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Notes on Little Egret breeding biology and on mercury content in egg shells and feathers

Loris Pietrelli · Massimo Biondi

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Abstract Data regarding the breeding biology and mercury concentration in egg shells and feathers of Little Egret, *Egretta garzetta*, collected since 2001 in the Tarquinia Salt-pans (Italy), are reported. The average number of breeding pairs was 20.5 with 2.92 hatched young per pair. Many fish species such as *Aphanius fasciatus* and *Atherina boyeri* were found in the diet. The hydrology of the saline ecosystem and disturbance by human activities play a major role in determining foraging habitat and reproductive success. The total Hg concentration ranged from 0.026 to 0.200 $\mu g\ g^{-1}$ and from 0.41 to 9.64 $\mu g\ g^{-1}$ dry weight in the egg shells and feathers, respectively. Eggshell thickness was not significantly correlated with Hg concentration.

Keywords *Egretta garzetta* · Breeding · Mercury · Egg shells · Feathers

1 Introduction

Salt-pans are semi artificial coastal ecosystems that have an high value because they combine their production process with the conservation of the environment: the saline themselves constitute an integrated ecosystems. Their ecological importance consists in the fact that they comprise the characteristics of both regular and hyper saline wetlands. Having in mind the environmental bio-monitoring of the saline ecosystem, long data on the mean clutch size, mean brood size and reproduction success of the breeding birds and, in addition, the pollutant control can be a sensitive indicator of wetland conditions. Considering water birds, a distinction between poor and good breeding seasons, in fact, can be due to abiotic factors such as the hydrological conditions of the habitat or to anthropic disturbance during breeding season such as during the feeding activity (Kushlan 1993).

L. Pietrelli (✉)
Environmental Department, ENEA CR Casaccia, Rome, Italy
e-mail: loris.pietrelli@enea.it

M. Biondi
GAROL, Via del Castello, 17, 00119 Rome, Italy

Fish-eating birds, moreover, are particularly exposed to Hg (Alleva et al. 2006) and therefore they are valuable for environmental monitoring because they are long-lived and top trophic level animals in the food search, consequently they are able to integrate pollutant levels over a broad area by bioaccumulation. Little Egret, *Egretta garzetta*, seems to be especially useful in this regard because Hg concentrations in its eggs, eggshells and feathers have already been reported from polluted and unpolluted sites (Hoffman and Curnow 1979; Dai et al. 1984).

In this study, data regarding the breeding biology and mercury concentration measured in egg shells and feathers of Little Egret collected since 2001, when the first colony was established in the Latium Region (Tarquinia Salt-pans), are reported.

2 Study area and methods

Data were gathered at the Tarquinia Salt pans Reserve, salt-operative until 1997, that were built along the Latium coast (Lat. 42°12′N, Log. 11°43′E) in the early 1800s (but utilized also by Etruscans) to supply mainly Rome city with salt for centuries. The overall site (about 200 ha) includes a variety of habitats such as saline, mudflats, sandy shores, forested river bank, *Tamarix* bush and pine-wood. The Little Egret colony was established since 2001 (Rigoli et al. 2001) in a pine wood ($\sim$70 ha) and it is still the sole heronry of Little Egret in the Latium region.

Year after year, the colony occupied the same place, a small artificial pine wood *Pinus halepensis* (4.5–7.1 m height), at about 250 m from the Tyrrhenian line coast, nests were placed on the top of mature tree: the mean value of the trunk circumference, at 150 cm height from the ground, of the utilized tree was 114.5 cm (range 80–122 cm).

We taken a census of the nests by total nest counts every year, during the reproductive season (May–August) from 2001 to 2007 and samples were collected on the ground under nests, large eggs fragments (mainly half egg) were often collected allowing to determine the egg thickness along the egg equator (or strictly close to it) in three random locations using a micrometer (0.001 mm).

The number of breeding pairs was defined as the maximum number of simultaneously occupied nests. We considered the number of hatched-young/nests counting *juvenes* at the age of 15–20 days along the colony. Data regarding the Little Egret diet were obtained by regurgitations analysis (data from 26 regurgitations collected during the 2003 breeding season and randomly some sample collected within 2004–2006) and the fish preys were determined by otoliths according to Martucci et al. (1993).

Concerning the chemical analysis, feathers and eggshells were placed in a beaker with distilled water and washed using an ultrasonic bath at room temperature to remove mud, feces, yolk remain, etc. The washed material was dried at 60°C for about 12 h, and the mercury analysis was performed by AMA 254 (Automatic solid/liquid Hg Analyzer) using solid samples, reference standard material BCR 422 by Community Bureau of Reference was used.

3 Results and discussion

The earliest Little Egret breeding season started with laying of the first egg on 1st decade of April (2005) and the latest on 3rd of May (2004, 2006) while the last fledging chick was usually recorded during 3rd decade of August (2003, 2005, 2007) (Table 1). The estimated

Table 1 Yearly data regarding the Little Egret colony breeding at Tarquinia Salt pans

	2001	2002	2003	2004	2005	2006	2007
1st laying (decades)	II May	II April	III April	III May	I April	III May	I May
Last fledging	?	II Aug	III Aug	I Aug	III Aug	II Aug	III Aug
Pairs	12	27	16	25	19	25	20
Nests	18	39	20	32	21	27	22
Hatching/pair	2.0	3.3	2.8	2.2	3.5	3.6	3.1
Utilized tree (n)	11	20	14	15	12	14	12
Area (m^2) ± 20%	200	1000	400	500	500	400	350
Eggshell thickness (n/SD)	0.230	0.229	0.214	0.222	0.208	0.219	0.215
	4/0.027	4/0.027	10/0.017	7/0.021	7/0.014	7/0.017	5/0.021

number of breeding pairs of Little Egret during the years of the study ranged between 12 and 27 (average 20.5) while the mean number of hatched young from all pairs nesting attempts was 2.92 (min 2.0, max 3.6). No data regarding the nests failed or predation (nocturnal mainly) were collected.

During the 2002 breeding season, the colony strongly increases (64.1% of new nests) and from 2004 the numbers of nesting pairs have been stable (19–25 pairs).

In particular, during the initial activities performed in the frame of the local "LIFE project" regarded the saline restoration (2003–2005), a decrease of the breeding pairs occurs probably due to the alterations of the salt pans conditions water levels (2003). When the hydrology of the saline changes significantly (high water level in particular), the greatest numbers of feeding adults were observed in the agricultural fields far from the colony or along the estuarine of close rivers. This suggests that hydrology of the salt pans plays a major role in determining foraging habitat selection and therefore the reproduction success.

Finally, areas close to the human activities in the salt pans seem to be avoided during feeding activity by Little Egret probably reflecting the negative impact of the strong disturbance on foraging sites.

Little Egret feeding behavior varied during the seasons: birds foraged closer to the nearest colony as the season progressed from April to June (as incubation and feeding of chicks constrained the birds to foraging closer to the colony).

In winter time, along the Latium coast Egrets, estimated mean population was 250–300 individuals (Brunelli et al. 2004), habitually foraged along the drainage canals (=51.4%), in particular where the land restoration was done during last century (especially along the Roman Littoral) (Biondi et al. 1999). Little Egret exploited prey types in an opportunistic way, mainly in relation to their availability (Fasola 1986): the saline offers the largest feeding area supporting the Little Egret during the breeding season. The feeding time was mainly spent within the salt works (about 90% from April to June till 2003) while since 2004, adults move in a larger range (5–12 km) to reach the freshwater canals around the site and secondarily along the mouth of the Marta and Mignone rivers spending only 55–60% of the feeding time into the saline. This behavior can be correlated to the disturbance (water level and human activities) occurred during the saline restoration project.

From the regurgitation analysis rests of many fish species such as *Aphanius fasciatus, Atherina boyeri, Mugil cephalus, Engraulis encrasicholos, Diplodus saragus, Anguilla anguilla* and *Gambusia affinis* as well the relevant presence of small *Crustacea*

(*Gammarus aequicauda*, *Idotea baltica* and *Cerphium insidiosum*) was found and in particular the presence of *Gammarus aequicauda* is particularly relevant: in spring time (June 2004) 361 ± 112 ind/m^2 was found (Blasi 2006). *Aphanius fasciatus* in particular due to its habitat characteristics (high salt content) seems to be mainly present in the Little Egret diet. Terrestrial *Arthropoda* and *Gasteropoda*, probably taken from grazing ground, moreover were eaten by Little Egret.

Eggshell thinning in birds was observed in the 1950s and 1960s as some of the first environmental consequences of the spreading of mercury and is still monitored. The mean eggshell thickness of 44 egg fragments of Little Egret collected around the heronry was 0.218 mm (SD 0.019) similar to the value found in Malaysia and Thailand (Keithmaleesatti et al. 2007). No significant difference, using Mann–Whitney *U* test, was observed comparing annual data and mean eggshell thickness was not significantly correlated with Hg concentration ($R^2 = 0.1225$).

Mercury is a metal that can exist in a range of organic and inorganic forms with varying degrees of toxicity and in particular in aquatic organisms. It adversely seems to affect reproduction, growth, behavior, osmo-regulation and oxygen exchange. A very important factor in the impacts of mercury to the environment is its ability to build up in the organisms and along the food chain.

Although all forms of Hg can accumulate to some degree, methyl mercury is absorbed and accumulates to a greater extent than other forms and its bio-magnification has a most significant influence on the impact on animals and humans. Fish appear to bind methyl mercury strongly and most of the methyl mercury in fish tissue is covalently bound to protein sulfhydryl groups as a consequence, there is a selective enrichment of methyl mercury and long half-life for elimination.

In Table 2, data regarding Hg concentrations are reported. All eggshells had detectable concentration of total Hg that ranged from 0.026 to 0.200 $\mu g\ g^{-1}$ dry weight and were similar to those in eggshells from China (Lam et al. 2004). Eggshell concentrations significantly change between some years ($U_{02–03} = 5$, $U_{02–04} = 0$, $U_{02–05} = 3$, $U_{02–06} = 7$, $P < 0.05$). Probably, changes in foraging habitat during the salt pans restoration activities (changes mainly in hydrology levels) could easily lead to a diet shift (e.g., variation in fish species or terrestrial prey such as Earthworms, *Gasteropodae*, etc.) reducing the Hg content.

Concentrations of Hg in feathers associated with adverse effects are reported in the range 5–65 $\mu g\ g^{-1}$ dry weight (Burger and Gochfeld 1997), within samples collected from Egret nestling the total Hg ranged from 0.41 to 9.64 $\mu g\ g^{-1}$ dry weight as reported in Table 2. The Hg concentration in feathers significantly change between years ($U_{03–05} = 8$, $U_{03–07} = 6$, $U_{05–07} = 10$, $P < 0.05$).

4 Conclusions

The hydrology of the saline ecosystem plays a major role in determining foraging habitat selection and therefore the reproduction success. In particular, the high water level and the large scale human activities occurred during the saline restoration plan, determined the reduction of the breeding pairs. This confirms the high vulnerability of the salt-pans ecosystems and that each action must be carefully programmed.

The levels of mercury found in the eggshells and feathers of Little Egrets suggest that the aquatic habitats of the Tarquinia marine salina have a background that must be considered in terms of environment monitoring. The collected data confirm the validity of the

Table 2 Concentration of Hg (μg g^{-1} dry wt) in eggshells and feathers of Little Egret

	2002	2003	2004	2005	2006	2007
Eggshells						
Mean ± SD	0.12 ± 0.045	0.068 ± 0.032	0.059 ± 0.016	0.072 ± 0.029	0.102 ± 0.041	0.095 ± 0.044
Range	0.109–0.147	0.032–0.132	0.042–0.094	0.036–0.122	0.063–0.200	0.026–0.179
Fragments (*n*)	9	10	9	12	10	10
Feathers						
Mean ± SD	2.81 ± 2.51	–	4.54 ± 3.25	–	5.12 ± 3.20	
Range	0.41–6.37		1.36–8.60		2.51–9.64	
n	4		5		5	

methodology as a tool to evaluate the status of an ecosystem and of environment in general (any bird has been killed to perform this study and, moreover, the analysis can be extended to other heavy metals and to organic compounds).

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