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Sensitivity of the European Kingfisher (*Alcedo atthis*) to global change: evidence from home range features and contaminations by trace elements and organic pollutants, a case study in the marshes of Western Europe

Raphaël Musseau^{1*}, Frédéric Angelier², Coraline Bichet², Maurice Millet³, Céline Rousselle¹, Jérôme Moreau^{2,4} & Paco Bustamante⁵

¹ BioSphère Environnement, 52 quai de l'Estuaire, 17120 Mortagne-sur-Gironde, France.

² Centre d'Etudes Biologiques de Chizé, UMR 7372, CNRS – La Rochelle Université, Villiers-en-Bois, 79360, France.

³ Institut de Chimie et Procédés pour l'Energie, l'Environnement et la Santé (ICPEES), UMR 7515, CNRS – Université de Strasbourg, Equipe de Physico-Chimie de l'Atmosphère, 67087 Strasbourg, France.

⁴ Biogéosciences, UMR 6282, CNRS – Université de Bourgogne, 6 Boulevard Gabriel, 21000 Dijon, France.

⁵ Littoral Environnement et Sociétés (LIENSs), UMR 7266, CNRS – La Rochelle Université, 2 rue Olympe de Gouges, 17000 La Rochelle, France.

*Corresponding author, e-mail: r.musseau@biosphere-environnement.com

Key words: apex predator, GPS tracking, mercury, pesticides, plant protection products, waterbirds, wetlands ecological networks.

Abstract

This study, conducted in western France from 2018 to 2021, aimed to examine the environmental disruption risks faced by the European Kingfisher, a species of conservation concern in Europe. We focused on examining the risks associated with habitat reduction, as well as the potential exposure of the species to trace elements and organic pollutants, and the effects of these pollutions on the health of individuals. Through a GPS tracking program, our results highlight the potential importance of wetlands with reduced surface areas and shallow water for the species. Furthermore, by analysing mercury and pesticide concentrations respectively in feathers and in blood samples, we also observed significant contamination of these pollutants in birds' tissues, leading to various negative health effects. Our study highlights: i) the necessity for conservation and ecological restoration policies dedicated to small wetlands to mitigate the potential detrimental effects of global changes on the conservation status of the species, and ii) the significance of apex predators, like the European Kingfisher, as keystone species, in assessing the risks of wetland contamination by trace elements or organic pollutants.

Introduction

Listed in Annex I of the European Union's "Birds Directive", the European Kingfisher (*Alcedo atthis*) is a small plunge-diving bird breeding within freshwater environments (Brooks *et al.*, 1985). The European population is currently undergoing rapid population decline and is therefore classified as vulnerable under Criterion "A" in Europe (BirdLife International, 2023). For this highly specialised species, the modification and the loss of habitats, as well as chemical and biological water pollution, are today identified as the most significant factors underlying the alarming population decline documented in Europe (Tucker & Heath, 1994). From 2018 to 2021, we conducted a study on a European Kingfisher population breeding in western France. The study aimed to assess, through a GPS tracking scheme, the spatial occupancy strategies of populations breeding within rural areas with different types of wetlands and to analyse the issues of the habitats they exploit. Additionally, we have searched for trace elements and molecules of plant protection products (PPPs) in feathers and blood samples, respectively, and studied their effects on various physiological markers of health status. Our study was conducted within various watersheds adjacent to the right bank of the Gironde estuary (France), which are part of the Special Protection Areas "Estuaire de la Gironde: marais de la rive nord" (SPA No. FR5412011) and "Estuaire de la Gironde: marais du Blayais" (SPA No. FR7212014). These areas are characterised by significant land agricultural coverage, including vast vineyards and cereal-cultivated surfaces. Our sampled sites ranged from the south of the city of Royan (45°34'37.85"N; 0°54'33.16"W) to the north of the city of Blaye (45°13'24.67"N; 0°37'2.68"W).

Material and methods

To assess the sensitivity of breeding birds towards habitat features of their home range, we equipped 16 adults in 2018 and 2019 with miniaturised and waterproof GPS archival tags deployed using leg-loop harnesses (total equipment mass = 1.4 g; \approx 3% of bird's average mass, see details in Musseau *et al.*, 2021 and Figure 1). Birds were captured and recaptured (to retrieve tags and download data) using mist nets positioned as close as possible to the nests previously located. The study aimed, through a GPS tracking scheme, to characterise the spatial occupancy strategies of populations breeding within rural areas interspersed by different types of wetlands and to analyse the issues related to the habitats exploited by birds. Over the two years, birds were equipped from early April to mid-June, with an average deployment on April 22nd. Birds' locations were collected every 30 minutes, between sunrise and sunset. Tags were programmed to initiate data collection one day after being deployed, allowing animals a minimum period of time to adapt to the equipment. On average, birds were recaptured 7.38 ± 1.30 days after being GPS tagged (95% CI = 5.85 – 11.64 days; maximum = 23 days). Home range size and fragmentation levels were assessed using a cluster analysis, following the multi-nuclear outlier-exclusive range core method defined by Kenward *et al.* (2001), excluding from the analysis locations in the largest 5% of the nearest-neighbour distance distribution. Analyses were performed using the software "Ranges 8" (Kenward *et al.*, 2008). An index of home range fragmentation (Cpart) was calculated adding areas of all clusters divided by the area of the single convex polygon encompassing all clusters. This index ranges from 0 to 1. A value close to 0 indicates a high fragmentation level of individual home range, while a value close to 1 corresponds to a single continuous cluster. In addition, diversity indices of home ranges exploited by birds were calculated to estimate the diversity of use of the different clusters (DivLocs) and the diversity of cluster areas (DivArea). These metrics were calculated using the Simpson's index: $1/\sum p_i^2$, where DivLocs p_i is the proportion of the total n locations in cluster i and DivArea p_i is the proportion of the area of the cluster i relative to the total area of the different clusters. The values of these metrics start at 1 if animals use a single cluster and increase with the diversity of habitat use (DivLocs) or the diversity of nuclei areas (DivArea).



Figure 1: Equipping a European Kingfisher (*Alcedo atthis*) with a miniaturised and waterproof GPS archival tag to assess home range features. Details in Musseau et al., 2021.

Given the particular risk of wetlands contamination by mercury (Hg), we studied the exposure level and the sensitivity of the European Kingfisher to this trace element. We sampled a total of 111 fledglings from 2018 to 2020 (25th May to 20th July) using mist nets stretched across watercourses within breeding sites. The effects of mercury exposure to the birds were assessed by measuring the concentration of total mercury (THg) in body feathers and analysing the measures obtained in relation to different biological markers (body condition index, feather density and leukocyte profile). The body condition index was determined as the residuals of a generalised linear model relating body mass to wing length of birds (Green, 2001), and the feather density as the mass of tail feathers divided by their mass (one tail feather collected by individual). Bird's Leukocyte profile was determined from blood smears stained with Giemsa R, and prepared by collecting blood through a prick of the ulnar vein using sterile hypodermic needles (27G, $\varnothing = 0.40$ mm) and heparinised microcapillary tubes (75 μ l) to capture the superficial blood flow. The THg concentration was measured from a total of 35 to 40 body feathers collected by plucking each sampled bird (≈ 50 mg). Feather samples were stored in sealed plastic bags and, before analysis, were washed for three minutes in an ultrasonic bath containing a mixture of chloroform and methanol (proportion respectively 2/1) and rinsed twice in pure methanol. After 24 hours of drying at 45°C in an oven, feathers were cut into tiny fragments with stainless steel scissors in order to obtain a homogenous powder. Total mercury concentrations of body feathers were then determined as described by Bustamante et al. (2006).

Finally, to assess the effect of plant protection products (PPPs) circulating within the trophic webs exploited by the species, from June 8th to July 7th of the year 2021, we sampled a total of 40 fledglings across four different important watersheds identified for their breeding population size of European Kingfishers. We sampled 10 birds from each of the four watersheds. Animals were captured in the same way as the fledglings sampled from 2018 to 2020. For each individual, we collected 50 μ l of blood using the method described above. The collected blood samples were then transferred into Eppendorf tubes, which were kept in an icebox until the end of the field day and subsequently stored at -80°C until analysis. The concentrations of 110 sought molecules in total blood were determined using the method developed by Rodrigues et al. (2023). Initial analyses were conducted to assess the impact of the identified molecules on the birds' health status by examining the relationship between the measured concentrations and the bird's body condition index (as described in the calculation method above), pending further analysis.

Results

From nine breeding birds recaptured with enough data to assess home range features (5 males and 4 females; ≥ 31 locations), we collected an average of 35.31 ± 6.66 locations usable for analyses. Data analyses highlighted rather limited home ranges exploited by birds (average = 2.50 ± 0.55 ha; 95% CI = 1.56 – 3.80 ha), composed on average by 2.78 ± 0.40 clusters. On average, fragmentation index (CPart) was 0.36 ± 0.08 (95% CI = 0.24 – 0.61), DivLocs 2.03 ± 0.30 (95% CI = 1.57 – 2.91) and DivArea 1.81 ± 0.27 (95% CI = 1.40 – 2.62). For the sampled population, the defined clusters corresponded to different types of wetlands with reduced surfaces: waterholes and forest ponds (40%), small channels and wet ditches (28%), small rivers (12%), small artificial ponds (8%), small basins (fountains: 8%) and water treatment plant basins (4%). See examples of data [Figure 2](#) and details in [Musseau *et al.*, 2021](#).

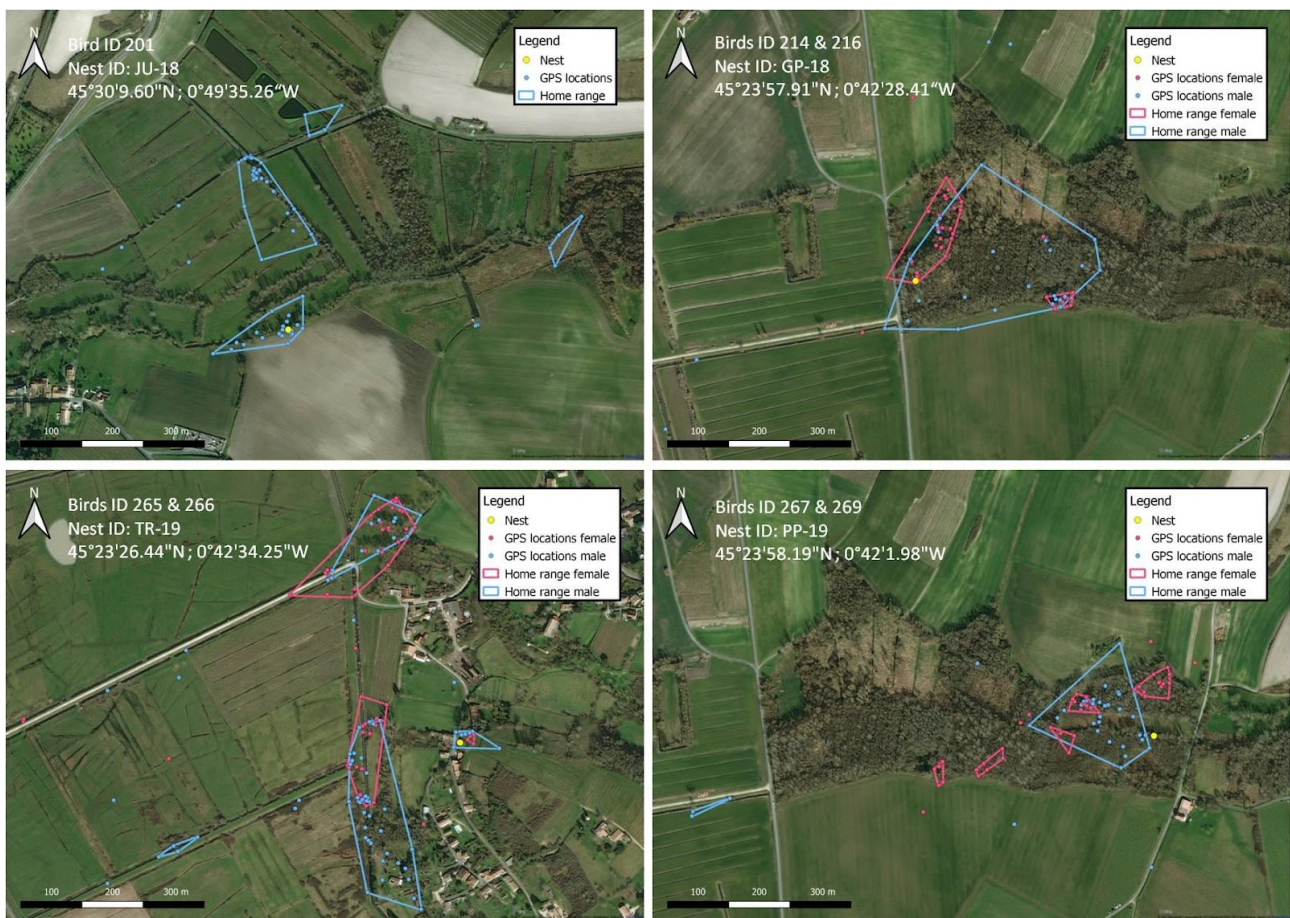


Figure 2: Examples of home ranges of seven European Kingfishers (*Alcedo atthis*) breeding within the marshes distributed along the right bank of the Gironde estuary (one male and three pairs). Bird ID 208: nest located in the bank of a small river; birds ID 214 & 216: nest located in the roots of a fallen tree within an alluvial forest; birds ID 265 & 268: nest located in a limestone cliff overhanging a small water basin; birds ID 267 & 269: nest located in the roots of a fallen tree within an alluvial forest. Details in [Musseau *et al.*, 2021](#).

The average concentration of THg in fledglings' feathers was $2.60 \pm 0.10 \mu\text{g.g}^{-1}$ (95% CI = 2.43 – 2.81; minimum = $1.05 \mu\text{g.g}^{-1}$; maximum = $6.00 \mu\text{g.g}^{-1}$). Using a Generalised Linear Model (GLM), we observed a negative relationship between the body condition index of birds and [THg] ($p < 0.01$; $F_{1,109} = 7.15$) as well as feather density and [THg] ($p = 0.02$; $F_{1,109} = 5.73$). The analysis of blood smears performed on 64 individuals sampled in 2018 and 2019 and the use of GLMs with the number of the different leukocyte types for 100 counted as the response variable and [THg] as the predictor, revealed a positive correlation between [THg] and the number of heterophils ($p = 0.01$; $\chi^2 = 6.51$; $df = 1$), as well as a negative correlation between [THg] and the number of lymphocytes ($p < 0.01$; $\chi^2 = 9.82$; $df = 1$). There was no significant association between the ratio of heterophils to lymphocytes and [THg] ($p = 0.30$; $F_{1,62} = 1.11$; $df = 1$).

Ultimately, for the 40 fledglings sampled during the year 2021, we detected 34 molecules of PPPs out of the 110 searched for in the total blood of individuals (mean = 12.18 ± 0.74 molecules per individual; 95% CI = 10.65 – 13.62, minimum = 2; maximum = 22). Thirteen of these molecules had an occurrence rate exceeding or equal to 50%. The average concentration of the detected molecules was $418.8 \pm 90.79 \text{ pg.mg}^{-1}$ per individual (95% CI = 317.9 – 812.5, see data summary in Table 1). We did not detect any correlation between the bird's body condition index and the total concentration of molecules (GLM: body condition index $\sim \Sigma$ (concentrations); $p = 0.27$; $F_{1,38} = 1.28$). However, a trend for a positive correlation was observed between the bird's body condition index and the total concentration of molecules weighted by the reciprocal value of their LD50 – lethal dose for oral ingestion in rats – (GLM: body condition index $\sim \Sigma$ (concentrations $i * \text{LD50}^{-1} i$), $i = 1$ to n ; $p = 0.08$; $F_{1,38} = 3.19$).

Table 1: Details of 34 plant protection product molecules (PPPs) detected in the blood of European Kingfisher (*Alcedo atthis*) fledglings from the marshes of the Gironde estuary, France: occurrence (proportion of birds where the molecule has been found), average concentration (mean, pg.mg^{-1}), maximum (max, pg.mg^{-1}), and minimum (min, pg.mg^{-1}).

MOLECULE	OCCURRENCE	MEAN	MAX	MIN	MOLECULE	OCCURRENCE	MEAN	MAX	MIN
Difenoconazole	95%	61,75	145,49	15,9	Deltamethrin	22,5%	722,13	2409,66	254,48
Sulcotrione	95%	1276,51	5967,21	497,38	Chlorpropham	20%	1269,10	2440,24	111,33
Tebutam	95%	23,39	74,48	2,14	Myclobutanil	12,5%	13,19	23,17	5,38
Metamitron	92,5%	39,11	103,45	3,39	Lambda-cyhalothrin	7,5%	236,75	319,05	164,19
Cyproconazole	90%	64,40	186,86	10,89	Carbetamide	5%	29,80	31,08	28,52
Prothioconazole	85%	41,84	284,87	8,73	Epoxiconazole	5%	7,84	11,56	4,12
Fluroxypyr	72,5%	1291,62	5812,28	453,85	Quinoxifen	5%	4,77	7,84	1,69
Chloridazon	70%	213,51	842,94	7,36	Azoxystrobin	2,5%	62,82	62,82	62,82
Fenpropidin	67,5%	215,91	477,66	75,58	Bifenthrin	2,5%	19,27	19,27	19,27
Cypermethrin	60%	142,31	353,77	26,21	Boscalid	2,5%	980,50	980,5	980,5
Indoxacarb	52,5%	598,11	1242,69	18,67	Clothianidin	2,5%	1664,00	1664	1664
Pyraclostrobin	52,5%	450,04	964,85	179,96	Dimoxystrobin	2,5%	66,25	66,25	66,25
Diflufenican	50%	121,51	298,5	29,73	Fenarimol	2,5%	111,85	111,85	111,85
Piperonyl-butoxide	40%	264,10	537,05	168,47	Kresoxim-methyl	2,5%	83,09	83,09	83,09
Prochloraz	37,5%	700,44	1327,86	167,25	Mecoprop-P	2,5%	1577,20	1577,2	1577,2
Propyzamide	32,5%	21,99	40,68	9,82	Metalaxyl-M	2,5%	111,80	111,8	111,8
Dimethomorph	27,5%	65,94	98,59	45,22	Pendimethalin	2,5%	2,92	2,92	2,92

Discussion

The indicators we measured regarding the breeding bird's spatial occupancy strategy indicate relatively small home ranges used by sampled individuals (2.50 ± 0.55 ha), along with a quite limited diversity in the utilisation of clusters defining the home ranges (DivLocs) and a restricted diversity of their corresponding areas (DivArea). The European Kingfisher is a plunge-diving bird for which prey capture is most efficient in shallow water (Viches *et al.*, 2013). Our study demonstrates the potential significance of wetlands with reduced areas, and shallow depths, such as wet ditches or small ponds, in maintaining trophic resources accessible to the species during the breeding period. This is especially crucial for populations with limited opportunities to exploit large water bodies, such as large rivers or ponds with restricted feeding possibilities due to potentially significant depths and/or high turbidity levels in these habitats.

Our findings highlight a negative association between the THg concentrations measured in the body feathers and variables such as body condition index and feather quality. Additionally, it appeared that THg concentrations had a positive influence on the abundance of heterophils in the blood, which are cells particularly involved in the inflammatory response in birds (Harmon, 1998). Once deposited on the Earth's surface, Hg can undergo complex, microbially mediated processes and be converted into methylmercury (MeHg), a toxic chemical complex that is assimilated and accumulated by living organisms, and biomagnifies in trophic webs (Evers *et al.*, 2016; Eagles-Smith *et al.*, 2018). Diminished body conditions and compromised feather integrity frequently characterise physiological stress in birds, as substantiated by previous researches (Lattin *et al.*, 2011; Kraft *et al.*, 2019). Similarly, an elevated number of heterophils (innate immunity) relative to lymphocytes (adaptive immunity) within avian leukocyte profiles reveals health disorders (Davis *et al.*, 2008; Clark, 2015). The established threshold of $5 \mu\text{g}\cdot\text{g}^{-1}$ for mercury concentration measured in avian feathers is widely acknowledged for its significant potential to impact the overall health status of adult birds (Burger & Gochfeld, 1997). Nonetheless, this threshold exhibits considerable variability across bird species (Grúz *et al.*, 2019), and adverse health effects have been reported in some species for concentrations ranging from 0.5 to $2.40 \mu\text{g}\cdot\text{g}^{-1}$ (Connell *et al.*, 2002; Jackson *et al.*, 2011).

Finally, our work illustrates that the European Kingfisher may face long-term exposure to a potentially significant number of molecules from plant protection products. For molecules like difenoconazole, the concentrations measured in our study (average = $61.75 \text{ pg}\cdot\text{mg}^{-1}$; maximum = $149.49 \text{ pg}\cdot\text{mg}^{-1}$) are comparable to those documented in the plasmas of a population of Eurasian Blackbirds (*Turdus merula*) sampled in the vineyards of the Cognac region where azoles are intensely used to protect vines and grapes against cryptogamic diseases (Angelier *et al.*, 2023). This highlights that wetlands and trophic webs of watersheds with extensive PPPs usage may become significantly contaminated by various molecules with different toxicity levels. Such contaminations of the trophic webs lead to complex mixture effects that could impact apex predator species, including piscivore species. Our analyses emphasize that the total concentration of molecules in birds cannot be attributed to variations in their body size. However, it appears that the body condition of birds may be positively influenced by the toxic load, which is defined as the sum of concentrations measured for each molecule weighted by the reciprocal value of their LD50. This illustrates the potential metabolic disruptions in juvenile European Kingfishers that may be associated with the toxic burden of PPPs. Notably, groups of molecules like azoles, which are still widely used as antifungal treatments for crops, have been shown to cause metabolic disruptions, including obesogenic effects in vertebrates (Li *et al.*, 2012; Thrikawala *et al.*, 2023). Considering the levels of pesticide circulation in surface waters across Europe (Mohaupt *et al.*, 2020), our results suggest that species such as the European Kingfisher may be exposed, over a significant portion of its distribution area, to mechanisms of endocrine disruption and physiological stress caused by significant toxic burden due to different molecule assemblages (Moreau *et al.*, 2022). These effects can be detrimental to individuals' health status and, consequently, population conservation. We are currently conducting additional analyses, including molecule dosages in bird feathers and telomere length measurements, which will soon enable us to gain a better understanding of these issues (Musseau *et al.*, in prep).

Conclusion

From a conservation perspective for the populations of European Kingfishers, which is currently classified as a species of conservation concern in Europe, our findings underscore the significance of restoring wetland ecological networks and developing conservation strategies that incorporate the preservation of small wetlands with shallow water, which are especially vulnerable to global changes such as anthropogenic alterations or climate change. Throughout the world, the contribution to biodiversity offered by wetlands with reduced surfaces is regularly overlooked or ignored, and often neglected in habitat conservation plans which tend to give priority to large ecological complexes (Semlitsch & Bodie, 1998; Melly *et al.*, 2018). Mercury is a trace element whose circulation on the planet has both natural phenomenon (such as volcanic eruptions or biomass burning) and human-driven activities (including fossil fuel combustion, mining, waste disposal or chemical production). In agricultural contexts, given the potential mercury enrichment they can generate in soils, special attention must be given regarding the spreading of sludge from water treatment plants and phosphatic mineral fertilisers (Wang *et al.*, 2016; Sánchez-Báscones *et al.*, 2017). Finally, in relation to plant protection products and the risks of molecule transfer to the surface waters, as well as environmentally conscious practices limiting product use in sensitive areas, it could be of interest to explore the potential role of vegetative buffer zones in mitigating diffuse pesticide pollution in wetland ecosystems (Gregoire *et al.*, 2009; Carlier *et al.*, 2017).

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Data Availability: The datasets used for the current study are available from the corresponding author upon reasonable request.

Conflicts of interest: The authors declare that they have no conflicts of interest.

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