Science of the Total Environment 996 (2025) 180109

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Long-term ecotoxicological assessment of a reintroduced osprey population: an integrated approach in coastal environments of Central Italy

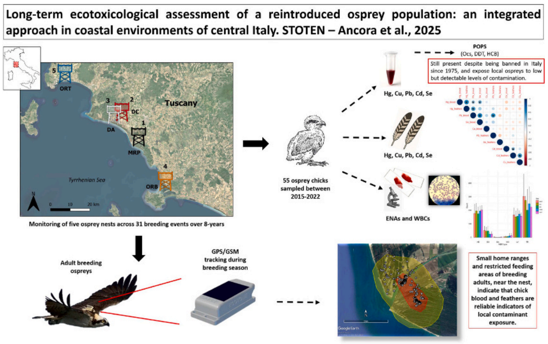
Stefania Ancora [a], Andrea Sforzi [b], Claudio Leonzio [a], Nicola Bianchi [a], Ilaria Caliani [a], Laura Giovanetti [a], Guia Consales [a], Letizia Marsili [a,c], Flavio Monti [d,*]

[a] *Department of Physical, Earth and Environmental Sciences, University of Siena, Via Mattioli, 4, 53100 Siena, Italy*
[b] *Maremma Natural History Museum, Strada Corsini 5, Grosseto 58100, Italy*
[c] *Centro Interuniversitario di Ricerca sui Cetacei, c/o DSFTA, Università di Siena, Strada Laterina 8, 53100 Siena, Italy*
[d] *Institute of Research on Terrestrial Ecosystems (IRET), National Research Council (CNR), Campus Ecotekne, 73100 Lecce, Italy*

HIGHLIGHTS

- Extensive ecotoxicological monitoring assessing health status of reintroduced ospreys.
- Feather and blood trace elements data generally do not appear to pose a concern.
- Ospreys are still exposed to detectable contamination from POPs banned since 1975.
- Genotoxicity biomarkers and hematological profiles suggest no genotoxic effects and good health.
- Long-term ecotoxicologically informed monitoring is key in conservation programs.

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Trace elements
ENA assay
Differential WBCs count
POPs
Non-destructive samples
Raptor
Reintroduction

ABSTRACT

Apex predators are particularly vulnerable to environmental stressors due to their high trophic position. Understanding both exposure and physiological impacts is essential to support the long-term success of conservation efforts. We investigated contaminant levels and health indicators in a recently reintroduced osprey *Pandion haliaetus* population in Central Italy, a top predator in aquatic food-webs. From 2015 to 2022, we applied a standardized, multidisciplinary monitoring framework to 55 wild-born osprey chicks, integrating non-destructive blood and feather sampling with trace element and POPs analyses, genotoxicity biomarkers, biological and behavioral metrics (e.g., sex, morphometrics, GPS-tracked adults' space use). Among trace elements, Se showed the highest blood concentrations (4.5 ± 2.1 mg/kg dw), while Cu predominated in feathers (11.5 ± 2.7 mg/kg dw); both Cd and Pb were consistently low. Hg and Se showed significant differences among breeding sites. Significant positive correlations were observed in blood for Hg—Se, Hg—Pb, Pb—Se, and in feathers for Cd—Cu. A low frequency of micronuclei (0.4 ± 1.1 ‰) was observed, with lobed nuclei accounting for 81 % of observed abnormalities (mean: 26.5 ± 24.1 ‰). Hematological profiles were dominated by heterophils, followed by lymphocytes, eosinophils, monocytes, and basophils, with a mean heterophil-to-lymphocyte ratio of 1.3 ± 0.6 and thrombocyte counts averaging 246.1 ± 132.9 ‰. The most abundant POPs were PCB153 > PCB138 >

---

* Corresponding author.
E-mail address: flavio.monti@cnr.it (F. Monti).

https://doi.org/10.1016/j.scitotenv.2025.180109
Received 7 May 2025; Received in revised form 18 June 2025; Accepted 17 July 2025
Available online 25 July 2025

PCB180 > PCB149 + 118 > PCB187, which together accounted for almost 40 % of total PCBs on average, confirming multi-year exposure in nestlings. Among DDTs, pp'DDE alone constituted between 15 % to 63 % of the total DDTs. We highlight the importance of integrative biomonitoring for assessing environmental quality and health in this critically endangered population, offering new insights into contaminant dynamics in reintroduced top predators and emphasizing the need for long-term, ecotoxicologically informed conservation strategies.

1. Introduction

Ecotoxicological monitoring studies have been increasingly used to evaluate the ecotoxicological status of wildlife populations and to detect the presence and the concentration of pollutants in the environment and in biota (Köhler and Triebskorn, 2013; García-Fernández, 2014; Espín et al., 2016). High-trophic level wildlife, such as apex predators, are particularly susceptible and vulnerable to the exposure and bioaccumulation of chemical contaminants through biomagnification processes (Thompson et al., 1998; Burger and Gochfeld, 2004; Scheuhammer et al., 2007; Elliott et al., 2009), representing excellent sentinel species for evaluating environmental health risks of related ecosystems (e.g. Lemarchand et al., 2011; García-Fernández et al., 2020). The assessment of ecotoxicological status of such target species can provide information on the potential health risks and subsequent ecological consequences that may occur both at individual and at the population level (Henny and Elliott, 2007), informing, in turn, on the quality of the environment where they live. Ecotoxicological monitoring is also essential to collect informative data to assist practitioners/managers in planning corrective measures in favour of endangered species, aiming at evidence-based conservation purposes (Rodriguez-Ramos Fernandez et al., 2011; Molenaar et al., 2017; Margalida et al., 2021). This is particularly relevant in the context of conservation translocation programs involving reintroductions or restockings (IUCN/SSC, 2013), where the health of individuals should be constantly monitored, particularly in the early stages of the translocation and/or during the first phases of the establishment of a new population (Sutherland et al., 2010). This approach helps to assess factors that could potentially affect the success of the conservation program and ensure that project efforts result in long-term self-sustaining populations (Sutherland et al., 2010; Herring et al., 2018). Raptors represent a particularly threatened group of birds (McClure et al., 2018; Buechley et al., 2019), especially vulnerable to high mortality rates and human-related threats (Sergio et al., 2008; De Pascalis et al., 2020), including environmental pollution (Gómez-Ramírez et al., 2014; Espín et al., 2016). More specifically, large raptors, occupying upper trophic levels in food webs and being long-lived, can accumulate, by biomagnification processes and over long time, significant concentrations of contaminants, potentially leading to negative consequences at individual (e.g. chick growth impairment, increased mortality) and population level (e.g. negative demographic trends and decline) (Lourenço et al., 2011).

The osprey *Pandion haliaetus* is a cosmopolitan apical raptor and a sentinel species of the trophic webs of aquatic ecosystems (Poole, 1989; Golden and Rattner, 2003; Grove et al., 2009). It is a specialist predator that exclusively feeds on live, epipelagic fish (Poole, 1989), within a feeding horizon corresponding to a subsurface area of 0–2 m depth. Despite its high specialization as a piscivore, it is an opportunistic forager that can feed in both freshwater and marine environments (Cramp and Simmons, 1980). High levels of many of these chemicals are found in marine and coastal ecosystems because of the influx from rivers, as well as runoff and other direct sources of pollution (Chakraborty and Chakraborty, 2021), and can accumulate in fish preyed upon by ospreys. As long-lived super-predator, osprey is prone to bioaccumulation of trace elements, organochlorine compounds and various other environmental contaminants (Espín et al., 2016), such as emerging contaminants, including polybrominated diphenyl ethers (PBDE) and perfluorooctane sulfonic acid (PFOS) (Grove et al., 2009; Chu et al., 2007; Eriksson et al., 2016). Several studies investigated the effects of contaminants on osprey populations of the New World (e.g. Elliott and Harris, 2001; Elliott et al., 2007; Henny et al., 2008), especially in relation to past widespread use of dichloro-diphenyl-trichloroethane (DDT). A dramatic eggshell thinning, as effect of this chemical compound and its metabolites, has been the cause of severe declines (e.g. Grove et al., 2009; Henny et al., 2010) in many American osprey populations during the 1960s and 1970s (Ames, 1966; Spitzer et al., 1978; Wiemeyer et al., 1988; Steidl et al., 1991). Following the ban of such pollutants (initiated in 1975), large-scale demographic and ecotoxicological studies re-evaluated exposure levels and spatial and temporal population trends, observing that American ospreys populations gradually recovered (Grove et al., 2009; Bierregaard et al., 2014; Lazarus et al., 2015).

In Europe, where strong demographic decreases have been recorded in the 20th century (Schmidt-Rothmund et al., 2014), ecotoxicological studies focused on the evaluation of heavy metals concentration in feathers, eggs and soft tissues samples from Scandinavian, French, German, Polish and Italian populations (Grove et al., 2009 and literature wherein; Lemarchand et al., 2011, 2012; Kalisinska et al., 2014; Odsjö and Sondell, 2014; Monti et al., 2020). Few studies are available on organic contaminants (e.g. Jiménez et al., 2007; Viluksela et al., 2024). Moreover, biochemical, molecular and behavioral biomarkers have been poorly used to evaluate the ecotoxicological status of the species in Europe, with the exception of plasma biochemistry parameters investigated in juvenile ospreys from Scotland (Meredith et al., 2012) and blood molecular sex determination of German nestling ospreys (Muriel et al., 2013). In the Mediterranean region, where the species underwent dramatic population contractions and is still in an unfavourable conservation status (Garrido et al., 2021; Westrip et al., 2022), only two exploratory studies have been conducted until now concerning trace elements and organochlorine compounds presence in eggs (Jiménez et al., 2007; Monti et al., 2020). There is therefore a lack of detailed information for these populations, especially in relation to their demographic trends. In this context, the species has been subject to various reintroduction projects in the last decades, especially in southern and Western Europe (Muriel et al., 2010; Monti et al., 2022). From a conservation point of view, these reintroduced populations are particularly vulnerable because of their relatively small numbers and because they are semi-dependent on human interventions (e.g. hacking facilities, artificial nests provisioning) at least in the first phases of the project and population establishment. For these reasons, an integrative and multidisciplinary monitoring approach is necessary to detect any adverse factor potentially threatening the success of the project itself and the persistence of the population in the long-term (Sutherland et al., 2010; Skujina et al., 2021; Monti et al., 2022).

To the best of our knowledge, only two ecotoxicological studies targeted reintroduced osprey populations in the Western Palearctic. Meredith et al. (2012) investigated hematologic and plasma biochemistry reference intervals in free-living wild osprey chicks from Scotland and England, before being successively translocated to Rutland Water (Central England) for release. Monti et al. (2020) analysed trace element variation by habitats and egg components in samples from Mediterranean populations in Corsica, Balearic Islands and the Italian reintroduced population. Monitoring studies using unhatched eggs as non-destructive samples have been extensively used and much useful data has been collected (e.g. in Finland; Viluksela et al., 2024). However, unhatched eggs could represent a biased sample in evaluating the presence and effects of environmental contaminants (Grove et al., 2009). For example, mercury may be acquired during migration/wintering and transferred to eggs and hatchlings, while mercury in feathers of older nestlings mainly reflects mercury concentrations in the local diet (e.g. Zabala et al., 2019). Chick feathers are generally better indicators of local contamination (Hughes et al., 1997; Zabala et al., 2019). Using other kinds of samples, such as feathers and blood from fledglings could be more informative providing more reliable estimates of contaminant levels. This is particularly significant in at-risk species, as blood and feather sampling is minimally invasive and allows for non-destructive monitoring of their health. Non-lethal biological techniques, such as blood biomarker analysis, have gained prominence in the study of endangered species (Muir et al., 1999; Vos et al., 2000; Champoux et al., 2002, 2006; Murvoll et al., 2006; Quirós et al., 2008). Given the critical role of physiological systems in enabling individuals to adapt to new selective pressures, it has been proposed that physiological biomarkers can serve as early warning indicators of potential long-term effects on populations (Mayer et al., 2018; Madliger et al., 2016; Giovanetti et al., 2024).

The main aim of this study was to assess the levels of environmental contaminants in the reintroduced Italian osprey population using a long-term, integrated approach based on complementary non-destructive sampling of blood and feathers. Multi-contaminant analyses and genotoxicity biomarkers were evaluated alongside biological and behavioral data, including sex, morphometric measurements, and adult space use at breeding sites. This comprehensive approach aimed to identify potential sources of environmental contamination, assess sub-lethal and long-term toxicological effects, and evaluate their impacts on the population's health.

The study also investigated potential correlations between trace elements concentrations and various factors: (i) sex of the birds, (ii) morphometric traits, (iii) genotoxicity and immunotoxicity markers, and (iv) differences in local contaminant level among breeding pairs within the study area where reintroduction efforts were carried out.

2. Material and methods

Between 2015 and 2022, blood and feather samples were collected from fifty-five osprey chicks to analyse trace elements (Hg, Cd, Pb, Se, Cu). Blood smears were used to assess erythrocytic nuclear abnormalities (ENA assay) and perform differential white blood cell (WBC) counts. Additionally, a subset of blood samples was analysed for persistent organic pollutants (POPs), including polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs: HCB, op'DDT, pp'DDT, op'DDE, pp'DDE, op'DDD and pp'DDD).

2.1. Study areas

The species returned to breed in Italy in 2011 as a result of a reintroduction project, following the first phase of translocation operations carried out between 2006 and 2010 in the Maremma Regional Park (Monti et al., 2022). Since 2011, the population gradually increased up to 7–8 pairs in 2024 (Monti et al., 2023; https://www.falcopescatore.it/), whereby five were the object of an extensive ecotoxicological monitoring program carried out during 8 years (2015–2022). Given the high philopatry of the species (Poole, 1989), each pair used the same nest year after year, therefore pairs and nesting sites coincide. The five osprey nest sites were regularly monitored within four protected areas, located in an extensive coastal wetland system in Southern Tuscany (coastal Central Italy; Fig. 1) in the following sites: Maremma Regional Park, Diaccia Botrona Natural Reserve, WWF Natural Reserve Orbetello Lagoon and WWF Natural Reserve Orti-Bottagone Marsh. The Maremma Regional Park is a regional protected area characterized by the Ombrone River which is flanked by brackish water wetlands and that contains a nest of ospreys, here defined with the code "MRP". The Diaccia Botrona Natural Reserve is a coastal marsh with shallow, brackish waters rich in fish. In this area are present two nests, coded "DC" and "DA". The WWF Natural Reserve Orbetello Lagoon contains a nest (coded "ORB") and it is one of the largest lagoons in the western Mediterranean, characterized by the presence of shallow waters and comprising two communicating basins (east and west basins). To be noted that this lagoon hosts a fish farm of great commercial interest, producing European sea bass and gilthead sea bream (Porrello et al., 2005) and where high levels of mercury were detected in fish (Miniero et al., 2013; Lenzi et al., 2021). The WWF Natural Reserve Orti-Bottagone Marsh is a coastal brackish water wetland where a nest (coded "ORT") is present. In all sites, the ospreys lay eggs on artificial nests built on three-pole structures (Sforzi et al., 2019).

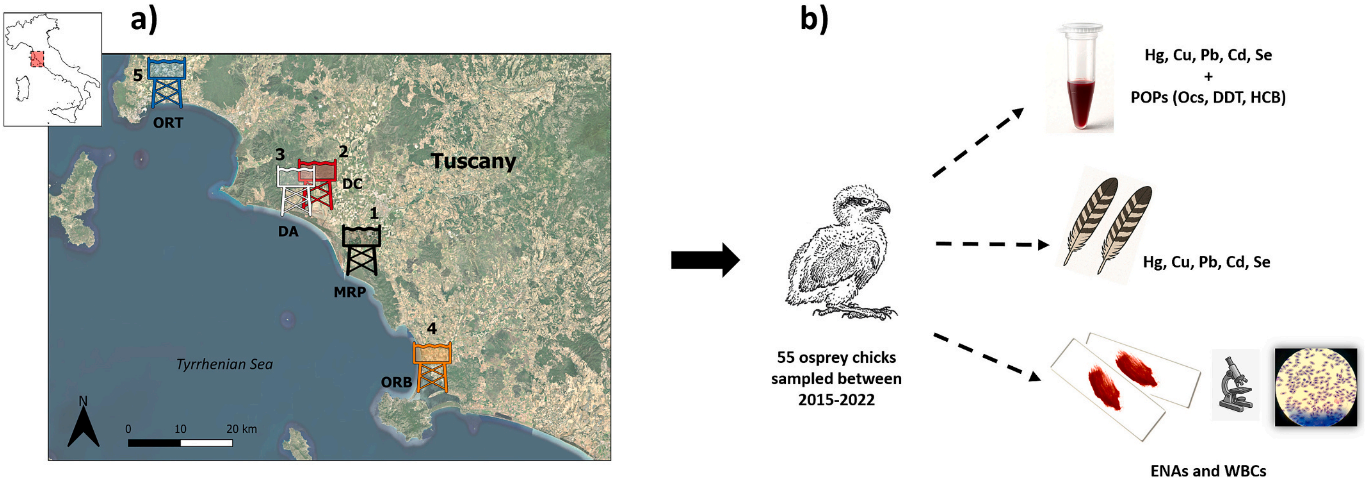


Fig. 1. Map of the study area in Southern Tuscany (central coastal Italy) and locations where osprey chicks' feather and blood samples were collected between 2015 and 2022. a) The five sites where the sampling took place are reported as follows: Maremma Regional Park (MRP, pair code 1, in black), the Diaccia Botrona Natural Reserve (DC, pair code 2 in red and DA, pair code 3 in white), WWF Natural Reserve Orbetello Lagoon (ORB, pair code 4 in orange) and WWF Natural Reserve Orti-Bottagone Marsh (ORT, pair code 5 in blue). b) Infographic representing the samples collected on each osprey chick: 1 mL of blood by venipuncture from the brachial vein of the wing, 6–8 body feathers and two blood smears per individual kept dry until laboratory analyses. Image readapted from Monti et al. (2023). The osprey chick is by Alessandro Troisi ©.

2.2. Biometric measurements and population monitoring

Population monitoring took place routinely: breeding events were continuously kept under daily surveillance through nest video recordings by means of video-surveillance systems, and accompanied by weekly field surveys, allowing recording of key breeding dates and parameters (Monti et al., 2019). Between 2015 and 2022, thirty-one reproductive events across the considered five nest sites were recorded, resulting in fifty-five newborn chicks sampled for the various analyses. All chicks were handled at an age of 5–8 weeks (range: 38–60 days according to the growth rate of the bird), and marked with both a metal and a coloured darvic ring (Monti et al., 2019). Capture, handling, and tagging procedures were carried out under formal authorization issued by the Tuscany Regional Administration, after the positive opinion expressed by the Italian Institute for Environmental Protection and Research (ISPRA), in accordance with Law157/1992 [Art.4 (1) and Art 7 (5)], which regulates research on wild bird and mammal species. Fieldwork was also conducted with the permission of management bodies of the nature reserves involved: Maremma Regional Park, Tuscany Region, WWF Natural Reserves. During ringing operations, biometric data were taken: a) body mass (MASS) measured with a 2500 g Pesola scale; b) tarsus length (TARSUS) with a digital calliper to the nearest 0.1 mm, from the front of the tarsometatarsal bone at the toe joint to the end of the bone below the ankle joint; c) the length of flattened wing chord (WING) using a metal ruler to the nearest 1 mm; d) head length (HL) measured as the distance between the tip of the bill and the back of the head; c) bill length (BL) as the distance between the tip of the bill and feathers. Bird handling (from capture to release) lasted about 30–40 min. The sex of the birds was assessed using molecular sexing, following Griffiths et al. (1998).

2.3. Sample collection for contaminant analysis

Blood and feather samples were collected from osprey chicks during ringing activities at nests. About 1 mL of blood was taken by venipuncture from the brachial vein of the wing with a syringe and transferred immediately in Vacutaner Lithium eparine-coated tubes. Two drops of blood were used to prepare two blood smears per individual (Fig. 1), which were kept dry until laboratory and the rest of the blood samples were stored at 4 °C during the field operations and then transferred in a freezer at −20 °C. About 6–8 breast feathers from each osprey chick were also collected and stored in paper bags at room temperature.

2.4. Adults' feeding areas and home ranges

During the entire duration of the chick-rearing period, osprey chicks are fed by parents that brought prey to the nest (Poole, 1989). Three breeding adults (2 males and 1 female) from three different nests were captured and fitted with back-pack mounted 24-g solar-powered GPS/GSM devices (model Duck-4, Ecotone, Gdynia, Poland), to estimate feeding areas and home ranges at breeding sites. These tags were programmed to collect GPS positions at hourly intervals during the day (05:00–21:00). The combined mass of the tracking device and harness never exceeded 3 % of the bird's body mass, which is within recommended limits (Kenward et al., 2001). All tags were attached as backpacks with a harness made of 7-mm-wide Teflon ribbon (Kenward et al., 2001). Data were extrapolated only for the active breeding period (assessed for each pair through video recordings and field surveys). The R "*adehabitatHR*" package (Calenge, 2006) was used to delineate 95 % and 50 % kernel density estimation (KDE) to estimate ospreys' home range (HR) and core area (CA), respectively. All GPS data analysed in this study are freely consultable in the Movebank database (www.movebank.org), project study name "Osprey in Mediterranean (Corsica, Italy, Balearics) - movement study ID: 20039459".

2.5. Contaminant analysis

2.5.1. Trace elements analysis in blood and feathers

All blood samples were lyophilized in a LIO 5Pascal, equipped with Edwards vacuum pump, for 48 h at 0.125 mbar and − 53 °C, while feathers were washed with ultrapure water and kept drying until analyses. Then, using a clean mortar and pestle, the dried materials were fully homogenized to a powder. Samples were processed for acid decomposition: about 0.100 g or 0.050 g of dry samples were added with nitric acid (HNO_3) and hydrogen peroxide (H_2O_2) 4:1 v/v and heated at 160 °C in a high-pressure hot block system (Teflon Bomb) for 9 h. Quality assurance measures included analysis of Standard Reference Materials namely, Fish Homogenate (IAEA-407), Fish muscle (DORM-4), treated and analysed under the same conditions as the samples. Recoveries of the element ranged from 92 to 105 %. Blanks were run during each set of tests to check the purity of the chemicals used and any sample contamination. Five elements (Hg, Cd, Pb, Cu and Se) were analysed using an atomic absorption spectrometer. For the determination of the various elements were used the following instruments: atomic absorption spectrometer (Analytik Jena ContrAA 700) with graphite furnace for Pb and Cd; cold vapour technique (Atomic Absorption Spectrometer Perkin Elmer Fims 400) for Hg; ICP-OES Plasma (Perkin Elmer 5100DV) for Cu; and Analytik Jena ContrAA 700 coupled to generation of hydride system (Hydrea HS 60) for Se. The precision of elements determination, expressed as the coefficient of variation on repeated assays of the same sample, was about 3 %. Concentrations are given as the mean of three replicates and expressed as mg/kg on dry weight basis (dw). For comparative purposes, to convert literature blood concentrations expressed as wet weight to dry-weight, we used the moisture content of 79.13 % indicated for other bird species by Eagles-Smith et al. (2008). Also, to convert blood volume-based μg/L to wet mass-based μg/kg concentrations, we used the density value of 1.06 kg/L of whole blood indicated by Langner et al. (2012) measured on a subset of fresh osprey blood samples, therefore a conversion factor of 0.943 was applied. Due to the preventive action of dietary Se from fish consumption against the toxicity of Hg (e.g. Cuvin-Aralar and Furness, 1991; Sørmo et al., 2011), the molar ratio between Se and Hg in blood and feathers was calculated.

2.5.2. Organochlorine compounds

A subsample of 13 individuals (out of the 55 chicks sampled) with a suitable amount of blood sample collected, was also analysed according to the modified U.S. Environmental Protection Agency (EPA) 8081/8082 method for determination of hexachlorobenzene (HCB), polychlorinated biphenyls (PCBs), and dichlorodiphenyltrichloroethane and its metabolites (DDTs) (Marsili, 2000). Precisely, lyophilized blood samples (0.1 g), fully homogenized to a fine powder, were extracted with n-hexane for pesticide residue analysis (PESTINORM, VWR Chemicals) using a Soxhlet apparatus. Before extraction, VWR cellulose thimbles (internal diameter: 25 mm, external diameter: 27 mm, length: 100 mm) were preheated at 110 °C for approximately 30 min and pre-extracted for 9 h in a Soxhlet apparatus with n-hexane to eliminate any potential organochlorine contamination. Each sample was spiked with a surrogate standard, 2,4,6-trichlorobiphenyl (IUPAC No. 30), before extraction. This compound was subsequently quantified and recovery rates ranged from 85 % to 95 %. Following the 9-h extraction with n-hexane, samples underwent a purification step with sulfuric acid 95 % (VWR Chemicals) for 12 h. The supernatant was concentrated to a volume of 10 mL using a rotary vacuum evaporator (Rotavapor 110) at 45 °C. To further remove residual lipid content, liquid chromatography was performed on a column containing Florisil (60–100 mesh, Merck) pre-dried at 110 °C for 1 h. The adsorption on Florisil was followed by sequential elutions with n-hexane until a final volume of 100 mL was reached. A second rotary evaporation was then performed to achieve a final volume of 100 μL. Decachlorobiphenyl (DecaPCB, IUPAC No. 209) was used as an internal standard, added to each sample before analysis, and included in the calibration standard mix (comprising Arochlor 1260, HCB, and both pp'- and op'-isomers of DDT, DDD, and DDE). High-resolution capillary gas chromatography analyses were conducted using an Agilent 6890 N series gas chromatograph, equipped with a 63Ni electron capture detector (ECD) and an on-column automatic injector. The column employed was an SBP-5 bonded phase capillary column (30 m length, 0.25 mm internal diameter, and 25 μm film thickness). Nitrogen was used as the carrier gas, with a head pressure of 15.5 psi (splitting ratio 50:1), while argon/methane (95:5) at 40 mL/min was used as the scavenger gas. The oven temperature was initially set at 100 °C for 10 min, then increased to 280 °C at a rate of 5 °C/min. The injector and detector temperatures were maintained at 200 °C and 280 °C, respectively. A mixture of specific isomers was used to calibrate the system, assess recovery, and confirm results. The standard injected contained 50 ng/mL of HCB, 100 ng/mL of DDT (op'DDT, pp'DDT, op'DDE, pp'DDE, op'DDD and pp'DDD), 200 ng/mL of op'DDT, and 2 μg/mL of Arochlor 1260. To evaluate the linearity in the instrumental response and the instrumental sensitivity, the following standard volumes were injected: 1, 2, and 4 μL. Capillary gas chromatography identified 29 PCB congeners (IUPAC No. 95, 99, 101, 118-pentachlorobiphenyls; 128, 135, 138, 144, 146, 149, 151, 153, 156-hexachlorobiphenyls; 170, 171, 172, 174, 177, 178, 180, 183, 187-heptachlorobiphenyls; 194, 195, 196, 199, 201, 202-octachlorobiphenyls; and 206-nonachlorobiphenyls). Total PCBs ($\sum$PCBs) were quantified as the sum of all congeners; total DDTs ($\sum$DDTs) were calculated as the sum of the isomers op'DDT, pp'DDT, op'DDE, pp'DDE, op'DDD and pp'DDD. To better characterize the degradation pattern and potential historical origin of DDT contamination, specific isomeric ratios were calculated, including pp'DDE/pp'DT, pp'DDE/DDTs, and (op'DDT + op'DDE + op'DDD)/DDTs. Results were expressed in ng/g dry weight (dw). The limit of detection (LOD) was calculated by measuring replicates ($n = 20$) of blank samples, determining the mean value and standard deviation (SD), and calculating the LOD as the mean + 2 SD. The LOD for all compounds analysed was 0.1 ng/kg (ppt).

2.6. Biomarker analysis

2.6.1. Erythrocytic nuclear abnormalities (ENA) assay

The ENA assay is a simple and cost-effective method to assess possible genotoxic effects that may occur in wildlife as result of exposure to environmental contaminants (Fenech et al., 2011). This test consists of the evaluation of DNA damage in erythrocytes by the identification and counting of nuclear abnormalities (kidney, segmented and lobed cells), including micronuclei (MN). The slides were fixed in methanol for 10 min and stained with Giemsa stain (5 %) for 30 min. The erythrocytic nuclear abnormalities were scored in 2000 mature erythrocytes per sample, according to the procedures of Maier and Schmid (1976) and Carrasco et al. (1990), adapted by Pacheco and Santos (1997). According to these authors, nuclear lesions were scored into one of the following categories: lobed nuclei, kidney-shaped nuclei, segmented nuclei and micronuclei. The results were expressed as the ENA frequency, the mean value (‰) of each abnormality and the sum of all the lesions observed.

2.6.2. Differential white blood cells (WBCs) count

The differential leukocytes count, an immune system biomarker, was performed on the same slides used for the ENA assay. White blood cells (WBCs) were identified and classified as lymphocytes, monocytes, eosinophils, heterophils or basophils according to the cellular morphology and staining characteristics of birds described by Giovanetti et al. (2024). Differential count was carried out on a total amount of 400 white blood cells per individual. Thrombocytes, as suggested in Davis et al. (2008), were considered only in their activated form. The ratio between the number of heterophils and lymphocytes was measured from each differential WBC, to obtain the H:L ratio, a stress indicator related to corticosteroid levels (Cīrule et al., 2012).

2.7. Statistical analyses

All analyses were carried out with R Studio software (v. 4.3.1; R Core Team, 2023). To investigate variation in trace elements concentration and erythrocyte abnormalities at the population level, we used generalized linear mixed models (GLMMs; Zuur and Ieno, 2016). Ten response variables were modelled to evaluate trace elements concentration in both ospreys' blood and feathers for the following five elements: Hg, Cd, Pb, Cu and Se. Other eight response variables were used to investigate abnormalities in: i) total ENA; ii-vi) heterophils, eosinophils, basophils, monocytes and lymphocytes; vii) number of thrombocytes, and viii) H:L ratio. Continuous response variables as trace element concentrations and the H:L ratio relationship were modelled with Gaussian error distributions and "identity link" functions, while discrete variables such as cell counts with the nbinom2 (link = "log") family function. In all models, we included the following predictors: "pair_code" (categorical; five levels: MRP, DC, DA, ORB, ORT; reference level: MRP; id of the breeding pair/nesting site), sex of the chick (categorical; two levels: male vs female) to investigate potential differences between sexes and tarsus length (numerical - as a proxy of the age and gradual growth of the bird during the chick rearing period) to evaluate response variables effects on the chick's status. When we found significant differences among the particular predictors, we used a Tukey pairwise post-hoc comparison through the *glth* function (*multcomp* package, v. 1.4–25; Hothorn et al., 2008). We did not consider the geographical location (or the nest site) as a predictor, because the "pair_code" itself already includes this information, being each breeding pair associated to a single nest within a specific location and distinct feeding areas of the adults (see section 3.1). We did not consider the weight of the chick as it could have been strongly dependent on the daily food intake at the time of ringing, thus representing a potentially confounding factor (e.g. Catitti et al., 2022). For this reason we used the tarsus length, for which we did not find significant differences between sexes (Independent Samples *t*-Test: $t = 0.150$ $p = 0.882$; $n = 53$; males: 58.4 ± 6.7 mm; females: 58.1 ± 6.6 mm), nor a significant correlation between its length and the sex of the bird (Spearman's rho = 0.01; $p = 0.94$). A preliminary data exploration showed that no multicollinearity was found among these predictor variables (sex and tarsus length). GLMMs were performed using the function *glmmTMB* (Brooks et al., 2017) for fitting models. Due to the presence of missing values (NAs) distributed across several variables, model selection and model comparison procedures (e.g., dredge or AIC-based selection) could not be reliably performed. Therefore, the analysis was confined to inference based on the full model, focusing on assessing the significance and direction of predictors' effects on the response variable. Models were validated through visual inspection of residual patterns (Zuur et al., 2009). Inference about the effects of predictors on each response variable was made by estimating *p*-values and 95 % confidence intervals (CIs) of predictors, assessing whether 95 % CIs overlapped "0" to identify informative predictors.

Possible correlations (Spearman test) among trace elements, in both blood and feathers, and bird morphometric, genotoxicity and immunotoxicity markers were investigated using the corrplot package (v0.92; Wei and Simko, 2017). Organochlorine pollutant values were not evaluated through GLMMs due to the limited sample size for this contaminant category ($n = 13$). Since data were not normally distributed, Kolmogorov-Smirnov tests were used to compare each OC pesticide between sexes. A Spearman-rank correlation test was also run to determine if any relationship existed between them. Tests were significant at $p < 0.05$. All descriptive statistical analyses are reported as mean ± standard deviation (SD), median and/or range (minimum-maximum).

3. Results

3.1. Home range and feeding areas of adult ospreys

Home range and core areas of GPS-tracked adult breeding ospreys were extremely small (HR = 6.4 ± 5.3 km^2; CA = 0.65 ± 0.63 km^2) and feeding areas mainly confined to zones adjacent to breeding sites (Fig. 2 and Table 1). Even in the case of the two adults of the two nests from the same wetland (DC and DA in the Diaccia Botrona Natural Reserve), used areas were totally disjointed and close to each nest site (Fig. 2). Chick feathers thus reflect most reliably conditions in the proximity of the nest, providing information on the presence of specific contaminants at a local scale. Details of corresponding spatial analyses of osprey movements are reported in Table 1.

Table 1
Information on movement components of three GPS-tracked adult breeding ospreys: ring code (ID), sex of the birds, tracking period, nest site code, number of GPS fixes, home range (HR) and core area (CA) expressed in km^2. Mean (± standard deviation) estimates are also reported.

ID	Sex	Tracking period	Site code	N fix	HR	CA
L7	Male	01/05/2015–31/07/2015	MRP	1148	9.36	1.61
IAC	Female	09/05/2016–31/07/2016	DA	3005	0.25	0.03
IAA	Male	01/05/2018–31/07/2018	DC	1276	9.9	0.70
Mean ± SD					*6.5 ± 5.4*	*0.78 ± 0.8*

3.2. *Trace elements in blood and feathers*

At population level, Se had the highest concentration in blood (4.5 ± 2.1 mg/kg dw) while in feathers, Cu was the most abundant (11.5 ± 2.7 mg/kg dw) (Table 2). Both Cd and Pb showed low levels in blood, respectively 0.008 ± 0.011 mg/kg dw and 0.092 ± 0.084 mg/kg dw (Table 2). Positive correlations were found between Hg—Pb ($r = 0.35$; $p < 0.05$), Hg—Se ($r = 0.40$; $p < 0.05$) and Pb—Se ($r = 0.61$; $p < 0.01$) in blood, while in feathers between Cd—Cu ($r = 0.54$; $p < 0.01$) (Fig. 3). For Cd, Hg and Pb blood and feathers were positively correlated ($r = 0.35$; $p < 0.05$ for Cd; $r = 0.62$; $p < 0.01$ for Hg; $r = 0.57$; $p < 0.01$ for Pb). Hg in feathers and Pb in blood had a positive correlation ($r = 0.53$; $p < 0.01$). Se in feathers was positively correlated with tarsus length ($r = 0.59$; $p < 0.01$).

Mercury significantly differed among breeding pairs, both in blood and feathers (Table 3; Table S1). Significantly higher values of mercury were found in chicks' blood belonging to MRP (1.9 ± 1.04 mg/kg dw;

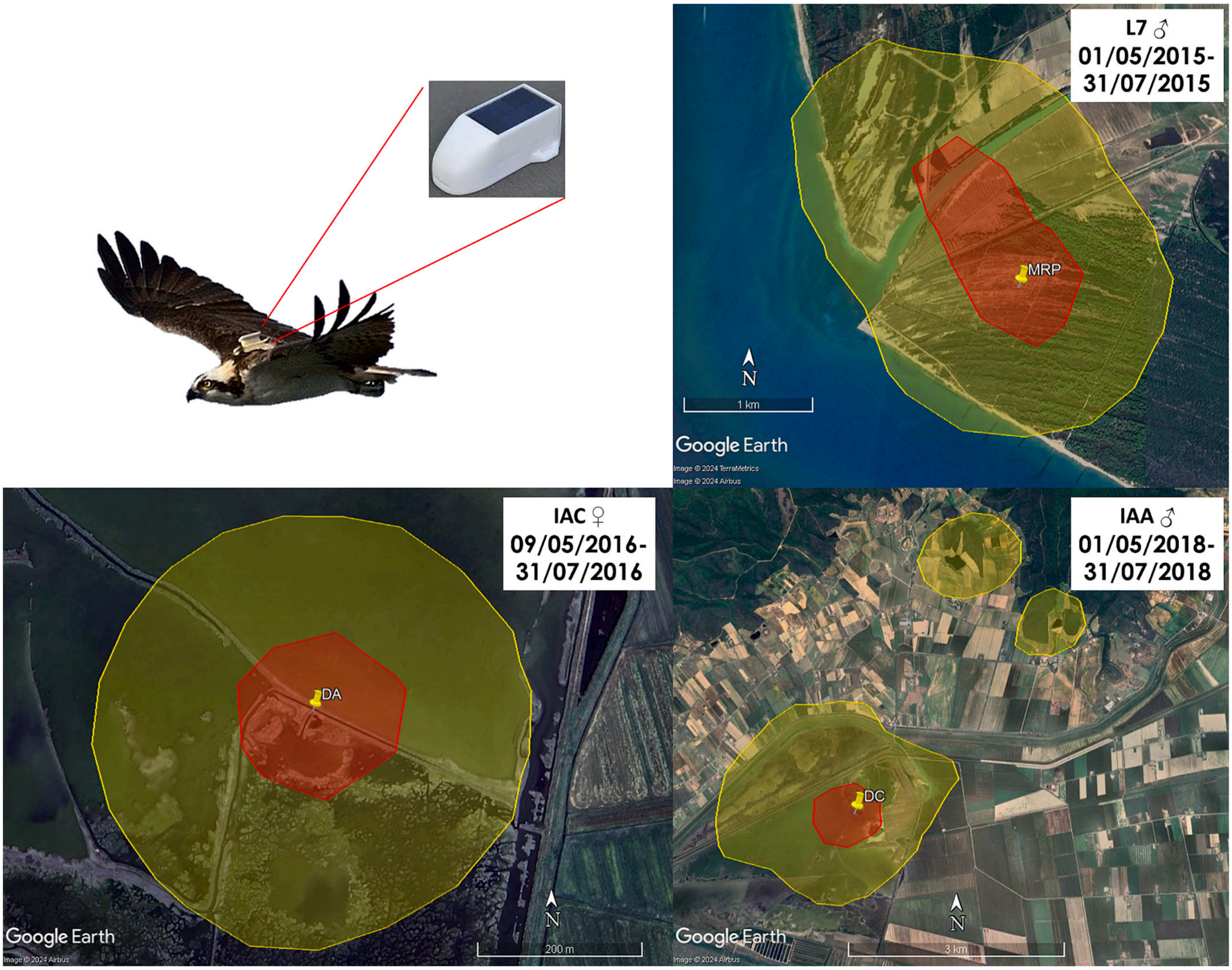


Fig. 2. Home ranges and core areas of breeding ospreys in coastal central Italy. Three adult ospreys (2 males - ring codes: L7 and IAA - and 1 female - ring code: IAC) were captured and fitted with 24-g solar-powered GPS/GSM devices (model Duck-4, Ecotone), to estimate feeding areas and home ranges during the chicks rearing period. The R "*adehabitatHR*" package (Calenge, 2006) was used to delineate 95 % and 50 % kernel density estimation for representing ospreys home range (in yellow) and core area (in red), respectively. Ring code of the bird (L7, IAA, IAC), sex and tracking period are reported on the white boxes.

Table 2
Mean ± SD and median values of trace elements (Hg, Cd, Pb, Se and Cu) from blood and feathers of Italian ospreys. Values are reported at population level and expressed as mg/kg on a dry weight (dw). The median is reported in square brackets, and the sample size is reported in round brackets.

Sample type	Hg	Cd	Pb	Se	Cu
Blood	1.132 ± 0.729 [0.972] (37)	0.008 ± 0.011 [0.004] (37)	0.092 ± 0.084 [0.081] (37)	4.554 ± 2.146 [4.156] (35)	3.983 ± 1.820 [3.601] (36)
Feathers	2.305 ± 1.306 [1.928] (54)	0.019 ± 0.027 [0.013] (54)	0.523 ± 0.352 [0.470] (49)	1.465 ± 0.545 [1.419] (45)	11.560 ± 2.779 [10.680] (43)

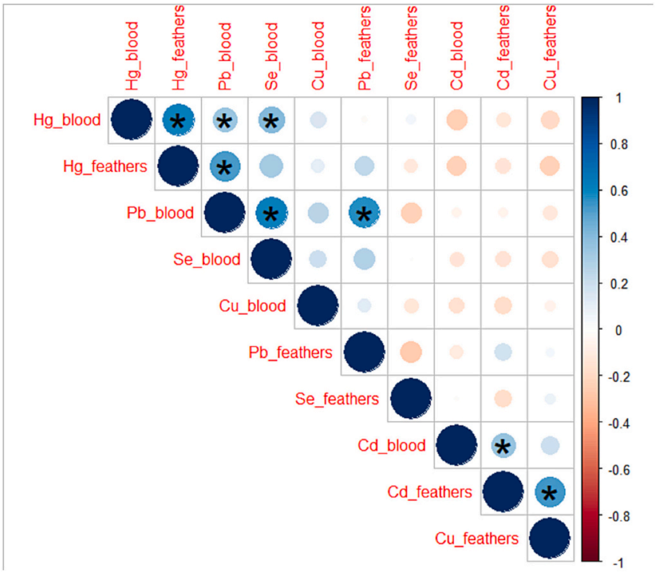


Fig. 3. Correlation matrix among trace elements concentrations (Hg, Cd, Pb, Se and Cu) in osprey blood and feathers. Positive correlations are displayed in blue and negative correlations in red. Color intensity and the size of the circle are proportional to the correlation coefficients. In the right side of the correlogram, the legend color shows the correlation coefficients and the corresponding colors. Significant correlations are labelled with an asterisk. Analysis of the data was done using the corrplot package (v0.92; Wei and Simko, 2017).

Table 3; Fig. 4a) with respect to DC, DA and ORT but not ORB that showed high levels as well (1.502 ± 0.312 mg/kg dw) (Table S1-S2). In feathers, higher values were recorded in ORB (Fig. 4b), with significant differences with all other nests except MRP (Table 3; Table S2). In both matrices investigated, Cd, Pb and Cu did not significantly differ between all fixed predictors (Table 3; Table S1). Se in feathers appeared to be significantly higher in birds with larger tarsus (Table 3). It also differed among pairs in blood: a significant higher concentration of Se was found in chicks from MRP with respect to those from DC, DA and ORT, but not ORB (Fig. 3; Table 3; Table S2). The Se:Hg molecular ratio for osprey feathers and blood samples is reported in Table S3.

3.3. *Levels of organochlorine pollutants*

The presence of xenobiotic contaminants was confirmed by the finding of polychlorinated biphenyls (PCBs) and organochlorine pesticides (HCB, op'DDT, pp'DDT, op'DDE, pp'DDE, op'DDD, pp'DDD) in osprey blood (Table 4). Due to the low and heterogeneous sample size for this contaminant category, correlation with other elements and differences between years and breeding pairs were not evaluated. Geometric mean and extremes (min-max) for each contaminant are reported in Table 4, along with the ratios between DDT isomers and/or PCBs. On average, among the 29 PCBs congeners analysed, the most abundant were PCB153 > PCB138 > PCB180 > PCB149 + 118 > PCB187, which jointly represent almost 40 % (39.93 %) on average of the total PCBs. Among DDTs, pp'DDE alone constitutes from 15 % to 63 % of DDTs. The mean ratios of pp'DDE/pp'DDT and of pp'DDE/DDTs were 7.53 and 0.45 respectively, while the ratio of the op' isomers sum (op'DDT + op'DDE+ op'DDD) to total DDT was on average 0.40. No statistical differences were found between OC pesticides and sexes (K-S: $p > 0.05$ for all), nor a significant correlation between them (Spearman test: $p > 0.05$ for all).

3.4. *ENAs and WBCs*

Erythrocytic nuclear abnormalities in ospreys' blood samples are shown in Table S4 and Fig. 5a. Mean values of ENAs at population level varied from the lowest number of micronucleis (0.41 ± 1.07 ‰), which account for only 1.22 % of total ENAs, while as for the other erythrocytic abnormalities, lobed nuclei represented 81.6 % among all anomalies with mean values of 26.5 ± 24.1 ‰ (Fig. 5a). The recurring ENAs were lobed nuclei, followed by segmented nuclei, kidney and micronuclei. Differences in total ENAs were not significant among the fixed predictors (Table 3), although values slightly differed among pairs with pairs from ORB showing highest mean values (Fig. 5a). Among the different white blood cells counted, heterophils and lymphocytes were the most abundant followed by eosinophils, monocytes and basophils. The mean H:L ratio was 1.3 ± 0.6. Thrombocytes showed mean values of 246.1 ± 132.9 ‰ (Table S4; Fig. 5b). WBCs levels were more similar among pairs, without statistical differences among them (Fig. 5b; Table 3).

No significant relationships were observed between tarsus length, genotoxicity, and immunotoxicity markers, including total nuclear abnormalities, eosinophils, basophils, monocytes, or lymphocytes. However, heterophils showed a significant association with tarsus length, consistent with findings for the H:L ratio (Table 3). Chicks with higher H:L ratio had significantly shorter tarsus too (Table 3). Regarding the possible statistical correlations of genotoxicity and immunotoxicity markers with trace elements, total ENAs was negatively correlated with Se and Cu in feathers (Table S5). In addition, eosinophils were negatively correlated with Pb in either blood or feathers, while being positively correlated with Se and Cu in feathers. Heterophils showed positive correlations with Cu and Pb in blood and with Pb in feathers. Heterophils were also inversely correlated with Se in feathers. The H:L ratio was positively correlated with Pb and Cu in blood, but negatively with Se in feathers (Table S5).

4. Discussion

4.1. *Osprey population ecotoxicological assessment*

In aquatic birds, especially top-predators, trace elements as well as other contaminants analysed in blood and feathers have been extensively used for monitoring programs and ecotoxicological studies (e.g. Burger and Gochfeld, 2004; Castro et al., 2011; Espín et al., 2016; Sabadková et al., 2024). To the best of our knowledge, this study is the first to use an approach that integrates multiple matrices (such blood and feathers), environmental contaminant analysis (as trace elements and POPs, including PCBs and DDTs), cellular markers (as ENA assay, WBCs), to investigate the health status of a reintroduced osprey population in Europe. Overall, we found that the concentration of trace elements found in blood samples and feathers appears above the range of causing negative effects to individuals' health and survival (Grove et al., 2009; Table S6). Organochlorine pollutants have been detected in the blood of sub-sampled chicks with levels that were below those found in other osprey populations from polluted areas, exposed both to agricultural and industrial activities (Grove et al., 2009; Table S6). The reintroduced Italian osprey population, since the first successful breeding

Table 3

Generalized linear mixed models (GLMMs) used to assess the effects of the "pair_code" (MRP, DC, DA, ORB, ORT), sex and tarsus length on trace elements concentration, components of erythrocytic abnormalities and differential white blood cells (WBCs) count of Italian osprey chicks, sampled between 2015 and 2022. The estimates and standard errors (SE) of all models are shown and the sample size is reported in brackets. Significant p-values (<0.05) are highlighted in bold. Significant codes are as follows: * - $p \leq 0.05$, ** - $p \leq 0.01$, *** -$p \leq 0.001$.

Response variable (sample size)	Predictor	Estimate	SE	p-value
Hg_feathers (*N* = 54)	(Intercept)	3.051368	1.096117	**0.00537****
	DC	−1.834273	0.368899	**6.62e-07*****
	DA	−1.0067	0.354052	**0.00446****
	ORB	1.1066	0.382498	**0.00381****
	ORT	−0.409479	0.458953	0.37228
	SexM	0.047635	0.246049	0.84649
	tarsus_mm	−0.004077	0.01803	0.82109
Cd_feathers (N = 54)	(Intercept)	0.0395895	0.034345	0.249
	DC	0.0256813	0.011559	**0.0263***
	DA	0.0116678	0.011094	0.2929
	ORB	0.008986	0.011985	0.4534
	ORT	0.0045957	0.01438	0.7493
	SexM	−0.011529	0.00771	0.1348
	tarsus_mm	−0.000404	0.000565	0.4751
Pb_feathers (*N* = 47)	(Intercept)	0.395845	0.497816	0.4265
	DC	0.180338	0.16526	0.2752
	DA	0.146278	0.151093	0.333
	ORB	0.32279	0.161743	**0.046***
	ORT	0.276705	0.191559	0.1486
	SexM	−0.173043	0.104424	0.0975
	tarsus_mm	0.001126	0.008142	0.89
Se_feathers (*N* = 43)	(Intercept)	−1.50417	0.67035	**0.0248***
	DC	−0.33949	0.21303	0.111
	DA	−0.21175	0.19163	0.2692
	ORB	−0.20843	0.20247	0.3033
	ORT	−0.61985	0.25761	**0.0161***
	SexM	0.14917	0.13822	0.2805
	tarsus_mm	0.05437	0.01094	**6.73E-07*****
Cu_feathers (*N* = 41)	(Intercept)	12.32494	4.54169	**0.00665****
	DC	2.23835	1.30845	0.08714
	DA	0.7269	1.16165	0.53148
	ORB	−0.07462	1.23377	0.95177
	ORT	1.31046	1.42671	0.35835
	SexM	−1.37092	0.84164	0.10334
	tarsus_mm	−0.01142	0.07651	0.88129
Hg_blood (*N* = 36)	(Intercept)	2.286902	0.968617	**0.01823***
	DC	−1.472594	0.26899	**4.39E-08*****
	DA	−1.24132	0.224099	**3.04E-08*****
	ORB	−0.57949	0.255986	**0.02359***
	ORT	−0.865241	0.293466	**0.00319****
	SexM	0.298901	0.173377	0.08471
	tarsus_mm	−0.008644	0.015969	0.5883
Cd_blood (*N* = 37)	(Intercept)	0.0100018	0.022814	0.661
	DC	0.0086941	0.006335	0.17
	DA	0.0047726	0.005278	0.366
	ORB	0.0031157	0.006029	0.605
	ORT	0.0065317	0.006912	0.345
	SexM	0.0020486	0.004084	0.616
	tarsus_mm	−0.000117	0.000376	0.756
Pb_blood (N = 37)	(Intercept)	0.393432	0.156346	**0.0119***
	DC	−0.082469	0.043418	0.0575
	DA	−0.043493	0.036172	0.2292
	ORB	−0.007715	0.041319	0.8519
	ORT	−0.037774	0.047369	0.4252
	SexM	0.002334	0.027985	0.9335
	tarsus_mm	−0.004783	0.002578	0.0635
Se_blood (*N* = 34)	(Intercept)	13.31577	3.66579	**0.000281*****
	DC	−3.33	0.9332	**0.000359*****
	DA	−2.84433	0.79725	**0.00036*****
	ORB	−1.47363	0.88734	0.096765
	ORT	−3.05407	1.01338	**0.00258****
	SexM	0.47729	0.6084	0.432747
	tarsus_mm	−0.12319	0.06064	**0.042194***
Cu_blood (*N* = 35)	(Intercept)	8.60408	3.68871	**0.0197***
	DC	−1.251	0.98554	0.2043
	DA	−0.1706	0.83612	0.8383
	ORB	−1.53738	0.93914	0.1016
	ORT	−0.52839	1.07401	0.6227
	SexM	0.21896	0.63439	0.73
	tarsus_mm	−0.07311	0.06109	0.2314

(*continued on next page*)

Table 3 (*continued*)

Response variable (sample size)	Predictor	Estimate	SE	p-value
Tot ENAs (*N* = 33)	(Intercept)	5.42617	1.46472	**0.000212** ***
	DC	0.40294	0.38783	0.298822
	DA	0.60689	0.33432	0.069473
	ORB	0.76582	0.3999	0.055492
	ORT	0.25388	0.4327	0.557385
	SexM	−0.25314	0.27788	0.362324
	tarsus_mm	−0.04018	0.02517	0.110378
Heterophiles (*N* = 27)	(Intercept)	6.484187	0.552923	**<2e-16** ***
	DC	0.040223	0.145634	0.7824
	DA	−0.020998	0.117372	0.858
	ORB	0.006692	0.132052	0.9596
	ORT	0.118901	0.142265	0.4033
	SexM	−0.0387	0.09317	0.6779
	tarsus_mm	−0.022608	0.009335	**0.0154** *
Eosinophils (27)	(Intercept)	2.11296	1.32705	0.1113
	DC	0.04537	0.3925	0.908
	DA	−0.12483	0.3249	0.7008
	ORB	−0.64604	0.35537	0.0691
	ORT	−0.68243	0.38329	0.075
	SexM	0.14167	0.26532	0.5934
	tarsus_mm	0.03023	0.02199	0.1693
Basophils (N = 27)	(Intercept)	1.74343	3.94543	0.6586
	DC	−1.80845	1.12543	0.1081
	DA	−1.52921	0.82357	0.0633
	ORB	−0.94828	0.8458	0.2622
	ORT	−0.22887	0.84542	0.7866
	SexM	−0.73587	0.72007	0.3068
	tarsus_mm	−0.01049	0.06349	0.8688
Monocytes (N = 27)	(Intercept)	2.28819	2.7888	0.4119
	DC	0.22671	0.74356	0.7604
	DA	0.19119	0.58364	0.7432
	ORB	0.96027	0.67172	0.1528
	ORT	1.24929	0.69047	0.0704
	SexM	−0.22057	0.47263	0.6407
	tarsus_mm	−0.01443	0.04761	0.7617
Lymphocytes (N = 27)	(Intercept)	4.326462	0.557748	**8.69E-15** ***
	DC	−0.06119	0.157248	0.697
	DA	−0.069695	0.124008	0.574
	ORB	0.099315	0.139489	0.476
	ORT	−0.04628	0.151252	0.76
	SexM	−0.032264	0.09878	0.744
	tarsus_mm	0.014168	0.009484	0.135
Thrombocites (N = 27)	(Intercept)	6.91425	1.43049	**1.34E-06** ***
	DC	−0.21084	0.36581	0.5644
	DA	−0.31776	0.29926	0.2883
	ORB	−0.27827	0.33493	0.4061
	ORT	−0.32936	0.36175	0.3626
	SexM	−0.44809	0.23812	0.0599
	tarsus_mm	−0.01655	0.02407	0.4917
H:L Ratio (*N* = 28)	(Intercept)	3.80778	1.23632	**0.00207** **
	DC	0.03861	0.33862	0.90921
	DA	0.01534	0.27068	0.95481
	ORB	−0.3222	0.30484	0.29054
	ORT	0.00986	0.32871	0.97607
	SexM	0.11125	0.21572	0.60605
	tarsus_mm	−0.04544	0.02085	**0.02929** *

event occurred in 2011, is showing good reproductive parameters and a continuous slight increasing trend (Monti et al., 2023). Specifically, within the scope of our study, between 2015 and 2022, a total of 31 breeding events were recorded among the five monitored pairs, resulting in 55 fledged chicks. Under the current contamination levels, these breeding pairs exhibited an average productivity of 1.77 chicks per pair per year and a linear growth rate of approximately 0.25 pairs per year. These figures indicate a good reproductive performance, suggesting that the present contaminant exposure does not appear to impair the population's reproductive output. Moreover, the population has continued to increase in recent years (2023–2025) and it is currently expanding into new areas (*unpubl. data*), further underscoring the positive trajectory of the reintroduction program. These findings highlight that, despite potential environmental stressors, the population demonstrates encouraging signs of establishment and growth.

4.2. *Trace elements in blood and feathers*

After ingestion, trace elements and other contaminants are absorbed by the gastrointestinal tract and enter systemic circulation, reaching various organs and tissues (e.g. Eens et al., 1998; Driscoll et al., 2013). Blood is an exchange compartment through the body, and the primary matrix for evaluating recent dietary uptake (e.g. Fournier et al., 2002). Feathers, on the other hand, incorporate trace elements from the bloodstream during their growth, making them useful indicators of pollutant exposure (Bracey et al., 2021). Since feather growth occurs over weeks (Lodenius and Solonen, 2013), the concentration of trace elements in feathers reflects recent environmental exposure and provides insights into local contamination sources (Renedo et al., 2018). In nestlings, feathers are a particularly reliable proxy for pollutant exposure during the growth period (Furness et al., 1986; Solonen and

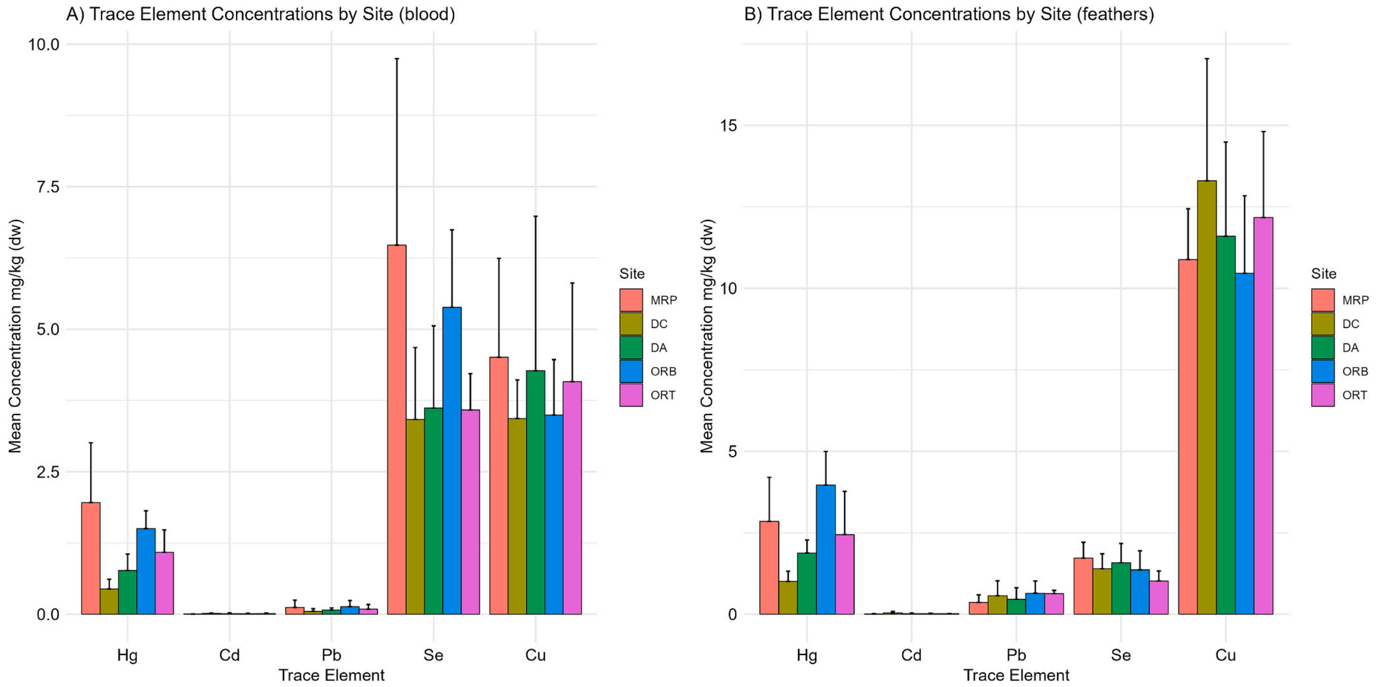


Fig. 4. Mean values of trace elements per breeding pair in A) blood and B) feathers. On the y-axis, the trace element concentrations are expressed as mg/kg (dw). Bars indicate standard deviation.

Lodenius, 1990; Hughes et al., 1997; Thompson et al., 1998; Espín et al., 2016; Bracey et al., 2021).

In this study, we found that Hg levels in blood were in line with those reported by Lounsbury-Billie et al. (2008) in juvenile ospreys from unpolluted areas of Everglades National Park in Florida (USA), and with those of Rattner et al. (2008) in individuals from Chesapeake and Delaware Bays (USA), where Hg is considered not a major environmental concern (Table S6). The nesting site of MRP and ORB were those with high Hg levels chicks' blood, with MRP showing the highest mean value of 1.94 mg/kg dw. Even this concentration is, however, in line with the lower literature values (Table S6) and do not exceed the highest values reported by DesGranges et al. (1998) for nestlings from polluted environments of constructed hydroelectric reservoirs in Quebec (Canada) of 1.8 mg/kg ww corresponding to about 9.2 mg/kg dw basis (Table S6). Jackson et al. (2016) using a combination of field and lab-based studies on Hg effect in a variety of species, including osprey, assessed avian mercury risk in blood across the western United States and Canada. This study indicated, for wet weight blood, five relative risk categories including background (<0.5 mg/kg), low (0.5–1 mg/kg), moderate (1–2 mg/kg), high (2–3 mg/kg), and extra high (>3 mg/kg) groups. These groups corresponding as dry weight basis to 2.4 mg/kg background, 2.4–4.8 mg/kg low, 4.8–9.6 mg/kg moderate, 9.6–4.4 mg/kg high, and > 14.4 mg/kg extra high. In this respect, none of the nestlings from our population in Italy exceeded the above-mentioned background group Hg effect threshold. Among breeding pairs, mercury significantly differed also in feathers. In particular, similarly to the findings obtained for blood, the two nesting sites with higher mean values were ORB and MRP. However, even the highest mean mercury level found for feathers from ORB chicks, felt generally below the literature values for nestling osprey from unpolluted areas (Table S6), varying between 0.1 and 5 mg/kg dry weight. As example, in aquatic ecosystems affected by Hg contamination in Finland, feathers of osprey chicks exhibit five to seven-fold mercury concentrations (exceeding 10.0 mg/kg dw in 26.6 %) than uncontaminated areas with 3.4–4.5 mg/kg dw (Häkkinen and Häsänen, 1980). In our case, osprey chicks from MRP and ORB, showed higher Hg concentrations both in blood and feathers compared to those from the other nestling sites (DA, DC, ORT), though at levels overlapping those of reference and unpolluted areas. Highest levels for the ORB site could be in part due to elevated Hg concentrations in fish from the Orbetello lagoon, where a study showed that in 90 % of the cases this element exceeded the regulatory maximum level of 0.5 mg/kg ww (Miniero et al., 2013; Lenzi et al., 2021). Moreover, from geographic point of view, it should be considered that in the South-Eastern Tuscany the nesting site of ORB and MRP are respectively close to Albegna and Ombrone rivers, both draining part of the Hg mining district of Mount Amiata, the third largest mercury district of the world (activity ceased in 1980), where is also located an active geothermal field. Future studies examining Hg content in specific prey of different nestling sites will shed insight into the real extent of the Hg input through the diet and the relative ecological risk for newborn osprey of those areas.

The positive and significant relationship found between Hg in blood and in feathers in Italian osprey chicks underlines the strong association for these matrices accounting for the same Hg source and informing about the recent intakes. However, levels found in feathers of Italian osprey chicks are lower even than those associated to no-observed deleterious Hg effects, at least for population reproductive parameters (Cahill et al., 1998; DesGranges et al., 1998). Mercury in feather samples was generally higher than in blood (Table S1) and other studies investigating the same biological matrices from nestling ospreys exhibited similar feathers/blood Hg ratios, also independently from the environmental concentration (DesGranges et al., 1998; Lounsbury-Billie et al., 2008; Rattner et al., 2008; Langner et al., 2012). This was expected due to the sequestration process of Hg in the highly keratinized tissues (feathers) (DesGranges et al., 1998; Furness et al., 1986; Rattner et al., 2008), acting as a complementary and efficient detoxification/elimination mechanism (Furness et al., 1986).

In addition to the Hg detoxification mechanism by sequestration in feathers, a better interpretation of the results requires evaluating Hg concentrations in conjunction with Se levels, due to Se protective role against Hg toxicity (Cuvin-Aralar and Furness, 1991; Wang and Wang, 2017). At the population level, we found a mean Se concentration in blood of 4.5 mg/kg dw. As an essential element, concentrations of Se in the blood are considered adequate for nutritional needs in birds at

Table 4

Concentrations (mean ± SD; range: min-max) of polychlorinated biphenyls (PCBs) and congeners, organochlorine pesticides (OCPs) and metabolites (ng/g dw) quantified in blood of 13 wild-born osprey chicks from the Italian osprey population monitored between 2016 and 2018 and in 2020.

	Mean ± St.Dev. Min-Max ng/g d.w.	Mean ± St.Dev. Min-Max %
EOM		**21.12 ± 11.68** **4.93–51.45**
HCB	**3.88 ± 4.29** **0.39–16.04**	
DDTs	**135.68 ± 98.70** **35.14–379.20**	
op'DDE	6.87 ± 6.36 0.91–22.05	5.69 ± 3.04 1.79–11.25
pp'DDE	66.76 ± 66.08 7.37–227.80	46.27 ± 22.28 14.92–74.12
op'DDD	11.49 ± 9.76 2.70–34.99	9.57 ± 4.40 3.15–16.32
pp'DDD	7.30 ± 8.13 1.36–33.33	5.65 ± 3.62 1.77–14.56
op'DDT	27.54 ± 19.07 3.44–63.22	22.46 ± 9.68 6.75–35.07
pp'DDT	12.56 ± 11.43 1.93–45.01	10.36 ± 5.65 4.30–19.67
PCBs	**203.99 ± 156.24** **67.46–692.83**	
95	8.63 ± 15.07 <LOD-57.48	2.90 ± 2.15 0.00–8.30
101	8.47 ± 5.69 <LOD-18.77	4.52 ± 3.23 0.00–9.32
99	4.78 ± 3.40 0.98–10.55	2.47 ± 1.25 0.66–4.70
151	5.35 ± 4.50 1.05–19.23	2.77 ± 1.52 0.89–7.28
144 + 135	4.95 ± 3.06 0.97–11.98	3.37 ± 2.12 0.14–8.23
149 + 118	10.27 ± 12.91 0.90–48.57	6.22 ± 10.43 0.00–41.01
146	7.63 ± 6.78 0.95–26.72	3.69 ± 1.66 0.65–6.43
153	32.18 ± 29.08 4.93–107.02	14.19 ± 6.30 7.12–24.81
138	17.06 ± 16.33 3.59–63.45	7.49 ± 2.47 4.61–12.71
178	5.30 ± 6.44 1.00–26.66	2.25 ± 0.88 1.08–3.85
187	10.48 ± 8.12 2.49–32.21	4.99 ± 1.65 2.58–8.06
183	4.93 ± 2.38 1.52–9.17	2.77 ± 1.01 1.28–4.37
128	2.03 ± 1.27 0.46–4.70	1.06 ± 0.39 0.34–1.67
174	6.93 ± 5.11 2.51–22.68	3.99 ± 2.81 1.39–13.08
177	4.03 ± 2.88 0.84–12.15	2.31 ± 1.23 0.50–4.26
156 + 171 + 202	4.81 ± 3.76 1.12–15.72	2.57 ± 1.20 0.95–4.89
172	3.51 ± 3.07 0.46–12.58	1.64 ± 0.64 0.39–2.89
180	16.95 ± 21.45 3.36–87.88	7.05 ± 3.03 3.60–13.09
199	3.75 ± 3.01 0.56–9.39	2.21 ± 1.96 0.38–6.64
170	9.92 ± 10.28 2.73–42.92	4.49 ± 1.36 2.12–6.26
196	4.94 ± 3.27 1.64–14.61	2.83 ± 1.37 1.26–5.72
201	7.02 ± 5.56 1.29–24.50	3.79 ± 1.79 1.09–7.45
195	9.19 ± 9.54 0.97–38.66	4.06 ± 1.88 0.99–7.14
194	6.14 ± 10.32 0.39–40.92	2.25 ± 1.43 0.33–5.91
206	7.42 ± 5.13 <LOD-17.85	4.12 ± 3.08 0.00–8.79
	Mean ± St.Dev. Min-Max ng/g l.w.	
HCB	**30.47 ± 48.81** **1.22–176.46**	
DDTs	**1098.80 ± 1511.60** **110.43–4981.14**	
PCBs	**1661.06 ± 2241.11** **217.63–7621.89**	
	Mean ± St.Dev. Min-Max	
DDTs/PCBs	0.68 ± 0.34 0.29–1.73	
pp'DDE/DDTs	0.45 ± 0.22 0.15–0.74	
pp'DDE/pp'DDT	7.53 ± 5.85 0.76–15.84	
Σop'DDTs/DDTs	0.40 ± 0.15 0.16–0.57	

0.62–0.95 mg/kg dw (Ohlendorf and Heinz, 2011), therefore osprey blood samples from all nestling sites are not at risk of Se depletion. Studies on blood Se concentration in nestling osprey for comparative purposes are scarce (Table S6). Values of the same order of magnitude are reported in the only one available study in Chesapeake and Delaware Bays, considered as reference areas (Rattner et al., 2008). Regarding Se in feathers, our finding of a mean population level of 1.46 mg/kg dw is similar to the background concentrations reported for birds, which range from 1 to 4 mg/kg and are typically below 2 mg/kg (Ohlendorf and Heinz, 2011). For nestling ospreys, literature studies (Table S6) indicate Se in feathers varying between 2.6 and 3.38 for American ospreys (Cahill et al., 1998; Rattner et al., 2008), and between 1.6 and 4.4 mg/kg dw for Scandinavian individuals (Norway; Odsjö et al., 2004). From a toxicological point of view, in feathers, levels of Se associated with effects vary among bird species and range from 1.8 mg/kg dw (sublethal) to 26 mg/kg dw (lethal) (Heinz, 1996; Hargreaves et al., 2010). Either as the mean of the osprey population or as the mean of different nesting sites, levels found in the present study fall below the threshold of sublethal effects and below the provisional threshold of 5 mg/kg dw, according to the Guidelines of United States Department of the Interior (1998). However, some nesting sites, specifically ORB and MRP, slightly exceed that threshold value, with Se blood levels of 5.38 and 6.47 mg/kg dw, respectively. Noteworthy, in both blood and feathers, these sites also showed elevated Hg concentrations and, especially for blood, the Se pattern among nest sites well mirrors that of Hg supported by the significant positive correlation found between these elements.

Numerous studies suggest that a fish Se to Hg molar ratio (Se:Hg) greater than 1 indicates a low risk for fish consumers (Pelletier, 1986; Cuvin-Aralar and Furness, 1991; Gerson et al., 2020; Manceau et al., 2021). In our study, the calculated Se:Hg molar ratio showed that only the ORB nesting site had a ratio below 1 in feathers, while Se was always in excess, particularly in blood, with levels that were approximately one order of magnitude higher than in feathers (Table S2). Overall, Se and Hg results indicated low Hg concentrations in both feathers and blood, falling within background levels and below known adverse effect thresholds (Grove et al., 2009), with Se potentially acting as an antagonist to Hg toxicity. Combined with the protective effect of Hg sequestration in feathers, these findings suggest that Hg is unlikely to pose a threat to the Italian osprey population, at present.

Interestingly, Se in feathers was positively correlated with tarsus length, which may suggest a relationship between this essential element

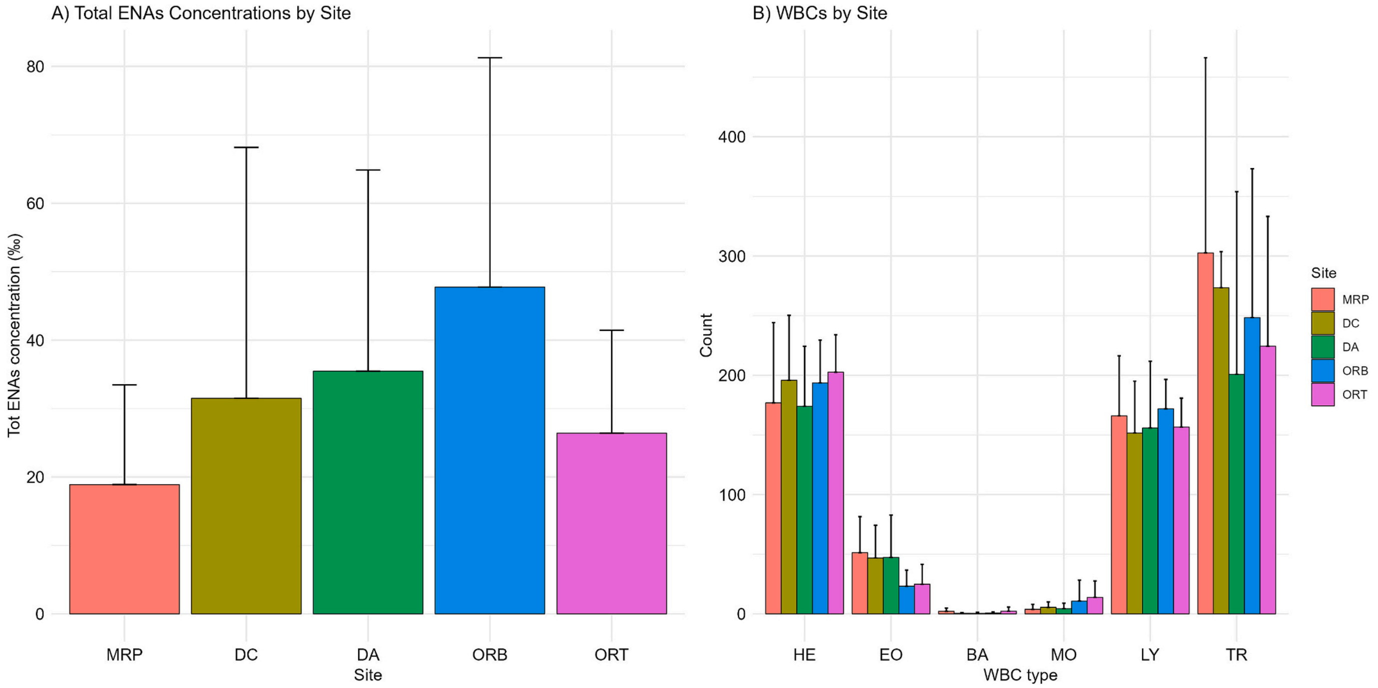


Fig. 5. Mean values of A) erythrocytes nuclear abnormalities (total ENAs) and B) WBCs detected in osprey chicks' blood, reported per each breeding pair (reference period 2015–2022). WBCs are reported as follows: HE = heterophils, EO = eosinophils, BA = basophils, MO = monocytes, LY = lymphocytes. TR indicates thrombocytes. Pairs are indicated by codes and different colors (refer to Fig. 1). Bars indicate standard deviations.

and the age of the chick as also supported by GLMMs results. Feathers, being a non-metabolic tissue, accumulate contaminants over time and reflect contamination deposited by the blood during feather growth in the medium to long-term, though some external contamination may persist. In this respect, larger nestlings with longer tarsi may be likely subject to higher intake and accumulation of Hg, consequently the elevated Se concentrations in feathers may reflect a protective mechanism against Hg toxicity. This indicates that Se may play a key role in buffering the potentially harmful effects of Hg, especially in larger nestlings with higher exposure to contaminated prey. However, the relationship in blood appears to be the opposite, with smaller birds showing higher Se concentrations. Indeed, abilities to absorb, store, and metabolize Se may varying among different individuals, nevertheless, as required for normal physiological function, this element distributed through the body by the circulating blood is subjected to different mechanism for maintaining homeostasis. Different dynamics of Se storage and transport in the body may not directly correlate with the long-term storage of Se in feathers.

Among the essential elements measured, Cu exhibited relatively high mean concentrations, particularly in feathers (11.5 mg/kg dw). This value slightly exceeds those previously reported for juvenile ospreys from southern Finland (5.6–8.6 mg/kg dw; Solonen et al., 1999) and from the Chesapeake and Delaware Bays (4.92–9.07 mg/kg dw; Rattner et al., 2008) (Table S6). Similarly, the blood Cu mean concentration observed in the Tuscan osprey population (3.983 mg/kg dw) was higher than values reported in previous studies, which are available only for North American populations (Table S6). Although Cu is an essential element for living organisms, it can become toxic when homeostatic regulatory mechanisms are overwhelmed (e.g. Gaetke et al., 2014). Elevated Cu tissue levels are often observed in birds inhabiting areas impacted by industrial or mining activities. Compounds of Cu are also widely used in agriculture to manage fungal, bacterial, and other plant diseases and are also released from antifouling paints used on boats and ships following the 1982 ban on tributyltin-based coatings (Soroldoni et al., 2017). Concentrations of Cu exceeding physiological requirements can induce oxidative stress, impair chick development, and cause damage to cerebral tissue, as well as degenerative changes in the liver and kidneys. These adverse effects have been documented primarily in poultry (Liu et al., 2020; Wang et al., 2023), but also on wildlife species (e.g. Eisler, 1998; Franson et al., 2012). Nevertheless, some bird species show a notable tolerance to metallic Cu, as observed in vultures (Risebrough et al., 2001), mallards (Sanderson and Bellrose, 1986) and American kestrels (Franson et al., 2012). Ospreys may similarly tolerate moderately elevated Cu levels due to their high trophic position and potential metabolic adaptations. However, elevated Cu concentrations, particularly found in feathers, likely indicate environmental exposure, possibly linked to contamination sources such as urban runoff, agricultural practices and/or industrial waste all of which are plausible within the study area, as per the rivers and channels communicating with coastal wetland sites.

Concentrations of Cd measured in the Tuscan osprey population were generally lower than those reported in previous studies (Table S6), particularly in feathers. Within Europe, the only available comparison dates back over three decades: Solonen et al. (1999) reported mean Cd levels in feathers of juvenile ospreys from southern Finland approximately one order of magnitude higher and with maximum values reaching nearly two orders of magnitude higher than those observed in the present study. In the United States, data from various rivers in the Chesapeake Bay (Maryland) showed mean concentrations up to four times higher, while other rivers in the same region presented similar values to ours. Importantly, none of the samples from our study exceeded, or even approached, the threshold Cd concentration of 2 mg/kg in feathers considered potentially harmful to avian populations (Burger and Gochfeld, 2000). With regard to blood, the Cd concentrations observed in our study were consistent with those reported in the literature (Table S6), which is currently limited to studies from the United States, including populations in Idaho (Henny et al., 1991), Chesapeake Bay and Delaware Bay (Rattner et al., 2008), and Montana (Langner et al., 2012). In all cases, levels were very low, often near the detection limit. It is important to note that Cd is a non-essential heavy metal with high toxicity, even at low concentrations. A blood Cd threshold of 0.05 μg/dL (equivalent to 0.0022 mg/kg dw) has been associated with disruptions in antioxidant enzyme activity (specifically glutathione peroxidase and catalase), as reported in Griffon vultures (*Gyps fulvus*) by Espín et al. (2014). The mean blood Cd concentration in our study population (Table 1) was approximately half this threshold.

No significant differences in Cd levels were found among the various nesting sites along the Tuscan coast, suggesting the absence of localized Cd pollution sources in the area. Moreover, the positive correlation observed between Cd concentrations in blood and feathers supports an endogenous origin, likely through dietary intake, rather than external deposition. Therefore, despite the known toxic potential of Cd, the concentrations detected in both feathers and blood samples from the Tuscan osprey population do not appear to pose a concern.

Existing studies on Pb concentrations in osprey blood report a broad range of values, from below detection limits to a maximum of approximately 0.43 mg/kg dw, recorded in a mining and smelter area in Idaho, USA (Henny et al., 1991) (Table S6). Excluding this exceptionally high value, Pb concentrations observed in the present study fall within the range reported in the literature (Table S6). Among the nesting sites surveyed, ORB and MRP exhibited the highest mean Pb levels in blood, while ORB and ORT showed the highest levels in feathers. Nevertheless, these concentrations remained below the maxima reported by Henny et al. (1991) for blood in nestlings exposed to industrial contamination, and below those observed by Rattner et al. (2008) for feathers in ospreys from the Chesapeake and Delaware Bays (USA). In our study, blood Pb concentrations ranged from 0.05 mg/kg dw at the DC site to 0.133 mg/kg dw at the ORB site. According to the classification by Franson and Pain (2011), these levels are consistent with baseline concentrations, typically associated with environments that are distant from major Pb emission sources.

Our study also revealed several significant positive correlations among trace element concentrations in osprey nestlings, suggesting potential co-exposure or shared sources of contamination. Positive correlations between Cd, Hg, and Pb were observed in both blood and feathers, supporting the hypothesis of simultaneous exposure and possibly common absorption or sequestration pathways for these elements in developing ospreys.

4.3. Levels of organochlorine pollutants

Contaminant profiling in the blood of osprey chicks revealed that the primary source of exposure is attributable to PCBs, as supported by a mean DDTs/PCBs ratio well below 1. This indicates a predominant industrial origin of contamination over agricultural sources. The most abundant PCBs congeners were in agreement with the literature that classifies these congeners as markers of exposure to polychlorinated biphenyls (Fierens et al., 2007): due to their high degree of chlorination, and therefore persistence in the environment, these are generally the most widespread (Carballo et al., 2008; García-Álvarez et al., 2014). A typical technical DDT consists of pp'DDT (77.1 %), op'DDT (14.9 %), pp'DDD (0.3 %), op'DDD (0.1 %), pp'DDE (4.0 %), op'DDE (0.1 %) and unidentified compounds (3.5 %) (WHO, 1989), with a pp'DDE/pp'DDT ratio of 0.05. While the pp'DDE/pp'DDT ratio was above the typical value observed in technical DDT formulations, suggesting the absence of recent DDT inputs and a high degree of degradation of the active principle, the pp'DDE/DDTs ratio remains below the critical threshold of 0.6 generally used to rule out recent inputs (Tsydenova et al., 2004). This may indicate recent or ongoing, albeit limited, exposure. Furthermore, the op'DDTs/DDTs ratio exceeded the 0.20 threshold typically associated with technical DDT, suggesting either the use of industrial DDT formulations or the presence of DDT-like insecticides such as dicofol, which contains higher proportions of op' isomers (Ricking and Schwarzbauer, 2012). Despite strict regulations from the Stockholm Convention on POPs on the use of Organochlorine pesticides (OCPs) and the past ban of certain metabolites (Tzanetou and Karasali, 2022: UNEP, 2025), our analysis revealed that DDT and its metabolites (DDE) are still present nowadays in our study area as they were found in all 13 osprey chicks sampled for this specific analysis. Nonetheless, it has been shown that these compounds can persist in the environment for extended periods and can be transported far from their original sources, even reaching pristine or isolated/remote areas. One example is the detection of organochlorine pesticide residues in the plasma of osprey nestlings from an isolated pristine area in Baja California Sur (Mexico), highlighting the ubiquitous presence of these contaminants (Rivera-Rodríguez and Rodríguez-Estrella, 2011).

In our case, levels of organochlorine pollutants were below the range found in other osprey populations from polluted areas, exposed both to agricultural and industrial activities (Grove et al., 2009). It should also be noted that Italian ospreys, and more broadly Mediterranean osprey populations, consists primarily of sedentary individuals or short-distance migrants that mostly winter within the Mediterranean basin (Monti et al., 2018). These populations may be less exposed to the long-term effects of POPs, such as DDT, compared to long-distance migratory populations that winter in sub-Saharan Africa (Viluksela et al., 2024). Indeed, DDT concentrations may be substantially higher in individuals from intercontinental migratory populations, as the use of DDT and similar compounds remains less strictly regulated in many non-European countries. Future studies should explore potential differences in contaminant exposure by comparing populations with contrasting migratory strategies and wintering locations.

4.4. ENAs and WBCs

It has been shown that an increase in the frequency of MN corresponds to an increase in the formation of nuclear abnormalities (Fenech et al., 2011). In addition, Khatun et al. (2021) showed that the frequency of ENAs, as well as MN, increases with exposure to contaminants over time.

To date, to the best of our knowledge, this method has not yet been applied specifically to ospreys in published studies. Thus, comparisons can still be drawn with findings from other bird species exposed to varying anthropogenic pressures. For example, the mean MN frequency found in our study (0.39/2000) was lower than that measured in blood of chicks of *Falco tinnunculus* sampled in rural areas (0.61/2000) (Giovanetti et al., 2024) and those measured in adult individuals of American Kestrel (19.2/1000 or 3.84/2000) from agricultural areas (Frixione et al., 2020). Stocker et al. (2022) found values much higher than ours in individuals of *Falco peregrinus* (1.62/2000) from urban settings in the city of São Francisco de Paula, Brazil. In our study, nuclear abnormalities were found in all the individuals sampled and bud nuclei were the most abundant abnormality. These comparisons with the bibliography suggest no genotoxic effects in our population.

Hematological parameters, such as white blood cell (leukocytes) count are an important diagnostic tool to better understand bird health (Mitchell and Johns, 2008; de Freitas et al., 2024) because their levels are closely related to those of the glucortocoid hormones. Increased levels of these hormones may lead to changes in the leukocyte profile (Cavalli et al., 2018).

In our case, the analysis of immune parameters revealed that the total number of WBC are within the normal range reported for birds (e.g. Kursa and Bezrukov, 2008). In agreement with the results obtained by Meredith et al. (2012), the osprey leukocyte count in our work showed higher values of heterophiles, which represent the most abundant leukocyte class, followed by lymphocytes, eosinophils, monocytes and basophils. A similar trend was also observed by Stocker et al. (2022) in the blood of Peregrine falcons sampled in a wildlife center located in Rio Grande do Sul (Brazil). On the contrary, free-living Burrowing owls *Athene cunicularia* from rural and urban areas in the Argentinean Pampas showed a different leukocyte profile (lymphocytes>heterophils) (Cavalli et al., 2018). The values of heterophils (65.71/200 or 131.42/400) and lymphocytes (121.68/200 or 243.36/400) highlighted by Giovanetti et al. (2024) on specimens of Common Kestrel sampled in natural areas in central Italy are in line with those of our work, while differed from those investigated in non-raptor species such as those found by de Freitas et al. (2024) in Common pauraque *Nyctidromus albicollis* (5.00/100 or 20.00/400) and Pale-breasted thrush *Turdus leucomelas* (4.00/100 or 16.00/400), that were lower in comparison to those we counted in osprey (185.67/400). However, the mean values of lymphocytes in our population were similar to those found by de Freitas et al. (2024) in these two species, being 50.17/100 or 200.68/400 and 58.00/100 or 232.00/400, respectively. In our study, eosinophil count, an indicator of helminth parasite load and activity, were in line with those measured in blood of osprey sampled in various locations in England and Scotland (Meredith et al., 2012) and higher than those of Giovanetti et al. (2024) (6.68/200 or 13.36/400) and de Freitas et al. (2024) (15.00/100 or 60/400) and (9.50/100 or 38/400) in their target species. Moreover, Cavalli et al. (2018) found values of eosinophils higher in comparison to our data, but other studies showed similar data for most healthy raptor species (e.g. Copete-Sierra, 2013). In addition, monocytes and basophils, typically involved in defense against infections and in mediating inflammatory responses, respectively, were present at low percentages, further supporting the conclusion that the monitored ospreys were in good health. Moreover, leukocyte profiles did not differ significantly among chicks from different nests, reinforcing the notion that no localized stressors are currently influencing the immune system of the population.

Interestingly, we found that a high H:L ratio was associated with a shorter tarsus length. As higher the H:L ratio and more the stress in the animal is accentuated (Davis et al., 2008), it is plausible that this had an effect on the regular growth of the osprey chick. The H:L ratio is widely used as a chronic stress index in animals and it is closely related to plasma levels of corticosteroid hormones (Davis et al., 2008). Together, heterophiles and lymphocytes represent nearly 80 % of the leukocyte population and are affected by stress in the opposite way, in response to glucocorticoids. While the former are recalled into the bloodstream, the latter adhere to vessel walls and then migrate to other tissues (lymph nodes, spleen, marrow, skin, etc.). Therefore, the higher the ratio between these two types of leukocytes, the more the animal is prone to stress (Davis et al., 2008). To date, to the best of our knowledge, there is no data on H:L ratio on osprey, so it is difficult to understand the basal levels of the species. Comparing our data with other studies, our values were higher than those found by Giovanetti et al. (2024) in Common kestrel (0.64 ± 0.64) from natural areas, and by Cavalli et al. (2018) in blood of Burrowing owls collected in rural areas (0.6 ± 0.1).

4.5. *Relationships among trace elements, ENAs and WBCs*

The correlation analysis revealed several significant associations between trace element concentrations and white blood cell profiles, suggesting potential immunomodulatory effects. In blood samples, Cd showed negative correlations with lobed cells and heterophils, indicating a possible immunosuppressive effect. In contrast, Pb and Cu were positively correlated with heterophils and the H:L ratio. Pb also showed a negative correlation with eosinophils. In feather samples, Se stood out with multiple significant correlations: it was negatively associated with lobed cells, total ENAs, and heterophils, but positively correlated with eosinophils, suggesting complex and cell-specific effects. Similarly, Cu showed negative correlations with lobed cells and ENAs, and a positive association with eosinophils. Pb followed a similar pattern as observed in blood, with positive correlations with heterophils and negative with eosinophils. However, these findings should be interpreted with caution. This is a preliminary analysis based on a relatively small number of samples collected across different areas and years, conditions that may introduce variability and lead to potentially spurious correlations. Nonetheless, the results provide valuable insights and form a useful basis for guiding future investigations into the immunological impacts of trace element exposure for this population.

5. Conclusions

This study represents the most extensive ecotoxicological monitoring program conducted on ospreys in Europe and the only one focusing on a reintroduced population. Our findings are significant not only for the management and for conservation of this small breeding population but also as an indirect assessment of the quality of the coastal habitats frequented by these birds. As a fish-eating raptor operating at the interface between marine and terrestrial environments, the osprey serves as an excellent bioindicator for evaluating environmental contamination in transitional ecosystems (Grove et al., 2009). By integrating analyses of multiple contaminants and biomarkers across complementary matrices, combined with morphometric and spatial data, this study provides a comprehensive assessment of the health status of the reintroduced osprey population in Italy. This approach is particularly important for small and vulnerable populations, where even localized changes in contamination levels can lead to adverse effects, exacerbating their susceptibility to stochastic events and extinction risks (e.g., Keller and Waller, 2002). Our results underscore the persistence of legacy pollutants such as DDTss, which, despite being banned in Italy since 1975, are still present in the environment and expose local ospreys to low but detectable levels of contamination. Regarding trace elements, particular attention is required at certain nest sites (e.g., ORB and MRP) where slightly elevated Hg levels were observed. These levels may reflect the ecological mobility of Hg, local fish farming activities, or the influence of rivers draining catchments associated with mining and geothermal areas inland. In addition, ospreys appears able to tolerate or adapt to moderately elevated levels of Cu from various sources, potentially possible in our study area. Standardized and long-term monitoring remains essential to detect changes in contaminant exposure and assess emerging threats. Such efforts are crucial for identifying potential risks and implementing timely conservation measures to ensure the long-term persistence of this reintroduced osprey population, as well as for informing adaptive management strategies in transitional habitats.

CRediT authorship contribution statement

Stefania Ancora: Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Investigation, Data curation, Conceptualization. **Andrea Sforzi:** Writing – review & editing. **Claudio Leonzio:** Writing – review & editing, Resources, Funding acquisition. **Nicola Bianchi:** Investigation. **Ilaria Caliani:** Writing – review & editing, Validation, Methodology, Investigation, Data curation. **Laura Giovanetti:** Writing – review & editing, Validation, Methodology, Investigation, Data curation. **Guia Consales:** Writing – review & editing, Validation, Methodology, Investigation, Data curation. **Letizia Marsili:** Writing – review & editing, Validation, Methodology, Investigation, Data curation. **Flavio Monti:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Financial support

This research has been supported by the Tuscan Archipelago National Park.

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.

Acknowledgements

We thank the Tuscan Archipelago National Park for funding this project. We also thank the Maremma Regional Park, the Diaccia Botrona

Natural Reserve, the WWF Orbetello Lagoon Natural Reserve and the WWF Orti-Bottagone Marsh Natural Reserve, under the Tuscany Region administration. We thank the "Progetto Falco pescatore" team: Giampiero Sammuri for coordinating the project, Francesco Pezzo the official ringer from ISPRA, Vincenzo Rizzo Pinna for the constant work with the video surveillance system at osprey nests, as well as Guido Alari and Alessandro Troisi for their help in fieldwork activities. We thank the Unit for Conservation Genetics (BIO-CGE) of the ISPRA for molecular sexing DNA analyses and Renato Ceccherelli and the staff of the Centro Recupero Uccelli Marini e Acquatici (CRUMA-LIPU) that provided important assistance in the recovery of debilitated or injured ospreys before release. The authors thank the two anonymous referees for their valuable suggestions on an earlier version of the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2025.180109.

Data availability

All data are reported in this study and its supplementary information. All GPS data analysed in this study are freely consultable in the Movebank database (www.movebank.org), project study name "Osprey in Mediterranean (Corsica, Italy, Balearics) - movement study ID: 20039459".

References

Ames, P.L., 1966. DDT residues in the eggs of the osprey in the North-Eastern United States and their relation to nesting success. J. Appl. Ecol. 3, 87–97. https://doi.org/10.2307/2401253.

Bierregaard, R.O., David, A.B., Gibson, L., Kennedy, R.S., Poole, A.F., Scheibel, M.S., Victoria, J., 2014. Post-DDT recovery of osprey (*Pandion haliaetus*) populations in southern New England and Long Island, New York, 1970–2013. J. Raptor Res. 48, 361–374. https://doi.org/10.3356/rapt-48-04-361-374.1.

Bracey, A.M., Etterson, M.A., Strand, F.C., Matteson, S.W., Niemi, G.J., Cuthbert, F.J., Hoffman, J.C., 2021. Foraging ecology differentiates life stages and mercury exposure in common terns (*Sterna hirundo*). Integr. Environ. Assess. Manag. 17, 398–410. https://doi.org/10.1002/ieam.4354.

Brooks, M.E., Kristensen, K., Van Benthem, K.J., Magnusson, A., Berg, C.W., Nielsen, A., Bolker, B.M., 2017. GlmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling.

Buechley, E.R., Santangeli, A., Girardello, M., Neate-Clegg, M.H., Oleyar, D., McClure, C. J., Şekercioğlu, Ç.H., 2019. Global raptor research and conservation priorities: tropical raptors fall prey to knowledge gaps. Divers. Distrib. 25 (6), 856–869.

Burger, J., Gochfeld, M., 2000. Metal levels in feathers of 12 species of seabirds from midway atoll in the northern Pacific Ocean. Sci. Total Environ. 257, 37–52.

Burger, J., Gochfeld, M., 2004. Marine birds as sentinels of environmental pollution. EcoHealth 1, 263–274.

Cahill, T.M., Anderson, D.W., Elbert, R.A., Perley, B.P., Johnson, D.R., 1998. Elemental profiles in feather samples from a mercury-contaminated lake in central California. Arch. Environ. Contam. Toxicol. 35, 75–81. https://doi.org/10.1007/s002449900365.

Calenge, C., 2006. The package adehabitat for the R software: a tool for the analysis of space and habitat use by animals. Ecol. Model. 197, 516–519. https://doi.org/10.1016/j.ecolmodel.2006.03.017.

Carballo, M., Arbelo, M., Esperón, F., Mendez, M., De La Torre, A., Muñoz, M.J., 2008. Organochlorine residues in the blubber and liver of bottlenose dolphins (Tursiops truncatus) stranded in the Canary Islands, North Atlantic Ocean. Environ. Toxicol.: Int. J. 23 (2), 200–210.

Carrasco, K.R., Tilbury, K.L., Myers, M.S., 1990. Assessment of the piscine micronucleus test as an in situ biological indicator of chemical contaminant effects. Can. J. Fish. Aquat. Sci. 47, 2123–2136. https://doi.org/10.1139/f90-237.

Castro, I., Aboal, J.R., Fernández, J.A., Carballeira, A., 2011. Use of raptors for biomonitoring of heavy metals: gender, age and tissue selection. Bull. Environ. Contam. Toxicol. 86, 347–351. https://doi.org/10.1007/s00128-011-0232-8.

Catitti, B., Grüebler, M.U., Kormann, U.G., Scherler, P., Witczak, S., van Bergen, V.S., Jenni-Eiermann, S., 2022. Hungry or angry? Experimental evidence for the effects of food availability on two measures of stress in developing wild raptor nestlings. J. Exp. Biol. 225, jeb244102. https://doi.org/10.1242/jeb.244102.

Cavalli, M., Baladrón, A.V., Juan, P., Isacch, J.P., D'Amico, V., Bó, M.S., 2018. Leukocyte profiles and body condition of free-living burrowing owls (Athene cunicularia) from rural and urban areas in the Argentinean pampas. Rev. Bras. Ornitol. 26, 45–51. https://doi.org/10.1007/BF03544347.

Chakraborty, S.K., Chakraborty, S.K., 2021. River pollution and perturbation: Perspectives and processes. In: Riverine Ecology Volume 2: Biodiversity Conservation, Conflicts and Resolution. Springer, pp. 443–530. https://doi.org/10.1007/978-3-030-68239-5_13.

Champoux, L., Rodrigue, J., DesGranges, J.L., Trudeau, S., Hontela, A., Boily, M., Spear, P., 2002. Assessment of contamination and biomarker responses in two species of herons on the st. Lawrence River. Environ. Monit. Assess. 79, 193–215. https://doi.org/10.1023/A:1020301003484.

Champoux, L., Rodrigue, J., Trudeau, S., Boily, M.H., Spear, P.A., Hontela, A., 2006. Contamination and biomarkers in the great blue heron, an indicator of the state of the St. Lawrence River. Ecotoxicology 15, 83–96. https://doi.org/10.1007/s10646-005-0045-8.

Chu, S., Henny, C.J., Kaiser, J.L., Drouillard, K.G., Haffner, G.D., Letcher, R.J., 2007. Dacthal and chlorophenoxy herbicides and chlorothalonil fungicide in eggs of osprey (*Pandion haliaetus*) from the Duwamish–Lake Washington–Puget Sound area of Washington State, USA. Environ. Pollut. 145, 374–381. https://doi.org/10.1016/j.envpol.2005.12.058.

Cīrule, D., Krama, T., Vrublevska, J., Rantala, M.J., Krams, I., 2012. A rapid effect of handling on counts of white blood cells in a wintering passerine bird: a more practical measure of stress? J. Ornithol. 153, 161–166. https://doi.org/10.1007/s10336-011-0728-6.

Copete-Sierra, M., 2013. Aspectos generales de la evaluación hematológica en fauna silvestre y no convencional. In: Memorias de la Conferencia Interna en Medicina y Aprovechamiento de Fauna Silvestre, Exótica y no Convencional, 9, pp. 17–55.

Cramp, S., Simmons, K.E.L., 1980. Handbook of the Birds of Europe, the Middle East and North Africa. The Birds of the Western Palearctic, 2. Oxford University Press, Oxford, pp. 265–277.

Cuvin-Aralar, M.L.A., Furness, R.W., 1991. Mercury and selenium interaction: a review. Ecotoxicol. Environ. Saf. 21 (3), 348–364.

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 (5), 760–772.

de Freitas, R.M.P., Benvindo-Souza, M., Sotero, D.F., de Carvalho Lopes, A.T., Santos, M. A., de Oliveira, J.M., e Silva, D. D. M., 2024. Ecotoxicological consequences of urbanization: a multi-biomarker approach to assessing sewage treatment plant effects on free-living birds. Environ. Res. 258, 119424.

De Pascalis, F., Panuccio, M., Bacaro, G., Monti, F., 2020. Shift in proximate causes of mortality for six large migratory raptors over a century. Biol. Conserv. 251, 108793.

DesGranges, J.-L., Rodrigue, J., Tardif, B., Laperle, M., 1998. Mercury accumulation and biomagnification in ospreys (*Pandion haliaetus*) in the James bay and Hudson Bay regions of Quebec. Arch. Environ. Contam. Toxicol. 35, 330–341.

Driscoll, C.T., Mason, R.P., Chan, H.M., Jacob, D.J., Pirrone, N., 2013. Mercury as a global pollutant: sources, pathways, and effects. Environ. Sci. Technol. 47, 4967–4983. https://doi.org/10.1021/es305071v.

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. Toxicol. Chem. 27 (10), 2136–2153.

Eens, M., Pinxten, R., Verheyen, R.F., 1998. Black-headed gulls (Larus ridibundus) as bioindicators of heavy metal contamination in Belgium: cadmium, lead and mercury accumulation. Sci. Total Environ. 209, 89–102. https://doi.org/10.1016/S0048-9697(97)00315-3.

Eisler, R., 1998. Copper Hazards to Fish, Wildlife, and Invertebrates: A Synoptic Review (no. 33). In: US Department of the Interior, US Geological Survey.

Elliott, J.E., Harris, M.L., 2001. An ecotoxicological assessment of chlorinated hydrocarbons in ospreys (*Pandion haliaetus*) and bald eagles (*Haliaeetus leucocephalus*) in Canada. Rev. Environ. Contam. Toxicol. 170, 1–46. https://doi.org/10.1007/978-1-4612-1186-1_1.

Elliott, J.E., Morrissey, C.A., Henny, C.J., Inzunza, E.R., Shaw, P., 2007. Satellite telemetry and prey sampling reveal contaminant sources to Pacific northwest ospreys. Ecol. Appl. 17, 1223–1233.

Elliott, K.H., Cesh, L.S., Dooley, J.A., Letcher, R.J., Elliott, J.E., 2009. PCBs and DDE, but not PBDEs, increase with trophic level and marine input in nestling bald eagles. Sci. Total Environ. 407, 3867–3875. https://doi.org/10.1016/j.scitotenv.2009.03.010.

Eriksson, U., Roos, A., Lind, Y., Hope, K., Ekblad, A., Kärrman, A., 2016. Comparison of PFASs contamination in the freshwater and terrestrial environments by analysis of eggs from osprey (Pandion haliaetus), tawny owl (Strix aluco), and common kestrel (Falco tinnunculus). Environ. Res. 149, 40–47.

Espín, S., Martínez-López, E., Jiménez, P., María-Mojica, P., García-Fernández, A.J., 2014. Effects of heavy metals on biomarkers for oxidative stress in griffon vulture (Gyps fulvus). Environ. Res. 147, 223–235. https://doi.org/10.1016/j.envres.2016.02.024.

Espín, S., García-Fernández, A.J., Herzke, D., Shore, R.F., van Hattum, B., Martínez-López, E., Coeurdassier, M., Eulaers, I., Fritsch, C., Gómez-Ramírez, P., Jaspers, V.L. B., Krone, O., Duke, G., Helander, B., Mateo, R., Movalli, P., Sonne, C., 2016. Tracking pan-continental trends in environmental contamination using sentinel raptors—what types of samples should we use? Ecotoxicology 25, 777–801. https://doi.org/10.1007/s10646-016-1636-8.

Fenech, M., Kirsch-Volders, M., Natarajan, A.T., Surralles, J., Crott, J.W., Parry, J., Thomas, P., 2011. Molecular mechanisms of micronucleus, nucleoplasmic bridge and nuclear bud formation in mammalian and human cells. Mutagenesis 26 (1), 125–132.

Fierens, S., Mairesse, H., Heilier, J.F., Focant, J.F., Eppe, G., Pauw, E.D., Bernard, A., 2007. Impact of iron and steel industry and waste incinerators on human exposure to dioxins, PCBs, and heavy metals: results of a cross-sectional study in Belgium. J. Toxicol. Environ. Health A 70 (3–4), 222–226.

Fournier, F., Karasov, W.H., Kenow, K.P., Meyer, M.W., Hines, R.K., 2002. The oral bioavailability and toxicokinetics of methylmercury in common loon (*Gavia immer*) chicks. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 133 (3), 703–714.

Franson, J.C., Pain, D.J., 2011. Lead in birds. In: Beyer, W.N., Meador, J.P. (Eds.), Environmental Contaminants in Biota, Interpreting Tissue Concentrations (2011), Secon edition. CRC Press, Boca Raton. 768 pp.

Franson, J.C., Lahner, L.L., Meteyer, C.U., Rattner, B.A., 2012. Copper pellets simulating oral exposure to copper ammunition: absence of toxicity in American kestrels (*Falco sparverius*). Arch. Environ. Contam. Toxicol. 62, 145–153.

Frixione, M.E., Rodríguez-Estrella, R., Rodríguez, C., 2020. Heavy metals in feathers of osprey (*Pandion haliaetus*) nestlings and their prey in southern Baja California: risk assessment. Environ. Sci. Pollut. Res. 27, 15037–15048. https://doi.org/10.1007/s11356-020-08041-w.

Furness, R.W., Muirhead, S.J., Woodburn, M., 1986. Using bird feathers to measure mercury in the environment: relationships between mercury content and moult. Mar. Pollut. Bull. 17 (1), 27–30.

Gaetke, L.M., Chow-Johnson, H.S., Chow, C.K., 2014. Copper: toxicological relevance and mechanisms. Arch. Toxicol. 88, 1929–1938.

García-Álvarez, N., Martín, V., Fernández, A., Almunia, J., Xuriach, A., Arbelo, M., Luzardo, O.P., 2014. Levels and profiles of POPs (organochlorine pesticides, PCBs, and PAHs) in free-ranging common bottlenose dolphins of the Canary Islands, Spain. Sci. Total Environ. 493, 22–31.

García-Fernández, A.J., 2014. Ecotoxicology, avian. In: Wexler, P., Wexler, P. (Eds.), Encyclopedia of Toxicology, 3rd edition 2. Elsevier Inc., Academic Press, pp. 289–294.

García-Fernández, A.J., Espín, S., Gómez-Ramírez, P., Martínez-López, E., Navas, I., 2020. Wildlife sentinels for human and environmental health hazards in ecotoxicological risk assessment. Ecotoxicol. QSARs 77-94.

Garrido, J.R., Numa, C., Barrios, V., Qninba, A., Riad, A., Haitham, O., Bakass, B., 2021. The Conservation Status and Distribution of the Breeding Birds of Prey of North Africa. IUCN, Gland, Switzerland.

Gerson, J.R., Walters, D.M., Eagles-Smith, C.A., Bernhardt, E.S., Brandt, J.E., 2020. Do two wrongs make a right? Persistent uncertainties regarding environmental selenium–mercury interactions. Environ. Sci. Technol. 54 (15), 9228–9234.

Giovanetti, L., Caliani, I., Damiani, G., Dell'Omo, G., Costantini, D., Casini, S., 2024. A blood-based multi-biomarker approach reveals different physiological responses of common kestrels to contrasting environments. Environ. Res. 251, 118674.

Golden, N.H., Rattner, B.A., 2003. Ranking terrestrial vertebrate species for utility in biomonitoring and vulnerability to environmental contaminants. Rev. Environ. Contam. Toxicol. 176, 67–136.

Gómez-Ramírez, P., Shore, R.F., Van Den Brink, N.W., Van Hattum, B., Bustnes, J.O., Duke, G., Sonne, C., 2014. An overview of existing raptor contaminant monitoring activities in Europe. Environ. Int. 67, 12–21.

Griffiths, R., Double, M.C., Orr, K., Dawson, R.J., 1998. A DNA test to sex most birds. Mol. Ecol. 7, 1071–1075.

Grove, R.A., Henny, C.J., Kaiser, J.L., 2009. Osprey: worldwide sentinel species for assessing and monitoring environmental contamination in rivers, lakes, reservoirs, and estuaries. J. Toxic. Environ. Health, Part B 12 (1), 25–44.

Häkkinen, I., Häsänen, E., 1980. Mercury in eggs and nestlings of the osprey (*Pandion haliaetus*) in Finland and its bioaccumulation from fish. Ann. Zool. Fenn. 17, 131–139.

Hargreaves, A.L., Whiteside, D.P., Gilchrist, G., 2010. Concentrations of 17 elements, including mercury, and their relationship to fitness measures in arctic shorebirds and their eggs. Sci. Total Environ. 408, 3153–3161.

Heinz, G.H., 1996. Selenium in birds. In: Beyer, W.M., Heinz, W.M. (Eds.), Environmental Contaminants in Wildlife: Interpreting Tissue Concentrations. Lewis Publishers; CRC Press, Boca Raton, FL, USA, pp. 447–458.

Henny, C.J., Elliott, J.E., 2007. Toxicology. In: Bird, D.M., Bildstein, K.L. (Eds.), raptor research and management techniques. Raptor Research Foundation.Surrey, British Columbia, Canada: Hancock house, pp. 329–350.

Henny, C.J., Blus, L.J., Hoffman, D.J., Grove, R.A., Hatfield, J.S., 1991. Lead accumulation and osprey production near a mining site on the Coeur D'alene River, Idaho. Arch. Environ. Contam. Toxicol. 21, 415–424.

Henny, C.J., Grove, R.A., Kaiser, J.L., 2008. Osprey distribution, abundance, reproductive success and contaminant burdens along lower Columbia River, 1997/1998 versus 2004. Arch. Environ. Contam. Toxicol. 5, 525–534.

Henny, C.J., Hill, E.F., Hoffman, D.J., Spalding, M.G., Grove, R.A., Johnson, B.L., 2010. North American osprey populations and contaminants: historic and contemporary perspectives. J. Toxicol. Environ. Health B 13 (7–8), 579–603.

Herring, G., Eagles-Smith, C.A., Varland, D.E., 2018. Mercury and lead exposure in avian scavengers from the Pacific northwest suggest risks to California condors: implications for reintroduction and recovery. Environ. Pollut. 243, 610–619.

Hothorn, T., Bretz, F., Westfall, P., 2008. Simultaneous inference in general parametric models. Biom. J. 50, 346–363.

Hughes, K.D., Ewins, P.J., Clark, K.E., 1997. A comparison of mercury levels in feathers and eggs of osprey (*Pandion haliaetus*) in the north American Great Lakes. Arch. Environ. Contam. Toxicol. 33, 441–452.

International Union for Conservation of Nature Species Survival Commission, 2013. Guidelines for reintroductions and other conservation translocations.

Jackson, A., Evers, D.C., Eagles-Smith, C.A., Ackerman, J.T., Willacker, J.J., Elliott, J.E., Bryan, C.E., 2016. Mercury risk to avian piscivores across western United States and Canada. Sci. Total Environ. 568, 685–696.

Jiménez, B., Merino, R., Abad, E., et al., 2007. Evaluation of organochlorine compounds (PCDDs, PCDFs, PCBs and DDTs) in two raptor species inhabiting a Mediterranean island in Spain. Environ. Sci. Pollut. Res. Int. 14 (Suppl. 1), 61–68.

Kalisinska, E., Gorecki, J., Lanocha, N., Okonska, A., Melgarejo, J.B., Budis, H., Golas, J., 2014. Total and methylmercury in soft tissues of white-tailed eagle (Haliaetus albicilla) and osprey (Pandion haliaetus) collected in Poland. Ambio 43, 858–870.

Keller, L.F., Waller, D.M., 2002. Inbreeding effects in wild populations. Trends Ecol. Evol. 17, 230–241.

Kenward, R.E., Pfeffer, R.H., Al-Bowardi, M.A., Fox, N.C., Riddle, K.E., Bragin, E.A., Levin, A., Walls, S.S., Hodder, K.H., 2001. Setting harness sizes and other marking techniques for a falcon with strong sexual dimorphism. J. Field Ornithol. 72, 244–257.

Khatun, M.M., Mostakim, G.M., Moniruzzaman, M., Rahman, U.O., Islam, M.S., 2021. Distortion of micronuclei and other peripheral erythrocytes caused by fenitrothion and their recovery assemblage in zebrafish. Toxicol. Rep. 8, 415–421.

Köhler, H.R., Triebskorn, R., 2013. Wildlife ecotoxicology of pesticides: can we track effects to the population level and beyond? Science 341 (6147), 759–765.

Kursa, M., Bezrukov, V., 2008. Health status in an Antarctic top predator: micronuclei frequency and white blood cell differentials in the south polar Skua. Polarforschung 77 (1), 1–5.

Langner, H.W., Greene, E., Domenech, R., Staats, M.F., 2012. Mercury and other mining-related contaminants in ospreys along the upper Clark Fork River, Montana, USA. Arch. Environ. Contam. Toxicol. 62, 681–695.

Lazarus, R.S., Rattner, B.A., McGowan, P.C., Hale, R.C., Schultz, S.L., Karouna-Renier, N. K., Ottinger, M.A., 2015. Decadal re-evaluation of contaminant exposure and productivity of ospreys (Pandion haliaetus) nesting in Chesapeake Bay regions of concern. Environ. Pollut. 205, 278–290.

Lemarchand, C., Rosoux, R., Berny, P., 2011. Semi-aquatic top-predators as sentinels of diversity and dynamics of pesticides in aquatic food webs: the case of Eurasian otter (Lutra lutra) and osprey (Pandion haliaetus) in Loire river catchment, France. In: Pesticides in the Modern World-Risks and Benefits, pp. 289–310.

Lemarchand, C., Rosoux, R., Pénide, M.E., Berny, P., 2012. Tissue concentrations of pesticides, PCBs and metals among ospreys, Pandion haliaetus, collected in France. Bull. Environ. Contam. Toxicol. 88, 89–93.

Lenzi, M., Franchi, E., Leporatti-Persiano, M., D'Agostino, A., Gennaro, P., Marsili, L., 2021. Assessment of the causes of hg bioaccumulation in the fish of a Mediterranean lagoon subject to environmental management interventions. Mar. Pollut. Bull. 162, 111907.

Liu, Y., Zhao, H., Wang, Y., Guo, M., Mu, M., Xing, M., 2020. Arsenic (III) and/or copper (II) induces oxidative stress in chicken brain and subsequent effects on mitochondrial homeostasis and autophagy. J. Inorg. Biochem. 211, 111201.

Lodenius, M., Solonen, T., 2013. The use of feathers of birds of prey as indicators of metal pollution. Ecotoxicology 22 (9), 1319–1334.

Lounsbury-Billie, M.J., Rand, G.M., Cai, Y., Bass, O.L., 2008. Metal concentrations in osprey (*Pandion haliaetus*) populations in the Florida Bay estuary. Ecotoxicology 17, 616–622.

Lourenço, R., Tavares, P.C., del Mar Delgado, M., Rabaça, J.E., Penteriani, V., 2011. Superpredation increases mercury levels in a generalist top predator, the eagle owl. Ecotoxicology 20, 635–642.

Madliger, C.L., Cooke, S.J., Crespi, E.J., Funk, J.L., Hultine, K.R., Hunt, K.E., Love, O.P., 2016. Success stories and emerging themes in conservation physiology. Conserv. Physiol. 4 (1), cov057.

Maier, P., Schmid, W., 1976. Ten model mutagens evaluated by the micronucleus test. Mutat. Res./Gen. Toxicol. 40 (4), 325–337.

Manceau, A., Bourdineaud, J.P., Oliveira, R.B., Sarrazin, S.L., Krabbenhoft, D.P., Eagles-Smith, C.A., Poulin, B.A., 2021. Demethylation of methylmercury in bird, fish, and earthworm. Environ. Sci. Technol. 55 (3), 1527–1534.

Margalida, A., Green, R.E., Hiraldo, F., Blanco, G., Sánchez-Zapata, J.A., Santangeli, A., Donázar, J.A., 2021. Ban veterinary use of diclofenac in Europe. Science 372 (6543), 694–695.

Marsili, L., 2000. Lipophilic contaminants in marine mammals: review of the results of ten years' work at the Department of Environmental Biology, Siena university (Italy). Int. J. Environ. Pollut. 13, 416–452.

Mayer, F.L., Versteeg, D.J., McKee, M.J., Folmar, L.C., Graney, R.L., McCume, D.C., Rattner, B.A., 2018. Physiological and nonspecific biomarkers. In: Mayer, F.L., et al. (Eds.), Biomarkers. CRC Press, pp. 5–86.

McClure, C.J., Westrip, J.R., Johnson, J.A., Schulwitz, S.E., Virani, M.Z., Davies, R., Butchart, S.H., 2018. State of the world's raptors: distributions, threats, and conservation recommendations. Biol. Conserv. 227, 390–402.

Meredith, A., Surguine, K., Handel, I., Bronsvoort, M., Beard, P., Thornton, S.M., Wesche, P., Hart, M., Anderson, D., Dennis, R., 2012. Hematologic and biochemical reference intervals for wild osprey nestlings (Pandion haliaetus). J. Zoo Wildl. Med. 43, 459–465.

Miniero, R., Beccaloni, E., Carere, M., Ubaldi, A., Mancini, L., Marchegiani, S., De Felip, E., 2013. Mercury (Hg) and methyl mercury (MeHg) concentrations in fish from the coastal lagoon of Orbetello, Central Italy. Mar. Pollut. Bull. 76 (1–2), 365–369.

Mitchell, E.B., Johns, J., 2008. Avian hematology and related disorders. Vet. Clin. North Am. Exot. Anim. Pract. 11, 141–154.

Molenaar, F.M., Jaffe, J.E., Carter, I., Barnett, E.A., Shore, R.F., Marcus Rowcliffe, J., Sainsbury, A.W., 2017. Poisoning of reintroduced red kites (*Milvus milvus*) in England. Eur. J. Wildl. Res. 63, 1–8.

Monti, F., Grémillet, D., Sforzi, A., Dominici, J.M., Bagur, R.T., Navarro, A.M., Duriez, O., 2018. Migration distance affects stopover use but not travel speed: contrasting patterns between long-and short-distance migrating ospreys. J. Avian Biol. 49 (10), e01839.

Monti, F., Cascio, P.L., Sforzi, A., 2019. Nocturnal activity of insect fauna in osprey nests: insights from video-cameras. J. Raptor Res. 53 (2), 212–214.

Monti, F., Bianchi, N., Sforzi, A., Leonzio, C., Ancora, S., 2020. Drawing the baseline of trace element levels in the vulnerable Mediterranean osprey *Pandion haliaetus*: variations by breeding location, habitats, and egg components. Environ. Sci. Pollut. Res. 27, 10236–10248.

Monti, F., Montgelard, C., Robert, A., Sforzi, A., Triay, R., Sarrazin, F., Duriez, O., 2022. Evolutionary risks of osprey translocations. Science 376 (6592), 468–469.

Monti, F., Mengoni, C., Sforzi, A., Pezzo, F., Serra, L., Sammuri, G., Mucci, N., 2023. Genetic variability and family relationships in a reintroduced osprey (*Pandion haliaetus*) population: a field-lab integrated approach. Diversity 15 (5), 622.

Muir, D., Braune, B., DeMarch, B., Norstrom, R., Wagemann, R., Lockhart, L., Hargrave, B., Bright, D., Addison, R., Payne, J., Reimer, K., 1999. Spatial and temporal trends and effects of contaminants in the Canadian Arctic marine ecosystem: a review. Sci. Total Environ. 230, 83–144.

Muriel, R., Ferrer, M., Casado, E., Calabuig, C.P., 2010. First successful breeding of reintroduced ospreys *Pandion haliaetus* in mainland Spain. Ardeola 57 (1), 175–180.

Muriel, R., Schmidt, D., Calabuig, C.P., Patino-Martinez, J., Ferrer, M., 2013. Factors affecting plasma biochemistry parameters and physical condition of osprey (*Pandion haliaetus*) nestlings. J. Ornithol. 154, 619–632.

Murvoll, K.M., Skaare, J.U., Moe, B., Anderssen, E., Jenssen, B.M., 2006. Spatial trends and associated biological responses of organochlorines and brominated flame retardants in hatchlings of North Atlantic kittiwakes (Rissa tridactyla). Environ. Toxicol. Chem. 25, 1648–1656.

Odsjö, T., Sondell, J., 2014. Eggshell thinning of osprey (*Pandion haliaetus*) breeding in Sweden and its significance for egg breakage and breeding outcome. Sci. Total Environ. 470, 1023–1029.

Odsjö, T., Roos, A., Johnels, A.G., 2004. The tail feathers of osprey nestlings (*Pandion haliaetus* L.) as indicators of change in mercury load in the environment of southern Sweden (1969–1998): a case study with a note on the simultaneous intake of selenium. Ambio 33, 133–137.

Ohlendorf, H.M., Heinz, G.H., 2011. Selenium in birds.

Pacheco, M., Santos, M.A., 1997. Induction of EROD activity and genotoxic effects by polycyclic aromatic hydrocarbons and resin acids on the juvenile eel (Anguilla anguilla L.). Ecotoxicol. Environ. Saf. 38 (3), 252–259.

Pelletier, E., 1986. Mercury-selenium interactions in aquatic organisms: a review. Mar. Environ. Res. 18 (2), 111–132.

Poole, A.F., 1989. Ospreys: A Natural and Unnatural History: 246. Cambridge University Press, Cambridge.

Porrello, S., Lenzi, M., Ferrari, G., Persia, E., Savelli, F., Tomassetti, P., 2005. Loading of nutrient from a land-based fish farm (Orbetello, Italy) at different times. Aquac. Int. 13, 97–108.

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 (1), 81–87.

R Core Team, 2023. R: A language and environment for statistical computing (Version 4.3.1). R Foundation for Statistical Computing. https://www.R-project.org/.

Rattner, B.A., Golden, N.H., Toschik, P.C., McGowan, P.C., Custer, W., 2008. Concentrations of metals in blood and feathers of nestling ospreys (*Pandion haliaetus*) in Chesapeake and Delaware bays. Arch. Environ. Contam. Toxicol. 54, 114–122.

Renedo, M., Amouroux, D., Duval, B., Carravieri, A., Tessier, E., Barre, J., Bustamante, P., 2018. Seabird tissues as efficient biomonitoring tools for hg isotopic investigations: implications of using blood and feathers from chicks and adults. Environ. Sci. Technol. 52 (7), 4227–4234.

Ricking, M., Schwarzbauer, J., 2012. DDT isomers and metabolites in the environment: an overview. Environ. Chem. Lett. 10, 317–323.

Risebrough, R.W., Valencia, R., Clendenen, D., Mason, A.Z., Bloom, P.H., Wallace, M.P., et al., 2001. Absence of demonstrable toxicity to Turkey vultures, Cathartes aura of copper and tungsten-tinbismuth-composite pellets. In: Final Report to US Fish and Wildlife Service, California Condor Recovery Program. The Bodega Bay Institute, Berkeley, CA.

Rivera-Rodríguez, L.B., Rodríguez-Estrella, R., 2011. Incidence of organochlorine pesticides and the health condition of nestling ospreys (Pandion haliaetus) at Laguna san Ignacio, a pristine area of Baja California Sur, Mexico. Ecotoxicology 20, 29–38.

Rodriguez-Ramos Fernandez, J., Höfle, U., Mateo, R., Nicolas de Francisco, O., Abbott, R., Acevedo, P., Blanco, J.M., 2011. Assessment of lead exposure in Spanish imperial eagle (Aquila adalberti) from spent ammunition in Central Spain. Ecotoxicology 20, 670–681.

Sabadková, T., Janiga, M., Korňan, J., Pitoňáková, T., 2024. Mercury in the feathers of Golden eagle (Aquila chrysaetos) from Western Carpathian, Slovakia. Environ. Sci. Pollut. Res. 31 (18), 26527–26535.

Sanderson, G.C., Bellrose, F.C., 1986. A review of the problem of lead poisoning in waterfowl. Illinois natural history survey special Publication No. 04.

Scheuhammer, A.M., Meyer, M.W., Sandheinrich, M.B., Murray, M.W., 2007. Effects of environmental methylmercury on the health of wild birds, mammals, and fish. Ambio 36, 12–19.

Schmidt-Rothmund, D., Dennis, R., Saurola, P., 2014. The osprey in the Western Palearctic: breeding population size and trends in the early 21st century. J. Raptor Res. 48 (4), 375–386.

Sergio, F., Caro, T., Brown, D., Clucas, B., Hunter, J., Ketchum, J., Hiraldo, F., 2008. Top predators as conservation tools: ecological rationale, assumptions, and efficacy. Annu. Rev. Ecol. Evol. Syst. 39 (1), 1–19.

Sforzi, A., Sammuri, G., Monti, F., 2019. From a regional reintroduction project to a country-wide conservation approach: scaling up results to promote osprey conservation in Italy. Avocetta J. Ornithol. 43 (1), 81–85.

Skujina, I., Ougham, H., Evans, E., Monti, F., Kalvāns, A., Cross, T., McKeown, N.J., 2021. Ecological and genetic monitoring of a recently established osprey (Pandion haliaetus) population in Wales. J. Raptor Res. 55 (4), 635–643.

Solonen, T., Lodenius, M., 1990. Feathers of birds of prey as indicators of mercury contamination in southern Finland. Ecography 13 (3), 229–237.

Solonen, T., Lodenius, M., Tulisalo, E., 1999. Metal levels of feathers in birds of various food chains in southern Finland. Ornis Fennica 76, 25–32.

Sørmo, E.G., Ciesielski, T.M., Øverjordet, I.B., Lierhagen, S., Eggen, G.S., Berg, T., Jenssen, B.M., 2011. Selenium moderates mercury toxicity in free-ranging freshwater fish. Environ. Sci. Technol. 45 (15), 6561–6566.

Soroldoni, S., Abreu, F., Castro, Í.B., Duarte, F.A., Pinho, G.L.L., 2017. Are antifouling paint particles a continuous source of toxic chemicals to the marine environment? J. Hazard. Mater. 330, 76–82.

Spitzer, P.R., Risebrough, R.W., Walker, W., Hernandez, R., Poole, A., Puleston, D., Nisbet, I.C., 1978. Productivity of ospreys in Connecticut—Long Island increases as DDE residues decline. Science 202 (4365), 333–335.

Steidl, R.J., Griffin, C.R., Niles, L.J., 1991. Contaminant levels of osprey eggs and prey reflect regional differences in reproductive success. J. Wildl. Manag. 601–608.

Stocker, J., Morel, A.P., Wolfarth, M., Dias, J.F., Niekraszewicz, B.A.L., Cademartori, C. V., Da Silva, F.R., 2022. Basal levels of inorganic elements, genetic damages, and hematological values in captive Falco peregrinus. Genet. Mol. Biol. 45 (2), e20220067.

Sutherland, W.J., Armstrong, D., Butchart, S.H.M., Earnhardt, J.M., Ewen, J., Jamieson, I., Tatayah, V., 2010. Standards for documenting and monitoring bird reintroduction projects. Conserv. Lett. 3 (4), 229–235.

Thompson, D.R., Furness, R.W., Monteiro, L.R., 1998. Seabirds as biomonitors of mercury inputs to epipelagic and mesopelagic marine food chains. Sci. Total Environ. 213, 299–305.

Tsydenova, O., Minh, T.B., Kajiwara, N., Batoev, V., Tanabe, S., 2004. Recent contamination by persistent organochlorines in Baikal seal (Phoca sibirica) from Lake Baikal, Russia. Mar. Pollut. Bull. 48, 749–758.

Tzanetou, E.N., Karasali, H., 2022. A comprehensive review of organochlorine pesticide monitoring in agricultural soils: the silent threat of a conventional agricultural past. Agriculture 12, 728. https://doi.org/10.3390/agriculture12050728.

United Nations Environment Programme, 2025. We Are all in this Together - Annual Report 2024.

Viluksela, M., Saurola, P., Koivusaari, J., Finnlund, M., Bignert, A., Airaksinen, R., et al., 2024. Temporal trends (1972–2017) and spatial differences of persistent halogenated aromatic hydrocarbons in osprey eggs in Finland. PLoS One 19 (9), e0308227. https://doi.org/10.1371/journal.pone.0308227.

Vos, J.G., Dybing, E., Greim, H.A., Ladefoged, O., Lambre, C., Tarazona, J.V., Brandt, I., Vethaak, A.D., 2000. Health effects of endocrine-disrupting chemicals on wildlife, with special reference to the European situation. Crit. Rev. Toxicol. 30, 71–133.

Wang, X., Wang, W.X., 2017. Selenium induces the demethylation of mercury in marine fish. Environ. Pollut. 231, 1543–1551.

Wang, X., Yang, F., Tian, X., et al., 2023. Toxic effects of copper on duck cerebrum: a crucial role of oxidative stress and endoplasmic reticulum quality control. Environ. Sci. Pollut. Res. 30, 98127–98138. https://doi.org/10.1007/s11356-023-29397-6.

Wei, T., Simko, V., 2017. R package "corrplot": visualization of a correlation matrix. https://github.com/taiyun/corrplot.

Westrip, J.R.S., Burfield, I.J., Allen, D.J., Numa, C., 2022. The Conservation Status of Breeding Raptors in the Mediterranean. IUCN, Málaga, Spain.

Wiemeyer, S.N., Bunck, C.M., Krynitsky, A.J., 1988. Organochlorine pesticides, polychlorinated biphenyls, and mercury in osprey eggs—1970–79—and their relationships to shell thinning and productivity. Arch. Environ. Contam. Toxicol. 17 (6), 767–787.

World Health Organization, 1989. DDT and its derivatives: environmental aspects/ published under the joint sponsorship of the United Nations Environment Programme, the International Labour Organisation, and the World Health Organization. In: DDT and its derivatives: environmental aspects/published under the joint sponsorship of the United Nations Environment Programme. the International Labour Organisation, and the World Health Organization.

Zabala, J., Rodriguez-Jorquera, I.A., Orzechowski, S.C., Frederick, P., 2019. Mercury concentration in nestling feathers better predicts individual reproductive success than egg or nestling blood in a piscivorous bird. Environ. Sci. Technol. 53 (3), 1150–1156.

Zuur, A.F., Ieno, E.N., 2016. A protocol for conducting and presenting results of regression-type analyses. Methods Ecol. Evol. 7 (6), 636–645.

Zuur, A.F., Ieno, E.N., Walker, N.J., Saveliev, A.A., Smith, G.M., 2009. Mixed Effects Models and Extensions in Ecology with r. Springer.