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RESEARCH ARTICLE

Drawing the baseline of trace element levels in the vulnerable Mediterranean osprey *Pandion haliaetus*: variations by breeding location, habitats, and egg components

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Abstract

Due to its peculiarity to accumulate environmental contaminants, the osprey *Pandion haliaetus* is a sentinel species for the biomonitoring of contaminants in aquatic ecosystems. Despite this, no information on trace element concentration exists for the Mediterranean area, where relict and vulnerable osprey populations exist. We evaluated the geographical patterns of heavy metals and selenium in osprey eggs from three different populations of the Mediterranean basin (Balearic Islands, Corsica, and Tuscany), to identify any possible contaminant sources. Pattern of metal concentration followed the order: Fe > Zn > Cu > Se > Hg > Pb > Cd. Differences in contaminant concentrations between habitats and among egg components were found. Egg content and inner membrane showed higher mercury levels (1.06 ± 0.89 and 0.67 ± 0.62 mg/kg dw, respectively) than those recorded in the eggshell. Mercury concentration was ca. two times higher in marine than in wetland samples, and even higher (3.6 times) when referred to the eggshell. Cu, Fe, Zn, and Se had higher concentration in the inner membrane. We stress how the choice of the biological material can have significant implications for the correct evaluation of contamination. Our study represents a first regional scale survey for the vulnerable Mediterranean osprey populations and provides baseline data for their long-term biomonitoring.

Keywords Eggs · Trace elements · Raptor · Bioindicators · Contamination · Mediterranean Sea · *Pandion haliaetus*

Introduction

Wildlife species have been historically used for ascertaining and monitoring the presence of persistent contaminants in natural ecosystems (Dmowski 1999; Burger 2006). Ecotoxicological analyses, conducted on such species with the role of ecological sentinels, have been used to detect the presence of specific contaminant levels and to identify polluted areas (Burger 2006; Jackson et al. 2016). Among birds, raptors have been largely used in this effort (e.g., Garcıa-Fernandez et al. 2008; Espín et al. 2016). As top predators, these birds are useful bioindicators for monitoring how certain substances move up in food web in progressively higher concentrations as they are incorporated into the diet of prey organisms (e.g., biomagnification process; Des Granges et al. 1998). Overall, there is an extensive set of studies examining the effects of contaminants on population parameters of different raptor species (Gómez-Ramírez et al. 2014). This is the case for the peregrine falcon *Falco peregrinus* (e.g., Garcıa-Fernandez et al. 2008; Fernie and Letcher 2010), the bald eagle *Haliaeetus leucocephalus* (e.g., Bechard et al. 2009), the white-tailed eagle *Haliaeetus albicilla* (e.g., Kitowski et al. 2017a), the common buzzard *Buteo buteo* (Kitowski et al. 2017b), and the osprey *Pandion haliaetus* (e.g., Grove et al. 2009).

The osprey is a long-lived fish-eating raptor at the top of the aquatic food web, which can adapt to anthropized environments (Poole 1989). Life history traits and other peculiar ecological characteristics make this species particularly

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suitable for biomonitoring and ecotoxicological research applied to aquatic ecosystems (Henny et al. 2004; Rattner et al. 2008; Grove et al. 2009; Jackson et al. 2016). Much of the research on this species has been carried out in the New World, especially in relation to population crashes caused by a widespread use of persistent pollutants in the environment (e.g., DDT and its congeners), documented during the '60s and '70s (Elliott et al. 2000; Henny et al. 2008). Recently, a large-scale ecotoxicological study of American ospreys has been even re-evaluated after decades to determine any spatial and temporal trend (Lazarus et al. 2015). In contrast, few studies were carried out in Europe (Grove et al. 2009; Lemarchand et al. 2009, 2011) and only one in the Mediterranean region (Jiménez et al. 2007). This latter study, in particular, focused on the evaluation of organochlorine compounds and being geographically restricted to one location (Mallorca-Balearic Islands). We are unaware of any published evaluation on heavy metal concentrations in the Mediterranean osprey population. This lack of information presents a barrier for the long-term biomonitoring of toxic metals for this population in the region and, ultimately, for developing conservation strategies for the species.

Being an important hotspot of biodiversity, the Mediterranean represents a geographic area of major concern for conservation (Myers et al. 2000). However, as a semi-closed basin surrounded by some of the most industrialized and heavily populated countries of the world, the Mediterranean Sea has reached alarming levels of contamination (Danovaro 2003) and most of the coastal areas have been classified as "problem areas" (EEA 2019). Furthermore, abundant natural reserves of mercury are present in this area. The majority of the mercury released into the marine environment is inorganic but can be converted to the methyl form (MeHg) by bacterial activity under anoxic conditions in upper layers of the sediments (Duran et al. 2008), as well as within the water column (Heimbürger et al. 2010).

In the Mediterranean, the osprey is still an uncommon breeding species with a vulnerable conservation status: after decades of direct persecution, the species survives here with relict populations, counting less than 80–100 breeding pairs distributed between Corsica, Balearic Islands, Morocco, and Algeria (Monti et al. 2018a). In the last decades, thanks to reintroduction programs, the species has returned to breed in mainland Spain (Muriel et al. 2010), Portugal (CIBIO 2011), and Italy (Monti et al. 2014). Moreover, although ospreys are migratory throughout most of their distributional range (Poole 1989), some populations, such as those of the Mediterranean, are characterized by short-distance migration, or even residents (Monti et al. 2018b). They are known to winter at temperate latitudes, thus remaining mostly in the Mediterranean basin, through the entire year and/or life-cycle (Monti et al. 2018b). This means that samples from these individuals can reflect the contaminant exposure at a very regional/local scale, being hence quite focused. In contrast, long distance migratory birds can absorb contaminants at wintering grounds, often located thousands of km away from breeding areas (e.g., Hughes et al. 1997; Elliott et al. 2007). Moreover, unlike to pelagic seabirds that forage offshore, the osprey feeding home range can be extremely small and largely confined to coastal areas adjacent to breeding sites (Monti et al. 2018a), thus providing information on the presence of specific contaminant in marine coastal habitats and wetland system interconnected with the sea.

The main objective of this study was to provide baseline data for the long-term biomonitoring of contaminants in the vulnerable Mediterranean osprey populations and, in particular (1) to investigate geographical patterns of mercury (Hg), cadmium (Cd), lead (Pb), iron (Fe), copper (Cu), zinc (Zn), and selenium (Se) in osprey eggs; (2) to evaluate any relationship between these elements' concentration and eggshell thickness (as a proxy for environmental pollution; i.e., Maurer et al. 2012); and (3) to evaluate differences in contaminant concentrations between samples from coastal marine environments and wetland habitats.

Furthermore, although eggs have been widely used as indicators of exposure to lipophilic contaminants (Ewins 1997), eggshells have been less examined especially for their trace element content (Peterson et al. 2017) and some studies pointed out that their concentrations are often too low to be detected by the instruments (Peterson et al. 2017). On the contrary, the egg content and the inner eggshell membrane may can contain markedly higher concentrations of some elements (i.e., mercury) than the eggshell (Peterson et al. 2017). It is therefore evident that the choice of the biological material for the analyses is of paramount importance, namely to precisely comparing data and areas of investigation. Differences in concentration values (being dependent also on instrument-specific detection limits) can have significant implications for the correct evaluation of contamination and, in turn, in the adequate management choices for a proper conservation (Espín et al. 2016; Peterson et al. 2017). For this reason, a final objective was added: (4) to investigate differences in contaminant concentrations in different parts of the egg (i.e., egg content, inner membrane, and eggshell) as methodological detail.

Material and methods

Study area and sampling

Overall, 24 unhatched osprey eggs were collected from three different Mediterranean osprey populations (Balearic Islands, Spain: $n = 10$ from five nest sites; Corsica, France: $n = 7$ from seven nest sites; coastal Tuscany, Italy: $n = 7$ from one nest site; Fig. 1). In the Balearic Islands and Corsica, the osprey is strictly associated to marine environments, where it breeds on rocky

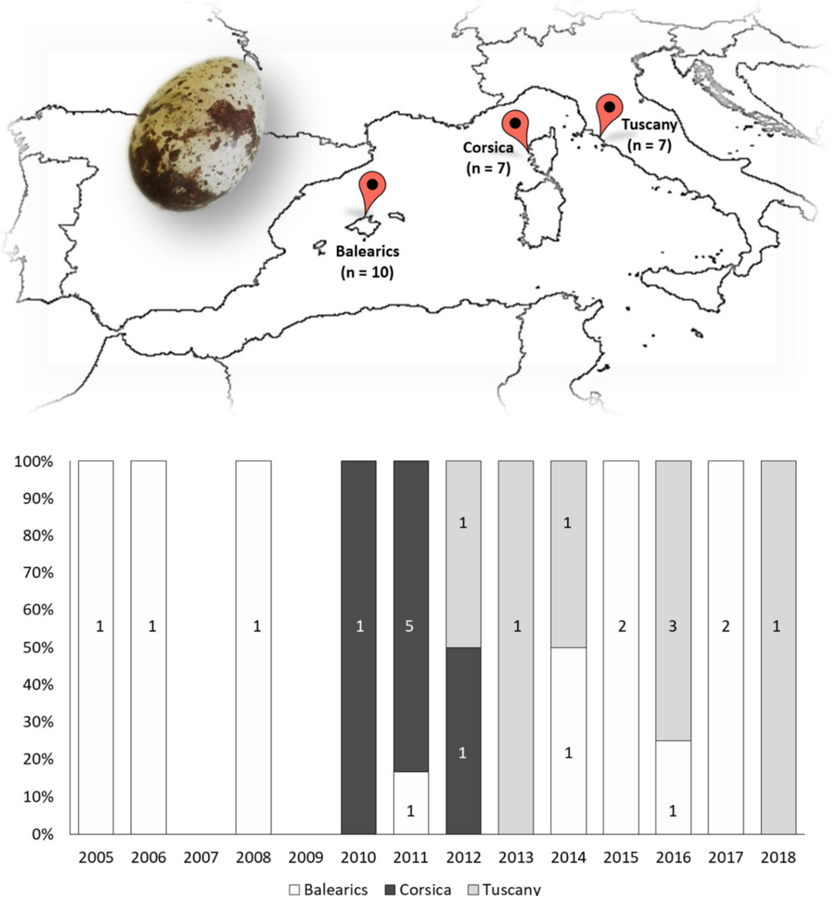


Fig. 1 On the map, location and number of eggs sampled. In the graph, the year repartition (%) of egg samples is reported: in white for Balearics, in black for Corsica, and in gray for Tuscany

pinnacles and cliffs. Conversely, the recently reconstituted Tuscan population attends coastal wetlands with brackish waters, where pairs breed on artificial nest structures. These populations are made up of a small number of breeding pairs: ca. 25 in the Balearics (R.B. Triay *pers.comm.*), 28–30 in Corsica (Monti et al. 2018b), and five in Tuscany (Sforzi et al. 2019). For this reason, on the contrary to studies conducted on larger osprey populations in America, where fresh eggs were removed from nests during early incubation (e.g., Elliott et al. 2000; Henny et al. 2008), we collected only abandoned addled eggs (eggs which failed to hatch) at the end of the reproductive cycle. The collection of a sample egg has been found in fact to reduce osprey productivity by about 0.36 to 0.42 young/nest, with an even wider range for individual years with small sample sizes (Henny and Martell 2017). Therefore, if on one hand our approach limited the number of samples available for the analyses, on the other hand this choice avoided to disturb breeding pairs and the risk of undermining the population stability over time. It is important to recognize that unhatched/failed eggs can be potentially biased sample, especially if a contaminant was involved with the nest failure. However, eggs were sampled in active nests (with young produced from any of the nests where an egg was collected) except for one case/year in Italy. Yet, because of the degraded status of the egg content at the time of collection, it was unlikely to distinguish between the albumen and yolk. Collected eggs cover a period of approximately 14 years (2005–2018). Eggs were kept dried at room temperature until analyses.

Laboratory procedures and statistical analyses

The eggs' exterior shells were firstly cleaned with deionized water and ethanol. Eggs were then opened to collect separately (a) egg content, (b) cleaned eggshell, and (c) inner membrane (material internally adhered to eggshells). We used clean stainless-steel scissors to cut a hole into the top of each egg and transferred the egg contents into a sterile polypropylene jar. The inner eggshell membrane was carefully removed from the eggshell by peeling it off with tweezers. Eggshell thickness, excluding membranes, was measured at three sites on the equator with a rounded contact point and the values were averaged (following Henny and Elliott 2007; Odsjö and Sondell 2014). All samples were stored frozen at − 20 °C. To prepare egg contents for analysis, we lyophilized the egg contents at − 50 °C for approximately 48 to 72 h. Then, using a clean mortar and pestle, we fully homogenized the dried egg contents to a powder. Samples were processed for acid decomposition 4:1 v/v nitric acid (HNO_3) and hydrogen peroxide

(H_2O_2) in high pressure hot block system (Teflon Bomb). Quality assurance measures included analysis of Standard Reference Materials (e.g., IAEA-407 Fish Homogenate, Egg Powder, Fish muscle-DORM-4). Blanks were run during each set of tests to check the purity of the chemicals used and any sample contamination. The elements were analyzed by using atomic absorption spectrometer. For the determination of the various essential elements were used the following instruments: atomic absorption spectrometer Analytik Jena Contra 700 graphite furnace for Pb and Cd; the cold vapor technique (Atomic Absorption Spectrometer Perkin Elmer Fims 400) for Hg; ICP-OES Plasma Perkin Elmer 5100DV for Cu, Fe, and Zn; and Analytik Jena Contra 700 coupled to generation of hydride system Hydrea (HS 60) for Se. Concentrations are given as mean of three replicates and were expressed in mg/kg on dry weight basis (dw). Since collected eggs varied from nearly fresh to nearly dry, an objective comparisons based on weight of the contents were not directly possible (Stickel et al. 1973). In order to account for moisture loss, a percent moisture value of 84% (as per Elliott et al. 1998) was used to convert data from dry weight basis into values expressed on a wet weight basis. This allowed comparing literature data and assess whether the species shows similar patterns of heavy metal and selenium exposure across its wide geographical range.

Shapiro-Wilk normality test was performed to determine whether the data were normally distributed. Variations in the eggshell thickness in relation to sampling location, habitats, and elements' concentration were tested through two-way analysis of variance (ANOVA). Due to the limited sample size, statistical analyses to examine possible differences between heavy metal concentrations and sampling locations and/or components of the eggs were tested through the non-parametric Kruskal-Wallis analysis of variance. The independent 2-group Mann-Whitney *U* test was used to test for differences between heavy metal concentrations in eggs belonging to individuals found in different habitat types: osprey sampling locations were pooled into marine (Balearics and Corsica) vs wetland (Tuscany) for statistical comparisons. We correlated elements' concentration with eggshell thickness, as well as the concentrations between different elements in egg components, through the Spearman test. The level of statistical significance was initially set as $p < 0.05$. Although the non-parametric Spearman test is quite robust, calculating numerous correlations increases the risk of a type I error and erroneously concludes the presence of a significant correlation. To avoid this, a Bonferroni correction was applied to correct for multiple comparisons (Rice 1989). After Bonferroni correction, the resulting α value was 0.05/7 tests = 0.007, which represented the new level of statistical significance. Statistical analyses were performed using SPSS 18.0 version. Data are reported as mean ± standard deviations.

Results

Morphometric and eggshell thickness

Mean eggs' width and length were 47.326 ± 1.11 mm and 64.235 ± 2.37 mm, respectively. Width and length did not vary across locations (ANOVA: $F = 4.068$, df = 2, $p = 0.32$; ANOVA: $F = 0.439$, df = 2, $p = 0.652$). Mean eggshell thickness was 0.449 ± 0.03 mm and it was normally distributed ($Z = 0.608$; $p = 0.853$; $n = 24$). Eggshell thickness did not vary significantly across locations (ANOVA: $F = 1.414$, df = 2, $p = 0.265$), being 0.441 ± 0.02 mm in the Balearic Islands ($n = 10$), 0.440 ± 0.04 mm in Corsica ($n = 7$), and 0.468 ± 0.03 mm in Tuscany ($n = 7$). All values are reported in Table 1. Similarly, eggshell thickness did not differ significantly between habitats (ANOVA: $F = 2.961$, df = 1, $p = 0.099$), being 0.441 ± 0.03 mm for samples collected in marine environments ($n = 17$) and 0.468 ± 0.03 mm for those in wetland habitats ($n = 7$). Overall, eggshell thickness was not significantly correlated with elements' concentration nor with any other egg measurements (i.e., length or width, in all cases).

Overall trace element concentrations

The overall mean element concentrations (± standard deviation) found in each egg component for the Mediterranean region and in the different breeding location are reported in Table 2. Data were pooled across years, since no significant differences between years were detected.

Trace element variation by location

Mercury concentration did not vary significantly across locations, regardless from the part of egg. Cadmium concentrations differed across locations only in the egg content ($\chi^2 = 10.908$, df = 2, $p = 0.004$) with highest levels in those from Tuscany, whereas showed similar concentrations when measured in the eggshell and inner membrane. Copper concentration did not vary except for the eggshell ($\chi^2 = 6.118$, df = 2, $p = 0.047$) reaching highest concentration in samples from Corsica. Lead concentration differed across locations only in the egg content ($\chi^2 = 9.917$, df = 2, $p = 0.007$) showing highest value in Corsica but not in the other parts of the egg. Concentrations of iron, zinc, and selenium did not vary for all parts of the egg ($p > 0.05$ in all cases).

Trace element variation by habitat

Peculiar differences emerged when testing for differences between samples collected in marine environments (grouping both Corsican and Balearic samples) and in wetland habitats (Tuscan samples) (Fig. 2). Eggshells of marine samples

Table 1 Mean (± SD) width, length, and eggshell thickness expressed *per* location and related habitat. Overall mean value (± SD) for the Mediterranean region is also reported. Sample size (n) is indicated within brackets

Location	Habitat type	Width (mm)	Length (mm)	Eggshell thickness (n)
Balearics	Marine	47.107 ± 1.06 (10)	64.687 ± 2.32 (10)	0.441 ± 0.02 (10)
Corsica	Marine	46.723 ± 1.23 (7)	64.200 ± 2.56 (4)	0.440 ± 0.04 (7)
Tuscany	Wetland	48.241 ± 0.78 (7)	63.505 ± 2.58 (6)	0.468 ± 0.03 (7)
Overall mean Mediterranean		47.326 ± 1.11 (24)	64.235 ± 2.37 (20)	0.449 ± 0.03 (24)

showed 3.6 times higher mercury concentration than those from wetlands ($U = 27.5$, $p = 0.042$) with mean mercury levels of 0.049 ± 0.04 mg/kg for marine and of 0.014 ± 0.010 mg/kg for wetland samples, respectively (Fig. 2a). No significant differences occurred for other parts of the egg for this element, despite the fact that in both cases mercury concentration was *ca.* two times higher in marine than in wetland samples. Significant differences were found also for the cadmium concentration in the egg content ($U = 25.0$, $p = 0.018$), which was 1.9 times higher for wetland samples (mean wetlands: 0.007 ± 0.004 mg/kg; Fig. 2b). It did not vary for other parts of the egg. Lead varied only for egg content ($U = 27.5$, $p = 0.035$) with higher values for marine samples (mean marine: 0.101 ± 0.078 mg/kg; Fig. 2c), but not for other parts of the egg. Copper did not vary in relation to habitat for any part of the egg, as well as iron and selenium. Zinc varied only for eggshell ($U = 25.0$, $p = 0.028$; Fig. 2d), but not for egg content and inner membrane.

Trace element variation by egg component

When investigating concentrations of heavy metals on different parts of the egg regardless the sampling location or habitat (Table 2), we found significant differences for mercury ($\chi^2 = 49.57$, df = 2, $p < 0.01$), with higher values recorded in the egg content and the inner membrane, which where 27.3 and 1.6 times higher than those of the eggshell, respectively. Between the different parts of the egg, no significant differences were detected for concentrations of cadmium and lead. Copper varied significantly ($\chi^2 = 38.90$, df = 2, $p < 0.01$) as well as iron ($\chi^2 = 40.01$, df = 2, $p < 0.01$), zinc ($\chi^2 = 53.82$, df = 2, $p < 0.01$), and selenium ($\chi^2 = 39.95$, df = 2, $p < 0.01$) with higher concentration detected in the inner membrane (Table 2).

Correlation between elements

The correlation between the concentrations of different elements was also evaluated. As showed in Fig. 3, a positive correlation between the concentration of Hg and Se ($r = 0.626$, $p = 0.001$) was found, as well as between Cu and Zn ($r = 0.741$, $p = 0.001$) in the egg content. Within eggshell, Hg also correlated with Pb ($r = 0.768$, $p = 0.001$) and Se with Fe ($r = 0.621$, $p = 0.004$). The relationship between Fe and Zn was significant in the membrane ($r = 0.650$, $p = 0.001$). Other correlations were found when testing for different habitats and/or egg components (all values are reported in Supplementary Information 1).

Discussion

Our study represents a first survey at Mediterranean regional scale and provides a first set of data for the long-term biomonitoring of heavy metals and selenium for the vulnerable osprey populations of the Mediterranean basin. It also gives insights on how such heavy metals concentrate in osprey eggs, in relation to different habitat types and across locations, and provide a focus on elements' pattern in different egg components as ecophysiological methodological additional issue.

Overall, trace element concentrations found in eggs seem not to affect the studied populations. In particular, both osprey populations in the Balearic Islands and Tuscany are showing stable-increasing trends (Siverio et al. 2018; Sforzi et al. 2019). In Corsica, the number of breeding pairs is stable, but breeding performance has dropped for pairs breeding in the Scandola Reserve, being affected by high touristic pressures (Monti et al. 2018a). Reproductive parameters are provided in Supplementary Information 2.

Morphometrics and eggshell thickness

Mean eggs' width and length were within the range of other literature studies (e.g., ARCADIS 2012), as well as the eggshell thickness (Table 3). However, it is noteworthy that we found eggshell values between 9.8 and 11.1% thinner than the pre-DDT era, as recorded in American osprey samples collected in Florida (0.498 mm) and eastern United States (0.505 mm) (Anderson and Hickey 1972; Wiemeyer et al. 1988). In our study, no clear relationships between egg biometric data, eggshell thickness, and trace element concentration were found. Therefore, other environmental contaminants, such as organochlorines, may had exert an interaction with metabolism during eggshell formation and affect shell thinning, as already described for American osprey populations (Grove et al. 2009). These highly toxic and persistent compounds undergo biomagnification and may have synergistic effects with organic mercury (Ackerman et al. 2013). Yet, selenium has been suggested to increase the sequestration

Table 2 Mean (± SD) trace element concentrations (mg/kg dw) in Mediterranean osprey eggs reported for different sampling locations (Balearic Islands, Corsica, and Tuscany) and in each part of the egg analyzed (egg content, eggshell, and inner membrane). Overall mean values (± SD) for each trace element are reported in italics. Sample size is also indicated within brackets. When values were under the instrument detection limits, we reported the LOD (limit of detection) for that element (i.e., Hg = 0.005; Cd = 0.003; Pb = 0.009)

Country	Egg content	Eggshell	Inner membrane
	Hg		
Balearics	1.22 ± 0.98 (10)	0.04 ± 0.04 (10)	0.74 ± 0.68 (10)
Corsica	1.27 ± 1.075 (7)	0.05 ± 0.04 (7)	0.91 ± 0.79 (6)
Tuscany	0.65 ± 0.45 (7)	0.01 ± 0.01 (7)	0.38 ± 0.25 (7)
Overall mean	*1.06 ± 0.89 (24)*	*0.03 ± 0.04 (24)*	*0.67 ± 0.62 (23)*
Cd			
Balearics	0.003 ± 0.001 (10)	0.008 ± 0.011 (10)	0.005 ± 0.003 (10)
Corsica	0.004 ± 0.001 (7)	0.01 ± 0.017 (7)	0.007 ± 0.003 (6)
Tuscany	0.007 ± 0.004 (7)	0.003 ± 0.001 (7)	0.005 ± 0.003 (7)
Overall mean	*0.005 ± 0.003 (24)*	*0.008 ± 0.01 (24)*	*0.006 ± 0.003 (23)*
Pb			
Balearics	0.07 ± 0.07 (10)	0.14 ± 0.17 (10)	0.04 ± 0.05 (10)
Corsica	0.15 ± 0.06 (7)	0.32 ± 0.29 (7)	0.13 ± 0.18 (6)
Tuscany	0.03 ± 0.04 (7)	0.09 ± 0.13 (7)	0.11 ± 0.08 (7)
Overall mean	*0.08 ± 0.07 (24)*	*0.18 ± 0.22 (24)*	*0.08 ± 0.11 (23)*
Cu			
Balearics	3.50 ± 0.65 (10)	1.80 ± 0.54 (10)	3.86 ± 1.08 (10)
Corsica	3.25 ± 1.32 (7)	2.11 ± 0.47 (7)	4.62 ± 1.87 (6)
Tuscany	3.26 ± 1.66 (7)	1.66 ± 0.11 (7)	4.21 ± 1.97 (7)
Overall mean	*3.36 ± 1.16 (24)*	*1.85 ± 0.46 (24)*	*4.16 ± 1.56 (23)*
Fe			
Balearics	51.44 ± 11.25 (10)	34.19 ± 18.32 (10)	125.33 ± 68.62 (10)
Corsica	42.75 ± 11.37 (7)	48.18 ± 32.04 (7)	144.31 ± 76.58 (6)
Tuscany	53.69 ± 6.72 (7)	27.56 ± 10.02 (7)	142.32 ± 84.25 (7)
Overall mean	*49.56 ± 10.76 (24)*	*36.34 ± 22.21 (24)*	*135.45 ± 72.65 (23)*
Zn			
Balearics	35.32 ± 8.37 (10)	11.75 ± 5.47 (10)	57.06 ± 27.06 (10)
Corsica	25.63 ± 10.66 (7)	9.93 ± 4.73 (7)	89.70 ± 50.15 (6)
Tuscany	29.81 ± 10.73 (7)	6.43 ± 1.71 (7)	64.43 ± 30.80 (7)
Overall mean	*30.89 ± 10.22 (24)*	*9.67 ± 4.84 (24)*	*67.82 ± 36.29 (23)*
Se			
Balearics	2.175 ± 0.738 (10)	0.325 ± 0.146 (7)	3.019 ± 1.356 (10)
Corsica	2.382 ± 0.943 (7)	0.554 ± 0.210 (7)	2.647 ± 1.167 (6)
Tuscany	1.901 ± 1.011 (7)	0.348 ± 0.279 (6)	2.012 ± 1.206 (7)
Overall mean	*2.155 ± 0.865 (24)*	*0.412 ± 0.230 (20)*	*2.616 ± 1.284 (23)*

of organic mercury within eggs decreasing the overall organic mercury toxic effects, thus positively influencing shell thickness (Bianchi et al. 2018). For Mediterranean ospreys, low eggshell thickness found in the present study and no clear relationships between eggshell thickness and trace element concentration suggest that this population may be at risk of deleterious effects due to exposure of other contaminants (e.g., OCs), in line with high values of OCs found in few nests of Mallorca Island (Jiménez et al. 2007).

Trace element levels in the Mediterranean area

Overall, Mediterranean osprey eggs did not contain trace element concentration at a level of concern. All of the 24 eggs analyzed in this study contained trace elements in the order (referring to egg content concentrations): Fe > Zn > Cu > Se > Hg > Pb > Cd. Indeed essential element such as Fe, Zn, Cu, and Se showed higher concentration than non-essential (Hg, Cd, and Pb). Since levels of most elements are reported for the first time in Mediterranean osprey eggs, and have been rarely investigated in the eggs of this species worldwide (e.g., ARCADIS 2012; Table 3), comparison with literature data is limited. However, this is not the case of Hg, largely examined in areas different from that of the present study (Grove et al. 2009). In our case, mercury concentrations were higher than those from literature data (Table 3). This is in line with other studies demonstrating that samples of waterbirds collected in the Mediterranean area accumulate remarkable amounts of

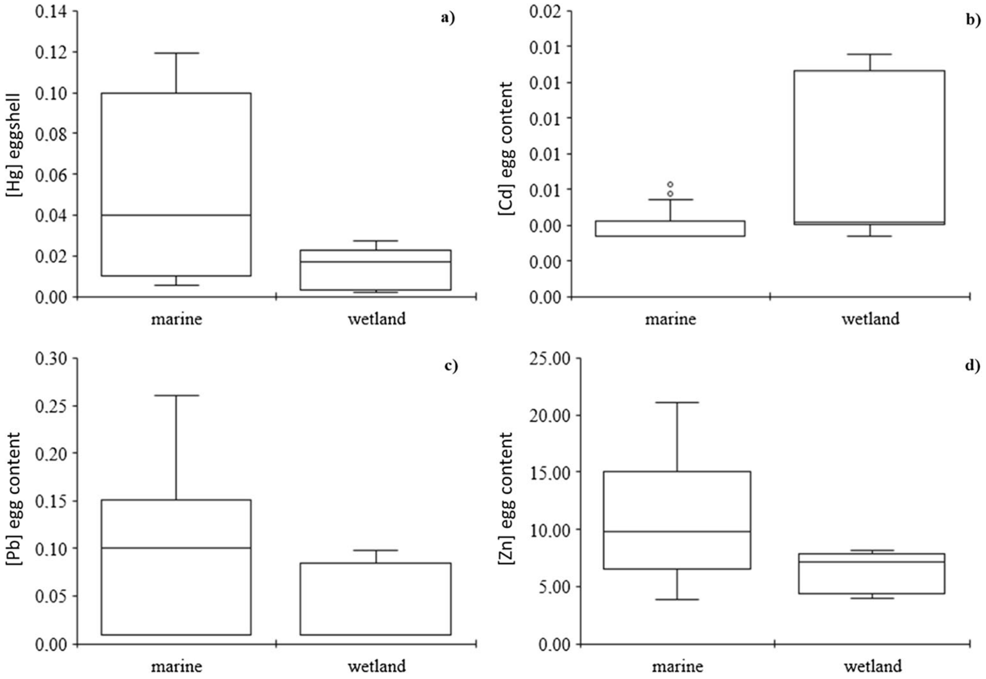


Fig. 2 Significant differences in trace element concentrations (calculated on different parts of the egg) between samples collected in marine vs wetland habitats. a) mercury concentration in the eggshell; b,c,d) cadmium, lead, zinc concentration in the egg content, respectively. Values are expressed in mg/kg dw

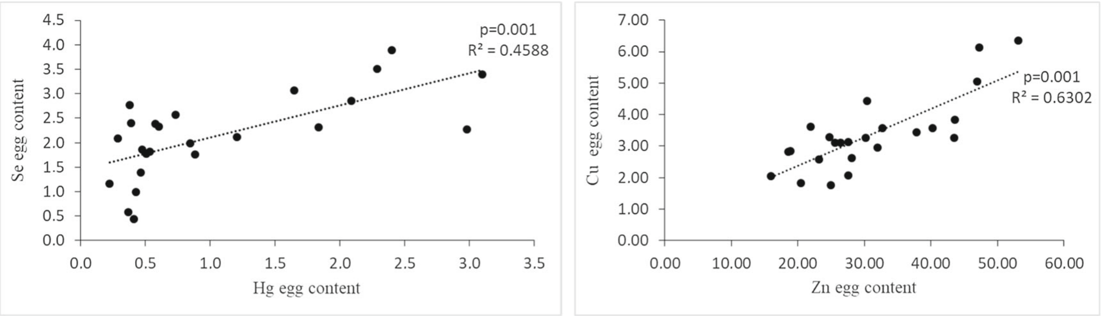
mercury, usually greater than those present in samples of the same species obtained from other areas (Leonzio et al. 1986). Threshold mercury concentrations that can cause reproductive impairment and/or possible adverse effects (i.e., mortality) vary by species as well as by the criteria used to assess effects (Scheuhammer et al. 2008). It has been suggested that mercury concentrations up to 2.5 mg/kg dw in eggs seem to be sufficient to induce impaired reproductive success in a variety of avian species (Thompson 1996; ARCADIS 2012). However, mean mercury concentration found in osprey eggs' content in the present study (1.06 ± 0.89 mg/kg dw; Table 2) was generally lower, suggesting a low-toxicological risk of reproductive impairment associated with levels of Hg. Among avian species, osprey is known having the highest sensitivity/risk to mercury (Heinz et al. 2009; Jackson et al. 2016). In a comparative egg dosing study of 21 bird species, the osprey was one of the most sensitive species to Hg in egg injections (Heinz et al. 2009). In spite of this, we recorded a maximum concentration of mercury above the effects value of 2.5 mg/kg dw (Thompson 1996) only in one case (e.g., 3.101 mg/kg dw in an egg from Corsica).

Concentrations of selenium found in osprey egg contents (2.155 ± 0.865 mg/kg dw; Table 2) were slightly higher than mercury levels as also indicated in other birds species (Furness 1993). Selenium concentrations were well under the threshold points of 6–7 mg/kg dw for deleterious effects, as well as under the threshold level of 4 mg/kg dw causing reproductive impairment, as indicated by Heinz et al. (1989). The significant positive correlation appearing between selenium and mercury in the egg content and the eggshell (Supplementary Information 1) is in line with other studies in wild animals assessing the role of selenium in protecting against the toxic effects of mercury (Leonzio et al. 1986; Burger et al. 2012). The interaction between the two elements has been reported in eggs, livers, kidneys, and other tissues of several fish-eating wildlife species (e.g., Odsjö et al. 2004; Scheuhammer et al. 2008; Ackerman et al. 2016). Likewise, concentrations of the other elements were below concentrations considered to influence breeding success and, in some cases, close to detection limits. More specifically, copper and zinc had similar concentrations to those found in both impacted and reference sites along the Tennessee River, in USA (ARCADIS 2012), whereas iron and lead even in a lower range. Cadmium was close to reporting limits. Finally, with the exception of Hg-Pb relationship in eggshell, the other significant relationships were found among essential elements (Supplementary information 1) which may likely related to physiological regulation processes (Goyer 1996).

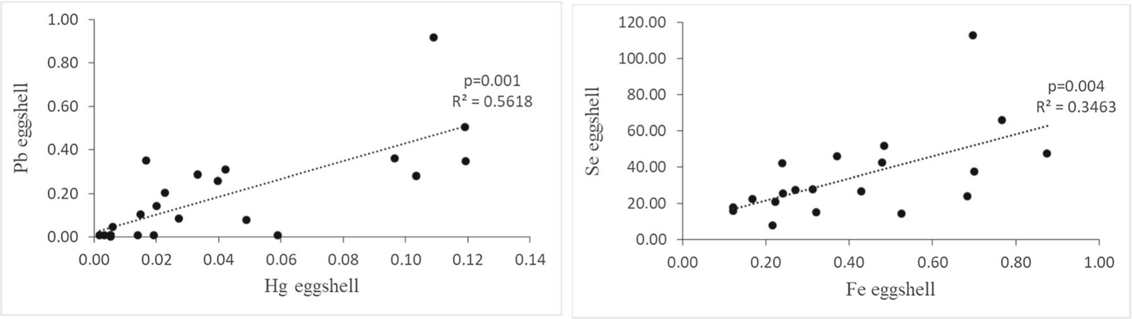
Differences by location

As other raptor species, the osprey is an income breeder that relies on food intake (locally acquired lipid and protein) than body reserves to service the energetic costs of egg production (Durant et al. 2000). Therefore, much of the contaminant residues in eggs should derive from local breeding ground sources and may reflect contaminant exposure within breeding territories (Elliott et al. 2007; Espín et al. 2016). In the present study, trace element concentrations in osprey eggs appear to be similar among locations (Table 3). Nevertheless, Balearic and Corsica samples reach Hg mean concentration higher than

a) Egg content



b) Eggshell



c) Inner membrane

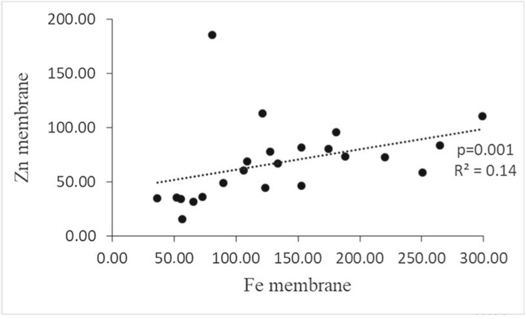


Fig. 3 Significant correlations (Bonferroni-corrected significance level $p \leq 0.007$) between elements' concentrations in different egg components calculated on all samples irrespective the location (Supplementary Information 1). **a** Egg content. **b** Eggshell. **c** Inner membrane. Data are expressed in terms of dry weight

Tuscany samples (about twice for egg content and even more for eggshell) (Table 2). However, probably due to high variability of results within each location and a low sample size, these differences were not significant. Essential elements likely undergo regulatory physiological processes especially when environmental concentrations are not affected by critical source of emission or impact. The absence of macroscopic differences at local scale between osprey populations may depend on metals (i.e., mercury) uniformly distributed throughout the food webs of the central Mediterranean Sea (Bianchi et al. 2018) and to the fact that Mediterranean ospreys tend to make use of similar areas throughout the year. In the Mediterranean, migratory female ospreys return at breeding sites in February–March and lay eggs a few weeks later (Monti et al. 2018b). Yet, in the case of slowly metabolized compounds, the female would retain some of the contaminant burden acquired on the wintering grounds (Henny et al. 2010). However, even in this case, they reflect the contaminant exposure at a very regional/local scale, being hence quite focused. This because the majority of Mediterranean ospreys is short-distance migratory (or even resident) wintering at temperate latitudes in the Mediterranean basin, unlike their conspecifics of northern and central Europe, that perform intercontinental migrations up to sub-Saharan Africa (Monti et al. 2018c). Yet, a larger sample size would probably be more effective to properly evaluate the role of certain elements on the mechanisms behind the exposure, their absorption, and the potential effects at local scale, as found for the Hg.

Table 3 Mean values (or range of means, min-max, and/or SD between brackets) of eggshell thickness, mercury, and lead concentration in osprey eggs as extrapolated from studies conducted in different regions across the species' distributional range. The * refers to studies that provided concentrations for different sites within the study area and that have been averaged in this table. For literature data expressed on wet weight basis (references indicated by the symbol $^{\$}$), values were adjusted for moisture loss (as per Elliott et al. 1998) and converted in dw, for comparative purposes. Thus, all values in the table are now expressed in mg/kg dw

Area	Study region	Habitat type	*N* samples	Sampling period	Eggshell thickness (mm)	Hg concentration	Pb concentration	Literature source
United States	CT	Marine/coastal	na	1964	na	0.875 (0.312–1.5)	na	Wiemeyer et al. (1975)$^{\$}$
United States	CT	Marine/coastal	8	1969	0.429	0.437–0.875*	na	Wiemeyer et al. (1975)$^{\$}$
United States	MD	Marine/coastal	3	1969	0.443	0.375–0.8125*	na	Wiemeyer et al. (1975)$^{\$}$
United States	ID, FL, MD, NC	Marine/coastal	58	1972–1974	0.330–0.501*	0.125–1.375*	na	Wiemeyer et al. (1988)$^{\$}$
United States	NJ	Marine/coastal	25	1985–1989	0.453–0482*	0.5625–1.437*	0.625–3.437*	Steidl et al. (1991)$^{\$}$
United States	MD	Marine/coastal/river	5	1973	na	0.312 (0.187–0.687)	na	Audet et al. (1992)$^{\$}$
United States	MD	Marine/coastal/river	5	1986	na	0.687 (0.4375–1.5)	na	Audet et al. (1992)$^{\$}$
United States	MA	Marine/coastal/river	3	1986	na	0.375 (0.3125–1.4375)	na	Audet et al. (1992)$^{\$}$
United States	VA	Marine/coastal/river	5	1987	na	0.687 (0.3125–1.3125)	na	Audet et al. (1992)$^{\$}$
United States	ID	Freshwater river	5	1986–1987	na	0.3125–0.75*	na	Henny et al. (1991)$^{\$}$
United States	NJ	Marine/coastal	17	1998	0.489	0.75 (0.25–1.625)	1.375–26.25*	Clark et al. (2001)$^{\$}$
United States	NJ	Freshwater lake	5	1991–1992	na	0.44–0.64*	na	Hughes et al. (1997)
United States	BC, OR, WA	Marine/coastal	67	1991–1997	na	0.341–1.18*	na	Elliott et al. (2000)
United States	OR, WA	Freshwater river	29	1997–1998	0.446	0.29 (0.12–0.94)	na	Henny et al. (2004)
United States	BC, AL	Freshwater alpine	93	1999–2003	na	0.39 (0.17)	na	Guigueno et al. (2012)
United States	MD, VA	Marine/coastal	75	2000–2001	na	0.04–0.06*	na	Rattner et al. (2004)
United States	DE, PA	Marine/coastal	39	2002	0.454–0.527*	0.04–0.09*	na	Toschik et al. (2005)
United States	WA	Marine/coastal	7	2003	na	0.72	na	Johnson et al. (2009)
United States	OR, WA	Freshwater river	40	2004	na	0.45 (0.16–1.01)	na	Henny et al. (2008)
United States	OR	Freshwater river	15	2008	0.393–0.567*	< 0.5	na	Buck and Kaiser (2011)
United States	TN	Freshwater river	19	2009–2010	na	0.16–0.89*	0.15–0.7*	ARCADIS (2012)
Canada	Québec	Marine/coastal	51	1989–1991	na	0.375–3.375*	na	Des Granges et al. (1998)$^{\$}$
Canada	Ontario	Freshwater lake	55	1991–1992	na	0.44–1.40*	na	Hughes et al. (1997)
Europe	Finland	Freshwater lake	16	1970–1972	na	0.625–2.5*	na	Häkkinen and Häsänen (1980)$^{\$}$
Europe	Russia	Freshwater lake	10	1992	0.487	0.5625 (0.125–1.125)	na	Henny et al. (1998)$^{\$}$
Europe	Mediterranean	Marine/coastal	24	2005–2018	0.449 ± 0.03	1.06 ± 0.89	0.08 ± 0.07	This study

Differences by habitat

Higher concentrations of mercury were recorded in eggs laid by adults frequenting marine environments, compared to eggs from wetland habitats even though significant difference was only found for eggshell (Fig. 2). This suggests that osprey populations living near the sea are much more exposed to mercury than those reproducing in wetland ecosystems. This agrees with high-level natural baseline values of mercury concentrations recorded in marine and oceanic waters worldwide (for a review: Gworek et al. 2016), which cause noticeable bioaccumulation in the food chain and, primarily, higher mercury contents in marine fishes. Differences observed between the two habitats are likely related mainly to different diet. In Corsica, the diet of breeding ospreys has been studied by identifying fish remains at nests; the identified species mainly belong to mullets (e.g., *Liza ramada*, *Liza aurata*, and *Chelon labrosus*) and breams (e.g., *Diplodus sargus* and *Diplodus vulgaris*) and to other less representative species (Francour and Thibault 1996). In the Balearic Islands, the most representative species detected by direct observation and remains of fish at nests are mullets (*Mugil* spp.), salema (*Sarpa salpa*), saddled bream (*Oblada melanura*), and gild-heat bream (*Sparus auratus*) (R.Triay, unpublished data). In Tuscany, where ospreys forage in coastal and inland wetlands, the flathead mullet *Mugil cephalus* represent the main prey item (Monti et al. 2014). Accordingly, osprey fishing in marine environments ingest prey items containing higher concentrations of mercury than those feeding into wetlands and is therefore subject to higher uptake of mercury through marine fishes (Chen et al. 2008; Eisler 2010). Similarly, two gull species feeding in different habitats (e.g., Audouin's gull *Larus audouinii* in pelagic environment and yellow-legged gull *Larus michahellis* on coastal and inland environments) showed also differences in mercury concentration, because of the different uptake of mercury through the diet (Bianchi et al. 2018; Pereira et al. 2019).

The marine origin of food may also have played a role in the element relationships found (Supplementary information 1): within the "sea" group, the egg content showed mercury significantly correlated to selenium. Conversely, within the "wetland" group, significant relationship was found only among essential elements and only in the eggshell.

Cadmium, differently from mercury, showed higher values for wetland samples, although it was recorded only for the egg content. In aquatic ecosystems, cadmium is not particularly mobile in the trophic web and does not tend to biomagnify, like mercury. It is associated with particulate matter and mainly found in the filtering organisms and in those associated with the sediment. It is therefore not surprising that in the coastal wetlands, typical settling environments, there may be more cadmium in prey (such as the flathead mullet) than in those from the marine ecosystem. *Mugil cephalus* in fact has a benthic feeding strategy, ingesting large amounts of organic matter, sand, or mud from the sediment of waterways (Waltham et al. 2013). Little Pb is transferred from the females to the eggshell (Leonzio and Massi 1989; Espín et al. 2016), because of the possibility to eliminate the metal through an efficient process of excretion. However, the few studies evaluating lead concentration in osprey eggs (Table 3) found greater values compared to those of the present study (0.08 ± 0.07 μg/g dw) that were close to detection limits. We do not exclude that other factors such as annual variations in the environmental conditions at local scale and/or differences at the inter-individual level would have had an effect on the exposure to this element for the species (e.g., Katzner et al. 2017).

Differences by egg component

All elements, except Pb and Cd, showed higher concentrations in the egg content (Hg) and in the inner membrane (Cu, Fe, Zn, and Se) than in the eggshell. Lead concentrations were 2-fold higher in eggshell than in the egg content, since it can act as a calcium analogue (Dauwe et al. 1999). In aquatic food webs, mercury (in its predominant chemical form, the methylmercury) can biomagnify and reach elevated concentrations in avian embryos, being transferred from the mother to her eggs (Stebbins et al. 2009; Ackerman et al. 2016). However, very few studies have estimated the contribution of different parts of the egg in mercury absorption (e.g., Brasso et al. 2012; Peterson et al. 2017), as well as that of other trace elements. By separating the content and inner membrane from the eggshells, we were able to differentiate the rate of contaminant concentration in different parts of the egg. In some cases, the mercury concentration measured in the eggshell was near the detection limits, being much lower than in other egg components. This is in agreement with the study by Peterson et al. (2017), conducted on 23 different avian species. Most of the intake of mercury deriving from the trophic web is found in the methylated form that shows particular affinity to the biological membranes and in general to the -SH groups of the proteins. This explains why it is more easily found in the protein material of the egg content and in the membrane than in the eggshell. However, several previous studies did not indicate if the inner membrane was separated or not from eggshells, causing possible misinterpretation for the comparison of data on the concentration of trace elements. This rings a bell in the choice of the tissue type, which is relevant when assessing contaminant levels in avian eggs: studies should harmonize the sampling protocol in order to properly evaluate the source and the order of magnitude of contamination (as suggested by Espín et al. 2016). This has significant implications in planning and performing biomonitoring study and management choices/corrections.

Conclusion

Being the Mediterranean basin one of the most contaminated regions of the globe, it is likely that ospreys breeding along Mediterranean shores might be the most polluted in Europe (Jiménez et al. 2007). Osprey populations have declined drastically in Mediterranean countries during recent decades, mainly because of the strong direct persecution (e.g., shooting, egg collection) and habitat destruction. However, contamination load and pollution could have contributed in playing a role in shaping the growth rate of certain populations. Despite its relevance, this factor has been poorly investigated to date in this region. This call for more attention and a better monitoring of the environmental factors potentially affecting these populations. This study constitutes a first survey at regional scale evaluating the heavy metals and selenium detected in osprey eggs; it provides baseline data for their concentration and revealed that different parts of the egg can serve as a biomonitor of local metal contamination (e.g., Hashmi et al. 2013). In the future, a greater sample size might allow to better investigating how environmental variations could affect the osprey population in the Mediterranean basin and the use of carbon and nitrogen stable isotopes' technique could give insights in exploring trophodynamics details. These findings might also provide possible interpretation of past and recent population trends. Given that pollution is likely to increase with urbanization and human activities in the next future, the current challenge is to find a solution to ensure the survival (and lay the groundwork for an increase) of the Mediterranean osprey populations in the long-term. "Unpolluted" protected areas can play an important role in this context, providing safe breeding places and foraging areas. Especially in coastal regions, the implementation of rigorous sustainable management practices within protected areas will surely contribute to the conservation of ospreys as well as a long list of wildlife species.

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References

Ackerman JT, Herzog MP, Schwarzbach SE (2013) Methylmercury is the predominant form of mercury in bird eggs: a synthesis. Environ Sci Technol 47:2052–2060

Ackerman JT, Eagles-Smith CA, Herzog MP, Hartman CA (2016) Maternal transfer of contaminants in birds: mercury and selenium concentrations in parents and their eggs. Environ Pollut 210:145–154

Anderson DW, Hickey JJ (1972) Eggshell changes in certain North American birds. Proc Internat Ornithol Congr 15:514–540

ARCADIS (2012) Trace element concentrations in great blue heron and osprey: 2009-2010. Tennessee Valley Authority, Ash Recovery Project, Kingston, Tennessee

Audet DJ, Scott DS, Wiemeyer SN (1992) Organochlorine and mercury in osprey eggs from the eastern United States. J Raptor Res 26:219–224

Bechard MJ, Perkins DN, Kaltenecker GS, Alsup S (2009) Mercury contamination in Idaho bald eagles, (*Haliaeetus leucocephalus*). Bull Environ Contam Toxicol 83:698–702

Bianchi N, Baccetti N, Leonzio C, Giovacchini P, Ancora S (2018) Temporal and geographical variations of mercury and selenium in eggs of *Larus michahellis* and *Larus audouinii* from central Mediterranean islands. Chem Ecol 34(7):595–609. https://doi.org/10.1080/02757540.2018.1482885

Brasso RL, Abel S, Polito MJ (2012) Pattern of mercury allocation into egg components is independent of dietary exposure in Gentoo penguins. Arch Environ Contam Toxicol 62:494–501

Buck J, Kaiser JL (2011). Contaminant concentrations in osprey (*Pandion haliaetus*) eggs from Portland harbor and surrounding areas: data summary report. Portland Natural Resources Trustee Council. Accessed online at: http://www.fws.gov/filedownloads/ftp_OFWO/PortlandHarborNRDAWebSupport/Documents/Osprey_FinalDataSummaryReport11.pd. on October 29, 2011

Burger J (2006) Bioindicators: a review of their use in the environmental literature 1970–2005. Environ Bioindic 1:136–144

Burger J, Gochfeld M, Jeitner C, Donio M, Pittfield T (2012) Interspecific and intraspecific variation in selenium: mercury molar ratios in saltwater fish from the Aleutians: potential protection on mercury toxicity by selenium. Sci Total Environ 431:46–56

Chen C, Amirbahman A, Fisher N, Harding G, Lamborg C, Nacci D, Taylor D (2008) Methylmercury in marine ecosystems: spatial patterns and processes of production, bioaccumulation, and biomagnification. EcoHealth 5(4):399–408. https://doi.org/10.1007/s10393-008-0201-1

CIBIO (Centro de Investigação em Biodiversidade e Recursos Genéticos) (2011) Reintroduction of the osprey (*Pandion haliaetus*) in Portugal. Annu Rep

Clark KE, Stansley W, Niles LJ (2001) Changes in contaminant levels in New Jersey osprey eggs and prey, 1989 to 1998. Arch Environ Contam Toxicol 40:277–284

Danovaro R (2003) Pollution threats in the Mediterranean Sea: an overview. Chem Ecol 19:15–32

Dauwe T, Bervoets L, Blust R et al (1999) Are eggshells and egg contents of great and blue tits suitable as indicators of heavy metal pollution? Belg J Zool 129:439–447

Des Granges JL, Rodrigue J, Tardif B, Laperle M (1998) Mercury accumulation and biomagnification in osprey (*Pandion haliaetus*) in the James Bay and Hudson Bay regions of Quebec. Arch Environ Contam Toxicol 35:330–341

Dmowski K (1999) Birds as bioindicators of heavy metal pollution: review and examples concerning European species. Acta Orn 34:1–23

Duran R, Ranchou-Peyruse M, Menuet V, Monperrus M, Bareille G, Gon MS, Salvado JC, Amouroux D, Guyoneaud R, Donard OFX, Caumette P (2008) Mercury methylation by a microbial community from sediments of the Adour Estuary (Bay of Biscay, France). Environ Pollut 156:951–958

Durant JM, Massemin S, Thouzeau C, Handrich Y (2000) Body reserves and nutritional needs during laying preparation in barn owls. J Comp Physiol B Biochem Syst Environ Physiol 170:253–260

EEA (2019) Contaminants in Europe's seas—moving towards a clean, non-toxic marine environment. Eur Environ Agency 2019, ISBN 978-92-9480-058-9. https://doi.org/10.2800/511375

Eisler R (2010) Compendium of trace metals and marine biota. Elsevier, Oxford

Elliott JE, Machmer MM, Henny CJ, Wilson LK, Norstrom RJ (1998) Contaminants in ospreys from the Pacific Northwest: I. Trends and patterns in polychlorinated dibenzo-pdioxins and dibenzofurans in eggs and plasma. Arch Environ Contam Toxicol 35:620–631

Elliott JE, Machmer MM, Wilson LK, Henny CJ (2000) Contaminants in ospreys from the Pacific Northwest: II. Organochlorine pesticides, polychlorinated biphenyls, and mercury, 1991–1997. Arch Environ Contam Toxicol 38:93–106

Elliott JE, Morrissey CA, Henny CJ, Inzunza ER, Shaw P (2007) Satellite telemetry and prey sampling reveal contaminant sources to Pacific Northwest ospreys. Ecol Appl 17:1223–1233

Espín S, García-Fernández AJ, Herzke D, Shore RF, van Hattum B, Martínez-López E, Coeurdassier M, Eulaers I, Fritsch C, Gómez-Ramírez P, Jaspers VL, Krone O, Duke G, Helander B, Mateo R, Movalli P, Sonne C, van den Brink N (2016) Tracking pan-continental trends in environmental contamination using sentinel raptors—what types of samples should we use? Ecotoxicology 25: 777–801

Ewins PJ (1997) Osprey (*Pandion haliaetus*) populations in forested areas of North America: changes, their causes and management recommendations. J Raptor Res 31:138–150

Fernie KJ, Letcher RJ (2010) Historical contaminants, flame retardants, and halogenated phenolic compounds in peregrine falcon (*Falco peregrinus*) nestling in the Canadian great lakes basin. Environ Sci Technol 44:3520–3526

Francour P, Thibault JC (1996) The diet of breeding osprey *Pandion haliaetus* on Corsica: exploitation of a coastal marine environment. Bird Study 43:129–133

Furness RW (1993) Birds as monitors of pollutants. In Birds as monitors of environmental change (eds Furness, R.W. & Greenwood, J.J.D.). London, Chapman & Hall, pp. 86–143

Garcıa-Fernandez AJ, Calvo JF, Martınez-Lopez E, Marıa-Mojica P, Martınez JE (2008) Raptor ecotoxicology in Spain: a review on persistent environmental contaminants. Ambio 37:432–439

Gómez-Ramírez P, Shore RF, van den Brink NW, van Hattum B, Bustnes JO, Duke G, Fritsch C, García-Fernández AJ, Helander BO, Jaspers V, Krone O, MartínezLópez E, Mateo R, Movalli P, Sonne C (2014) An overview of existing raptor contaminant monitoring activities in Europe. Environ Int 67:12–21

Goyer RA (1996) Toxic effects of metals. In Casarett and Doull's toxicology: the basic science of poisons, fifth edition (C. D. Klaassen, M. O. Amdur, and J. Doull, Editors). McGrawHill, New York, pp. 691–736

Grove RA, Henny CJ, Kaiser JL (2009) Osprey: worldwide sentinel species for assessing and monitoring environmental contamination in rivers, lakes, reservoirs and estuaries. J Toxicol Environ Health B 12:25–44

Guigueno M, Elliott K, Levac J, Wayland M, Elliott J (2012) Differential exposure of alpine ospreys to mercury: melting glaciers, hydrology or deposition patterns? Environ Int 40:24–56

Gworek B, Bemowska-Kalabun O, Kijenska M, Wrzosek-Jakubowska J (2016) Mercury in marine and oceanic waters: a review. Water Air Soil Pollut 227:371

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

Hashmi MZ, Malik RN, Shahbaz M (2013) Heavy metals in eggshells of cattle egret (*Bubulcus ibis*) and little egret (*Egretta garzetta*) from the Punjab province, Pakistan. Ecotoxicol Environ Saf 89:158–165

Heimbürger LE et al (2010) Methyl mercury distributions in relation to presence of nano- and picophytoplankton in an oceanic water column (Ligurian Sea, North-western Mediterranean). Geochim Cosmochim Acta 74:5549–5559

Heinz GH, Hoffman DJ, Gold LG (1989) Impaired reproduction of mallards fed an organic form of selenium. J Wildl Manag 53:418–428

Heinz GH, Hoffman DJ, Klimstra JD, Stebbins KR, Kondrad SL, Erwin CA (2009) Species differences in the sensitivity of avian embryos to methylmercury. Arch Environ Contam Toxicol 56:129–138

Henny CJ, Elliott JE (2007) Toxicology. In Raptor research and management techniques, eds. D.M. Bird and K.L. Bildstein pp. 329–350. Raptor Research Foundation.Surrey, British Columbia, Canada: Hancock House

Henny CJ, Martell MS (2017) Satellite-tagged osprey nearly sets longevity record and productivity response to initial captures. J Raptor Res 51(2):180–183

Henny CJ, Blus LJ, Hoffman DJ, Grove RA, Hatfield JS (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 CJ, Galushin VM, Kuznetsov AV (1998) Organochlorine pesticides, PCBs, and mercury in osprey *Pandion haliaetus* eggs from the Upper Volga River, Russia. In: Chancellor RD, Meyberg B-U, Ferrero JJ (eds) Holarctic birds of prey. Proceeding of an International Conference, Badajoz, pp 525–534

Henny CJ, Grove RA, Kaiser JL, Bentley VR (2004) An evaluation of osprey eggs to determine spatial residue patterns and effect along the Lower Columbia River, U.S.A. In: Chancellor RD, Meyberg B-U (eds) Raptors worldwide. World working group on birds of prey and owls, Budapest, pp 369–388

Henny CJ, Grove RA, Kaiser JL (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 CJ, Grove RA, Kaiser JL, Johnson BL (2010) North American osprey populations and contaminants: historic and contemporary perspectives. J Toxicol Environ Health B 13(7–8):579–603

Hughes KD, Ewins PJ, Clark KE (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

Jackson A, Evers DC, Eagles-Smith CA, Ackerman JT, Willacker JJ, Elliott JE, Lepak JM, Vander Pol SS, Bryan CE (2016) Mercury risk to avian piscivores across western United States and Canada. Sci Total Environ 568:685–696

Jiménez B, Merino R, Abad E, Rivera J, Olie K (2007) Organochlorine compounds (PCDDs, PCDFs, PCBs and DDTs) in two raptor species inhabiting a Mediterranean Island in Spain. Environ Sci Pollut Res 14:61–68

Johnson B, Henny C, Kaiser J, Davis J, Schulz E (2009) Assessment of contaminant exposure and effects on ospreys nesting along the Lower Duwamish River, Washington, 2006–07. U. S Geological Survey Open-File Report 2009–1255, pp. 88

Katzner TE, Stuber MJ, Slabe VA, Anderson JT, Cooper JL, Rhea LL, Millsap BA (2017) Origins of lead in populations of raptors. Anim Conserv. https://doi.org/10.1111/acv.12379

Kitowski I, Jakubas D, Wiącek D, Sujak A (2017a) Concentrations of lead and other elements in the liver of the white-tailed eagle (*Haliaeetus albicilla*), a European flagship species, wintering in

Eastern Poland. Ambio. 46:825–841. https://doi.org/10.1007/s13280-017-0929-3

Kitowski I, Jakubas D, Wiącek D, Sujak A, Pitucha G (2017b) Trace element concentrations in livers of common buzzards *Buteo buteo* from eastern Poland. Environ Monit Assess 189:421. https://doi.org/10.1007/s10661-017-6135-8

Lazarus RS, Rattner BA, McGowan PC, Hale RC, Schultz SL, Karouna-Renier NK, Ottinger MA (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 (2009) Etude toxicologique du basin de la Loire à l'aide de bioindicateurs, dans le contexte des effets prévisibles du réchauffement climatique. Plate-Forme Recherche/Données/Informations, Plan Loire Grandeur Nature 2007-2013, www.plan-loire.fr

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 European otter (*Lutra lutra*) and osprey (*Pandion haliaetus*) in Loire River catchment, France. *In*: Stoytcheva, M. (ed.), Pesticides in the modern world: risks and benefits. InTech, ISBN 978-953-307-458-0

Leonzio C, Massi A (1989) Metal biomonitoring in birds eggs: a critical experiment. Bull Environ Contain Toxicol 43:402–406

Leonzio C, Fossi C, Focardi S (1986) Heavy metals and selenium variations in a migratory bird wintering in a mercury polluted area. Bull Environ Contam Toxicol 37:219–225

Maurer G, Portugal SJ, Cassey P (2012) A comparison of indices and measured values of eggshell thickness of different shell regions using museum eggs of 230 European bird species. Ibis 154:714–724

Monti F, Dominici JM, Choquet R, Duriez O, Sammuri G, Sforzi A (2014) The osprey reintroduction in Central Italy: dispersal, survival and first breeding data. Bird Study 61:465–473

Monti F, Duriez O, Dominici JM, Sforzi A, Robert A, Fusani L, Grémillet D (2018a) The price of success: integrative long-term study reveals ecotourism impacts on a flagship species at a UNESCO site. Anim Conserv. https://doi.org/10.1111/acv.12407

Monti F, Grémillet D, Sforzi A, Sammuri G, Dominici JM, Triay R, Munoz A, Fusani L, Duriez O (2018b) Migration and wintering strategies in vulnerable Mediterranean osprey populations. Ibis. https://doi.org/10.1111/ibi.12567

Monti F, Grémillet D, Sforzi A, Dominici JM, Triay Bagur R, Muñoz Navarro A, Fusani L, Klaassen RHG, Alerstam T, Duriez O (2018c) Migration distance affects stopover use but not travel speed: contrasting patterns between long- and short-distance migrating ospreys. J Avian Biol. https://doi.org/10.1111/jav.01839

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

Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GAB, Kent J (2000) Biodiversity hotspots for conservation priorities. Nature 403:853–858

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 AG (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

Pereira MG, Lawlor A, Bertolero A, Díez S, Shore RF, Lacorte S (2019) Temporal and spatial distribution of mercury in gulls eggs from the Iberian Peninsula. Arch Environ Contam Toxicol 76:394–404. https://doi.org/10.1007/s00244-018-0584-0

Peterson SH, Ackerman JT, Eagles-Smith CA, Hartman CA, Herzog MP (2017) A critical evaluation of the utility of eggshells for estimating mercury concentrations in avian eggs. Environ Toxicol Chem. https://doi.org/10.1002/etc.3777

Poole AF (1989) Ospreys: a natural and unnatural history. Cambridge University Press, Cambridge, p 246

Rattner BA, McGowan PC, Golden NH, Hatfield JS, Toschik PC, Lukei RF et al (2004) Contaminant exposure and reproductive success of ospreys (*Pandion haliaetus*) nesting in Chesapeake Bay regions of concern. Arch Environ Contam Toxicol 47:126–140

Rattner BA, Golden NH, Toschik PC, McGowan PC, Custer TW (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

Rice WR (1989) Analyzing tables of statistical tests. Evolution 43:223–225

Scheuhammer AM, Basu N, Burgess NM, Elliott JE, Campbell GD, Wayland M, Champoux L, Rodrigue J (2008) Relationships among mercury, selenium, and neurochemical parameters in common loons (*Gavia immer*) and bald eagles (*Haliaeetus leucocephalus*). Ecotoxicology 17(2):93–101

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

Siverio M, Siverio F, Rodríguez B, Del Moral JC (Eds.) (2018) El águila pescadora en España y Portugal: población invernante 2016–2017, reproductora en 2018 y método de censo. SEO/BirdLife. Madrid

Stebbins KR, Klimstra JD, Eagles-Smith CA, Ackerman JT, Heinz GHA (2009) Non-lethal micro-sampling technique to monitor the effects of mercury on wild bird eggs. Environ Toxicol Chem 28:465–470

Steidl RJ, Griffin CR, Niles LJ (1991) Contaminant levels of osprey eggs and prey reflect regional differences in reproductive success. J Wildl Manag 55:601–608

Stickel LF, Wiemeyer SN, Blus LJ (1973) Pesticide residues in eggs of wild birds: adjustment for loss of moisture and lipid. Bull Environ Contam Toxicol 9:193–196

Thompson DR (1996) Mercury in birds and terrestrial mammals. In: Beyer W, Heinz G, Redmon-Norwood A (eds) Environmental contaminants in wildlife: interpreting tissue concentrations. Lewis Publishers, CRC Press, Boca Raton, pp 341–356

Toschik PC, Rattner BA, McGowan PC, Christman MC, Carter DB, Hale RC, Matson CW, Ottinger MA (2005) Effects of contaminant exposure on reproductive success of ospreys (*Pandion haliaetus*) nesting in Delaware River and Bay, USA. Environ Toxicol Chem 24:617–628

Waltham NJ, Teasdale PR, Connolly RM (2013) Use of flathead mullet (*Mugil cephalus*) in coastal biomonitor studies: review and recommendations for future studies. Mar Pollut Bull 69(1e2):195e205

Wiemeyer SN, Spitzer PR, Krantz WC, Lamont TG, Cromartie E (1975) Effects of environmental pollutants on Connecticut and Maryland ospreys. J Wildl Manag 39:124–139

Wiemeyer SN, Bunck CM, Krynitsky AM (1988) Organochlorine pesticides, polychlorihated biphenyls, and mercury in osprey eggs (1970-79) and their relationships to shell thinning and productivity. Arch Environ Contain Toxicol 17:767–787