ACCUMULATION OF TRACE ELEMENTS IN FEATHERS OF THE KENTISH PLOVER *CHARADRIUS ALEXANDRINUS*

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Abstract

A non-invasive study of trace element accumulation in tail feathers of the Kentish plover (*Charadrius alexandrinus*) was performed along the coastline of the northern littoral strip of the Venice Lagoon, with the aim to verify whether contamination may be a factor affecting conservation status of Kentish plover populations.

Body burdens in feathers of 11 trace elements including toxic metals/metalloids and essential elements (As, Cd, Co, Cr, Cu, Hg, Ni, Pb, Se, V, Zn) were quantified by ICP-MS, then concentrations were normalized to feather's age calculated using ptilochronology in order to obtain daily deposition rates.

Mercury emerged as a major threat to the conservation of the species, since average feather concentration was clearly above the adverse-effect threshold associated with impairment in the reproductive success in a number of bird species. Also Cd and Se occurred at levels that may impact on the conservation status of the studied species at local scale, even if to a lesser extent than Hg.

Gender-related differences in trace element accumulation emerged only for As, although for this element the risks associated to environmental exposure seem to be negligible.

Key words – bioaccumulation, mercury, selenium, cadmium, water birds conservation, ptilochronology,

1 Introduction

The Kentish plover *Charadrius alexandrinus* (hereafter KP) is a small wader of Family Charadriidae, breeding in wetlands and coastal areas of Europe, North-Africa, Middle-East and Central-Asia (Delany et al., 2009). Population size of KP is declining all over its distribution range, and the cause of the decline has been recognized in habitat loss and fragmentation, increased human uses of the sandy coastal areas for commercial and recreational purposes, and increased predation by birds and mammals taking advantage of human activities (Delany et al., 2009; Domínguez and Vidal, 2003; Scarton, 2017). For this reasons, the species was listed in the Annex I of the Directive 2009/147/EC and was included in the IUCN Italian Red List as an endangered (EN) species (Rondinini et al., 2013).

Biology, breeding ecology and behaviour of KP have been widely studied in all its distribution range (Argüelles-Ticó et al., 2016; Fraga and Amat, 1996; Kosztolányi et al., 2009; Lessells, 1984). Nevertheless, no researches were performed up to date to assess the impact of environmental contamination on its conservation status, and few data are available concerning bioaccumulation (Kim and Koo, 2008; Zheng et al., 2018).

In coastal areas and wetlands KP feeds mostly on polychaetes (nereidae), small crustaceans (isopods, ostracods and amphipods), small bivalves, coleopterans and dipterans (Castro et al., 2009; Cramp and Simmons, 1983; Perez-Hurtado et al., 1997). Although KP is not a top predator in coastal food webs, its dietary habits make it susceptible to bioaccumulation and, possibly, biomagnification of toxicants. In estuarine and coastal areas, trace elements are of concern due to their ubiquitous distribution and the potential detrimental effects they can exert on birds physiology (Furness, 1996; Ohlendorf and Heinz, 2011). Toxic effects from trace elements includes changes in behaviour, impairments in development and reproduction. Mercury (Hg) has been proven to cause embryo malformations, lowered hatchability, decreased chick growth, reduced survival of young, changes in reproductive behavior and endocrine disruption in waterbirds (Burger and Gochfeld, 1997; Jayasena et al., 2011). Lead (Pb) may disrupt the heme biosynthetic pathway and cause also death (Blus et al., 1995 and citation herein). Cadmium (Cd) may induce growth retardation, anaemia, suppression of egg production, kidney damage and marrow hyperplasia (Furness, 1996; Spahn and Sherry, 1999). Selenium (Se) is highly toxic for the early developmental and embryonic stages and causes reproductive impairments, reduction of hatching success and teratogenicity (Ohlendorf and Heinz, 2011; Spallholz and Hoffman, 2002). For many other elements little is known about possible detrimental effects in birds.

Trace elements concentration in birds may be measured in various organs (liver, kidneys, brain), tissues (muscle, bones, fat), excrements, feathers and eggs (Dauwe et al., 2000; Markowski et al., 2013; Zheng et al., 2018).

Feather analysis is a non-destructive and ethically preferable techniques as compared with organ and tissue analysis, since feathers may be easily collected and, if necessary, repeatedly sampled to study the accumulation of trace elements without affecting welfare or survival of the individuals (Adout et al., 2007). A feather is connected with the blood circulation only during its growth period; in this phase elements taken up through the food are incorporated into the sulphydryl rich keratinous matrix of the developing feather, where they are stored. When the feather has completely grown, the vascular connection shrivels up and trace elements cannot be further allocated into the keratin (Burger, 1993). Thus, their accumulation in feathers may be associated with a definite time-span, corresponding to the time needed to fully develop the feather, variable from species to species (García-Fernández et al., 2013; Grubb, 2006). This time-span can be determined using ptilochronology, the study of the alternating pale and dark bands of feathers which is indicative of the daily growth (Grubb, 2006), so that the deposition rates of the contaminants can be calculated (García-Fernández et al., 2013), and the dilution artefact due to feather growth rates and mass reported by Bortolotti (2010) can be minimized. The author, indeed, postulated that for elements and compounds functionally incorporated in the feather (as sulphur and other elements), the deposition should follow a *mass-dependent* model, whilst the deposition of non-structural elements, as contaminants and hormones, occurs incidentally and should be *time-dependent*, so that growth rate of the feather becomes a critical parameter (Bortolotti, 2010). Moreover, in the case of resident populations and non-migratory species, the determination of trace elements in feathers represents a reliable method for monitoring the uptake of contaminants though the local food web (Rothschild and Duffy, 2005).

To verify whether KPs breeding in the area of the Venice Lagoon are exposed to levels of trace elements that may, directly or indirectly, contribute to the local decline of the species - estimated by Scarton (2017) as loss of 30% of the breeding pairs in the last three decades - an accumulation study using feathers collected from specimens breeding along the littoral strips of the lagoon was performed.

The focus was mainly on those elements known to cause detrimental effects on birds, for which threshold concentrations for adverse effects are available in the literature (such as Hg, Cd, Pb and Se); the concentrations of metals and metalloids possibly involved in toxic effects, but for which no threshold data are available (including As, Co, Cr, Cu, Ni, V, Zn) were measured as well.

The extent to which KP deposited contaminants in the feathers was also assessed by comparing trace element concentrations in KP feathers with literature data concerning other waterbirds in different geographical areas.

2 Materials and methods

2.1 Study area

Feathers have been collected in the Cavallino-Treporti peninsula, the northernmost littoral strip separating the Lagoon of Venice from Adriatic Sea (Figure 1). Along the 13 km of the coasts of the peninsula, develops a fragmented system of natural and restored dunes covering a surface of about 350,000 m^2 (Cecconi & Nascimbeni 1997). Here the sandy substrate is characterized by the typical herbaceous vegetation of drift lines (habitat "1210 - Annual vegetation of drift lines" according to the Directive 92/43/EEC), embryo and shifting dunes (habitat "2110 - Embryonic shifting dunes" and "2120 - Shifting dunes along the shoreline with *Ammophila arenaria* (white dunes)"). The semifixed dune system is occupied by the habitat "2230 - Malcolmietalia dune grasslands" and by the occurrence of the endemic, priority habitat "2130* - Fixed coastal dunes with herbaceous vegetation (grey dunes)" (Del Vecchio et al., 2015).

In this area a small population of KP, estimated in 13-16 pairs in the period 2016-2018, nests regularly on breeding grounds overlapping drift lines, embryonic and shifting dunes, and partially also semifixed dunes. KPs feed all over the year on invertebrate assemblages colonizing shallows of the northern basin of the Lagoon (mostly infaunal polychaetes and crustaceans), but also on invertebrate communities of shores (infaunal polychaetes, crustaceans, and occasionally insects), and on drift materials deposited by the sea.

Trace elements concentrations in shallow sediments within the Lagoon are well known for As, Cd, Cu, Hg, Pb and V, whilst data are deficient (or lacking) for many others, such as Se. Shallows and mudflats of the northern basin are generally characterized by low concentrations of metals as compared with the industrial district and urban areas (Picone et al., 2018), with only As and Hg occurring at concentrations above effect range low (ERL) and effect range median (ERM) (Long et al., 2006). Nevertheless, recent studies evidenced that trace elements may biomagnify (Dominik et al., 2014) and exert toxic effects toward aquatic invertebrates (Picone et al., 2016, 2018) also in shallows far from the urban centres and industrial districts, and used by birds for feeding. In contrast, very few data are available for sediments of littoral strips, and in most cases the information refers to old studies (Donazzolo et al., 1981).

2.2 Sampling

Fully grown feathers of KP were collected during the breeding season, from March to September 2018. Adult birds were trapped at the nest during incubation of clutches, females in the morning and males at dusk. Trapped bird were sexed, weighted at the nearest gram and measured for the standard determination of head, bill, wing and tarsus length. A metal ring and a yellow colour ring with a black, 3-digit alphanumeric code were also applied on the left and right tarsus, respectively, to univocally identify birds and avoid duplicate sampling during the same season. A total of 13 individuals were captured (8 females and 5 males) representing the 48% of the total population nesting in the area during the 2018 breeding season. This sample size is also very similar to that used by Lucia et al. (2012, 2014) for studying accumulation patterns in other Charadriidae species.

The right outermost feather of the tail was removed from each bird. Tail feathers were chosen since growth bars are more evident in the outermost rectrices (Grubb, 1989). Once collected, feathers were individually placed in paper envelopes labelled with date, ring codes and sampling location, and stored at room temperature until growth rate determination and chemical analyses (Jaspers et al., 2006).

Removal of the feather caused no visible trauma to birds and did not affect their flight ability.

2.3 Ptilochronology

Growth bands were identified and measured following the procedure reported in Grubb (1989, 2006). Each feather was taped by its calamus to a millimetre graph paper sheet. An insect-mounting pin was perpendicularly pushed at the proximal and distal ends of each feather to mark on the millimetre graph paper its total length. Then, the pin was also pushed through the feather at the distal edge of each dark bar, to mark on the paper the distance between the consecutive growth bars. According to Grubb (1989), the point at 2/3 of the feather's length from the proximal end was also marked. The width of growth bars was determined for a minimum of 5 to a maximum of 10 growth bars centred at the 2/3 point, using a software for digital images analysis (Motic Images Plus 2.0). The total length of the feather was measured to the nearest 0.01 mm, using a digital caliper. The average width of the growth bars was calculated for each feather; the time needed to fully develop each feather was then calculated by dividing total length of the feather by average width of growth bars.

2.4 Chemical analyses

KP feathers needed to be completely mineralized and then analysed by ICP-MS (Agilent 7500Ce), in order to evaluate body burdens of the trace elements of interest. Microwave digestion (Ethos 1, Milestone) is widely acknowledged as the most robust sample preparatory technique for ICP-MS.

Every feather was weighed as a whole and then decontaminated before microwave digestion; all the instruments, watch glasses and Petri capsules were previously decontaminated, rinsed with ultrapure water (Elga) and dried prior to be used. In order to remove any impurity, every specimen was thoroughly washed, alternating ultrapure water (Elga) and a solution of acetone 58.08 g l-1 (1M) (Acetone 99.9 SpS Romil); to remove any residual trace of acetone, every feather was lastly rinsed with ultrapure water and put inside the mineralization vessels.

For the mineralization of feather, the vessel-inside-vessel technology^{TM} was employed (Milestone, Ethos 1); the secondary quartz vessels contained the feather and digestion reagents (Nitric Acid HNO3 69% Plasma Pure Plus, SCP Science, and Hydrogen Peroxide H_2O_2 30-32% Ultrapure Romil, ratio 4:1), while the primary TFM^{TM} vessel contained the solution required to accomplish accurate temperature monitoring (Ultrapure water, Elga, and H2O2 Ultrapure Romil, ratio 4,5:1). Reagent blanks were similarly prepared, excluding the sample, and were placed in the vessels in a random position, to test any matrix effects. Microwave digestions were performed at controlled temperature with a high pressure rotor. Once the mineralization was done and cooled, every digested sample was recovered, weighed, suitably diluted (with ultrapure water, in triplicate) and stored frozen at -20°C till the analysis in ICP-MS.

Primary and secondary vessels were cleaned after every digestion; after being thoroughly rinsed with ultrapure water, quartz vessels were filled with ultrapure HNO_3 and primary vessels were filled with the above described mix. An appropriate digestion procedure was then performed. Then all vessels were thoroughly rinsed with ultrapure water and let to dry, prior to the next digestion. All the steps were performed under a cleaned fume hood, in order to minimize any contamination.

A Certified Reference Material, ERM-CE278k (Joint Research Centre Institute for Reference Materials and Measurements, Geel, Belgium), was analyzed to check for the accuracy of the procedure. Results obtained showed good recoveries for all trace elements analysed in feathers (> 85%), thus the procedure was considered as accurate.

Measurement error, expressed as the percentage of the relative standard deviation (σ*, RSD) was <10% for all elements of interest; measurement uncertainty, expressed as the percentage of the standard error (SEM) was < 5% for all elements studied and the confidence interval was ≥ 95%. According to the results obtained, the method resulted repeatable and reliable.

Daily deposition rate (DDR) was calculated according to the following equation:

$$DDR = \frac{trace\ element\ concentration\ in\ feather\ (ng\ g^{-1}) \cdot feather\ mass\ (g)}{feather's\ age\ (d)}$$

2.5 Data analysis

One-way analysis of variance (ANOVA) was used to compare the average width of growth bars among individuals of KP; prior to ANOVA, normality and variance homoscedasticity of raw data were verified using Kolmogorov-Smirnov and Bartlett's test, respectively. Non-parametric Mann-Whitney U-test (α = 0.05) for independent samples comparison was used to compare the average concentration of trace elements in KP's feathers between sexes. Spearman's non-parametric correlation was used to assess possible correlations among trace elements in feathers of KP, and between trace elements and weight. *Post-hoc* power analysis was used to assess power variability as a function of sample size (n = 13), difference between males and females trace element concentrations and standard deviation.

3 Results

3.1 Ptilochronology

The average length of the sixth rectrix of KP was 51.6 ± 1.6 mm (n = 13). Nevertheless, growth bars were clearly discernible only in six rectrices (4 retrieved from males, 2 from females); since no significant differences have been observed among individuals (one-way ANOVA: $F_{5,32} = 0.729$, p = 0.232), the pooled average width of 2.38 ± 0.30 mm was used as daily growth rate for estimating feather age. According to these data, the average feather age for rectrices of KP has been estimated to be 21.7 d (min. = 20.6 d; max. = 22.9 d).

3.2 Trace elements in feathers of Kentish plover

All analysed trace elements occurred at detectable concentrations in tail feathers of KP (Table 1). As expected, trace elements deposited at higher concentrations were essential metals (Zn and Cu), but relevant concentrations of potentially toxic elements as Hg and Se were observed too. Feather's content and deposition rates of Co were negligible. Gradient for deposition in feathers was as follows: Zn > Cu > Hg > Se > Cr > Ni > Pb > As = Cd = V > Co.

3.3 Gender differences in Kentish plover

Significant difference between males and females of KP were detected only for As (Mann-Whitney U-test: Z = -2.103, p = 0.041), with females characterized by As feather concentration about 3 times higher than males. No significant differences were observed for the other trace elements (Table 1). Plots showing the variability of the power as a function of the difference in concentration between sexes and standard deviation are reported in Supplementary Material, and allowed for an estimation of the power achieved with available data.

3.4 Correlation among trace elements in Kentish plover

Several positive intercorrelations among trace elements occurred in KP feathers (Table 2). In particular, Co, Se, Pb V and Zn were strongly and positively correlated. Similarly, Cd was correlated with Co, Se and V, but not with Pb and Zn. A significant correlation was also observed between Cr and Cu, whilst for As, Hg, and Ni no significant correlation were detected.

No correlations between bird weight and trace element concentrations were observed.

4 Discussion

Accumulation of trace elements in feathers is a topic that was treated in several studies, but the possible contribution of environmental contamination to the conservation status of the threatened species *Charadrius alexandrinus* was never assessed before. The use of surrogate species to estimate exposure in a study area, although it is a feasible and often exploited approach, should be avoided, whenever possible. In facts, as stated by Miller et al. (2019) "surrogate species of varying body mass and life history traits may not consistently reflect levels of exposure across *taxa*, or even among congeners or sex-age classes in the same areas". Biomonitoring of contaminants should focus on specific *taxa* of concern (Miller et al., 2019), especially when threatened and/or declining species (as KP) are target of the study.

Although sample size may appear low, it should be noted that 13 specimens represent the 48% of the nesting population of KP in the study area in 2018, and 43% of the nesting specimens censed in 2017. Studies involving Charadriidae are generally characterized by a limited sample size, also when identification of gender-related differences is of concern (Lucia et al., 2014, 2012; see Supplementary Material), probably due to the less gregarious behaviour of waders as compared with Laridae and Anatidae. For these reasons, we believe that sample size is adequate to assess the possible exposure of the local population of KP to trace elements. However, we are aware that the power of the statistical analysis achieved with 13 individuals may be low to effectively explore possible gender-related differences for some trace element, due to both sample size and variability among individuals of the same sex.

Trace elements in feathers may derive both from local exposure to contaminants through food ingestion and mobilization from internal tissues at the time of feather formation (Burger and Gochfeld, 1991, 1996); for this reason it is fundamental to know when a given species moults and, if a migratory species is of concern, where it is when it moults (Burger and Gochfeld, 1996).

In KP, the molt of tail feathers occurs in the post-nuptial period (Ginn and Melville, 1983). In most of European migrant populations the molt generally occurs partly in breeding areas and partly in staging/stopover area, after a first post-breeding migration (Newton, 2009). However, in areas where KP is resident or short-distance migrant, the molt may occur in breeding grounds. Monitoring and ring recoveries collected over a period of 4 years on birds ringed in the Cavallino-Treporti peninsula confirmed that KP individuals spent also their post-nuptial and overwintering period in the breeding area. Thus, trace elements accumulation in feathers may be ascribed to local exposure to contaminants, although the lack of GPS tracking studies cannot exclude the occurrence of exceptions to this main trend.

4.1 Gender differences in Kentish Plover

The distinct plumage of males and females during breeding allows for the investigation of sex-related differences in accumulation of trace elements. The present study represents the first experience with KP on this topic.

Statistical analysis highlighted that significant differences between males and females occurred only for As. Nevertheless, the power of the analysis may be too low to allow for an accurate assessment of such differences for most of the analyzed trace elements. In any case, literature data concerning

Charadriidae suggested that gender-related differences in trace element accumulation in feathers are an exception rather than a rule. In facts, Lucia et al. (2014, 2012) observed differences only for Cu in dunlin (*Calidris alpina*) and Hg in redshank (*T. totanus*), whilst Ashbaugh et al. (2018) reported significantly higher concentrations of Se in female snowy plovers (*C. nivosus*) than in males.

As concern As, Lucia et al. (2010) observed that sex was a significant factor only for greylag goose (*Anser anser*), with females characterized by higher burdens than males.

Little is known about As in birds, also regarding intraspecific factors that may influence accumulation as gender, age and size (Sánchez-Virosta et al., 2015). Some authors pointed out that As - and trace element concentrations in general - may differ when size dimorphism is relevant (Janssens et al., 2001), but this hypothesis seems to be not applicable to KP, due to the minimal size and weight differences between males and females. Ashbaugh et al. (2018) postulated that higher concentrations of Se in females of *C. nivosus* may be due to sex-based susceptibility to uptake or metabolic differences between sexes, but no evidence supporting these hypotheses were adduced. Dissimilarity in prey type may contribute to differential accumulation in females and males (Janssens et al., 2001), since type of food eaten plays a predominant role in the As amounts that birds may accumulate (Lebedeva, 1997). Further studies on diet of KP, possibly involving stable isotope analysis, are needed to determine whether differences in As concentrations may be due to different diet composition between males and females, or whether other variables should be taken into consideration, including gender-related metabolic differences.

4.2 Correlation among trace elements in KP tail feathers

Spearman's correlations evidenced that accumulation of essential elements in tail feathers of KP is often positively correlated with deposition of non-essential metals. These correlations suggest that non-essential metals (i.e. Pb and Cd) may be taken up through the same mechanism that allow the assimilation of essential trace elements (including Se, Zn and Cu), and/or that essential and toxic elements may come from same prey items.

Except for Hg (Dominik et al., 2014), there is a general lack of information concerning the transfer of contaminants along the benthic food web of the Venice Lagoon, so it is not possible to identify whether these correlations are effectively due to contaminant transfer from sediments to biota.

Comparison with literature data is of little help in this context, since too many variables differed among studies, including contamination levels in study areas, feeding habits of the species, type of feather, and trace elements analyzed. Consequently, correlation data are often not consistent and, in some cases, also contrasting. As an example, Karimi et al. (2016) observed correlations between Pb and Zn in primary and tails feathers of waterfowls in Iran (as in the present study), but same elements were not correlated in waterfowls from wetlands of Korea (Kim and Oh, 2014). Similarly, Pb and Cd were positively correlated in seabirds as pigeon guillemots *Cepphus columba* and flesh-footed shearwaters (*Puffinus carneipes*) (Bond and Lavers, 2011; Burger et al., 2007), but not in KP (present work), Laughing gull *Larus atricilla*, and waterfowls (Gochfeld et al., 1996b; Karimi et al., 2016). Correlation between Cu and Cr were not observed in previous studies.

Interestingly, data concerning KP highlighted also the lack of correlation between Se and Hg, a condition often observed in birds (Ashbaugh et al., 2018; Burger et al., 2007). Since Se is reported to mitigate the effects due to Hg in birds (Burger et al., 1993; Ohlendorf and Heinz, 2011), the lack of this correlation may be symptomatic of an increased risk of Hg-related toxicity towards KP, especially for those specimens characterized by higher Hg/Se molar concentration ratio.

4.3 Ecotoxicological significance of trace element levels for Kentish plover

Very little is known concerning accumulation of trace elements in KP, so the assessment of the significance of trace elements accumulated in KP feathers was based on: 1) available adverse-effects thresholds for birds, and 2) comparison with literature data regarding waders and seabirds sampled in different geographical areas. These latter data are reported in detail in *Supplementary Material*.

Adverse-effects thresholds are available for main elements known to exert toxicity towards birds (Hg, Cd and Pb) and Se. Besides these elements, there is a general lack of laboratory experiments relating feather concentrations with adverse effects; other available thresholds (i.e. for Cr, Zn and Ni) are most often values empirically derived from internal tissue concentrations.

4.3.1 Mercury

The trace element causing major concern for the conservation of KP is surely Hg. Feathers are known to represent a major pathway for birds to eliminate the accumulated Hg, as they may contain up to 70-90% of the total Hg body burden (Braune and Gaskin, 1987; Honda et al., 1986). Relevant Hg concentrations were thus expected for KP, also in consideration of its invertivorous feeding habits (Castro et al., 2009; Cramp and Simmons, 1983) and evidences that benthic macrofauna of Venice lagoon accumulates Hg to a significant extent (Dominik et al., 2014). Nevertheless, it was unexpected to measure Hg concentrations above the toxicity threshold of 5000 μg kg^{-1} (Eisler, 2000, 1987) in 11 out of 13 analyzed KP. This outcome is critical and of great ecological relevance: even if sample size is relatively small, these data underline that a relevant part of the nesting population (at least 39%) was surely exposed to Hg at levels that may have detrimental effects on its conservation. In facts, according to Eisler (2000), feather concentrations above 5000 ppb are symptom of a severe exposure to Hg, and are associated with possible impairments in reproduction and sterility in a number of species. In facts, dietary exposure to Hg or methyl-mercury does not affect survival of adults birds (Whitney and Cristol, 2017), but may depress reproductive success by reducing the clutch size (Tartu et al., 2013), reducing the number of hatched and fledged chicks per brood (Hallinger and Cristol, 2011; Taylor and Cristol, 2015; Varian-Ramos et al., 2014), increasing latency to renest after loss of a clutch (Varian-Ramos et al., 2014) or changing endocrine expression, reproductive behavior and partner selection (Jayasena et al., 2011). All these sub-lethal effects may significantly contribute to the depletion of the population at local scale, by reducing hatching and survival of hatchlings and fledglings.

Moreover, Hg concentrations measured in KP are among the highest reported in the literature for water birds. Feather concentrations similar or higher than those observed in Venice Lagoon were reported only for some piscivorous species as cormorants, terns, and petrels (Bond and Lavers, 2011; Eagles-Smith et al., 2008; Hribšek et al., 2017; Monteiro et al., 1995; Ochoa-acunã et al., 2002). In waders, Hg occur at levels generally lower than those observed for KP, also in contaminated areas as the Derwent estuary, Australia (Einoder et al., 2018). An exception to this trend is the Black-necked stilt *Himantopus mexicanus* (Eagles-Smith et al., 2008).

These data strengthen the hypothesis that exposure to inorganic Hg and/or monomethyl-Hg in the Venice Lagoon is of concern, not only for KP but also for other species feeding on macrobenthic community. Although the available data do not allow to establish whether reproduction failures observed in the Cavallino-Treporti peninsula, such as increased number of unfertilized and unhatched eggs (Picone, unpublished data) may be due to dietary exposure to Hg, they indicate that trophic transfer of Hg needs a continuous monitoring.

4.3.2 Cadmium

In birds, Cd taken up through food ingestion is mainly deposited in kidney and liver, and only secondarily in feathers. According to Burger and Gochfeld (2000), toxic effects of Cd towards seabirds may occur at feather concentration ranging from 100 up to 2,000 μg kg^{-1}. These thresholds, however, were empirically derived from adverse-effect concentrations in kidney obtained in laboratory (10,000 μg kg^{-1} of Cd) (Eisler, 2000) and kidney:feathers ratios in pelagic birds. Thus, these thresholds should be considered as guidance values rather than adverse-effect concentrations. In any case, average concentration in KP exceeds 100 μg kg^{-1}, suggesting that some individuals may have been exposed to Cd to an extent that may lead to sub-lethal effects including growth inhibition, disruption of Ca metabolism, anemia, renal and testicular damages (Burger, 2008; Burger and Gochfeld, 2000; Eisler, 2000 and citations herein).

Concentrations of Cd measured in KP in the present work are lower than those reported by Kim and Koo (2008) for the same species (910 μg kg^{-1}), and also than those measured in ducks and waders in Iran (Karimi et al., 2016) and Korea (Kim and Oh, 2012). Nevertheless, Cd levels in KP feathers are considerably higher than those observed in waders and ducks collected in wetlands reported to be contaminated by Cd and/or other elements in France and Australia (Einoder et al., 2018; Lucia et al., 2010).

Based on a liver-to-kidney ratio, Lucia et al. (2010) have estimated a low-chronic exposure to Cd for graylag goose (*Anser anser*), red knot (*Calidris canutus*) and grey plover (*Pluvialis squatarola*), with minor threat for survival. Since mean Cd concentrations in feathers of these birds ranged in the interval of 40-90 μg kg^{-1} (Lucia et al. 2010), according to these data the exposure of KP to Cd may be considered from low to moderate, suggesting that long term effects cannot be excluded, as also evidenced by the comparison with the adverse-effect threshold.

4.3.3 Lead

KP exposure to Pb in the Venice Lagoon seems to be negligible and occurrence of toxicosis unlikely. Concentrations of Pb in tail feathers of KP are by far lower than the 4,000 μg kg^{-1} toxicity threshold reported by Burger and Gochfeld (2000), associated with drooped wings, loss of appetite, lethargy, weakness, impaired locomotion, lowered reproductive success and survival (Burger and Gochfeld, 2004). Kim and Koo (2008) for the same species observed an average Pb concentration of 9,840 μg kg^{-1}, one order of magnitude higher than that measured in the present work, and more than twice the adverse-effect threshold reported by Burger and Gochfeld (2000).

In general, Pb concentrations measured in this work are among the lowest reported in bird's feathers. Transfer of Pb through the trophic web seems not to be a factor for conservation of KP.

4.3.4 Selenium

Selenium is essential in ultra-trace, but may become toxic at concentrations slightly higher than those required for homeostasis (Ohlendorf and Heinz, 2011). Background concentrations in feathers are reported to be typically less than 2,000 µg kg^{-1} but a range of 1,000-4,000 µg kg^{-1} is most often considered, due to interspecific variability (Ohlendorf and Heinz, 2011). A Se concentration of 5,000 µg kg^{-1} was identified as a provisional threshold of toxicity by a number of researchers (Ashbaugh et al., 2018; Burger et al., 1993; Ohlendorf and Heinz, 2011), although concentrations that may be directly related to impairments in adults or offspring are not available. Two specimens of KP exceeded the 5,000 µg kg^{-1} threshold, suggesting that possible adverse effects due to Se cannot be excluded. Nevertheless, in migratory waders sampled at middle latitudes (including the KP's sister species *C. nivosus*), Se occurs often at concentrations higher than 5,000 µg kg^{-1} (Ashbaugh et al., 2018; Burger et al., 1993; Lucia et al., 2010; St. Clair et al., 2015). Thus, it cannot be excluded that background concentration in Charidriidae and other shorebirds may be naturally higher than those observed in seabirds and arctic birds. Further research is needed to establish background values for waders and identify adverse-effect threshold for Se.

4.3.5 Chromium

Burger and Gochfeld (2000) suggested that impairments due to Cr may occur at feather concentrations above 2,800 µg kg^{-1}. Since mean concentration in tail feathers of KP is lower than this value, and only a single female (2,745 µg kg^{-1}) approximates this adverse-effect concentration derived from liver concentrations, Cr does not seem to be of concern for the conservation status of KP in the Venice Lagoon.

As compared with other Charadriidae, feather concentrations of Cr in KP are low; more difficult is the comparison with other families, since concentrations are very variable among sites and species.

4.3.6 Nickel

Nickel is an essential metal required by organisms for growth; nevertheless, bird exposed to high concentrations of this element through diet may suffer adverse effects including metabolic upset, altered bone density, inhibition of growth (Eisler, 2000). Literature data showed that KP feather concentrations are slightly higher than levels occurring in a number of seabird species. However, exposure to Ni in the Venice Lagoon appears to be not of concern since the adverse-effect threshold proposed by Outridge and Scheuhammer (1993) is 4000 µg kg^{-1}, about 3 times higher than the average concentration measured for KP.

4.3.7 Zinc

Zinc is an essential element for birds, playing a key role in enzymatic reactions, bone structure and growth and for cellular immunity (Park et al., 2004). Nevertheless, it may become toxic at concentrations overwhelming homeostasis. In the case of Zn, the occurrence of pigments able to bind metals as eumelanin (Klasing, 1998; Lodenius and Solonen, 2013) may increase the feather concentration, providing "false positive" responses concerning exposure to and accumulation of Zn.

This is not the case of KP. Firstly, the sixth rectrix of KP lacks of pigmentation. Secondly, the adverse-effect thresholds for Zn reported by Einoder et al. (2018) - based on data provided by Gasaway and Buss (1972) - is at 200,000 µg kg^{-1}, a level more than 3 times higher than the average Zn concentration measured in KP tail feathers. Thirdly, Zn concentrations in feathers of KP are among the lowest observed in literature. Hazard due to Zn is thus improbable in Venice Lagoon for KP.

4.3.8 Other trace elements (As, Co, Cu and V)

Although As is carcinogen and may cause behavioral and physical effects in birds, fate and toxicology of As in water birds are poorly known, and consequently no theoretical nor empirical adverse-effect threshold is available (Bond and Lavers, 2011; Burger et al., 2007). In any case, feathers are not expected to be a major structure for As deposition in birds (Fujihara et al., 2004).

Concentrations of As in KP are the lowest observed for Charadriidae, with a mean value about one order of magnitude lower than the concentrations measured in dunlin (*Calidris alpina*) and grey plover (*P. squatarola*) (Goede et al., 1989; Lucia et al., 2010). On the basis of these data, hazard due to exposure to As in the area of Venice Lagoon seems to be negligible for KP.

Information concerning effects of Co towards birds are scarce, also due to the lack of controlled laboratory studies relating Co concentrations in feathers and other tissue with adverse effects (Burger et al., 2018). However, average concentrations observed in KP are among the lowest observed in water birds for Co; most probably this element does not pose any risk to conservation of KP in Venice Lagoon area.

Due to its essential role in many biochemical and enzymatic reactions, Cu tends to be rather uniform among bird species, and deviations from this trend are most often due to some species-specific bioaccumulation pattern rather than to habitat contamination (Kim et al., 1996). According to Eisler (2000), in birds from contaminated sites (near smelters) Cu in feathers usually ranges in the interval 23,000 - 53,000 µg kg^{-1}. Feather concentrations in KP are clearly lower than this values (only a single male approximates 20,000 µg kg^{-1}), suggesting a low exposure to Cu. Nevertheless, predictions concerning possible toxic effects are not feasible with the available data. Feather concentrations of Cu measured in KP are consistent with values reported for other Charadriidae and waterbirds, but higher than those observed by Kim and Koo (2008) for the same species in Korea.

Deposition of V in feathers has been largely disregarded by ecotoxicologists, although there are evidences of vanadium-induced toxicity in birds, including gastrointestinal hemorrhage, loss of weight and hearth failures (Rattner et al., 2006). However, in the case of KP, V is not expected to be of concern: feather concentrations are low as compared with shearwaters and waterfowls (Bond and Lavers, 2011; Hosseini Alhashemi et al., 2011). Moreover, uptake of sediment-bound V from rooted plants seems to be a preferential pathway for V transfer into the food web (Hosseini Alhashemi et al., 2011).

4.3.9 Daily deposition rates (DDRs)

The present work is the first experience with KP and also one of the few studies concerning daily deposition rates in birds. Normalizing the content of contaminant by age of feather is potentially more biologically meaningful than reporting data normalized to unit of feather weight, as stated by Clarkson and Riscassi (2011). These authors agree with the theory of Bortolotti (2010) and argue that potential biological impacts of ingested contaminants may be masked in a healthy individual with structurally dense feathers, as the normalization per unit mass of feather will be low, whilst analyzing a feather's content by daily deposition may potentially correct for this effect. Moreover, determining the average daily deposition into growing feathers provide a higher sensitivity for detecting differences among species than normalizing the content of trace elements per unit of feather weight (Clarkson and Riscassi, 2011). Nevertheless, although its relevance, this approach was rarely applied in ecotoxicology.

Deposition rate of Hg in KP resulted lower than the DDR observed in nestlings of double-crested cormorant *Phalacrocorax auritus* (12 ng d^{-1}) but higher than that measured in the glossy ibis *Plegadis falcinellus* (2 ng d^{-1}) along the coasts of New York and Virginia (Clarkson and Riscassi, 2011). A lower DDR as compared with double-crested cormorant was expected, due to the strictly piscivorous feeding habits of *P. auritus*. On the other hand, a deposition rate about 2 times higher than chicks of glossy ibis, that feed mostly on aquatic invertebrates and insects as KP, is of concern and evidences a significant exposure to Hg.

For elements other than Hg, deposition rate are not available in literature, so further comparisons cannot be performed. However, some inferences can be made. High deposition rates observed for essential elements as Cu and Zn are most probably due to their structural role in the keratinous composition of the feather (Howell et al., 2017). Due to the lack of pigmentation in the sixth rectrix of KP, deposition rates of Zn and Cu cannot be related with the occurrence of eumelanin or pheomelanin, known to serve as metal reservoir also in feathers (Edwards et al., 2016). Low deposition rate observed for Co indicates negligible blood concentration during feather growth and thus a limited availability of Co through the food web. Deposition rates for As, Cd and V are quite low, as compared with other trace elements. However, in the case of Cd, despite a low DDR the feather concentration exceeded the adverse-effect threshold of 100 µg kg^{-1}, indicating that DDR alone cannot be used as a predictor of metal-related toxic effects.

Further studies are needed to acquire more data concerning DDR in a number of species with different feeding habits, in order to obtain benchmarks to estimate exposure.

5 Conclusions

This work represents the first study aimed at identifying possible disturbances related to environmental contamination on the conservation status of the threatened species *Charadrius alexandrinus*, by using a non-invasive approach. Although the sample size was relatively small, it allowed to gain an insight into possible harmful exposure of the local population of KP to trace elements, especially as concern the identification of main threats for the species.

Mercury emerged as a major threat to the conservation of the species at local scale; feather concentrations above the adverse-effect threshold in 11 out of 13 analyzed birds underlined a probable risk of Hg-related toxicosis, that may lead to impairments in the reproductive success of KP, and of other water birds with similar feeding habits. Further studies are needed to verify whether reproductive failures already observed in the field may be linked to Hg-related toxicosis or other causes.

Even if to a lesser extent than Hg, also exposure to Cd and Se may be of concern for KP conservation. For these elements there is the need to identify which are the compartments of the benthic food web along which the toxicant may be transferred from the sediment to the birds (i.e. polychaetes, amphipods, small bivalves, etc.).

Daily deposition rates provided a measure of the exposure to contaminants that may be more biologically meaningful than the mass/mass ratio and can provide useful insights concerning the transfer of contaminants along the food web that may be directly related with dietary intakes in an ecological risk assessment approach.

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7 References

Adout, A., Hawlena, D., Maman, R., Paz-Tal, O., Karpas, Z., 2007. Determination of trace elements in pigeon and raven feathers by ICPMS. Int. J. Mass Spectrom. 267, 109–116. doi:https://doi.org/10.1016/j.ijms.2007.02.022

Argüelles-Ticó, A., Küpper, C., Kelsh, R.N., Kosztolányi, A., Székely, T., van Dijk, R.E., 2016. Geographic variation in breeding system and environment predicts melanin-based plumage ornamentation of male and female Kentish plovers. Behav. Ecol. Sociobiol. 70, 49–60. doi:10.1007/s00265-015-2024-8

Ashbaugh, H.M., Conway, W.C., Haukos, D.A., Collins, D.P., Comer, C.E., French, A.D., 2018. Evidence for exposure to selenium by breeding interior snowy plovers (*Charadrius nivosus*) in saline systems of the Southern Great Plains. Ecotoxicology 27, 703–718. doi:10.1007/s10646-018-1952-2

Blus, L.J., Henny, C.J., Hoffman, D.J., Grove, R.A., 1995. Accumulation in and effects of lead and cadmium on waterfowl and passerines in northern Idaho. Environ. Pollut. 89, 311–318. doi:10.1016/0269-7491(94)00069-P

Bond, A.L., Lavers, J.L., 2011. Trace element concentrations in feathers of Flesh-footed Shearwaters (*Puffinus carneipes*) from across their breeding range. Arch. Environ. Contam. Toxicol. 61, 318–326. doi:10.1007/s00244-010-9605-3

Bortolotti, G.R., 2010. Flaws and pitfalls in the chemical analysis of feathers: bad news — good news for avian chemoecology and toxicology. Ecol. Appl. 20, 1766–1774. doi:10.1890/09-1473.1

Braune, B.M., Gaskin, D.E., 1987. A mercury budget for the Bonaparte’s gull during autumn moult. Ornis Scand. 18, 244. doi:10.2307/3676891

Burger, J., 2008. Assessment and management of risk to wildlife from cadmium. Sci. Total Environ. 389, 37–45. doi:10.1016/J.SCITOTENV.2007.08.037

Burger, J., 1993. Metals in avian feathers: bioindicators of environmental pollution. Rev. Environ. Toxicol. 5, 203–311.

Burger, J., Gochfeld, M., 2004. Marine Birds as Sentinels of Environmental Pollution. Ecohealth 1, 263–274. doi:10.1007/s10393-004-0096-4

Burger, J., Gochfeld, M., 2000a. Metals in Albatross Feathers from Midway Atoll: Influence of Species, Age, and Nest Location. Environ. Res. 82, 207–221. doi:10.1006/enrs.1999.4015

Burger, J., Gochfeld, M., 2000b. Metal levels in feathers of 12 species of seabirds from Midway Atoll in the northern Pacific Ocean. Sci. Total Environ. 257, 37–52. doi:10.1016/S0048-9697(00)00496-4

Burger, J., Gochfeld, M., 1997. Risk, mercury levels, and birds: relating adverse laboratory effects to field biomonitoring. Environ. Res. 75, 160–172. doi:10.1006/ENRS.1997.3778

Burger, J., Gochfeld, M., 1996. Heavy metal and selenium levels in Franklin's Gull (*Larus pipixcan*) parents and their eggs. Arch. Environ. Contam. Toxicol. 30, 487–491. doi:10.1007/s002449900067

Burger, J., Gochfeld, M., 1991. Lead, mercury, and cadmium in feathers of tropical terns in Puerto Rico and Australia. Arch. Environ. Contam. Toxicol. 21, 311–315. doi:10.1007/BF01055351

Burger, J., Gochfeld, M., Sullivan, K., Irons, D., 2007. Mercury, arsenic, cadmium, chromium lead, and selenium in feathers of pigeon guillemots (*Cepphus columba*) from Prince William Sound and the Aleutian Islands of Alaska. Sci. Total Environ. 387, 175–184. doi:10.1016/J.SCITOTENV.2007.07.049

Burger, J., Mizrahi, D., Tsipoura, N., Jeitner, C., Gochfeld, M., 2018. Mercury, lead, cadmium, cobalt, arsenic and selenium in the blood of Semipalmated sandpipers (*Calidris pusilla*) from Suriname, South America: Age-related differences in wintering site and comparisons with a stopover site in New Jersey, USA. Toxics 6. doi:10.3390/toxics6020027

Burger, J., Seyboldt, S., Morganstein, N., Clark, K., 1993. Heavy metals and selenium in feathers of three shorebird species from Delaware bay. Environ. Monit. Assess. 28, 189–198. doi:10.1007/BF00547037

Castro, M., Masero, J.A., Pérez-Hurtado, A., Amat, J.A., Megina, C., 2009. Sex-related seasonal differences in the foraging strategy of the Kentish plover. Condor 111, 624–632. doi:10.1525/cond.2009.080062

Cecconi, G., Nascimbeni, P., 1997. Ricostruzione e naturalizzazione delle dune artificiali sul litorale del Cavallino.

Clarkson, C.E., Riscassi, A., 2011. Using ptilochronology to determine daily mercury deposition in feathers of nestling waterbirds. Environ. Toxicol. Chem. 30, 2081–2083. doi:10.1002/etc.591

Cramp, S., Simmons, K.E.L., 1983. Handbook of the birds of Europe, the Middle East and North Africa. The birds of the Western Palearctic: 3. Waders to gulls. Oxford University Press, Oxford.

Dauwe, T., Bervoets, L., Blust, R., Pinxten, R., Eens, M., 2000. Can excrement and feathers of nestling songbirds be used as biomonitors for heavy metal pollution? Arch. Environ. Contam. Toxicol. 39, 541–546. doi:10.1007/s002440010138

Delany, S., Scott, D.A., Dodman, T., Stroud, D.A., 2009. The wader atlas: An atlas of wader populations in Africa and western Eurasia. Wetlands International, Wageningen.

Del Vecchio, S., Pizzo, L., Buffa, G., 2015. The response of plant community diversity to alien invasion: evidence from a sand dune time series. Biodivers. Conserv. 24, 371–392. doi:10.1007/s10531-014-0814-3

Domínguez, J., Vidal, M., 2003. Influencia del investigador en el éxito reproductivo del Chorlito Patinegro *Charadrius alexandrinus*. Ardeola 50, 15–19.

Dominik, J., Tagliapietra, D., Bravo, A.G., Sigovini, M., Spangenberg, J.E., Amouroux, D., Zonta, R., 2014. Mercury in the food chain of the Lagoon of Venice, Italy. Mar. Pollut. Bull. 88, 194–206. doi:10.1016/J.MARPOLBUL.2014.09.005

Donazzolo, R., Merlin, O.H., Vitturi, L.M., Orio, A.A., Pavoni, B., Perin, G., Rabitti, S., 1981. Heavy metal contamination in surface sediments from the Gulf of Venice, Italy. Mar. Pollut. Bull. 12, 417–425. doi:10.1016/0025-326X(81)90160-0

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, 2136. doi:10.1897/08-038.1

Edwards, N.P., van Veelen, A., Anné, J., Manning, P.L., Bergmann, U., Sellers, W.I., Egerton, V.M., Sokaras, D., Alonso-Mori, R., Wakamatsu, K., Ito, S., Wogelius, R.A., 2016. Elemental characterisation of melanin in feathers via synchrotron X-ray imaging and absorption spectroscopy. Sci. Rep. 6, 34002. doi:10.1038/srep34002

Einoder, L.D., MacLeod, C.K., Coughanowr, C., 2018. Metal and Isotope Analysis of Bird Feathers in a Contaminated Estuary Reveals Bioaccumulation, Biomagnification, and Potential Toxic Effects. Arch. Environ. Contam. Toxicol. 75, 96–110. doi:10.1007/s00244-018-0532-z

Eisler, R., 2000. Handbook of Chemical Risk Assessment. CRC Press. doi:10.1201/9781420032741

Eisler, R., 1987. Mercury hazards to fish, wildlife, and invertebrates: A synoptic review, Fish and Wildlife Service Biological Report 85 (1.10).

Fraga, R.M., Amat, J.A., 1996. Breeding biology of a Kentish plover (*Charadrius alexandrinus*) population in an inland saline lake. Ardeola 43, 69–85.

Fujihara, J., Kunito, T., Kubota, R., Tanaka, H., Tanabe, S., 2004. Arsenic accumulation and distribution in tissues of black-footed albatrosses. Mar. Pollut. Bull. 48, 1153–1160. doi:10.1016/J.MARPOLBUL.2004.03.007

Furness, R.W., 1996. Cadmium in Birds, in: Beyer, W.N., Heinz, G.H., Redmon-Norwood, A.W. (Eds.), Environmental Contaminants in Wildlife: Interpreting Tissue Concentrations. Lewis Publishers, Boca Raton, FL, pp. 389–404.

García-Fernández, A.J., Espín, S., Martínez-López., E., 2013. Feathers as a biomonitoring tool of polyhalogenated compounds: A review. Environ. Sci. Technol. 47, 3028–3043. doi:10.1021/es302758x

Gasaway, W.C., Buss, I.O., 1972. Zinc Toxicity in the Mallard Duck. J. Wildl. Manage. 36, 1107. doi:10.2307/3799239

Ginn, H.B., Melville, D.S., 1983. Moult in birds. British Trust for Ornithology.

Gochfeld, M., Belant, J.L., Shukla, T., Benson, T., Burger, J., 1996. Heavy metals in laughing gulls: gender, age and tissu differences, Environmental Toxicology and Chemistry.

Goede, A., Nygard, T., De Bruin, M., Steinnes, E., 1989. Selenium, mercury, arsenic and cadmium in the life-cycle of the Dunlin, *Calidris alpina*, a migrant wader. Sci. Total Environ. Elsevier Sci. Publ. B.V 78, 205–218.

Grubb, T.C.J., 2006. Ptilochronology. Feather time and the biology of birds. Oxford University Press.

Grubb, T.C.J., 1989. Ptilochronology: feather growth bars as indicators of nutritional status. Auk

106, 314–320.

Hallinger, K.K., Cristol, D.A., 2011. The role of weather in mediating the effect of mercury exposure on reproductive success in tree swallows. Ecotoxicology 20, 1368–1377. doi:10.1007/s10646-011-0694-1

Honda, K., Nasu, T., Tatsukawa, R., 1986. Seasonal changes in mercury accumulation in the black-eared kite, *Milvus migrans lineatus*. Environ. Pollut. Ser. A, Ecol. Biol. 42, 325–334. doi:10.1016/0143-1471(86)90016-4

Hosseini Alhashemi, A.S., Karbassi, A.R., Hassanzadeh Kiabi, B., Monavari, S.M., Nabavi, S.M.B., Sekhavatjou, M.S., 2011. Bioaccumulation of trace elements in trophic levels of wetland plants and waterfowl birds. Biol. Trace Elem. Res. 142, 500–516. doi:10.1007/s12011-010-8795-x

Hribšek, I., Jovičić, K., Karadžić, B., Skorić, S., 2017. Allocation of metals and trace elements in different tissues of piscivorous species *Phalacrocorax carbo*. Arch. Environ. Contam. Toxicol. 73, 533–541. doi:10.1007/s00244-017-0452-3

Janssens, E., Dauwe, T., Bervoets, L., Eens, M., 2001. Heavy metals and selenium in feathers of great tits (*Parus major*) along a pollution gradient. Environ. Toxicol. Chem. 20, 2815–2820. doi:10.1002/etc.5620201221

Jaspers, V.L.B., Voorspoels, S., Covaci, A., Eens, M., 2006. Can predatory bird feathers be used as a non-destructive biomonitoring tool of organic pollutants? Biol. Lett. 2, 283–285. doi:10.1098/rsbl.2006.0450

Jayasena, N., Frederick, P.C., Larkin, I.L. V, 2011. Endocrine disruption in white ibises (*Eudocimus albus*) caused by exposure to environmentally relevant levels of methylmercury. Aquat. Toxicol. 105, 321–327. doi:https://doi.org/10.1016/j.aquatox.2011.07.003

Karimi, M.H.S., Hassanpour, M., Pourkhabbaz, A.R., Błaszczyk, M., Paluch, J., Binkowski, Ł.J., 2016. Trace element concentrations in feathers of five Anseriformes in the south of the Caspian Sea, Iran. Environ. Monit. Assess. 188, 1–7. doi:10.1007/s10661-015-5015-3

Kim, E.-Y., Ichihashi, H., Saeki, K., Atrashkevich, G., Tanabe, S., Tatsukawa, R., 1996. Metal accumulation in tissues of seabirds from Chaun, northeast Siberia, Russia. Environ. Pollut. 92, 247–252. doi:10.1016/0269-7491(96)00007-3

Kim, J., Koo, T.-H., 2008. Heavy Metal Concentrations in Feathers of Korean Shorebirds. Arch. Environ. Contam. Toxicol. 55, 122–128. doi:10.1007/s00244-007-9089-y

Kim, J., Oh, J.-M., 2014a. Concentration of trace elements in feathers of waterfowl, Korea. Environ. Monit. Assess. 186, 8517–8525. doi:10.1007/s10661-014-4021-1

Kim, J., Oh, J.-M., 2014b. Concentration of trace elements in feathers of waterfowl, Korea. Environ. Monit. Assess. 186, 8517–8525. doi:10.1007/s10661-014-4021-1

Kim, J., Oh, J.-M., 2012. Monitoring of heavy metal contaminants using feathers of shorebirds, Korea. J. Environ. Monit. 14, 651. doi:10.1039/c2em10729e

Klasing, K.C., 1998. Comparative avian nutrition. Cab International.

Kosztolányi, A., Javed, S., Küpper, C., Cuthill, I.C., Shamsi, A. Al, Székely, T., 2009. Breeding ecology of Kentish plover *Charadrius alexandrinus* in an extremely hot environment. Bird Study 56, 244–252. doi:10.1080/00063650902792106

Lebedeva, N.V., 1997. Accumulation of heavy metals by birds in Southwest of Russia. Russ. J. Ecol. 28, 41–46. doi:10.06

Lessells, C.M., 1984. The mating system of Kentish plovers *Charadrius alexandrinus*. Ibis (Lond. 1859). 126, 474–483.

Lodenius, M., Solonen, T., 2013. The use of feathers of birds of prey as indicators of metal pollution. Ecotoxicology 22, 1319–1334. doi:10.1007/s10646-013-1128-z

Long, E.R., Ingersoll, C.G., MacDonald, D.D., 2006. Calculation and uses of mean sediment quality guideline quotients: A critical review. Environ. Sci. Technol. 40, 1726–1736. doi:10.1021/es058012d

Lucia, M., André, J.M., Gontier, K., Diot, N., Veiga, J., Davail, S., 2010. Trace element concentrations (mercury, cadmium, copper, zinc, lead, aluminium, nickel, arsenic, and selenium) in some aquatic birds of the southwest atlantic coast of France. Arch. Environ. Contam. Toxicol. 58, 844–853. doi:10.1007/s00244-009-9393-9

Lucia, M., Bocher, P., Chambosse, M., Delaporte, P., Bustamante, P., 2014. Trace element accumulation in relation to trophic niches of shorebirds using intertidal mudflats. J. Sea Res. 92, 134–143. doi:10.1016/J.SEARES.2013.08.008

Lucia, M., Bocher, P., Cosson, R.P., Churlaud, C., Bustamante, P., 2012. Evidence of species-specific detoxification processes for trace elements in shorebirds. Ecotoxicology 21, 2349–2362. doi:10.1007/s10646-012-0991-3

Markowski, M., Kaliński, A., Skwarska, J., Wawrzyniak, J., Bańbura, M., Markowski, J., Zieliński, P., Bańbura, J., 2013. Avian feathers as bioindicators of the exposure to heavy metal contamination of food. Bull. Environ. Contam. Toxicol. 91, 302–305. doi:10.1007/s00128-013-1065-9

Miller, M.W.C., Lovvorn, J.R., Matz, A.C., Taylor, R.J., Latty, C.J., Brooks, M.L., Hollmén, T.E., 2019. Interspecific patterns of trace elements in sea ducks: Can surrogate species be used in contaminants monitoring? Ecol. Indic. 98, 830–839. doi:10.1016/J.ECOLIND.2018.11.023

Monteiro, L.R., Furness, R.W., Del Nevo, A.J., 1995. Environmental Contamination and

Toxicology Mercury Levels in Seabirds from the Azores, Mid-North Atlantic Ocean. Arch. Environ. Contam. Toxicol 28, 304–309.

Newton, I., 2009. Moult and plumage. Ringing Migr. 24, 220–226. doi:10.1080/03078698.2009.9674395

Ochoa-acunã, H., Sepúlveda, M.S., Gross, T.S., 2002. Mercury in feathers from Chilean birds: influence of location, feeding strategy, and taxonomic affiliation. Mar. Pollut. Bull. 44, 340–345. doi:10.1016/S0025-326X(01)00280-6

Ohlendorf, H.M., Heinz, G.H., 2011. Selenium in Birds, in: Beyer, W.N., Meador, J.P. (Eds.), Environmental Contaminants in Biota: Interpreting Tissue Concentrations, 2nd Edition. Lewis Publishers, Boca Raton, FL, pp. 669–701.

Ohlendorf, H.M., Heinz, G.H., n.d. Selenium in Birds.

Outridge, P.M., Scheuhammer, A.M., 1993. Bioaccumulation and toxicology of nickel: implications for wild mammals and birds. Environ. Rev. 1, 172–197. doi:10.1139/a93-013

Park, S.Y., Birkhold, S.G., Kubena, L.F., Nisbet, D.J., Ricke, S.C., 2004. Review on the role of dietary zinc in poultry nutrition, immunity, and reproduction. Biol. Trace Elem. Res. 101, 147–164. doi:10.1385/BTER:101:2:147

Perez-Hurtado, A., Goss-Custard, J.D., Garcia, F., 1997. The diet of wintering waders in Cádiz Bay, southwest Spain. Bird Study 44, 45–52. doi:10.1080/00063659709461037

Picone, M., Bergamin, M., Delaney, E., Ghirardini, A.V., Kusk, K.O., 2018. Testing lagoonal sediments with early life stages of the copepod *Acartia tonsa* (Dana): An approach to assess sediment toxicity in the Venice Lagoon. Ecotoxicol. Environ. Saf. 147. doi:10.1016/j.ecoenv.2017.08.042

Picone, M., Bergamin, M., Losso, C., Delaney, E., Arizzi Novelli, A., Ghirardini, A.V., 2016. Assessment of sediment toxicity in the Lagoon of Venice (Italy) using a multi-species set of bioassays. Ecotoxicol. Environ. Saf. 123. doi:10.1016/j.ecoenv.2015.09.002

R. Howell, N., Lavers, J.L., Uematsu, S., Paterson, D., Howard, D.L., Spiers, K., Jonge, M.D. de, Hanley, T., Garrett, R., Banati, R.B., 2017. The Topobiology of Chemical Elements in Seabird Feathers. Sci. Rep. 7, 1998. doi:10.1038/s41598-017-01878-y

Rattner, B.A., McKernan, M.A., Eisenreich, K.M., Link, W.A., Olsen, G.H., Hoffman, D.J., Knowles, K.A., McGowan, P.C., 2006. Toxicity and hazard of vanadium to Mallard ducks (*Anas platyrhynchos*) and Canada geese (*Branta canadensis*). J. Toxicol. Environ. Heal. Part A 69, 331–351. doi:10.1080/15287390500398265

Rondinini, C., Battistoni, A., Peronace, V., Teofili, C., 2013. Lista Rossa IUCN dei Vertebrati Italiani. Roma.

Rothschild, R.F.N., Duffy, L.K., 2005. Mercury concentrations in muscle, brain and bone of Western Alaskan waterfowl. Sci. Total Environ. 349, 277–83. doi:10.1016/j.scitotenv.2005.05.021

Sánchez-Virosta, P., Espín, S., García-Fernández, A.J., Eeva, T., 2015. A review on exposure and effects of arsenic in passerine birds. Sci. Total Environ. 512–513, 506–525. doi:10.1016/J.SCITOTENV.2015.01.069

Scarton, F., 2017. Long-term trend of the waterbird community breeding in a heavily man-modified coastal lagoon: the case of the important bird area ``Lagoon of Venice''. J. Coast. Conserv. 21, 35–45. doi:10.1007/s11852-016-0470-8

Spahn, S.A., Sherry, T.W., 1999. Cadmium and lead exposure associated with reduced growth rates, poorer fledging success of little blue heron chicks (*Egretta caerulea*) in south Louisiana wetlands. Arch. Environ. Contam. Toxicol. 37, 377–84.

Spallholz, J.E., Hoffman, D.J., 2002. Selenium toxicity: cause and effects in aquatic birds. Aquat. Toxicol. 57, 27–37. doi:https://doi.org/10.1016/S0166-445X(01)00268-5

St. Clair, C.T., Baird, P., Ydenberg, R., Elner, R., Bendell, L.I., 2015. Trace elements in pacific Dunlin (*Calidris alpina pacifica*): patterns of accumulation and concentrations in kidneys and feathers. Ecotoxicology 24, 29–44. doi:10.1007/s10646-014-1352-1

Tartu, S., Goutte, A., Bustamante, P., Angelier, F., Moe, B., Clément-Chastel, C., Bech, C., Gabrielsen, G.W., Bustnes, J.O., Chastel, O., 2013. To breed or not to breed: endocrine response to mercury contamination by an Arctic seabird. Biol. Lett. 9, 20130317. doi:10.1098/rsbl.2013.0317

Taylor, C.E., Cristol, D.A., 2015. Tissue mercury concentrations and survival of Tree swallow embryos, nestlings and young adult females on a contaminated site. Bull. Environ. Contam. Toxicol. 95, 459–464. doi:10.1007/s00128-015-1643-0

Varian-Ramos, C.W., Swaddle, J.P., Cristol, D.A., 2014. Mercury reduces avian reproductive success and imposes selection: An experimental study with adult- or lifetime-exposure in Zebra finch. PLoS One 9, e95674. doi:10.1371/journal.pone.0095674

Whitney, M.C., Cristol, D.A., 2017. Impacts of sublethal mercury exposure on birds: A detailed review. Springer, Cham, pp. 113–163. doi:10.1007/398_2017_4

Zheng, S., Wang, P., Sun, H., Matsiko, J., Hao, Y., Meng, D., Li, Y., Zhang, G., Zhang, Q., Jiang, G., 2018. Tissue distribution and maternal transfer of persistent organic pollutants in Kentish Plovers (*Charadrius alexandrinus*) from Cangzhou Wetland, Bohai Bay, China. Sci. Total Environ. 612, 1105–1113. doi:10.1016/J.SCITOTENV.2017.08.323