



# Mercury Exposure in Birds Linked to Marine Ecosystems in the Western Mediterranean

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## Abstract

Mercury (Hg), particularly as methylmercury (MeHg), is a nonessential, persistent, and bioaccumulative toxic element with high biomagnification capacity and is considered a threat to marine environments. We evaluated total Hg concentrations in liver, kidney, and brain in 62 individuals of 9 bird species linked to marine ecosystems from western Mediterranean admitted in a Wildlife Rehabilitation Center (WRC) (Alicante, Spain, 2005–2020). Age- and sex-related differences in Hg levels, as well as the cause of admission to the WRC, were also evaluated in certain species. The species studied were: northern gannet (*Morus bassanus*), European shag (*Phalacrocorax aristotelis*), great cormorant (*Phalacrocorax carbo*), osprey (*Pandion haliaetus*), Balearic shearwater (*Puffinus mauretanicus*), yellow-legged gull (*Larus michahellis*), razorbill (*Alca torda*), common tern (*Sterna hirundo*), and black-headed gull (*Chroicocephalus ridibundus*). Concentrations in feathers of 27 individuals, and concentrations in internal tissues in 7 other individuals of 7 different species were also reported but not statistically evaluated due to the limited number of samples. Results suggest that individuals were chronically exposed to Hg through diet. The differences in Hg concentrations among species may be explained by their diet habits. Mercury concentrations strongly correlated between tissues ( $r=0.78-0.94$ ,  $p<0.001$ ,  $n=61-62$ ). Some individuals of certain species (i.e., European shag, northern gannet, and great cormorant) showed Hg concentrations close to or above those described in the literature as causing reproductive alterations in other avian species. Consequently, certain individuals inhabiting western Mediterranean could be at risk of suffering long-term, Hg-related effects. Some of the species evaluated are listed within different categories of threat according to the International Union for Conservation of Nature (IUCN) and are endangered at a national level, so this study will provide valuable information for assessors and authorities in charge of the management of the environment and pollution.

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Marine ecosystems are threatened by pollutants such as mercury (Hg), especially in its organic form as methylmercury (MeHg), a persistent, bioaccumulative, and toxic, nonessential element that is distributed worldwide (Cherel et al. 2018; Kenney et al. 2018). Natural processes and anthropogenic activities participate in the continuous release of Hg into the environment (Kenney et al. 2018; Ruus et al. 2015), which enters marine ecosystems mostly through wet and dry atmospheric deposition processes and runoff from industrial emissions (Carravieri et al. 2018; Ishii et al. 2017; Zamani-Ahmadmoodi et al. 2010). In the marine environment, the inorganic Hg is methylated and converted into MeHg, the most toxic and bioavailable form (Cherel et al. 2018; Kenney et al. 2018; Ruus et al. 2015). Methylmercury is assimilated by phytoplankton and zooplankton, becoming part of the food chain, where it bioaccumulates and biomagnifies as the

trophic level increases. Consequently, top predators, such as seabirds achieve higher concentrations of this contaminant in their organs and tissues (Carravieri et al. 2018; Misztal-Szkudlinska et al. 2018).

Methylmercury is a neurotoxic and endocrine disruptor element that also alters behaviour, reproductive success, nestlings' growth and development, metabolism, and immune responses (Carravieri et al. 2018; Fort et al. 2015; García-Fernández 2014), affecting principally reproduction in seabirds (Carravieri et al. 2018). Against this toxicity, organisms have protective mechanisms, such as the synthesis and binding to metallothioneins (MT), demethylation and formation of nontoxic complexes with selenium, or MeHg elimination through moult (Espín et al. 2012, 2016). These processes seem to be particularly effective in seabirds, explaining the tolerance of these predators to higher Hg concentrations compared with other bird species (García-Fernández 2014).

It is essential to conduct Hg biomonitoring studies in wildlife inhabiting marine ecosystems and, for this purpose, seabirds and other piscivorous birds (e.g., osprey, *Pandion haliaetus*) are considered good bioindicators of Hg-polluted marine environments because they are long-lived species, they bioaccumulate MeHg in their organism, and they are in a high trophic position in the food web (Carravieri et al. 2018; Espín et al. 2012; García-Fernández 2014; García-Fernández et al. 2020; Kojadinovic et al. 2007; Moura et al. 2018a; Ribeiro et al. 2009). In this sense, collecting tissues from birds that have died in massive mortality events or from dead specimens stored at Research or Wildlife Rehabilitation Centers may provide interesting data to examine Hg concentrations and the relationships between internal tissues in a broad range of species (Espín et al. 2012; Fort et al. 2015; Mallory et al. 2018).

The Mediterranean is a semiclosed sea with restricted water exchange and surrounded by industrialized countries, which entails a greater risk of Hg contamination (Espín et al. 2012; Pereira et al. 2019). However, data are scarce about the concentrations of this metal in certain bird species of the western Mediterranean. The purpose of this study was to evaluate the exposure to Hg in different seabird and aquatic bird species linked to marine ecosystems in eastern Spain. The specific objectives are: (1) to provide data on total Hg concentrations in liver, kidney, and brain of different seabird and aquatic bird species as well as in feathers of some individuals, and (2) to assess differences in total Hg concentrations among nine species, as well as between sexes, age groups and causes of admission in the Wildlife Rehabilitation Center (WRC) for four species where a sufficient number of samples was available. Based on the available literature, we hypothesize that larger species, as well as male and adult individuals, will present higher Hg concentrations. In addition, we expect to find higher Hg concentrations in

internal tissues of those specimens suffering non-traumatic pathologies (i.e., individuals with symptoms of undernutrition due to infectious or parasitic diseases).

## Materials and Methods

### Species and Study Area

In this study, Hg exposure was evaluated in 62 individuals of 9 species of birds linked to marine ecosystems: 13 European shags (*Phalacrocorax aristotelis*), 13 yellow-legged gulls (*Larus michahellis*), 12 northern gannets (*Morus bassanus*), 8 great cormorants (*Phalacrocorax carbo*), 5 razorbills (*Alca torda*), 3 common terns (*Sterna hirundo*), 3 Balearic shearwaters (*Puffinus mauretanicus*), 3 osprey (*Pandion haliaetus*), and 2 black-headed gulls (*Chroicocephalus ridibundus*). Table 1 reports the main characteristics of these 9 species, including their habitat, diet, body weight, and conservation status. Mercury concentrations in 7 individuals of 7 other different species are also reported: Atlantic puffin (*Fratercula arctica*), ruddy turnstone (*Arenaria interpres*), Audouin's gull (*Ichthyaetus audouinii*), Mediterranean gull (*Ichthyaetus melanocephalus*), Scopoli's shearwater (*Calonectris diomedea*), little tern (*Sternula albirostris*), and grey heron (*Ardea cinerea*). Data for those 7 species where only one individual was available are presented for information purposes but are not included in the statistics nor discussed due to limitations in number of samples. All of these animals were found dead or injured along the Occidental Mediterranean coastline, at different locations in the province of Alicante, and were admitted in the WRC of Santa Faz (Alicante, eastern Spain; Fig. 1) between 2005 and 2020. The causes of admission in the WRC were trauma, drowning, fish-hook ingestion, electrocution, entanglement in fishing line and fishing net, and undernutrition as a result of other pathologies (e.g., infectious diseases).

### Sampling

Necropsies of the 69 individuals were performed in the WRC. A total of 206 samples of liver ( $n=69$ ), kidney ( $n=68$ , no kidney sample was retained in a cormorant individual), and brain ( $n=69$ ) were collected in Eppendorf tubes, transported under cold conditions to the Toxicology laboratory at the University of Murcia, and stored frozen at  $-20\text{ }^{\circ}\text{C}$  until analysis. Sterile Eppendorf tubes were used so that there was no possibility of contamination from the containers. Back feathers were only collected in 27 individuals and were kept in sterile sealed plastic bags at room temperature. In most cases, the age ( $n=63$  individuals), sex ( $n=61$ ), and body mass ( $n=55$ ) of the individuals were recorded. Age was determined through plumage patterns

**Table 1** Characteristics of the nine study species linked to marine ecosystems and number of individuals analysed

Common name	Scientific name	Order	Habitat	Diet	Adult weight (g)	Conservation status* N**
Northern gannet	<i>Morus bassanus</i>	Suliformes	Strictly marine, within the limits of the continental shelf <sup>fr</sup>	Pelagic fish and fishery discards <sup>b,i</sup>	2400–3600 <sup>d,i</sup>	LC/SP <sup>a,b,j</sup> 12 (5M/6F, 2J/10A)
European shag	<i>Phalacrocorax aristotelis</i>	Suliformes	Marine and coastal, near the coast, on stretches of rocky coast <sup>a</sup>	Benthic, pelagic and demersal fish <sup>b</sup>	1760–2154 <sup>h</sup>	LC/VU <sup>a,b,j</sup> 13 (2 M/9F, 11 J/1A) <sup>m</sup>
Great cormorant	<i>Phalacrocorax carbo</i>	Suliformes	Preferably open water surfaces (coast and inland): lakes, lagoons, reservoirs, rivers, deltas, marshes, etc <sup>a</sup>	Benthic fish, crustaceans, amphibians, molluscs and bird chicks <sup>b</sup>	1300–3100 <sup>b</sup>	LC/N <sup>a,b</sup> 8 (3 M/5F, 2 J/5A)
Osprey	<i>Pandion haliaetus</i>	Accipitriformes	Spanish breeding populations: closely linked to marine environment. They breed on cliffs and fish in nearby areas: bays, estuaries, inland lagoons. Migratory or wintering individuals: aquatic complexes (bays, inland lagoons, reservoirs, estuaries) <sup>a</sup>	Exclusively piscivorous, mostly live fish. Juveniles during the dispersion period feed on fish from inland waters <sup>a,b,i</sup>	1120–2050 <sup>d</sup>	LC/VU <sup>a,b,j</sup> 3 (3 M, 1 J/2A)
Balearic shearwater	<i>Puffinus mauretanicus</i>	Procellariiformes	Strictly marine, in waters near the coast <sup>b</sup>	Small pelagic fish, fishery discards and planktonic organisms <sup>b,k</sup>	≤ 500 <sup>f</sup>	CR/EN <sup>a,b,j</sup> 3
Yellow-legged gull	<i>Larus michahellis</i>	Charadriiformes	Marshes, salt pans, beaches, coastal or inland wetlands, coastal urban centres. Breeding season: preferably marine cliffs and islands near the coast <sup>a</sup>	Fish, invertebrates, reptiles, small mammals, waste, bird eggs and chicks, and fishery discards <sup>b,c</sup>	750–1500 <sup>d</sup>	LC/N <sup>a,b</sup> 13 (7 M/5F, 8 J/5A)
Razorbill	<i>Alca torda</i>	Charadriiformes	Summer period: coastal places with rocky cliffs Winter period: open sea <sup>a</sup>	Pelagic fish, krill and invertebrates <sup>b,f</sup>	524–890 <sup>d</sup>	NT/SP <sup>a,b,j</sup> 5 (5F, 5A)

Table 1 (continued)

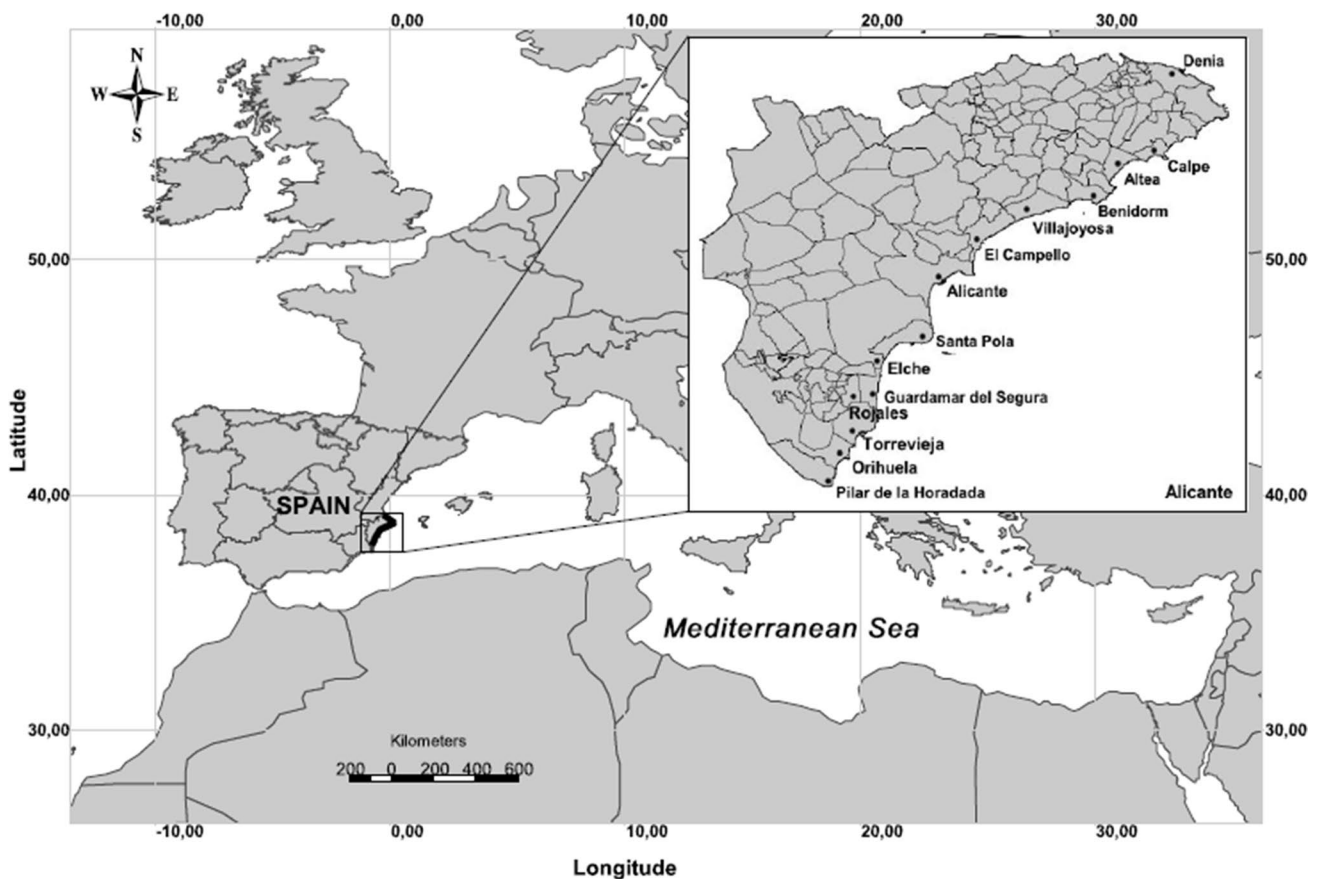
Common name	Scientific name	Order	Habitat	Diet	Adult weight (g)	Conservation status* N**
Common tern	<i>Sterna hirundo</i>	Charadriiformes	On the coast and inland: sandy beaches, dune systems, estuarine islands, lakes and rivers, cliffs with herbaceous vegetation. Breeding season: coastal environments, preferably with shallow waters and sandy bottom <sup>a</sup>	Small pelagic fish, often juveniles, and occasionally crustaceans and planktonic insects <sup>b,e</sup>	110–150 <sup>d</sup>	LC/SP <sup>a,b,j</sup> 3 (2F, 1 J/1A)
Black-headed gull	<i>Chroicocephalus ridibundus</i>	Charadriiformes	Nesting: near humid areas (fresh and brackish water) in estuaries, deltas, marshes, lagoons, lakes, rivers with low flow, gravel pits, reservoirs. Feeding: fishing ports, garbage dumps, agricultural land inland, rice fields, salt pans <sup>a</sup>	Mostly foods of animal origin (aquatic and terrestrial insects, earthworms and marine invertebrates, fish, rodents), waste <sup>a,b</sup>	200–400 <sup>d</sup>	LC/N <sup>a,b,f</sup> 2 (1 M/1F, 2 J)

N not threatened, LC least concern, NT near threatened, VU vulnerable, EN endangered, CR critically endangered, SP special protection

<sup>a</sup>SEO/Birdlife (2008), <sup>b</sup>BirdLife International (2018), <sup>c</sup>Vizuete et al. (2018), <sup>d</sup>Hemb (2019), <sup>e</sup>Szostek and Becker (2015), Fundación CRAM, (2019), <sup>f</sup>Miles et al. (2015), <sup>h</sup>Misztal-Szkudlińska et al. (2018), <sup>i</sup>Hamer et al. (2000), <sup>j</sup>BOE, (2011), <sup>k</sup>Louzao et al. (2012), <sup>l</sup>Triay and Siverio (2004) and <sup>m</sup>12 (2 M/8F, 10 J/1A) in the case of the kidney

\*Conservation status is indicated in the following order: Global (International Union for Conservation of Nature, IUCN)/National (Spain)

\*\*Total number of individuals in this study, indicating in parentheses numbers by gender and age when they could be registered. F female, M male, A adult, J juvenile



**Fig. 1** Map of the sampling area (coastline of Alicante, Spain). Three individuals were found in different locations in the province of Valencia (i.e., Montaverner, Oliva, and Valencia; not shown in map)

and morphological criteria and sex by direct visualization of the gonads during the necropsy.

### Mercury Analysis

Total Hg (hereafter Hg) was analysed using a Milestone DMA-80 direct Hg analyser based on atomic absorption spectrophotometry, with a detection limit of 0.005 ng. Each sample (0.05 g wet weight for internal tissues and 0.005 g dry weight for feathers) was loaded in a quartz boat. The precision and accuracy of the method were previously evaluated using certified reference material (CRM; TORT-2, lobster hepatopancreas, National Research Council Canada), and blanks were also run in each sample set. A recovery percentage of  $108.9 \pm 4.1\%$  (mean  $\pm$  standard deviation, SD) and a coefficient of variation for repeatability of 3.7% were obtained. Feathers were washed using distilled water, Milli-Q water, and acetone before analytical determination to remove external contamination from the surface.

The percentage of humidity of the internal tissues was calculated in an Infrared Moisture Analyser MA35 (Sartorius) in order to express the results of total Hg in both wet

weight (ww) and dry weight (dw) and compare them with other published studies.

### Statistical Analysis

The results obtained were analysed using the IBM SPSS v.24 statistical package. A descriptive statistical analysis was performed by obtaining the mean  $\pm$  SD and median (min–max) Hg concentrations. Species with only one individual available ( $n=7$  species; Table 2) and results from feathers (27 samples from 11 species; Table 2) were excluded to perform statistical tests and discuss results due to limitations in number of samples. The normality of the variables was tested using a Kolmogorov–Smirnov test and Hg concentrations in liver, kidney, and brain were log-transformed, obtaining a normal distribution after the transformation. ANOVA followed by Tukey’s tests for multiple comparison were performed to test significant differences in Hg concentrations between tissues and species ( $n=9$  species; Table 2). The relationships between the Hg concentrations in liver, kidney, and brain and their correlation with body mass were tested using Pearson’s correlation coefficient. For those species where male, female, juvenile, and

**Table 2** Mercury concentrations (mean  $\pm$  SD, median and range) in  $\mu\text{g/g}$  (wet weight) of the species studied

Species	N*	Liver	Kidney	Brain	N**	Feathers
<i>Morus bassanus</i>	12	7.16 $\pm$ 4.60 <sup>a</sup>	6.62 $\pm$ 2.85 <sup>a</sup>	1.27 $\pm$ 0.81 <sup>a</sup>	3	15.45 $\pm$ 5.84
		6.16 (1.96–19.33)	6.58 (3.03–10.98)	1.13 (0.32–2.97)		13.86 (10.58–21.93)
<i>Phalacrocorax aristotelis</i>	13	14.56 $\pm$ 29.63 <sup>a</sup>	16.12 $\pm$ 37.89 <sup>a,c</sup>	0.98 $\pm$ 0.62 <sup>a</sup>	5	3.25 $\pm$ 1.99
		4.74 (1.43–110.57)	3.17 (1.30–134.17)	0.88 (0.29–1.92)		3.04 (0.64–6.26)
<i>Phalacrocorax carbo</i>	8	5.65 $\pm$ 3.62 <sup>a,c</sup>	4.40 $\pm$ 4.75 <sup>a,c,d</sup>	0.53 $\pm$ 0.27 <sup>a,b</sup>	1	5.53
		4.40 (2.34–10.81)	2.88 (1.71–16.06)	0.50 (0.28–1.14)		
<i>Pandion haliaetus</i>	3	1.98 $\pm$ 0.02 <sup>a,b</sup>	3.12 $\pm$ 3.28 <sup>a,b</sup>	0.88 $\pm$ 0.56 <sup>a</sup>	3	2.87 $\pm$ 1.19
		1.99 (1.95–2.00)	1.42 (1.03–6.91)	0.94 (0.28–1.41)		2.83 (1.70–4.08)
<i>Puffinus mauretanicus</i>	3	1.81 $\pm$ 0.04 <sup>a,b</sup>	1.14 $\pm$ 0.51 <sup>b,c</sup>	0.40 $\pm$ 0.06 <sup>a,b</sup>	–	
		1.83 (1.76–1.85)	1.08 (0.67–1.68)	0.43 (0.33–0.46)		
<i>Larus michahellis</i>	13	1.31 $\pm$ 0.74 <sup>b</sup>	1.01 $\pm$ 0.66 <sup>b</sup>	0.27 $\pm$ 0.15 <sup>b</sup>	8	4.91 $\pm$ 2.74
		1.03 (0.35–2.47)	0.90 (0.30–2.50)	0.20 (0.10–0.60)		4.55 (1.61–10.59)
<i>Alca torda</i>	5	1.27 $\pm$ 0.90 <sup>b</sup>	1.11 $\pm$ 0.97 <sup>b,d</sup>	0.54 $\pm$ 0.42 <sup>a,b</sup>	–	
		0.96 (0.63–2.87)	0.70 (0.55–2.84)	0.37 (0.27–1.29)		
<i>Sterna hirundo</i>	3	1.19 $\pm$ 0.29 <sup>b,c</sup>	0.74 $\pm$ 0.37 <sup>b,d</sup>	0.20 $\pm$ 0.03 <sup>b</sup>	–	
		1.15 (0.93–1.50)	0.54 (0.51–1.17)	0.20 (0.17–0.24)		
<i>Chroicocephalus ridibundus</i>	2	0.41 $\pm$ 0.14 <sup>b</sup>	0.28 $\pm$ 0.08 <sup>b</sup>	0.15 $\pm$ 0.08 <sup>b</sup>	2	1.61 $\pm$ 0.58
		0.41 (0.30–0.51)	0.28 (0.22–0.34)	0.15 (0.09–0.21)		1.61 (1.20–2.02)
<i>Fratereula arctica</i>	1	1.98	1.26	0.58	–	
<i>Ichthyaelus audouinii</i>	1	10.07	6.43	1.36	1	14.15
<i>Ichthyaelus melanocephalus</i>	1	0.88	1.01	0.35	1	29.23
<i>Calonectris diomedea</i>	1	8.54	3.32	1.10	1	10.54
<i>Sternula albifrons</i>	1	0.90	0.66	0.13	1	2.12
<i>Ardea cinerea</i>	1	2.13	0.52	0.22	1	5.23
<i>Arenaria interpres</i>	1	0.68	0.52	0.22	–	

N\* number of samples for liver, kidney and brain; N\*\* number of samples for feathers

Means sharing the same letter within each sample type do not show significant differences (Tukey test comparing Hg concentrations between species for each tissue type, species with one individual were excluded in the analysis)

The mean water content in the liver, kidney, and brain was 69.5  $\pm$  2.4%, 72.6  $\pm$  2.7%, and 80.6  $\pm$  2.0%, respectively

adult individuals were available, as well as different causes of admission to the WRC ( $n = 4$  species, i.e., *Morus bassanus*, *Phalacrocorax aristotelis*, *Phalacrocorax carbo*, and *Larus michahellis*), ANOVA was used to test differences in Hg concentrations according to sex, age and cause of admission. The causes of admission were classified into two groups, based on the probability to be related to loss of body mass: (1) traumatic type entry, which included trauma, drowning, hook ingestion, fishing line entanglement, and fishing net entanglement, and (2) nontraumatic type entry, which included individuals with symptoms of undernutrition as a result of other pathologies (e.g., parasitic or infectious diseases). For all analyses, the level of significance was set at  $p \leq 0.05$ .

## Results

Hg concentrations in liver, kidney, brain, and feathers for the different study species are shown in Table 2, and Hg concentrations reported in internal tissues of the same species in some publications are provided in Table 3 for comparison purposes. Mercury concentrations differed significantly between the nine species for the three internal tissue types (ANOVA test for liver:  $F = 10.09$ , kidney:  $F = 9.5$  and brain:  $F = 7.8$ ,  $p < 0.001$ ; Table 2; Fig. 2). Tukey's test results comparing Hg concentrations among species within each sample type show that, in general, northern



**Table 3** Mercury concentration ( $\mu\text{g/g}$ ) reported in the literature in liver, kidney and brain of birds linked to marine ecosystems

Species	Age (Sex)	Wet/dry weight	Liver (mean $\pm$ SD)	Kidney (mean $\pm$ SD)	Brain (mean $\pm$ SD)	Sampling area	Year	References
<i>Alca torda</i>	A (F)	w.w.	1.27 $\pm$ 0.90	1.11 $\pm$ 0.97	0.54 $\pm$ 0.42	Alicante (Spain)	2005–2020	Present study
<i>Alca torda</i>	A (F)	d.w.	4.17 $\pm$ 2.97	4.06 $\pm$ 3.57	2.79 $\pm$ 2.19	Alicante (Spain)	2005–2020	Present study
<i>Alca torda</i>	A (M/F)	d.w.	3.0 $\pm$ 0.87	2.54 $\pm$ 0.82	1.67 $\pm$ 0.52	Alicante (Spain)	2007	Espín et al. (2012)
<i>Alca torda</i>	J (M/F)	d.w.	2.68 $\pm$ 0.86	1.80 $\pm$ 0.65	1.22 $\pm$ 0.40			
<i>Alca torda</i>	A	d.w.	4.25 $\pm$ 1.68	3.77 $\pm$ 1.50	2.05 $\pm$ 0.84	Bay of Biscay (France)	2006	Fort et al. (2015)
<i>Alca torda</i>	A	d.w.	10.13 $\pm$ 4.71	6.48 $\pm$ 2.14	2.22 $\pm$ 0.99	Isle of Rhé and Orégon (France)	2014	Fort et al. (2015)
<i>Alca torda</i>	A	d.w.	9.63 $\pm$ 2.81	4.90 $\pm$ 1.12	–	Aveiro and Peniche (Portugal)	2005–2007	Ribeiro et al. (2009)
<i>Alca torda</i>	J	d.w.	5.00 $\pm$ 1.53	2.99 $\pm$ 0.82	–			
<i>Alca torda</i>	A (M/F)	d.w.	1.71 $\pm$ 0.42	–	–	Kuvshin Island (Russia)	1992	Savinov et al. (2003)
<i>Pandion haliaetus</i>	A/J (M)	w.w.	1.98 $\pm$ 0.02	3.12 $\pm$ 3.28	0.88 $\pm$ 0.56	Alicante (Spain)	2005–2020	Present study
<i>Pandion haliaetus</i>	A/J (M/F)	w.w.	3.40	–	–	France	2007	Lemarchand et al. (2012)
<i>Pandion haliaetus</i>	w.w.	w.w.	3.61 $\pm$ 2.16	5.28 $\pm$ 3.08	1.01 $\pm$ 0.59	Build-up environments, Quebec (Canada)	1989–1991	DesGranges et al. (1998)
<i>Pandion haliaetus</i>	w.w.	w.w.	0.72 $\pm$ 0.36	0.91 $\pm$ 0.51	0.23 $\pm$ 0.11	Natural environments, Quebec (Canada)		
<i>Pandion haliaetus</i>	w.w.	w.w.	4.70	4.30	–	Norway	1972–1977	Norheim and Frösille (1978)
<i>Pandion haliaetus</i>	C	w.w.	0.20	0.20	0.10	Inari (Finland)	1970–1972	Häkkinen and Häsänen (1980)
<i>Pandion haliaetus</i>	C	w.w.	–	0.40	0.10	Ranua (Finland)	1970–1972	Häkkinen and Häsänen (1980)
<i>Pandion haliaetus</i>	C	w.w.	1.80	1.40	0.30	Hämeenkyrö (Finland)	1970–1972	Häkkinen and Häsänen (1980)
<i>Pandion haliaetus</i>	A/J (M)	d.w.	6.50 $\pm$ 0.09	11.42 $\pm$ 12.00	4.54 $\pm$ 2.93	Alicante (Spain)	2005–2020	Present study
<i>Pandion haliaetus</i>	A/J (F)	d.w.	5.38 $\pm$ 2.45	4.65 $\pm$ 0.87	0.49 $\pm$ 0.23	Pomerania (Poland)	2003–2006	Kalinska et al. (2014)
<i>Pandion haliaetus</i>	C	d.w.	3.50	1.2	0.8	South Carolina (USA)	2003–2005	Hopkins et al. (2007)
<i>Pandion haliaetus</i>	J	d.w.	8.60	1.6	1.6			
<i>Pandion haliaetus</i>	A	d.w.	24.40	49.1	2.1			
<i>Pandion haliaetus</i>	C	d.w.	0.80	0.80	0.40	Inari (Finland)	1970–1972	Häkkinen and Häsänen (1980)
<i>Pandion haliaetus</i>	C	d.w.	–	2.20	1.20	Ranua (Finland)	1970–1972	Häkkinen and Häsänen (1980)
<i>Pandion haliaetus</i>	C	d.w.	7.10	6.80	2.90	Hameenkyro (Finland)	1970–1972	Häkkinen and Häsänen (1980)

Table 3 (continued)

Species	Age (Sex)	Wet/dry weight	Liver (mean ± SD)	Kidney (mean ± SD)	Brain (mean ± SD)	Sampling area	Year	References
<i>Chroicocephalus ridibundus</i>	J (M/F)	w.w.	<b>0.41 ± 0.14</b>	<b>0.28 ± 0.08</b>	<b>0.15 ± 0.08</b>	Alicante (Spain)	2005–2020	Present study
<i>Chroicocephalus ridibundus</i>	J (M/F)	d.w.	<b>1.35 ± 0.49</b>	<b>1.04 ± 0.30</b>	<b>0.81 ± 0.46</b>	Alicante (Spain)	2005–2020	Present study
<i>Larus ridibundus</i> <sup>a</sup>	A/J (M/F)	d.w.	0.67 ± 0.34*	0.66 ± 0.35*	0.23 ± 0.13*	Gdańsk Gulf (Poland)	2009–2012	Szumilo-Pilarska et al. (2016)
<i>Chroicocephalus ridibundus</i>	d.w.	–	0.69 ± 0.51	–	–	Southeast Poland	2010–2011	Kitowski et al. (2015)
<i>Larus ridibundus</i> <sup>a</sup>	d.w.	3.20**	–	–	–	Caspian Sea (Iran)	2008	Rajaei et al. (2010)
<i>Larus ridibundus</i> <sup>a</sup>	d.w.	2.58 ± 1.37	2.49 ± 1.71	0.99 ± 0.80	0.99 ± 0.80	Inland dump site, Tuscany (Italy)	1984–1985	Leonzio et al. (1986)
<i>Larus ridibundus</i> <sup>a</sup>	d.w.	3.19 ± 2.75	4.02 ± 2.00	3.78 ± 1.78	3.78 ± 1.78	Coastal area, Tuscany (Italy)	1984–1985	Leonzio et al. (1986)
<i>Larus michahellis</i>	A/J (M/F)	w.w.	<b>1.31 ± 0.74</b>	<b>1.01 ± 0.66</b>	<b>0.27 ± 0.15</b>	Alicante (Spain)	2005–2020	Present study
<i>Larus michahellis</i>	A/J (M/F)	d.w.	<b>4.32 ± 2.45</b>	<b>3.69 ± 2.43</b>	<b>1.40 ± 0.82</b>	Alicante (Spain)	2005–2020	Present study
<i>Larus cachinnans</i>	A	d.w.	2.98	2.17	–	Ebro Delta (Spain)	1997–1999	Arcos et al. (2002)
<i>Morus bassanus</i>	A/J (M/F)	w.w.	<b>7.16 ± 4.60</b>	<b>6.62 ± 2.85</b>	<b>1.27 ± 0.81</b>	Alicante (Spain)	2005–2020	Present study
<i>Morus bassanus</i>	w.w.	0.85 ± 0.10	0.53 ± 0.07	–	–	Portugal	2007–2008	Mendes et al. (2013)
<i>Morus bassanus</i>	w.w.	1.88 ± 1.52	–	–	–	Galicia (Spain)	2005	Carbonell et al. (2007)
<i>Morus bassanus</i>	A/J (M/F)	d.w.	<b>23.49 ± 15.11</b>	<b>24.18 ± 10.40</b>	<b>6.58 ± 4.21</b>	Alicante (Spain)	2005–2020	Present study
<i>Morus bassanus</i>	A (M)	d.w.	7.30	–	–	Firth of Forth (United Kingdom)	1972	Parslow et al. (1973)
<i>Morus bassanus</i>	A (M/F)	d.w.	18.40	–	–	Lancashire (United Kingdom)	1972	Parslow et al. (1973)
<i>Sula bassana</i> <sup>d</sup>	A	d.w.	12	–	3	Pembroke (Britain)	1969–1974	Parslow and Jefferies (1977)
<i>Sula bassana</i> <sup>d</sup>	A	d.w.	17	–	6	Northumberland (Britain)	1969–1974	Parslow and Jefferies (1977)
<i>Sula bassana</i> <sup>d</sup>	A	d.w.	17	–	4	Norfolk (Britain)	1969–1974	Parslow and Jefferies (1977)
<i>Sula bassana</i> <sup>d</sup>	J	d.w.	8	–	3	Somerset (Britain)	1969–1974	Parslow and Jefferies (1977)
<i>Sula bassana</i> <sup>d</sup>	J	d.w.	6	–	2	S. Devon (Britain)	1969–1974	Parslow and Jefferies (1977)
<i>Morus bassanus</i>	d.w.	–	–	–	2.23	Nova Scotia (North America)	–	Mallory et al. (2018)
<i>Morus bassanus</i>	A	d.w.	2.60 ± 0.80	3.42 ± 0.65	–	Ovar and Peniche (Portugal)	2003–2006	Mendes et al. (2008)
<i>Morus bassanus</i>	J	d.w.	1.29 ± 0.18	0.95 ± 0.13	–	–	–	–
<i>Morus bassanus</i>	A/J (M/F)	d.w.	7.03 ± 0.87	2.76 ± 0.47	–	Galicia (Spain)	2015–2017	Nardiello et al. (2019)
<i>Phalacrocorax aristoteleis</i>	A/J (M/F)	w.w.	<b>14.56 ± 29.63</b>	<b>16.12 ± 37.89</b>	<b>0.98 ± 0.62</b>	Alicante (Spain)	2005–2020	Present study



Table 3 (continued)

Species	Age (Sex)	Wet/dry weight	Liver (mean ± SD)	Kidney (mean ± SD)	Brain (mean ± SD)	Sampling area	Year	References
<i>Phalacrocorax aristotelis</i>		w.w.	4.24 ± 3.01	–	–	Galicia (Spain)	2005	Carbonell et al. (2007)
<b><i>Phalacrocorax aristotelis</i></b>	<b>A/J (M/F)</b>	<b>d.w.</b>	<b>47.77 ± 97.17</b>	<b>58.85 ± 138.31</b>	<b>5.06 ± 3.23</b>	<b>Alicante (Spain)</b>	<b>2005–2020</b>	<b>Present study</b>
<i>Phalacrocorax aristotelis</i>	A	d.w.	5.32	5.60	–	Ebro Delta (Spain)	1997–1999	Arcos et al. (2002)
<i>Phalacrocorax aristotelis</i>	A (M/F)	d.w.	28.83 ± 17.84	–	–	Galicia (Spain)	2002–2003	Sanpera et al. (2008)
<i>Phalacrocorax aristotelis</i>	J (M/F)	d.w.	19.90 ± 12.84	–	–	Galicia (Spain)	2005	Sanpera et al. (2008)
<i>Phalacrocorax aristotelis</i>	J	d.w.	5.36 ± 3.79	–	–	Galicia (Spain)	2005	Sanpera et al. (2008)
<i>Phalacrocorax aristotelis</i>		d.w.	65.58 ± 83.70	–	–	Capraia Island (Italy)	–	Lambertini and Leonzio (1986)
<b><i>Phalacrocorax carbo</i></b>	<b>A/J (M/F)</b>	<b>w.w.</b>	<b>5.65 ± 3.62</b>	<b>4.40 ± 4.75</b>	<b>0.53 ± 0.27</b>	<b>Alicante (Spain)</b>	<b>2005–2020</b>	<b>Present study</b>
<i>Phalacrocorax carbo</i>		w.w.	2.12 ± 0.22	2.23 ± 0.30	–	Trebon (Czech Republic)	2015	Kral et al. (2017)
<i>Phalacrocorax carbo</i>	A/J	w.w.	10.00 ± 8.30	2.70 ± 2.10	–	Zahlnice (Czech Republic)	2003	Houserová et al. (2005)
<i>Phalacrocorax carbo</i>	A/J/C (M/F)	w.w.	1.20 ± 0.50	0.90 ± 0.70	–	Tokyo (Japan)	1993–1994	Saeki et al. (2000)
<i>Phalacrocorax carbo</i>	A (M/F)	w.w.	1.70 ± 0.80	1.50 ± 1.00	–	Lake Biwa (Japan)	1993	Saeki et al. (2000)
<i>Phalacrocorax carbo</i>		w.w.	30.35	36.90	–	Belgium	1970–1981	Delbeke et al. (1984)
<b><i>Phalacrocorax carbo</i></b>	<b>A/J (M/F)</b>	<b>d.w.</b>	<b>18.55 ± 11.88</b>	<b>16.09 ± 17.37</b>	<b>2.78 ± 1.39</b>	<b>Alicante (Spain)</b>	<b>2005–2020</b>	<b>Present study</b>
<i>Phalacrocorax carbo</i>	(M/F)	d.w.	5.70 ± 0.91	3.60 ± 2.24	–	Caspian Sea (Iran)	2009	Aazami et al. (2011)
<i>Phalacrocorax carbo</i>	A	d.w.	42.20 ± 6.28	7.20 ± 1.00	–	Záhlnice (Czech Republic)	2003	Houserová et al. (2007)
<i>Phalacrocorax carbo</i>	J	d.w.	7.50 ± 1.63	4.10 ± 0.49	–	–	–	–
<i>Phalacrocorax carbo</i>	A/J (M/F)	d.w.	8.32	9.25	–	Caspian Sea (Iran)	2004	Mazloomi et al. (2008)
<i>Phalacrocorax carbo</i>	A (M/F)	d.w.	15.51 ± 17.30	30.21 ± 47.93	–	Gdańsk (Poland)	2006	Misztal-Szkudlinska et al. (2011)
<i>Phalacrocorax carbo</i>	A (M/F)	d.w.	12.00 ± 9.00	14.00 ± 9.00	1.40 ± 1.10	Lake Biwa (Japan)	1993–2003	Nam et al. (2005)
<i>Phalacrocorax carbo</i>	A	d.w.	3.18 ± 1.11	–	–	Autonomous Province of Vojvodina (Serbia)	2010	Skoric et al. (2012)
<i>Phalacrocorax carbo</i>	J	d.w.	6.18 ± 2.21	–	–	–	–	–

Table 3 (continued)

Species	Age (Sex)	Wet/dry weight	Liver (mean ± SD)	Kidney (mean ± SD)	Brain (mean ± SD)	Sampling area	Year	References
<i>Phalacrocorax carbo</i>		d.w.	5.71 ± 1.85	3.79 ± 0.71	–	Caspian Sea (Iran)	2011	Aazami and KianiMehr (2017)
<i>Phalacrocorax carbo</i>		d.w.	–	8.95 ± 1.23	–	Southeast Poland	2010–2011	Kitowski et al. (2015)
<i>Phalacrocorax carbo</i>	A/J (M/F)	d.w.	3.58 ± 2.91	–	–	Kis-Balaton (Hungary)	2009	Lehel et al. (2013)
<i>Phalacrocorax carbo</i>	A (M/F)	d.w.	4.48 ± 3.34	–	–	Kis-Balaton (Hungary)	2009	Lehel et al. (2013)
<i>Phalacrocorax carbo</i>	J (M/F)	d.w.	2.68 ± 2.09	–	–	Kis-Balaton (Hungary)	2009	Lehel et al. (2013)
<i>Phalacrocorax carbo</i>	A/J (M/F)	d.w.	8.32 ± 1.32	9.25 ± 1.71	–	Caspian Sea (Iran)	2006	Mollazadeh et al. (2011)
<i>Phalacrocorax carbo</i>	A	d.w.	36.24 ± 30.56***	7.61 ± 5.79***	–	Záhlnice (Czech Republic)	2003	Houserová et al. (2005)
<i>Phalacrocorax carbo</i>	J	d.w.	5.77 ± 3.08***	4.61 ± 0.67***	–	–	–	–
<i>Phalacrocorax carbo</i>		d.w.	0.28 ± 0.33	0.34 ± 0.34	0.07	Kanto (Japan)	2001–2002	Horai et al. (2007)
<i>Phalacrocorax carbo</i>	A/J (M/F)	d.w.	8.09*	–	–	Axios Delta, Evros (Greece)	1999–2002	Goutner et al. (2011)
<i>Phalacrocorax carbo</i>	(M/F)	d.w.	3.39 ± 1.39	4.05 ± 2.18	0.65 ± 0.38	Biwa Lake (Japan)	1993	Saeki et al. (2000)
<i>Puffinus mauretanicus</i>		w.w.	<b>1.81 ± 0.04</b>	<b>1.14 ± 0.51</b>	<b>0.40 ± 0.06</b>	Alicante (Spain)	2005–2020	Present study
<i>Puffinus mauretanicus</i>	(M/F)	w.w.	1.00 ± 0.53	0.50 ± 0.23	–	Aveiro and Peniche (Portugal)	2010–2011	Costa et al. (2016)
<i>Puffinus mauretanicus</i>		d.w.	<b>5.96 ± 0.16</b>	<b>4.20 ± 1.86</b>	<b>2.11 ± 0.34</b>	Alicante (Spain)	2005–2020	Present study
<i>Sterna hirundo</i>	A/J (F)	w.w.	<b>1.19 ± 0.29</b>	<b>0.74 ± 0.37</b>	<b>0.20 ± 0.03</b>	Alicante (Spain)	2005–2020	Present study
<i>Sterna hirundo</i>	A	w.w.	1.25	1.51	0.19	New Brunswick (Canada)	1978–1984	Braune (1987)
<i>Sterna hirundo</i>	A/J (F)	d.w.	<b>3.93 ± 0.96</b>	<b>2.71 ± 1.35</b>	<b>1.07 ± 0.18</b>	Alicante (Spain)	2005–2020	Present study
<i>Sterna hirundo</i>	A	d.w.	0.99	–	–	Ebro Delta (Spain)	1997–1999	Arcos et al. (2002)

Bold values indicate results from the present study

A adult, J juvenile, C chick, M male, F female

\*Transformed units with respect to published data for comparison purposes

\*\*Approximate value (exact data is not provided)

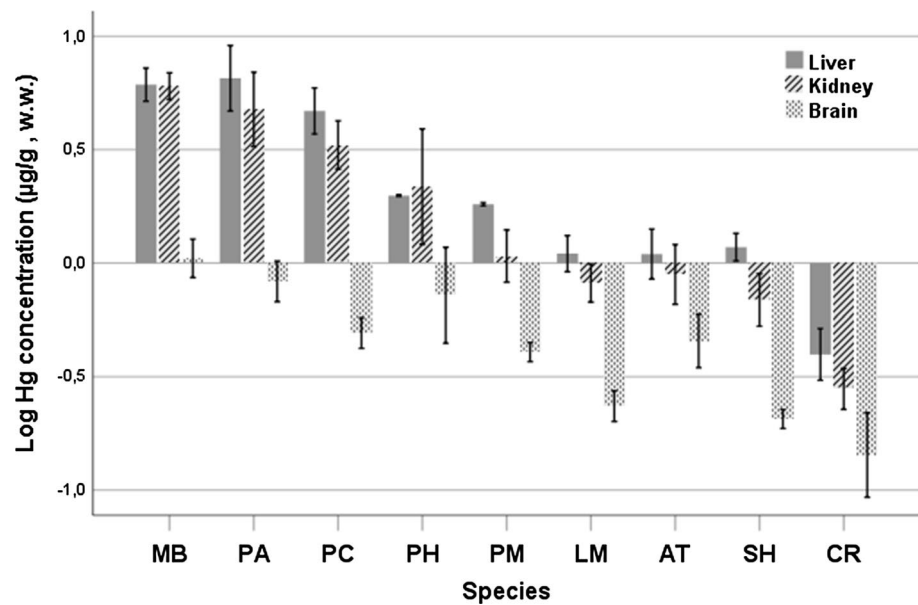
\*\*\*Median value

<sup>a</sup>Currently listed as *Chroicocephalus ridibundus*

<sup>b</sup>Currently listed as *Larus michahellis*

<sup>c</sup>Currently listed as *Morus bassanus*

**Fig. 2** Log mercury concentrations ( $\pm$  SE;  $\mu\text{g/g}$ , wet weight) in liver, kidney, and brain of the species studied. MB *Morus bassanus*; PA *Phalacrocorax aristotelis*; PC *Phalacrocorax carbo*; PH *Pandion haliaetus*; PM *Puffinus mauretanicus*; LM *Larus michahellis*; AT *Alca torda*; SH *Sterna hirundo*; CR *Chroicocephalus ridibundus*



gannet, European shag, and great cormorant—the greater species—were the ones that presented the highest Hg concentrations (mean Hg in liver: 7.16, 14.56, and 5.65  $\mu\text{g/g}$  ww, respectively; Table 2), coinciding with our initial hypothesis. The osprey was the next species with the highest Hg concentration (mean Hg in liver: 1.98  $\mu\text{g/g}$  ww; Table 2) but did not show significant differences with the rest of the species except for yellow-legged gull, razorbill, common tern, and black-headed gull in brain (Table 2). Mercury levels also differed among tissues (ANOVA test:  $F = 46.7$ ,  $p < 0.001$ ). Tukey's test showed no differences between liver and kidney ( $p = 0.386$ ), whereas the concentrations in these tissues were significantly higher than those found in the brain ( $p < 0.001$ ) for the nine species studied (Table 2). For these nine species, the mean ratio of  $\text{Hg}_{\text{liver}}:\text{Hg}_{\text{kidney}}$  was 1.03 (0.63–1.61,  $n = 62$ ; coefficient of variation, CV, of 26%), reflecting that liver and kidney values were similar, whereas the ratio of  $\text{Hg}_{\text{liver}}:\text{Hg}_{\text{brain}}$  was 8.28 (2.25–14.86,  $n = 62$ , CV 70%) similar to the ratio of  $\text{Hg}_{\text{kidney}}:\text{Hg}_{\text{brain}}$  (8.01, 1.87–16.45,  $n = 62$ , CV 87%), showing the higher Hg levels in the liver and kidney compared with the brain.

Pearson's correlation coefficients showed that Hg concentrations in tissues were positively correlated with the body mass of the individuals ( $r_{\text{Hg liver-Body mass}} = 0.450$ ,  $r_{\text{Hg kidney-Body mass}} = 0.537$ ,  $r_{\text{Hg brain-Body mass}} = 0.565$ ,  $p < 0.005$ ,  $n = 48$ ). In addition, strong significant positive correlations were observed for Hg concentrations between tissues ( $r_{\text{liver-kidney}} = 0.937$ ,  $r_{\text{liver-brain}} = 0.787$ ,  $r_{\text{kidney-brain}} = 0.784$ ,  $p < 0.001$ ,  $n = 61$ –62; Fig. 3).

Differences in Hg concentrations according to sex, age, and cause of admission were evaluated in four species (i.e., northern gannet, European shag, great cormorant, and

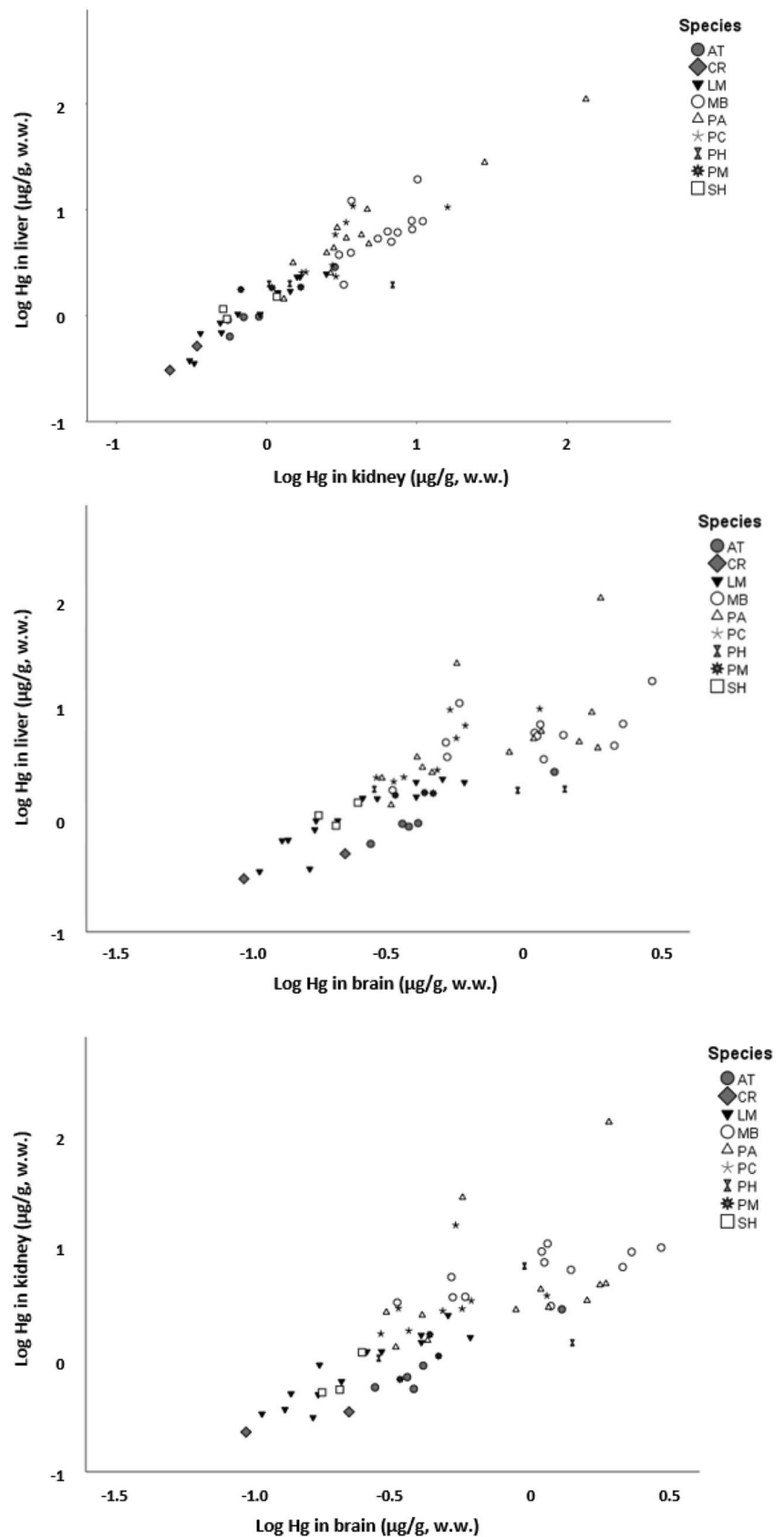
yellow-legged gulls). Adult European shags showed significantly higher Hg concentrations in liver and kidney than juvenile birds ( $F = 35.3$  and  $68.9$ , respectively,  $p < 0.001$ ), as expected, whereas the opposite trend was found in the three tissue types in yellow-legged gulls ( $F = 28.5$ ,  $35.0$ , and  $16.6$  in liver, kidney, and brain, respectively,  $p < 0.003$ ; Fig. 4). Sex-related differences were only observed in yellow-legged gulls. Females had lower Hg concentrations in tissues than males ( $F = 8.0$ ,  $p = 0.018$  in liver;  $F = 8.8$ ,  $p = 0.014$  in kidney;  $F = 5.8$ ,  $p = 0.037$  in brain; Fig. 4), which is in line with the literature data. Finally, significant differences in Hg concentrations according to the cause of admission to WRC were only found in liver for northern gannets ( $F = 6.3$ ,  $p = 0.033$ ) and European shags ( $F = 6.7$ ,  $p = 0.029$ ), birds suffering nontraumatic pathologies showing higher hepatic Hg concentrations than birds admitted due to traumatic causes (Fig. 5), which was expected according to our hypothesis.

## Discussion

### Tissue Hg Concentrations and Interspecific Differences

The pattern of Hg distribution in tissues of nine species linked to marine ecosystems was similar to other studies: liver  $\geq$  kidney  $>$  brain (Table 3). Chronic exposure to Hg entails a balance in concentrations between compartments in the body, which explains the distribution pattern observed and the strong correlations found between Hg concentrations in liver, kidney, and brain (Fig. 3). The distribution of Hg in different organs depends on the form of Hg to which the individual is exposed, and the ratio

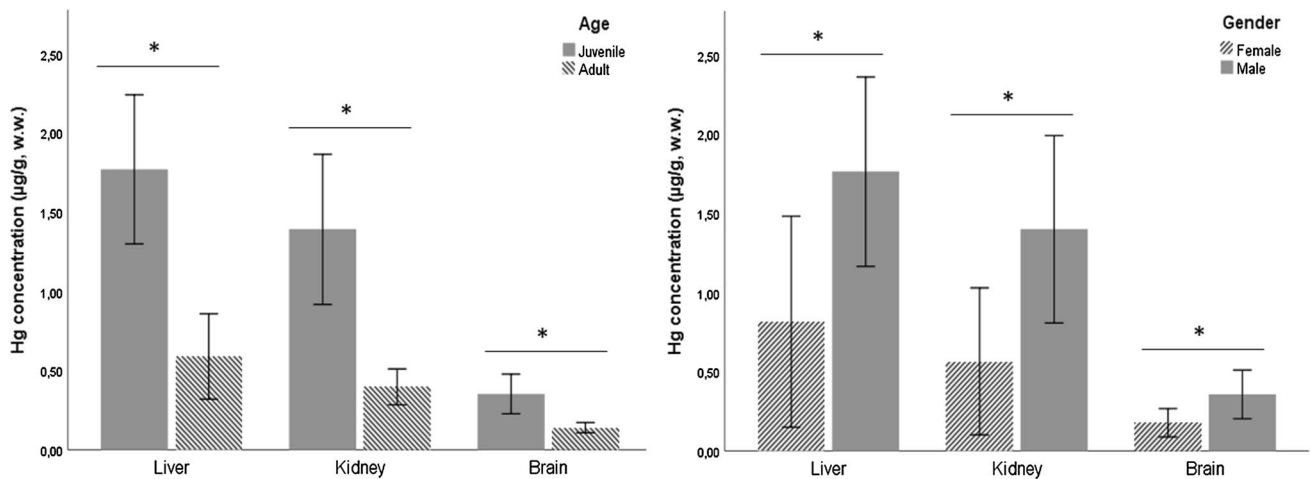
**Fig. 3** Correlations between (log) mercury concentrations ( $\mu\text{g/g}$ , wet weight) in tissues of 9 wild bird species ( $r_{\text{liver-kidney}}=0.937$ ,  $r_{\text{liver-brain}}=0.787$ ,  $r_{\text{kidney-brain}}=0.784$ ,  $p<0.001$ ,  $n=61-62$ ). *AT* *Alca torda*; *CR* *Chroicocephalus ridibundus*; *LM* *Larus michahellis*; *MB* *Morus bassanus*; *PA* *Phalacrocorax aristotelis*; *PC* *Phalacrocorax carbo*; *PH* *Pandion haliaetus*; *PM* *Puffinus mauretanicus*; *SH* *Sterna hirundo*



of Hg in kidney and liver may be used to distinguish a chronic exposure to MeHg or inorganic Hg (Scheuhammer 1987). Thus, a kidney:liver ratio markedly greater than 1 reflects an exposure to inorganic Hg, whereas a ratio close to 1 (and  $< 2$ ) is characteristic of MeHg exposure. In this study, the kidney:liver ratio was within the range 0.62–1.58 (mean ratio: 0.88) depending on the species, probably reflecting that the individuals evaluated were mainly exposed to MeHg. This is consistent with the fact that almost 100% of the total Hg detected in muscle of different fish species was in the form of MeHg (Scheuhammer 1987).

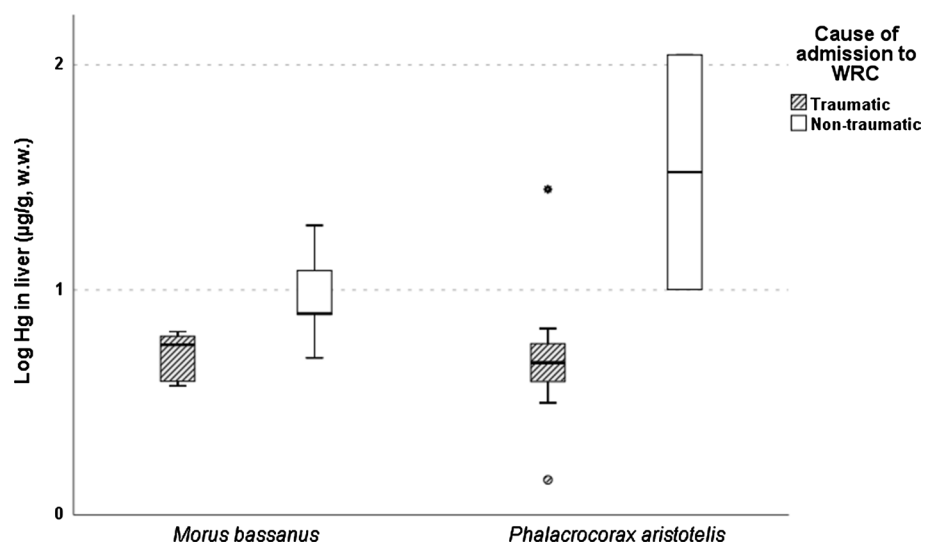
Several factors may explain variations in Hg concentrations between species (Table 2), some of them interspecific, such as detoxification capacity, size, diet, or

migratory habits, and others intraspecific, such as age, sex, or body condition (Moura et al. 2018a; Ramos et al. 2013). One of the main factors that determine the interspecific differences in the pollutant load in the organism is the diet, being the main route of Hg exposure in marine vertebrates (Carravieri et al. 2018; Kojadinovic et al. 2007; Moura et al. 2018b; Ribeiro et al. 2009). Although the study area is an essential factor to consider due to the potential differences in Hg contamination, it has not been discussed in this study, because all individuals were found dead or injured along the Occidental Mediterranean coastline. Also, the exact origin of the migratory individuals before their arrival to the coast of Alicante is unknown. Despite this, it should be considered that the origin could partly explain the differences in Hg concentrations found in certain species. This may be critical in some cases, and an



**Fig. 4** Mean (and 95% CI) mercury concentrations ( $\mu\text{g/g}$ , w.w.) in tissues of *Larus michahellis* by age and gender. \*Significant differences between ages and sexes were found in the three tissue types ( $p < 0.05$ )

**Fig. 5** Mean (and 95% CI) (log) mercury concentration ( $\mu\text{g/g}$ , w.w.) in liver of *Morus bassanus* and *Phalacrocorax aristotelis* according to cause of admission to the WRC (traumatic or nontraumatic). Significant differences according to the cause of admission were found in both species ( $p < 0.05$ )



approach to relate Hg concentrations in an abiotic matrix with those in bird tissues is recommended for future studies (e.g., the Biota Sediment Accumulation Factor, Calle et al. 2015).

In general, the species studied are mainly piscivorous, which means that they are exposed to higher Hg levels than species with different diet habits, since fish accumulate high levels of this metal, especially as MeHg (Kojadinovic et al. 2007). Depending on the species of fish they ingest, they will be exposed to a different Hg amount. Demersal and benthic fish have higher Hg concentrations than pelagic fish because they occupy higher trophic levels and are closer to the bottom sediments (Arcos et al. 2002; Vizuete et al. 2018). The study species that presented a larger size (northern gannet, European shag, and great cormorant) showed the highest Hg concentrations (Table 2), which was supported by a positive correlation between Hg concentrations in tissues and body mass. This could be due to the consumption of larger prey, which can contain higher Hg levels than smaller prey of the same species (Zamani-Ahmadmahmoodi et al. 2014). Although the northern gannet feeds on pelagic fish, it ingests larger prey than the cormorants by feeding farther from the coast. Also, it selectively looks for places where it can take advantage of trawl fishery discards (BirdLife International 2018; Hamer et al. 2000; Kubetzki et al. 2009), which may lead to greater Hg exposure, because birds can consume species that they cannot access in a natural way, such as demersal or benthic fish. One of its main prey is the Atlantic mackerel (*Scomber scombrus*), a large size fish (215–455 mm) that feeds on plankton but mostly on smaller fish as its size increases, being more exposed to Hg than other fish species (Hamer et al. 2000; Olaso et al. 2005). High Hg levels in cormorants (i.e., European shag and great cormorant) can also be explained by the diving capacity of both species, which allows them to feed on benthic fish (Arcos et al. 2002; BirdLife International 2018, Misztal-Szkudlinska et al. 2018). The osprey feeds exclusively on fish, and the Balearic shearwater takes advantage of commercial fishery discards and ingests pelagic fish but feeds on smaller prey, so less Hg exposure can be expected (BirdLife International 2018; Louzao et al. 2012). Both species showed slightly (but not significant for most tissues) lower Hg concentrations than northern gannets, European shags, and great cormorants. Although the diet of razorbills and common terns are mainly based on fish, they presented lower Hg concentrations than the northern gannet and European shag, probably because these species ingest smaller and pelagic prey (BirdLife International 2018; Szostek and Becker 2015). Some fish included in the diet of these species are sardines (*Sardina pilchardus*) for razorbills and also anchovies (*Engraulis encrasicolus*) in the case of common terns, being small and pelagic fish

species that can be found in the Mediterranean Sea (Costalago et al. 2015; Espín et al. 2012; Szostek and Becker 2015). Sardines present a size < 250 mm and anchovies from 10 to 130 mm. They mainly feed on phytoplankton and zooplankton, respectively, so they occupy a low trophic level (Borme et al. 2009; Costalago et al. 2015; Tudela and Palomera 1997). In addition, the common tern ingests mostly juvenile fish, so they are expected to accumulate a smaller amount of Hg (Szostek and Becker 2015). The yellow-legged and black-headed gulls showed lower Hg levels than European shags and cormorants, probably because they are opportunistic species also ingesting terrestrial and freshwater food, which have less Hg load than prey of marine origin (BirdLife International 2018; Ramos et al. 2013; Vizuete et al. 2018). In future studies, it would be interesting to analyse the stable isotope Nitrogen 15 ( $^{15}\text{N}$ ) to determine the trophic level of each study species so that a comparison of Hg concentrations versus the trophic position can be made.

In general, Hg concentrations found in liver, kidney, and brain were similar to or lower than those observed in the same species from other countries, particularly for razorbill, osprey, black-headed gull, or Balearic shearwater (Table 3). However, for certain species (mainly northern gannet, European shag, and great cormorant) concentrations found in this study were higher than levels reported in the literature (Table 3).

Mercury concentrations in internal tissues are a key indicator of bioaccumulation. Measuring both liver and kidney simultaneously can provide information on the nature of exposure (i.e., chronic exposure to MeHg or inorganic Hg). Threshold concentrations (mainly in liver and kidney) associated with adverse effects in birds have been suggested for interpretation (Espín et al. 2016). However, due to ethical and legal reasons, sampling is generally possible where carcasses are found in the field or injured animals are euthanased for welfare reasons. In addition, metabolism, demethylation and health condition (starvation versus healthy individuals) can influence the balance (e.g., remobilization of Hg) and alter tissue Hg concentrations. On the other hand, feathers are considered a good matrix for Hg determination since they can be obtained as moulted feathers, from carcasses or be plucked without permanently damaging the bird, being a minimally invasive matrix. Moreover, MeHg is uniformly deposited in feathers and they are a more stable matrix. However, this deposition only occurs during feather growth, reflecting Hg concentration in blood during this period, while internal tissues maintain a continuous exchange with blood, so they provide updated information even though Hg levels are affected by changes in diet and/or fat mobilization. In addition, feathers can be contaminated on the surface (although Hg external contamination is typically small), and moulting periods and patterns are different



among species (Espín et al. 2016), which may pose some difficulties when comparing results. Although Hg concentrations were also analysed in feathers from some species in this study, a proper statistical analysis could not be done due to limitations in the number of samples. Mercury concentrations in feathers of most species were, in general, similar to those reported in other studies (Arcos et al. 2002; Cotín et al. 2012; Mazloomi et al. 2008; Misztal-Szkudlinska et al. 2012; Monteiro et al. 1999; Moreno et al. 2013; Otero et al. 2018; Paiva et al. 2008; Rumbold et al. 2001; Sanpera et al. 2008; Szumiło-Pilarska et al. 2016, 2017; Zolfaghari et al. 2009), whereas they were lower in the case of osprey (Cahill et al. 1998; DesGranges et al. 1998; Lounsbury-Billie et al. 2008) and higher for northern gannet, black-headed gull, Audouin's gull and Scopoli's shearwater (Arcos et al. 2002; Goutner et al. 2000, 2013; Mendes et al. 2008; Monteiro et al. 1995, 1999; Nardiello et al. 2019).

### Sex, Age, and Cause of Admission to WRC

In this study, the influence of sex, age, and cause of admission on Hg exposure was evaluated in four species (i.e., northern gannet, European shag, great cormorant, and yellow-legged gulls). Sex-related differences in tissue Hg concentrations were only found in yellow-legged gulls, females showing lower Hg levels compared to males (Fig. 4). Different studies (Ishii et al. 2017; Vizuete et al. 2018) have demonstrated that, in adult individuals, females have lower Hg levels than males justified by their excretion capacity through egg laying. Regarding age differences, adult European shags showed higher Hg concentrations in liver and kidney than juvenile birds. Several authors agree that adult individuals have higher Hg concentrations than juveniles of the same species because of the greater accumulation of Hg in their body during their life (Moura et al. 2018b; Ribeiro et al. 2009; Saeki et al. 2000; Vizuete et al. 2018). However, the opposite trend was found in yellow-legged gulls in this study (Fig. 4), which might be due to their opportunistic diet habits (Table 1) and a different diet source between juvenile and adult birds. However, further studies with higher number of samples would be needed to better evaluate these sex and age-related differences.

Northern gannets and European shags suffering non-traumatic pathologies (i.e., specimens with symptoms of undernutrition as a result of pathologies such as infectious or parasitic diseases) showed higher hepatic Hg concentrations than birds admitted to the WRC due to traumatic causes (Fig. 5). In this regard, Sanpera et al. (2008) have observed that dehydrated individuals, with poor body condition and a state of weakness had higher Hg concentrations in their tissues as a result of a general redistribution of metals in the organs. Further studies with a larger number of samples within each cause of admission type would be necessary in

order to evaluate deeply the effect of the cause of admission on Hg concentrations in the study species.

### Risk Assessment

In the majority of cases, the individuals studied showed Hg concentrations below the critical levels related to reproductive disturbances in black ducks (*Anas rubripes*) (i.e., reduced egg production, hatchability, and survival of ducklings; liver: 23 µg/g, ww; kidney: 16 µg/g, ww; brain: 3.79 µg/g, ww; Finley and Stendell 1978) or marked behavioural changes in pigeons (i.e., declined rate of pecking, changes in posture and coordination; brain: 12–16 µg/g, Evans et al. 1982). However, two individuals of European shag showed tissue concentrations exceeding those critical levels in liver and kidney (liver: 27.94 and 110.57 µg/g, ww; kidney: 28.40 and 134.17 µg/g, ww; brain: 0.57 and 1.92 µg/g, ww). A northern gannet (liver: 19.33 µg/g, ww; kidney: 10.13 µg/g, ww; brain: 2.97 µg/g, ww) and a great cormorant (kidney: 16.06 µg/g, ww; liver: 10.58 µg/g, ww; brain: 0.53 µg/g, ww) had concentrations close to that levels. In addition, all the species studied showed mean hepatic Hg levels similar to or higher than those associated with altered behaviour and decreased reproductive success in laboratory-reared ducklings (liver: 1–2 µg/g, ww; reviewed by Zillioux et al. 1993). It is clear that these comparisons should be interpreted with caution due to the interspecific differences in tolerance to contaminants. In addition, total Hg is not the best indicator of toxic effects, and more importance should be given to the more toxic form, MeHg concentrations (Wolfe et al. 1998). However, these results suggest that Hg concentrations in the marine ecosystems of the western Mediterranean could constitute a risk situation for certain seabird individuals, especially for endangered species (at national level), such as the European shag, with only 49–55 breeding pairs in the Valencian Community in 2018 (D. G. Medi Natural i Avaluació Ambiental 2018), or the northern gannet under Special Protection in Spain (Table 1).

### Conclusions

The results of this study suggest that individuals of nine bird species linked to marine ecosystems found dead in the western Mediterranean coasts were chronically exposed to MeHg. Mercury concentrations differed among species, which can be explained by their different dietary habits. In general, Hg concentrations found are similar to or higher than those reported in other studies worldwide. Some individuals of certain species (i.e., European shag, northern gannet, and great cormorant) showed Hg concentrations close to or higher than those described in the literature as causing reproductive alterations in other avian species. These



comparisons should be made with caution due to the possible difference in sensitivity between species. However, our results suggest that certain individuals inhabiting marine ecosystems in the western Mediterranean could be at risk of suffering long-term, Hg-related effects on physiology, reproduction, and behaviour. Some of the species evaluated are listed within different categories of threat according to the International Union for Conservation of Nature (IUCN) (including Near Threatened and Critically Endangered species) and are endangered at a national level, so this study will provide valuable information for risk assessors and authorities in charge of the management of the environment and pollution. Further studies with a greater number of specimens of each species are necessary to better evaluate the effect of sex, age, and cause of admission to WRCs on Hg concentrations in the study species. The cause of admission to the WRC is essential, because it helps to relate the Hg concentrations found with the history and symptoms of the individuals. Therefore, this factor should be described and evaluated in future research.

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**Availability of Data and Materials** Available upon request.

## Compliance with Ethical Standards

**Conflicts of interest** The authors declare that they have no conflict of interest.

**Ethics Approval** Not applicable. Carcasses from dead individuals admitted in the Wildlife Rehabilitation Centre were used in this study.

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