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Assessing the impacts of trace element contamination on the physiology and health of seabirds breeding along the western and southern coasts of Portugal^{\star}

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ABSTRACT

Coastal seabirds serve as sentinels of ecosystem health due to their vulnerability to contamination from human activities. However, our understanding on how contaminant burdens affect the physiological and health condition of seabirds is still scarce, raising the uncertainty on the species' vulnerability vs tolerance to environmental contamination. Here, we quantified 15 Trace Elements (TE) in the blood of gull (yellow-legged gull Larus michahellis and Audouin's gull Ichthyaetus audouinii) and shearwater (Cory's shearwater Calonectris borealis) adults, breeding in five colonies along the Portuguese coastline. Additionally, stable isotopes of carbon (δ^{13} C) and nitrogen (δ^{15} N) were quantified to elucidate foraging habitat and trophic ecology of adults, to identify potential patterns of TE contamination among colonies. We used immuno-haematological parameters as response variables to assess the influence of TE concentrations, stable isotope values, and breeding colony on adults' physiological and health condition. Remarkably, we found blood mercury (Hg) and lead (Pb) concentrations to exceed reported toxicity thresholds in 25% and 13% of individuals, respectively, raising ecotoxicological concerns for these populations. The breeding colony was the primary factor explaining variation in five out of six models, underlining the influence of inherent species needs on immuno-haematological parameters. Model selection indicated a negative relationship between erythrocyte sedimentation rate and both Hg and selenium (Se) concentrations, but a positive relationship with $\delta^{13}C$. The number of immature erythrocyte counts was positively related to Hg and Se, particularly in yellow-legged gulls from one colony, highlighting the colony-site context's influence on haematological parameters. Further research is needed to determine whether essential TE concentrations, particularly copper (Cu) and Se, are falling outside the normal range for seabirds or meet speciesspecific requirements. Continuous monitoring of non-essential TE concentrations like aluminium (Al), Hg, and Pb, is crucial due to their potential hazardous concentrations, as observed in our study colonies.

1. Introduction

Over the past century, marine ecosystems have faced cumulative threats due to the worldwide intensification of anthropogenic disturbances. The overexploitation of metal ores for several human activities (mining, metallurgical industry, construction) has led to growing concentrations of trace elements (TEs) in the marine environment (Rauch and Pacyna, 2009; Sen and Peucker-Ehrenbrink, 2012). TEs can readily integrate marine food webs through different mechanisms: *via* adsorption onto suspended particles or dissolved organic matter, *via* their

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uptake by microorganisms and phytoplankton, or directly from the water through gills and skin (Budko and Martynova, 2019; Galindo et al., 2012). Once prey are ingested, TEs can be assimilated through the intestine in marine consumers and be transferred from lower trophic levels to higher trophic levels (Szynkowska et al., 2018). Thus, TEs may undergo bioaccumulation within consumers over time, and some of these elements like mercury (Hg) can also undergo biomagnification throughout food webs (Seco et al., 2021). This process can lead to harmful TE concentrations in top predators, such as seabirds (Bearhop et al., 2000; Campbell et al., 2005).

Seabirds are likely to be contaminated by TEs mainly through their diet (Correia et al., 2023; Fromant et al., 2016). Coastal seabirds are especially vulnerable to contamination by TEs due to increased anthropogenic activities, such as the growing urbanisation and the increase in the number of fishing vessels, which have contributed to the increased release of elements into their foraging habitats (e.g., cadmium (Cd), copper (Cu), nickel (Ni), lead (Pb), zinc (Zn); Richir and Gobert 2016; Richir et al., 2021; Ye et al., 2022). This makes coastal seabirds good for biomonitoring local-scale contamination (Correia et al., 2023; Jouanneau et al., 2022; Thorne et al., 2021). Notably, Hg concentrations have been linked to a higher reliance on marine prey by seabirds (Anderson et al., 2009; Bustamante et al., 2016; Jouanneau et al., 2022). Meanwhile, Pb concentrations have been positively correlated with a higher exposure to urbanised environments (Bauerová et al., 2017; Callendar and Rice, 2000; Hitt et al., 2023). Blood is useful for assessing short-term exposure to contaminants, e.g., during the breeding season (i. e, when seabirds are accessible for sampling; Jouanneau et al., 2022; Laranjeiro et al., 2021), and may be a good proxy of contamination in other internal tissues (see Albert et al., 2019 for Hg). Also, the use of stable isotopes of carbon (δ^{13} C) and nitrogen (δ^{15} N) as proxies of foraging habitat and trophic position, respectively, has proven valuable in unravelling sources of contamination in seabirds, highlighting geographical and species-specific variations in the bioaccumulation of contaminants (Anderson et al., 2010; Carravieri et al., 2020, 2017; Laranjeiro et al., 2020).

While considerable attention has been directed towards blood Hg and its toxicity in seabirds (see the reviews of Ackerman et al., 2016; Chastel et al., 2022), our understanding of the exposure of seabirds to essential TEs - Cu, iron (Fe), selenium (Se), and Zn - or non-essential elements such as Cd and Pb remains limited (but see Carravieri et al., 2020, 2017, 2014; Carvalho et al., 2013; Laranjeiro et al., 2021; Sebastiano et al., 2017, 2016; Voulgaris et al., 2019). Essential TEs play crucial roles in biological processes, within a specific range of concentrations, but can become double-edge swords when deviating from their optimal range. For instance, Se is required as a cofactor for enzymes that fight oxidants, e.g., glutathione peroxidase, which protect organisms against oxidative stress (Gamble et al., 1997). However, excessive levels of Se can be toxic to birds, leading to adverse effects during embryonic development (Spallholz and Hoffman, 2002), impaired immunity, and teratogenesis (Franson et al., 2000; Janz et al., 2010). Additionally, Se is recognized for its capacity to mitigate Hg toxicity by obstructing Hg binding sites, forming an insoluble and inorganic selenous form - mercury selenide - thereby hindering Hg interaction with organic molecules (Manceau et al., 2021; Nigro and Leonzio, 1996). Thus, it is recommended to consider Se alongside Hg, employing the Se:Hg molar ratio, when assessing the impacts of Hg and its associated toxicity risks to wildlife (Carravieri et al., 2017; Cruz-Flores et al., 2024; Goutte et al., 2014a).

Conversely, non-essential TEs can be toxic at very low doses; for instance, seabirds with blood-equivalent Hg concentrations above 0.95 μ g g⁻¹ dry weight (dw) (*i.e.*, 0.2 μ g g⁻¹ wet weight, ww; Ackerman et al., 2016) showed higher rates of egg neglect (Tartu et al., 2015a), a 50% reduction in fledging success (Tartu et al., 2015b), and reduced breeding success in the subsequent year (Goutte et al., 2014a, b). Indeed, several adverse health impacts stemming from excess TE concentrations, beyond Hg, have been documented in terrestrial and aquatic birds (see

Ancora et al., 2008; Pain et al., 2019; Vallverdú-Coll et al., 2019). Some frequently reported sub-lethal effects include immune system disruption (Ibañez et al., 2024; Vallverdú-Coll et al., 2019), physiological impairment (Bauerová et al., 2020, 2017), oxidative stress (Costantini et al., 2014; Espín et al., 2014a, 2014b), or mortality when reaching lethal concentrations (De Francisco et al., 2003; Pain et al., 2019). For instance, higher blood concentrations of arsenic (As), Cd, chromium (Cr), Cu, and Pb were linked to decreased absolute erythrocyte counts and increased haematopoiesis (evidenced by increased immature erythrocyte frequencies) in the great tit Parus major (Bauerová et al., 2017). This may reduce haemoglobin (Hb) concentrations, impairing the oxygen-carrying capacity of adults (Minias, 2015), which was also reported for urban and suburban bird populations (Goodchild et al., 2022; Herrera-Dueñas et al., 2014). In another study, urban-living great tits with increased concentrations of Cd, Pb, and Zn exhibited increased total leukocyte counts and heterophil/lymphocyte ratio (H/L) (Bauerová et al., 2020), a general indicator of stress (Norte et al., 2022) which also increases in response to infection (Davis et al., 2008). Yet, the effects may vary according to the contaminant, the level of exposure, and species-specific tolerance to contaminants (e.g., Espín et al., 2016), underlining that the ecological context of the species/population must be considered.

In this study, we quantified TE concentrations in adults of three seabird species: yellow-legged gull (Larus michahellis), Audouin's gull (Ichthyaetus audouinii), and Cory's shearwater (Calonectris borealis) in five colonies located in the west and south coasts of Portugal, comprising four natural colonies and one urban colony. Yellow-legged gulls are generalist, highly opportunistic, and exhibits a mixed-foraging strategy taking advantage of both marine and terrestrial resources (Calado et al., 2021; Pais de Faria et al., 2021b). Within urban colonies, these gulls heavily rely on anthropogenic-derived resources such as urban waste or items sourced from landfills (Fernandes, 2022; Pais de Faria et al., 2021b). In natural colonies, while they may also forage in nearby landfills, they primarly rely on marine prey, acquired either naturally or through interactions with fishing vessels, i.e., fishery discards (Calado et al., 2021). In contrast, Audouin's gulls and Cory's shearwaters are strict marine foragers, although they exploit different marine habitats: Audouin's gulls predominantly forage within coastal marine habitats (Matos et al., 2018), whereas Cory's shearwaters use both coastal marine (i.e., neritic regions) and oceanic habitats (Paiva et al., 2010; Pereira et al., 2020). Consequently, their diets reflect these differences, with Audouin's gulls primarily consuming pelagic fish, occasionally supplemented by demersal fish sourced from fishery discards (Calado et al., 2021; Matos et al., 2018), while Cory's shearwaters target epipelagic fish, although cephalopods may also constitute a significant part of their diet (Alonso et al., 2014; Paiva et al., 2010).

This study capitalised on the diverse foraging strategies of these three species to investigate TE exposure across geographically proximate seabird colonies and evaluate potential impacts on adults' physiology. The two main objectives were: firstly, to determine the blood TE concentrations in adults across five colonies, compare them with closely related species occupying similar ecological niches, and highlight geographical exposure patterns. Secondly, to evaluate the potential impacts of TEs on adults' physiological condition using general immunohaematological parameters. Using yellow-legged gulls breeding in different colonies facilitated the comparison of TEs contamination between urban vs natural ecological contexts, while Audouin's gulls and Cory's shearwaters enabled comparisons among species, but most importantly, between coastal marine vs oceanic habitats. We expect species and colony to influence blood TE concentrations and immunohaematological parameters. Specifically, yellow-legged gulls breeding in urban environments should have elevated concentrations of Cd, Ni, and Pb which are related to industrial processes, urban runoff, and historical use of leaded gasoline (Ye et al., 2022). These elevated TE concentrations may alter physiological and immune responses, evidenced by decreased Hb concentrations, increased immature

erythrocyte counts, and higher H/L ratios (*e.g.*, Bauerová et al., 2020, 2017). Conversely, we expect higher concentrations of As, Hg, and Se in strictly marine foragers, especially those relying on fishery discards, such as gulls breeding in natural colonies (Calado et al., 2021). Additionally, Cory's shearwaters should exhibit higher Cd concentrations due to the inclusion of cephalopods in their diet (Bustamante et al., 1998). Elevated levels of As, Cd, and Hg concentrations may increase the incidence of genotoxic events, particularly on erythrocytes, leading to nuclear abnormalities. Overall, this study contributes to provide baseline values of haematological parameters in wild seabirds, as well as new information about the TEs toxicity thresholds in these species.

2. Materials and Methods

2.1. Study areas and study species

Fieldwork was carried out during the 2021 breeding season at three locations along the West and South coast of Portugal (Fig. 1): Porto (41°08′N, 8°36′W), a large urban and industrial area, that lies ~8 km from a major fishing harbour (Matosinhos); Berlenga Island (39°24′N, 9°30′W), a small neritic island that lies ~11 km from Peniche, a small city with a medium-sized fishing harbour; Deserta Island (36°57′N, 7°53′W), an uninhabited sandy island sited within the lagoon system of Ria Formosa, Algarve, that lies ~7 km from a large fishing harbour (Olhão). Yellow-legged gull (YLG) breeds in all study sites, although population densities are largely different among breeding colonies: 593–813 breeding pairs in Porto, *ca.* 2400 breeding pairs in Berlenga Island, and *ca.* 540 breeding pairs in Deserta Island (Oliveira et al., 2023). The colony of Porto represented an urban population (Fernandes, 2022), the colony of Deserta represented a coastal population with a pronounced mixed-foraging strategy (Matos et al., 2018), and the colony of Berlenga represented a coastal population but with a marine based foraging strategy (Mendes et al., 2018). Audouin's gull (AG) only breeds in Deserta Island, with a current increase of its breeding population, *ca.* 5340 breeding pairs (ICNF, unpublished data), while Cory's shearwater (CS) only breeds in Berlenga Island and has a current stable population of *ca.* 300 breeding pairs (Oliveira et al., 2020).

2.2. Fieldwork and sample collection

At each breeding colony, incubating gulls were caught using a walking trap placed over the nest (from late April in Deserta Island to early and late May in Berlenga Island and Porto, respectively), while shearwaters were caught by hand at night, during the chick-rearing period (in late August), when adults return to their nesting burrow to feed their chick. All birds handled were ringed (N = 75), weighted using a Pesola Spring© balance (±5 g), measured with a calliper (tarsus, ±1 mm), and a ruler (flattened wing, ±1 mm). A blood sample (0.8–1.2 mL, <2% of the body mass of AG, the smallest species in this study) was collected from the tarsal vein using heparinized syringes coupled to a 25-G needle, transferred into a 1.5 mL microtube, and immediately stored in a cool box. At the end of the day, blood was centrifuged and stored at -20 °C. Additionally, two heparinized capillary tubes of whole blood (~150 µL) were collected for the analysis of immuno-haematological parameters (see below for a more detailed description).



Fig. 1. Locations of the 5 breeding colonies (coloured stars) along the western and southern coasts of Portugal. Symbols: orange star, yellow-legged gull; pink star, Cory's shearwater; blue star, Audouin's gull. Photo credits M.I. Laranjeiro. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

2.3. Trace elements analysis

Blood cells (hereafter 'blood') were freeze-dried and used to measure total mercury (Hg) and 14 other elements at the laboratory Littoral, Environment and Societies (LIENSs, CNRS-La Rochelle Université). Total Hg was quantified with an Altec AMA 254 spectrophotometer (N = 75, aliquots mass: 0.86 \pm 0.69 mg dw). Samples were analysed in duplicate-triplicate until reaching a relative standard deviation < 10%(Bustamante et al., 2006). TORT-3 lobster hepatopancreas (Hg certified concentration: 0.292 \pm 0.022 $\mu g~g^{-1}$ dw) was used as Certified Refer ence Material (CRM) to check the accuracy of the method at each 15 samples. The measured values were 0.287 \pm 0.004 $\mu g~g^{-1}$ dw (N = 8), thus corresponding to a recovery rate of 99.1 \pm 1.5%. Blanks were run at the beginning of each set of samples and the limit of detection (LoD) was 0.1 ng. Aluminium (Al), As, Cd, cobalt (Co), Cr, Cu, Fe, manganese (Mn), Ni, Pb, Se, tin (Sn), strontium (Sr), and Zn were analysed by Inductively Coupled Plasma (ICP) Mass Spectrometry (Thermo Fisher Scientific X Series 2 ICP-MS) and ICP-Optical Emission Spectrometry (Agilent Technologies 5800 VDV ICP-OES) (aliquot mass: 80–200 mg, N = 75) following Kojadinovic et al. (2011). Briefly, prior to analysis, blood samples were microwave digested in a mixture of 3 mL of suprapure nitric acid (HNO₃) and 1 mL of suprapure chloridric acid (HCl), and then diluted to 25 mL with deionized water. Accuracy and reproducibility of the preparation was tested through analytical blanks and replicates of CRMs (TORT-3 Lobster Hepatopancreas and DOLT-5 Dogfish Liver, both NRC, Canada) along each set of samples. Recovery rates of CRMs ranged from 84 to 101% more specifically, equal to 96 \pm 11% for Al, 95 \pm 6% for As, 92 \pm 2% for Cd, 88 \pm 4% for Co, 97 \pm 5% for Cr, 101 \pm 4% for Cu, 94 \pm 4% for Fe, 97 \pm 7% for Mn, 92 \pm 3% for Ni, 84 \pm 3% for Pb, $100\pm8\%$ for Se, 88 \pm 7% for Sn, 90 \pm 8% for Sr, and 101 \pm 2% for Zn. Results are given as mean \pm SD in $\mu g~g^{-1}$ dw.

2.4. Stable isotopes analysis

Freeze-dried blood samples were weighted (between 0.2 and 0.4 mg dw, N = 71) with a microbalance and packed into tin capsules for combustion. Relative abundances of δ^{13} C and δ^{15} N were measured using a continuous flow mass spectrometer (Delta V Plus with a Conflo IV interface, Thermo Scientific, Bremen, Germany) coupled to an elemental analyser (Thermo Scientific EA 1112) at the laboratory LIENSs. Results were expressed using the delta (δ) notation and computed using the following equation: $\delta X = [(R_{Sample} / R_{Standard}) -1] \times 1000$, where X stands for ¹³C or ¹⁵N, and R being the respective ratio, *i.e.*, ¹³C/¹²C or ¹⁵N/¹⁴N. Standard values correspond to Vienna PeeDee Belemnite and atmospheric N₂ for carbon and nitrogen, respectively. Replicate measurements of reference materials (USGS-61 and USGS-63, US Geological Survey) were used to check the accuracy of the isotopic results, and analytical precision was <0.10 ‰ for both δ^{13} C and δ^{15} N values.

2.5. Immuno-haematological parameters analysis

Erythrocyte sedimentation rate (ESR) was measured by collecting one heparinized capillary tube filled with whole blood and stored vertically at 4 $^{\circ}$ C for 4 h (Heylen and Matthysen, 2008). ESR is the ratio of the volume of the capillary tube not occupied by erythrocytes and the total blood volume in the tube (Heylen and Matthysen, 2008).

Haemoglobin (Hb) concentration (g dL⁻¹) was measured in whole blood (20 μ L) following Pais de Faria et al. (2022) using the Haemoglobin Assay commercial kit obtained from SPINREACT according to the manufacturer's instructions. Hb analyses were conducted at the Marine and Environmental Sciences Centre laboratory (MARE, University of Coimbra).

A small drop of whole blood was smeared on a slide in the field, fixed in methanol (99% vol) for 3 min within 4 h after collection, left to dry at room temperature (20 °C), and stained with the Giemsa procedure (Palhares and Grisolia, 2002). The analysis of each blood smear was carried in a two-step's procedure: identify monolayer fields with similar density of well-stained erythrocytes under 400× magnification; proceed to the identification and counts of leukocytes (i.e., WBC), immature erythrocytes (IE), and erythrocyte nuclear abnormalities (ENA) under 1000× magnification following Zuñiga-González et al. (2000). WBC were classified as basophiles, eosinophils, heterophils, lymphocytes, and monocytes, according to nuclear size, lobules, and colouration intensity and/or granularity, and overall cell dimension (Fokidis et al., 2008; Mallory et al., 2015; Norte et al., 2008). WBC was estimated by counting the number of leukocytes per 10,000 erythrocytes (Thrall et al., 2012), and the ratio between heterophil/lymphocyte (H/L) was calculated in a total of 100 WBC (Davis et al., 2008). IE were identified based on their less oval to round nuclei and polychromatic cytoplasm. ENA were classified as bilobated/binucleated, budding, kidney-shape, micronucleus, notched, nucleoplasmatic bridge, tailed, vacuolated nucleus, and unknown nuclear malformation (Pacheco and Santos, 1996; Skarphedinsdottir et al., 2010). The sum of total ENA was calculated for each blood smear. IE and ENA were estimated per 10,000 erythrocytes. Blood smears were examined under a Leica DMLS microscope at the laboratory MARE by a single operator to mitigate bias linked with different operators. For more details about the aim of each parameter analysed consult Table S1 in Supplementary Material.

2.6. Data analysis

First, to explore contaminant co-variance and to identify the TEs reflecting most of the total variance of data, a linear discriminant analysis (LDA) was computed on normalised and scaled TEs with concentrations above the limit of quantification (LOQ) in at least 70% of the total number of individuals. Concentrations below this threshold were replaced with a half of the LOQ value (LOQ/2) and included in the analysis (Hites, 2019). Thus, Cd, Co, Cr, Ni, Sn, and Sr were excluded from the LDA. Replacements occurred for 15 individuals for Pb (20%) and one individual for As (1.3%). δ^{13} C and δ^{15} N values were also added to LDA to help disentangling patterns of TE concentrations among species breeding on the different study sites. δ^{13} C was used to explore TEs relationships with the feeding habitat, as an indicator of the use of terrestrial or oceanic habitats and pelagic food webs (lower δ^{13} C values) vs. exploitation of marine or coastal habitats and benthic food webs (higher δ^{13} C values) (Newsome et al., 2007); while δ^{15} N was used to identify co-variance of TEs with trophic level (Carravieri et al., 2017). Since δ^{13} C has a trophic component, a linear regression was carried out between blood $\delta^{13}{\rm C}$ and $\delta^{15}{\rm N}$ with species/colony as a fixed categorical variable (F_{5, 65} = 80.5, p < 0.001, $r^2 = 0.85$; Fig. S1 in Supplementary Material), and the residuals were then used instead of raw δ^{13} C values (Ceia et al., 2012). All predictors (Al, As, Cu, Fe, Hg, Mn, Pb, Se, Zn, δ^{15} N, and residual δ^{13} C) were inspected for multicollinearity issues prior to LDA, through the calculation of the variation inflation factor (VIF > 2) and spearman correlation coefficients ($r_s > 0.50$) under the usdm R package (Naimi, 2017). This inspection resulted in the exclusion of Cu, Zn, and δ^{15} N from discriminant analysis (Table S2 and Table S3 in Supplementary Material). The Se:Hg molar ratio was calculated for all individuals according to Cruz-Flores et al. (2024), although not included in LDA since it is highly correlated to Hg and Se concentrations. Since only YLG breeds in all the three study species, the lack of overlapping breeding sites for AG and CS prevents simultaneous comparisons across species and colonies. Consequently, we opted to create five categories based on the species and study site (hereafter 'colony-site' factor). One-way ANOVAs or Kruskal-Wallis were computed to compare the concentrations of each TE, Se:Hg molar ratios, and stable isotopes among colony-sites followed by post-hoc Tukey tests or pairwise tests on significant results (significance level of α < 0.05, Table 1). *P*-values were adjusted using the Bonferroni correction to counteract potential Type I errors.

To facilitate comparisons with published data, we used a conversion factor of 4.76 by multiplying the wet mass concentrations to obtain the

Table 1

Trace elements (TEs) concentrations (μ g g⁻¹ dw) measured in adults' blood, Se:Hg molar ratio, stable isotope values (δ^{13} C and δ^{15} N, \ll) and Bayesian estimation of standard ellipses (SEA_B; 95% confidence intervals, CI), and immuno-haematological parameters calculated for each colony. Values are mean \pm SD and quantifiable sample sizes (N). Differences in TEs concentrations and stable isotopes were evaluated using one-way ANOVA or Kruskal-Wallis followed by post-hoc Tukey or pairwise tests are indicated by different letters ($\alpha < 0.05$). Cr and Sn were not tested for differences due to low detection frequency (<10 adults in total).

| | Cory's shearwater (CS) | | Audouin's gull (AG) | | Yellow-legged gull (YLG) | | | | | |
|---------------------------------|------------------------|-----------------------------|---------------------|-----------------------------|--------------------------|---------------------------|----------|---------------------------------------|------|-------------------------|
| | | | | | Porto | | Berlenga | | Dese | rta |
| | N | $\text{Mean} \pm \text{SD}$ | N | $\text{Mean} \pm \text{SD}$ | N | $\text{Mean}\pm\text{SD}$ | N | $\text{Mean} \pm \text{SD}$ | N | $Mean \pm SD$ |
| Trace elements ($\mu g g^{-1}$ | dw) | | | | | | | | | |
| Al | 15 | 6.60 ± 3.25^a | 15 | 7.62 ± 3.34^{ab} | 15 | 10.28 ± 2.79^{bc} | 15 | $11.36\pm4.11^{\rm c}$ | 15 | 9.12 ± 2.50^{abc} |
| As | 15 | 1.32 ± 0.68^{a} | 15 | 6.40 ± 4.84^{c} | 14 | 1.69 ± 2.52^{ab} | 15 | $\textbf{7.48} \pm \textbf{2.25}^{c}$ | 15 | 5.27 ± 5.69^{bc} |
| Cd | 14 | 0.047 ± 0.024^{b} | 9 | 0.013 ± 0.003^{a} | 2 | 0.022 ± 0.008^{ab} | 12 | 0.029 ± 0.016^{ab} | 15 | All < 0.01 |
| Со | 4 | 0.23 ± 0.15^a | 3 | $0.23\pm0.02^{\rm a}$ | 2 | 0.01 ± 0.01^{a} | 2 | 0.02 ± 0.01^a | 7 | 0.02 ± 0.01^a |
| Cr | 5 | 0.52 ± 0.45 | 15 | All < 0.10 | 15 | All < 0.20 | 1 | 0.79 | 1 | 0.12 |
| Cu | 15 | 1.69 ± 0.61^a | 15 | $2.44\pm0.37^{\rm b}$ | 15 | $3.27\pm0.69^{\rm c}$ | 15 | 3.24 ± 0.80^c | 15 | $3.17\pm0.53^{\rm c}$ |
| Fe | 15 | 2264 ± 76^b | 15 | 2328 ± 99^{b} | 15 | 2097 ± 213^a | 15 | $2300\pm117^{\rm b}$ | 15 | 2048 ± 149^a |
| Hg | 15 | $2.08\pm0.52^{\rm b}$ | 15 | $6.58\pm2.71^{\rm c}$ | 15 | 1.08 ± 0.75^{a} | 15 | $2.69\pm1.12^{\rm b}$ | 15 | $5.63\pm2.73^{\rm c}$ |
| Mn | 15 | 0.15 ± 0.11^a | 15 | 0.10 ± 0.04^{a} | 15 | 0.15 ± 0.06^a | 15 | $0.11\pm0.03^{\rm a}$ | 15 | $0.11\pm0.03^{\rm a}$ |
| Ni | 6 | 0.34 ± 0.25^a | 2 | 0.08 ± 0.05^a | 1 | 0.09 ^a | 3 | 0.42 ± 0.59^a | 3 | 0.20 ± 0.10^a |
| Pb | 9 | 0.03 ± 0.02^a | 8 | $0.03\pm0.02^{\rm a}$ | 15 | $0.83\pm0.82^{\rm b}$ | 13 | 0.04 ± 0.04^a | 15 | 0.07 ± 0.06^a |
| Se | 15 | $56.2 \pm \mathbf{17.9^d}$ | 15 | $56.8 \pm 12.7^{\rm d}$ | 15 | $6.6\pm3.9^{\rm a}$ | 15 | $20.1\pm9.0^{\rm b}$ | 15 | $31.3 \pm 12.9^{\rm c}$ |
| Sn | 15 | All < 0.03 | 2 | 0.10 ± 0.06 | 15 | All < 0.04 | 15 | All < 0.04 | 15 | All < 0.02 |
| Sr | 5 | 0.08 ± 0.03^a | 7 | 0.09 ± 0.05^a | 6 | 0.11 ± 0.04^{a} | 13 | 0.15 ± 0.10^a | 12 | 0.11 ± 0.05^a |
| Zn | 15 | 23.5 ± 4.5^a | 15 | $25.2\pm3.1^{\rm a}$ | 15 | $25.5\pm3.0^{\rm a}$ | 15 | 30.2 ± 8.4^{a} | 15 | 25.0 ± 2.2^a |
| Se:Hg | 15 | $73.6\pm31.9^{\rm b}$ | 15 | $27.1\pm14.9^{\rm a}$ | 15 | 19.4 ± 11.9^{a} | 15 | 20.2 ± 8.8^a | 15 | $18.4 \pm 13.1^{\rm a}$ |
| Stable isotopes | | | | | | | | | | |
| δ^{13} C (‰) | 13 | $-19.2\pm0.4^{\rm b}$ | 15 | $-19.4\pm0.4^{\rm b}$ | 15 | -20.9 ± 1.1^a | 13 | $-19.4\pm0.5^{\rm b}$ | 15 | $-18.8\pm1.0^{\rm b}$ |
| δ^{15} N (‰) | 13 | $12.1\pm0.5^{\rm b}$ | 15 | $11.6\pm0.4^{\rm b}$ | 15 | 9.3 ± 1.1^{a} | 13 | $13.5\pm0.5^{\rm c}$ | 15 | $12.2\pm1.0^{\rm b}$ |
| SEA _B [95% CI] | 13 | 0.57 ± 0.17 | 15 | 0.26 ± 0.07 | 15 | 3.20 ± 0.89 | 13 | 1.08 ± 0.33 | 15 | 2.20 ± 0.61 |
| | | [0.27-0.90] | | [0.14-0.40] | | [1.71-4.92] | | [0.53–1.68] | | [1.16-3.38] |
| Immuno-haematological | param | eters | | | | | | | | |
| ESR (Pi) | 13 | 0.30 ± 0.14 | 15 | 0.14 ± 0.05 | 15 | 0.05 ± 0.03 | 13 | 0.08 ± 0.05 | | - |
| Hb (g dL^{-1}) | 13 | 12.16 ± 3.34 | 12 | 10.47 ± 1.41 | 15 | 11.91 ± 2.11 | 12 | 9.96 ± 1.53 | 7 | 9.37 ± 3.52 |
| H/L ratio | 13 | 1.27 ± 0.49 | 14 | 1.02 ± 0.72 | 13 | 1.17 ± 0.47 | 13 | 1.76 ± 0.71 | 15 | 1.61 ± 0.75 |
| WBC (counts) | 13 | 79 ± 10 | 14 | 61 ± 15 | 13 | 71 ± 12 | 13 | 69 ± 14 | 15 | 65 ± 13 |
| IE (counts) | 13 | 215 ± 68 | 13 | 133 ± 38 | 11 | 166 ± 73 | 13 | 170 ± 75 | 15 | 154 ± 51 |
| ENA (counts) | 13 | 12.3 ± 6.5 | 13 | 0.9 ± 1.1 | 11 | 5.7 ± 3.3 | 12 | 0.9 ± 0.9 | 15 | 1.9 ± 2.2 |

dry mass equivalents. This assumption is based on an estimated humidity of approximately 79% in wet tissues such as blood, as indicated by Eagles-Smith et al. (2008). These results must be interpreted cautiously, considering that the conversion factor may vary among species and individuals.

Second, to explore the occurrence of patterns on immunohaematological parameters among colony-sites, a second LDA was carried out using those quantified in at least 50% of the individuals. ESR was not included in the LDA because it was not quantified in YLG breeding in Deserta. One individual was excluded from this LDA because none of immuno-haematological parameters was quantified. Isolated missing values were replaced by the average value computed using the available values for each colony-site, corresponding to 16% of missing values for Hb, 10% for IE and ENA counts, and 4% for H/L ratio and WBC counts. Since the first two axes of the LDA explained more than 95% of total variance of immuno-physiological parameters among colony-sites (see Fig. S2 and Table S4 in Supplementary Material), this factor was added as a predictor in subsequent regression-type analyses (see below).

Third, univariate analyses (linear or generalized linear models, GLM) were used to test the effect of the colony-site (fixed categorical variable with five levels), LD scores of axes 1 and 2 extracted from LDA of TE and stable isotopes (fixed continuous variables), and their interactions on each immuno-haematological parameter – ESR, Hb, H/L, WBC, IE, and ENA (response variables) – in a total of six models (Table S5 in Supplementary Material). Full models were adjusted to the family data distribution (*e.g.*, Gaussian, Gamma (log link function), Poisson (log link function), Negative binomial) and the shearwater was used as the reference colony-site, as the representative for coastal and oceanic habitats. Models were checked for residuals normality, homoscedasticity, and outliers using 'check_model' function within the *performance* R

package (Lüdecke et al., 2021), before going through model selection. Overdispersion was checked to ensure the appropriateness of the chosen modelling approach in regression models of variables with Poisson distribution, i.e., counting data variables (WBC, IE, and ENA). Whenever overdispersion was observed, the negative binomial family was chosen and adjusted for skewness with θ shape parameter (Zuur et al., 2009). Models were run using the 'dredge' function within the MuMIn R package (Barton, 2023), and model selection was carried out following the Akaike's Information Criterion corrected for small sample sizes (AICc) to identify the most parsimonious models (Δ AIC < 2; Burnham and Anderson, 2002). AICc weights - representing the normalised weight of evidence in support of a particular model - along with the explained deviance (reflecting the proportion of variation explained by the model, Zuur et al., 2009) were employed for deducing the effect of explanatory variables on the response variables (Burnham and Anderson, 2002). When several models outperformed the null model and had Δ AICc < 2, we employed model averaging for inference. This process generated averaged parameter estimates (β) for the predictor variables included in the models, weighted by their respective AICc weights. If the null model ranked as the optimal model, all explanatory variables were statistically deemed non-significant. Predicted values and confidence intervals (CI) were extracted from the most parsimonious model or average models using the 'ggpredict' function within the ggeffects R package (Lüdecke, 2018) and plotted using ggplot2 R package (Wickham et al., 2023). Candidate models with both significant and non-significant effects are presented in Table 2.

Fourth, scaled and centred isotopic data were employed with the *SIBER* R package – Stable Isotope Bayesian Ellipses in R – to calculate Bayesian estimates of standard ellipse areas (SEA_B) and draw bivariate standard ellipses for each colony (Jackson et al., 2011). For visualisation purposes, standard ellipse areas corrected for small sample sizes (SEA_C)

Table 2

Summary outputs including model specification, sample size, and Akaike's Information Criteria (AIC). This table includes all the six response variables tested showing both significant (in bold) and non-significant effects. Only up to the best ranked models and the null model are shown. The best model outputs are specified when only one model was selected, while model average (full average) outputs are specified ($\beta \pm$ SE [95% CI]) when two or more models were selected (Δ AICc < 2). Abbreviations: LD1 and LD2, scores on axis 1 and 2 of a LDA of TEs (Al, As, Fe, Hg, Mn, Pb, and Se) and residual carbon isotopes (residual δ^{13} C); *k*, number of parameters; AICc, Akaike's information criterion corrected for small sample sizes; Δ AICc, difference between AICc of the specific model and the best model; *w_i*, AICc weights; Exp. Dev., explained deviance; AG, Audouin's gull; YLG, yellow-legged gull; BER, Berlenga Island; DES, Deserta Island; POR, Porto.

| | 1 | | | | |
|-----------------------------------------------------------|-----------------------------------------------------------------|---------|-------------------|-----------|-----------------|
| Immuno-naematological parameter | ĸ | AICC | ⊿AICc | Wi | Exp. Dev. |
| Erythrocyte sedimentation rate (ESR) (N = 53) | | | | | |
| GLM Gamma family ('log' link) | | | | | |
| | | 154.0 | 0 | 0.55 | 0.66 |
| Colony + LDI + LD2 | 6 | -176.3 | 0 | 0.55 | 0.66 |
| Null model | | -128.9 | 4/.42 | 0 | 0 |
| Intercent | $p \pm 3E [95\% G]$ | z value | p (> z) | *** | |
| ColonyAC | -1.21 ± 0.19 [-1.60 [0 -0.78] | -0.45 | p < 0.001 | | |
| ColonyVIC BEP | $-0.29 \pm 0.20 [-0.08 - 0.10]$ | -1.42 | 0.10 | * | |
| ColonyVIC DOP | $-0.92 \pm 0.37 [-1.72 - 0.13]$ | 2.30 | 0.02 | *** | |
| LD1 | $-2.93 \pm 0.40 [-3.81 - 2.00]$ $-0.18 \pm 0.07 [-0.320.03]$ | -2.62 | p < 0.001 0.02 | * | |
| LD2 | -0.10 ± 0.07 [-0.32 = -0.03] | 3.53 | n < 0.02 | *** | |
| | 0.20 - 0.07 [0110 0.00] | 0.00 | p (01001 | | |
| Haemoglobin concentration (Hb) ($N = 58$) | | | | | |
| Linear model (Gaussian family) | | | | | |
| Colony | 5 | 648.4 | 0 | 0.38 | 0.21 |
| Colony + LD2 | 6 | 648.8 | 0.41 | 0.31 | 0.23 |
| Null model | 2 | 655.8 | 7.46 | 0.01 | 0 |
| Model average (full average) | $\beta \pm SE$ [95% CI] | z value | p (> z) | | |
| Intercept | $122.30 \pm 6.29 \; [109.98 134.61]$ | 19.46 | p < 0.001 | *** | |
| ColonyAG | -17.54 ± 8.52 [-34.250.84] | 2.06 | 0.04 | * | |
| ColonyYLG BER | -28.03 ± 12.83 [-53.182.88] | 2.18 | 0.03 | * | |
| ColonyYLG DES | -27.55 ± 8.48 [-44.1710.93] | 3.25 | 0.001 | ** | |
| ColonyYLG POR | -0.36 ± 9.12 [-18.23 – 17.52] | 0.04 | 0.97 | | |
| LD2 | -1.66 ± 2.60 [-9.08 – 1.66] | 0.64 | 0.52 | | |
| Heterophil/lymphocyte ratio (H/L) (N = 68) | | | | | |
| GLM Gaussian ('log' link) | | | | | |
| Colony ^a | 5 | 95.8 | 0 | 0.38 | 0.20 |
| $Colony + 1D1^{a}$ | 5 | 95.8 | 1.97 | 0.38 | 0.20 |
| Colony $+$ LD2 ^a | 6 | 97.2 | 1.07 | 0.19 | 0.21 |
| Null model | 2 | 102.3 | 6.51 | 0.19 | 0.21 |
| | - | 102.0 | 0.01 | 0.01 | 0 |
| White Blood Cells counts (WBC) ($N = 68$) | | | | | |
| GLM negative binomial | | | | | |
| Colony | 5 | 572.4 | 0 | 0.51 | 0.20 |
| Null model | 2 | 578.5 | 6.17 | 0.02 | 0 |
| | $\beta \pm SE$ [95% CI] | z value | p (> z) | | |
| Intercept | $4.38 \pm 0.05 \ [4.28 - 4.47]$ | 89.95 | p < 0.001 | *** | |
| ColonyAG | -0.27 ± 0.07 [-0.400.13] | -3.93 | <i>p</i> < 0.001 | *** | |
| ColonyYLG BER | -0.14 ± 0.07 [-0.28–0.01] | -2.05 | 0.04 | * | |
| ColonyYLG DES | -0.20 ± 0.07 [-0.330.06] | 2.90 | 0.004 | ** | |
| ColonyYLG POR | -0.12 ± 0.07 [-0.25 – 0.02] | -1.73 | 0.08 | | |
| Immature erythrocytes (IE) (N = 61) | | | | | |
| GLM negative binomial | | | | | |
| Colony + LD1 + LD2 + Colony:LD1 | 11 | 704.5 | 0 | 0.25 | 0.41 |
| Colony + LD1 + Colony:LD1 | 10 | 705.2 | 0.40 | 0.17 | 0.38 |
| Colony + LD1 + LD2 + Colony: LD1 + Colony: LD2 + LD1: LD2 | 16 | 705.6 | 1.85 | 0.14 | 0.54 |
| Null model | 2 | 709.0 | 4.58 | 0.02 | 0 |
| Model average (full average) ^b | $\beta \pm SE$ [95% CI] | z value | p (> z) | | |
| Intercept | $5.29 \pm 0.10 \; [5.09 – 5.49]$ | 51.79 | p < 0.001 | *** | |
| ColonyAG | $-0.05 \pm 0.34 \; [\text{-}0.67 - 0.61]$ | 0.15 | 0.88 | | |
| ColonyYLG BER | -0.23 ± 0.22 [-0.71 – 0.21] | 1.01 | 0.31 | | |
| ColonyYLG DES | -0.47 ± 0.13 [-0.73–0.21] | 3.56 | p < 0.001 | *** | |
| ColonyYLG POR | $-0.33 \pm 0.31 [\text{-}0.93 - 0.28]$ | 1.06 | 0.29 | | |
| ColonyAG:LD1 | -0.13 ± 0.13 [-0.39 – 0.13] | 0.98 | 0.33 | | |
| ColonyYLG BER:LD1 | -0.10 ± 0.16 [-0.41 – 0.21] | 0.62 | 0.53 | | |
| ColonyYLG DES:LD1 | 0.31 ± 0.09 [0.14–0.48] | 3.60 | <i>p</i> < 0.001 | *** | |
| COLONYYLG POR:LD1 | 0.01 ± 0.10 [-0.20 – 0.21] | 0.07 | 0.95 | | |
| Erythrocyte nuclear abnormalities (ENAs) (N = 59) | | | | | |
| GLM negative binomial | | | | | |
| Colony | 5 | 269.8 | 0 | 0.41 | 0.63 |
| Colony + LD1 | 6 | 270.4 | 0.68 | 0.29 | 0.64 |
| INUII MODEL | 2 | 323.2 | 53.47 | 0 | U |
| | | | | (continue | d on next page) |

Table 2 (continued)

| Immuno-haematological parameter | k | AICc | ⊿AICc | Wi | Exp. Dev. |
|---------------------------------|-------------------------------------------|---------|-----------|-----|-----------|
| Model average (full average) | $\beta \pm SE$ [95% CI] | z value | p (> z) | | |
| Intercept | $2.29 \pm 0.15 \; [2.00 – 2.57]$ | 15.73 | p < 0.001 | *** | |
| ColonyAG | -1.61 ± 0.24 [-2.081.13] | 6.67 | p < 0.001 | *** | |
| ColonyYLG BER | -1.70 ± 0.30 [-2.271.11] | 5.72 | p < 0.001 | *** | |
| ColonyYLG DES | -1.20 ± 0.20 [-1.600.80] | 5.85 | p < 0.001 | *** | |
| ColonyYLG POR | -0.52 ± 0.30 [-1.11 - 0.08] | 1.71 | 0.09 | | |
| LD1 | $-0.03 \pm 0.05 \; [\text{-}0.19 - 0.04]$ | 0.60 | 0.55 | | |

^a There were no significant predictors in model averaging (full average).

^b LD1, LD2, their interaction, and Colony:LD2 were not significant (p > 0.05).

were computed using 40% of the data (Fig. S3 in Supplementary Material). Data exploration, visualisation, and model computations were carried out in R version 4.3.1 (R Core Team, 2023).

3. Results

3.1. TE concentrations and stable isotopes

Among the 15 targeted TEs, essential elements including Cu (overall range: 1.18–4.92 µg g⁻¹ dw), Fe (1608–2501 µg g⁻¹ dw), Mn (0.07–0.53 µg g⁻¹ dw), Se (2.2–89.5 µg g⁻¹ dw), Zn (18.8–57.0 µg g⁻¹ dw), and nonessential TEs like Al (2.40–18.27 µg g⁻¹ dw) and Hg (0.30–15.47 µg g⁻¹ dw) were detected in all individuals (Table 1). The highest concentrations of Cu were found in YLG populations and the lowest in CS, while Al concentrations were higher in YLG breeding in Berlenga rather than in CS (ANOVA, F_{4, 70} = 5.29, *p* = 0.02). Se concentrations were higher in AG and in CS compared with YLGs, while Hg concentrations were the highest in AG and YLG breeding in Deserta and the lowest in YLG breeding in Porto (38.67 < F_{4, 70} = 15.83, *p* < 0.001). Se:Hg molar ratio was the highest in CS (F_{4, 70} = 15.83, *p* < 0.001; Table 1) and not different among the other colonies (*p* > 0.05). As concentrations (0.13–18.45 µg g⁻¹ dw) were detected in all individuals, apart from one YLG breeding in Porto and were higher in AG and YLG breeding in Berlenga compared with CS and YLG breeding in Porto (F_{4, 69} = 8.38, *p*



Fig. 2. Biplot of adult scores extracted by LDA on the two principal axes (LD1 and LD2) with log-transformed and scaled trace elements (TEs) and stable isotopes (residual δ^{13} C and δ^{15} N) measured in adults' blood of gulls and shearwaters breeding along the western and southern coasts of Portugal. Ellipses include 70% of data. δ^{15} N, Cu, and Zn were excluded due to multicollinearity issues (VIF >2, $r_s > 0.50$) and As was removed from the plot for a clearer representation. Dashed lines indicate the axes where LD1 and LD2 are equal to zero. Abbreviations: AG, Audouin's gull; CS, Cory's shearwater; YLG: yellow-legged gull; BER, Berlenga Island; DES, Deserta Island; POR: Porto. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

< 0.001). The detection frequencies of Cd (0.010–0.099 µg g⁻¹ dw), Pb (0.01–2.63 µg g⁻¹ dw), and Sr (0.04–0.46 µg g⁻¹ dw) varied widely among colonies (Table 1). Cd was higher in CS and not detected in YLG breeding in Deserta, while Pb concentrations were the highest in YLG breeding in Porto (Kruskal-Wallis, $\chi^2 = 47$, df = 4, p < 0.001). Co, Cr, and Ni were detected in low concentrations in a few individuals, while Sn was only detected in two AGs (Table 1).

Blood δ^{13} C and δ^{15} N values varied widely among adults from different colonies (ANOVA, F_{4, 66} = 18.1 and 59.7, respectively for carbon and nitrogen, both p < 0.001). Notably, urban-dwelling YLGs and YLGs breeding in Berlenga occupied distinct isotopic niches compared to the other colonies, as evident from non-overlapping standard ellipses (Table 1, Figs. S3–A in Supplementary Material). Urban YLGs and those breeding in Deserta exhibited the broadest isotopic niches, while the strictly marine AG and CS exhibited narrower niches (Figs. S3–B in Supplementary Material).

3.2. TE and isotopic patterns in the ordination space

Among the 7 TEs (Al, As, Fe, Hg, Mn, Pb, and Se) and the residual δ^{13} C values included in the LDA, Hg and Se contributed most to LD1, while the residual δ^{13} C values contributed most to LD2 (Table S6 in Supplementary Material). The LDA highlighted a clear segregation of urban YLGs along axis 1, strongly associated to the lower blood Hg and Se concentrations in this population. On the other hand, YLG breeding in Berlenga was clearly segregated along axis 2, strongly associated to the



Fig. 3. Predicted erythrocyte sedimentation rate (ESR) increased with increasing LD2 scores ($\beta \pm$ SE: slope 0.23 \pm 0.07, intercept -1.21 ± 0.19 , N = 53, Δ AICc_{Null model} = 47.42, see Table 2) expressive of a negative relationship with δ^{13} C values. Solid lines represent the predicted lines; dashed lines represent the 95% confidence intervals, CI.



Fig. 4. Predicted immature erythrocyte counts (IE) increased with increasing LD1 scores within the population of YLG breeding in Deserta (model average, $\beta \pm$ SE: slope 0.31 \pm 0.09, intercept 5.29 \pm 0.10, N = 71, Δ AICc_{Null model} = 4.58, see Table 2) expressive of a positive relationship with Hg and Se concentrations. Solid coloured points represent data from the model for each species breeding at each colony. Solid line represents the predicted line of the significant interaction.

higher residual δ^{13} C values (Fig. 2). LD scores of the two main axes were retained for subsequent univariate analyses, and TEs here reported were used for further inferences (Table S6 in Supplementary Material).

3.3. Association of TEs and trophic ecology with immuno-haematological parameters

The best model explaining ESR (range: 0.01-0.29) included the colony-site, LD1, and LD2 as predictors, with no interaction (Table 2). ESR was higher in CS than in urban YLGs, and those breeding in Berlenga exhibited a moderate negative association with LD1, and a very strong positive association with LD2 (Fig. 3, Table 2). This suggests an overall increase of ESR with δ^{13} C values. Model averaging indicated that there was a positive and strong effect of the interaction between YLG breeding in Deserta and LD1 on the number of IE (Table 2, Fig. 4). This suggests an increase in the number of IE in YLGs breeding in Deserta with the increase on Hg and Se concentrations. Hb concentrations, WBC, and ENA counts only included the colony-site as significant predictor in the best model (WBC counts) or average models (Hb and ENA counts) (Table 2). More specifically, CS presented higher concentrations of Hb, higher numbers of WBC and ENAs than AG and YLGs breeding in natural colonies, while urban YLGs presented higher numbers of ENAs than all other gull populations. Heterophil/lymphocyte ratio (H/L) exhibited no association with either the colony-site or any of the LD axes (Table 2).

4. Discussion

This study analysed blood concentrations of 15 trace elements (TEs) in three seabird species breeding along the western and southern coasts of Portugal. It indicates a diverse exposure to essential and non-essential TEs, possibly driven by colony-site and species-specific trophic ecology and foraging habitats, as evidenced by the distinct isotopic niches and their sizes. Immuno-haematological parameters were strongly influenced by the colony-site, suggesting that species-specific requirements together with the ecological context of the breeding colony could be the primary factors explaining these differences. Intriguingly, ESR and IE counts exhibited moderate and strong relationships with δ^{13} C values and

Hg and Se concentrations, respectively, rather than solely the colonysite, which might warrant further investigation.

4.1. Comparison to previous studies on blood TE concentrations in seabirds

The majority of ecotoxicological studies on TEs have been carried out using feathers collected from adult seabirds, while only a few studies have used blood (but see Anderson et al., 2010; Carravieri et al., 2014; Fromant et al., 2016; Sebastiano et al., 2017, 2016). Different tissues have diverse turnover rates, preventing direct comparison between them, thus here we focus on blood studies' comparisons. In the present study, Se and Hg were the elements which contributed the most for separation among colony-sites in the ordination space along the LD1, evidencing a clear segregation of the urban YLGs from the natural colonies. In fact, the range of Se concentrations was very wide among colonies, being 3- to 10-fold lower in the urban-dwellers than in other Larids (Correia et al., 2023; Sebastiano et al., 2017). Notably, most urban YLGs had Se concentrations in the range of seabird chicks reared in natural habitats (<10 $\mu g~g^{-1}$ dw, Carravieri et al., 2020; Kim et al., 2013; Sebastiano et al., 2017, 2016). This underlines the lower bioavailability of Se in terrestrial and urban habitats, highly linked to the lower intake of marine prey by YLGs from Porto (Pais de Faria et al., 2021b), when compared to species strictly foraging on marine resources (range 30–200 μ g g⁻¹ dw; Anderson et al., 2010; Carravieri et al., 2014; Correia et al., 2023; Fromant et al., 2016; Sebastiano et al., 2017).

Overall, CS had generally low Hg concentrations, comparable to those found in other small and medium-sized Procellariiformes (range $0.3-3.3 \ \mu g \ g^{-1} \ dw$; Anderson et al., 2009; Carravieri et al., 2021, 2018; Carvalho et al., 2013; Fromant et al., 2016). YLGs and AGs breeding in natural colonies had similar Hg concentrations to those of western gulls (L. occidentalis) breeding along the west coast of the USA (Clatterbuck et al., 2021) and to YLGs and lesser black-backed gulls (L. fuscus) breeding in the Île de Ré on the west coast of France (Jouanneau et al., 2022), although not exceeding records reported for the great black-backed gull (L. marinus) breeding in the Île de Ré (range 7.0-21.5 $\mu g g^{-1}$ dw; Jouanneau et al., 2022). Conversely, our urban-dwelling YLGs had low Hg concentrations, similarly to gulls breeding in the Baltic Sea (Szumiło-Pilarska et al., 2017). The variation in Hg concentrations detected in our study aligns with the prevalent diet at gull colonies: YLGs and AGs from Deserta feed on demersal and mesopelagic fish made available via trawler discards, highly abundant in Algarve (Bueno-Pardo et al., 2017), whereas YLGs from Berlenga and Porto typically rely on crustaceans and anthropogenic-derived resources, respectively (Calado et al., 2021; Pais de Faria et al., 2021b). This highlights the role of diet, trophic position, and foraging habitat in Hg exposure (Anderson et al., 2009; Jouanneau et al., 2022; Mills et al., 2022, 2020), evidencing Hg biomagnification (Seco et al., 2021). Demersal and mesopelagic fishes are known to be of higher trophic position (δ^{15} N-enriched; Navarro et al., 2010), and more enriched in Hg (Blum et al., 2013; Chouvelon et al., 2012; Choy et al., 2009) compared to crustaceans and anthropogenic-derived resources (Chouvelon et al., 2012; Thorne et al., 2021). The higher rate of microbial-mediated methylation of Hg in low oxygen waters, such as the mesopelagic layer, prompts the formation and accumulation of methyl-Hg (MeHg) (Choy et al., 2009). This may help explaining the higher Hg concentrations found for AGs and YLGs breeding in Deserta when compared to CS, which rely on cephalopods and epipelagic fish, typically lower in Hg (Choy et al., 2009; Minet et al., 2021).

 $\delta^{13} C$ contributed most to the separation of YLGs breeding in Berlenga from those breeding in the urban colony along LD2. As previously noted for $\delta^{15} N, \delta^{13} C$ is generally higher in marine than in terrestrial food webs, and in demersal than in pelagic food webs (Newsome et al., 2007). The lower $\delta^{13} C$ and $\delta^{15} N$ values (r_s = 0.623), indicating a diet poorer in marine resources, together with higher Pb concentrations, distinguished urban YLGs from other populations in the ordination space. Pb is a

non-essential element associated with higher exposure to urban and landfill environments (Kim and Williams, 2017; Meillère et al., 2016), explaining why YLGs showed the highest detection frequencies of Pb (85-100%). This is likely due to their use of both marine and terrestrial/anthropogenic resources (Pais de Faria et al., 2021b). Markedly, 13% of the individuals surpassed the lowest benchmarks of Pb reported to elicit inhibitory effects on delta-aminolaevulinic acid dehydratase (ALAD) in Baltic eiders (0.24 µg g⁻¹ dw; Franson et al., 2000; Franson and Deborah, 2011). This enzyme is essential in the initial steps of heme synthesis - a precursor of Hb necessary to bind oxygen (Gibson et al., 1998) – and its inhibition occurs due to the replacement of Zn atoms by Pb atoms, required for ALAD activity (Astrin et al., 1987). All YLGs exceeding 0.24 μ g g⁻¹ dw of Pb in blood were urban dwellers, except one individual from Deserta Island. Thus, these results underscore the elevated Pb exposure in the urban centres, attributable to YLG's use of anthropogenic facilities such as landfills (Fernandes, 2022; Lopes et al., 2021; Matos et al., 2018), but also suggest a high resilience of YLGs to contamination.

Other TEs, such as Co, Fe, Mn, Ni, and Zn were within the typical seabird concentration ranges (Anderson et al., 2010; Carravieri et al., 2014; Correia et al., 2023; Fromant et al., 2016; Sebastiano et al., 2017), denoting their role in homeostatic processes (Walsh, 1990). Despite including cephalopods in their diet, a major source of Cd for predators (Bustamante et al., 1998), CS had low concentrations of this metal in its blood, suggesting a low exposure risk (e.g., Carravieri et al., 2014; Sebastiano et al., 2017, 2016; but see Carvalho et al., 2013; Voulgaris et al., 2019). Al and Cu concentrations found in YLGs were higher compared to those from other seabirds (Al: range 0.7–6.9 μ g g⁻¹ dw, Cu: range 0.5–1.3 µg g⁻¹ dw; Correia et al., 2023; Finger et al., 2016, 2015; Fromant et al., 2016; Kim et al., 2013; Sebastiano et al., 2017, 2016). YLGs are known to feed on considerable amounts of the Henslow's swimming crab (Polybius henslowii), especially in Berlenga Island (Alonso et al., 2015; Calado et al., 2021; Pais de Faria et al., 2021b). In fact, crustaceans usually contain high Cu and Zn enzymatic requirements (mean Cu: 26.3 μ g g⁻¹ dw, mean Zn: 34.5 μ g g⁻¹ dw; White and Rainbow, 1985); in crustaceans, haemocyanin is the respiratory pigment used to transport oxygen (Taylor and Anstiss, 1999), leading to an increased requirement of Cu (White and Rainbow, 1985). Nevertheless, Cu concentrations were similar across all YLG colony-sites, suggesting that these elevated levels of Cu may be related to the foraging ecology of YLG (i.e., species-specific factors) or potentially to a metabolic disturbance. Usually, Cu concentrations are expected to remain constant due to homeostatic processes (Walsh, 1990). Conversely, As concentrations varied widely among and within colony-sites (range 0.3–18.5 μ g g⁻¹ dw), with higher concentrations in gulls than shearwaters, consistent with previous reports (Carravieri et al., 2014; Carvalho et al., 2013; Correia et al., 2023; Fromant et al., 2016; Sebastiano et al., 2017). Multiple sources of As challenge the tracking of its origin, especially for wide-ranging foragers like shearwaters or flexible foragers like gulls. These species rely on diverse foraging habitats and prey, e.g., marine vs terrestrial and pelagic vs demersal, which may 'dilute' As concentrations in blood, making it difficult to detect contamination patterns. Despite the uncertainty surrounding As toxicity thresholds, concentrations in our study did not exceed 50 µg g⁻¹ dw (Eisler, 1994; Neff, 1997; Sánchez-Virosta et al., 2015), suggesting minimal threat to these populations. Therefore, when assessing TE concentrations, we must acknowledge the multiple sources of each element (natural and anthropogenic), as well as the potential species-specific requirements for essential elements that may influence the overall concentrations.

4.2. Hg toxicity and Se:Hg molar ratio

Remarkably, 25% of the individuals, primarily AGs and YLGs from Deserta, exceeded the internationally defined toxicity threshold for Hg (1 μ g g⁻¹ ww), which poses a moderate risk to Hg toxicity (range

4.8–14.3 μ g g⁻¹ dw; Ackerman et al., 2016). Such concentrations are known to impact physiology, behaviour, and reproduction in several seabird species (Ackerman et al., 2016; Chastel et al., 2022; Goutte et al., 2015, 2014a, b; Tartu et al., 2015a, b). However, it is worth noting that all individuals, with the exception of one urban-dwelling YLG (Se:Hg = 3.9), exhibited a Se:Hg molar ratio above 4, a recent threshold outlined as the Se-Hg relationship needed to effectively demethylate MeHg, the most toxic organic form of Hg (Manceau et al., 2021). This suggests an overall effective protection against Hg toxicity (Cuvin-Aralar and Furness, 1991; Nigro and Leonzio, 1996). In particular, AGs and YLGs had similar mean Se:Hg molar ratios, despite elevated Se concentrations found in AGs. If we consider a 1:1 stoichiometry for our study species, it suggests that AG and YLG populations may have lower protection against the toxic effects of Hg compared to CS. This contrasts with expectations based solely on the absence of differences in Hg concentrations observed between CS and YLG from Berlenga. These results underscore the importance of considering Hg and Se levels at the same time in predators (Cruz-Flores et al., 2024) and prey to validate our findings, and assess potential Hg exposure risks for coastal breeding seabirds.

4.3. Impact of TEs on physiology and health of seabirds

Our study found that immuno-haematological parameters of gulls and shearwaters were not related to TEs exposure. The parameters used here did not show any evidence of negative health impacts on the immune response and physiological condition of breeding adults. Despite widely used in ecology, it should be noted that these parameters are relatively non-specific, thus it is possible that they simply could not detect certain impacts due to the lack of specificity to TE contamination. Yet, it is worth noting the elevated levels of Hg and Pb in some individuals, which were correlated with demographic consequences in other species (Ackerman et al., 2016; Chastel et al., 2022; Franson and Deborah, 2011; Goutte et al., 2014a, b).

Of particular interest was the overall negative relationship between ESR and δ^{13} C values, as well as the relationship between IE, Hg and Se concentrations within YLG breeding in Deserta. A higher ESR often indicates systemic inflammation, due to infections, chronic diseases, or acute inflammatory responses (Heylen and Matthysen, 2008; Norte et al., 2022). However, it can also reflect physiological stress, including exposure to pollutants or other stressors (aggressions, competition, starvation, etc.) (Norte et al., 2022). Our results suggested that higher δ^{13} C values favoured lower ESR. In gulls, higher δ^{13} C values are often linked to a marine foraging strategy, while lower δ^{13} C values are usually linked to a more terrestrial foraging strategy (Garthe et al., 2016; Mendes et al., 2018; Pais de Faria et al., 2021b). Thus, it would be predicted that urban YLGs exhibit higher ESR than the other YLG populations. This prediction is contradictory to a previous study that assessed several immuno-haematological parameters in YLG adults and chicks in the same natural and urban colonies (Pais de Faria et al., 2022). Here, adults and chicks followed the same pattern: individuals from urban colonies exhibited lower ESR than individuals from natural colonies. In fact, in natural colonies the nest density was much higher than in urban environments, causing more intraspecific negative interactions. Higher amounts of IE indicate a necessity for replenishing erythrocytes due to haemorrhage or haemolysis, typical of regenerative anaemias caused by ectoparasites (Boyd, 1951; Leighton et al., 1983). Interestingly, our results revealed that within YLG breeding in Deserta, the number of IE was higher in individuals with higher Hg and Se concentrations. This indicates that YLG with a more marine foraging strategy had higher numbers of IE. A higher amount of IE reveals that the individual is replacing the erythrocytes that were depleted due to a haemorrhagic event (Minias, 2015). Haemorrhagic events can occur when competing for food resources while at sea, or during interactions with fishing vessels that prompt aggressions among individuals (Costa et al., 2020). Indeed, the amount of Hb synthesised in IE is less than 20% of that found in mature erythrocytes, meaning that Hb concentrations are lower and are expected to be replenished with a substantial delay (Minias, 2015). Moreover, Hg is known to induce oxidative stress by generating reactive oxygen species (Henry et al., 2015), which damage cells including the erythrocytes, thereby further lowering Hb levels. Despite the significance of these relationships, we recommend caution when interpreting these results, since the number of studies analysing these parameters in wild seabirds is still limited, which challenges the establishment of baseline values, even more with low sample sizes (but see Pais de Faria et al., 2022).

WBC and ENA counts were notably higher in CS than in the other two species examined here, suggesting increased immune responses and greater genotoxicity (i.e., DNA damage), respectively. However, these values were comparable to those reported for other seabirds (Colominas-Ciuró et al., 2022; D'Amico et al., 2016; Olmastroni et al., 2024, 2019). Among gulls, urban-dwelling YLGs exhibited higher ENA counts than AGs and YLGs breeding in natural colonies. This is in line with previous reports on the higher prevalence of genotoxicity, e.g., DNA adducts, micronucleus and other ENAs, in urban and suburban birds (Baesse et al., 2019, 2015; Skarphedinsdottir et al., 2010) or in more polluted areas (Quirós et al., 2008), when compared to more 'natural' environments. We should not overlook the potential genotoxic effect of the high Pb concentrations on urban-dwelling YLGs, despite the low number of ENAs found in our study (range 0-27), when compared with previous studies (D'Amico et al., 2016; Olmastroni et al., 2024, 2019). A higher frequency of ENAs is often indicative of sensitivity to contaminants such as essential and non-essential TEs (Christopher et al., 2004; Shah et al., 2021), however it might not be directly related to overall exposure. Instead, ENAs have been linked to increased oxidative stress, infectious diseases, nutritional deficiencies, and physiological stress (Bourgeon et al., 2012; Keilen et al., 2022).

Other factors such as age, sex, body condition, breeding stage, and the presence and loads of other stressors, like organic pollutants or parasites, may influence individual physiological and immunological responses (Bustnes et al., 2004; Davey et al., 2000; D'Amico et al., 2016; Wells et al., 2024). This should be particularly critical during the breeding period when adults are more vulnerable. For instance, Hb concentrations tend to increase from the egg-laying to the chick-rearing period due to the high demands of oxygen-carrying capacity while feeding the chicks (Davey et al., 2000), which may partly explain the increasing pattern found for Hb between gulls (incubation) and shearwaters (chick-rearing). On the one hand, we advise caution when interpreting immuno-haematological parameters alone as indicators of individual or populational vulnerability to contaminants. We advocate for a critical look when interpreting these relationships in the context of environmental contamination. On the other hand, the lack of values outside the normal ranges and the lack of species-specific toxicity benchmarks for most of TEs do not preclude potential physiological effects, as the species may play a pivotal role in such cause-effect relationships.

5. Conclusion

This study represents a significant contribution in our understanding of blood TE contamination in coastal and oceanic seabirds. A key finding was the large variation in TE concentrations among colony-sites, including some essential elements which may reflect species-specific requirements. Notably, differences found in Hg, Pb, and Se concentrations among colony-sites underscore the influential role of diet and trophic ecology in shaping TE accumulation patterns in adult breeding seabirds. The marked impact of the colony-site effect highlights the necessity for additional research on the toxicity thresholds of TEs, within the specific ecological context of each population, *i.e.*, urban vs natural, coastal marine vs inshore. Our findings raise ecotoxicological concerns, particularly regarding Pb concentrations in urban-dwelling YLGs and Hg concentrations in AGs and YLGs which rely on fishery discards (Calado

et al., 2021). Continuous monitoring during both breeding and non-breeding periods is essential, especially for AGs, which are classified as Vulnerable according to the IUCN Red List (BirdLife International, 2024). Finally, the limited associations observed between TEs concentrations and immuno-haematological parameters emphasises the need for more comprehensive investigations and larger sample sizes. Such studies could shed light on physiological and immunological responses to environmental contamination. This appears to be particularly relevant in light of the expanding presence of gulls in urban environments (Pais de Faria et al., 2021a), raising questions into the physiological adaptations of generalist animals inhabiting urbanised areas (García et al., 2023; Nos et al., 2024).

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CRediT authorship contribution statement

Ivo dos Santos: Writing – original draft, Visualization, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. Vitor H. Paiva: Writing – original draft, Supervision, Resources, Funding acquisition, Conceptualization. Ana C. Norte: Writing – original draft, Resources, Methodology, Conceptualization. Carine Churlaud: Writing – review & editing, Methodology. Filipe R. Ceia: Writing – review & editing, Investigation. Joana Pais de Faria: Writing – review & editing, Investigation. Jorge M. Pereira: Writing – review & editing, Investigation. Lara R. Cerveira: Writing – review & editing, Investigation. Maria I. Laranjeiro: Writing – review & editing, Investigation. Sara N. Veríssimo: Writing – review & editing, Investigation. Sara N. Veríssimo: Writing – review & editing, Investigation. Gara S. Writing – original draft, Supervision, Resources, Funding acquisition, Conceptualization. Paco Bustamante: Writing – original draft, Supervision, Resources, Funding acquisition, Conceptualization.

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. Supplementary data

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References

- Ackerman, J.T., Eagles-Smith, C.A., Herzog, M.P., Hartman, C.A., Peterson, S.H., Evers, D.C., Jackson, A.K., Elliott, J.E., Vander Pol, S.S., Bryan, C.E., 2016. Avian mercury exposure and toxicological risk across western North America: a synthesis. Sci. Total Environ. 568, 749–769. https://doi.org/10.1016/j.scitotenv.2016.03.071.
- Albert, C., Renedo, M., Bustamante, P., Fort, J., 2019. Using blood and feathers to investigate large-scale Hg contamination in Arctic seabirds: a review. Env. Res. 177, 108588 https://doi.org/10.1016/j.envres.2019.108588.
- Alonso, H., Almeida, A., Granadeiro, J.P., Catry, P., 2015. Temporal and age-related dietary variations in a large population of yellow-legged gulls *Larus michahellis*: implications for management and conservation. Eur. J. Wildl. Res. 61, 819–829. https://doi.org/10.1007/s10344-015-0958-9.
- Alonso, H., Granadeiro, J.P., Waap, S., Xavier, J., Symondson, W.O., Ramos, J.A., Catry, P., 2014. An holistic ecological analysis of the diet of Cory's shearwaters using prey morphological characters and DNA barcoding. Mol. Ecol. 23, 3719–3733. https://doi.org/10.1111/mec.12785.
- Ancora, S., Bianchi, N., Leonzio, C., Renzoni, A., 2008. Heavy metals in flamingos (*Phoenicopterus ruber*) from Italian wetlands: the problem of ingestion of lead shot. Environ. Res. 107, 229–236. https://doi.org/10.1016/j.envres.2008.02.004.
- Anderson, O.R.J., Phillips, R.A., McDonald, R.A., Shore, R.F., McGill, R.A.R., Bearhop, S., 2009. Influence of trophic position and foraging range on mercury levels within a seabird community. Mar. Ecol. Prog. Ser. 375, 277–288. https://doi.org/10.3354/ meps07784.
- Anderson, O.R.J., Phillips, R.A., Shore, R.F., McGill, R.A.R., McDonald, R.A., Bearhop, S., 2010. Element patterns in albatrosses and petrels: influence of trophic position, foraging range, and prey type. Environ. Pollut. 158, 98–107. https://doi.org/ 10.1016/j.envpol.2009.07.040.
- Astrin, K.H., Bishop, D.F., Wetmur, J.G., Kaul, B., Davidow, B., Desnick, R.J., 1987. δ-Aminolevulinic acid dehydratase Isozymes and lead toxicity. Annals New York Aca. Sci. 514, 23–29.
- Baesse, C.Q., de Magalhães Tolentino, V.C., da Silva, A.M., de Andrade Silva, A., Ferreira, G.Å., Paniago, L.P.M., Nepomuceno, J.C., de Melo, C., 2015. Micronucleus as biomarker of genotoxicity in birds from Brazilian Cerrado. Ecotox. Environ. Safety 115, 223–228. https://doi.org/10.1016/j.ecoenv.2015.02.024.
- Baesse, C.Q., de Magalhães Tolentino, V.C., Morelli, S., Melo, C., 2019. Effect of urbanization on the micronucleus frequency in birds from forest fragments. Ecotox. Environ. Safety 171, 631–637. https://doi.org/10.1016/j.ecoenv.2019.01.026. Barton, K., 2023. Package 'MuMIn' Multi-Model Interface. R Interface.
- Bauerová, P., Krajzingrová, T., Těšický, M., Velová, H., Hraníček, J., Musil, S., Svobodová, J., Albrecht, T., Vinkler, M., 2020. Longitudinally monitored lifetime changes in blood heavy metal concentrations and their health effects in urban birds. Sci. Total Environ. 723, 138002 https://doi.org/10.1016/j.scitotenv.2020.138002.
- Bauerová, P., Vinklerová, J., Hraníček, J., Čorba, V., Vojtek, L., Svobodová, J., Vinkler, M., 2017. Associations of urban environmental pollution with health-related physiological traits in a free-living bird species. Sci. Total Environ. 601, 1556–1565. https://doi.org/10.1016/j.scitotenv.2017.05.276.
- Bearhop, S., Ruxton, G.D., Furness, R.W., 2000. Dynamics of mercury in blood and feathers of great skuas. Environ. Toxicol. Chem.: Int. J. 19, 1638–1643. https://doi. org/10.1002/etc.5620190622.
- BirdLife International, 2024. Species Factsheet: Larus Audouinii. https://datazone.bir dlife.org/species/factsheet/audouins-gull-larus-audouinii. (Accessed 23 February 2024).
- Blum, J.D., Popp, B.N., Drazen, J.C., Anela Choy, C., Johnson, M.W., 2013. Methylmercury production below the mixed layer in the North Pacific ocean. Nat. Geosci. 6, 879–884. https://doi.org/10.1038/ngeo1918.
- Bourgeon, S., Leat, E.H., Magnusdottir, E., Fisk, A.T., Furness, R.W., Strøm, H., Hanssen, S.A., Petersen, Æ., Olafsdóttir, K., Borgå, K., Gabrielsen, G.W., Bustnes, J. O., 2012. Individual variation in biomarkers of health: influence of persistent organic pollutants in Great skuas (*Stercorarius skua*) breeding at different geographical locations. Environ. Res. 118, 31–39. https://doi.org/10.1016/j. envres.2012.08.004.

Boyd, E.M., 1951. The external parasites of birds: a review. Wilson Bull. 63, 363-369.

- Budko, D.F., Martynova, D.M., 2019. Trace element uptake assessment in the planktonic biofiltration system. Oceanology 59, 27–36. https://doi.org/10.1134/ S0001437019010016.
- Bueno-Pardo, J., Ramalho, S.P., García-Alegre, A., Morgado, M., Vieira, R.P., Cunha, M. R., Queiroga, H., 2017. Deep-sea crustacean trawling fisheries in Portugal: quantification of effort and assessment of landings per unit effort using a Vessel Monitoring System (VMS). Sci. Rep. 7, 40795 https://doi.org/10.1038/srep40795.
- Burnham, K.P., Anderson, D.R., 2002. Model Selection and Multi-Model Inference: A Practical Information-Theoretic Approach, second ed. Springer, New York.
- Bustamante, P., Carravieri, A., Goutte, A., Barbraud, C., Delord, K., Chastel, O., Weimerskirch, H., Cherel, Y., 2016. High feather mercury concentrations in the wandering albatross are related to sex, breeding status and trophic ecology with no demographic consequences. Environ. Res. 144, 1–10. https://doi.org/10.1016/j. envres.2015.10.024.
- Bustamante, P., Caurant, F., Fowler, S.W., Miramand, P., 1998. Cephalopods as a vector for the transfer of cadmium to top marine predators in the north-east Atlantic Ocean. Sci. Total Environ. 220, 71–80. https://doi.org/10.1016/S0048-9697(98)00250-2.
- Bustamante, P., Lahaye, V., Durnez, C., Churlaud, C., Caurant, F., 2006. Total and organic Hg concentrations in cephalopods from the North Eastern Atlantic waters: influence of geographical origin and feeding ecology. Sci. Total Environ. 368, 585–596. https://doi.org/10.1016/j.scitotenv.2006.01.038.
- Bustnes, J.O., Hanssen, S.A., Folstad, I., Erikstad, K.E., Hasselquist, D., Skaare, J.U., 2004. Immune function and organochlorine pollutants in arctic breeding glaucous gulls. Arch. Environ. Contam. Toxicol. 47, 530–541. https://doi.org/10.1007/ s00244-003-3203-6.
- Calado, J.G., Veríssimo, S.N., Paiva, V.H., Ramos, R., Vaz, P.T., Matos, D., Pereira, J.M., Lopes, C., Oliveira, N., Quaresma, A., Ceia, F.R., Velando, A., Ramos, J.A., 2021. Influence of fisheries on the spatio-temporal feeding ecology of gulls along the western Iberian coast. Mar. Ecol. Prog. Ser. 661, 187–201. https://doi.org/10.3354/ meps13601.
- Callendar, E., Rice, K.C., 2000. The urban environmental gradient: anthropogenic influences on the spatial and temporal distributions of lead and zinc in sediments. Environ. Sci. Technol. 34, 232–238. https://doi.org/10.1021/es990380s.
- Campbell, L.M., Norstrom, R.J., Hobson, K.A., Muir, D.C., Backus, S., Fisk, A.T., 2005. Mercury and other trace elements in a pelagic Arctic marine food web (Northwater Polynya, Baffin Bay). Sci. Total Environ. 351, 247–263. https://doi.org/10.1016/j. scitotenv.2005.02.043.
- Carravieri, A., Bustamante, P., Labadie, P., Budzinski, H., Chastel, O., Cherel, Y., 2020. Trace elements and persistent organic pollutants in chicks of 13 seabird species from Antarctica to the subtropics. Environ. Int. 134, 105225 https://doi.org/10.1016/j. envint.2019.105225.
- Carravieri, A., Bustamante, P., Tartu, S., Meillère, A., Labadie, P., Budzinski, H., Peluhet, L., Barbraud, C., Weimerskirch, H., Chastel, O., Cherel, Y., 2014. Wandering albatrosses document latitudinal variations in the transfer of persistent organic pollutants and mercury to Southern Ocean predators. Environ. Sci. Technol. 48, 14746–14755. https://doi.org/10.1021/es504601m.
- Carravieri, A., Cherel, Y., Brault-Favrou, M., Churlaud, C., Peluhet, L., Labadie, P., Budzinski, H., Chastel, O., Bustamante, P., 2017. From Antarctica to the subtropics: contrasted geographical concentrations of selenium, mercury, and persistent organic pollutants in skua chicks (*Catharacta* spp.). Environ. Pollut. 228, 464–473. https:// doi.org/10.1016/j.envpol.2017.05.053.
- Carravieri, A., Fort, J., Tarroux, A., Cherel, Y., Love, O.P., Prieur, S., Brault-Favrou, M., Bustamante, P., Descamps, S., 2018. Mercury exposure and short-term consequences on physiology and reproduction in Antarctic petrels. Environ. Pollut. 237, 824–831. https://doi.org/10.1016/j.envpol.2017.11.004.
- Carravieri, A., Warner, N.A., Herzke, D., Brault-Favrou, M., Tarroux, A., Fort, J., Bustamante, P., Descamps, S., 2021. Trophic and fitness correlates of mercury and organochlorine compound residues in egg-laying Antarctic petrels. Environ. Res. 193, 110518 https://doi.org/10.1016/j.envres.2020.110518.
- Carvalho, P.C., Bugoni, L., McGill, R.A.R., Bianchini, A., 2013. Metal and selenium concentrations in blood and feathers of petrels of the genus *Procellaria*. Environ. Toxicol. Chem. 32, 1641–1648. https://doi.org/10.1002/etc.2204.
- Ceia, F.R., Phillips, R.A., Ramos, J.A., Cherel, Y., Vieira, R.P., Richard, P., Xavier, J.C., 2012. Short-and long-term consistency in the foraging niche of wandering albatrosses. Mar. Biol. 159, 1581–1591. https://doi.org/10.1007/s00227-012-1946-1.
- Chastel, O., Fort, J., Ackerman, J.T., Albert, C., Angelier, F., Basu, N., Blévin, P., Brault-Favrou, M., Bustnes, J.O., Bustamante, P., Danielsen, J., Descamps, S., Dietz, R., Erikstad, K.E., Eulaers, I., Ezhov, A., Fleishman, A.B., Gabrielsen, G.W., Gavrilo, M., Gilchrist, G., Gilg, O., Gíslason, S., Golubova, E., Goutte, A., Grémillet, D., Hallgrimsson, G.T., Hansen, E.S., Hanssen, S.A., Hatch, S., Huffeldt, N.P., Jakubas, D., Jénsson, J.E., Kitaysky, A.S., Kolbeinsson, Y., Krasnov, Y., Letcher, R.J., Linnebjerg, J.F., Mallory, M., Merkel, F.R., Moe, B., Montevecchi, W.J., Mosbech, A., Olsen, B., Orben, R.A., Provencher, J.F., Ragnarsdottir, S.B., Reiertsen, T.K., Rojek, N., Romano, M., Søndergaard, J., Strøm, H., Takahashi, A., Tartu, S., Thórarinsson, T.L., Thiebot, J.-B., Will, A.P., Wilson, S., Wojczulanis-Jakubas, K., Yannic, G., 2022. Mercury contamination and potential health risks to Arctic seabirds and shorebirds. Sci. Total Environ. 844, 156944 https://doi.org/10.1016/j.scitotenv.2022.156944.
- Chouvelon, T., Spitz, J., Caurant, F., Mèndez-Fernandez, P., Autier, J., Lassus-Débat, A., Chappuis, A., Bustamante, P., 2012. Enhanced bioaccumulation of mercury in deepsea fauna from the Bay of Biscay (north-east Atlantic) in relation to trophic positions identified by analysis of carbon and nitrogen stable isotopes. Deep Sea Res. Part I: Ocean. Res. Pap. 65, 113–124. https://doi.org/10.1016/j.dsr.2012.02.010.

- Choy, C.A., Popp, B.N., Kaneko, J.J., Drazen, J.C., 2009. The influence of depth on mercury levels in pelagic fishes and their prey. Proc. Nat. Acad. Sci. 106, 13865–13869. https://doi.org/10.1073/pnas.0900711106.
- Christopher, M.M., Shooshtari, M.P., Levengood, J.M., 2004. Assessment of erythrocyte morphologic abnormalities in mallards with experimentally induced zinc toxicosis. Amer. J. Vet. Res. 65, 440–446. https://doi.org/10.2460/ajvr.2004.65.440.
- Clatterbuck, C.A., Lewison, R.L., Orben, R.A., Ackerman, J.T., Torres, L.G., Suryan, R.M., Warzybok, P., Jahncke, J., Shaffer, S.A., 2021. Foraging in marine habitats increases mercury concentrations in a generalist seabird. Chemosphere 279, 130470. https:// doi.org/10.1016/j.chemosphere.2021.130470.
- Colominas-Ciuró, R., Cianchetti-Benedetti, M., Michel, L., Dell'Omo, G., Quillfeldt, P., 2022. Foraging strategies and physiological status of a marine top predator differ during breeding stages. Comp. Bioch. Physio. Part A: Mol. Integ Physio. 263, 111094 https://doi.org/10.1016/j.cbpa.2021.111094.
- Correia, E., Granadeiro, J.P., Vale, C., Catry, T., 2023. Trace elements in relation to trophic ecology of long-distance migratory shorebirds and seabirds in West Africa. Environ. Pollut. 316, 120674 https://doi.org/10.1016/j.envpol.2022.120674.
- Costa, R.A., Sá, S., Pereira, A.T., Angelo, A.R., Vaqueiro, J., Ferreira, M., Eira, C., 2020. Prevalence of entanglements of seabirds in marine debris in the central Portuguese coast. Mar. Pollut. Bull. 161, 111746 https://doi.org/10.1016/j. marnolbul 2020 111746

Costantini, D., Meillère, A., Lecomte, V., Sorci, G., Faivre, B., Weimerskirch, H., Bustamante, P., Labadie, P., Budzinski, H., Chastel, O., 2014. Oxidative stress in relation to reproduction, contaminants, gender and age in a long-lived seabird. Oecologia 175, 1107–1116. https://doi.org/10.1007/s00442-014-2975-x.

Cruz-Flores, M., Lemaire, J., Brault-Favrou, M., Christensen-Dalsgaard, S., Churlaud, C., Descamps, S., Elliott, K., Erikstad, K.E., Ezhov, A., Gavrilo, M., Grémillet, D., Guillou, G., Hatch, S., Huffeldt, N.P., Kitaysky, A.S., Kolbeinsson, Y., Krasnov, Y., Langset, M., Leclaire, S., Linnebjerg, J.F., Lorentzen, E., Mallory, M.L., Merkel, F.N., Montevecchi, W., Mosbech, A., Patterson, A., Perret, S., Provencher, J.F., Reiertsen, T.K., Renner, H., Strøm, H., Takahashi, A., Thiebot, J.-B., Thórarinsson, T. L., Will, A., Bustamante, P., Fort, J., 2024. Spatial distribution of selenium-mercury in Arctic seabirds. Environ. Pollut. 343, 123110 https://doi.org/10.1016/j. envpol.2023.123110.

- Cuvin-Aralar, M.L.A., Furness, R.W., 1991. Mercury and selenium interaction: a review. Ecotox. Environ. Saf. 21, 348–364. https://doi.org/10.1016/0147-6513(91)90074-Y.
- Davey, C., Lill, A., Baldwin, J., 2000. Variation during breeding in parameters that influence blood oxygen carrying capacity in shearwaters. Aust. J. Zool. 48, 347–356. https://doi.org/10.1071/Z000047.
- Davis, A.K., Maney, D.L., Maerz, J.C., 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.x.
- De Francisco, N., Ruiz Troya, J.D., Agüera, E.I., 2003. Lead and lead toxicity in domestic and free living birds. Avian Pathol. 32, 3–13. https://doi.org/10.1080/ 0307945021000070660.
- D'Amico, V.L., Coria, N., Palacios, M.G., Barbosa, A., Bertellotti, M., 2016. Physiological differences between two overlapped breeding Antarctic penguins in a global change perspective. Pol. Biol. 39, 57–64. https://doi.org/10.1007/s00300-014-1604-9.
- Eagles-Smith, C.A., Ackerman, J.T., Adelsbach, T.L., Takekawa, J.Y., Miles, A.K., Keister, R.A., 2008. Mercury correlations among six tissues for four waterbird species breeding in San Francisco Bay, California, USA. Environ. Toxic. Chem: Int. J. 27, 2136–2153. https://doi.org/10.1897/08-038.1.
- Eisler, R., 1994. A review of arsenic hazards to plants and animals with emphasis on fishery and wildlife resources. In: Nriagu, J.O. (Ed.), Arsenic in the Environment, Part II: Human Health and Ecosystem Effects, vol. 27. John Willey and Sons, New York, NY, USA, pp. 185–259.
- Espín, S., Martínez-López, E., Jiménez, P., María-Mojica, P., García-Fernández, A.J., 2014a. Effects of heavy metals on biomarkers for oxidative stress in Griffon vulture (*Gyps fulvus*). Environ. Res. 129, 59–68. https://doi.org/10.1016/j. envres.2013.11.008.
- Espín, S., Martínez-López, E., Jiménez, P., María-Mojica, P., García-Fernández, A.J., 2016. Interspecific differences in the antioxidant capacity of two Laridae species exposed to metals. Environ. Res. 147, 115–124. https://doi.org/10.1016/j. envres.2016.01.029.
- Espín, S., Martínez-López, E., León-Ortega, M., Martínez, J.E., García-Fernández, A.J., 2014b. Oxidative stress biomarkers in Eurasian eagle owls (*Bubo bubo*) in three different scenarios of heavy metal exposure. Environ. Res. 131, 134–144. https:// doi.org/10.1016/j.envres.2014.03.015.
- Fernandes, R., 2022. Spatial Ecology and Diel Rhythms of Natural and Urban-Dwelling Gulls. Universidade de Coimbra, Portugal. MSc thesis.
- Finger, A., Lavers, J.L., Dann, P., Nugegoda, D., Orbell, J.D., Robertson, B., Scarpaci, C., 2015. The little penguin (*Eudyptula minor*) as an indicator of coastal trace metal pollution. Environ. Pollut. 205, 365–377. https://doi.org/10.1016/j. envpol.2015.06.022.
- Finger, A., Lavers, J.L., Orbell, J.D., Dann, P., Nugegoda, D., Scarpaci, C., 2016. Seasonal variation and annual trends of metals and metalloids in the blood of the Little Penguin (*Eudyptula minor*). Mar. Pollut. Bull. 110, 261–273. https://doi.org/ 10.1016/j.marpolbul.2016.06.055.
- Fokidis, H.B., Greiner, E.C., Deviche, P., 2008. Interspecific variation in avian blood parasites and haematology associated with urbanization in a desert habitat. J. Avian Biol. 39, 300–310. https://doi.org/10.1111/j.0908-8857.2008.04248.x.
- Franson, J.C., Deborah, J.P., 2011. Lead in Birds, Environmental Contaminants in Biota. CRC Press, pp. 563–593.
- Franson, J.C., Hollmen, T., Poppenga, R.H., Hario, M., Kilpi, M., Smith, M.R., 2000. Selected trace elements and organochlorines: some findings in blood and eggs of

nesting common eiders (*Somateria mollissima*) from Finland. Environ. Toxic. Chem. 19, 1340–1347. https://doi.org/10.1002/etc.5620190517.

- Fromant, A., Carravieri, A., Bustamante, P., Labadie, P., Budzinski, H., Peluhet, L., Churlaud, C., Chastel, O., Cherel, Y., 2016. Wide range of metallic and organic contaminants in various tissues of the Antarctic prion, a planktonophagous seabird from the Southern Ocean. Sci. Total Environ. 544, 754–764. https://doi.org/ 10.1016/j.scitotenv.2015.11.114.
- Galindo, M.D., Jurado, J.A., García, M., de Canales, M.L.G., Oliva, M., López, F., Granado, M.D., Espada, E., 2012. Trace metal accumulation in tissues of sole (*Solea senegalensis*) and the relationships with the abiotic environment. Inter. J. Environ. Anal. Chem. 92, 1072–1092. https://doi.org/10.1080/03067319.2010.548092.
- Gamble, S.C., Wiseman, A., Goldfarb, P.S., 1997. Selenium-dependent glutathione peroxidase and other selenoproteins: their synthesis and biochemical roles. J. Chem. Tech. Biotech. 68, 123–134. https://doi.org/10.1002/(SICI)1097-4660(199702)68: 2<123::AID-JCTB641>3.0.CO;2-O.
- García, G.O., Zumpano, F., Mariano y Jelicich, R., Favero, M., 2023. Effect of urbanization on individual condition of a threatened seabird: the Olrog's Gull Larus atlanticus. Urban Ecosys 26, 411–424. https://doi.org/10.1007/s11252-023-01347-7
- Garthe, S., Schwemmer, P., Paiva, V.H., Corman, A.M., Fock, H.O., Voigt, C.C., Adler, S., 2016. Terrestrial and marine foraging strategies of an opportunistic seabird species breeding in the Wadden Sea. PLoS One 11, e0159630. https://doi.org/10.1371/ journal.pone.0159630.
- Gibson, S.L., Cupriks, D.J., Havens, J.J., Nguyen, M.L., Hilf, R., 1998. A regulatory role for porphobilinogen deaminase (PBGD) in δ-aminolaevulinic acid (δ-ALA)-induced photosensitization? Br. J. Cancer 77, 235–242. https://doi.org/10.1038/ bic1298.30
- Goodchild, C.G., VanDiest, I., Lane, S.J., Beck, M., Ewbank, H., Sewall, K.B., 2022. Variation in hematological indices, oxidative stress, and immune function among male song sparrows from rural and low-density urban habitats. Front. Ecol. Evol. 10, 817864 https://doi.org/10.3389/fevo.2022.817864.
- Goutte, A., Barbraud, C., Herzke, D., Bustamante, P., Angelier, F., Tartu, S., Clement-Chastel, C., Moe, B., Bech, C., Gabrielsen, G.W., Bustnes, J.O., Chastel, O., 2015. Survival rate and breeding outputs in a high Arctic seabird exposed to legacy persistent organic pollutants and mercury. Environ. Pollut. 200, 1–9. https://doi. org/10.1016/j.envool.2015.01.033.
- Goutte, A., Bustamante, P., Barbraud, C., Delord, K., Weimerskirch, H., Chastel, O., 2014a. Demographic responses to mercury exposure in two closely related Antarctic top predators. Ecology 95, 1075–1086. https://doi.org/10.1890/13-1229.1.
- Goutte, A., Barbraud, C., Meillère, A., Carravieri, A., Bustamante, P., Labadie, P., Budzinski, H., Delord, K., Cherel, Y., Weimerskirch, H., Chastel, O., 2014b. Demographic consequences of heavy metals and persistent organic pollutants in a vulnerable long-lived bird, the wandering albatross. Proc. Roy. Soc. B 281, 20133313. https://doi.org/10.1098/rspb.2013.3313.
- Henry, K.A., Cristol, D.A., Varian-Ramos, C.W., Bradley, E.L., 2015. Oxidative stress in songbirds exposed to dietary methylmercury. Ecotoxicology 24, 520–526. https:// doi.org/10.1007/s10646-014-1400-x.
- Herrera-Dueñas, A., Pineda, J., Antonio, M.T., Aguirre, J.I., 2014. Oxidative stress of house sparrow as bioindicator of urban pollution. Ecol. Indicat. 42, 6–9. https://doi. org/10.1016/j.ecolind.2013.08.014.
- Heylen, D.J.A., Matthysen, E., 2008. Effect of tick parasitism on the health status of a passerine bird. Funct. Ecol. 22, 1099–1107. https://doi.org/10.1111/j.1365-2435.2008.01463.x.

Hites, R.A., 2019. Correcting for censored environmental measurements. Environ. Sci. Technol. 53, 11059–11060. https://doi.org/10.1021/acs.est.9b05042.

- Hitt, L.G., Khalil, S., Blanchette, A., Finkelstein, M.E., Iverson, E.N., McClelland, S.C., Ribeiro, R.D., Karubian, J., 2023. Lead exposure is correlated with reduced nesting success of an urban songbird. Environ. Res. 227, 115711 https://doi.org/10.1016/j. envres.2023.115711.
- Ibañez, A., Mills, W., Bustamante, P., Morales, L., Torres, D., D'Astek, B., Mariano-Jelicich, R., Phillips, R., Montalti, D., 2024. Deleterious effects of mercury contamination on immunocompetence, liver function and egg volume in an Antarctic seabird. Chemosphere 346, 140630. https://doi.org/10.1016/j. chemosphere.2023.140630.
- Jackson, A.L., Inger, R., Parnell, A.C., Bearhop, S., 2011. Comparing isotopic niche widths among and within communities: SIBER – stable Isotope Bayesian Ellipses in R. J. Ecol. 595–602. https://doi.org/10.1111/j.1365-2656.2011.01806.x.

Janz, D.M., De Forest, D.K., Brooks, M.L., Chapman, P.M., Gilron, G., Hoff, D., Hopkins, W.A., McIntyre, D.O., Mebane, C.A., Palace, V.P., Skorupa, J.P., Wayland, M., 2010. Selenium toxicity to aquatic organisms. In: Ecological Assessment of Selenium in the Aquatic Environment.

- Jouanneau, W., Sebastiano, M., Rozen-Rechels, D., Harris, S.M., Blévin, P., Angelier, F., Brischoux, F., Gernigon, J., Lemesle, J.-C., Robin, F., Cherel, Y., Bustamante, P., Chastel, O., 2022. Blood mercury concentrations in four sympatric gull species from South Western France: insights from stable isotopes and biologging. Environ. Pollut. 308, 119619 https://doi.org/10.1016/j.envpol.2022.119619.
- Keilen, E.K., Borgå, K., Thorstensen, H.S., Hylland, K., Helberg, M., Warner, N., Bæk, K., Reiertsen, T.K., Ruus, A., 2022. Differences in trophic level, contaminant load, and DNA damage in an urban and a remote herring gull (*Larus argentatus*) breeding colony in coastal Norway. Environ. Toxicol. Chem. 41, 2466–2478. https://doi.org/ 10.1002/etc.5441.
- Kim, M., Park, K., Park, J.Y., Kwak, I.S., 2013. Heavy metal contamination and metallothionein mRNA in blood and feathers of black-tailed gulls (*Larus crassirostris*) from South Korea. Environ. Monit. Assess. 185, 2221–2230. https://doi.org/ 10.1007/s10661-012-2703-0.

I. dos Santos et al.

Kim, M.A., Williams, K.A., 2017. Lead levels in landfill areas and childhood exposure: an integrative review. Publ. Health Nurs. 34, 87–97. https://doi.org/10.1111/ phn.12249.

- Kojadinovic, J., Jackson, C.H., Cherel, Y., Jackson, G.D., Bustamante, P., 2011. Multielemental concentrations in the tissues of the oceanic squid *Todarodes filippovae* from Tasmania and the southern Indian Ocean. Ecotoxicol. Environ. Saf. 74, 1238–1249. https://doi.org/10.1016/j.ecoenv.2011.03.015.
- Laranjeiro, M.I., Alves, L.M., Silva, J.M., Calado, J.G., Norte, A.C., Paiva, V.H., Lemos, M. F.L., Ramos, J.A., Novais, S.C., Ceia, F.R., 2020. Assessment of environmental health based on a complementary approach using metal quantification, oxidative stress and trophic ecology of two gull species (*Larus michahellis & Larus audouinii*) breeding in sympatry. Mar. Pollut. Bull. 159, 111439 https://doi.org/10.1016/j. marpolbul.2020.111439.
- Laranjeiro, M.I., Alves, L.M., da Silva, J.M., Pereira, J.M., Norte, A.C., Paiva, V.H., Lemos, M.F.L., Ramos, J.A., Novais, S.C., Ceia, F.R., 2021. Year-round element quantification of a wide-ranging seabird and their relationships with oxidative stress, trophic ecology, and foraging patterns. Environ. Pollut. 284, 117502 https://doi. org/10.1016/j.envpol.2021.117502.
- Leighton, F.A., Peakall, D.B., Butler, R.G., 1983. Heinz-body hemolytic anemia from the ingestion of crude oil: a primary toxic effect in marine birds. Science 220, 871–873. https://doi.org/10.1126/science.6844918.
- Lopes, C.S., Paiva, V.H., Vaz, P.T., Pais de Faria, J., Calado, J.G., Pereira, J.M., Ramos, J. A., 2021. Ingestion of anthropogenic materials by yellow-legged gulls (*Larus michahellis*) in natural, urban, and landfill sites along Portugal in relation to diet composition. Environ. Sci. Pollut. Res. 28, 19046–19063. https://doi.org/10.1007/ s11356-020-12161-5.
- Lüdecke, D., 2018. ggeffects: tidy data frames of marginal effects from regression models. J. Open Source Soft 3, 772. https://doi.org/10.21105/joss.00772.
- Lüdecke, D., Ben-Shachar, M.S., Patil, I., Waggoner, P., Makowski, D., 2021. performance: an R package for assessment, comparison and testing of statistical models. J. Open Source Software 6. https://doi.org/10.21105/joss.03139.
- Mallory, M.L., Little, C.M., Boyd, E.S., Ballard, J., Elliott, K.H., Gilchrist, H.G., Hipfner, J. M., Petersen, A., Shutler, D., 2015. Leucocyte profiles of Arctic marine birds: correlates of migration and breeding phenology. Conserv. Physiol. 3, cov28 https:// doi.org/10.1093/conphys/cov028.
- Manceau, A., Gaillot, A.-C., Glatzel, P., Cherel, Y., Bustamante, P., 2021. In vivo formation of HgSe nanoparticles and Hg-tetraselenolate complex from methylmercury in seabirds—implications for the Hg–Se antagonism. Environ. Sci. Technol. 55, 1515–1526. https://doi.org/10.1021/acs.est.0c06269.
- Matos, D.M., Ramos, J.A., Calado, J.G., Ceia, F.R., Hey, J., Paiva, V.H., 2018. How fishing intensity affects the spatial and trophic ecology of two gull species breeding in sympatry. ICES J. Mar. Sci. 75, 1949–1964. https://doi.org/10.1093/icesjms/ fsy096.
- Meillere, A., Brischoux, F., Bustamante, P., Michaud, B., Parenteau, C., Marciau, C., Angelier, F., 2016. Corticosterone levels in relation to trace element contamination along an urbanization gradient in the common blackbird (*Turdus merula*). Sci. Total Environ. 566, 93–101. https://doi.org/10.1016/j.scitotenv.2016.05.014.
- Mendes, R.F., Ramos, J.A., Paiva, V.H., Calado, J.G., Matos, D.M., Ceia, F.R., 2018. Foraging strategies of a generalist seabird species, the yellow-legged gull, from GPS tracking and stable isotope analyses. Mar. Biol. 165, 1–14. https://doi.org/10.1007/ s00227-018-3421-0.
- Mills, W.F., Bustamante, P., McGill, R.A., Anderson, O.R., Bearhop, S., Cherel, Y., Votier, S.C., Phillips, R.A., 2020. Mercury exposure in an endangered seabird: longterm changes and relationships with trophic ecology and breeding success. Proc. Roy. Soc. B. 287, 20202683 https://doi.org/10.1098/rspb.2020.2683.
- Mills, W.F., Ibañez, A.E., Bustamante, P., Carneiro, A.P., Bearhop, S., Cherel, Y., Mariano-Jelicich, R., McGill, R.A.R., Montalti, D., Votier, S.C., Phillips, R.A., 2022. Spatial and sex differences in mercury contamination of skuas in the Southern Ocean. Environ. Pollut. 297, 118841 https://doi.org/10.1016/j.envpol.2022.118841.
- Minet, A., Manceau, A., Valada-Mennuni, A., Brault-Favrou, M., Churlaud, C., Fort, J., Nguyen, T., Spitz, J., Bustamante, P., Lacoue-Labarthe, T., 2021. Mercury in the tissues of five cephalopods species: first data on the nervous system. Sci. Total Environ. 759, 143907 https://doi.org/10.1016/j.scitotenv.2020.143907.
- Minias, P., 2015. The use of haemoglobin concentrations to assess physiological condition in birds: a review. Conserv. Physiol. 3, 1–15. https://doi.org/10.1093/ conphys/cov007.
- Naimi, B., 2017. Package 'usdm': Uncertainty Analysis for Species Distribution Models, pp. 1–18. R package version 1.
- Navarro, J., Oro, D., Bertolero, A., Genovart, M., Delgado, A., Forero, M.G., 2010. Age and sexual differences in the exploitation of two anthropogenic food resources for an opportunistic seabird. Mar. Biol. 157, 2453–2459. https://doi.org/10.1007/s00227-010-1509-2.
- Neff, J.M., 1997. Ecotoxicology of arsenic in the marine environment. Environ. Toxicol. Chem.: An Inter. J. 16, 917–927. https://doi.org/10.1002/etc.5620160511.
- Newsome, S.D., Martinez del Rio, C., Bearhop, S., Phillips, D.L., 2007. A niche for isotopic ecology. Front. Ecol. Environ. 5, 429–436. https://doi.org/10.1890/ 060150.1.
- Nigro, M., Leonzio, C., 1996. Intracellular storage of mercury and selenium in different marine vertebrates. Mar. Ecol. Prog. Ser. 135, 137–143. https://doi.org/10.3354/ meps135137.
- Norte, A.C., Araújo, P.M., Costantini, D., 2022. Physiological toolbox to explore the relationships between seabirds and their changing environments. In: Ramos, J.A., Pereira, L. (Eds.), Seabird Biodiversity and Human Activities. CRC Press, Boca Raton, pp. 36–58.
- Norte, A.C., Ramos, J.A., Araújo, P.M., Sousa, J.P., Sheldon, B.C., 2008. Health-state variables and enzymatic biomarkers as survival predictors in nestling great tits

(Parus major): effects of environmental conditions. Auk 125, 943–952. https://doi.org/10.1525/auk.2008.07188.

- Nos, D., Montalvo, T., Cortés-Francisco, 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 https://doi.org/ 10.1016/j.jhazmat.2023.133129.
- Oliveira, N., Abreu, P., Bores, J., Fagundes, A.I., Alonso, H., Andrade, J., 2020. Evaluating the potential of artificial nests as a conservation measure for Cory's Shearwaters *Calonectris borealis* breeding in Berlengas Archipelago, Portugal. Airo 27, 3–19.
- Oliveira, N., Alonso, H., Encarnação, V., Menezes, D., Magalhães, M., Carreira, G., Heber, S., Pimentel, R., Medeiros, V., Bairos, J., Raposo, P., Coelho, R., Rufino, R., Neves, R., Nascimento, T., Silva, E., Andrade, J., 2023. Changes in numbers and distribution of Yellow-legged Gull *Larus michahellis* nesting in Portugal during the last two decades. Airo 31, 20–37.
- Olmastroni, S., Pompeo, G., Jha, A.N., Mori, E., Vannuccini, M.L., Fattorini, N., Ademollo, N., Corsi, I., 2019. Erythrocytes nuclear abnormalities and leukocyte profile of the immune system of Adélie penguins (*Pygoscelis adeliae*) breeding at Edmonson Point, Ross Sea, Antarctica. Polar Biol. 42, 1343–1352. https://doi.org/ 10.1007/s00300-019-02522-3.
- Olmastroni, S., Simonetti, S., Fattorini, N., D'Amico, V., Cusset, F., Bustamante, P., Cherel, Y., Corsi, I., 2024. Living in a challenging environment: monitoring stress ecology by non-destructive methods in an Antarctic seabird. Sci. Total Environ., 171249 https://doi.org/10.1016/j.scitotenv.2024.171249.
- Pacheco, M., Santos, M., 1996. Induction of micronuclei and nuclear abnormalities in the erythrocytes of *Anguilla anguilla* L. exposed either to cyclophosphamide or to bleached kraft pulp mill effluent. Fresenius Environ. Bull. 5, 746–751.
- Pain, D.J., Mateo, R., Green, R.E., 2019. Effects of lead from ammunition on birds and other wildlife: a review and update. Ambio 48, 935–953. https://doi.org/10.1007/ s13280-019-01159-0.
- Pais de Faria, J., Paiva, V.H., Veríssimo, S., Gonçalves, A.M., Ramos, J.A., 2021a. Seasonal variation in habitat use, daily routines and interactions with humans by urban-dwelling gulls. Urban Ecosys 24, 1101–1115. https://doi.org/10.1007/ s11252-021-01101-x.
- Pais de Faria, J., Paiva, V.H., Veríssimo, S.N., Lopes, C.S., Soares, R., Oliveira, J., dos Santos, I., Norte, A.C., Ramos, J.A., 2022. Plenty of rooftops with few neighbours occupied by young breeding Yellow-legged Gulls (*Larus michahellis*): does this occur at the expense of their health condition? Ibis 165, 312–321. https://doi.org/ 10.1111/ibi.13123.
- Pais de Faria, J., Vaz, P.T., Lopes, C.S., Calado, J.G., Pereira, J.M., Veríssimo, S.N., Paiva, V.H., Gonçalves, A.M.M., Ramos, J.A., 2021b. The importance of marine resources in the diet of urban gulls. Mar. Ecol. Prog. Ser. 660, 189–201. https://doi. org/10.3354/meps13599.
- Paiva, V.H., Xavier, J., Geraldes, P., Ramirez, I., Garthe, S., Ramos, J.A., 2010. Foraging ecology of Cory's shearwaters in different oceanic environments of the North Atlantic. Mar. Ecol. Prog. Ser. 410, 257–268. https://doi.org/10.3354/meps08617.
- Palhares, D., Grisolia, C.K., 2002. Comparison between the micronucleus frequencies of kidney and gill erythrocytes in tilapia fish, following mitomycin C treatment. Genet. Mol. Biol. 25, 281–284. https://doi.org/10.1590/S1415-47572002000300005.
- Pereira, J.M., Paiva, V.H., Ceia, F.R., Ramos, J.A., 2020. Facing extremes: Cory's shearwaters adjust their foraging behaviour differently in response to contrasting phases of North Atlantic Oscillation. Reg. Environ. Change 20, 1–13. https://doi. org/10.1007/s10113-020-01662-1.
- Quirós, L., Ruiz, X., Sanpera, C., Jover, L., Piña, B., 2008. Analysis of micronucleated erythrocytes in heron nestlings from reference and impacted sites in the Ebro basin (NE Spain). Environ. Pollut. 155, 81–87. https://doi.org/10.1016/j. envpol.2007.10.030.

Rauch, J.N., Pacyna, J.M., 2009. Earth's global Ag, Al, Cr, Cu, Fe, Ni, Pb, and Zn cycles. Glob. Biogeochem. Cycles 23, GB2001. https://doi.org/10.1029/2008GB003376.

- R Core Team, 2023. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna.
- Richir, J., Bray, S., McAleese, T., Watson, G.J., 2021. Three decades of trace element sediment contamination: the mining of governmental databases and the need to address hidden sources for clean and healthy seas. Environ. Int. 149, 106362 https:// doi.org/10.1016/j.envint.2020.106362.
- Richir, J., Gobert, S., 2016. Trace elements in marine environments: occurrence, threats and monitoring with special focus on the coastal Mediterranean. J. Environ. Anal. Toxicol. 6, 1–19. https://doi.org/10.4172/2161-0525.1000349.
- Sánchez-Virosta, P., Espín, S., García-Fernández, A.J., Eeva, T., 2015. A review on exposure and effects of arsenic in passerine birds. Sci. Total Environ. 512, 506–525. https://doi.org/10.1016/j.scitotenv.2015.01.069.
- Sebastiano, M., Bustamante, P., Costantini, D., Eulaers, I., Malarvannan, G., Mendez-Fernandez, P., Churlaud, C., Blévin, P., Hauselmann, A., Dell'Omo, G., Covaci, A., Eens, M., Chastel, O., 2016. High levels of mercury and low levels of persistent organic pollutants in a tropical seabird in French Guiana, the Magnificent frigatebird, *Fregata magnificens*. Environ. Pollut. 214, 384–393. https://doi.org/ 10.1016/j.envpol.2016.03.070.
- Sebastiano, M., Bustamante, P., Eulaers, I., Malarvannan, G., Mendez-Fernandez, P., Churlaud, C., Blévin, P., Hauselmann, A., Covaci, A., Eens, M., Costantini, D., Chastel, O., 2017. Trophic ecology drives contaminant concentrations within a tropical seabird community. Environ. Pollut. 227, 183–193. https://doi.org/ 10.1016/j.envpol.2017.04.040.
- Seco, J., Aparício, S., Brierley, A.S., Bustamante, P., Ceia, F.R., Coelho, J.P., Philips, R.A., Saunders, R.A., Fielding, S., Gregory, S., Matias, R., Pardal, M.A., Pereira, E., Stowasser, G., Tarling, G.A., Xavier, J.C., 2021. Mercury biomagnification in a

I. dos Santos et al.

Southern Ocean food web. Environ. Pollut. 275, 116620 https://doi.org/10.1016/j.envpol.2021.116620.

- Sen, I.S., Peucker-Ehrenbrink, B., 2012. Anthropogenic disturbance of element cycles at the Earth's surface. Environ. Sci. Technol. 46, 8601–8609. https://doi.org/10.1021/ es301261x.
- Shah, N., Khan, A., Habib Khan, N., Khisroon, M., 2021. Genotoxic consequences in common grass carp (*Ctenopharyngodon idella* Valenciennes, 1844) exposed to selected toxic metals. Biol. Trace Elem. Res. 199, 305–314. https://doi.org/10.1007/ s12011-020-02122-x.
- Skarphedinsdottir, H., Hallgrimsson, G.T., Hansson, T., Hägerroth, P.Å., Liewenborg, B., Tjärnlund, U., Åkerman, G., Baršienė, J., Balk, L., 2010. Genotoxicity in herring gulls (*Larus argentatus*) in Sweden and Iceland. Muta. Res./Genet. Toxicol. Environ. Mutag. 702, 24–31. https://doi.org/10.1016/j.mrgentox.2010.07.002.
- Spallholz, J.E., Hoffman, D.J., 2002. Selenium toxicity: cause and effects in aquatic birds. Aquat. Toxicol. 57, 27–37. https://doi.org/10.1016/S0166-445X(01)00268-5.
- Szumiło-Pilarska, E., Falkowska, L., Grajewska, A., Meissner, W., 2017. Mercury in feathers and blood of gulls from the Southern Baltic coast, Poland. Water, Air. Soil Pollut 228, 1–13. https://doi.org/10.1007/s11270-017-3308-6.
- Szynkowska, M.I., Pawlaczyk, A., Maćkiewicz, E., 2018. Bioaccumulation and biomagnification of trace elements in the environment. In: Chojnacka, K., Saeid, A. (Eds.), Recent Advances in Trace Elements. John Wiley & Sons Ltd, New Jersey, pp. 251–276. https://doi.org/10.1002/9781119133780.ch13.
- Tartu, S., Angelier, F., Wingfield, J.C., Bustamante, P., Labadie, P., Budzinski, H., Weimerskirch, H., Bustnes, J.O., Chastel, O., 2015a. Corticosterone, prolactin and egg neglect behavior in relation to mercury and legacy POPs in a long-lived Antarctic bird. Sci. Total Environ. 505, 180–188. https://doi.org/10.1016/j. scitotenv.2014.10.008.
- Tartu, S., Bustamante, P., Angelier, F., Lendvai, Á.Z., Moe, B., Blévin, P., Bech, C., Gabrielsen, G.W., Bustnes, J.O., Chastel, O., 2015b. Mercury exposure, stress and prolactin secretion in an Arctic seabird: an experimental study. Funct. Ecol. https:// doi.org/10.1111/1365-2435.12534.
- Taylor, H.H., Anstiss, J.M., 1999. Copper and haemocyanin dynamics in aquatic invertebrates. Mar. Freshw. Res. 50, 907. https://doi.org/10.1071/MF99117.
- Thorne, L.H., Fuirst, M., Veit, R., Baumann, Z., 2021. Mercury concentrations provide an indicator of marine foraging in coastal birds. Ecol. Indicat. 121, 106922 https://doi. org/10.1016/j.ecolind.2020.106922.

- Thrall, M.A., Weiser, G., Allison, R.W., Campbell, T.W. (Eds.), 2012. Veterinary Hematology and Clinical Chemistry. John Wiley & Sons.
- Vallverdú-Coll, N., Mateo, R., Mougeot, F., Ortiz-Santaliestra, M.E., 2019. Immunotoxic effects of lead on birds. Sci. Total Environ. 689, 505–515. https://doi.org/10.1016/j. scitotenv.2019.06.251.
- Voulgaris, M.D., Karris, G., Xirouchakis, S., Pedro, P.Z., Asimakopoulos, A.G., Grivas, K., Bebianno, M.J., 2019. Trace metal blood concentrations in Scopoli's shearwaters (*Calonectris diomedea*) during 2007–2014: a systematic analysis of the largest species colony in Greece. Sci. Total Environ. 691, 187–194. https://doi.org/10.1016/j. scitotenv.2019.07.082.
- Walsh, P.M., 1990. The use of seabirds as monitors of heavy metals in the marine environment. In: Furness, R.W., Rainbow, P.S. (Eds.), Heavy Metals in the Marine Environment. CRC, Boca Raton, FL, USA.
- Wells, M.R., Coggan, T.L., Stevenson, G., Singh, N., Askeland, M., Lea, M.-A., Philips, A., Carver, S., 2024. Per-and polyfluoroalkyl substances (PFAS) in little penguins and associations with urbanisation and health parameters. Sci. Total Environ. 912, 169084 https://doi.org/10.1016/j.scitotenv.2023.169084.
- White, S.L., Rainbow, P.S., 1985. On the metabolic requirements for copper and zinc in molluscs and crustaceans. Mar. Environ. Res. 16, 215–229. https://doi.org/10.1016/ 0141-1136(85)90139-4.
- Wickham, H., Chang, W., Henry, L., Pedersen, T.L., Takahashi, K., Wilke, C., Woo, K., Yutani, H., Dunnington, D., 2023. Package 'ggplot2' - Create Elegant Data Visualisations Using the Grammar of Graphics.
- Ye, J., Li, J., Wang, P., Ning, Y., Liu, J., Yu, Q., Bi, X., 2022. Inputs and sources of Pb and other metals in urban area in the post leaded gasoline era. Environ. Pollut. 306, 119389 https://doi.org/10.1016/j.envpol.2022.119389.
- Zuñiga-González, G., Torres-Bugarín, O., Luna-Aguirre, J., González-Rodríguez, A., Zamora-Perez, A., Gómez-Meda, B.C., ventura-Aguilar, A.J., Ramos-Ibarra, M.L., Ramos-Mora, A., Ortíz, G.G., Gallegos-Arreola, M.P., 2000. Spontaneous micronuclei in peripheral blood erythrocytes from 54 animal species (mammals, reptiles and birds): part two. Mutat. Res. 467, 99–103. https://doi.org/10.1016/s1383-5718(00) 00021-8.
- Zuur, A.F., Ieno, E.N., Walker, N., 2009. Mixed Effects Models and Extensions in Ecology with R. Springer.