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Mercury in Egg and Eggshell of Whiskered Tern (*Chlidonias hybrida*) from Anzali Wetlands of the Caspian Sea, Iran

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Abstract Mercury content of egg and eggshell from Whiskered Terns of Anzali wetlands of the Caspian Sea were determined. Mercury levels in egg content of both abandoned (433 ± 4) and non-abandoned (459 ± 15) eggs were 150 times greater than eggshells. Eggshell thickness differed between non-abandoned (0.5 ± 0.03) and abandoned eggs (0.6 ± 0.5) ($p < 0.001$). No significant association was found between shell Hg and shell thickness in this study. There was significantly more Hg in egg content than in shell ($p < 0.001$). Mercury levels in eggs did not exceed the levels associated with adverse reproductive effects.

Keywords Mercury · Egg and eggshell · Whiskered Tern (*Chlidonias hybrida*) · Caspian Sea · Iran

Metal pollution and one of its challenging consequences, habitat destruction, has gained much attention recently. Environmental contamination by mercury (Hg) has become a growing concern (Wiener et al. 2003) because it has increased as much as two to tenfold during the past one and half centuries (Swain et al. 1992). Despite the seriousness of worldwide Hg pollution, no international legally-binding agreements exist to curb Hg release. Major conservation plans and actions depend on accurate bio-monitoring data from vulnerable habitats that are in danger of further Hg contamination. Anzali wetlands of Iran are stretched along the southern coast of the Caspian Sea. These wetlands host many resident and migrating birds and provide important nesting habitat for many species including Whiskered Terns (*Chlidonias hybrida*). Selkeh Wildlife Refuge (37°23′N, 49°27′E) is a protected area of 365,000 m^2 within Anzali Wetlands located along the southern coast of the Caspian Sea (37°0.8′N, 49°25′E) (Fig. 1).

We opted to look at eggs and eggshell Hg in Whiskered Terns of Anzali (a noninvasive method) since this bird is high up on the food chain, most common in the area, and a suitable bio indicator of Hg contamination. One advantage of studies such as this is that they provide baseline data which can be used in future to evaluate differences within a specific geographical area. Our study was also in response to the increasing interest in bird conservation in Iran; as part of a worldwide recognition that maintaining and protecting habitat integrity and diversity is critical in preventing further loss of habitat, environmental degradation, and ecosystem destabilization and collapse. In addition, we were interested in examining the issue of the relationship between Hg and eggshell thickness further. At present, conflicting data exists about effects of Hg on bird eggshell thickness. Some experimental data show that Hg causes eggshell thinning (Stoewsand et al. 1971), but others suggest that the effects of mercury do not include eggshell thinning (Peakall and Lincer 1972).

Materials and Methods

Anzali Wetlands cover an area of about 200 km^2 and are one of the most important wetlands of Iran (Fig. 1). The Convention on Wetlands of International Importance is an intergovernmental treaty that provides the framework for

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Fig. 1 Location of Selkeh Wildlife Refuge within the Anzali International Wetlands of Northern Iran

national action and international cooperation for conservation and wise use of wetlands and their resources. This treaty was adopted in the Iranian city of Ramsar in 1971 and came into force in 1975 when Anzali Wetlands were registered and recognized for the important role they play in ecology of migratory birds and as especial habitat for waterfowl. Anzali Wetlands have four distinct parts, (1) the Western lagoon, (2) the Eastern area, (3) the Central area [with Selkeh Wildlife Refuge], and (4) Siahkeshim protected area. Industrial, agricultural and domestic wastewaters enter the Eastern area of Anzali which support a variety of plant-life and where water depth ranges from 0.8 to 1.5 m. The Central Area encompasses Selkeh Wildlife Refuge (water depth ~ 1 m). Recent studies document that each day over six million m^3 of polluted water enter Anzali, and currently there are no control of physico-chemical parameters of these waters (Jafari 2009). Such discharges increase nutrient load and heavy metal content of the Anzali. Temperatures of Anzali range from 4.5°C in February to 27.5°C in August.

Eggs and eggshells were collected from breeding colonies of Whiskered Tern in June and July 2008 from Selkeh, under license of Environmental Protection Agency of Gilan. The area was monitored for eight weeks. Sixty eight nests in which egg-laying had started were marked with small plastic tags and were monitored for nesting and hatching success. Timing of initiation of clutches was determined. Whole-eggs [abandoned ($n = 20$) or non-abandoned ($n = 16$) and broken eggshells ($n = 19$)] were collected. Non-abandoned eggs were collected from nests containing two or more eggs between Day Zero to Day Fifteen of egg-laying (Burger and Eichhorst 2005). To avoid pseudo replication, eggs were removed from random areas of the breeding colony (Braune 2007). Sample preservation and preparation has been described (Ohlendorf 1986), eggs were weighed, length was measured, egg breadth was calculated, volume estimated (Coulson 1963) and eggs were stored at −18°C for later use.

Later, eggs were cracked open, content weighed, homogenized and freeze-dried at 60° C; content of eggs were ground to a powder in mortar and pestle and kept for later use. Eggshell preparation procedure is published (Elliott et al. 1996). Mercury was measured by the LECO AMA 254 Advanced Mercury Analyzer (USA) according to ASTM, Standard No, D-6722. Accuracy of total Hg analysis was checked by running three samples of Standard Reference Materials (SRM), (Al-Majid and Preston, 2000). Recovery varied between 94.8% and 105%; detection limit was 0.001 mg/kg of dry weight.

SPSS (version 15) was used. Data was tested for normality and homogeneity of variance (ANOVA); normality and homogeneity was confirmed. Paired t-test was used for analysis of data. Mercury level in egg content was compared to Hg in eggshell. Spearman' rank correlation coefficient was used to measure the strength of the association between breadth/length ratio and mercury concentration in eggs (Ikemoto et al. 2005). Ratcliffe's index (*eggshell weight/(egg length*egg width*) was calculated as a surrogate for eggshell density for each shell (Anthony et al. 2007). p was set at <0.05.

Results and Discussion

Marked nests (n = 68) were visited at least twice a week during the 2008 breeding season for 8 successive weeks. Egg length, breadth, mass, and volume are indicated in Table 1. Table 2 indicates Hg (ng/g, dry weight ± SEM) in abandoned, non-abandoned eggs and broken eggshells; and Table 3 summarizes clutch sizes. To assess reproductive success in each nest we monitored (1) clutch size (total number of eggs in each nest), (2) hatchability or number of eggs that hatched divided by the total number of eggs in the nest during the entire incubation period (Keithmaleesatti et al. 2007); and (3) nesting success, defined as the number of nests that produced at least one chick divided by total number of nests (Rumbold et al. 2001). Overall we observed 127 cases of lost eggs. Hatchability was 26.2% during the breeding season and nesting success was 66.2%.

No significant relationship was detected between egg breadth to egg length ratio and Hg concentration (Fig. 2), or between eggshell Hg and egg content Hg (Fig. 3). There was significantly more Hg (150 times greater) in eggshell, than in egg content; in both abandoned (433.2 ± 4) and non-abandoned (459 ± 15) eggs ($p < 0.001$). Eggshell density differed between non-abandoned (0.5 ± 0.03) and abandoned eggs (0.6 ± 0.5) ($p < 0.001$). Abandoned eggs had significantly thicker shells. But when considering relationship between Hg content of non-abandoned and abandoned eggs and the shell thickness; we found no significant associations. No significant association was found between shell Hg and shell thickness in any of the eggs either. In regards to eggshell thickness our field data does not corroborate with lab experiments that suggest Hg to be the cause of eggshell thinning. While it is possible that a species difference is involved, it is more likely that the form of Hg is critical. We know that methylation of Hg occurs in biological systems and the top predators probably receive most of their Hg in the form of methyl mercury.

Egg breadth/length ratio is known to increase with the age of the mother bird (Ikemoto et al. 2005). Relationship between breadth/length ratio and Hg levels was examined to investigate any possible influence that mother bird's age may have had on egg Hg. Mercury levels in egg content and eggshell did not increase with an increase in breadth/length ratio ($p > 0.05$) (Fig. 2).

In general, species that are higher on the food chain accumulate higher levels of contaminants and seabirds are particularly vulnerable because they are long lived and are often at the top of their food chains. In biological systems, Hg levels are suggested to reflected trophic-level relationships. Since Tern's diet consists of fish, water insects and algae, they are considered high up on the food chain. Bald eagles and gulls are also high on food chain, but they eat much larger prey items and their eggs have been shown to have 5–13 times more Hg than Whiskered Terns from the Caspian (Anthony et al. 2007). Levels of Hg in waterfowl of South Carolina, Hawaii, Florida Everglades, and coastal Maine resemble those found in Whiskered Terns of the Caspian (Ohlendorf 1986; Rumbold et al. 2001). Lab data shows that mercury levels of 1.5 ppm in eggs are associated with adverse effects, such as impaired reproduction.

Table 3 Clutch sizes of Whiskered Tern

Clutch size	Number of nests	% of total nests
1 egg	6	8.8
2 eggs	24	35.3
3 eggs	34	50
4 eggs	4	5.9
Total	68	

Table 1 Statistical description of eggs of Whiskered Tern's from Anzali Wetlands of the Caspian Sea, Iran

	Egg length (mm)	Egg breadth (mm)	Breadth/length ratio	Mass (gm)	Weight (gm)	Volume (cm^3)
Abandoned eggs	36 ± 0.35 (15)	25.6 ± 0.25 (15)	0.71 (13)	14 ± 0.34 (16)	5.6 ± 0.6 (11)	11.5 ± 0.3 (13)
Non-abandoned eggs	36.2 ± 0.3 (16)	26 ± 0.1 (16)	0.71 (16)	14.4 ± 0.15 (16)	4.7 ± 0.3 (15)	11.6 ± 0.3

Values are (mean ± SEM). Sample number indicated in the parentheses

Table 2 Mercury in eggs and eggshells of Whiskered Tern (ng/g, dry weight ± SEM)

	Hg in egg content (ng/g, dry weight ± SEM)	Hg in eggshell (ng/g, dry weight ± SEM)	Content to Eggshell ration
Abandoned (n = 20)	433 ± 39	2.6 ± 0.5	165.3
Non-abandoned (n = 16)	459 ± 15	2.9 ± 0.3	156.7
Broken eggshells (n = 19)		2.6 ± 0.9	

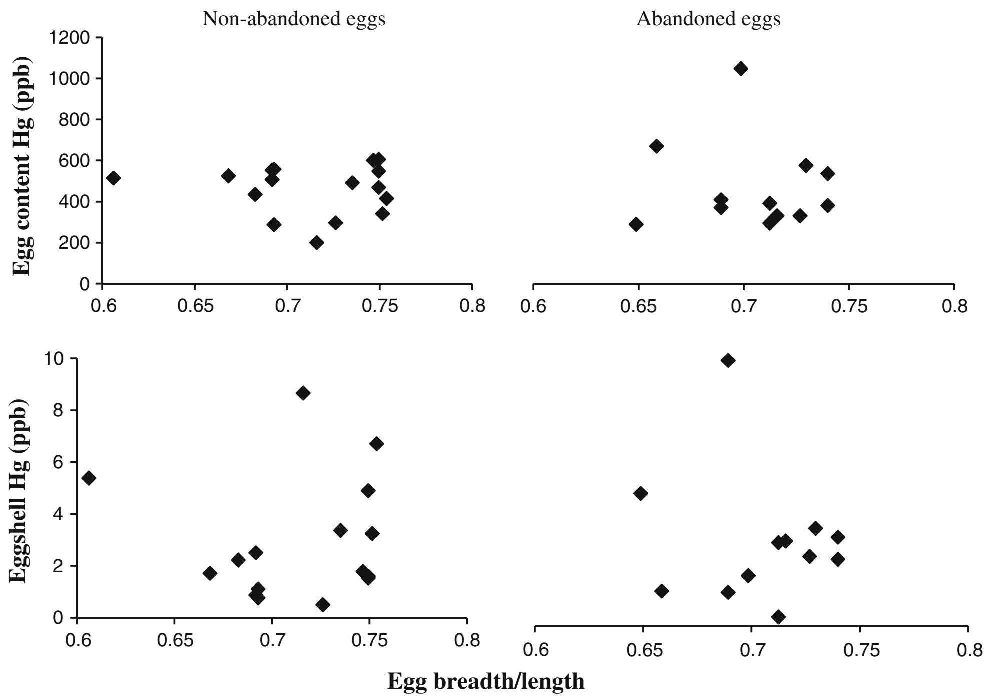

Fig. 2 Egg breadth to egg length ratio and mercury concentration in Whiskered Tern from Anzali International Wetlands of the Caspian Sea, Iran

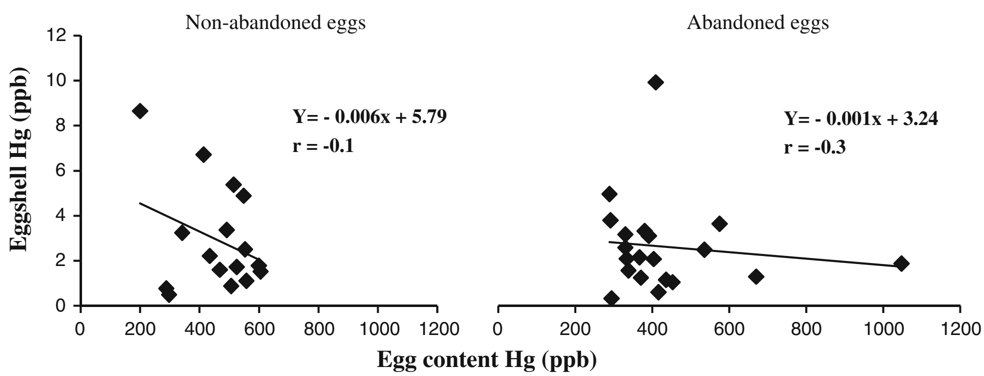

Fig. 3 Correlation between mercury concentration (ppb, dry weight) in the egg content and eggshell of Whiskered Tern from Anzali International Wetlands of the Caspian Sea, Iran

Mercury levels in our terns were lower than levels considered damaging to reproductive health in birds. However, continued release of untreated waste-water into Anzali Wetlands will be harmful to bird populations in these wetlands. For conservation efforts to be effective, measures have to be put in place immediately to protect these valuable wetlands from further contamination by anthropomorphic contaminants including Hg.

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