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Baseline

Exceptionally high Cd levels and other trace elements in eggshells of American oystercatcher (*Haematopus palliatus*) from the Bahía Blanca Estuary, Argentina

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ABSTRACT

Concentrations of six trace metals were determined in the eggshells of American oystercatchers (*Haematopus palliatus*) from the Bahía Blanca estuary, Argentina. All metals measured in selected samples presented concentrations above the detection limit. Means found for Cu, Pb, Cr, Zn and Ni were as follow: 2.02 ± 0.52, 7.23 ± 2.33, 0.78 ± 0.03, 2.22 ± 1.13 and 6.05 ± 0.89 mg/kg dw. The mean concentrations of Cd found were surprisingly high: 13.28 ± 3.38 mg/kg dw. Previous studies made on prey items of the American oystercatchers showed low to medium concentrations of the six trace metals. This may indicate a possible transfer of the metals that are available in the environment through food chains. Our study indicates that American oystercatchers sequester heavy metals in their eggshells. Therefore the eggshells may be useful as biomonitors for trace metal contamination in the Bahía Blanca estuary.

Organisms that are trophically top on the food chain, as seabirds, shorebirds and wading birds, are exposed to higher levels of organic and inorganic contaminants than are organisms in a lower trophic level (Monteiro and Furness, 1995; Furness and Camphuysen, 1997). In particular, heavy metals may be acquired by external contact, by inhalation and particularly by ingestion of food and water (Burger and Gochfeld, 1991, 2002). Once these contaminants enter the body of birds they can be accumulated in tissues or eliminated through deposition in the uropygial gland, salt gland and in feathers. Moreover, females can also sequester several metals (e.g. Mn, Hg, Cr, Pb and Cd) in eggs and eggshells (Burger, 1994; Burger et al., 2000; Burger and Gochfeld, 2002; Lam et al., 2005), where metal concentrations represent recent exposure as well as mobilization from stored materials in females at the time of egg formation (Burger and Gochfeld, 1995, 1996).

The Bahía Blanca Estuary, located southwest of the coast of Buenos Aires Province, Argentina (38°45′–39°40′ S and 61°45′–62°30′ W), is an excellent feeding and breeding site for several species, including shorebirds (Delhey et al., 2001; Petracci, 2002). Prey abundance and physical characteristics of the intertidal areas are the main determinants of the use of this estuarial environment by the different shorebird species. This ecosystem is under constant and increasing anthropogenic pressure, because of impact produced by urban settlements (350,000 habitants), industrial developments (petrochemical complex, industrial park, oil refinery) and harbors (Andrade et al., 2000; Ferrer et al., 2000; Tombesi et al., 2000; Marcovecchio et al., 2009). Raw sewage and runoff from the intensively used agricultural areas also generate an impact on the environment (Perillo et al., 2001). Previous studies of trace metals made on benthic invertebrates (e.g.,. crabs, polychaetes) which are known to be prey items of several bird species (Bachmann and Martinez, 1999; Palomo et al., 2003; Petracci et al., 2004; Herrera et al., 2005; Iribarne et al., 2005) showed low to medium concentrations (Simonetti, 2012; Simonetti et al., 2012, 2013).

Monitoring contaminant concentrations in eggshells of shorebirds may reveal the exposure levels in the breeding ground where the birds usually spend several weeks before laying their eggs.

In this context, concentrations of six heavy metals (Cu, Cd, Cr, Zn, Ni and Pb) were measured in eggshells of American oystercatcher (*Haematopus palliatus*), a resident species that reproduces and feeds in the Bahía Blanca estuary (Delhey and Petracci, 2004). The objectives of this study are (i) to report reference values of heavy metals for birds in the Bahía Blanca Estuary; and (ii) to assess the suitability and feasibility of using eggshell of shorebirds for monitoring heavy metals in this estuarine ecosystem.

Since there was no intention to interfere with the reproductive success of American oystercatchers, only infertile eggs (i.e. not hatched) and eggs from abandoned nests were collected during the reproductive

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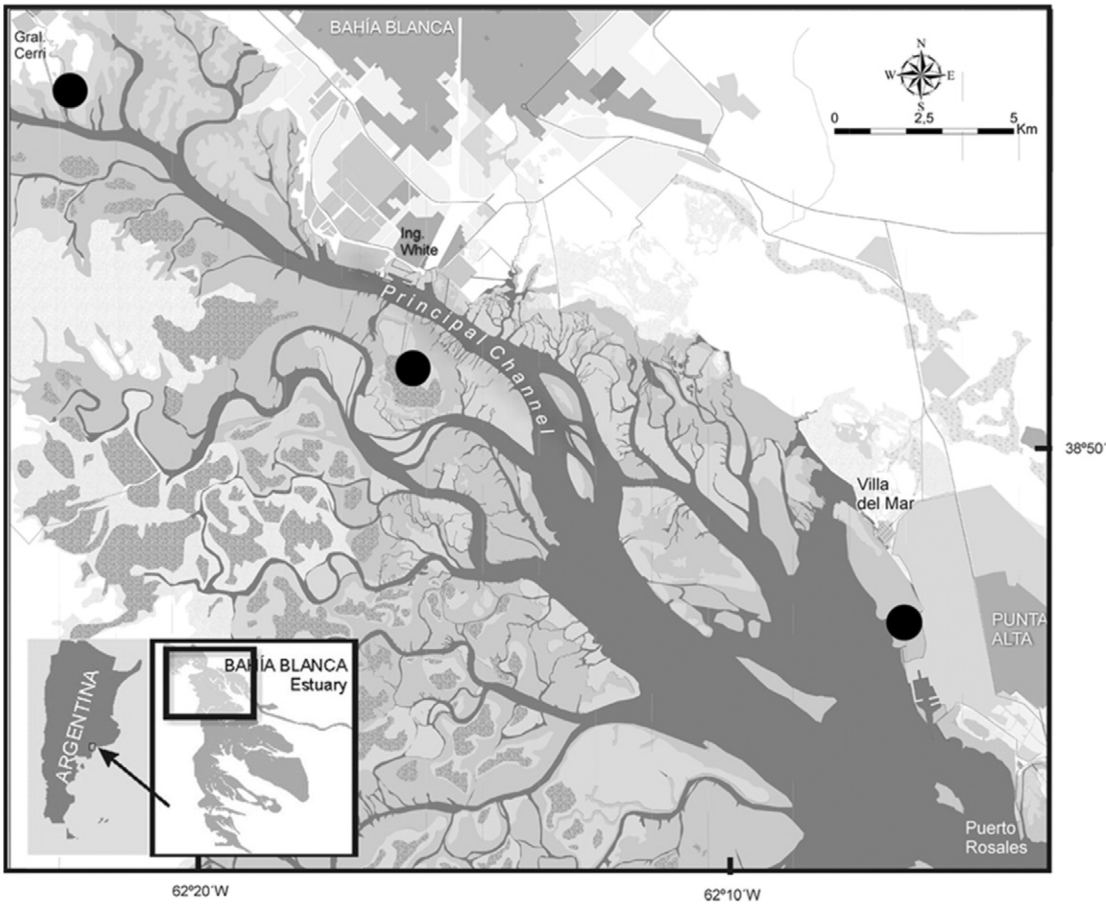

Fig. 1. Map of the study area. Black points represent the sampling areas in the Bahía Blanca Estuary.

season (September–December) of 2008 and 2009 in the Bahía Blanca estuary (Fig. 1). Collected eggs corresponded to different nests to avoid pseudoreplication. The eggs (n = 11) were kept in polyethylene bags and transported to the laboratory where the eggshells were separated from the egg content. Afterwards the inside and outside of the eggshells were washed with distillated water to remove loosely adherent internal and external contamination (Burger, 1994) and preserved at −20 °C in the freezer until analysis. Eggshells were thawed at room temperature and then analyzed for trace elements. Acid digestion of eggshell samples was performed according to the methodology described by Marcovecchio and Ferrer (2005). Eggshell samples were mineralized with 3 ml concentrated HNO_3 and 1 ml concentrated $HClO_4$ and placed in a heated glycerin bath at a temperature of 120 ± 10 °C. After acid digestion, the residue was transferred to centrifuge tubes and completed with diluted nitric acid (0.7%) up to 10 ml.

Trace metal concentrations (Cu, Cd, Cr, Zn, Ni, and Pb) were determined by atomic absorption spectroscopy with air acetylene flame using a Perkin-Elmer AA-2380. All concentrations are expressed in parts per million (mg/kg) on a dry weight (dw) basis. The method detection limit (MDL) was experimentally calculated as the SD of 12 blank replicates. The MDLs, expressed in mg/kg, were as follows: Cu 0.77, Cd 0.27, Cr 0.29, Zn 0.88, Ni 1.54, and Pb 0.29. The %RSD (percentage of relative SD) of the replicate samples were between 10 and 20%. For both the analytical quality control and reagent blanks, as well as calibration-curve build-up, certified reference materials [CRMs (mussel tissue flour, reference material No. 6, provided by the National Institute for Environmental Studies, Tsukuba, Japan)] and analytical grade reagents (Merck or Baker) were used. The recovery percentages for the six metals in CRM were >90%.

The concentrations of Cu, Cd, Cr, Ni, Zn and Pb in the eggshells of American oystercatchers from the Bahía Blanca estuary, with mean and standard deviation, are shown in Table 1.

Eggs are a good indicator of local exposure as the birds that breed in temperate and tropical regions spend many weeks on the breeding grounds before laying their eggs, acquiring sufficient resources (and heavy metals) to produce eggs (Burger, 2002). This also applies to resident species, such as the American oystercatcher. Overall, females sequester metals in their eggs during formation thereof not only in content but also in the shell, and this is another route of excretion of metals (Burger, 1994, 2002). Our study clearly showed that American oystercatchers do sequester heavy metals in their eggs. Moreover, the six metals showed concentrations above the detection limit in all eggshells samples.

Table 1
Trace metal concentrations (mg/kg dry weight) in eggshells (n = 11) of American oystercatcher, *Haematopus palliatus*, from the Bahía Blanca estuary.

Eggshell number	Cu	Cd	Cr	Ni	Zn	Pb
1	1.91	14.87	0.78	6.15	1.25	6.40
2	1.86	9.95	0.76	5.40	1.14	9.09
3	2.57	1.70	0.74	6.12	3.23	4.64
4	1.97	10.09	0.80	5.73	2.11	8.12
5	2.63	10.77	0.76	5.93	1.05	7.60
6	2.35	21.07	0.75	7.32	3.29	8.26
7	2.20	11.53	0.78	6.06	1.02	5.71
8	1.57	14.54	0.76	4.84	3.81	8.21
9	2.56	11.62	0.82	7.59	2.08	9.79
10	1.66	11.77	0.80	6.63	3.86	9.63
11	0.90	12.91	0.84	4.78	1.60	2.13
Mean	2.02	13.28	0.78	6.05	2.22	7.23
Std dev	0.52	3.38	0.03	0.89	1.13	2.33
Median	1.97	11.62	0.78	6.06	2.08	8.12

Table 2
Comparison of means and concentration ranges of metals (mg/kg dry weight) in eggshells reported in the present study with the literature.

Authors	Study area	Species	Cu	Cd	Cr	Ni	Zn	Pb
Present study	Bahía Blanca estuary, Argentina	American oystercatcher	2.02 (0.90–2.63)	13.28 (1.70–21.07)	0.78 (0.74–0.84)	6.05 (4.78–7.59)	2.22 (1.02–3.86)	7.23 (2.13–9.79)
Hashmi et al. (2013)	Punjab province, Pakistan	Cattle and Little egrets	0.01–0.31/0.05–0.31	0–2.8/0–1.60	0–2/0–2	0–0.18/0–0.24	0.06–75.58/0.53–9.27	0.05–8.55/0–4.55
Al-Obaidi et al. (2012)	Baghdad city, Iraq	Several wild birds	–	–	2.33–3.76	–	10.21–10.82	0.40–0.44
Dev et al. (2010)	Barak valley, Assam, India	Sixspecies of Ardeidae	–	0.05–0.78	0.22–0.26	–	–	0.58–0.91
Ayas et al. (2008)	Aydincik and Karaburun Islands, Turkey	Audouins's gull	nd–49.95	–	–	–	–	nd–18.42
Ayas (2007)	Ankara, Turkey	Grey heron and Black-crowned night heron	1.67–14.32/nd–3.66	0.42–1.97/nd–0.61	–	nd–0.94/nd–0.78	–	2.42–11.42/nd–2.52
Dauwe et al. (2005)	Antwerp, Belgium	Great tit	0.42–7.54	nd–0.75	0.05–0.74	0.05–13.40	0.15–20.20	0.03–23.80
Ikemoto et al. (2005)	Tarishima Island, Japan	Short-tailed and Black-footed albatrosses	0.69–0.88/0.64–1.23	0.006–0.02/0.001–2.05	0.04–0.11/0.04–0.24	–	0.71–10.2/0.37–13.5	0.004–0.017/0.003–0.12
Swaileh & Sansur 2006	West Bank, Palestina	House sparrow	0.90–1.20	0.01–0.02	–	–	14.30–25.50	1.50–4.80
Lam et al. (2004)	Hong Kong, China	Black-crowned night heron and Little egret	0.78–1.83/0.95–2.85	–	0.05–0.13/0.09–0.70	–	0.76–12.40/0.83–33.08	0.02–0.05/0.04–0.56
Mora (2003)	Arizona, USA	Yellow-breasted chat and Willow flycatcher	6.20 ± 8/3.00 ± 1.70	–	–	4.10 ± 3.60/6.50 ± 5.70	9.50 ± 10/46.2 ± 10.7	0.60 ± 0.70/0.90 ± 0.60
Dauwe et al. (1999)	Hoboken, Belgium	Blue tit and Great tit	2.80 ± 1.10/3.20 ± 0.50	0.15 ± 0.02/0.31 ± 0.08	–	–	32 ± 8/28 ± 5	7.40 ± 1.10/15 ± 4
Spahn and Sherry (1999)	South Louisiana wetlands, USA	Little blue heron	nd	nd	nd	nd	nd	nd
Morera et al. (1997)	Ebro Delta, Spain	Audouin'sgull	2.14 ± 0.70	nd	–	–	6.58 ± 2.12	nd
Burger (1994)	Long Island, USA	Herring gull and Roseate tern	–	0.05 ± 0.008/0.10 ± 0.04	1.60 ± 0.70/1.20 ± 0.30	–	–	0.30 ± 0.05/1.20 ± 0.30

nd = not detected.

Levels of the non-essential metals Pb and Cd on eggshells in the literature are quite controversial. Furness (1993) suggested that these metals are poorly transferred from the female to the eggs. Morera et al. (1997) found levels below the detection limit for both metals in eggshells of Audouin's gull (*Larus audouinii*); the same results were found in the study of Spahn and Sherry (1999) working with eggshells of Little blue heron (*Egretta caerulea*). However, more recent studies have found detectable levels of Pb and Cd in several bird species (Burger, 1994; Dauwe et al., 1999; Lam et al., 2004; Mora, 2003; Ikemoto et al., 2005; Ayas, 2007; Ayas et al., 2008; Dev et al., 2010; Al-Obaidi et al., 2012; Hashmi et al., 2013). Scheuhammer (1987) suggested that metals such as Cd and Pb may interact with Ca metabolic pathways, the main component of eggshells. Consequently these metals may be more easily incorporated into the shell during its formation. Besides, this author also stated that transfer of heavy metals to the eggs could only take place when there is an excessive accumulation of these elements in the organs of adult female birds. The results of this study showed that Cd concentrations found in the eggshells of American oystercatchers ranged from 1.70 to 21.07 mg/kg dw. These values were greater than those described in the literature for several bird species (Table 2). However for Pb concentrations, whose range was between 2.13 and 9.79 mg/kg dw, these values were within the range reported for other bird species (Table 2).

The levels of essential metals, such as Cu and Zn are metabolically regulated in bird tissues (Savinov et al., 2003). Zn has an important role in many metabolic processes, especially the activation of enzymes and the regulation of gene expression (Savinov et al., 2003); moreover it is a component of a number of metalloenzymes such as carbonic anhydrase which is essential for eggshell formation (Scheideler, 2008). In the present study, the levels of this metal found in the eggshells of American oystercatchers ranged from 1.02 to 3.86 m/kg dw. These levels were within the values described in the literature for different species of birds like egrets, herons, albatrosses and passerines (Lam et al., 2004; Dauwe et al., 2005; Ikemoto et al., 2005; Hashmi et al.;, 2013; Table 2). Nevertheless, it is worth mentioning that other studies have detected higher Zn levels than those found in this study, as in the case of Cattle egrets (*Bubulcus ibis*) from Pakistan with values up to 75.58 mg/kg dw (Hashmi et al., 2013; Table 2).

Cu also plays an important role in the metabolism of birds. This metal is closely associated with iron metabolism, being essential in blood cell formation (Scheideler, 2008). The range of Cu concentrations found in the eggshells of American oystercatcher was between 0.90 and 2.63 mg/kg dw. These values were very similar to those reported in eggshells of several bird species (Table 2). The greatest concentrations of Cu described in the literature were 14.32 mg/kg dw for Grey heron (*Ardea cinerea*) (Ayas, 2007) and 49.95 mg/kg dw for Audouin's gull (Ayas et al., 2008) and are quite far from the concentrations found in this study.

Ni is generally regarded as an essential micronutrient, although to date there are no studies addressing specific essentiality requirements of Ni in birds (Phipps et al., 2002). There is a small number of researches which have been measured the essential metal Ni concentrations in bird eggshells (Mora, 2003; Dauwe et al., 2005; Ayas, 2007; Hashmi et al., 2013). In this study, the range of Ni concentrations in was from 4.78 to 7.59 mg/kg dw. Comparing these values with those found in the literature, it appears that Ni levels in eggshells of American oystercatchers were higher than levels found in Little blue herons (*E. caerulea*) (Spahn and Sherry, 1999) Grey and Black-crowned night herons (*Nycticorax nycticorax*) (Ayas, 2007), Cattle egrets (*B. ibis*) and Little egrets (*Egretta garzetta*) (Hashmi et al., 2013). Meanwhile concentrations found for Great tit (*Parus major*), Yellow-breasted chat (*Icteria virens*) and Willow flycatcher (*Empidonax traillii extimus*) were similar and even superior to those found for the American oystercatcher (Mora, 2003; Dauwe et al., 2005; Table 2).

Cr was the last essential metal measured in this study. This element appears to have a beneficial role in the regulation of insulin's action on carbohydrate, protein and lipid metabolism. In addition, Cr is thought to be essential for activating certain enzymes and for stabilization of proteins and nucleic acids (Sahin et al., 2009). The levels of Cr obtained in this study ranged from 0.74 to 0.84 mg/kg dw. In general, these values are consistent with those reported in the literature, with the exception of some species such as House sparrow (*Passer domesticus*; 3.76 ± 0.39 mg/kg dw), White-eared bulbul (*Pycnonotus leucotis*; 2.33 ± 0.42 mg/kg dw), Collared dove (*Streptopelia decaocto*; 2.69 ± 0.38 mg/kg dw), Rock dove (*Columba livia*; 2.67 ± 0.41 mg/kg dw), Herring gull (*Larus argentatus*; 1.6 ± 0.7 mg/kg dw) and Roseate tern (*Sterna dougallii*; 1.2 ± 0.3 mg/kg dw) (Burger, 1994; Al-Obaidi et al., 2012; Table 2) were levels of Cr in the eggshells of these species were greater.

Nowadays, it is widely recognized that dietary exposure is a major route for the transfer of metals in marine food webs (Rainbow, 2002; Wang, 2002). The diet of American oystercatcher is based mainly on bivalves, molluscs and worms (Nol and Humphrey, 1994). In Argentina, this species feeds mainly on clams, crabs and polychaetes (Bachmann and Martinez, 1999; Simonetti, personal observation). Previous studies of heavy metals in prey items of American oystercatchers (i.e., crabs, polychaetes) in the Bahía Blanca estuary showed detectable levels for the six metals analyzed in this study. To complement this study, in Table 3 are shown the concentrations of the six metals in two prey items of the American oystercatcher.

Analyzing the metals that are essential for birds in general, and for the American oystercatcher in particular, the following is observed: in the case of Cu and Zn concentrations in eggshells of the oystercatchers were lower than those found in the prey items. On the other hand, while Cr and Ni were found below the detection limit in the polychaetes, in crabs both metals were detectable and concentrations were again greater than those found in eggshells of the oystercatchers (Table 3). These results could be related to the metabolic needs of this species. Anyway, considering that eggshells are an excretion mechanism of metals, levels of these four elements found therein would be linked to environmental levels, which would exceed the metabolic requirements and they would be incorporated, among other routes, through the diet.

As for the non-essential metals, the concentrations found in the eggshells of the oystercatchers may be related with environmental levels and their bioaccumulation through the diet.

In the case of Cd, where concentrations were exceptionally high in the eggshells, it is observed that in crabs, and to a lesser extent in the polychaetes, the Cd was above the detection limit (Table 3). Therefore one possible way of incorporating this metal could be through diet. Noteworthy that in surface sediments and particularly in the SPM (Suspended Particulate Matter) that match the period of this study, concentrations were above the detection limit. Cd concentrations found in surface sediments were <0.1 mg/kg dw (Botté et al., 2010). Meanwhile, the levels found for SPM values were between <0.1 and 32.83 mg/kg dw (Fernandez Severini, 2008), where the author suggests an increase of this metal in SPM over time. Another possible source for this metal could be through the atmospheric deposition, but so far there are no studies proving the entry through atmosphere. A third possibility could be from agricultural sources because of the utilization of phosphate fertilizers containing Cd between other metals.

For Pb, although in polychaetes this metal was not detected, crabs showed concentrations above the detection limit. For this metal, the levels found in the SPM were between <0.5 and 68.6 mg/kg dw (Fernandez Severini, 2008), while the concentrations found in surface sediments were about 15 mg/kg dw (Botté et al., 2010). Again, the environmental levels could be the main source of Pb.

The present work provides novel information about accumulation of trace metals such as Pb, Cd, Cu, Zn, Ni and Cr in eggshells of American oystercatcher from the Bahía Blanca estuary, Argentina. Moreover, to date it appears to be the first study of metals in bird eggshells of Latin America. Both, essential and non-essential elements are accumulated in the eggshells. Our data suggest that eggshells of American oystercatchers are suitable as biomonitors within the studied environment.

The presence of trace metals so much in the low links (crabs and polychaetes) as the superiors (eggshells of American oystercatchers)

Table 3
Trace metals concentrations in benthic organisms from the intertidal zone of the Bahía Blanca Estuary, Argentina.

Organism		Cu	Cd	Cr	Ni	Zn	Pb
Crabs (*Neohelice granulata*)[a]	Min–max	81.40–172.52	4.89–11.94	nd–1.93	9.52–19.33	28.82–37.99	nd–4.66
	Mean	135.64	8.77	–	14.25	33.77	–
	SD	29.42	2.48	–	3.53	3.05	–
Polychaetes[b]	Min–max	1.12–4.63	0.59–1.42	nd	nd	6.53–8.26	nd
	Mean	2.94	0.96	–	–	7.42	–
	SD	1.03	0.28	–	–	0.42	–

nd = not detected.
[a] Simonetti et al. (2012, 2013). Data are expressed in mg/kg dry weight.
[b] Simonetti (2012). Data are expressed in mg/kg wet weight.

suggests that metals which are bioavailable in the environment may be transferred to populations of American oystercatchers of the Bahía Blanca estuary across trophic chains.

Given the toxicity of these elements, appears a potential risk in this species, since many of these metals may have effects on the development and the nervous system of birds. In this sense, it is necessary to determine the critical levels of these metals to predict quantitatively the effect it can have on these populations. Particularly Cd levels should be taken into account because the concentrations found are relatively high, which would indicate bioaccumulation of this metal in this species.

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