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Research Paper

Sources of persistent organic pollutants and their physiological effects on opportunistic urban gulls

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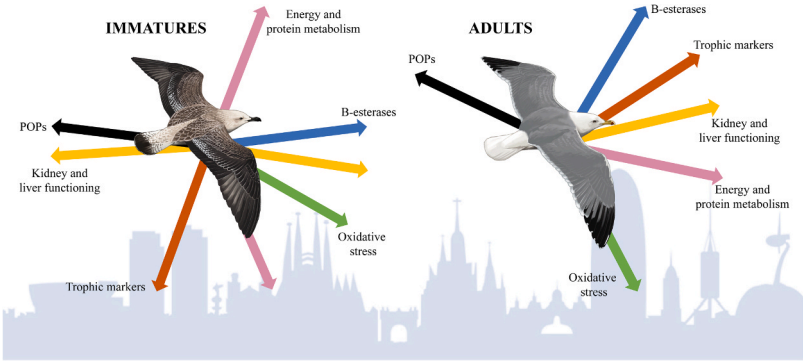
HIGHLIGHTS

- In urban ecosystems, gulls face high exposure to PCBs and PBDEs.
- Immature males presented a higher physiological response to PCBs and PBDEs.
- Carboxylesterases were negatively correlated to bioaccumulated PCBs and PBDEs.
- To assess pollutant exposure and effects, a multi-biomarker approach is encouraged.
- Yellow-legged gulls are proposed sentinels for monitoring urban pollution.

GRAPHICAL ABSTRACT



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ABSTRACT

Urbanization is associated with drastic shifts in biodiversity. While some species thrive in urban areas, the impact of inhabiting these human-altered environments on organism physiology remains understudied. We investigated how exposure to polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) affects the physiology of yellow-legged gulls (*Larus michahellis*) inhabiting a densely populated, industrialized city. We analyzed blood samples from 50 gulls (20 immatures and 30 adults) and assessed 27 physiological parameters and biomarkers related to xenobiotic protection, health, and feeding habits in these same individuals. We also tracked the movements of 25 gulls (15 immatures and 10 adults) to identify potential sources of persistent organic pollutants (POPs). Both adult and immature gulls primarily inhabited urban areas, followed by marine habitats. Immature gulls spent more time in freshwater, landfills, and agricultural areas. Bioaccumulated ΣPCB (median = 92.7 ng g^{-1} ww, 1.86–592) and ΣPBDE (median = 1.44 ng g^{-1} ww, 0.022–9.58) showed no significant differences between age and sex groups. Notably, immature males exhibited the highest correlations with POP concentrations, particularly with the activity of carboxylesterases (CEs), suggesting a higher sensitivity than

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adults. These findings highlight the potential of plasmatic CEs in immature yellow-legged gulls as effective tracers of POPs exposure and effects, offering insights into the anthropogenic impacts on urban biodiversity.

1. Introduction

Urbanization is a clear and widespread example of human impact on the natural environment, which has led to a worldwide loss of biodiversity [1-3]. However, while most wildlife species are unable to live in urban areas, others may persist [4] or even thrive in these environments [5]. As a result, these species may benefit from predictable food resources and the absence of natural predators [6-9]. However, urban wildlife faces other hazards such as exposure to chemical pollutants and the subsequent effects on health [10-13].

Persistent organic pollutants (POPs), including industrial chemicals such as the polychlorinated biphenyls (PCBs), and polybrominated diphenyl ethers (PBDEs) are ubiquitous in cities [14]. PCBs have been freely used for decades in different commercial products and processes and it was not until the 1970 s, that public awareness arose about the risk that they pose to human and animal health [15]. Decades passed before most European countries restricted their use, and it was not until 2004 for PCBs and 2010 for PBDEs, that global restrictions were implemented at the Stockholm Convention [16,17]. POPs exposure occurs through air, water and food [18] and despite the restrictions, these chemicals are still present in wildlife and humans at levels of concern due to their persistent characteristics. Urban and industrial areas display the highest levels of exposure to POPs [19], which can have detrimental effects on wildlife and human health. This can be particularly severe for long-lived species occupying higher positions within food webs because of bioaccumulation and biomagnification processes [20-24].

Persistent organic pollutants interfere with the reproductive system and reduce the survival of birds, mammals, and fishes through endocrine disruption and immune suppression effects [25,26]. For example, reproductive failure and eggshell thinning mainly due to DDT and DDE exposure was observed in white-tailed eagles (*Haliaeetus albicilla*), leading to the near collapse of the Swedish population in the second half of the 20th century [27,28], which subsequently recovered after the ban on POPs [29]. Understanding the health implications of inhabiting urban areas and the associated pollutant exposures is important in terms of detecting its effects before they are evident at population or ecosystem levels [30]. In this sense, the use of multiple biomarkers and physiological parameters provide valuable information [31,32]. Biomarkers of exposure such as B-esterases (*e.g.*, carboxylesterases and cholinesterases) can be helpful in determining the extent of organism exposure to environmental pollutants in living organisms [33], including exposure to POPs [34,35]. Assessing these exposure biomarkers together with blood biochemistry parameters related to physiological condition [36], can aid in determining the potential impacts of such pollutants on wildlife health and immune systems [37,30,38-41].

Patterns of movement in wildlife are highly variable between species or even individuals of a particular population, as they are one of the main factors determining the degree of exposure to different types and concentrations of pollutants [42-47]. Urban areas provide a high diversity of foraging habitats, which can include adjacent marine (in the case of coastal cities) or forested areas, but also areas of industrial activity and landfills. For example, wildlife species feeding on landfills are more exposed to flame retardants and other POPs [48,49], while those foraging and living in intensive crop production areas are exposed to higher PCBs levels [50]. Therefore, the integration of knowledge given by biomarkers, contaminant levels, and spatial movements can help to identify sources of pollution and its potential effects on wildlife health [51-53].

Our main aim was to unravel how exposure to POPs (PCBs and PBDEs) affects the physiology of an opportunistic predator, the yellow-legged gull (*Larus michahellis*), inhabiting a highly populated and industrialized city (Barcelona, Spain). Similar to other opportunistic species, the yellow-legged gull is a clear example of a species adapted to breeding and foraging in humanized habitats [8,54,55]. Considering its high foraging plasticity [56-59] and use of different anthropic environments, this species could be highly exposed to anthropogenic chemical pollutants. Specifically, our objectives were: 1) to identify the primary habitats of yellow-legged gulls and assess their blood POP levels, 2) to identify the most sensitive indicators of POPs exposure and discuss their suitability as biomarkers, and 3) to assess the role of yellow-legged gulls as sentinel species in urban environments. This study provides valuable insights into how exposure to POPs affects the physiology of an urban wild bird, while also suggesting its potential as a sentinel species for monitoring pollutants in urban areas.

2. Materials and methods

2.1. Fieldwork procedures

Sampling was conducted during the breeding season of the yellow-legged gull in the years 2019 (GPS tracking) and 2020 (POPs and physiological analysis) in the city of Barcelona (northeastern Spain, Fig. 1A). The metropolitan area of Barcelona is one of the most densely populated cities in Spain (and Europe), where industry is the most important sector. Industry is mainly dedicated to chemical and pharmaceutical products, followed by automotive, food, paper and graphic arts, and waste treatment clusters [60]. All these activities are potential sources of POPs [61-63].

Barcelona is home to a population of around 200–300 yellow-legged gull breeding pairs [64], with an estimated 1686 ± 273 breeders and immature individuals [65]. In order to investigate the movements of yellow-legged gulls in this urban population, we fitted 15 immatures and 10 adults with GPS devices (Perthold Engineering LLC; 16 g of weight), attached by means of a conventional Teflon harness [66], which represented less than 3% of their body mass. The individuals were captured at the beginning of the 2019 breeding period in a fish-baited trap located in a metropolitan park in Barcelona and their position was recorded every 5 min, 24 h a day (Fig. 1B). To examine blood concentrations of POPs, physiological parameters and trophic markers (i.e. stable isotopes) we sampled 2 mL of blood from 20 immatures and 30 adult yellow-legged gulls captured during the breeding period of 2020 in the same baited trap used during the GPS instrumentation. Blood samples were collected from the brachial vein with insulin syringes and then immediately centrifuged after extraction (at 4ºC, 3000 rpm, 10 min); the plasma was stored at − 80 ºC until analysis. Foraging locations during 2019 were not obtained for the same individuals analyzed during 2020. Therefore, direct comparisons between POPs, physiology and spatial movements were not possible.

For all individuals sampled, we measured body mass (g), wing length (mm), tarsus length (mm), and bill length (mm) (Table 1). The sex of each individual was determined molecularly [67]. The age (immature and adult) of each yellow-legged gull was determined on the basis of their plumage characteristics [68].

2.2. POPs determination

An optimized sample extraction treatment method was used for the analysis of congeners (PCBs 28, 52, 101, 118, 138, 153, 180, and PBDEs 28, 47, 49, 99, 100, 138, 153, PBB-153, 154, 183). For the PCBs, the non-dioxin-like PCBs considered as indicators were chosen and PCB-118 was included for practical reasons. For the PBDEs, the congeners that have been considered of primary interest by the European Food Safety

Authority and the European Commission [74,75] were included in the study. All the standards and reagents employed, as well as the extraction procedure are detailed in Table S1, Table S2 and Table S3. All experiments were performed on a GC Trace 1310 coupled to a Q Exactive GC mass spectrometer (GC-Q-Orbitrap) (Thermo Fisher Scientific, Bremen, Germany) operated in electron ionization mode (EI). Please refer to Supplementary Table S1 for a full description of the method.

2.3. Physiological and trophic markers

A total of 27 physiological parameters in blood plasma were determined (Table 1), grouped as follows: B-esterases (cholinesterases–ChEs, carboxylesterases–CEs), oxidative stress (catalase–CAT, glutathione peroxidase–GPX, glutathione reductase–GR, superoxide dismutase–SOD, thiobarbituric acid reactive substances–TBARS, total antioxidant capacity–TAC), kidney and liver functioning (alanine aminotransferase–ALT, albumin–ALB, alkaline phosphatase–ALP, aspartate aminotransferase–ASTL, bilirubin–BILT, creatinine–CREJ, gamma-glutamyltransferase–GGT), energy and protein metabolism (cholesterol–CHOL, lactate dehydrogenase–LDH, total protein–TP, triglycerides–TRIGL, urea–UREA, uric acid–UA), health and aging (telomere length–TEL), and three trophic markers (stable isotopes of nitrogen–$\delta^{15}N$, carbon–$\delta^{13}C$, and sulfur–$\delta^{34}S$).

In brief, B-esterases were measured using the substrates 4-nitrophenyl butyrate (4NPB) and 1-naphthyl butyrate (1NB) for CEs, and acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) for ChEs. 4NPB-CE activity was measured according to Hosokawa and Satoh [76] and 1NB-CE activity according to Mastropaolo and Yourno [77]. AChE and BChE were measured following the Ellman et al. [78] protocol. All methods were adapted to microplate format and read in a spectrophotometer (Table S1). Oxidative stress, kidney and liver functioning and energy and protein metabolism parameters were determined in a Cobas integra 400 Automated Analyzer using the commercial enzymatic colorimetric methods (Roche Diagnostics S.L., San Cugat del Vallés, Barcelona, Spain) (Table S1). For the telomere length determination we followed the methodology of Badás et al. [79] (Table S1). SIA analyses were conducted at the Stable Isotopes Lab of the Estación Biológica de Doñana (EBD-CSIC; www.ebd.csic.es/lie/index.html). See Vez-Garzón et al. [59] and Table S1 for more information.

2.4. Statistical analysis

2.4.1. Movements of yellow-legged gulls

To characterize the habitat used by the GPS-tracked yellow-legged gulls, we estimated the percentage of use of each habitat type by each individual, as the number of positions in the habitat in relation to the total number of GPS fixes for each gull. Habitat type at each GPS position was determined using the land-cover layer Urban Atlas (European

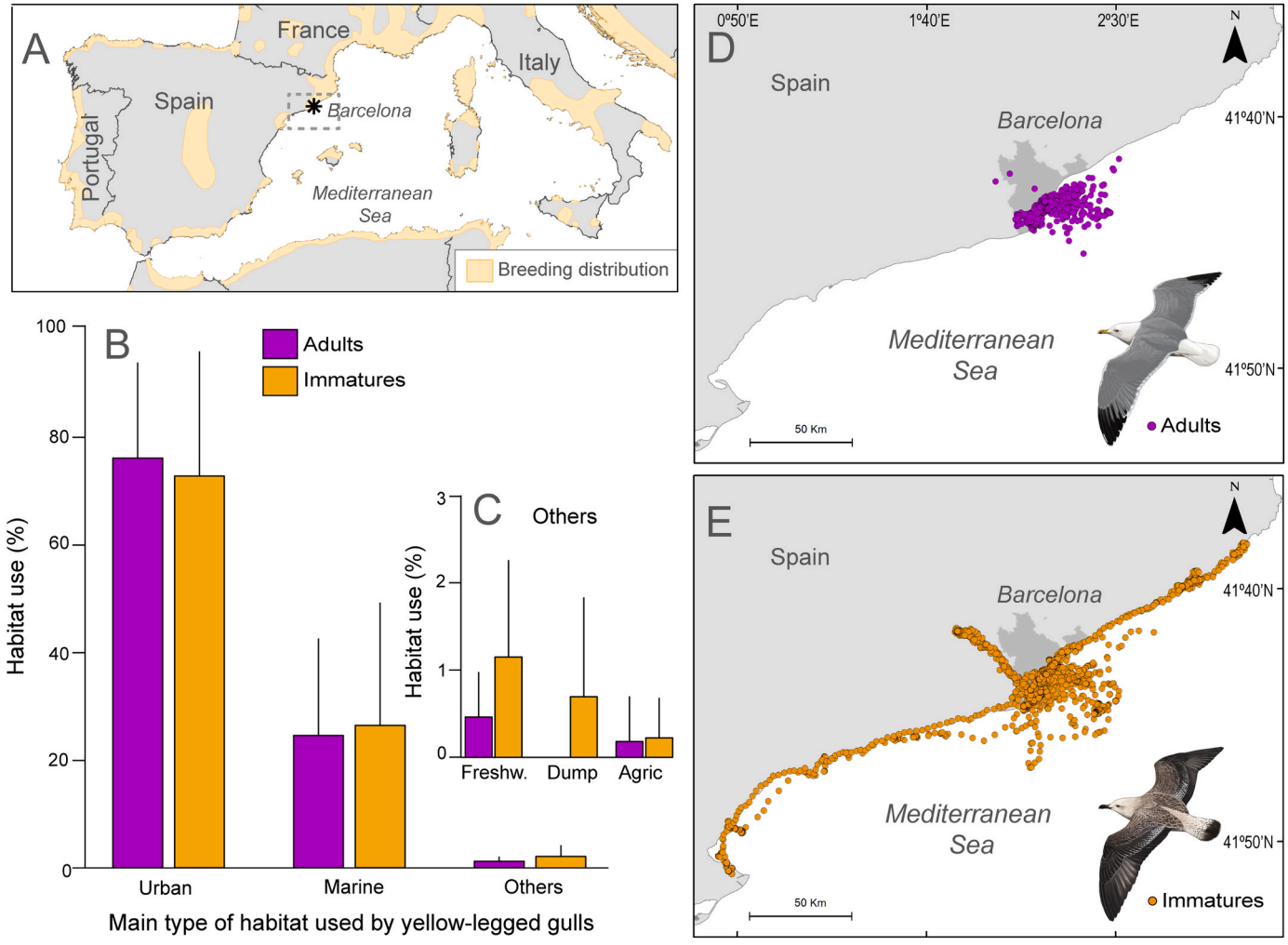

Fig. 1. Location of Barcelona city and breeding distribution of the yellow-legged gull within the western Mediterranean basin (from BirdLife International and NatureServe, http://www.birdlife.org) (A). Main habitat use (B), detailed information for Others (Freshw = freshwater, Dump = garbage and Agric = agriculture habitats) (C) and spatial distribution of 10 adult (D) and 15 immature (E) GPS-tracked yellow-legged gulls during the breeding period 2019 inhabiting the city of Barcelona (Spain). Gulls drawing by Martí Franch.

Environment Agency; Copernicus Land Monitoring Services 2016; < 5 m resolution), grouped into 5 categories: urban areas, marine domain (including fishing ports, open sea and beach habitats), freshwater, garbage dumps, and agriculture. Each of these habitats could represent a different exposure of POPs. To perform the spatial analysis, we used QGIS 3.30.2 (QGIS Development 2019). Non-parametric Kruskal-Wallis and U-Mann Whitney tests were used to compare the type of habitat used by immature and adult yellow-legged gulls.

2.4.2. POPs and physiological parameters

POP exposure was calculated as the sum of all the different congeners, ΣPCBs and ΣPBDEs, because a preliminary analysis found few differences between congeners in relation to gulls age and sex (Fig. 2 and Table S4). Spearman's correlations were performed to determine the correlation among the different biomarkers and physiological indicators with ΣPCB and ΣPBDE. Correlation matrices were built using the 'rcorr' package. Principal Component Analysis (PCA) was performed separately for the two age groups (immatures and adults), with the data being centered and scaled using the 'prcomp' package. Table S5 displays the eigenvectors of each variable for every component. Generalized Linear Models (GLMs) were conducted using a Gamma distribution with a *log* link function to assess the effects of age and sex and the two-way interaction on each of the different physiological parameters (Table S6). Similarly, GLMs were built to assess the relationship between the physiological parameters and stable isotopes on ΣPCB and ΣPBDE concentrations. GLMs were conducted separately for each group of parameters as classified in Table 1 according to bibliography. The results of the model deviance analysis are presented in Table S7 (ΣPCB) and Table S8 (ΣPBDE). Statistical analyses and plots were performed and generated using R software (version 4.2.2).

3. Results

3.1. Spatial movements and habitat use

The spatial movements of yellow-legged gulls throughout the city of Barcelona and its surroundings differed between immatures and adults (Fig. 1). Adult yellow-legged gulls showed lower displacement from the city of Barcelona (Fig. 1D). In contrast, immature individuals showed wider explorative movements, with wider movement within the city, reaching distant habitats, as well as covering a larger marine area up to 20 km from the coast (Fig. 1E). We found differences in the use of the main type of habitats (urban, marine and others) between adults (Kruskal-Wallis Test, $H = 25.6$, $p < 0.001$, $n = 10$) and immatures ($H = 30.2$, $p < 0.001$, $n = 15$). Specifically, urban habitat was the main foraging habitat used by both adult (mean and standard deviation = 75.1 ± 17.5%) and immature (71.81 ± 22.85%) yellow-legged gulls, followed by the marine environment (adults = 24.3 ± 17.8; immatures

Table 1
Mean and standard deviation of body mass, morphological parameters, biomarkers and physiological parameters of yellow-legged gulls captured at the city of Barcelona according to age (immature and adult) and sex (male and female). The full name, abbreviation, units and number of samples are also reported. Letters in superscript correspond to the references according to which the parameters were grouped.

Parameter	Abbreviation - units	Immature Males		Immature Females		Adult Males		Adult Females	
		n	Mean ± SD	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD
Morphology									
Body weight	g	10	1080 ± 61.1	10	897 ± 94.3	19	1091 ± 47.7	11	898 ± 58.1
Wing length	mm	10	460 ± 12.8	10	437 ± 8.82	19	468 ± 9.36	11	438 ± 13.7
Tarsus length	mm	7	73.0 ± 2.56	9	68.7 ± 3.27	12	70.7 ± 2.87	6	68.9 ± 3.61
Bill length	mm	7	58.4 ± 2.17	9	53.3 ± 3.51	12	59.4 ± 3.05	6	51.2 ± 3.18
B-Esterases[a]									
Acetylcholinesterase	AChE - nmol mL^{-1}	10	1222 ± 195	10	1160 ± 139	19	1178 ± 197	11	1048 ± 192
Buthyrylcholinesterase	BChE - nmol mL^{-1}	10	1429 ± 213	10	1383 ± 201	19	1401 ± 292	11	1223 ± 248
Carboxylesterase	4NPB-CE nmol mL^{-1}	10	257 ± 61.7	10	218 ± 46.2	19	301 ± 119	11	238 ± 55.4
Carboxylesterase	1NB-CE nmol mL^{-1}	10	919 ± 109	10	839 ± 66.1	19	969 ± 175	11	850 ± 123
Oxidative stress[b]									
Catalase	CAT - U mg $prot^{-1}$	6	536 ± 138	8	493 ± 145	18	434 ± 135	8	517 ± 161
Glutathione peroxidase	GPx - mU mg $prot^{-1}$	7	594 ± 155	8	504 ± 85.7	19	507 ± 73.3	11	480 ± 92.1
Glutathione reductase	GR - mU mg $prot^{-1}$	7	6.97 ± 1.76	8	7.02 ± 2.13	19	7.05 ± 3.07	11	8.01 ± 4.13
Superoxid dismutase	SOD - U mg $prot^{-1}$	7	2.26 ± 0.54	8	2.17 ± 0.32	19	2.15 ± 0.64	11	2.40 ± 0.51
Thiobarbituric acid-reactive subs.	TBARS - nmol MDA mL^{-1}	7	9.87 ± 1.09	8	10.2 ± 1.28	19	10.2 ± 1.18	11	10.1 ± 0.52
Total Antioxidant Capacity	TAC - μM L^{-1}	9	978 ± 361	9	937 ± 224	18	1384 ± 646	10	1305 ± 766
Kidney and liver functioning[b]									
Alanine aminotransferase	ALT - U L^{-1}	10	52.3 ± 21.0	10	52.3 ± 28.9	19	63.9 ± 21.4	11	57.6 ± 23.3
Albumin	ALB - g dL^{-1}	10	1.40 ± 0.21	10	1.44 ± 0.15	19	1.49 ± 0.18	11	1.47 ± 0.11
Alkaline phosphatase	ALP - U L^{-1}	10	121 ± 50.3	10	102 ± 38.6	19	122 ± 79.4	11	79.8 ± 47.4
Aspartate aminotransferase	ASTL - U L^{-1}	10	246 ± 141	10	274 ± 208	19	331 ± 122	11	258 ± 63.0
Bilirubin	BILT - μmol L^{-1}	10	0.16 ± 0.05	9	0.59 ± 0.32	18	0.48 ± 0.18	9	0.53 ± 0.26
Creatinine	CREJ - mg dL^{-1}	10	0.24 ± 0.07	10	0.21 ± 0.06	19	0.20 ± 0.05	10	0.21 ± 0.03
Gamma-Glutamyltransferase	GGT - U L^{-1}	10	5.77 ± 1.44	10	5.45 ± 3.08	19	5.21 ± 2.85	11	5.18 ± 2.97
Energy and protein metabolism[b,c,d]									
Cholesterol	CHOL - mg dL^{-1}	10	270 ± 43.3	10	255 ± 38.9	19	345 ± 47.0	11	311 ± 36.0
Lactate dehydrogenase	LDH - U L^{-1}	10	261 ± 146	10	269 ± 205	19	194 ± 68.7	11	192 ± 61.3
Total Protein	TP - mg mL^{-1}	10	24.2 ± 2.06	10	24.8 ± 0.67	19	25.2 ± 1.43	11	25.0 ± 1.05
Triglycerides	TRIGL - mg dL^{-1}	10	149 ± 74.9	10	131 ± 92.2	19	98.7 ± 34.7	11	98.3 ± 21.5
Urea	UREA - mg dL^{-1}	10	7.66 ± 3.05	10	5.40 ± 2.01	19	10.0 ± 4.88	11	9.36 ± 3.66
Uric acid	UA - mg dL^{-1}	10	9.98 ± 4.34	10	9.35 ± 3.32	19	12.4 ± 6.35	11	14.0 ± 9.31
Health and aeging[e]									
Telomere length	TEL - NRQ Total	7	2.06 ± 0.74	8	1.46 ± 0.89	19	1.66 ± 0.99	11	1.72 ± 1.11
Trophic markers[f]									
$\delta^{13}C$	$\delta^{13}C$ - ‰	10	50.8 ± 0.92	10	50.7 ± 0.35	19	50.3 ± 2.83	11	50.3 ± 2.22
$\delta^{15}N$	$\delta^{15}N$ - ‰	10	15.4 ± 0.39	10	15.4 ± 0.10	19	14.8 ± 0.97	11	14.4 ± 1.24
$\delta^{34}S$	$\delta^{34}S$ - ‰	10	16.0 ± 1.64	10	17.2 ± 1.06	19	14.4 ± 3.06	11	15.2 ± 1.45

[a] [69], [b] [70], [c] [71], [d] [72], [e] [73], [f] [24]

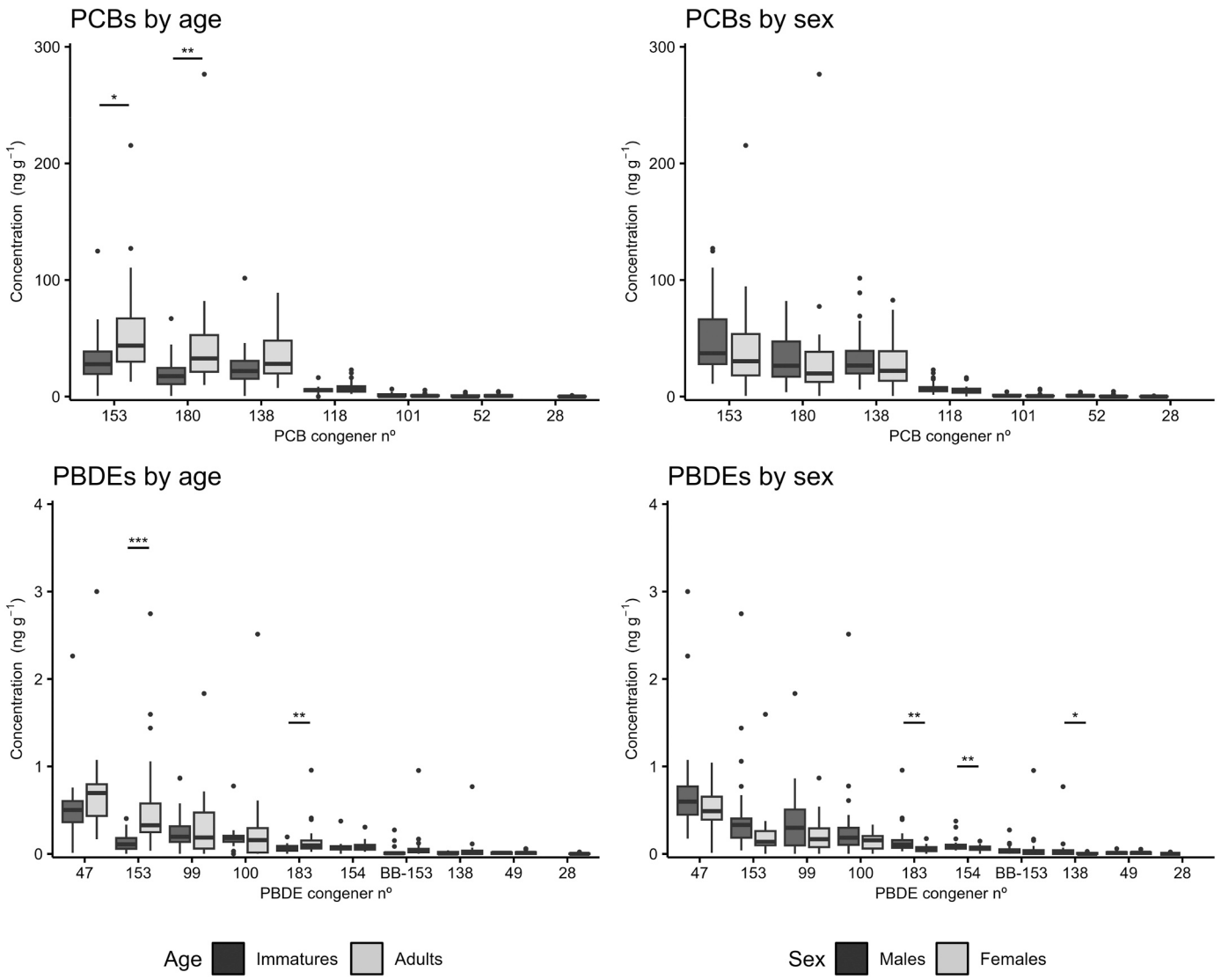

Fig. 2. Concentration in ng g^{-1} (wet weight) of PCBs (top panels) and PBDEs (bottom panels) congeners of yellow-legged gull inhabiting the city of Barcelona by age (immatures and adults) and sex (males and females). Based on the statistical analysis, the p-values is expressed as: * $p < 0.05$–0.01, * * $p < 0.01$–0.001, * ** $p < 0.001$.

= 26.1 ± 22.5), and habitats that included freshwater (adults = 0.46 ± 0.56; immatures = 1.14 ± 1.11), garbage dumps (adults = 0; immatures = 0.64 ± 1.14) and agricultural areas (adults = 0.18 ± 0.51; immatures = 0.22 ± 0.45). Between ages, we did not find differences in the use of urban (U-Mann-Whitney test; U = 75, $p = 0.99$) and marine environments (U = 72, $p = 0.89$) (Fig. 1B-C), but differences were found between immatures and adults in the use of other habitats (U = 38; $p = 0.03$), including freshwater, garbage dumps and agricultural areas (Fig. 1B-C).

3.2. POPs

Yellow-legged gulls exhibited a median ΣPCB concentration of 92.7 ng g^{-1} ww (wet weight), ranging from 1.86 to 591.56, which was much higher than the median ΣPBDE concentration of 1.44 ng g^{-1} ww, ranging from 0.022 to 9.58 (Fig. 3). The ΣPCBs and ΣPBDEs concentrations did not show significant variations based on age and sex, primarily due to data dispersion (Fig. 3).

3.3. Physiological parameters

PCA analysis including physiological plasmatic parameters, trophic markers and pollutant concentrations was conducted separately for the two age groups (immatures and adults), as the data presented a high degree of overlap when grouped (Fig. S1). The deviation explained by the first two components of the PCA was 39.1% for immatures and 36.0% for adults (Fig. 4A and B, respectively). Despite the high degree of overlap between males and females in both age groups, immature individuals (Fig. 4A, eigenvalues in Table S5) showed some differences with higher dispersion of males in PC1 and females in PC2. PC1 showed a positive association between B-esterases and the oxidative stress biomarkers SOD, GR and TAC, which was opposite to that of the kidney and liver functioning parameters ALT, ASTL and LDH (Fig. 4A). Significant correlations were observed between 4NPB-CE and TAC (Spearman's correlation coefficient: $\rho = 0.71$, $p = 0.030$, $n = 18$) and ALB ($\rho = 0.68$–0.73, $p = 0.028$–0.016, $n = 20$), the negative association of B-esterases with kidney and liver parameters did not reach statistical significance. PC1 also showed a negative association of B-esterases and oxidative stress with POPs, which was stronger in immature males (Fig. 4A) with significant and negative correlations in the case of CEs

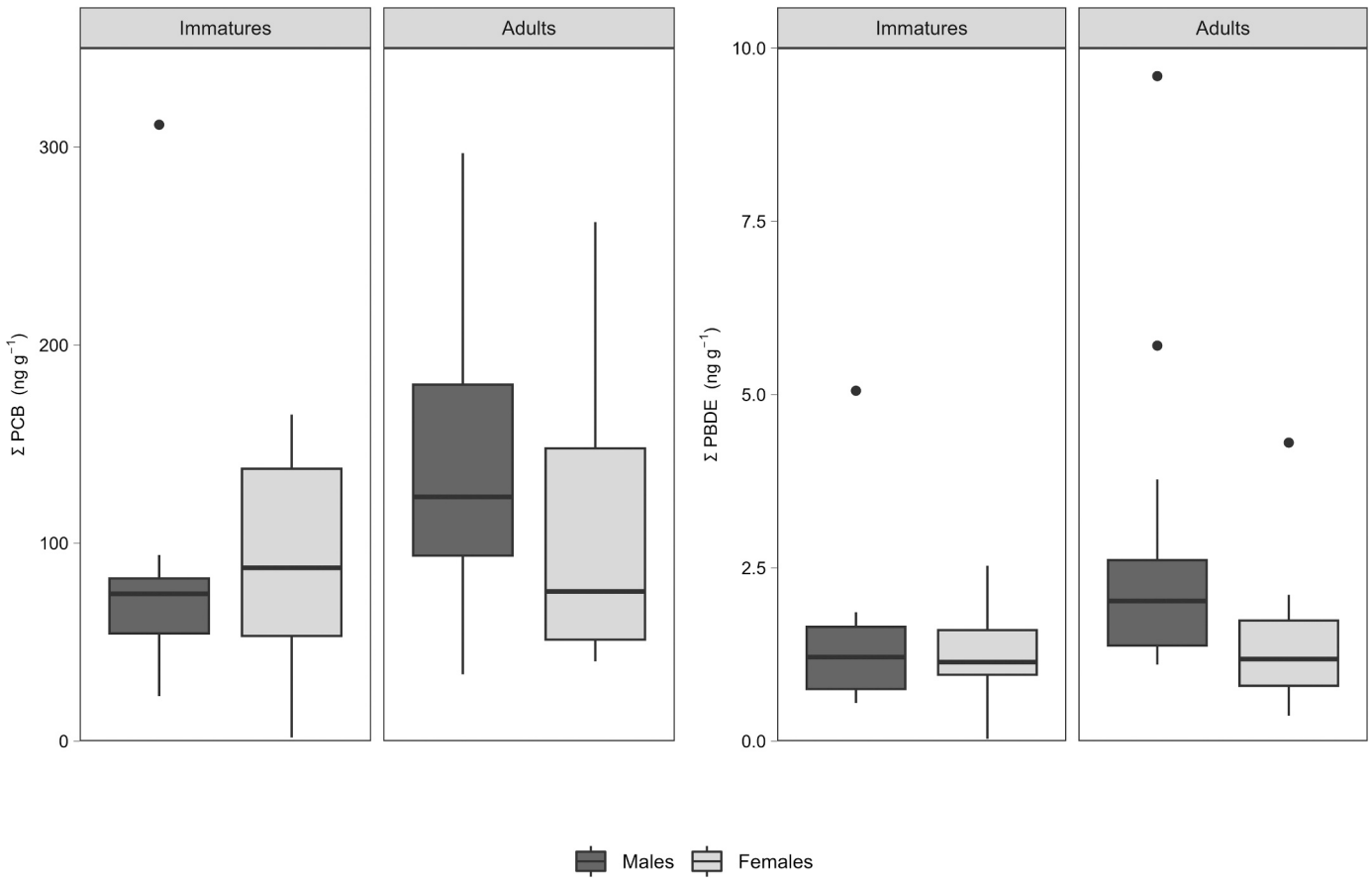

Fig. 3. Box plot of the total concentrations (ng g^{-1}) of POPs corresponding to the sum of all the PCBs (ΣPCB) and PBDEs (ΣPBDE) congeners of yellow-legged gulls inhabiting the city of Barcelona by age (immatures, adults) and sex.

(ρ = −0.66 to −0.82, p = 0.038–0.004, n = 20). In contrast, the main parameters contributing to this separation in PC2 were the energy and protein metabolism parameters CHOL, TRIGL, UREA and UA, the trophic markers $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$, along with BILT, ALB, CREJ and ALP. The correlation analysis did not show relevant interaction among these groups of parameters. In adult individuals (Fig. 4B), the oxidative stress biomarkers were positively associated with energy and protein metabolism parameters, except for TBARS and LDH, which were related to B-esterases. In this regard, only significant Spearman correlations were found between CAT and UREA (ρ = 0.60, p = 0.008, n = 26), GPx (ρ = 0.66, p = 0.002, n = 26) and UA (ρ = 0.79, p < 0.001, n = 26). In adults, the concentrations of POPs showed an opposite relationship with oxidative stress and energy and protein metabolism, but not with B-esterases (Fig. 4B). When analyzing correlations, this association was only observed between GPx and PCBs in males (ρ = −0.50, p = 0.03, n = 19).

3.4. Differences in blood biomarkers by age and sex

Regarding B-esterases, both 4NPB-CE ($F_{1,49}$ = 5.46, p = 0.02) and 1NB-CE ($F_{1,49}$ = 7.22, p = 0.024) activities showed significantly higher values in males than females (Fig. 5; Table S6). Among all oxidative stress parameters, only TAC showed significant variation with age ($F_{1,45}$ = 6.46, p = 0.015), presenting higher levels in adult individuals. BILT (kidney and liver functioning) values showed a significant interaction between age and sex ($F_{1,45}$ = 20.3, p<0.001), and a significant difference between males and females was found in immatures, with higher values in females ($F_{1,45}$ = 22.5, p < 0.001) (Fig. 5). Among the energy and protein metabolism biomarkers, CHOL ($F_{1,49}$ = 33.4, p < 0.001) and UREA ($F_{1,49}$ = 10.2, p= 0.003) showed higher values in adults than immatures, whereas LDH ($F_{1,45}$ = 4.94, p= 0.03) and TRIGL ($F_{1,49}$ = 7.40, p= 0.009) showed the opposite trend (Fig. 5). The values of $\delta^{13}C$ ($F_{1,49}$ = 5.01, p=0.03) and $\delta^{34}S$ ($F_{1,49}$ = 7.95, p= 0.007) were higher in immatures than in adults (Fig. 5).

3.5. Physiological response to POPs exposure

The correlations between the levels of some PCB and PBDE individual congeners and the physiological parameters revealed a stronger correlation with CE activities in immature males (supplementary Fig. S2 and Fig. S3 for immatures and adults, respectively). Higher negative correlations were observed with PCBs 118, 138, and 153, and PBDEs 100, 154, 47 and 99 (ρ = −075 to −0.92, p < 0.001–0.048) (Fig. S2A). Also, the correlations of the different congeners between age groups and sex was not consistent. On this basis, to integrate all the data and considering the fact that the measured concentrations of POPs did not present age- or sex-related differences, the same analysis was performed using the total values of PCBs and PBDEs. Considering ΣPCB and ΣPBDE values, the strongest and most significant correlations between the analyzed physiological parameters and POPs (Fig. 6, Fig. S4) were also found in immature males, where B-esterases showed a negative correlation with POPs. Specifically, 4NPB-CE was negatively correlated with ΣPCB (ρ = −0.66, p = 0.03), and ΣPBDE correlated with 4NPB-CE (ρ = −0.82, p = 0.004) and 1NB-CE (ρ = −0.81, p = 0.005) (Fig. 6). According to the GLMs (Table S7 and Table S8), ΣPCB values were related to 4NPB-CE activity and this effect was dependent on age ($F_{1,49}$ = 4.45, p = 0.04) and sex ($F_{1,49}$ = 6.64, p = 0.01), suggesting a higher response in immatures and males, respectively. ΣPBDE concentration was related to the interaction of NPB-CE and sex ($F_{1,49}$ = 5.37, p= 0.02), which was significant only for males.

GPx (oxidative stress) was negatively correlated with ΣPCB

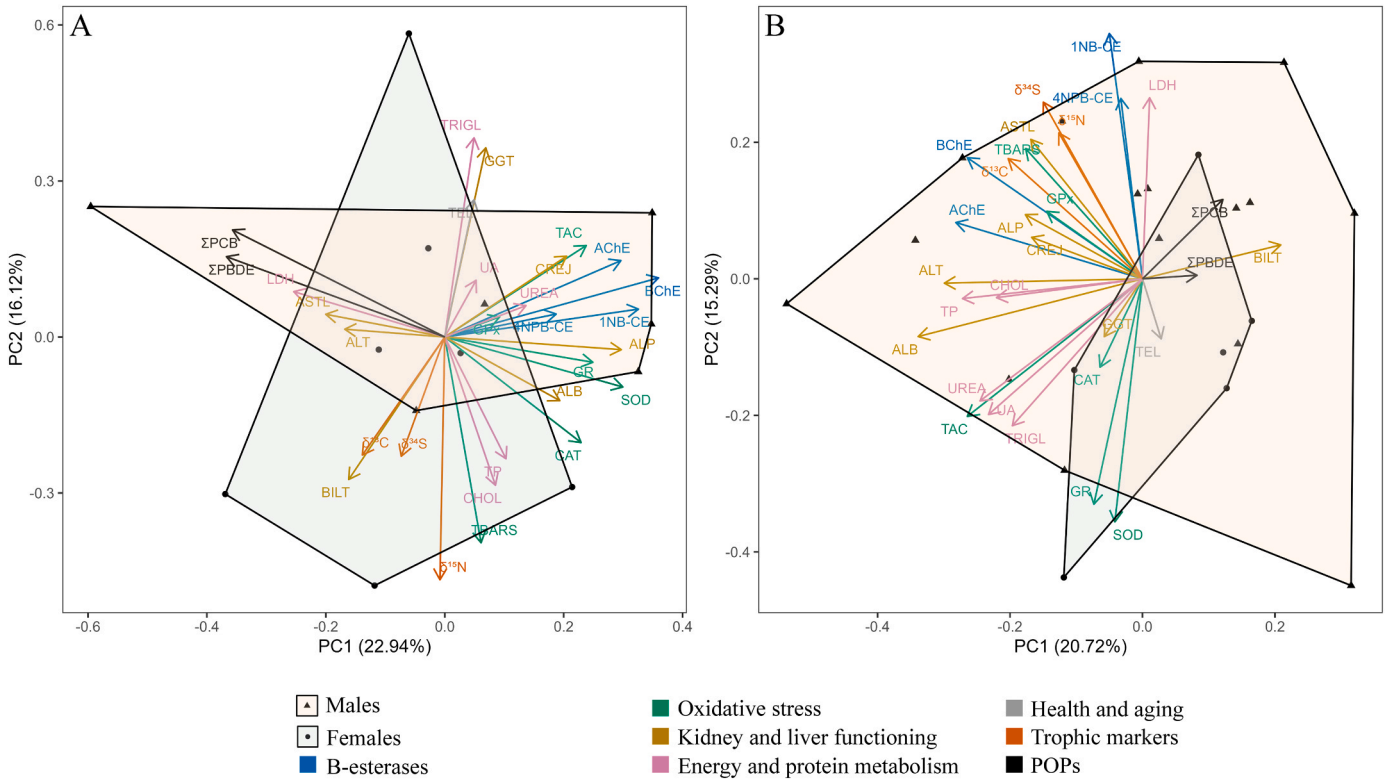

Fig. 4. Principal component analysis including biomarkers and physiological parameters of A) immature and B) adult yellow-legged gulls inhabiting the city of Barcelona. The shadowed polygons integrate the male and female individuals. The color of the arrows indicates the aggrupation of the parameters. Arrow length is proportional contribution of a particular variable to a principal component.

($\rho = -0.50$, $p = 0.03$) in adult males (Fig. 6). The GLM results also showed a significant interaction of GPx with sex ($F_{1,44} = 10.2$, $p = 0.003$), because the relationship was significant only for males. The ΣPBDE values were significantly influenced by TAC ($F_{1,38} = 6.86$, $p = 0.01$) and an age-dependent effect of CAT ($F_{1,38} = 4.25$, $p = 0.04$).

The liver and kidney parameters, indicative of their function, did not show a significant correlation with POPs values. However, according to the GLM analysis, the interaction between ALT and sex ($F_{1,45} = 6.05$, $p = 0.01$) indicated a significant response to PCBs only in males. The interaction of ALB and age ($F_{1,45} = 4.29$, $p = 0.02$) was significantly related to ΣPCB only in adults. Additionally, ALT also showed a significant effect on ΣPBDE ($F_{1,45} = 8.01$, $p = 0.007$) and also the interaction of ALT with sex ($F_{1,45} = 4.90$, $p = 0.03$), due to a positive and significant effect only in males. Also, BILT was positively related to ΣPBDE concentrations ($F_{1,38} = 6.86$, $p = 0.01$).

Within energy and protein metabolism variables, ΣPCB was related to CHOL ($F_{1,49} = 7.22$, $p = 0.011$) and total protein (TP) ($F_{1,49} = 5.75$, $p = 0.022$). In the case of TP the interaction with sex was positive and only significant in males ($F_{1,49} = 8.12$, $p = 0.007$). The interactions of UREA with age ($F_{1,49} = 4.18$, $p = 0.048$) and sex ($F_{1,49} = 6.51$, $p = 0.015$) were positive and significant for immatures and males, respectively. ΣPBDE concentration was related to CHOL ($F_{1,49} = 8.89$, $p = 0.005$) and to the interaction of UA and sex ($F_{1,49} = 5.77$, $p = 0.006$), because the effect of UA on ΣPBDE is weaker in males than in females.

The GLM for trophic markers revealed age as a significant factor, both for ΣPCB ($F_{1,49} = 7.44$, $p = 0.009$) and ΣPBDE ($F_{1,49} = 5.64$, $p = 0.02$). The interaction of $\delta^{15}N$ and sex had a significant negative effect on ΣPCBs in males ($F_{1,49} = 8.03$, $p = 0.007$). According to the GLM for trophic markers, ΣPBDEs were also significantly related to age, with higher levels expected in immature individuals ($F_{1,49} = 5.45$, $p = 0.025$).

4. Discussion

In this study, the concentrations of PCBs and PBDEs and 27 biochemical parameters were analyzed in the blood plasma of the yellow-legged gull, a species known for its ability to live in urban and highly humanized environments. GPS tracking information served to qualitatively identify potential habitats that could be sources of POPs within the urban - marine ecosystem of Barcelona. Furthermore, we investigated the physiological consequences of POPs exposure and identified reliable biomarkers of pollution within the parameters considered. Our findings revealed that immature yellow-legged gull males presented a higher relationship of their physiological parameters with POPs load, with the most significant correlations with plasmatic CEs, which suggests the potential use of immatures as bioindicators and CEs as biomarkers of anthropogenic pollution in urban environments and their adjacent marine areas. On the other hand, the physiological response to pollutants is not so clear in immature females and adults probably because other ontogenetic and sex-related processes are masking the effects of pollution. This approach is useful for the evaluation of exposure and associated health risks that POPs may pose to gulls, other nearby wildlife and humans.

Predatory birds, including the yellow-legged gull, frequently exhibit high levels of PCBs, PBDEs, and other POPs [80,81]. Most studies with yellow-legged gulls have mainly focused on eggs [82-84] and less frequently on other tissues [85], including blood [86]. A comparable study in the Mediterranean region (Turkey, eastern Mediterranean Sea) found lower levels of ΣPCBs (same congeners as in our study) than those observed in our study (mean ± standard deviation, 8.0 ± 4.0 ng g^{-1}). The reported ΣPBDEs (only PBDE 17, 47, 66 and 100) concentration (1.6 ± 0.4 ng g^{-1}) was also similar to our values [86]. Other studies have found higher environmental and bioaccumulated concentrations of PCBs in the western Mediterranean Sea, and particularly in Spain, compared to the eastern Mediterranean region [82,87]. These

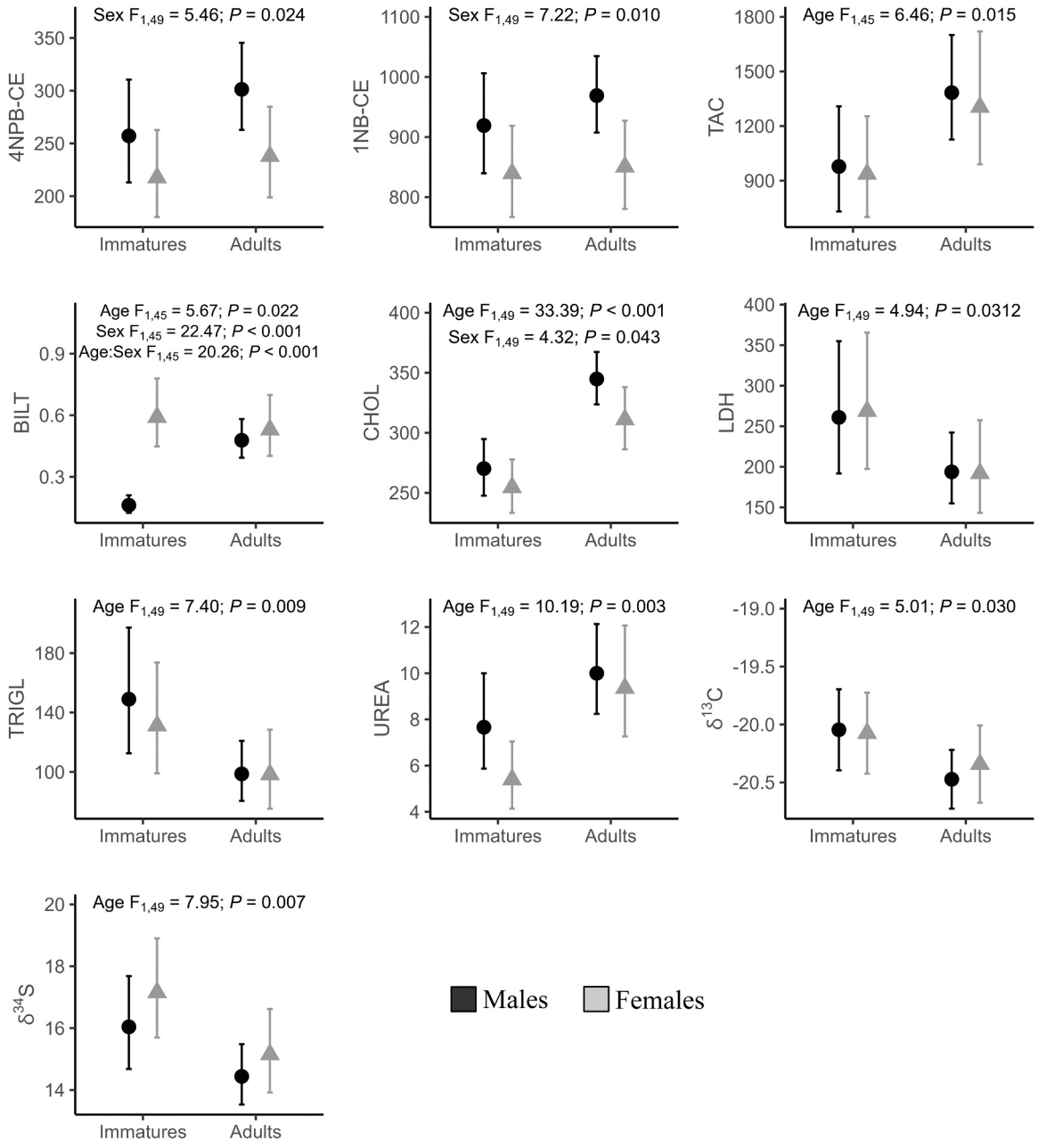

Fig. 5. Mean and standard deviation for physiological parameters of yellow-legged gulls inhabiting the city of Barcelona that presented significant difference according to age (immatures and adults) and/or sex (male and female). See supplementary Table S6 for more information.

differences surely reflect different environments and background pollution levels, in which bird foraging and nesting areas play a key role in the magnitude of exposure to POPs [88]. Accordingly, some studies have revealed higher PCBs levels (of industrial origin) in the eggs of individuals inhabiting coastal areas and consuming fish, while migratory birds contained higher levels of organochlorine pesticides associated with agricultural activities [42]. These elevated values of POPs found in blood are consistent with the historically high levels of environmental chemicals present in our sampling region [89].

Immature yellow legged gulls exhibited a higher physiological response to POPs that could also reflect their habitat preference, potentially spending more time than adults foraging in marine areas and landfills, both habitats being important sources of POPs [49]. As a result, GPS data indicated the presence of yellow-legged gulls near primary sources of POPs. These findings emphasize the potential of immature males of this species as effective indicators for monitoring POPs in urban and coastal regions. However, it is important to state that in our particular case, foraging movements were not concurrently studied with the assessment of POPs and physiological parameters. This limitation arose from the fact that the GPS tracks did not correspond to the same individuals utilized for the other measurements. Consequently, this constraint hindered our ability to establish a quantitative connection between individual behavior and pollution levels. In addition to spatial variations in pollutants exposure, sex is an important factor to take into account, as mature females have the ability to remove POPs from their body through laying eggs [90-92]. Although statistical significance was not reached, this tendency was also observed in our individuals, with lower POPs concentrations in adult females than in adult males, which was not observed in immatures.

The bioaccumulation of POPs in yellow-legged gulls may impact on several physiological processes, whose ultimate effects on the species' fitness are still poorly understood [93]. To effectively detect and

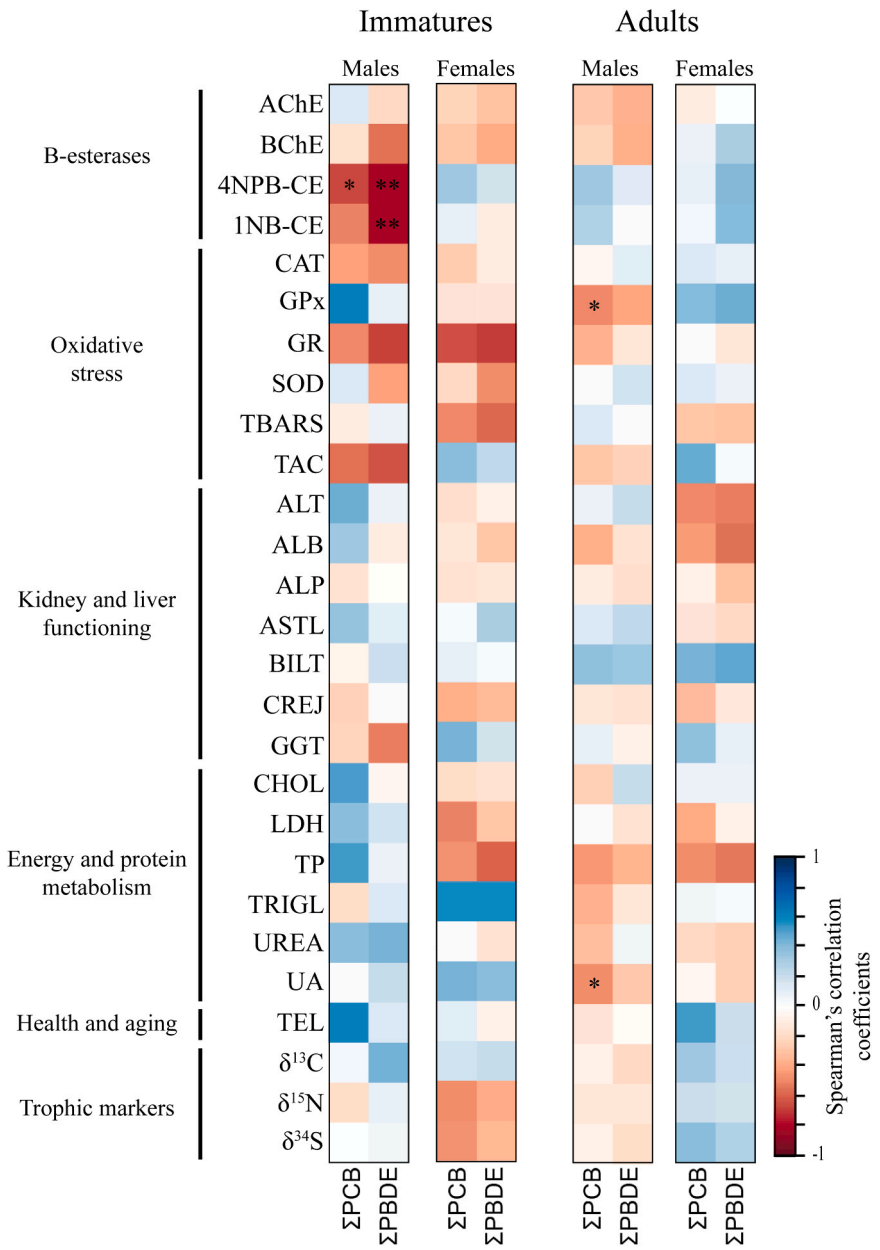

Fig. 6. Correlation between blood biochemistry and POPs of yellow-legged gulls inhabiting the city of Barcelona by sex and age. The color scale corresponds to the Spearman correlation coefficients and the p-value is expressed as: * $p < 0.05$–0.01, * * $p < 0.01$–0.001, * ** $p < 0.001$.

understand the effects of pollution in wildlife, long-term monitoring strategies are essential. A critical component of such strategies is the identification of reliable biomarkers that can assess the health risks associated with environmental contamination [94], embracing well-known legacy pollutants and those of emerging environmental concern [81]. The biomarkers measured in this study were analyzed in blood, which yielded reliable and consistent results for most of the parameters. This non-lethal matrix, is highly informative in vertebrates as it provides insights into an organism's general functioning within a shorter time frame compared to other tissues [95]. Also, blood is more adequate for long-term pollution monitoring when employing a multi-biomarker approach [96], being cost effective and requiring small sample volumes [97-100].

Among the biochemical parameters selected in this study, plasmatic CEs proved to be the enzymatic activities more related to POPs levels in plasma, with strong and significant negative correlations mostly in immature males (4NPB-CE: ΣPCB $\rho = -0.66$; 1NB-CE: $\rho = -0.81$, ΣPBDE $\rho = -0.82$). CEs are metabolic enzymes that possess high scavenging capacity and substrate promiscuity [101], which allows them to bind and hydrolyze a wide range of pollutants, facilitating their elimination from the organism, including the metabolic hydroxylated forms of POPs (i.e. PCB 101) [102,103]. In this context, it has also been shown that CEs can play a major role as scavengers, protecting AChE from inhibition and therefore mitigating potential neurotoxic effects [104,85, 105,106]. Additionally, the sex- and age-dependent differences observed in the response of CEs to bioaccumulated ΣPCB and ΣPBDE, can be explained by the overexpression of CEs in males of most taxonomic groups, which is believed to take part in the sperm protection, maturation and use, maximizing its fertilizing potential [107] and other reproduction-related processes in adult individuals [108-111]. This highlights the relevance of focusing on immature individuals, and particularly immature males, in monitoring programs to avoid the influence of ontogenetic and sex derived traits on biomarker activities.

Pollution, mainly during particular physiological processes, can

contribute to the enhancement of reactive oxygen species (ROS) which, if not counter balanced, could lead to oxidative damage [112,113]. It has already been established in birds that the oxidative stress levels caused by ROS are influenced by the different stages of the species' life cycle and sex [34,114]. Reproduction was one of the main drivers of oxidative stress in thick-billed murres (*Uria lomvia*), especially in females during reproduction, which translated into reduced SOD, GPx, and CAT activities [115]. Hence, the selection of immature males would avoid these confounding factors. A variable influence of the biological traits was observed in the six oxidative stress parameters analysed in the present study with no consistent correlation with the measured chemical burden. Other studies showed significant differences in antioxidant enzymes responses when analyzing liver or feather pollutant levels, but no clear response was observed between these enzymes and PCB and PBDE load measured in blood [86,112]. In addition, pollutants can indirectly affect some biochemical parameters as a result of disease and immunological status [26]. Oxidative stress has been clearly associated to the immune response, being key in the protection against immunopathological disorders [116,117]. These indirect effects of pollution-derived chemicals through immune system modulation could also affect not only the oxidative stress response but also other parameters such as the abovementioned B-esterases. In fact, the genetic expression of CEs can be suppressed by the presence of inflammatory cytokines, which can impact their hydrolytic activity and the associated detoxification processes [37,118,119]. Moreover, AChE and its physiological substrate, acetylcholine (ACh), play important roles in the regulation of immune-mediated inflammation in humans and fish [120,121]. Consequently, the close relationship between oxidative stress and B-esterases regulation and, in turn, with the immune system can also provide additional valuable information on the indirect health consequences deriving from pollution in gulls.

Changes in blood biochemistry are commonly used to assess the health status of wild animals [122,123]. In the present study, a significant positive link between ΣPCB and ΣPBDE levels in plasma with ALT, a kidney and liver mal-functioning indicator, was observed with a varying impact depending on the sex of the gulls. Previous research on American kestrels (*Falco sparverius*) exposed to PCB 126, also reported an increase in ALT activity [124]. Furthermore, higher levels of ALT, ALP, and AST were observed in our study compared to Garcia et al. [122], which measured the same parameters in yellow-legged gulls from the Chafarinas Islands (southwestern Mediterranean Sea). These results suggest that our individuals could present some liver and kidney distress, what is not surprising given that our study was conducted in a more populated and industrialized area than the more pristine Chafarinas Islands. Bilirubin content is also associated with higher levels of ΣPBDEs in gulls, as also observed in other bird studies [39,125], and it was proposed as a proxy of hepatic lesions in raptor species [40]. The parameters related to energy and protein metabolism showed a great variability with age in the present study. Cholesterol levels were higher in adult individuals, which was contrary to expectations considering the normal reduction caused by the investment of energy stores in the maturation process and during reproduction [122,123]. High levels of plasma cholesterol pose a risk of developing arterial disease and atherosclerosis for adult birds [126,127] and seem to be associated with the ingestion of low-quality food from anthropogenic sources. The content of UA, the main nitrogenous waste by-product excreted in birds, was also associated to ΣPBDEs concentrations. This parameter can increase due to dehydration or during fasting, and abnormally high levels are indicative of renal disease [128]. In this study, UA content was higher than in previous studies on yellow-legged gulls [97,122] but lower than in Orlog's gulls [31], which suggests species-dependence background levels. Urea, which was related to ΣPCB content and is highly sensitive to dehydration is used as a pre-renal disease indicator [129].

The trophic marker $\delta^{15}N$ was only associated with ΣPCB load. Previous studies have reported increased $\delta^{13}C$ and $\delta^{15}N$ levels in seabird eggs sampled in those areas with higher human population density, which at the same time have increased concentrations of PCBs and PBDEs, in comparison to rural areas [130,131]. The fact that our results are not so clear may be due to several factors, including the fact that stable isotopes in blood can only inform on diet and foraging areas up to four weeks prior to sampling. Also, despite the use of different habitats by the yellow-legged gulls in Barcelona, in comparison to Morrissey et al. [131], our study area was more geographically restricted and thus, isotopic differences might not be so marked. Despite the limitations and the fact that a direct link between stable isotopes and POPs content with GPS data was not possible, the clear relationship between $\delta^{15}N$ and ΣPCBs, together with the recognized habitat and prey plasticity of the species, reinforces the hypothesis that the diet of the individuals likely dictated their contaminant's burden. Other studies conducted in liver as the main metabolic tissue and isotope analysis, which entails the sacrifice of individuals, already confirmed a sound $\delta^{15}N$-ΣPCBs correlation in other bird species [11].

Taken together, all the above-mentioned results point to immature yellow-legged gulls as appropriate candidates to be sentinel bird species in urban areas, and highlight the relevance of using multiple biomarkers and particularly plasmatic B-esterases as general stress markers to forecast the consequences of anthropogenic pollution on gulls.

5. Conclusions

This study advanced and contributed to the understanding of the adverse health effects of POPs on wild bird populations [132]. The results evidenced extensive PCBs but also PBDEs exposure in yellow-legged gulls inhabiting Barcelona and support the adoption of a multi-biomarker approach for monitoring its effects. The simultaneous use of biomarkers capable of detecting exposure to complex mixtures of chemicals (e.g., CEs), along with complementary biochemical parameters that assess health status, is highly valuable for evaluating the direct and indirect consequences of chemical pollution exposures on wildlife. The comprehensive dataset used, combined with trophic markers and spatial information on the local distribution and foraging areas of the species (GPS-tracked individuals), also offers qualitative information on the identification of pollution sources, an aspect that should be further explored and fine-tuned in order to develop effective management strategies. The findings of this study emphasize the use of the yellow-legged gull, particularly immature males, as sentinels of chemical pollution, specifically PCBs and PBDEs, in urban areas and their surrounding marine environments. This is highly relevant within the One Health framework [133], which aims to enhance human, animal, and environmental health by considering the connectivity of these systems.

Environmental Implications

This manuscript addresses the study of environmentally relevant pollutants, specifically polychlorinated biphenyls (PCBs, 7 congeners) and polybrominated diphenyl ethers (PBDEs, 10 congeners), in blood and their impact on gulls' physiology. These pollutants are well-known for their persistence in the environment, posing a significant threat to long-lived predators due to their capacity to bioaccumulate. Furthermore, this study is also relevant as it was conducted in a densely populated urban area, where both humans and wildlife co-exist. Therefore, in accordance with the One Health approach, gulls could serve as sentinel species for assessing pollutant exposure in cities.

CRediT authorship contribution statement

David Nos: Conceptualization, Investigation, Formal analysis, Writing – original draft. **Tomas Montalvo**: Resources, Investigation, Writing – review & editing. **Núria Cortés**: Investigation, Resources. **Jordi Figuerola**: Resources, Investigation, Writing – review & editing. **Raül Aymí**: Resources, Investigation. **Joan Giménez**: Investigation,

Writing – review & editing. **Montserrat Solé:** Conceptualization, Supervision, Investigation, Resources, Writing – review & editing. **Joan Navarro:** Conceptualization, Supervision, Investigation, Validation, Funding acquisition, Resources, Writing – review & editing.

Declaration of Competing Interest

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

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2023.133129.

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