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Morphometric parameters and total mercury in eggs of snowy egret (*Egretta thula*) from Cartagena Bay and Totumo Marsh, north of Colombia

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ABSTRACT

Eggs from egrets (*Egretta thula*) were collected from Cartagena Bay and Totumo Marsh, two sites at the north of Colombia with different pollution background, and measured their morphometric parameters as well as total mercury (T-Hg) and calcium levels in eggshell. Statistically significant differences were observed for egg weight and size between the two sampling locations. T-Hg and calcium concentrations in eggshell were greater in eggs from Cartagena Bay, the industrial site, compared to Totumo Marsh, a non-industrial location. The opposite was observed for eggshell thickness (3.6% less in the bay). Pearson correlation analysis showed eggshell T-Hg negatively correlated with eggshell weight in eggs from the marsh ($R = -0.795$, $P < 0.006$), but not from the bay ($R = 0.387$, $P = 0.269$), probably suggesting greater susceptibility to Hg in birds from the non-polluted site. In short, results suggest eggs from *E. thula* at Cartagena Bay have greater T-Hg concentrations and less eggshell thickness than those from Totumo Marsh.

1. Introduction

During the last decades the survival of several bird species has been threatened by different environmental factors including global warning, urbanization and pollution. Many environmental pollutants have been recognized as endocrine disruptors with proven adverse effects on reproduction, including organic molecules, and heavy metals (Sugni et al., 2010). Among these chemicals, mercury (Hg) is of particular concern due to its ubiquitous nature, toxicity, and capacity to be transferred from one trophic level to the next, being biomagnified through aquatic and terrestrial food webs (Bryan et al., 2012; Haines et al., 2003; Dolbec et al., 2001), and a cause for concern regarding breeding bird populations (Hargreaves et al., 2011). Therefore, it represents a key factor for biodiversity conservation, especially for endangered species (Ackerman et al., 2012). This is particularly important in tropical coastal ecosystems, as stated by the United Nations document "The Future We Want", signed in Rio de Janeiro in June 2012 (Costa et al., 2012).

In the Caribbean region of Colombia, a tropical coastal environment, tourism is one of the main economical activities, and in cities such as Cartagena, the natural surroundings definitively are an added value. In this scenario, the snowy egret (*Egretta thula*) and other bird species coexist in the urban area, taking advantage of the remaining available green spots. *E. thula* is a small, snow-white heron with a slim, pointed, black bill, yellow eyes, bright yellow feet, and long, black legs. However, entropic pressure on these and other bird species is a challenging problem, as it has been reported that Cartagena Bay, the main water body in the city, has pollution issues related to biological agents (Olivero et al., 2005a), new contaminants (Olivero et al., 2005b), some of which have been already reported in humans (Kannan et al., 2004), and mercury (Olivero-Verbel et al., 2009; Carvajal et al., 2000), among others.

Despite the fact that the snowy egret is one of the most abundant bird species in Cartagena Bay, few data have been collected on the biology, ecology and toxicology associated with these birds in this area. However, considering that its diet is based on fish, shrimps, and sometimes insects, it occupies a high level in the trophic chain, making it susceptible to the accumulation of heavy metals and other endocrine dysfunction agents. As in marine ecosystems, eggshell Hg is a marker for previous Hg exposure (Xu et al., 2011), the objective of this research was to evaluate the presence of Hg and morphological characteristics of the eggs of *E. thula*, as an indicator of the pollution status of the egrets, and the possible implications in the survival ability for this species in Cartagena Bay.

2. Materials and methods

2.1. Sample collection

Eggs from *E. Thula* were obtained from active nests in two different locations at the north of Colombia: Cartagena Bay and Totumo Marsh (Fig. 1) on May–September, 2005. The bay has been

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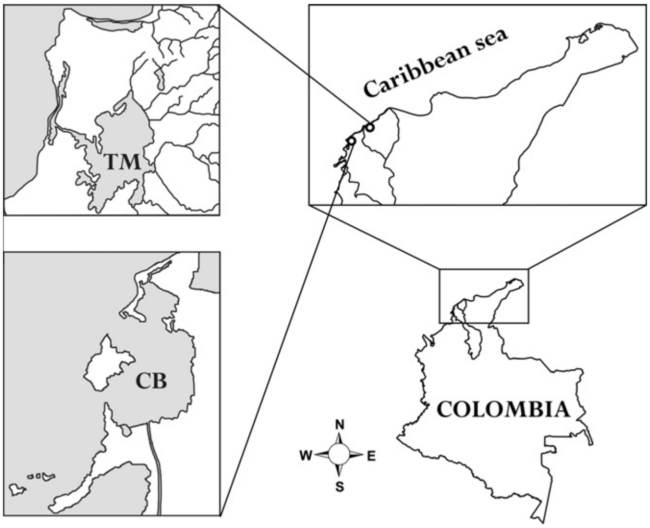

Fig. 1. Map of Colombia showing the geographic localization of sampling areas. Totumo Marsh (TM) and Cartagena Bay (CB).

largely recognized as an ecosystem with Hg-related pollution (Olivero-Verbel et al., 2009, 2008; Olivero et al., 2005a,b; Carvajal et al., 2000), whereas the Totumo Marsh, a coastal marsh, located approximately 50 km north from the bay, is considered a low-polluted area, with no Hg inputs from industrial sources. At each sampling point, 20 eggs were manually collected, taking one egg from nests containing at least three of them. Eggs were maintained under refrigeration until analysis, no more than 1 week after collection.

2.2. Morphometric parameters

Once collected, eggs were carefully washed with distilled water and dried at room temperature on paper towels. Egg length, and width, in the middle of the length, was measured with a vernier caliper with a resolution of ±0.05 mm (Helander et al., 2002). Total egg and eggshell weights were measured using an analytic scale to a ±0.01 g resolution. Eggshell thickness was recorded on at least five randomly taken fractions using a digital micrometer with a precision of ±0.001 mm (Helander et al., 2002).

2.3. Total mercury in the eggshells and egg content

Individual eggshell fragments were carefully washed with a toothbrush and deionized water to remove any organic membranes or remnants of albumen or yolk. These were allowed to air dry for 24 h and stored in plastic bags until Hg analysis. In the case of egg content, both egg white and yolk were homogenized using a vortex, the product transferred to plastic vials and kept at −20 °C until analysis. Total Hg (T-Hg) concentrations were assessed using 150 mg of previously pulverized eggshell, or 60 mg of egg content. Samples were pirolyzed at 800 °C and the vaporized Hg was detected by a RA-915+ Zeeman Mercury Spectrometer (Sholupov et al., 2004), and quantified using a calibration curve obtained from certified standards. Calibration curves were considered optimal if the value of R was equal or greater than 0.99. The accuracy of the method was quantified by analysis of blanks, and the use of the certified sample DORM-2, dogfish muscle, from the National Research Council of Canada. The measured concentration of T-Hg in DORM-2 was 4.46 ± 0.25 μg T-Hg/g (certified value 4.47 ± 0.32 μg T-Hg/g). The precision of the assay was estimated as the coefficient of variation for two independent measurement values. In all cases, it was always below 10%.

2.4. Calcium concentration in the eggshells

The calcium contents of eggshell samples were measured by X-ray fluorescence (XRF) as previously described (Leoni and Saitta, 1976; Tamponi et al., 2003). One-gram samples of *E. thula* eggshells were pulverized and mixed with 14 g of powdered polypropylene homopolymer (PP) unused resin. The mixture was homogenized by manual shaking during 3 min, placed in a stainless steel mold, and then put under hydraulic press at 230 °C and 100 psi of pressure, obtaining a 6 cm diameter disk. Readings were performed on a Philips PW 1480 fluorescence instrument equipped with a Sc anode tube, glass of LiF200, and a flow detector, with analytic software X40. Calibration curves were prepared with calcium carbonate in PP and reported values are the averages of at least five determinations.

2.5. Statistics

The results were reported as the average ± SE of the mean. Statistical differences between sampling sites were carried out using *T*-test, after examining the data for normality (Kolmogorov–Smirnov) and homoscedasticity (Barlett). Otherwise, variable transformations were performed. Relationships between variables were assessed using Pearson correlations. In all cases, statistical significance was set at $P < 0.05$ (Walpole et al., 2001).

3. Results

Length, width and total weights of eggs from *E. thula* nesting in Totumo Marsh and Cartagena Bay are shown in Fig. 2. Eggs from

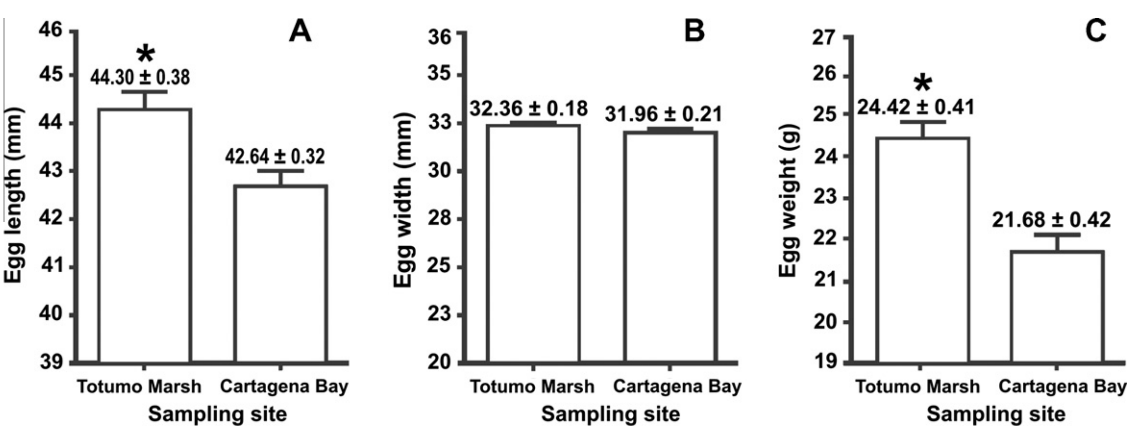

Fig. 2. Length (A), width (B) and total weight (C) of the eggs from *Egretta thula* collected in Totumo Marsh and Cartagena Bay.

egrets living at Totumo Marsh presented greater length (44.30 ± 0.38 mm) and weight (24.42 ± 0.41 g) than those from Cartagena Bay (42.64 ± 0.32 mm and 21.68 ± 0.42 g, respectively). No statistical differences in egg widths were observed for the sampling sites.

Eggshell thickness and weights are presented in Fig. 3. Eggs from Totumo Marsh have significantly greater eggshell thickness (196 ± 2.21 μm) than those from Cartagena Bay (189 ± 2.43 μm), with a mean difference of 3.6%. Although, in average, eggshell weights from Totumo Marsh area were 2.7% greater than those from Cartagena Bay, the differences were not significant.

T-Hg levels in eggshell, egg content, and total calcium concentrations in eggshell are presented in Fig. 4. Eggshells from Cartagena Bay had greater Hg and Calcium concentrations than those from Totumo Marsh. In the case of Hg, detected levels were more than twofold greater in the bay. However, T-Hg concentrations obtained in egg contents did not show statistically differences between sampling sites.

Correlation analysis between different studied morphometric and chemical parameters for eggs from *E. thula* are presented in Table 1. Results showed associations between studied variables varied depending on the sampling site. Interestingly, in Cartagena Bay, all morphometric parameters were significantly correlated. In contrast, in Totumo Marsh, significant correlations were found for egg weight, length and width, but not between these variables and eggshell weight or thickness. In the bay, a significant positive association was found between T-Hg in egg content and egg width ($R = 0.455$, $P = 0.044$). In the marsh, T-Hg in eggshell inversely correlated with eggshell weight ($R = -0.795$, $P = 0.006$), and T-Hg in egg content also negatively correlated with eggshell thickness

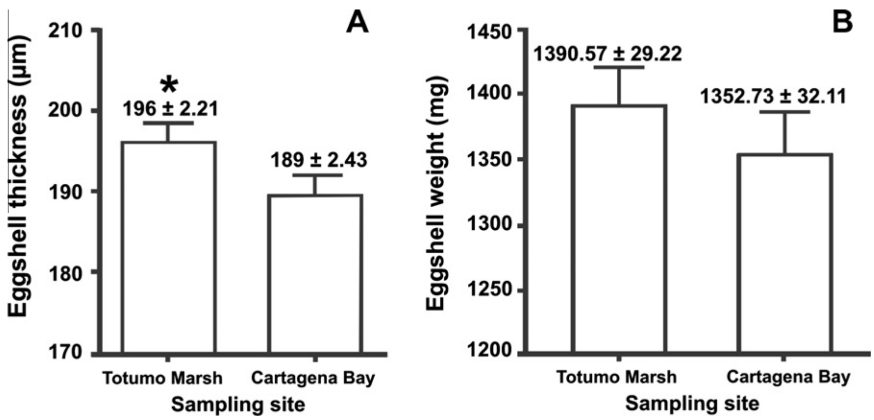

Fig. 3. Thickness (A) and weight (B) in eggshells of *Egretta thula* from Totumo Marsh and Cartagena Bay.

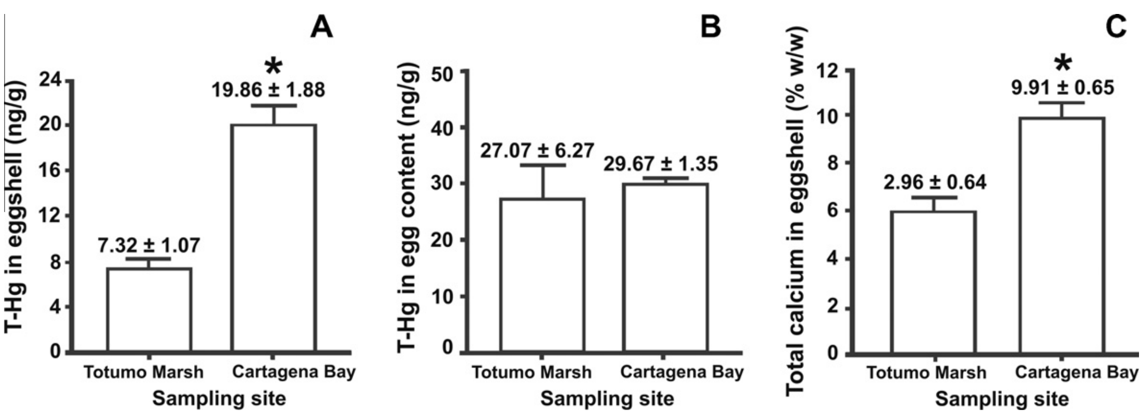

Fig. 4. Total mercury concentrations in eggshell (A), egg content (B), and total calcium in eggshell (C) of *Egretta thula* from Totumo Marsh and Cartagena Bay.

Table 1
Pearson correlation for mercury content and morphometric variables studied in eggs from *Egretta thula* living at Totumo Marsh (left side) and Cartagena Bay (right side, underlined).

Parameters	Egg weight	Egg length	Egg width	Eggshell weight	Eggshell thickness	T-Hg in eggshell	T-Hg in egg content
Egg weight	1	0.743 ($P \leqslant 0.001$)	0.912 ($P \leqslant 0.001$)	0.793 ($P < 0.001$)	0.523 ($P = 0.018$)	0.063 ($P = 0.863$)	0.365 ($P = 0.114$)
Egg length	0.772 ($P < 0.001$)	1	0.677 ($P = 0.001$)	0.756 ($P < 0.001$)	0.555 ($P = 0.011$)	0.020 ($P = 0.957$)	0.137 ($P = 0.564$)
Egg width	0.840 ($P < 0.001$)	0.475 ($P = 0.035$)	1	0.826 ($P < 0.001$)	0.518 ($P = 0.019$)	0.002 ($P = 0.995$)	0.455 ($P = 0.044$)
Eggshell weight	0.236 ($P = 0.317$)	0.099 ($P = 0.677$)	0.251 ($P = 0.285$)	1	0.803 ($P < 0.001$)	0.387 ($P = 0.269$)	0.354 ($P = 0.126$)
Eggshell thickness	−0.275 ($P = 0.240$)	−0.263 ($P = 0.262$)	−0.147 ($P = 0.537$)	0.566 ($P = 0.009$)	1	0.548 ($P = 0.101$)	0.164 ($P = 0.489$)
T-Hg in eggshell	−0.037 ($P = 0.919$)	−0.042 ($P = 0.909$)	−0.114 ($P = 0.754$)	−0.795 ($P = 0.006$)	−0.392 ($P = 0.263$)	1	0.134 ($P = 0.712$)
T-Hg in egg content	−0.098 ($P = 0.689$)	−0.120 ($P = 0.625$)	−0.287 ($P = 0.234$)	−0.140 ($P = 0.568$)	−0.596 ($P = 0.007$)	0.143 ($P = 0.714$)	1

($R = -0.596$, $P = 0.007$). In both locations, a significant association was observed between eggshell weight and eggshell thickness (Cartagena Bay, $R = 0.803$, $P < 0.001$; Totumo Marsh, $R = 0.566$, $P = 0.009$).

4. Discussion

E. Thula is a high-trophic level bird susceptible to pollution exposure derived from its environment. In this study, several differences in both egg morphometric and chemical parameters were found between specimens collected at two places with different history of Hg contamination at the north of Colombia. As expected, eggshells from egrets living at Cartagena Bay had greater T-Hg concentrations than those from Totumo Marsh. This distribution of Hg, based on proximity to anthropogenic sources, has been extensively described for other bird species (Day et al., 2012; Hothem et al., 2008; Anthony et al., 2007).

The mean difference in eggshell thickness registered in eggs from Totumo Marsh in comparison with those from Cartagena Bay reached 7 μm (3.6%). A reduction in eggshell thickness has been associated with several factors, including exposure to high concentrations of organochlorinated pesticides, such as dichlorodiphenyldichloroethylene (DDE), polychlorinated biphenyls, brominated flame retardants (Miljeteig et al., 2012; Gómez-Ramírez et al., 2012; Malik et al., 2011; Fernie et al., 2009; Bouwman et al., 2008), metals, such as magnesium, copper, zinc, lead and Hg (Rodriguez-Navarro et al., 2002). Moreover, it has been widely recognized that Hg reduces eggshell thickness and calcium content of the egg (Lundholm, 1987). However, such negative associations have also been absent in other studies (Miljeteig et al., 2012). Despite these contradictory findings, there is a general consensus that Hg is a metal with a concern for breeding bird populations (Hargreaves et al., 2011), and it has been suggested that thinning above 16–20% is associated with a decline in bird populations (Miljeteig et al., 2012). Although such reductions in eggshell thickness were not found in this study, additional studies should be conducted to monitor this problem.

T-Hg concentrations found in egret eggshells from Cartagena Bay (19.86 ± 1.88 ng/g) are similar to those reported for another egret species, *Egretta alba* (21.02 ± 3.17 ng/g), from Northeast China (Wang et al., 2012), and do not surpass the levels that may produce harmful effects on reproduction (Henny et al., 2002). The levels of Hg in the eggs, which can be greater in egg content than in eggshells (Aliakbari et al., 2011), as also observed here, may represent the level of the pollutant deposited by the females during the egg formation period (Burger and Gochfeld, 1996), and that could also have been derived from recent exposure or mobilization from other tissues in the females (Burger et al., 1999).

It has been reported that lower egg weights may be associated with Hg levels between 0.5 and 6 ppm (Burger and Gochfeld, 1997). However, although the concentrations reported here were much lower than those affecting egg development, the differences between sampling sites imply some degree of affectation in these organisms. The differences may be the result of several factors. First, the environmental conditions in both sites are quite unsimilar. The bay is recognized as a source of Hg pollution, as more than 30 years ago, an extinct chlor-alkali plant released elemental Hg into the bay as part of its operations. This contamination has been documented for several environmental matrices, including sediments, fish, crabs and humans (Olivero-Verbel et al., 2009, 2008; Carvajal et al., 2000). Second, other chemicals may also be involved, as the bay is surrounded by several types of industries, and chemicals such as perfluorooctyl sulphonates and polycyclic aromatic hydrocarbons have been found in fish and sediments, respectively (Johnson-Restrepo et al., 2008; Olivero et al., 2005b). Third, it is possible that those egrets living in Cartagena may undergo stress derived from human activities. Ecosystem pollution by industries, transport, sewage disposal and deforestation linked to urbanization may be pushing *E. thula* to increase their reproduction rate, probably by laying more eggs per clutch or season, but with less time for the development of an optimal eggshell thickness.

Results from Pearson correlation revealed different relationships when data were analyzed for each sampling site. In the bay, associations between morphometric variables seem more consistent than in the reference site. This may be, in part, a consequence of greater calcium content in eggshells from the polluted site, as it has been shown that the structure of the shell is related to crystal size and orientation (Dunn et al., 2012), and calcium levels could be critical for adequate crystal formation. Greater calcium concentrations in eggshells from the bay, in comparison with the marsh, may derive from the food source for the egrets. On the bay, these birds rely mostly on sea food, whereas in the marsh, available food is impacted by freshwater inputs that reduce water salinity, and therefore, calcium content.

In Cartagena Bay, T-Hg in egg content seems to be a function of egg width ($R = 0.455$, $P = 0.044$), suggesting a possible bioaccumulation process. Interestingly, although in this place, there is no association between T-Hg in eggshell and eggshell weight ($R = 0.387$, $P = 0.269$), in the marsh, eggshell weight decreased with an increase in T-Hg ($R = -0.795$, $P = 0.006$). Moreover, at this less polluted site, T-Hg in egg content also negatively correlated with eggshell thickness ($R = -0.596$, $P = 0.007$), results that probably imply that in the marsh, despite T-Hg levels are smaller than in the bay, egrets are more susceptible to Hg exposure, probably as a consequence of less calcium availability; whereas birds from the bay, may have reached some tolerance to this pollutant.

As eggshell thickness strongly influences bird survival (Castilla et al., 2010), actions should be taken to decrease Hg levels in the bay, including recovering mangrove areas for egrets and other birds to nest, deforestation prevention and avoiding ecosystem damage by littering or pollution.

5. Conclusion

The results found on this study showed, for the first time, the impact of environmental contamination on *E. thula* living at Cartagena Bay. T-Hg and calcium concentrations were greater than those found in Totumo Marsh, the reference site, and this pollutant may be one of the factors responsible for the smaller eggshell thickness observed in eggs from *E. thula* in the bay.

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