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The influence of colony habitat and egg components on lead and cadmium concentrations of great egrets and roseate spoonbills in southern Brazil

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Abstract

Birds are important sentinels of environmental contamination due to their well-known response to fluctuations in the concentration of trace elements and foraging preferences. Lead (Pb) and cadmium (Cd) are environmental contaminants that can cause lethal or sublethal effects, such as neurological, physiological, and reproductive dysfunction in a range of bird species. Here, we examined Pb and Cd concentrations in eggshells and egg contents of great egrets (*Ardea alba*) and roseate spoonbills (*Platalea ajaja*), waterbirds with contrasting foraging strategies, from two habitats, estuarine and limnetic, in southern Brazil. We found that colony habitat had an important influence on Pb and Cd concentrations in eggshells, confirming that environmental abiotic factors play a role in the uptake and bioavailability of these elements. Great egrets had higher Pb concentrations in eggshells and egg contents in the estuary habitat, as well as higher Cd in egg contents at the limnetic colony compared to roseate spoonbills. Contrastingly, spoonbills from the limnetic habitat had the highest Pb concentrations. Differences in concentrations of Pb and Cd among breeding sites and egg components may be related to the distinct foraging sites by females of egrets and spoonbills before the egg laying. Our results indicate ongoing estuarine and freshwater contamination by Pb and Cd in southern Brazilian coastal areas. Indeed, Cd concentrations in waterbirds were higher than the environmental standards, which is a concern for wildlife health and environmental quality. Finally, eggshells proved to be a sensitive, efficient, and low-impact sampling method to monitor low levels of contamination in waterbirds.

Keywords Ardea alba · Bioindicator · Environmental sentinel · Metal contamination · Platalea ajaja · Waterbirds

Introduction

Waterbirds are a diverse group of species that can act as sentinels of environmental pollution. They allow for long-term monitoring and assessment of biomagnification and bioaccumulation of multiple compounds by sampling both resident and migratory species, across many individuals and species (Burger and Gochfeld, 2004). Pelecaniformes, i.e. egrets, herons, spoonbills, storks, ibises, and allies, are wading birds, long-legged and long-billed waterbirds (Gill and Prum, 2019), that occur in aquatic habitats that may drain urban or agricultural areas, environments often severely modified by human activities, and in more natural areas (Burger and Gochfeld, 1997; Kushlan and Hancock, 2005; Corriveau et al. 2022). Wading birds are central-place foragers during the breeding period, which means they forage in areas near their nest sites, bringing food back to the nest to feed their chicks (Bell, 1990; Craig et al. 2015;

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Winkler et al. 2020a, 2020b). As a result, eggs and chicks have often been studied for various contaminants in breeding sites worldwide (e.g. Burger and Gochfeld, 1995, 2005; Abdullah et al. 2015; Burger and Elbin, 2015; Janaydeh et al. 2018; Silva et al. 2018).

Great egrets (*Ardea alba*) and roseate spoonbills (*Platalea ajaja*) are wading birds with a wide distribution in the Americas (Kushlan and Hancock, 2005; McCrimmon-Jr et al. 2011). In southern Brazil, great egrets occur year-round (Belton, 1994), roosting in urban areas during the non-breeding season. Roseate spoonbills are partially resident, while part of the population migrates from Pantanal and lower Paraná River at Entre Ríos, Santa Fé, and Corrientes Provinces in Argentina (Antas, 1994; Belton, 1994; Santos et al. 2008). The great egret is a visual predator, relying on larger prey, while the roseate spoonbill is a tactile forager, filtering small prey and vegetation through lateral movements of the bill (Dumas, 2000; McCrimmon-Jr et al. 2011; Britto and Bugoni, 2015). Both species nest in estuarine and freshwater habitats in mixed-species colonies with other waterbirds, but spoonbills from southern Brazil feed their chicks at early stages with a diet exclusively from freshwater sources while egrets, even those nesting in the brackish estuary, feed them with both estuarine and limnetic food items (Britto and Bugoni, 2015). Egrets and spoonbills frequently nest near urban areas and forage in rice fields (Weller, 1999; Craig et al. 2015; Wolfe et al. 2020), being exposed to a range of contaminants, including non-essential trace elements such as lead (Pb) and cadmium (Cd), which are often elevated in the environment due to anthropogenic activities (Baird and Cann, 2011; Williamson et al. 2013).

Lead is a toxic element present in ecosystems both naturally, through processes such as erosion and volcanic activity, and anthropogenically, from sources including fuel spills, corrosion of aging pipelines, improper disposal of urban and industrial effluents, waste from ceramic and dye production, and hunting and fishing activities (Russel, 1994; Corseuil and Marins, 1997; Duarte and Pascal, 2000; Spiro and Stigliani, 2008; Baird and Cann, 2011). In southern Brazilian wetlands, sources of Pb can be related to anthropogenic activities, including the disposal of lead shot from hunting (Guadagnin et al. 2007), as hunting was allowed until 2006 and banned only in 2008 (TRF4, 2008), but it still occurs illegally. Increases in Pb concentration from ammunition or direct ingestion of shot pellets were also reported for other wetlands in South America (Ferreyra et al. 2015; Plaza et al. 2018), the USA (Zwank et al. 1985), and Europe (Newth et al. 2013). The bioavailability of Pb in aquatic environments depends on water pH and hardness. High concentrations of calcium carbonate ($CaCO_3$) can decrease Pb bioavailability, while low water pH increases it (Baird and Cann, 2011). Negative effects of Pb on avian health have been reported and include tumors and genetic, embryological, reproductive, neurological, and behavioral disorders (Burger and Gochfeld, 1995, 2005; Moreira and Moreira, 2004).

Cadmium, another toxic and non-essential element, was widely used as paint pigmentation (Fiedler and Bayard, 1986; Baird and Cann, 2011) and is still present in plastic products (Angelin et al. 2020). It is naturally available in association with zinc (Zn), as well as anthropogenically deposited in aquatic ecosystems due to industrial waste and the use of fertilizers (Russel, 1994; Spiro and Stigliani, 2008; Baird and Cann, 2011). As observed for Pb, Cd can also alter avian development and metabolism (Burger and Gochfeld, 1997), forming stable halides and replacing calcium (Ca) in bones, eggshells, and other Ca-rich tissues, which weakens their structure (Spiro and Stigliani, 2008). The toxic effects of Cd in wading birds are similar to those reported for Pb. Cd can affect linkages in cell membranes, interfering with the normal functioning of the brain and causing impairments in breeding success and embryo development and survival, usually resulting in tumors and embryonic malformations (Kim and Koo, 2007; Malik and Zeb, 2009). The toxicity of Pb and Cd contaminants depends on absorbed concentrations, which are proportional to those present in the ingested prey (Carvalho et al. 2013; Abdullah et al. 2015). Depending on the timing, duration, and magnitude of exposure, these elements can lead to mortality or, in most cases, to sublethal toxicity (Richardson et al. 1974; Hoffman et al. 1985; Scheuhammer, 1987; Burger and Gochfeld, 2005).

Despite the ecological importance of southern Brazilian wetlands for waterbirds, as well as the potential risk of pollution and disturbance to these wetlands from activities such as hunting, industry emissions, irrigated rice fields, and urbanization, there is a paucity of studies on exposure of Brazilian waterbirds to toxic trace elements (Plaza et al. 2018; Silva et al. 2018). Similarly, high levels of metals (particularly Pb and Cd) have been reported in other ecosystem components, such as water, sediments, fish, and mammals (Waldemarin, 1999; Santos et al. 2003; Barbosa, 2007; Caldas and Sanches-Filho, 2013; Sanches-Filho et al. 2013), which suggests that southern Brazilian coastal environments are potential hotspots of trace element contamination.

In order to assess contaminants in avian wildlife, the use of non-destructive or minimally invasive sampling techniques is of increasing interest (Sánchez-Virosta et al. 2015). Egg sampling is a method with a minor short-term impact on individual birds. The analysis of eggshells, albumin, and yolk is a useful tool for the determination of organic and inorganic contaminants and their potential risk factors for waterbirds. In this study, we compared Pb and Cd levels in eggshells and egg contents of great egrets and roseate spoonbills breeding in estuarine and limnetic habitats. We

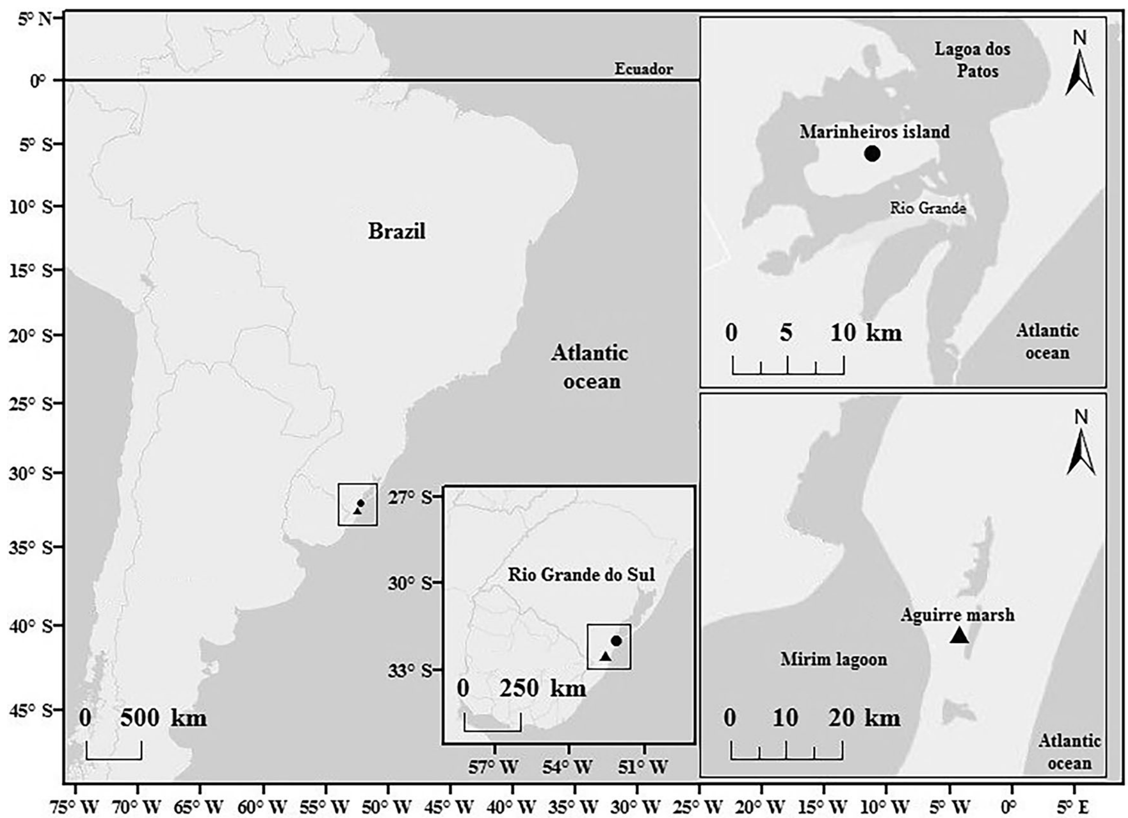

Fig. 1 Study sites in southern Brazil showing the location of the estuarine colony at Marinheiros Island (circle), Lagoa dos Patos estuary, and the limnetic colony in Aguirre marsh (triangle), near Taim Ecological Reserve and Mirim lagoon

hypothesized that egrets would have higher exposure to contaminants than spoonbills, reflecting it in concentrations in eggs, as egrets forage in estuarine and limnetic habitats and upward along the trophic chain during early chick-rearing while spoonbills forage exclusively in limnetic environments (Britto and Bugoni, 2015). We further hypothesized that eggshells would have higher concentrations of Pb (e.g. Mora, 2003) and Cd (e.g. Ashkoo et al. 2020) than egg contents, due to competitive binding and replacement of Ca during eggshell formation and early stages of embryogenesis (e.g. Orłowski et al. 2019). We also compared Pb and Cd concentrations between two colony habitats, one estuarine and one limnetic, to investigate the range of wading birds' metal exposures in the region. We expected to find higher Pb and Cd concentrations in the estuarine habitat compared to the limnetic one, as the estuarine colony is located near an industrial and urban zone. In addition, as the limnetic habitat is near an ecological reserve, but with historical hunting activities, we expected to find higher Pb concentrations in spoonbills compared to egrets, even those showing lower values compared to the estuarine colony, due to spoonbills' tactile foraging technique, which may increase their exposure to accumulated lead pellets in the sediment.

Materials and methods

Study area

The coastal plain in Rio Grande do Sul state, the southernmost state in Brazil, stretches for 600 km in NE-SW directions and is composed of siliceous sand (Farion, 2007). The terrain is flat, resulting in lakes and lagoons with few or no tributaries (Mirlean et al. 2005). One of the most important barrier systems in the region is the limnetic-estuarine Patos-Mirim hydrological system, which spreads along most of the plain.

The Lagoa (lagoon) dos Patos occupies an area of 10,300 km^2, with the estuarine portion occupying about 10% of the total area, i.e. ~970 km^2 (Garcia and Vieira, 2001). Water level and salinity are variable in the estuary and are strongly influenced by rainfall and wind, which determine discharge levels and make waters in the estuarine portion slightly saline year-round, with salinity higher in the summer (Fontoura, 2004). The Lagoa dos Patos estuary is a key area for Pelecaniformes in South America (Kushlan and Hancock, 2005). Marinheiros Island (32°04′S, 52°10′W, Fig. 1) is located 18 km from the mouth of Lagoa dos Patos in the Atlantic Ocean and 6 km northeast of Rio Grande city. Every spring-summer, great egrets, roseate spoonbills, cocoi herons (*Ardea cocoi*), snowy egrets (*Egretta thula*), little blue egrets (*Egretta caerulea*), yellow-crowned night herons (*Nyctanassa violacea*), cattle egrets (*Bubulcus ibis*), black-crowned night herons (*Nycticorax nycticorax*) (Gianuca et al. 2008), and bare-faced ibises (*Phimosus infuscatus*) (Barreto, Faria, and Bugoni, unpubl. data), nest in a large mixed colony, hereafter termed "estuarine habitat".

South of Rio Grande city is the Aguirre marsh, near Mirim lagoon, and the Estação Ecológica do Taim (ESEC Taim), an ecological reserve and Ramsar Site approximately 33,000 ha in size. Irrigated rice cultivation is the predominant economic activity in the region, interspersed with pasture cattle ranching (Marques and Villanueva, 2001). The waterbird colony at Aguirre marsh is in a limnetic environment (32°29′S, 52°32′W), 66 km south from the estuarine colony and 60 km south of Rio Grande city (Fig. 1), hereafter termed "limnetic habitat". The same species occur here as in the estuarine colony, except for the absence of estuarine species such as the little blue egret and the yellow-crowned night heron, and the presence of nesting plumbeous ibis (*Theristicus caerulescens*). Nests are located 5.3 km from Mirim lagoon and 16 km straight line distance from the ocean. This colony had been previously studied by Miño et al. (2009), Britto and Bugoni (2015), and Faria et al. (2016), and breeding occurs there every year, except when water levels are very low (Barreto, Faria, and Bugoni, unpubl. data).

Sampling and sample preparation

We obtained 48 eggs (12 from each species and habitat) from November 2011 to February 2012, during the austral spring and summer. We randomly sampled a single egg from each nest with three or more eggs to avoid the removal of the whole clutch and to maintain independence between samples. We only collected eggs that were within eight days of being laid, determined by the water flotation method (Westerskov, 1950; Liebezeit et al. 2007), representing early incubation stages (i.e. up to 14 days of development; White et al. 1982; Kushlan and Hancock, 2005). We kept the eggs in egg boxes, to avoid breakage, and refrigerated (up to 4 °C) until analysis. In the laboratory, we opened the eggs and visually determined the presence/absence of an embryo, using only the samples with no visible embryo. Our final sample size was 9 eggs of great egrets and 10 eggs of roseate spoonbills from the estuarine habitat, and 10 eggs of great egrets and 12 eggs of roseate spoonbills from the limnetic habitat.

Egg sample preparation consisted of separating egg contents (albumin+yolk representing a single sample) from eggshells. The analysis of separate components allowed us to interpret the differential metal detoxification patterns, as well as provided insights on potential exposure to the embryo. We washed the eggshells in an ultrasonic bath with hydrogen peroxide for 10 min, followed by deionized water for another 10 min, to ensure the removal of surficial residuals. We then oven-dried eggshells and contents at 60 °C for 24 to 36 h, weighed, and acid-digested them for 24 h with 2 mL of nitric acid per 0.1 g of dry sample.

We diluted the digested eggs to a final volume of 20 mL with ultra-pure deionized water. We determined Cd concentrations with a flame atomic absorption spectrophotometer (model AAS-932 Plus, GBC, Hampshire, Illinois, USA) at the Zoophysiology Laboratory, Biological Sciences Institute, Universidade Federal do Rio Grande - FURG, Rio Grande, Rio Grande do Sul, Brazil. The concentration determinations were based on a standard curve ($R^2adj = 0.9651$) built with known standard solutions (NIST, USA, R ≤ 100%). We analyzed the concentrations of Pb in a graphite furnace atomic absorption spectrophotometer (model ContrAA 800, Analytik Jena, Germany). The concentration determinations were based on a standard curve ($R^2adj = 0.9896$) built with a multi-element standard solution (1000 mg/L, Merck, Germany, R ≤ 88%). Procedures for quality control and assurance included running blanks (0.02, 0.05, and 0.10 mg/kg), spiking matrices, and digesting and measuring concentrations of certified reference materials of water, fish muscle and liver (DORM-4, DOLT-5), and lobster hepatopancreas (TORT-3, National Research Council, Canada), as well as a modified matrix for each metal in the graphite furnace AAS. The limit of detection (LOD) was three times the standard deviation (SD) value of the blank signals (3 × SD; $n = 10$), corresponding to 0.050 mg/kg for Cd and 0.005 mg/kg for Pb. The limit of quantification (LOQ) was ten times the SD value of the blank signals (10 × SD; $n = 10$), corresponding to 0.167 mg/kg for Cd and 0.017 mg/kg for Pb. Concentrations of Pb and Cd are presented in mg/kg, dry weight (dw).

Data analysis

We calculated the concentrations of Pb and Cd for each egg component and presented the results as minimum, maximum, and geometric mean ± 1 standard deviation (SD). We compared Pb and Cd concentrations in eggshells and egg contents between species (2 levels; egret and spoonbill), habitat (2 levels; estuary and limnetic), and their interactions. We used a full Generalized Linear Model (GLM) for each element and tissue, using gamma distribution (chosen by the data distribution, which had right-skewed data values) and inverse linking function (McCullagh and Nelder, 1989). We used ANOVA (*F* test) as a post-test to identify the explicability of the variables in each model. To test the normality of the residuals, we performed a Shapiro-

Wilks test for both elements, also considering the graphical representations (Knief and Forstmeier, 2021). For homoscedasticity verification, we analyzed the graphs (Residuals vs. Fitted and Scale-location) of each model's residuals. Finally, we performed a Durbin-Watson test for independence of the residuals. We then investigated correlations in concentrations between tissues (eggshell and egg content of the same egg), using a non-parametric Spearman rank correlation coefficient (Zar, 2010), as the data did not meet parametric presumptions. All analyses were carried out in R software version 4.2.1 (R Core Team, 2022), using the packages *car* version 3.1-0 (Fox and Weisberg, 2019) and *FSA* version 0.9.3 (Ogle et al. 2022). The *p*-values were regarded as significant if <0.05. For graphical representation, we used the packages *ggplot* version 3.3.6 (Wickham, 2016) and *ggpubr* (Kassambara, 2020) in R software.

Results

Lead and cadmium concentrations in great egrets and roseate spoonbills were above the detection limit for all eggshell and egg content samples in both estuarine and limnetic habitats (Fig. 2). Geomean concentrations of Pb were higher in great egrets from the estuary for both eggshell (0.25 ± 0.09 mg/kg dw) and egg content (0.22 ± 0.09 mg/kg dw) compared to egrets from the limnetic habitat (eggshell 0.17 ± 0.03 mg/kg dw, egg content 0.19 ± 0.05 mg/kg dw) and roseate spoonbills from both habitats (eggshell: estuary 0.24 ± 0.07 mg/kg dw, limnetic 0.21 ± 0.05 mg/kg dw, egg content: estuary 0.19 ± 0.05 mg/kg dw, limnetic 0.19 ± 0.08 mg/kg dw). Great egrets from the estuary also had the highest measured values for eggshell concentrations (min. = 0.18 mg/kg dw, max. = 0.46 mg/kg dw, Fig. 2) in comparison to egrets from the limnetic habitat (min. = 0.12 mg/kg dw, max. = 0.22 mg/kg dw) and spoonbills from both habitats (estuary: min. = 0.17 mg/kg dw, max. = 0.42 mg/kg dw; limnetic: min. = 0.14 mg/kg dw, max. = 0.35 mg/kg dw). On the other hand, roseate spoonbills had the highest egg content concentrations, with the highest measured Pb concentration at the limnetic habitat (min. = 0.09 mg/kg dw, max. = 0.40 mg/kg dw) and at the estuary (min. = 0.13 mg/kg dw, max. = 0.38 mg/kg dw) compared to great egrets from both habitats (estuary: min. = 0.12 mg/kg dw, max. = 0.35 mg/kg dw; limnetic: min. = 0.11 mg/kg dw, max. = 0.28 mg/kg dw).

Habitat was a significant factor in predicting Pb levels in eggshells ($p < 0.01$; Table 1). The model explained 33.3% of the variability in the concentrations (Table 2), with

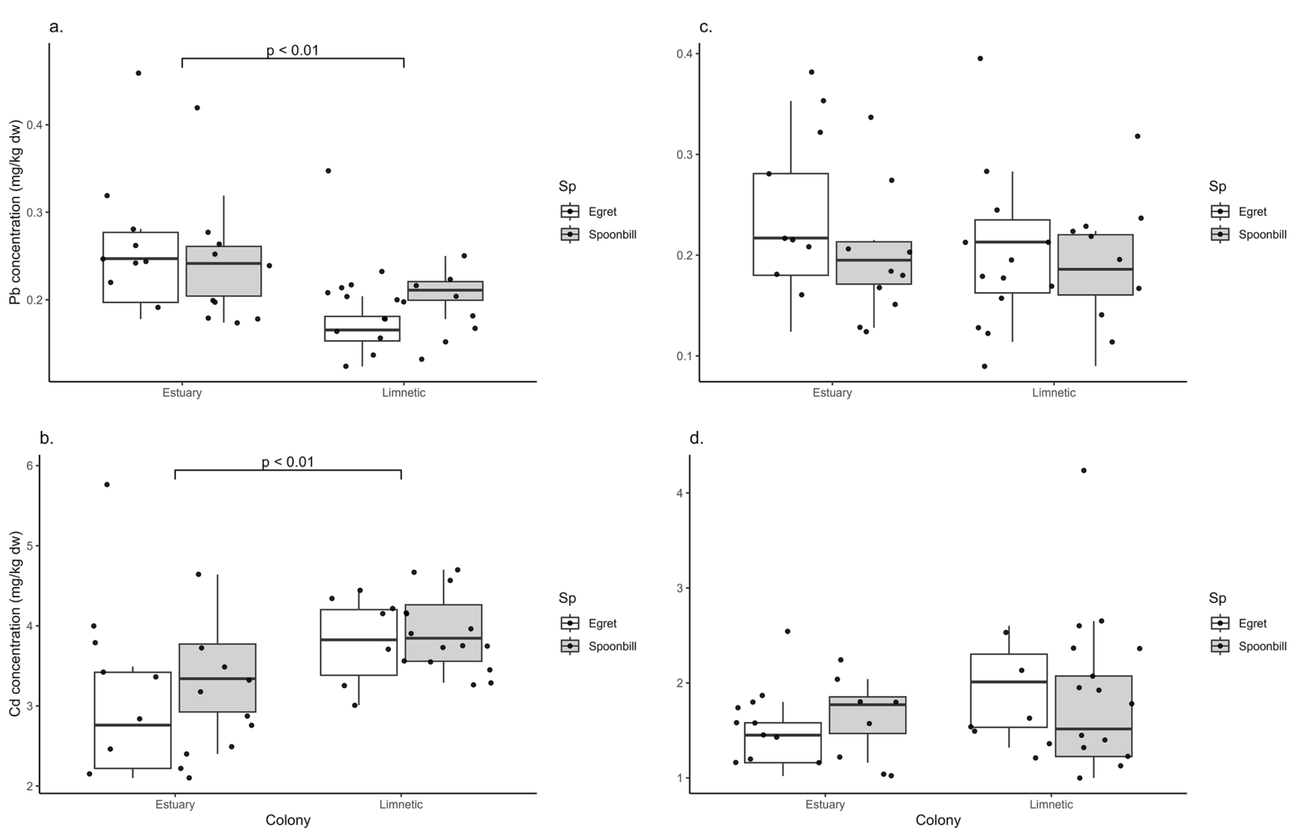

Fig. 2 Mean Pb (**a**) and Cd (**b**) concentrations (mg/kg dry weight) in eggshells and mean Pb (**c**) and Cd (**d**) concentrations (mg/kg dry weight) in egg content (albumen+yolk) of great egrets *Ardea alba* (egret) and roseate spoonbills *Platalea ajaja* (spoonbill) sampled at the estuary and limnetic colonies in southern Brazil. Differences among habitats are seen only for Pb and Cd concentrations in eggshells

colony habitat explaining the bulk of the variance (22.9%). Unlike the eggshells, neither species, colony habitat, nor their interaction were significant factors in predicting Pb concentrations in egg contents (Table 1). The model explained only 3.4% of the variability for Pb concentrations (Table 2).

For Cd, geomean values were higher at the limnetic habitat. Spoonbills had the highest geomean for eggshell (3.93 ± 0.49 mg/kg dw) compared to egrets from both habitats (estuary 2.88 ± 1.15 mg/kg dw, limnetic 3.78 ± 0.50 mg/kg dw) and spoonbills from the estuary (3.31 ± 0.70 mg/kg dw). For egg content, egrets had the highest geomean (1.90 ± 0.45 mg/kg dw) compared to egrets from the estuary (1.41 ± 0.40 mg/kg dw) and spoonbills from both habitats (estuary 1.68 ± 0.41 mg/kg dw, limnetic 1.67 ± 0.92 mg/kg dw). Similar to the patterns observed for Pb concentrations, great egrets from the estuary had the highest Cd eggshell concentrations (min. = 2.10 mg/kg dw, max. = 5.76 mg/kg dw, Fig. 2) in comparison to egrets from the limnetic habitat (min. = 3.01 mg/kg dw, max. = 4.44 mg/kg dw) and spoonbills from both habitats (estuary: min. = 2.40 mg/kg dw, max. = 4.64 mg/kg dw; limnetic: min. = 3.29 mg/kg dw, max. = 4.70 mg/kg dw). Conversely, as observed for Pb, roseate spoonbills had the highest Cd concentrations in egg content at the limnetic habitat (min. = 1.00 mg/kg dw, max. = 4.24 mg/kg dw) and at the estuary (min. = 1.16 mg/kg dw, max. = 2.54 mg/kg dw) compared to great egrets from both habitats (estuary: min. = 1.02 mg/kg dw, max. = 2.24 mg/kg dw; limnetic: min. = 1.32 mg/kg dw, max. = 2.60 mg/kg dw).

Similar to Pb models, habitat was again a significant factor in predicting Cd concentrations in eggshells ($p = 0.04$, Table 3). The model explained 21.1% of the variability in Cd levels (Table 4) and, as observed for Pb, colony habitat explained most of the variance (17.8%). Unlike the eggshells, species, colony habitat, and their interaction were not significant factors in predicting Cd concentrations in egg contents (Table 3). The model explained only 10.4% of Cd concentrations (Table 4).

Lead and cadmium showed a significant positive correlation in egg contents ($r_s = 0.565$; $p < 0.01$), indicating that both metals may be 'excreted' together during egg formation (Table 5). On the other hand, concentrations of the same element between eggshell and egg content, from the same egg (Table 5), were not correlated for Pb ($r_s = -0.133$; $p = 0.41$) nor Cd ($r_s = 0.025$; $p = 0.88$).

Table 1 Coefficients from the GLMs analysis explaining Pb concentrations (mg/kg dry weight) in eggshell and egg content samples

Term	Estimate	Standard Error (SE)	*t*	*p*
Eggshell				
Intercept	**3.88**	**0.33**	**11.61**	**<0.01**
Species (Spoonbill)	0.09	0.47	0.20	0.85
Colony (Limnetic)	**2.07**	**0.59**	**3.51**	**<0.01**
Species:Colony	−1.42	0.76	−1.88	0.07
Egg Content				
Intercept	**4.28**	**0.51**	**8.43**	**<0.01**
Species (Spoonbill)	0.33	0.73	0.46	0.65
Colony (Limnetic)	0.73	0.76	0.96	0.34
Species:Colony	−0.37	1.05	−0.35	0.73

Factors in each model included species (spoonbill, *Platalea ajaja*, and great egret, *Ardea alba*), colony habitat (estuary and limnetic), and the interactions between them. The intercept represents the Pb concentrations in great egret at the estuarine colony, in relation to the other factors

Significant terms ($p < 0.05$) are in bold

Table 2 Summary of results of concentrations of Pb models in eggshell and egg content samples of Brazilian egrets (*Ardea alba*) and roseate spoonbills (*Platalea ajaja*) obtained for the GLM

Source of variation	df	Deviance	% explained	df residuals	Residual deviance	*F*	*p*
Eggshell							
Null				40	3.28		
Species	1	0.10	3.08	39	3.18	1.51	0.23
Colony	**1**	**0.75**	**22.95**	**38**	**2.42**	**11.26**	**<0.01**
Species:Colony	1	0.24	7.32	37	2.18	3.60	0.07
Total Explained	3	1.09	33.35				
Egg Content							
Null				40	4.66		
Species	1	0.01	0.28	39	4.65	0.10	0.75
Colony	1	0.13	2.87	38	4.51	1.06	0.31
Species:Colony	1	0.02	0.32	37	4.50	0.12	0.73
Total Explained	3	0.16	3.48				

The % explained is calculated as deviance/residual deviance of the null model × 100

Significant terms ($p < 0.05$) are in bold

Concentrations of Pb and Cd in eggshells were also not significantly correlated ($r_s = -0.106$; $p = 0.51$; Table 5).

Discussion

Waterbird nesting habitat was the main predictor of concentrations of Pb and Cd in eggshells of egrets and spoonbills. Our results showed higher Pb concentrations in eggshells and egg contents from the estuarine habitat, partially matching our hypothesis of higher contamination at the estuary due to its proximity to an industrial and urbanized area. However, Cd concentrations in eggs were lower at the estuary compared to the limnetic habitat, which is the opposite of what we expected. An increased concentration of pollutants in the limnetic habitat compared to the estuary is possibly explained by wind dispersion. In a previous study, elevated Pb concentrations in the air were found south of Rio Grande city during the spring and summer (Vanz et al. 2003), influenced by the predominant northeast winds (Braga and Krusche, 2000), which explains higher concentrations of Cd at the limnetic habitat. Eggshells had higher concentrations compared to egg contents, as predicted, likely due to metals' ability to interact with calcium-binding sites (Spiro and Stigliani, 2008) thereby depositing these non-essential elements preferentially in eggshells. Great egrets had the highest Pb concentrations in both tissues at the estuary, while spoonbills had the higher values at the limnetic colony, corroborating our expectations. Great egrets only showed higher Cd values for egg content at the limnetic colony, partially corroborating our

Table 3 Coefficients from the GLMs analysis explaining Cd concentrations (mg/kg dry weight) in eggshell and egg content samples

Term	Estimate	Standard Error (SE)	*t*	*p*
Eggshell				
(Intercept)	**0.33**	**0.03**	**13.40**	**<0.01**
Species (Spoonbill)	−0.03	0.03	−1.04	0.31
Colony (Limnetic)	**−0.07**	**0.03**	**−2.19**	**0.04**
Species:Colony	0.02	0.04	0.58	0.57
Egg Content				
(Intercept)	**0.69**	**0.08**	**8.71**	**<0.01**
Species (Spoonbill)	−0.11	0.10	−1.06	0.30
Colony (Limnetic)	−0.18	0.10	−1.80	0.08
Species:Colony	0.14	0.13	1.09	0.28

Factors in each model included species (spoonbill, *Platalea ajaja*, and great egret, *Ardea alba*), colony habitat (estuary and limnetic), and the interactions between them. The intercept represents the Cd concentrations in great egret at the estuarine colony, in relation to the other factors

Significant terms ($p < 0.05$) are in bold

Table 5 Spearman correlations (r_s) of Pb and Cd concentrations (mg/kg dry weight) between eggshells and egg contents (from the same egg), and Cd *versus* Pb in eggshells and egg contents, of great egret (*Ardea alba*) and roseate spoonbill (*Platalea ajaja*) sampled in estuarine and limnetic environments in southern Brazil

Trace element	Eggshell vs. Egg content		Eggshell		Egg content	
	r_s	*p*	r_s	*p*	r_s	*p*
Pb	−0.13	0.41	–	–	–	–
Cd	0.03	0.88	–	–	–	–
Pb vs. Cd	–	–	−0.12	0.45	**0.55**	**<0.01**

Values from both colony habitats and species were pooled for analysis

Significant correlations after Bonferroni correction ($p < 0.05$) are in bold

Table 4 Summary of results of concentrations of Cd models in eggshell and egg content samples of Brazilian egrets (*Ardea alba*) and roseate spoonbills (*Platalea ajaja*) obtained for the GLM

Source of variation	df	Deviance	% explained	df residuals	Residual deviance	*F*	*p*
Eggshell							
Null				40	2.07		
Species	1	0.05	2.46	39	2.02	1.02	0.32
Colony	**1**	**0.37**	**17.82**	**38**	**1.65**	**7.37**	**0.01**
Species:Colony	1	0.02	0.82	37	1.63	0.33	0.57
Total Explained	3	0.44	21.10				
Egg Content							
Null				40	4.19		
Species	1	0.02	0.36	39	4.17	0.13	0.72
Colony	1	0.28	6.66	38	3.90	2.35	0.13
Species:Colony	1	0.14	3.39	37	3.75	1.20	0.28
Total Explained	3	0.44	10.41				

The % explained is calculated as deviance/residual deviance of the null model × 100

Significant terms ($p < 0.05$) are in bold predictions that egrets would have higher Cd and Pb concentrations than spoonbills due to their distinct feeding habits.

The concentrations of Pb in eggshells analyzed in our study sites were, in general, lower than those recorded in other colonies of waterbirds worldwide. Mean concentrations of Pb in eggshells of great egrets from the estuary in our study were 27 times lower than recorded for grey herons (*Ardea cinerea*; 6.827 mg/kg dw) and 4 times lower than eggshells of black-crowned night heron (3.81 mg/kg dw) from a heronry close to a thermal power plant in Turkey (Ayas, 2007). Mean Pb concentrations of great egrets from the limnetic habitat were twice as low as eggshell concentrations of grey herons from a heronry with reports of active hunting in Poland (0.39 mg/kg dw; Kitowski et al. 2013; 0.47 mg/kg dw; Kitowski et al. 2014).

Contrastingly, Cd concentrations in eggshells analyzed in our study sites were much higher than documented for other waterbird colonies across the globe. Mean concentrations of Cd in eggshells of great egrets from the estuary in our study are up to four times higher than in eggshells of grey herons (0.931 mg/kg dw), and 16 times higher than eggshells of black-crowned night heron from Turkey (3.81 mg/kg dw; Ayas, 2007). Cd concentrations of eggshells of great egrets from the limnetic habitat in our study were 63 times higher than grey herons from Poland (0.06 mg/kg dw; Kitowski et al. 2013, 2014). The Pb mean concentrations in egg contents of great egrets from the estuary in our study were up to 45 times higher than the means reported in great egrets in the New York City harbor in the USA (5.1 ng/g dw; Burger and Elbin, 2015), but up to four times lower than in the little egret (*Egretta garzetta*) in an environment close to urban and industrial activities in Pakistan (0.89 µg/g dw; Shabahz et al. 2013). Concentrations of Cd in egg contents of great egrets from the estuary in our study were more than 400 times higher than in great egrets in the USA (3.5 ng/g dw; Burger and Elbin, 2015), and 6 times higher than in little egrets from Pakistan (0.25 µg/g dw; Shabahz et al. 2013).

Different environmental characteristics and their interactions influence the bioavailability of trace elements in different geographical locations (Riba et al. 2003; Dutton and Fisher, 2011) and, consequently, the chances for absorption of metals through diet (Carvalho et al. 2013; Abdullah et al. 2015). While egrets are known to feed in both limnetic and estuarine environments when breeding in the estuarine colony, spoonbills obtain food only in limnetic environments when foraging and feeding chicks in southern Brazil (Britto and Bugoni, 2015). This spatial difference in dietary prey sources was confirmed in another study at our same sampling sites using isotope values of carbon ($\delta^{13}C$) of great egrets and roseate spoonbills, which is used as a proxy for identification of foraging sites (see Britto and Bugoni, 2015 for detailed information). Regardless, both species had similar nitrogen isotopic signatures ($\delta^{15}N$), used as a proxy for trophic level (Britto and Bugoni, 2015). Consequently, considering that egrets and spoonbills are central-place foragers (Craig et al. 2015; Winkler et al. 2020a, 2020b), arriving at the colony several weeks before reproduction for the defense of territories, courtship, and nest construction, and eggshell production occurs a few hours after copulation (Nys et al. 2004), we suggest the potential foraging site as a strong driver of trace elements concentrations in eggshells and egg contents. As spoonbills forage only at limnetic environments, they have higher exposure to Cd in both habitats and higher exposure to Pb at the limnetic site.

The absorption of contaminants by egrets in southern Brazil seems related directly to trace element bioavailability in their foraging areas and consumption of contaminated prey. There is evidence of Pb and Cd contamination in fish species usually consumed by egrets (Sanches-Filho et al. 2013), as well as in the sediment of the Lagoa dos Patos estuary (Santos et al. 2003; Barbosa, 2007; Caldas and Sanches-Filho, 2013). Although we are unaware of studies of Cd concentrations in prey or sediment at the limnetic habitat, our results showed higher concentrations of this element in eggs of great egrets and spoonbills from the Aguirre marsh colony, with values even higher for spoonbills. Studies of Cd concentrations are needed to provide information on contamination sources and the effects of non-essential elements on fish and waterbirds in the limnetic habitat. The area is important not only from an ecological perspective but also economically, especially for rice production and cattle ranching activities (Marques and Villanueva, 2001). Consequently, the bioavailability of non-essential elements exposes other animals, and even humans, to contamination.

Another potential source of contamination is the deposition of Pb through spent ammunition from hunting, especially near the limnetic habitat (Guadagnin et al. 2007). As Pb and Cd levels in egg contents were highly correlated, females of great egrets and spoonbills may be absorbing these elements. As the non-essential elements are bioavailable at their prey and foraging sites, females may then be ingesting shot pellets and bioavailable elements and then excreting both metals through egg laying. Females are known to increase their calcium (Ca) ingestion pre-laying (Perrins, 2008), which may increase the uptake and assimilation of Pb and Cd due to competition for Ca binding sites (Spiro and Stigliani, 2008). Females may then excrete metals into the egg using the same detoxification pathways for both elements (Koster et al. 1996). Toxic elements can still become available to the embryos due to binding competition with essential elements such as Ca, Zn, and copper (Cu) (Spiro and Stigliani, 2008), leading to an increase of non-essential and a decrease of essential elements in bird tissues (Nunes et al. 2021). Metal uptake by embryos can be gradually absorbed from the egg (Orłowski et al. 2016, Orłowski et al. 2019) and affect developing organs (Yamamoto et al. 2012; Ali et al. 2024), leading to embryonic toxicity and reduced survival (Orłowski et al. 2016; Vallverdú-Coll et al. 2016).

Chicks are potentially exposed to chronic effects in cases where the concentration of toxic elements is not high enough to replace Zn and, consequently, decrease egg viability, as Zn is directly related to egg quality (Sahin et al. 2002). Neurological effects, such as the decreased ability to thermoregulate, difficulty in locomotion, and decreased learning and recognition were reported in gull chicks exposed to a dose of 100 mg/kg of Pb (Burger and Gochfeld, 2005). Moreover, as Cu and Zn have a role in gene activation and homeostasis in eukaryotic cells (Rutherford and Bird, 2004), and both Pb and Cd can disrupt the optimal concentrations of both essential elements in the organism (Spiro and Stigliani, 2008; Nunes et al. 2021), effects such as homeostatic dysregulation and a decrease in immune responses may be observed if the chicks absorb and accumulate Pb and Cd in concentrations high enough to replace Cu and Zn in their bloodstream. The detoxification pathways (feathers, eggshells, and excreta) also contribute to the transport of matter from the aquatic to the terrestrial environments (Shoji et al. 2019), which was observed in our estuarine habitat (Caseiro-Silva et al. 2023). Consequently, high concentrations of Pb and Cd absorbed by egrets and spoonbills through diet can lead to an increase of non-essential elements in terrestrial environments, as eggshells and carcasses may increase the concentrations of trace elements in the terrestrial environment where colonies are located, leading to a biochemical disequilibrium in these environments and their trophic chain.

Even lacking a long-term measurement of the avian exposure to non-essential elements, our findings provide some of the first evidence that egrets and spoonbills are exposed to toxic trace elements, especially Cd, representing chronic intoxication risk. Pb levels in eggshells and egg contents exceeded environmental regulations in Brazil (up to 0.033 mg/L in freshwater ecosystems; Brasil, 2012), and in the USA (up to 0.015 mg/L in drinking water; EPA, 1984), for example, which also happened with Cd thresholds in both Brazil (0.01 mg/L in freshwater ecosystems; Brasil, 2012) and the USA (up to 0.002 mg/L in freshwater ecosystems; EPA, 2016). Since the aquatic environments studied and where wading birds forage and breed have been historically used for agricultural or hunting purposes, receive drainage from urban areas, and have abiotic properties that favor trace elements uptake, such as pH and dissolved organic matter, there is a concern for avian health, as well as other species. Therefore, studies involving the determination of trace elements in other ecosystemic components (e.g. water and soil), as well as considering different sampling approaches (e.g. account for egg laying order [Orłowski et al. 2016] and in different embryo stages [Orłowski et al. 2019]), which are lacking in our study, can provide important information regarding the direct effects of contaminants on the region. Besides, higher eggshell concentrations compared to egg content and body tissues also suggest that eggshells are an ideal indicator of avian contamination by metals (Ayas, 2007; Burger and Elbin, 2015) as they can be obtained non-lethally, particularly if using eggshells left behind after chick hatching. We recommend the use of eggs, notably eggshells, for monitoring the contamination of birds, as concentrations in eggs can provide sensitive measures to indicate environmental health.

Conclusion

Great egrets and roseate spoonbills from two habitats, an estuarine and a limnetic colony, studied in southern Brazil are exposed to toxic trace elements, likely from anthropogenic sources. The eggshells and egg contents analyzed in our study show elevated concentrations of the non-essential elements Pb and Cd when compared to similar waterbird species and environments worldwide. Our results suggest ongoing contamination in the region, especially by Cd, as the concentrations in eggs are higher than the published environmental standards (Pb: 0.033 mg/L in Brazil and 0.015 mg/L in the USA, Cd: 0.01 mg/L in Brazil and 0.002 mg/L in the USA; Brasil, 2012; EPA, 1984; EPA, 2016). Colony habitat is an important predictor for Pb and Cd concentrations, especially in eggshells. Moreover, the use of distinct environments and different feeding strategies by waterbirds suggests that abiotic environmental conditions, foraging techniques, and consumed prey can influence the bioavailability and absorption of trace elements during egg laying. Therefore, Pb and Cd contamination in waterbirds breeding in estuarine and limnetic areas can be considered ubiquitous. Long-term monitoring using integrative methods, such as trace elements analysis in different embryo stages and considering the egg laying order, combined with stable isotopes of eggs, consumed prey, and sediment, coupled with stronger environmental regulations, would provide information about the health and conservation of wildlife, trends in contamination sources, and hopefully improvements in ecosystem quality in the region. Moreover, eggshells seem to be sensitive bioindicators, even at low concentrations of inorganic contaminants, as they represent a short period of ingestion, detect contamination close to the colony, are easy to collect, and avoid handling and disturbing birds. Eggs reliably indicate environmental conditions, especially for species with distinct diets, allowing for inferences about trace element contamination in species of conservation concern.

Data availability

The data that support the findings of this study are available from the authors upon request.

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Author contributions CTB and LB contributed to the study conception and design. Sample collection was performed by CTB, FAF, and LB. Material preparation and sample analysis were performed by CTB and AB. Data analysis was performed by CTB. The first draft of the manuscript was written by CTB, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

Ethical approval All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted (Comissão de Ética em Uso Animal - CEUA), Universidade Federal do Rio Grande - FURG (#P037/2011); Instituto Chico Mendes de Conservação da Biodiversidade - ICMBio (SISBIO #29999-1).

References

Abdullah M, Fasola M, Muhammad A, Malik SA, Bostan N, Bokhari H, Kamran MA, Shafqat MN, Alamdar A, Khan M, Ali N, Eqani SAMAS (2015) Avian feathers as non-destructive bio-monitoring tool of trace metals signatures: a case study of severely contaminated areas. Chemosphere 119:553–561

Ali RA, Awadalla EA, Hamed AS, Mostafa DEF (2024) Cardiotoxicity of cadmium and its effects on heart efficiency during early and late chick embryogenesis. Cardiovasc Toxicol 24:982–1003

Angelin EM, Ghirardello M, Babo S, Picollo M, Chelazzi L, Melo MJ, Nevin A, Valentini G, Comelli D (2020) The multi-analytical in situ analysis of cadmium-based pigments in plastics. Microchem J 157:105004

Antas PTZ (1994) Migration and other movements among the lower Paraná River valley wetlands, Argentina, and south Brazil/Pantanal wetlands. Bird Conserv Int 4:181–190

Ashkoo A, Amininasab SM, Zamani-Ahmadmahmoodi R (2020) Bioaccumulation of heavy metals in eggshell and egg content of seabirds: lesser (*Thalasseus bengalensis*) and greater Crested tern (*Thalasseus bergii*). Mar Pollut Bull 154:111126

Ayas Z (2007) Trace element residues in eggshells of grey heron (*Ardea cinerea*) and black-crowned night heron (*Nycticorax nycticorax*) from Nallihan Bird Paradise, Ankara-Turkey. Ecotoxicology 16:347–352

Baird C, Cann M (2011) Química ambiental, 4th ed. Bookman, Porto Alegre

Barbosa FG (2007) Metais traço nas águas de superfície do porto da cidade do Rio Grande (estuário da Lagoa dos Patos). Rev Urutágua. http://www.urutagua.uem.br/011/11barbosa.htm Accessed 08 December 2019

Bell WJ (1990) Central place foraging. In: Searching behavior Chapman and Hall Animal Behavior Series. Springer, Dordrecht. https://doi.org/10.1007/978-94-011-3098-1_12 Acessed 30 January 2022

Belton W (1994) Aves do Rio Grande do Sul: distribuição e biologia. Unisinos, São Leopoldo

Braga MFS, Krusche N (2000) Padrão de ventos em Rio Grande, RS, no período de 1992 a 1995. Atlântica 22:27–40

Brasil (2012) Resolução N°. 357, de 17 de Março de 2005, Conselho Nacional do meio Ambiente – CONAMA. Dispõe sobre a classificação dos corpos de água e diretrizes ambientais para o seu enquadramento, bem como estabelece as condições e padrões de lançamento de efluentes e dá outras providências. http://conama.mma.gov.br/images/conteudo/LivroConama.pdf Accessed 18 April 2019

Britto VO, Bugoni L (2015) The contrasting feeding ecology of great egrets and roseate spoonbills in limnetic and estuarine colonies. Hydrobiologia 744:187–210

Burger J, Gochfeld M (1995) Growth and behavioral effects of early postnatal chromium and manganese exposure in herring gull (*Larus argentatus*) chicks. Pharmachol Biochem Behav 50:607–612

Burger J, Gochfeld M (1997) Heavy metal and selenium concentrations in feathers of egrets from Bali and Sulawesi, Indonesia. Arch Env Contam Toxicol 32:217–221

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

Burger J, Gochfeld M (2005) Effects of lead on learning in herring gulls: an avian wildlife model for neurobehavioral deficits. Neurotoxicology 26:615–624

Burger J, Elbin S (2015) Metal levels in eggs of waterbirds in the New York Harbor (USA): trophic relations and possible risk to human consumers. J Toxicol Environ Health A 78:78–91

Caldas JS, Sanches-Filho PJ (2013) Determinação de Cu, Pb e Zn no sedimento da região do Pontal da Barra, Laranjal (Lagoa dos Patos, Pelotas – RS, Brasil). Braz J Aquat Sci Technol 17:13–18

Carvalho PC, Bugoni L, McGill RAR, Bianchini A (2013) Metal and selenium concentrations in blood and feathers of petrels of the genus *Procellaria*. Env Toxicol Chem 32:1641–1648

Caseiro-Silva F, Faria FA, Barreto CT, Fernandez CN, Bugoni L (2023) Colonial waterbirds provide persistent subsidies to swamp forests along an estuarine island food chain. Oecologia 202:113–127

Corriveau A, Klaassen M, Garnett ST, Kaestli M, Power MW, Mousavi-Derazmahalleh, Coghlan ML, Christian K, Bunce M, Campbell HA (2022) Using dietary metabarcoding analysis to characterize waterbirds-agriculture interaction. J Appl Ecol 59:2756–2766

Corseuil HX, Marins MDM (1997) Contaminação de águas subterrâneas por derramamentos de gasolina: O problema é grave? Eng Sanit Ambient 2:50–54

Craig EC, Elbin SB, Sparks JP, Curtis PD (2015) Identifying important foraging habitats for colonial waterbirds in an urban estuary: a stable isotope approach. Waterbirds 38:330–338

Duarte RPS, Pascal A (2000) Avaliação do cádmio (Cd), chumbo (Pb), níquel (Ni) e zinco (Zn) em solos, plantas e cabelos humanos. Energ Agric 15:1–13

Dumas JV (2000) Roseate spoonbill (*Platalea ajaja*), version 2.0. In: Poole A, Gill FB (eds). The birds of North America. Cornell Lab of Ornithology, Ithaca. https://doi.org/10.2173/bna.490 Accessed 08 December 2019

Dutton J, Fisher NS (2011) Salinity effects on the bioavailability of aqueous metals for the estuarine killfish *Fundulus heteroclitus*. Env Toxicol Chem 30:2107–2114

EPA (1984) Ambient water quality criteria for Cadmium. Environmental Protection Agency, United States of America. https://www.epa.gov/sites/production/files/2019-03/documents/ambient-wqc-cadmium-1984.pdf Accessed 18 April 2019

EPA (2016) Lead and copper rule revisions. White paper. Environmental Protection Agency, United States of America. https://www.epa.gov/sites/production/files/2016-10/documents/508_lcr_revisions_white_paper_final_10.26.16.pdf Accessed 18 April 2019

Faria FA, Silva-Costa A, Gianuca D, Bugoni L (2016) Cocoi heron (*Ardea cocoi*) connects estuarine, coastal, limnetic and terrestrial environments: an assessment based on conventional dietary and stable isotope analysis. Estuaries. Coast 39:1271–1281

Farion SRL (2007) Litoral do Rio Grande do Sul: rio, lago, lagoa, laguna. Ágora 13:167–186

Ferreyra H, Beldomenico PM, Marchese K, Romano M, Caselli A, Correa AI, Uhart M (2015) Lead exposure affects health indices in free-ranging ducks in Argentina. Ecotoxicology 24:735–745

Fiedler I, Bayard MA (1986) Artists' pigments: a handbook of their history and characteristics, vol 1. Cambridge University Press, Cambridge

Fontoura JAS (2004) Hidrodinâmica costeira e quantificação do transporte longitudinal de sedimentos não coesivos na zona de surfe das praias adjacentes aos molhes da barra do Rio Grande, RS, Brasil. PhD. Thesis, Universidade Federal do Rio Grande do Sul

Fox J, Weisberg S (2019) An *R* companion to applied regression. 3rd ed. Sage, Thousand Oaks

Garcia AM, Vieira JP (2001) O aumento da diversidade de peixes no estuário da Lagoa dos Patos durante o episódio *El Niño* 1997-1998. Atlântica 23:85–96

Gianuca D, Quintela FM, Barros J, Gomes-Jr A, Gianuca NM (2008) Ocorrência regular da garça-azul *Egretta caerulea* (Ciconiiformes, Ardeidae) no estuário da Lagoa dos Patos, Rio Grande do Sul, Brasil. Pan-Amer J Aquat Sci 3:328–334

Gill FB, Prum RO (2019) Ornithology, 4th ed. WH Freeman, Macmillan Learning, New York

Guadagnin DL, Perelló LFC, Menegheti JO (2007) A situação atual da caça de lazer e manejo de áreas úmidas no Rio Grande do Sul. Neotrop. Biol Conserv 2:63–70

Hoffman DJ, Franson JC, Pattee OH, Bunck CM, Anderson A (1985) Survival, growth, and accumulation of ingested lead in nestling American kestrels (*Falco sparverius*). Arch Environ Contam Toxicol 14:89–94

Janaydeh M, Ismail A, Omar H, Zulkifli SZ, Bejo MH, Aziz NAA (2018) Relationship between Pb and Cd accumulations in house crow, their habitat, and food content from Klang area, Peninsular Malaysia. Env Monit Assess 190:47

Kassambara A (2020) 'ggplot2' Based publication ready plots. https://cran.r-project.org/web/packages/ggpubr/ggpubr.pdf Accessed 28 October 2022

Kim E, Koo T (2007) Heavy metal distribution in chicks of two heron species from Korea. Arch Env Contam Toxicol 54:740–747

Kitowski I, Sujak A, Strobel W, Wiacek D, Rymarz M (2013) Trace elements in eggshells of the grey heron (*Ardea cinerea*) from the Roztocze Hills (South East Poland). Zool Ecol 23:240–244

Kitowski I, Sujak A, Wiacek D, Strobel W, Rymarz M (2014) Trace element residues in eggshells of grey heron (*Ardea cinerea*) from colonies of East Poland. North-Western. J Zool 10:346–354

Knief U, Forstmeier W (2021) Violating the normality assumption may be the lesser of two evils. Behav Res 53:2576–2590

Koster MD, Ryckman DP, Weselohb DVC, Strugerc J (1996) Mercury levels in great lakes herring gull (*Larus argentatus*) eggs, 1972-1992. Env Pollut 93:261–270

Kushlan JA, Hancock JA (2005) The herons. Oxford Academic Press, Oxford

Liebezeit JR, Smith PA, Lanctot RB, Schekkerman H, Tulp I, Kendall S, Tracy DM, Rodrigues RJ, Meltofte H, Robinson JA, Gatto-Trevor C, McCaffery B, Morse J, Zack SW (2007) Assessing the development of shorebirds eggs using the flotation method: species-specific and generalized regression models. Condor 109:32–47

Malik RN, Zeb N (2009) Assessment of environmental contamination using feathers of *Bubulcus ibis* L., as a biomonitor of heavy metal pollution, Pakistan. Ecotoxicology 18:522–536

Marques DM, Villanueva AON (2001) Regime hidrológico de banhados e sua conservação. Cad Pesq Sér. Biol 13:63–79

McCrimmon-Jr DA, Ogden JC, Bancroft GT (2011) Great egret (*Ardea alba*), version 2.0. In: Poole AF (ed). The birds of North America. Cornell Lab of Ornithology, Ithaca. https://doi.org/10.2173/bna.570. Accessed 08 December 2019

McCullagh P, Nelder JA (1989) Generalized linear models, 2nd ed. Chapman and Hall/CRC, Boca Raton

Miño CI, Sawyer GM, Benjamin RC, del-Lama SN (2009) Parentage and relatedness in captive and natural populations of the roseate spoonbill (Aves: Ciconiiformes) based on microsatellite data. J Exp Zool Part A 311:453–464

Mirlean N, Larned ST, Nikora V, Kütter VT (2005) Mercury in lakes and lake fishes on a conservation-industry gradient in Brazil. Chemosphere 60:226–236

Mora MA (2003) Heavy metals and metalloids in egg contents and eggshells of passerine birds from Arizona. Env Pollut 125:393–400

Moreira FR, Moreira JC (2004) A importância da análise de especiação do chumbo em plasma para a avaliação dos riscos à saúde. Quím Nova 27:251–260

Newth JL, Cromie RL, Brown MJ, Delahay RJ, Meharg AA, Deacon C, Norton GJ, O'Brien MF, Pain DJ (2013) Poisoning from lead gunshot: still a threat to wild waterbirds in Britain. Eur J Wildl Res 59:195–204

Nunes GT, Efe MA, Barreto CT, Gaiotto JV, Silva AB, Vilela F, Roy A, Bertrand S, Costa PG, Bianchini A, Bugoni L (2021) Ecological trap for seabirds due to the contamination caused by the Fundão dam collapse. Brazil Sci Tot Env 807:151486

Nys Y, Gautron J, Garcia-Ruiz JM, Hincke MT (2004) Avian eggshell mineralization: biochemical and functional characterization of matrix proteins. CR Oalevol 3:549–562

Ogle DH, Doll JC, Wheeler P, Dino A (2022) FSA: fisheries stock analysis. R package version 0.9.3. https://github.com/fishR-Core-Team/FSA Accessed 28 October 2022
Orłowski G, Hałupka L, Pokorny P, Klimczuk E, Sztwiertnia H, Dobicki W (2016) Variation in egg size, shell thickness, and metal and calcium content in eggshells and egg contents in relation to laying order and embryonic development in a small passerine bird. Auk 133:470–483
Orłowski G, Merta D, Pokorny P, Łukaszewicz E, Dobicki W, Kobielski A, Rzońca Z, Krzywiński A (2019) Eggshell resorption, and embryonic mobilization and accumulation of calcium and metals in eggs of wild and captive capercailles *Tetrao urogallus*. Env Pollut 249:152–162
Perrins CM (2008) Eggs, egg formation and the timing of breeding. Ibis 138:2–15
Plaza PI, Uhart M, Caselli A, Wiemeyer G, Lambertucci SA (2018) A review of lead contamination in South American birds: the need for more research and policy changes. Perspect. Ecol Conserv 16:201–207
R Core Team (2022) R: a language and environment for statistical computing. R Foundation for Statistical Computing. Vienna. https://www.R-project.org/ Accessed 09 August 2022
Riba I, García-Luque E, DelValls TA (2003) Bioavailability of heavy metals bound to estuarine sediments as a function of pH and salinity values. Chem Speciat Bioavailab 15:101–114
Richardson ME, Fox MRS, Fry-Jr BE (1974) Pathological changes produced in Japanese quail by ingestion of cadmium. J Nutr 104:323–338
Rutherford JC, Bird AJ (2004) Metal-responsive transcription factors that regulate iron, zinc and copper homeostasis in eukaryotic cells. Eukaryot Cell 3:1–13
Russel (1994) Química geral, 2nd ed., vol 1. Makron Books, São Paulo
Sahin N, Onderci M, Sahin K (2002) Effects of dietary chromium and zinc on egg production, egg quality, and some blood metabolites of laying hens reared under low ambient temperature. Biol Trace Elem Res 85:47–58
Sanches-Filho PJ, Fonseca VK, Holbig L (2013) Avaliação de metais em pescado da região do Pontal da Barra, Laguna dos Patos, Pelotas-RS. Ecotoxicol Env Contam 8:105–111
Sánchez-Virosta P, Espín S, García-Fernández AJ, Eeva T (2015) A review on exposure and effects of arsenic in passerine birds. Sci Total Env 512-513:506–525
Santos IR, Baisch P, Lima GTNP (2003) Metais pesados em sedimentos superficiais da lagoa Mirim, fronteira Brasil-Uruguai. Geochim Bras 17:37–47
Santos MH, Lopes IF, del Lama SN (2008) Population genetic study of mitochondrial DNA in roseate spoonbill (Aves; *Platalea ajaja*) breeding in colonies from Pantanal wetlands, Brazil. Biochem Genet 46:492–505
Scheuhammer AM (1987) The chronic toxicity of aluminum, cadmium, mercury, and lead in birds: a review. Env Pollut 46:263–295
Shabahz M, Hashmi MZ, Malik RN, Yasmin A (2013) Relationship between heavy metals concentrations in egret species, their environment and food chain differences from two Headworks of Pakistan. Chemosphere 93:274–282
Shoji A, Elliott KH, Aris-Brosou S, Mizukawa H, Nakayama SMM, Ikenaka Y, Ishizuka M, Kuwae T, Watanabe K, Gonzalez JE, Watanuki Y (2019) Biotransport of metallic trace elements from marine to terrestrial ecosystems by seabirds. Env Toxicol Chem 38:106–114
Silva RCA, Saiki M, Moreira EG, Oliveira PTMS (2018) The great egret (*Ardea alba*) as a bioindicator of trace element contamination in the São Paulo Metropolitan Region, Brazil. J Radioanal Nucl Chem 315:447–458
Spiro TG, Stigliani WM (2008) Química ambiental, 2nd ed. Pearson Prentice Hall, São Paulo
TRF4 (2008) Tribunal Regional Federal 4ª Região, Rio Grande do Sul. TRF4 proíbe caça amadora no Rio Grande do Sul. Consulta Processual Unificada. https://www.trf4.jus.br/trf4/controlador.php?acao=noticia_visualizar&id_noticia=5702 Accessed 08 December 2019
Vanz A, Mirlean N, Baisch P (2003) Avaliação de poluição do ar por chumbo particulado: uma abordagem geoquímica. Quím Nova 26:25–29
Vallverdú-Coll N, Mougeot F, Ortiz-Santaliestra ME, Castano C, Santiago-Moreno J, Mateo R (2016) Effects of lead exposure on sperm quality and reproductive success in an avian model. Env Sci Technol 50:12484–12492
Waldemarin HF (1999) Análise da contaminação da lontra (*Lontra longicaudis*) por metais pesados em uma unidade de conservação e uma área com forte influência antrópica. MSc. Dissertation, Universidade Federal do Rio Grande do Sul
Weller MW (1999) Wetland birds: habitat resources and conservation implications. Cambridge University Press, New York
Westerskov K (1950) Methods for determining the age of game bird eggs. J Wildl Manage 14:56–67
White DH, Mitchell CA, Cromartie E (1982) Nesting ecology of roseate spoonbills at Nuences Bay, Texas. Auk 99:275–284
Wickham H (2016) ggplot2: elegant graphs for data analysis. Springer-Verlag, New York
Williamson L, Hudson M, O'Connell M, Davidson N, Young R, Amano T, Székely T (2013) Areas of high diversity for the world's inland-breeding waterbirds. Biodivers Conserv 22:1501–1512
Winkler DW, Billerman SM, Lovette IJ (2020a) Herons, egrets, and bitterns (*Ardeidae*), version 1.0. In: Billerman SM, Keeney BK, Rodewald PG, Schulenberg TS (eds). Birds of the world. Cornell Lab of Ornithology, Ithaca. https://doi.org/10.2173/bow.ardeid1.01 Accessed 31 January 2022
Winkler DW, Billerman SM, Lovette IJ (2020b). Ibises and spoonbills (*Threskiornithidae*), version 1.0. In: Billerman SM, Keeney BK, Rodewald PG, Schulenberg TS (eds). Birds of the world. Cornell Lab of Ornithology, Ithaca. https://doi.org/10.2173/bow.thresk1.01 Accessed 31 January 2022
Wolfe EP, Lefevre KL, Lang K (2020) Expansion of roseate spoonbill breeding distribution in southwest Florida. Fla Field Nat 48:19–24
Yamamoto FY, Neto FF, Freitas PF, Ribeiro CAO, Ortolani-Machado CF (2012) Cadmium effects on early development of chick embryos. Env Toxicol Pharmacol 34:548–555
Zar JH (2010) Biostatistical analysis, 5th ed. Pearson International Edition, New Jersey
Zwank J, Wright VL, Shealy PM, Newsom JD (1985) Lead toxicosis in waterfowl on two major wintering areas in Louisiana. Wildl Soc Bull 13:17–26