4% of turtles sampled in Esquineau, all contaminant levels were below the limit of quantification. Eleven compounds were never detected at levels above the LQ in the plasma samples: PeCB, Lindane, Quintozene, Heptachlor, and the dioxin-like PCBs: PCB-118, -105, -114, -126, -156, -157 and -167. The following seven compounds were detected at levels above the LQ at least in one sample from Esquineau, but not in samples from Faïsses: alpha-HCH, 44'-DDT and PCBs 28, 101, 123, 169 (Table 1). Overall, the contaminant concentrations in the plasma samples were very low (Table 1).

3.2. Influence of individual traits, sampling site, and date on POPs levels and occurrence

Only three POPs (HCB, PCB-52 and -153) occurred commonly, being quantified in more than 10% of individuals (Table 1). Plasma occurrences of PCB-52 and HCB were significantly higher in turtles captured at Faïsses compared to Esquineau, while the occurrence of PCB-153 was higher in turtles captured at Esquineau (Tables 1 and 2). HCB occurrence slightly increased with sampling date (Table 2). Sex, mass, and age class did not explain POPs occurrence in turtles, except for PCB-153 whose detection frequency was higher in older individuals (Table 2).

Levels of HCB and PCB-52 were significantly higher in turtles from Faïsses, while PCB-153 levels tended to be higher in turtles from Esquineau (Tables 1 and 3). Moreover, levels of PCB-153 were lower in the youngest turtles (Table 3, Fig. 2). Sex, mass, and date did not explain variation in levels of POPs among turtles (Table 3).

4. Discussion

The aim of this study was to characterize legacy levels of POPs (OCPs and PCBs) in the plasma of European pond turtles in the Camargue, France, and to determine whether these levels were a function of individual traits and habitat type. Plasma levels of POPs were low and often below limits of quantification (from 0.2 to 3.1 ng/mL). We found significant differences in burdens of POPs between turtles from the two sampling sites and these differences could be attributed to the hydraulic system (drainage/irrigation), keeping in mind that we lack site replication to conclude firmly to an effect of the hydraulic system. The occurrence and levels of PCB-153 were higher in older turtles.

The Rhône River has been historically contaminated, leading to an accumulation of POPs in sediments downstream (Mourier et al. 2014). Previous studies conducted on several animal taxa in the Camargue confirmed high exposure during the last decades, with high concentrations of PCBs in muscles of eels (*Anguilla anguilla*) fished between 1997 and 2000 (Roche *et al.*, 2004), but also in eggs of little egrets (*Egretta garzetta*) collected in 1996 (Berny *et al.*, 2002). POP levels were low in the pond turtle in Camargue, which could indicate a decline in legacy POPs exposure for wild species in the Camargue. Although a longitudinal study of the same species at the same locations would be required to test this hypothesis, decreasing PCB concentrations have been observed in the Rhône River sediments in previous decades (Liber *et al.*, 2019) and water analyses conducted by the Nature Protection National Society (SNPN) have not detected PCBs and OCPs in the Fumemorte canal since 2011 (Cheiron, 2019). Moreover, organochlorine contamination across food webs tended to diminish through time in the Vaccarès lagoon (Roche et al., 2009a), into which the Fumemorte canal flows. Exotic red swamp crayfish (*Procambarus clarkii*), the main prey of European pond turtles in the Camargue (Ottonello *et al.*, 2005), were not contaminated by POPs in 2019 (i.e., < LQ, with LQ ranging from 0.9. to 1.98 ng/g dw, unpublished data).

One of the first studies looking at the plasma concentration of POPs in turtles documented high PCBs and OCPs levels in snapping turtles (*Chelydra serpentina*) in Ontario, Canada in 2001–2004 (Letcher *et al.*, 2015): plasma concentrations of OCPs (sum of 17 contaminants) ranged from 0.2 to 236 ng/g w.w.(wet weight) and the most abundant pesticide was p,p'-DDE (mean ± SE: 27 ± 6 ng/g w.w.). In our study, plasma concentrations of POPs (sum of 16 contaminants) were much lower, ranging from 0 to 2 ng/g w.w and p,p'-DDE levels did not exceed 2.3 ng/g ww. Snapping turtles are freshwater turtles with similar feeding habits to that of European pond turtle: snapping turtles also consume plant and animal matter, including aquatic invertebrates, fish, frogs, and reptiles (Ernst et al. 1994). Other studies of turtles documented plasma concentrations of POPs comparable to those in our study; for instance, for PCB 153 and HCB in another freshwater species, the Western pond turtle (*Actinemys marmorata*) in Sequoia National Park, USA, in 2012 (Meyer *et al.*, 2016), as well as in marine turtles such as Loggerhead sea turtles (*Caretta caretta*) in the Eastern Atlantic Ocean in 2011-2012 (Bucchia *et al.*, 2015), and in Green turtles (*Chelonia mydas*) and Hawksbill turtles (*Eretmochelys imbricata*) in Cape Verde in 2009-2011 (Camacho *et al.*, 2014).

In terms of occurrence of POPs, our results in European pond turtles were low compared to other studies (Bucchia et al., 2015; Meyer et al., 2016). The occurrences of dioxin-like PCBs in 8% of European pond turtles, as well as p,p'-DDE in 2% of individuals, were much lower than those found in plasma of Loggerhead sea turtles that ranged from 63% in the Atlantic Ocean to 100% in the Adriatic Sea for dioxin-like PCBs and 100 % for p,p'-DDE (Bucchia *et al.*, 2015).

Despite low detection frequencies and levels, we found differences in POPs burdens between turtles from the two sampling sites. Turtles living in the drainage waters of Faïsses site exhibited significantly higher occurrence and levels of HCB compared to turtles from Esquineau, likely because of the remobilisation of trapped OCPs in soils. On the other hand, the occurrence and levels of PCB 153 were higher in turtles from Esquineau, a site receiving water from irrigation channels. A previous study has documented higher concentrations of PCBs in the aquatic fauna in irrigation canals compared to the drainage canals downstream of rice fields (Roche *et al.*, 2009b). In contrast to the situation for PCB-153, concentrations of PCB-52 were higher in turtles in Faïsses, which may be due to the latter PCB's lower sedimentation rate associated with its lower molecular weight and a greater solubility due to a lower level of chlorination (Gong *et al.*, 1998; Alkhatib & Weigand, 2002). The processes of deposition and release of PCBs from the irrigation part to the drainage part of the canals need to be further studied and other populations of pond turtles in the two types of hydraulic systems should be studied to confirm this pattern.

The occurrence and levels of PCB-153 were lower in the youngest turtles (4-8 years old) which could be attributed to a shorter exposure period. PCB-153 is a recalcitrant chemical with a high hydrophobicity (high octanol-water partition coefficient), high resistance to metabolic transformation, and slow respiratory elimination through air-exhalation (high octanol-air partition coefficient). PCB-153 is also more prone to bioaccumulation than is the case for many other PCB congeners (Kelly *et al.* 2007), especially in long-lived air-breathing predatory species (Rowe, 2008). It is also possible that there is a dietary shift during growth in pond turtles leading to the consumption of higher trophic-level prey with age, and this diet would be more PCB contaminated due to biomagnification. This hypothesis, however, is not consistent with dietary studies of *E. orbicularis* in Camargue, that instead documented a shift to a more herbivorous diet with age based on prey identification in faecal samples (Ottonello *et al.*, 2005) or no difference in the proportion of plants, invertebrates, and vertebrates in the diet based on metabarcoding (Duccotterd et al. 2020).

We did not detect differences in POPs levels between male and female pond turtles, despite males often being more contaminated than females in other turtle species (Guirlet *et al.*, 2010; Bangma *et al.*, 2019; Lambiase e*t al.*, 2021) due to a transfer of pollutants from the mother to the eggs through vitellogenesis (Moss *et al.*, 2009; de Solla, 2010).

Conclusion

The concentrations of POPs were very low in European pond turtles, probably much lower than they were several decades ago in wild vertebrates in Camargue, France. The occurrence and concentrations of POPs may have been influenced by the hydraulic system where the turtles were captured and were influenced by turtle age. Further studies are needed to assess the impact of current contaminants on biodiversity, especially for aquatic top predators such as turtles.

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Ethics Statement—This study was performed in accordance with laws relative to capture, transport, and experiments on E. orbicularis (DREAL permit # CERFA_13616-01) and all procedures were approved by an independent ethical committee (APAFIS#17899-201812022345423 v2).

Disclaimer—The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability statement—Data, associated metadata, and calculation tools are available from the corresponding author (aurelie.goutte@ephe.psl.eu).

Author contributions statement—AO, LB, CLG and NM conducted the field work and collected the blood samples. LB and FA conducted the laboratory work to prepare plasma samples for analysis with gas chromatography and tandem mass spectrometry. LB, OL, AG, AO, GBD, and MV conceived and coordinated the study, participated in data analysis, and in writing the manuscript. All authors gave final approval for publication and agree to be held accountable for the work performed therein.

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Figures

Figure 1: Sampling locations for European pond turtles in the Regional Nature Reserve of Tour du Valat, Camargue, France. The two capture locations are Esquineau (in blue), irrigated with water pumped from the Rhône River, and Faïsses (in orange), consisting of drainage canals of agricultural parcels.

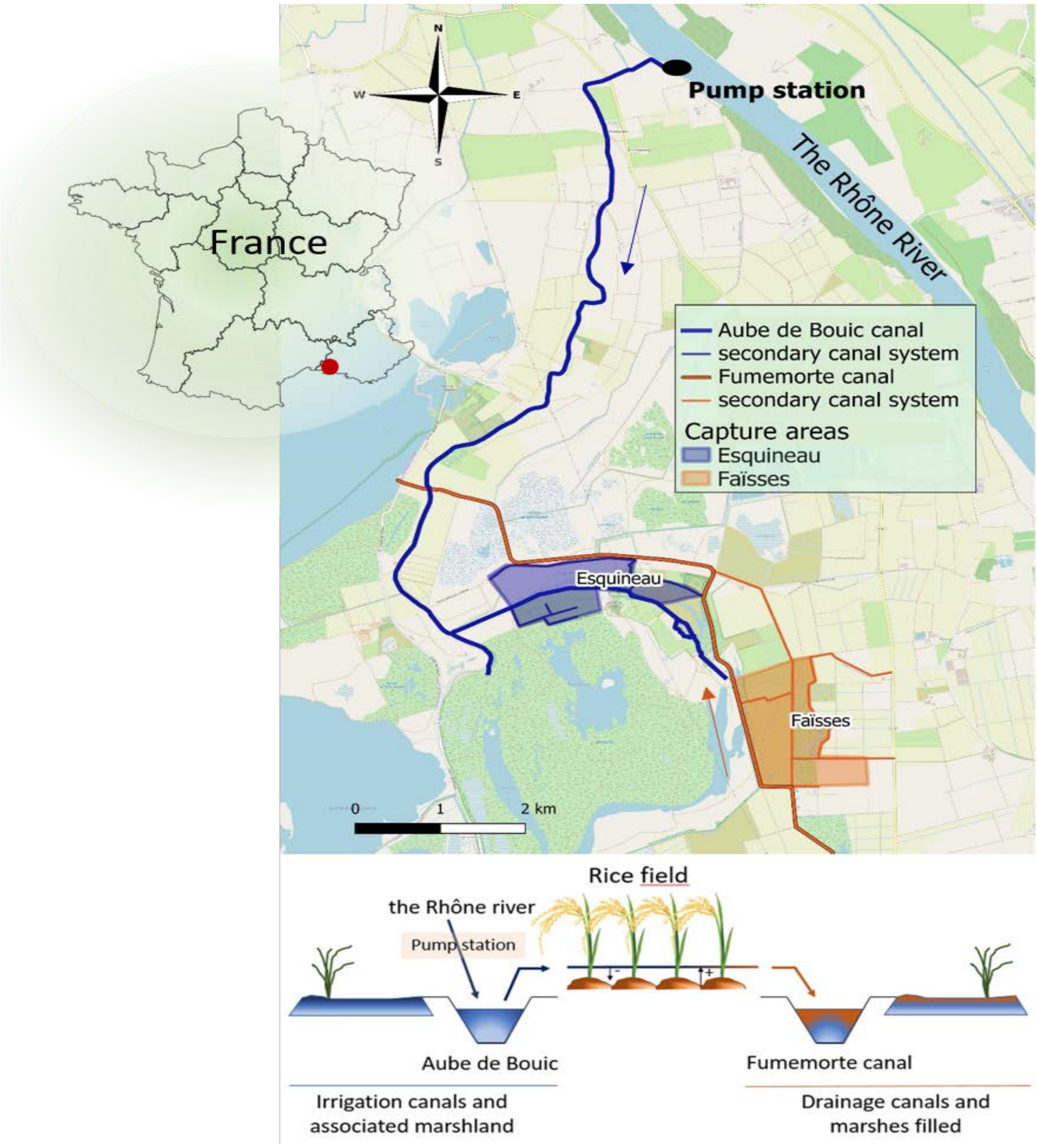
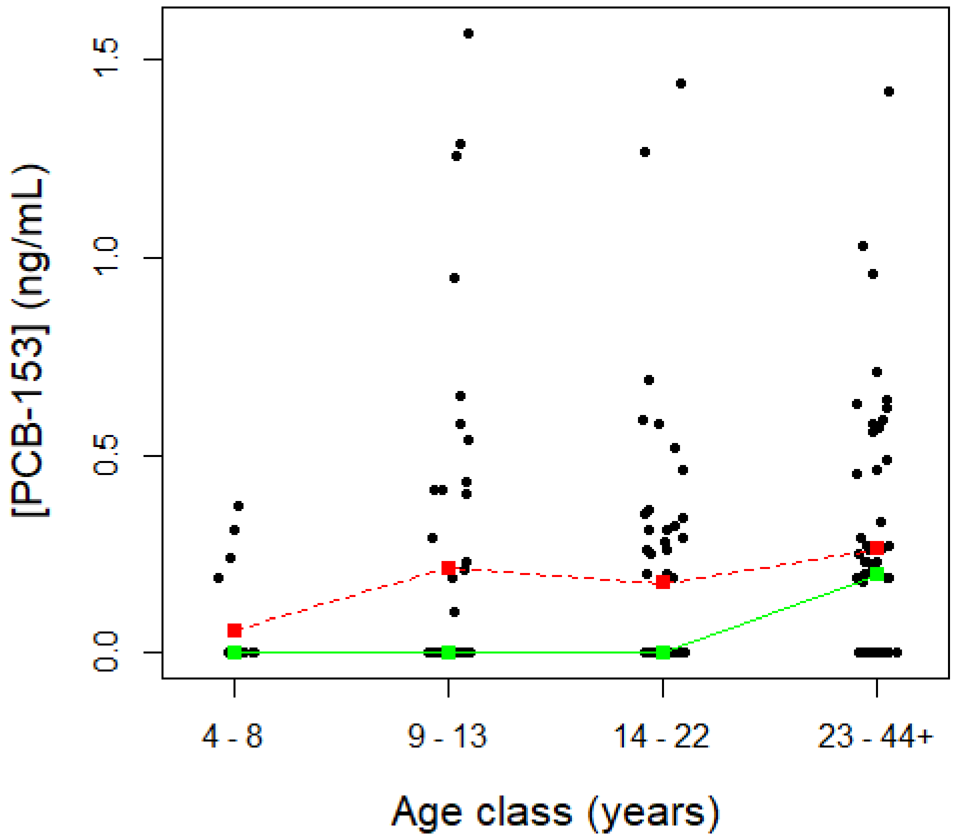


Figure 2: Plasma PCB 153 (ng/mL) levels in European pond turtles increase with age class (4-8, 9-13, 14-22, 23-+44 years old). Each point represents an individual. Red and green squares correspond to mean and median levels for age class, respectively. Age class was determined based on growth streaks on the plastron (see methods).



Tables

Table 1. Concentrations of organochlorine compounds (ng/ml w.w.) in plasma samples of European pond turtles from the two sites: Esquineau and Faïsses. Means and SD are calculated considering all the data (i.e. levels > LQ and data < LQ by assigning them zero for value). SD: standard deviation; Df: Detection frequency; LQ: limit of quantification (calculated as nine times the signal-to-noise ratio using the spiked matrices).

Variable	Esquineau (n=126)						Faïsses (n=48)				
	LQ	Mean	±	SD	Max	Df (%)	Mean	±	SD	Max	Df (%)
Organochlorine pesticides											
Alpha HCH	1.9	0.04	±	0.29	2.39	2	0.00	±	0.00	0.00	0
beta HCH	0.9	0.08	±	0.39	3.22	5	0.02	±	0.14	0.98	2
Delta HCH	1.3	0.03	±	0.23	2.19	2	0.08	±	0.37	2.22	4
HCB	0.2	0.02	±	0.10	0.77	7	0.07	±	0.18	1.00	19
Aldrin	1	0.01	±	0.09	1.03	1	0.02	±	0.17	1.16	2
Isobenzan	0.6	0.02	±	0.11	0.79	2	0.17	±	0.50	2.02	13
44'-DDE	1.1	0.04	±	0.29	2.33	2	0.05	±	0.34	2.30	2
44'-DDT	2.4	0.05	±	0.41	3.62	2	0.00	±	0.00	0.00	0
PCBs											
PCB-28	1	0.14	±	0.53	3.16	8	0.00	±	0.00	0.00	0
PCB-52	0.2	0.54	±	1.30	10.18	36	0.73	±	0.91	3.80	69
PCB-81	0.9	0.02	±	0.21	2.10	2	0.02	±	0.17	1.16	2
PCB-101	2.3	0.06	±	0.48	4.82	2	0.00	±	0.00	0.00	0
PCB-123	2.6	0.02	±	0.23	2.61	1	0.00	±	0.00	0.00	0
PCB-138	1.8	0.23	±	0.94	7.28	8	0.07	±	0.27	1.27	6
PCB-153	0.2	0.22	±	0.34	1.57	46	0.13	±	0.25	1.03	29
PCB-169	1.4	0.01	±	0.17	1.86	1	0.00	±	0.00	0.00	0
PCB-180	0.3	0.02	±	0.10	0.72	2	0.03	±	0.14	0.83	4

Table 2. Results from a generalized linear models (GLM) with binomial distribution and a logit link function model with POP levels having an occurrence greater than 10% as dependent variables (PCB-52, -153 and HCB), as a function of sex, sampling site, and their interaction, sampling date, age, and mass.

	PCB-52		PCB-153		HCB	
	χ^2	p value	χ^2	p value	χ^2	p value
Sex	1.699	0.192	0.162	0.688	0.433	0.511
Site	14.347	< 0.001	4.488	0.034	4.052	0.044
Date	1.289	0.256	0.951	0.329	3.828	0.050
Sex x Site	2.659	0.103	0.285	0.594	0.306	0.580
Age class*	4.870	0.182	10.603	0.014	0.840	0.840
Mass*	2.119	0.146	0.264	0.608	0.760	0.383

* Age class and mass were not tested in the same GLM since these two variables were highly correlated (see methods).

Table 3. The effects for sex, sampling site, age, date or mass on pollutant levels were tested, using Peto-Prentice tests (group comparisons) and tobit models (linear regressions).

	HCB	PCB-52	PCB-153
	Peto-Prentice tests		

	χ^2	p value	χ^2	p value	χ^2	p value
Sex	0.7	0.403	1.1	0.300	0.6	0.444
Site	5.2	0.022	12.3	< 0.001	3.7	0.054
Age class	1.0	0.800	6.7	0.082	9.5	0.023
	Tobit models					
	Wald	p value	Wald	p value	Wald	p value
Mass	0.498	0.481	0.086	0.769	1.265	0.261
Date	2.814	0.093	0.191	0.662	0.607	0.436