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Physiological biomarkers and hemoparasites in two gull species from human-altered landscapes

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Abstract

Human-altered landscapes expose wildlife to interacting stressors, including contamination, habitat modification, and altered food resources, potentially causing sublethal and often overlooked effects on health, immune function, and parasite dynamics. We assessed these effects by evaluating the physiological condition and hemoparasite prevalence and intensity of two gull species breeding in contrasting anthropogenic contexts in eastern Spain: yellow-legged gull (*Larus michahellis*, YLG) and Audouin's gull (*Ichthyaetus audouinii*, AG). We quantified hematological parameters, plasma B-esterase activities, and hemoparasite occurrence and metrics. Principal component analyses revealed species-specific multivariate responses. YLG showed weak inter-colony differentiation in hematological variables and similar B-esterase activities across sites. In contrast, AG exhibited clear habitat-associated differences. Individuals from the highly agriculture-modified Ebro Delta, where rice fields made up much of the habitat used, had elevated total leukocyte counts, eosinophils, and monocytes, consistent with innate immune activation potentially related to parasite exposure. They also showed consistently lower activities of all six B-esterases measured compared with other colonies. Erythrocyte abnormalities did not vary among sites within species but were higher in AG than in YLG. Both species hosted *Haemoproteus* sp., while *Babesia* sp. was detected only in AG. Hemoparasite prevalence was similar between species, whereas infection intensity was positively associated with monocytes and immature erythrocytes. Integrative blood biomarkers revealed subtle, species-specific physiological responses to human-altered environments, with greater inter-colony physiological and hemoparasite variation in AG than in YLG. Combining physiological biomarkers with hemoparasite analyses provides sensitive early-warning indicators of environmental stress, supporting wildlife health assessments and evidence-based urban planning.

INTRODUCTION

Human population growth and the expansion of human-altered landscapes have reshaped natural ecosystems and posed new challenges and opportunities for wildlife (Isaksson 2020; Moreau et al. 2022). Humans subsidize terrestrial and marine resources, supporting population growth of several taxa (Parra and Tellería 2004; Méndez et al. 2020; Laurich et al. 2019). Concurrently, anthropogenic environments expose wildlife to multiple stressors, including pollution, pathogens, intensified predation, habitat loss, and altered food resources, that can compromise wildlife health and population dynamics (Isaksson 2018; Sinkovics et al. 2021; Solís et al. 2023). While numerous studies have examined how wildlife exploit urban and other anthropogenic habitats through behavioral adjustments (e.g., Ferrer Vilaplana et al. 2024; Navarro et al. 2024), far fewer have explored the physiological consequences of exploiting human-modified environments, including potential exposure to parasites (Nos et al. 2024; Oliveira et al. 2025).

Because these physiological effects may arise from interacting stressors, a multi-method integrative approach is required to understand how human-driven environmental modifications translate into measurable changes in avian physiology. Biomarkers are powerful tools to quantify biological effects, evaluate environmental quality, and detect toxicities associated with complex chemical mixtures (Martínez-Haro et al. 2015). Biochemical biomarkers can act as early warning signs of long-term population-level consequences (Salama and Chennaoui 2024) and help assess wildlife sensitivity or resilience to urbanization (Isaksson 2020). In birds, pollution-related biomarkers (i.e., blood-related ones) have also been proposed as indicators of environmental health (Serafini et al. 2026).

In this context, multi-marker approaches combining immune parameters, genotoxicity metrics, neurotoxicity assays and parasite screening provide complementary information on different dimensions of physiological condition. Cellular immune responses—such as leukocyte profiles and heterophil/lymphocyte ratios—can reflect immune status, infection, inflammation and physiological stress. Genotoxic biomarkers, including erythrocyte nuclear abnormality assays, can indicate DNA damage and genomic instability associated with chemical contamination. Neurotoxicity can be assessed through enzyme activity measures such as plasma B-esterase activity, which is sensitive to exposure to mainly

pesticides. Finally, blood-borne parasite screening allows quantification of infection occurrence and intensity, providing insight into host–parasite dynamics and potentially into the health consequences of exposure to anthropogenic stressors. Using biomarkers selected based on their demonstrated sensitivity to environmental quality and individual condition in wild birds (Valkiūnas 2004; Atkinson et al. 2001; Casini et al. 2006; Oudi et al. 2019; Nwaogu et al. 2023; Solé et al. 2022), comparisons across human-altered landscapes can identify environment-specific physiological challenges related to pollution, habitat modification, and parasite exposure (Isaksson 2020; Oliveira et al. 2025).

Gulls, as synanthropic species that exploit human-derived food subsidies, exemplify how human-related alterations of natural landscapes and increased food availability can drive population growth and modulate shifts in breeding distribution (Ouled-Cheikh et al. 2021; Fernández-Chacón et al. 2013; Oro et al. 2023). These changes have facilitated the expansion of breeding populations into novel habitats, including urban environments, where many gull species now reproduce successfully and establish persistent colonies (Coccon et al. 2022; Goumas et al. 2024; Martín-Vélez et al. 2026).

The yellow-legged gull (*Larus michahellis*), henceforth YLG, is a paradigmatic example of an opportunistic, overpopulated, and human-commensal gull. Its European population is close to 1,000,000, and it breeds widely across the Mediterranean Sea and northwestern Atlantic Ocean (BirdLife International 2021), including highly anthropogenic areas. Its population has surged due to its flexible diet and dependence on human-derived food (Ouled-Cheikh et al. 2021), exploiting resources in landfills and agricultural areas (Martín-Vélez et al. 2024). Many gulls have colonized cities, breeding and foraging on diverse urban resources (Méndez et al. 2020; Martín-Vélez et al. 2026).

The Audouin's gull (*Ichthyaetus audouinii*), henceforth AG, is historically endemic to the Mediterranean region and is currently classified as “Vulnerable” (BirdLife International 2021). Although traditionally considered a specialist feeder, in recent decades it has adapted to exploit fisheries discards and freshwater resources (Ferrer Vilaplana et al. 2024). It is now colonizing areas in the Atlantic Ocean. From the 2000s to the early 2010s, most of the global population (70%, ≈ 15,396 breeding pairs) was concentrated in a relatively natural area in the Ebro Delta (NE Spain) (Fernández-Chacón et al. 2013). Since 2017, the population has progressively dispersed to other breeding areas (Oro et al. 2023), including human-modified environments like industrial ports and industrial sites. This expansion is likely a survival mechanism (Payo-Payo et al. 2017), and although their adaptability to new resources and feeding opportunities during dispersal has recently been studied (Ferrer Vilaplana et al. 2024), their physiological and health conditions during this process remain largely unknown.

The study aimed to evaluate the physiological condition of two gull species (YLG and AG) breeding in colonies located in human-altered landscapes with different levels of anthropogenic pressure in the eastern Iberian Peninsula, Spain. By applying a conservative multi-biomarker approach, we provide a framework for assessing the physiological consequences of wildlife exploitation of human food subsidies and the use of human-altered habitats.

MATERIAL AND METHODS

Study area and fieldwork

Fieldwork was conducted along the western Mediterranean (Spain, Fig. 1). The selected breeding colonies were surrounded by diverse degrees of anthropogenic foraging habitats, significantly altered and shaped by human activities. These included Barcelona (BCN) and Ivars Lake (IV) for YLG, and BCN, the Ebro Delta (ED), Castelló (CAS) and Torrevieja (TV) for AG (Fig. 1). These colonies were in contrasting anthropogenic landscapes, including urban, agricultural and livestock areas, fishing ports, rice fields, saltpans, and protected natural areas, where both gull species can exploit marine and terrestrial resources near their breeding colonies (Ferrer Vilaplana et al. 2024; Navarro et al.

2024; Martín-Vélez et al. 2024). Specifically, BCN was mainly associated with urban and port environments, including human garbage sources; IV with agricultural landscapes, small towns, livestock farms, irrigation ponds, and small dumps; ED with rice fields, agriculture/livestock areas, and fishing ports; CAS with urban areas, fishing ports, and agriculture/livestock habitats; and TV with urban, saltpans and agriculture/livestock habitats.

In April 2022, during the breeding season, 26 YLG (12 in BCN and 14 in IV) and 47 AG (12 in BCN, 8 in CAS, 7 in TV, and 20 in ED) breeding adults were captured from their respective nests using walk-in wire mesh traps. Individuals were captured and sampled late in the incubation period from synchronous colonies, selecting nests with three eggs. Blood samples (0.5–1.0 mL) were collected from the brachial vein using 2 mL syringes with 23G needles and stored on ice (4 ºC) for approximately 3–4 h until centrifugation in the laboratory. Thin blood smears were prepared with a drop of fresh blood and fixed with ethanol for 3 min. All birds were released at the capture site after sampling (CSIC Ethics Committee: REF 28-04-15-237; Generalitat de Catalonia REF: AC/059 – 23, SF/0068/23).

Hematological parameters

Hematological analyses included leukocyte profile (an indicator of cellular immune activity and infection/inflammation), the physiological stress index (typically based on the heterophil-to-lymphocyte ratio (H/L), reflecting chronic stress), thrombocyte counts (related to blood clotting and, in birds, also immune and inflammatory responses), immature erythrocyte counts (reflecting erythropoietic activity and red blood cell regeneration), and erythrocytic nuclear abnormality counts (ENA; a proxy of genotoxic damage; Van Ngan et al. 2007, Davis et al. 2008). These analyses were performed under a light microscope by analyzing the monolayer regions of the blood smear previously stained with Diff-Quick stain (Mgg Quick Stain, Bio-optica). The leukocyte profile was assessed by estimation of the total leukocyte count (or total white blood cell count, tWBC) and its five leukocyte types (heterophils, eosinophils, basophils, lymphocytes, and monocytes). The tWBC was estimated by counting all leukocytes in ten consecutive 40x monolayer fields (D'Amico et al. 2010). Leukocyte type proportions were obtained as the proportion of each leukocyte type in a sample of 100 leukocytes under 100x microscope magnification (Campbell 1995). This count includes an estimate of the number of thrombocytes. The immature erythrocytes percentage was estimated by counting all immature cells in relation to 2000 mature erythrocytes under 100x microscope magnification. The number of erythrocytic nuclear abnormalities (ENAs) was scored for each blood smear in relation to 2000 mature erythrocytes (D'Amico et al. 2016). Nuclear abnormalities included amorphous nuclei (with undefined and irregular edges), displaced nuclei, notched nuclei, kidney-shaped nuclei, bilobulated nuclei, and micronuclei. Results are expressed as the frequency of the total number of abnormalities per 2000 cells (D'Amico et al. 2016). Sample sizes differ between hematological and enzymatic parameters across colonies due to a combination of factors: blood smears and plasma samples were not always obtained from the same subset of individuals, and in some cases plasma volume was insufficient for B-esterase analysis due to hemolysis or sample degradation (see Table 2).

Table 2

Physiological parameters for Audouin's gull (*Ichthyaetus audouinii*) adults sampled across four colonies in 2022 in the eastern Iberian Peninsula (Spain). tWBC: total white blood cells; H/L ratio: heterophil/lymphocyte ratio; ImmEr: immature erythrocytes; ENAs: erythrocytes nuclear abnormalities. Leucocyte types are expressed as %. Enzymatic activities are expressed as nmol/min/mL plasma. AChE: acetylcholine esterase; BChE: butyrylcholine esterase; CE: carboxylesterase; pNPA: p-nitrophenyl acetate; pNPB: p-nitrophenyl butyrate; αNA: α-naphthyl acetate; αNB: α-naphthyl butyrate.

Ichthyaetus audouinii	**Ebro Delta**			**Castelló**			**Torrevieja**			**Barcelona**		
Parameter	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
tWBC	128684	59151	19	81000	33299	6	88714	31837	7	73750	30899	12
Lymphocytes	42	8	19	46	12	6	48	7	7	47	11	12
Heterophils	44	11	19	43	16	6	40	5	7	43	8	12
Eosinophils	3	2	19	2	1	6	1	1	7	1	1	12
Basophils	0	0	19	0	0	6	0	0	7	0	0	12
Monocytes	16	7	19	9	2	6	11	5	7	13	5	12
Thrombocytes	122	58	19	75	48	6	130	39	7	164	56	12
H/L ratio	1.10	0.44	19	1.06	0.69	6	0.85	0.19	7	0.99	0.38	12
ImmEr	25	11	19	17	10	6	16	12	7	20	9	12
ENAs	2.51	1.14	19	2.7	1.57	6	1.69	0.49	7	2.92	1.1	12
AChE	495.3	155.6	7	644.6	222.0	8	746.8	210.55	7	732.2	166.9	12
BChE	603.1	240.0	7	856.3	311.8	8	967.2	297.21	7	988.1	238.5	12
pNPA-CE	260.5	21.8	7	301.0	443.0	8	340.4	62.57	7	531.5	375.5	12
pNPB-CE	205.0	38.8	7	260.7	68.0	8	300.9	80	7	442.1	352.8	12
αNA-CE	318.6	64.8	7	494.9	165.2	8	560.4	129.24	7	665.8	241.9	12
αNB-CE	552.1	116.2	7	670.9	193.6	8	740.2	213.82	7	970.4	421.5	12

N indicates the number of individuals analyzed for each parameter. Sample sizes differ between hematological and enzymatic determinations because blood smears and plasma samples were not always obtained from the same individuals within each colony.

Plasma B-esterases activity

B-esterases (selected as indicators of neurotoxicity) comprised acetylcholine esterase (AChE) and butyrylcholine esterase (BChE) as cholinesterases, while the carboxylesterases (CE) were measured using p-nitrophenyl acetate (pNPA), p-nitrophenyl butyrate (pNPB), α-naphthyl acetate (αNA), and α-naphthyl butyrate (αNB) as substrates. All these B-esterases were measured from the plasma obtained after blood sample centrifugation at 2,000 g (Multispin 12, Argo LAB).

AChE was measured using 25 µL of 1/10 diluted plasma following the protocol outlined in Ellman et al. (1961). Specifically, we added to the sample 180 µM of 5,5'-dithiobis-2-nitrobenzoic acid (DTNB). After 2 min of pre-incubation, the substrates acetylthiocholine (ATC) or butyrylthiocholine (BTC) were added, each at a 1 mM final assay concentration to indicate AChE and BChE activities, respectively. CE activity was measured using pNPA or pNPB as

substrates. The reaction consisted of adding 25 µL of 1/10 diluted plasma to 200 µL of phosphate buffer 100 mM pH 8.0, containing a final concentration of 1 mM pNPA or pNPB. The formation of p-nitrophenol was recorded at 405 nm as described in Hosokawa and Satoh (2001). The substrates αNA or αNB, each at 0.25 mM concentration, were incubated with the same phosphate buffer and 25 µL of a 1/20 diluted plasma. Readings were done at 235 nm in the UV mode (Mastropaolo and Yourno 1981). Linearity of the measures (in triplicate) was ensured during the 5-min kinetics at 25 ºC in a TECAN Infinite 200 microplate reader using transparent 96-well microplates. Only linear reactions were considered, and these were registered using the kinetic assays mode of Magellan V6.0 data analysis software. Enzyme activity was expressed as nmol/min/mL plasma.

Hemoparasites analyses

Blood smears were used to identify protozoan hemoparasites and quantify them in relation to 2000 erythrocytes under a light microscope at 100x magnification (Godfrey et al. 1987). The prevalence (the percentage of individuals infected with a specific parasite), mean intensity (the average number of parasites -erythrocytes infected by merozoites or gametocytes- of a particular species on infected hosts), and mean abundance (the average number of parasites per host in the total sample, including both infected and uninfected hosts) of parasites were obtained (Bush et al. 1997). For the specific determination of hemoparasites (*Haemoproteus* sp., *Plasmodium* sp., and *Leucocytozoon* sp.), molecular analyses were performed on stored blood samples. DNA was extracted using the DNeasy Blood & Tissue Kit (Qiagen, Germany) according to the manufacturer's instructions, and nested PCR of the cytochrome b region of mitochondrial DNA was performed with primers specific for different hemoparasites following the protocol of Hellgren et al. (2004).

Prevalence was calculated using the combined blood smear/PCR analyses, whereas intensity and abundance were calculated exclusively from blood smears. For YLG, 17 available smears were examined (BCN = 10; IV = 7), and PCR analyses were performed on stored blood samples from the 17 individuals (BCN = 10; IV = 7). For AG, 44 individuals were screened for hemoparasites using available blood smears (BCN = 12; TV = 7, CAS = 6, ED = 19), and PCR analyses were conducted on stored blood samples from 46 individuals (BCN = 12; TV = 7, CAS = 7, ED = 20).

Statistical analysis

Firstly, principal component analyses (PCA) were performed separately for YLG and AG to evaluate multivariate patterns in hematological and plasma B-esterase activity among populations. Site differences were visualized using individual scores and population centroids (mean ± SD) on the first two principal components. Variable loadings were examined to identify the physiological parameters contributing most strongly to site differentiation. Then, for each species and for each physiological parameter, the differences between sites were analyzed. Preliminary assessments of normality for each variable were conducted using the Shapiro-Wilk test and Q-Q plots, and homogeneity of variances was evaluated with Levene's test. As in most of the cases, variables exhibited non-normal distributions and outliers; non-parametric correlations were prioritized, and the Mann–Whitney (U) or the Kruskal-Wallis tests were employed. Post-hoc comparisons among sites were also used.

Differences in hemoparasites prevalence among the four colonies of AG were assessed using Fisher's Exact Test, as 37.5% of cells in the contingency table had expected frequencies below 5, violating chi-square test assumptions. Parasite abundance among colonies was compared using non-parametric Kruskal-Wallis tests. For YLG, differences in prevalence between the two studied colonies (BCN vs IV) were analyzed using Fisher's Exact Test, appropriate for 2×2 contingency tables with small sample sizes (N = 17). Differences in parasite abundance were assessed using the Mann-Whitney test, reporting the exact bilateral p-value given the small sample (N = 17). Associations between hematological parameters and hemoparasites were evaluated using Spearman's rank correlation coefficient (rho) for variables that did

not meet the assumption of normality, and Pearson's correlation coefficient (r) for variables that satisfied this assumption, as assessed by the Shapiro-Wilk test. All statistical analyses were performed using SPSS 15.0.1 (SPSS Inc., Chicago, IL, USA) and R version 4.5.2 (R Core Team 2026).

RESULTS

Hematological parameters

For YLG, the PCA explained 56% of the total variance (PC1 = 39.4%, PC2 = 16.6%; Fig. 2A). PC1 primarily separated individuals along a gradient associated with monocytes (M), immature erythrocytes (ImmEr), and basophils (B), while PC2 reflected additional variation in the hematological profile. Individuals from IV showed a relative positioning toward higher eosinophils (E) and thrombocytes (Throm), whereas individuals from BCN were more closely aligned with monocytes (M) and immature erythrocytes (ImmEr). Despite these tendencies, substantial overlap between populations and large within-site dispersion indicated only weak differentiation between localities. For AG, the PCA also explained 49.8% of the total variance (PC1 = 30.4%, PC2 = 19.4%; Fig. 2B). But in this case, PC1 separated ED individuals from those of TV and BCN. Individuals from ED were positioned toward higher total white blood cell counts (tWBC), monocytes (M), basophils (B), and immature erythrocytes (ImmEr), whereas TV and BCN individuals showed a closer alignment with lymphocytes (L). Individuals from CAS occupied an intermediate position and were aligned partly with the thrombocytes (Throm) vector. As for YLG, substantial overlap among AG populations reflected considerable within-site variability and only moderate differentiation among localities. Overall, the PCA structure suggested that local conditions may influence the multidimensional hematological profile more clearly in AG than in YLG, where between-location differences appeared subtler. In addition, the strong overlap between BCN and IV in YLG suggested a relatively homogeneous physiological structure between these sites for the analyzed hematological parameters.

The leukocyte profile of YLG did not show significant differences between sites (Table 1), indicating a broadly similar hematological status across habitats for this species. In contrast, AG exhibited significant variation in tWBC, eosinophils, and monocytes (Table 2). The counts of the three parameters were higher in ED than in the other three colonies (all post-hoc comparisons $p \leq 0.006$) (Table 2). No significant differences were detected for the remaining leukocyte types of AG among sites (Table 2).

Table 1
Physiological parameters of yellow-legged gull (YLG) adults sampled in BCN and IV during 2022 in the eastern Iberian Peninsula (Spain). tWBC: total white blood cells; H/L ratio: heterophil/lymphocyte ratio; ImmEr: immature erythrocytes; ENAs: erythrocytes nuclear abnormalities. Leucocyte types are expressed as %. Enzymatic activities are expressed as nmol/min/mL plasma. AChE: acetylcholine esterase; BChE: butyrylcholine esterase; CE: carboxylesterase; pNPA: p-nitrophenyl acetate; pNPB: p-nitrophenyl butyrate; αNA: α-naphthyl acetate; αNB: α-naphthyl butyrate.

Larus michahellis	**Barcelona (BCN)**			**Ivars Lake (IV)**		
Parameter	Mean	SD	N	Mean	SD	N
tWBC	110700	28581	12	105500	51244	14
Lymphocytes	48	8	12	48	9	14
Heterophils	43	8	12	44	10	14
Eosinophils	3	2	12	4	2	14
Basophils	1	1	12	1	1	14
Monocytes	7	3	12	5	2	14
Thrombocytes	166	73	12	138	45	14
H/L ratio	0.95	0.36	12	0.99	0.48	14
ImmEr	13	10	12	8	3	14
ENAs	1.28	0.49	12	1.5	0.59	14
AChE	1505.9	303.5	12	1626.7	351.8	14
BChE	2062.6	529.8	12	2276.7	475.4	14
pNPA-CE	801.5	541.2	12	594.1	337.7	14
pNPB-CE	875.8	535.9	12	714.8	339.2	14
αNA-CE	1227.9	360.2	12	1186.1	253.0	14
αNB-CE	1428.1	537.3	12	1302.2	287.20	14

Thrombocytes showed no statistical differences between sites for YLG (Table 1). However, thrombocyte counts in AG were statistically higher in BCN (Table 2). In fact, thrombocyte counts differed significantly between the two industrial colonies, with counts in BCN being 2.6 times higher than in CAS (Table 2). Regarding immature erythrocyte counts, both YLG and AG showed similar patterns, with no significant differences among sites (Tables 1 and 2).

Erythrocyte nuclear abnormalities (ENAs) did not show any difference between sites for YLG. (Table 1). Similarly, no significant differences in ENAS were detected among sites for AG (Table 2). However, ENAs occurrence differed significantly between species (YLG and AG) regardless of location ($U = 119.5$, $p < 0.001$).

Plasma B-esterase activities

For YLG, the PCA explained 97.6% of the total variance (PC1 = 75.7%; PC2 = 21.9%; Fig. 2C). Individuals from BCN and IV showed partial overlap in the ordination space, indicating generally similar enzymatic profiles between sites. However, samples from IV tended to cluster toward positive PC2 values, associated with higher acetylcholine esterase (AChE)

and butyrylcholine esterase (BChE) activities. In contrast, individuals from BCN displayed greater dispersion across the ordination space, suggesting higher inter-individual variability. For AG, PCA accounted for 89.1% of the total variance (PC1 = 68.3%; PC2 = 20.8%; Fig. 2D). Gulls from ED, CAS, and TV showed high overlap, indicating similar enzymatic profiles. However, individuals from TV and CAS tended to associate with positive PC2 values and higher AChE, BChE, and αNA-CE. Conversely, ED individuals were aligned to positive PC1 and displayed lower PC2 scores. Individuals from BCN exhibited the greatest dispersion, including an extreme outlier, indicating high inter-individual variability.

Overall, no significant differences in the six B-esterase activities were seen for YLG individuals between the two sampled sites (Table 1). In contrast, AG specimens exhibited significant differences across all six B-esterases when comparing sites (Table 2). Those from ED showed consistently lower enzymatic activities for all six B-esterases (AChE, BChE, pNPA-CE, pNPB-CE, αNA-CE, and αNB-CE) compared to those from the other locations (Table 2).

Hemoparasite analyses

Haemoproteus sp. was found in YLG (64.71% prevalence, 2.73 mean intensity, and 1.76 mean abundance, Table 3), identified morphologically in blood smears and detected by PCR. The average number of parasites counted in the blood smears was 1.9 per 2,000 erythrocytes (range: 0–24; SD = 6.1; N = 17). Overall, YLG tended to have a higher hemoparasite prevalence in IV than in BCN (Table 3); however, no statistical differences were found between sites (U = 24, p = 0.56). Hemoparasite intensity was significantly positively correlated with the number of monocytes (rho = 0.53, p = 0.03) and immature erythrocytes (rho = 0.52, p = 0.03), while no significant correlations were observed between parasitic intensity and total leukocytes, lymphocytes, heterophils, basophils, eosinophils, thrombocytes, H/L ratio, or ENAs (all Spearman correlations p > 0.05).

Table 3
Hemoparasites metrics for yellow-legged gull (*Larus michahellis*). Prevalence was calculated using the combined blood smear/PCR analyses, whereas intensity and abundance were calculated exclusively from blood smears.

***Haemoproteus* sp.**	**Prevalence %**	**Intensity**	**Abundance**
Barcelona	60.00	5.00	3.00
Ivars Lake	71.43	0	0
Total (2 sites)	64.71	2.73	1.76
Intensity and abundance for Ivars Lake are 0 because no hemoparasite was found in blood smears. *Haemoproteus* sp. was only found by PCR			

In comparison, AG hosted two species of hemoparasites, *Haemoproteus* sp. and *Babesia* sp., presenting in total a prevalence of 71.74%, a mean intensity of 24.09, and a mean abundance of 18,07 (Table 4). The average number of parasites counted in the blood smears was 18.1 per 2000 erythrocytes (range 0–116, SD = 31.9, N = 44). *Haemoproteus* sp. was present in all sampled sites, while *Babesia* sp. was present only in one individual from ED (Table 4). Prevalence of *Haemoproteus* sp. did not show statistical differences among sites (Fisher's Exact Test: p = 0.278), although it varied from 58.3% in BCN to 100% in TV. Hemoparasite intensity was positively correlated with monocyte counts (rho = 0.79, p < 0.0001), basophil counts (rho = 0.36, p = 0.014), and immature erythrocytes (r = 0.67, p < 0.0001). No significant correlations were observed with total leukocytes, lymphocytes, heterophils, eosinophils, thrombocytes, H/L ratio, or ENAs (all Spearman correlations p > 0.05).

Table 4
Hemoparasites metrics for Audouin's gull (*Ichthyaetus audouinii*). Prevalence was calculated using the combined blood smear/PCR analyses, whereas intensity and abundance were calculated exclusively from blood smears. H: *Haemoproteus* sp., B: *Babesia* sp.

	Prevalence %		**Intensity**		**Abundance**	
	H	**B**	**H**	**B**	**H**	**B**
Barcelona	58.33		26.43		15.42	
Torrevieja	100		14.14		14.14	
Castelló	71.43		0.6		0.43	
Ebro Delta	65	5	39.2	116.0	20.63	6.10
Total (4 sites)	71.74		24.09		18.07	

No significant differences in hemoparasite prevalence were found between both species, YLG (64.71%) and AG (71.74%) (Fisher's Exact Test: $p = 0.758$; $\chi^2 = 0.29$, $df = 1$, $p = 0.589$). The odds ratio of infection in AG relative to YLG was 1.38 (95% CI: 0.42–4.52, $p = 0.59$), indicating similar prevalences between species.

DISCUSSION

The present study reveals that the suite of environmental impacts associated with anthropogenic habitats affected the physiological status and parasite dynamics of two opportunistic gulls. Using a comprehensive hematology-based, multi-biomarker approach, we showed that Audouin's gulls (AG) exhibited pronounced differences among the 4 contrasted colonies in leukocyte profiles, B-esterase activities, and hemoparasite metrics, whereas yellow-legged gulls (YLG) appeared more physiologically consistent. These responses highlight that human-altered landscapes do not affect all bird species equally, and that species-specific ecology and life history modulate vulnerability to human-associated stressors. More broadly, our integrative framework, which combines hematology, enzymatic biomarkers, and hemoparasite screening, is particularly relevant in the current context of rapid human expansion and increasing contact between wildlife and humans, as it links physiological challenges and pathogen exposure to human impacts.

Integrated interpretation of blood-related parameters in two gull species

Hematological parameters and plasma B-esterases constitute sensitive and widely applied tools for assessing physiological condition, immune function, exposure to pathogens, parasitic infections, contaminants, chronic stress, and the effects of environmental disturbances on wildlife populations (Campbell 1995). As they integrate responses occurring at multiple physiological levels, they also provide valuable insight into how species cope with habitat alteration, pathological and environmental pressures. In this context, variation in leukocyte profiles, thrombocyte counts, erythrocyte parameters (immature erythrocytes and erythrocyte nuclear abnormalities (ENAs)), and B-esterase activities integrated into the hemoparasites analyses can help identify physiological resilience, early signs of stress, and general health conditions associated with ongoing urbanization.

Our results revealed consistent immune metrics in YLG of both highly anthropogenic sites sampled, BCN and IV, suggesting a stable baseline cellular immune status in this gull species. Likewise, thrombocyte counts, which are closely linked to avian innate immunity and inflammatory responses, remained comparable among sites, reinforcing the idea of a relatively high tolerance to anthropogenic environments. Supporting this interpretation, YLG also showed

similar B-esterase activities between the two habitats, indicating limited biochemical adjustment to urban conditions (in the case of BCN). Together, these physiological indicators suggest greater resilience to contaminant exposure, uniform baseline detoxification capacity, and/or species-specific tolerance to urban-associated stressors (Walker 2003).

In terms of hemoparasite occurrence, the presence of *Haemoproteus* sp. in YLG, together with the lack of differences between the two sites, further reinforces that habitat-related factors had limited influence on host–parasite dynamics in this species. Although parasite intensity was positively correlated with some hematological parameters, indicating localized immune and hematopoietic responses, no associations were detected with broader indicators of physiological stress or immune activation. Hence, hemoparasite infection appeared to impose only minor physiological costs, reinforcing the view that YLG can maintain physiological stability under varying environmental conditions, including human-modified habitats.

Overall, the integration of blood-related parameters and hemoparasite analyses revealed that this gull species could maintain physiological homeostasis despite habitat modifications. Such physiological stability may reflect adaptive or phenotypically flexible responses that buffer individuals against urban-related challenges (Chamberlain et al. 2009; Evans et al. 2010), resulting in lower physiological costs in urban-breeding populations (Seoane et al. 2011; Sol et al. 2013).

In contrast, AG individuals displayed particularities among habitats in several physiological parameters, highlighting the sensitivity of hematological biomarkers for detecting environmentally associated changes in immune function and health condition. In this sense, individuals from ED, a largely agricultural but also protected area (75% arable land, 20% natural area), exhibit a significantly different hematological profile from the rest of the colonies. The leukocytosis, accompanied by eosinophilia and relative monocytosis, suggests a coordinated immune response to parasitic challenges. It was reported that Hemosporidian infections, particularly *Haemoproteus* infections, in turn, increase total leukocyte counts and impose measurable immune costs (Figuerola et al. 1999; Ricklefs and Sheldon 2007; Dunn et al. 2013). Thus, the increased eosinophil counts in individuals from ED further support this, aligning with general antiparasitic defenses that involve the release of peroxidase, lysozyme, and arginine-rich granules (Mitchell and Johns 2008), despite eosinophilia often indicating anthelmintic responses (Campbell 1995). Similar eosinophil responses have been observed in Eurasian Tree Sparrows (*Passer montanus*) with *Haemoproteus sp.* infections (Vega et al. 2025). Additional evidence of immune activation was provided by a higher count of monocytes in ED and the positive association between monocytes and hemoparasites. As monocytes and granulocytes are key components of innate immunity and early nonspecific defense, their increase represents an informative indicator of host physiological condition and infection status (Davis et al. 2008; Masello et al. 2009; Schat et al. 2014). To support this last observation, avian monocyte levels typically range from 0% to 3% (Gálvez Martínez et al. 2009); the elevated values recorded in this study, particularly in ED individuals (> 10%), may indicate chronic inflammatory and/or parasitic processes (Charles-Smith et al. 2014). Similarly, the observed association between basophils and hemoparasite infections is consistent with evidence suggesting that basophils contribute to antiparasitic immune responses (Eberle and Voehringer 2016).

Notably, lymphocytes, heterophils, and H/L counts in ED were similar to those from other colonies, indicating that the leukocytosis is specific to innate immune cells related to parasitosis, not a nonspecific stress response. Together, these results emphasize the usefulness of leukocyte profiles as indicators of subtle physiological responses to parasitic and environmental pressure.

The habitat-dependent physiological patterns observed in AG further highlight the value of physiological biomarkers as sensitive indicators of ecological exposure and physiological sensitivity. In line with this interpretation, thrombocyte counts also differed among habitats in this species. Beyond their role in hemostasis, thrombocytes are major

components of avian innate immunity and inflammatory processes; consequently, variation in their abundance may reflect differences in immune activation, tissue repair, or exposure to environmental stressors. The higher thrombocyte counts observed in AG at the metropolitan BCN area likely indicate greater exposure to stressors associated with highly human-altered environments, such as pollutants, disturbance, or altered diet. Therefore, taken together, these findings suggest that thrombocyte levels may represent a potentially informative biomarker for differentiating habitats according to anthropogenic pressure and for detecting species-specific physiological responses to environmental change (Sparling et al. 1998).

Immature erythrocyte counts also supported physiological responses to stressors, such as parasite infection in both species. In birds, hemoparasite infections often increase erythrocyte destruction, which is typically compensated through accelerated erythropoiesis. Microscopically, this process is reflected by increased proportions of immature erythrocytes (Campbell 1995), a parameter widely used to evaluate physiological responses to Hemosporidian infections (Soares et al. 2016). Although immature erythrocyte counts did not differ significantly among habitats in either gull species, their values showed a strong positive correlation with hemoparasites, and they ranged from 5% to 50%, clearly exceeding the expected baseline of 1–5% (Thrall et al. 2012), indicating active erythropoiesis. These patterns likely reflect compensatory bone marrow activity associated with hemoparasite infection intensity, which showed a strong positive correlation with immature erythrocyte proportions. Thus, even in the absence of clear habitat differences, erythrocyte parameters proved valuable for detecting underlying physiological adjustments linked to infection pressure.

The blood biomarker ENAs is a widely recognized indicator of cytogenetic damage and physiological disruption (Van Ngan et al. 2007), providing complementary information on sublethal stress responses. ENA frequencies were similar among colonies within each species, suggesting that urbanized environments did not produce detectable site-related differences in this biomarker during the sampling period. However, despite the absence of habitat-related effects, ENA levels remained consistently higher in AG than in YLG, indicating an interspecific difference in cytogenetic status independent of urbanization level. This pattern may reflect species-specific sensitivity to environmental stressors, including differences in contaminant exposure pathways, erythrocyte turnover dynamics, or intrinsic mechanisms regulating nuclear stability.

Plasma B-esterase activities further supported the hematological results as physiological measurements to single out habitat- and species-specific responses to anthropogenic stressors, mostly in the case of AG. Individuals from ED, as well as CAS, showed lower hydrolysis rates in B-esterase measurements (potentially indicative of pesticide exposure), whereas individuals from TV exhibited higher enzymatic activities that could reflect basal values. The lower activity of all six measured B-esterases in ED suggests a generalized enzymatic downregulation in this agricultural environment largely dominated by rice cultivation, likely associated with exposure to pesticide-like compounds derived from crop management practices. Agricultural and industrial colonies (ED, CAS, and BCN) face distinct but greater toxicological pressure, potentially affecting their long-term viability, while TV serves as a valuable reference population for pollution-related studies with this gull species.

Previous studies with YLG from other Spanish regions demonstrated that B-esterase activity varies according to age, sex, and tissue type (Martínez Morcillo et al. 2018). Moreover, plasma has been proposed as a particularly informative and conservative matrix for biomonitoring in urban populations, such as those from Barcelona, where plasmatic pNPB-CE and αNB-CE were shown to be more sensitive indicators of polybrominated diphenyl ether (PBDE) exposure than cholinesterases (Nos et al. 2024). Although species sensitivity to neurotoxic compounds has sometimes been associated with the ratio between AChE and protective B-esterases (Giovanetti et al. 2024), the relationship between these enzymes remained similar in the two contrasted species. In general, these findings suggest that the biochemical

impact of land use on neurotoxicant-related enzymes is strongly species-dependent, with AG appearing more responsive to neurotoxic stressors associated with agricultural management.

Hemoparasite characterization in the two gull species

Haemoproteus has been reported in erythrocytes of YLG and AG in the Ebro Delta, likely due to high densities or activity of its *Culicoides* vectors (Martínez-Abraín et al. 2002). *Haemoproteus* comprises 128 species of avian parasites transmitted by biting midges, generally considered relatively benign (Hubálek 2021). *Haemoproteus* infection was confirmed in this study using both blood smear and PCR analyses. Microscopic examination revealed the presence of young, developing, and fully grown gametocytes, which are typical of the genus (Valkiūnas and Iezhova 2022). The parasite morphology was consistent with gametocyte features used to identify *Haemoproteus* species in Charadriiformes birds (Valkiūnas and Iezhova 2022). Based on plates in Valkiūnas and Iezhova (2022), the species could be *H. larae* or *jenniae*, both previously found in gulls (Vanstreels et al. 2019). Findings of severe tissue damage in infected birds, caused by *Haemoproteus* parasites (meronts and megalomeronts) in organs such as the brain, heart, lungs, kidneys, and skeletal muscles, highlight the need for further research into the diversity and development of these pathogens (Atkinson et al. 2008; Valkiūnas and Iezhova 2017; Ortiz-Catedral et al. 2019). Identifying parasite species at the species level and analyzing molecular data are crucial. Combining microscopic and PCR-based methods is the preferred approach, especially in wildlife studies, as it enhances the significance of research on Hemosporidian parasite diversity (Bensch and Hellgren 2020). However, advancements in the morphological identification of *Haemoproteus* species are lagging behind the growth of DNA sequence data. While microscopy and PCR yield similar *Haemoproteus* prevalence estimates, PCR may detect 25–35% more infections than blood smears (Fallon and Ricklefs 2008). Therefore, smear analyses alone could underestimate blood parasite prevalence. Because only infective gametocytes circulate in the blood, while asexual stages reside in tissues (Bennett and Peirce 1988), our findings likely represent active infections.

In the present study, *Babesia sp*. was detected in one individual of AG from ED; this parasite has not been previously reported in this species. *Babesia bennetti* was described as a new species parasitizing YLG on Benidorm Island (Merino 1998). This remains the only report of *B. bennetti* in Laridae (Peirce 2000), and its pathogenic effects on gulls are unknown (Hubálek 2021). More recently, *Babesia sp*. belonging to the Peircei clade in the new classification of Piroplasms has been reported in YLG chicks and named *Babesia* sp. YLG (Bonsergent et al. 2022). We could not identify this parasite at the species level based on morphology because only early ring forms and trophozoites were present in erythrocytes (Peirce and Parsons 2012); therefore, we report it as *Babesia* sp. YLG as described for YLG (Bonsergent et al. 2022; Pollet 2023). Future research should use molecular methods. While *Babesia* sp. infection in birds may not always cause clinical signs, it can increase morbidity and mortality, especially with concurrent infections or other health issues (Peirce and Parsons 2012). In penguins, for example, *Babesia* infection has been linked to mild anemia, leukocytosis, and impaired liver function (Sergent et al. 2004; Parsons et al. 2016).

CONCLUSIONS

Overall, gulls showed distinct physiological profiles and hemoparasite infection patterns, with greater inter-colony differences in AG than in YLG, suggesting species-specific responses to habitat. AG from the Ebro Delta exhibited broader hematological changes and higher hemoparasite metrics, potentially reflecting anthropogenic and infection-related pressures. In contrast, YLG appeared more tolerant of urban stressors, although their physiological profile was influenced by pollution, disturbance, altered food resources, and parasite exposure.

These findings alert against generalizing about the suitability of modified environments for gulls and highlight the need to determine whether physiological differences translate into population-level effects on survival, reproduction, and long-term health. Human development may affect gull species differently depending on parasite burden, contaminant

exposure, energetic constraints, and species-specific sensitivity. Integrating physiological biomarkers with hemoparasite analyses can detect subtle responses to environmental stressors, supporting their use as early-warning indicators of wildlife health and informing evidence-based urban planning.

Declarations

Competing Interests:

The authors have no relevant financial or non-financial interests to disclose.

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Author Contribution

All authors contributed to the study conception and design. Material preparation and data collection were performed by V.M.V., J.N., and F.R.; hematological analysis was performed by V.D. and M.B.; B-esterase analyses were performed by M.S. and V.D.; and hemoparasites analyses were performed by V.D. and C.G. The first draft of the manuscript was written by V.D., and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data Availability

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

References

1. Atkinson CT, Dusek RJ, Lease JK (2001) Serological responses and immunity to superinfection with avian malaria in experimentally-infected Hawaii Amakihi. J Wildl Dis 37:20–27
2. Atkinson CT, Thomas NJ, Hunter DB (2008) Parasitic diseases of wild birds. Wiley-Blackwell, Oxford
3. Bennett GF, Peirce MA (1988) Morphological form in the avian Haemoproteidae and an annotated checklist of the genus Haemoproteus Kruse, 1890. J Nat Hist 22:1683–1696

4. Bensch S, Hellgren O (2020) The use of molecular methods in studies of avian haemosporidians. In: Santiago-Alarcón D, Marzal A (eds) Avian malaria and related parasites in the tropics: ecology, evolution and systematics. Springer, Berlin, pp 113–136
5. BirdLife International (2021) European Red List of Birds 2021. Publications Office of the European Union
6. Bonsergent C, Vittecoq M, Leray C, Burkart L, McCoy KD, Malandrin L (2022) Characterization and diversity of Babesia sp. YLG, a new member of the Peircei group infecting Mediterranean yellow-legged gulls (Larus michahellis). Ticks Tick Borne Dis 13(1):101852. https://doi.org/10.1016/j.ttbdis.2021.101852
7. Bush AO, Lafferty KD, Lotz JM, Shostak AW (1997) Parasitology Meets Ecology on Its Own Terms: Margolis. Revisit J Parasitol 83(4):575–583. https://doi.org/10.2307/3284227
8. Campbell TW (1995) Avian hematology and cytology. Iowa State University, Ames
9. Casini S, Marsili L, Fossi MC, Mori G, Bucalossi D, Porcelloni S, Caliani I, Stefanini G, Ferraro M, Di Catenaja CA (2006) Use of biomarkers to investigate toxicological effects of produced water treated with conventional and innovative methods. Mar Environ Res 62:S347–S351. https://doi.org/10.1016/j.marenvres.2006.04.060
10. Chamberlain DE et al (2009) Avian productivity in urban landscapes: a review and meta-analysis. Ibis 151(1):1–18
11. Charles-Smith LE, Rutledge ME, Meek CJ, Baine K, Massey E, Ellsaesser LN, DePerno CS, Moorman CE, Degernes LA (2014) Hematologic parameters and hemoparasites of nonmigratory Canada geese (Branta canadensis) from Greensboro, North Carolina, USA. J Avian Med Surg 28:16–23. https://doi.org/10.1647/2012-072
12. Coccon F, Vanni L, Dabalà C, Giunchi D (2022) The abundance of yellow-legged gulls Larus michahellis breeding in the historic centre of Venice, Italy and the initial effects of the new waste collection policy on the population. Urban Ecosyst 25(2):643–656. https://doi.org/10.1007/s11252-021-01175-7
13. D'Amico VL, Bertellotti M, Baker AJ, González PM (2010) Hematological and plasma biochemistry values for endangered red knots (Calidris canutus rufa) at wintering and migratory sites in Argentina. J Wildl Dis 46:644–648
14. D'Amico VL, Coria N, Palacios MG, Barbosa A, Bertellotti M (2016) Physiological differences between two overlapped breeding Antarctic penguins in a global change perspective. Polar Biol 39:57–64
15. Davis AK, Maney DL, Maerz JC (2008) The use of leukocyte profiles to measure stress in vertebrates: a review for ecologists. Funct Ecol 22:760–772. https://doi.org/10.1111/j.1365-2435.2008.01467
16. Dunn JC, Goodman SJ, Benton TG et al (2013) Avian blood parasite infection during the non-breeding season: an overlooked issue in declining populations? BMC Ecol 13:30. https://doi.org/10.1186/1472-6785-13-30
17. Eberle JU, Voehringer D (2016) Role of basophils in protective immunity to parasitic infections. Semin Immunopathol 38:605–613. https://doi.org/10.1007/s00281-016-0563-3
18. Ellman GL, Courtney KD, Andres V Jr., Featherstone RM (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 7(2):88–95. https://doi.org/10.1016/0006-2952(61)90145-9
19. Evans KL et al (2010) A conceptual framework for the colonization of urban areas: the blackbird as a model. Biol Rev 85(3):643–667
20. Fallon SM, Ricklefs RE (2008) Host specialization and geographic localization of avian malaria parasites: a regional analysis in the Lesser Antilles. Am Nat 171(5):674–689
21. Fernández-Chacón A, Genovart M, Pradel R, Tavecchia G, Bertolero A, Piccardo J, Forero MG, Afán I, Muntaner J, Oro D (2013) When to stay, when to disperse, and where to go: survival and dispersal patterns in a spatially structured seabird population. Ecography 36(10):1117–1126. https://doi.org/10.1111/j.1600-0587.2013.00246.x
22. Ferrer Vilaplana A, Afán I, Oro D, Bécares J, Illa M, Gil M, Bartolero A, Forero M, Ramírez Benítez F (2024) Distribution and habitat use by the Audouin's Gull (Ichthyaetus audouinii) in anthropized environments. Sci Total Environ 954:176555. https://doi.org/10.1016/j.scitotenv.2024.176555

23. Figuerola J, Muñoz E, Gutiérrez R, Ferrer D (1999) Blood parasites, leucocytes and plumage brightness in the Cirl Bunting, Emberiza cirlus. Funct Ecol 13:594–601
24. Gálvez Martínez CF, Ramírez Benavides GF, Osorio JH (2009) El laboratorio clínico en hematología de aves exóticas. Biosalud 8:178–188
25. Giovanetti L, Caliani I, Damiani G, Dell'Omo G, Costantini D, Casini S (2024) A blood-based multi-biomarker approach reveals different physiological responses of common kestrels to contrasting environments. Environ Res 251(2):118674. https://doi.org/10.1016/j.envres.2024.118674
26. Godfrey RD Jr, Fedynich AM, Pence DB (1987) Quantification of hematozoa in blood smears. J Wildl Dis 23(4):558–565. https://doi.org/10.7589/0090-3558-23.4.558
27. Goumas M, Berkin CR, Rayner CW, Boogert NJ (2024) From the sea to the city: explaining gulls' use of urban habitats. Front Ecol Evol 12:1256911
28. Hellgren O, Waldenström J, Bensch S (2004) A new PCR assay for simultaneous studies of Leucocytozoon, Plasmodium, and Haemoproteus from avian blood. J Parasitol 90(4):797–802
29. Hosokawa M, Satoh T (2001) Measurement of Carboxylesterase (CES) Activities. Curr Protoc Toxicol, 10: 4.7.1–4.7.14.
30. Hubálek Z (2021) Pathogenic microorganisms associated with gulls and terns (Laridae), Journal of Vertebrate Biology 70(3):21009.1–98, (4 August 2021)
31. Isaksson C (2018) Impact of urbanization on birds. In: Tietze DT (ed) Bird Species, Fascinating Life Sciences. Springer International Publishing, Cham, pp 235–257. https://doi.org/10.1007/978-3-319-91689-7_13
32. Isaksson C (2020) Urban ecophysiology: beyond costs, stress and biomarkers. J Exp Biol 223:jeb203794. https://doi.org/10.1242/jeb.203794
33. Laurich B, Drake C, Gorman OT, Irvine C, MacLaurin J, Chartrand C, Hebert CE (2019) Ecosystem change and population declines in gulls: shifting baseline considerations for assessing ecological integrity of protected areas. J Great Lakes Res 45(6):1215–1227. https://doi.org/10.1016/j.jglr.2019.08.009
34. Martín-Vélez V, Montalvo T, Giralt D, Ramírez F, Giménez J, Morral-Puigmal C, Navarro J (2024) Evil and allies: Opportunistic gulls as both spreaders and sentinels of antibiotic-resistant bacteria in human-transformed landscapes. J Appl Ecol 61(11):2809–2821
35. Martín-Vélez V, Navarro J, Montalvo T (2026) Nesting the city: long-term spatiotemporal dynamics of nest distribution in a synanthropic gull within a high-density European city. Urban Ecosyst 29(1):22
36. Martínez Morcillo S, Perego MC, Vizuete J, Caloni F, Cortinovis C, Fidalgo LE, López-Beceiro A, Míguez MP, Soler F, Pérez-López M (2018) Reference intervals for B-esterases in gull, Larus michahellis (Naumann, 1840) from Northwest Spain: Influence of age, gender, and tissue. Environ Sci Pollut Res 25(2):1533–1542. https://doi.org/10.1007/s11356-017-0630-x
37. Martínez-Abraín A, Merino S, Oro D, Esparza B (2002) Prevalence of blood parasites in two western-Mediterranean local populations of the yellow-legged gull Larus cachinnans michahellis. Ornis Fenn 79:34–40
38. Martínez-Haro M, Beiras R, Bellas J, Capela R, Coelho JP, Lopes I, Moreira- Santos M, Reis-Henriques AM, Ribeiro R, Santos MM, Marques JC (2015) A review on the ecological quality status assessment in aquatic systems using community-based indicators and ecotoxicological tools: what might be the added value of their combination? Ecol Indic 48:8–16. https://doi.org/10.1016/j.ecolind.2014.07.024
39. Masello JF, Choconi RG, Helmer M, Kremberg T, Lubjuhn T, Quillfeldt P (2009) Do leucocytes reflect condition in nestling burrowing parrots Cyanoliseus patagonus in the wild? Comp Biochem Physiol Mol Integr Physiol 152:176–181

40. Mastropaolo W, Yourno J (1981) An ultraviolet spectrophotometric assay for α-naphthyl acetate and α-naphthyl butyrate esterases. Anal Biochem 115(1):188–193
41. Méndez A, Montalvo T, Aymí R, Carmona M, Figuerola J, Navarro J (2020) Adapting to urban ecosystems: unravelling the foraging ecology of an opportunistic predator living in cities. Urban Ecosyst 23(5):1117–1126. https://doi.org/10.1007/s11252-020-00995-3
42. Merino S (1998) *Babesia bennetti* n. sp. from the yellow-legged gull (*Larus cachinnans*, Aves, Laridae) on Benidorm Island, Mediterranean Sea. J Parasitol 84:422–424
43. Mitchell EB, Johns J (2008) Avian hematology and related disorders. Vet Clin North Am Exot Anim Pract 11:501–522
44. Moreau J, Rabdeau J, Badenhausser I, Giraudeau M, Sepp T, Crépin M, Gaffard A, Bretagnolle V, Monceau K (2022) Pesticide impacts on avian species with special reference to farmland birds: a review. Environ Monit Assess 194:790. https://doi.org/10.1007/s10661-022-10394-0
45. Navarro J, Martín-Vélez V, Giménez J, Antonio Garcia J, Cermeño P, Figuerola J, Aymí R, Montalvo T (2024) Ontogeny of foraging behaviour in an opportunistic gull inhabiting urban marine ecosystems. https://doi.org/10.1002/wlb3.01406. Wildl Biol e01406
46. Nos D, Montalvo T, Cortés N, Figuerola J, Aymí R, Giménez J, Solé M, Navarro J (2024) Sources of persistent organic pollutants and their physiological effects on opportunistic urban gulls. J Hazard Mater 465:133129
47. Nwaogu CJ, Amar A, Nebel C, Isaksson C, Hegemann A, Sumasgutner P (2023) Innate immune function and antioxidant capacity of nestlings of an African raptor covary with the level of urbanisation around breeding territories. J Anim Ecol 92:124–141. https://doi.org/10.1111/1365-2656.13837
48. Oliveira CF, Solé M, Martín-Vélez V, Rico M, Montalvo T, Navarro J (2025) Physiological impact of air quality in urban gulls and its implications for their use as pollution sentinels in coastal cities. J Hazard Mater, 140518
49. Oro D, Alsedá L, Hastings A, Genovart M, Sardanyes J (2023) Social copying drives a tipping point for nonlinear population collapse. Proc Natl Acad Sci USA 120(11):e2214055120. https://doi.org/10.1073/pnas.2214055120
50. Ortiz-Catedral L, Brunton D, Stidworthy MF, Elsheikha HM, Pennycott T, Schulze C (2019) Haemoproteus minutus is highly virulent for Australasian and South American parrots. Parasit Vectors 12:40
51. Oudi A, Chokri MA, Hammouda A, Chaabane R, Badraoui R, Besnard A, Santos R (2019) Physiological impacts of pollution exposure in seabird's progeny nesting in a Mediterranean contaminated area. Mar Pollut Bull 142:196–205. https://doi.org/10.1016/j.marpolbul.2019.02.056
52. Ouled-Cheikh J, Morera-Pujol V, Bahillo Á, Ramírez F, Cerdà-Cuéllar M, Ramos R (2021) Foraging in the Anthropocene: Feeding plasticity of an opportunistic predator revealed by long-term monitoring. Ecol Indic 129:107943. https://doi.org/10.1016/j.ecolind.2021.107943
53. Parra J, Tellería JL (2004) The increase in the Spanish population of griffon vulture Gyps fulvus during 1989–1999: effects of food and nest site availability. Bird Conserv Int 14(01). https://doi.org/10.1017/S0959270904000048
54. Parsons NP, Gous TA, Schaefer AM, Vanstreels RET (2016) Health evaluation of African penguins (Spheniscus demersus) in southern Africa. Onderstepoort J Vet Sci 83. https://doi.org/10.4102/ojvr.v83i1.1147
55. Payo-Payo A, Genovart M, Sanz-Aguilar A, Greño JL, García-Tarrasón M, Bertolero A, Piccardo J, Oro D (2017) Colonisation in social species: the importance of breeding experience for dispersal in overcoming information barriers. Sci Rep 7(1):42866. https://doi.org/10.1038/srep42866
56. Peirce MA (2000) A taxonomic review of avian piroplasms of the genus Babesia Starcovici, 1893 (Apicomplexa: Piroplasmorida: Babesiidae). J Nat Hist 34:317–332
57. Peirce MA, Parsons NJ (2012) Babesia ugwidiensis, a new species of avian piroplasm from Phalacrocoracidae in South Africa. Parasite 19:375–379

58. Pollet T (2023) A four-year study reveals the potential role of the soft tick Ornithodoros maritimus in the transmission and circulation of Babesia sp. YLG in Yellow-legged gull colonies. Peer Community Infections, 100082
59. R Core Team (2026) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/
60. Ricklefs RE, Sheldon KS (2007) Malaria prevalence and white-blood-cell response to infection in a tropical and in a temperate thrush. Auk 124:1254
61. Salama Y, Chennaoui M (2024) A critical review of biomarkers in toxicology and risk assessment of environmental pollutants. Ann Environ Sci Toxicol 8(1):6–18
62. Schat KA, Kaspers B, Kaiser P (2014) Avian immunology, 2nd edn. Academic, San Diego
63. Seoane J et al (2011) Urban tolerance and diet specialization determine the responses of birds to urbanization. Urban Ecosyst 14(2):241–259
64. Serafini PP, Dias VHV, Lima D, da Costa DM, da Silveira GEE, Geraldo ME, Deconto VS, Bugoni L, Bainy AC, Lüchmann D K H (2026) A systematic review of aquatic contamination biomarkers in seabirds and their potential for ocean health monitoring. Mar Pollut Bull 225:119234. https://doi.org/10.1016/j.marpolbul.2026.119234
65. Sergent N, Rogers T, Cunningham M (2004) Influence of biological and ecological factors on haematological values in wild Little Penguins, Eudyptula minor. Comp Biochem Physiol Part A 138:333–339
66. Sinkovics C, Seress G, Pipoly I, Vincze E, Liker A (2021) Great tits feed their nestlings with more but smaller prey items and fewer caterpillars in cities than in forests. Sci Rep 11:24161. https://doi.org/10.1038/s41598-021-03504-4
67. Soares L, Ellis VA, Ricklefs RE (2016) Co-infections of haemosporidian and trypanosome parasites in a North American songbird. Parasitology 143:1930–1938
68. Sol D, Lapiedra O, González-Lagos C (2013) Behavioural adjustments for a life in the city. Anim Behav 85(5):1101–1112. https://doi.org/10.1016/j.anbehav.2013.01.023
69. Solé M, Figueres E, Mañanós E, Rojo-Solís C, García-Párraga D (2022) Characterisation of plasmatic B-esterases in bottlenose dolphins (Tursiops truncatus) and their potential as biomarkers of xenobiotic chemical exposures. Environ Pollut 313:120149. https://doi.org/10.1016/j.envpol.2022.120149
70. Solís I, Álvarez E, Barba E (2023) Global warming modifies the seasonal distribution of clutches on a Mediterranean great tit population. Int J Biometeorol 67:367–376. https://doi.org/10.1007/s00484-022-02415-x
71. Sparling DW, Vann S, Grove RA (1998) Blood changes in mallards exposed to white phosphorus. Environ Toxicol Chem 17(12):2521–2529
72. Thrall MA, Weiser G, Allison R, Campbell TW (2012) Veterinary hematology and clinical chemistry, 2nd edn. Wiley-Blackwell
73. Valkiūnas G (2004) Avian Malaria Parasites and other Haemosporidia, 1st edn. CRC. https://doi.org/10.1201/9780203643792
74. Valkiūnas G, Iezhova TA (2017) Exo-erythrocytic development of avian malaria and related haemosporidian parasites. Malar J. 2017;16:101
75. Valkiūnas G, Iezhova TA (2022) Keys to the avian Haemoproteus parasites (Haemosporida, Haemoproteidae). Malar J 21:269. https://doi.org/10.1186/s12936-022-04235-1
76. Van Ngan P, Gomes V, Passos MJA, Ussami KA, Campos DY, Rocha AJDS, Pereira BA (2007) Biomonitoring of the genotoxic potential (micronucleus and erythrocyte nuclear abnormalities assay) of the Admiralty Bay water surrounding the Brazilian Antarctic research station 'Comandante Ferraz'. King George Island Polar Biol 30. https://doi.org/10.1007/s00300-006-0174-x

77. Vanstreels RET, Hurtado R, Snyman A, Roberts DG, Parsons NJ, Pistorius PA (2019) Empirical Primaquine Treatment of Avian Babesiosis in Seabirds. J Avian Med Surg 33(3):258–264
78. Vega A, Yabsley MJ, Hernández SM, Garrett KB, Aguirre JI, Banda E (2025) Assessing Parasite Prevalence and Health Status of the Eurasian Tree Sparrow (Passer montanus) in Green Urban Areas of a Southern European City. Birds 6(3):43. https://doi.org/10.3390/birds6030043
79. Walker CH (2003) Neurotoxic pesticides and behavioural effects upon birds. Ecotoxicology 12:307–316

Figures

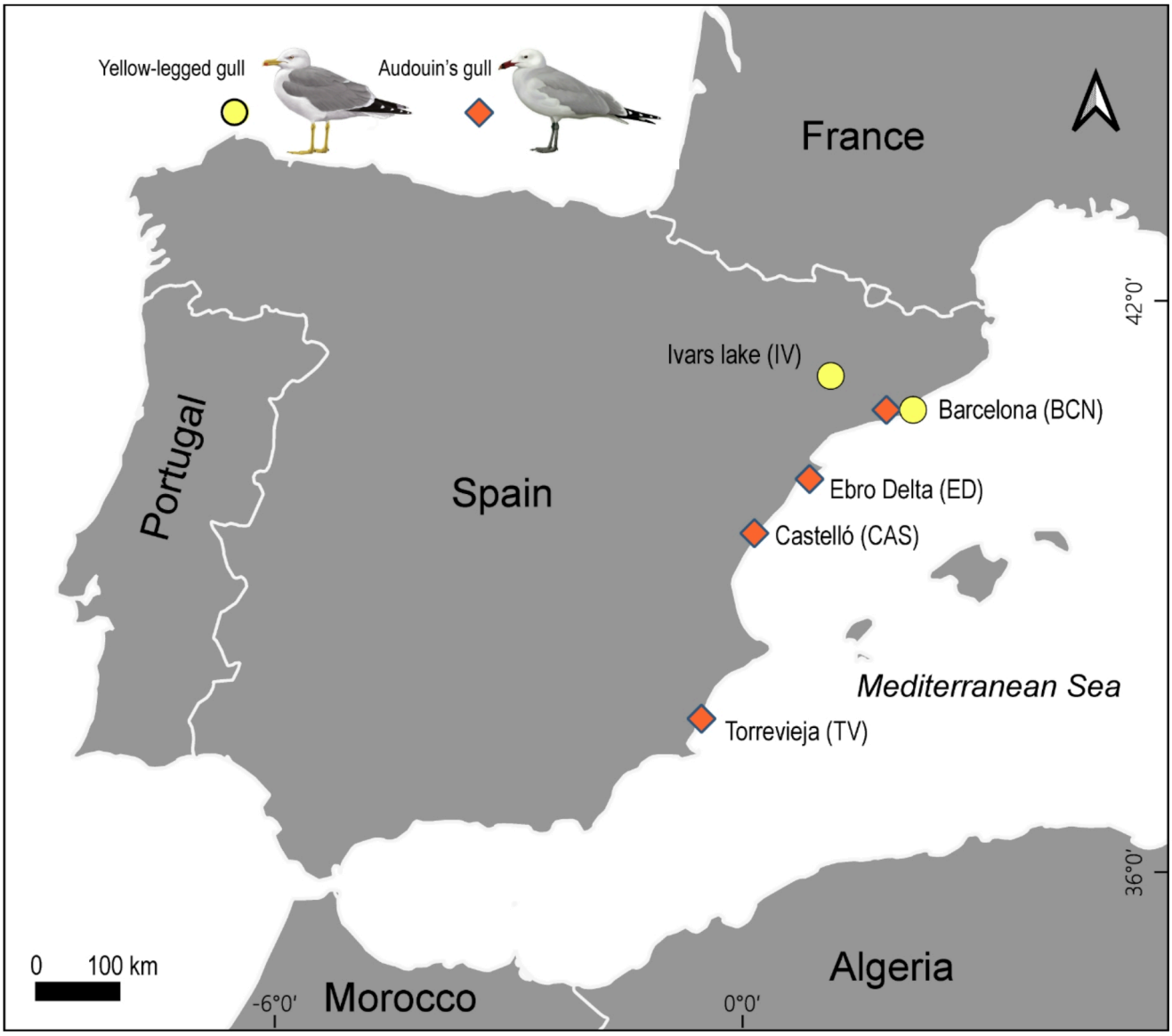


Figure 1

Sampling sites of yellow-legged gull (YLG) and Audouin's gull (AG) along the western Mediterranean coast of Spain. BCN=Barcelona, IV=Ivars Lake, ED=Ebro Delta, CAS=Castelló, TV=Torrevieja. The illustrations of the gulls were made by Martí Franch.

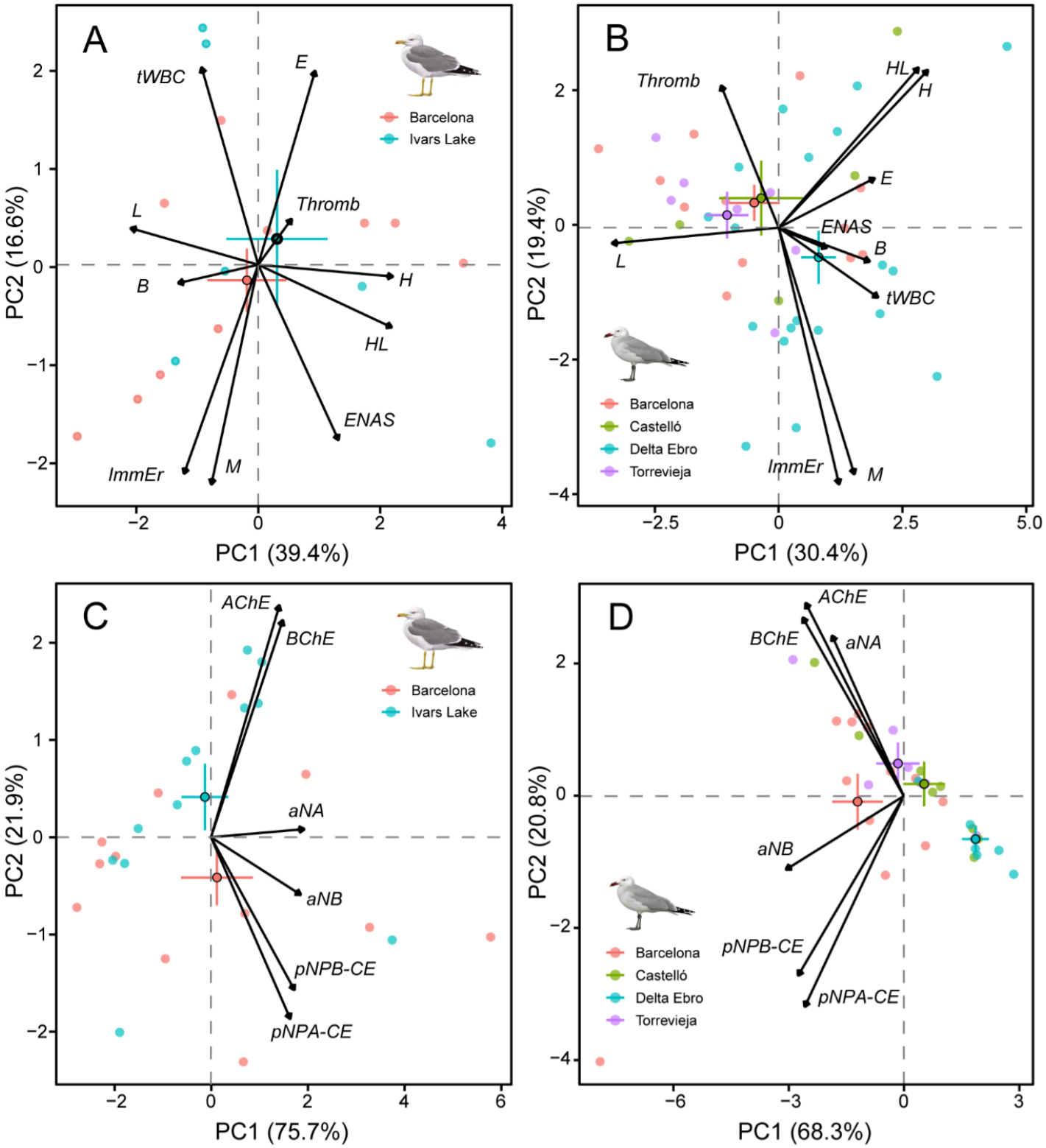


Figure 2

Principal component analyses (PCA) of hematological parameters (A and B) and B-esterase activity (C and D) in YLG and AG. In all plots, sample points represent individuals (colored by locality/population), arrows indicate the direction and relative contribution of variables, and dashed lines represent the PCA axes (PC1 and PC2). The percentages on axes denote the variance explained by each component. Hematological parameters: tWBC (total white blood cells), L (lymphocytes), H (heterophils), E (eosinophils), B (basophils), M (monocytes), thrombocytes, HL ratio (heterophil/lymphocyte ratio), ImmEr (immature erythrocytes), and ENAs (erythrocytes nuclear abnormalities). B-

esterases: AChE (acetylcholine esterase), BChE (butyrylcholine esterase), pNPA-CE (p-nitrophenyl acetate carboxylesterase), pNPB-CE (p-nitrophenyl butyrate carboxylesterase), αNA-CE (α-naphthyl acetate carboxylesterase), and αNB-CE (α-naphthyl butyrate carboxylesterase). The illustrations of the gulls were made by Martí Franch.