

Mercury Pollution in Three Species of Waders from Shadegan Wetlands at the Head of the Persian Gulf

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Abstract Persian Gulf supports diverse ecosystems and biota in need of remediation and protection, and metal data from this region is needed. Mercury (Hg) in tissues of three waders (Black-winged Stilt *Himantopus himantous*, Red-wattled Plover *Hoplopterus indicus*, and White-tailed Plover *Vanellus leucurus*) from Shadegan Wetlands is reported. Black-winged Stilt had higher Hg in feather (6.6 ± 0.6 µg/g dry weight), liver (3.5 ± 1 µg/g dry weight), kidney (4.5 ± 0.8 µg/g dry weight) and muscle (1.2 ± 0.2 µg/g dry weight) (not statistically significant). Differences in Hg among waders could have resulted from diverse feeding habitats and dissimilar foraging sites.

Keywords Mercury · Black-winged Stilt (*Himantopus himantous*) · Red-wattled Plover (*Hoplopterus indicus*) · White-tailed Plover (*Vanellus leucurus*)

Persian Gulf is a semi-enclosed formation and heavy discharges of the surrounding industries have been ongoing

for many decades. Other sources of Persian Gulf pollution include invasions and bombardments that have been staggering in the recent years and are yet to be fully investigated. Although heavy metals in general and Hg in particular are very toxic to both humans and the wildlife, limited research is available on Hg pollution in the Persian Gulf area. Aquatic environments, such as Shadegan, are especially at high risk for Hg contamination since much of the atmospheric deposition and all of the industrial water-runoffs culminate in these ecosystems.

Shadegan wetlands are located at the head of the Persian Gulf near the city of Abadan in southwestern Iran (30°20'N 48°20'E) (Fig. 1). Shadegan is the largest area of wetland in Iran covering ~400,000 ha; encircling the southern tip of an extensive floodplain and delta system. To date, 149 species of birds have been spotted and documented in this area. In the northeastern parts of these wetlands there are large rice fields, date orchards and residential areas. Harvest of fish, birds, and wetland reeds are of considerable importance for the local economy. Reeds are cut on a large scale in mid-summer to provide materials for thatching and weaving.

Large areas of agricultural lands, local fisheries, oil export facilities, a sugar cane plant, and a petrochemical plant operate in the general area. Recent studies show that Shadegan's natural water regime has been disrupted by excessive withdrawal of water for irrigation, and the saline discharges from the local sugar cane industries. Moreover, an overall reduction in wetland water quality and quantity has ensued, leading to changes in plant community composition which can be expected to have affected aquatic and terrestrial organisms of the area. Effects of recently introduced war-pollution on these wetlands and their inhabitants, while still not fully known, may be severe and irreversible. Known sources of damage to these wetlands

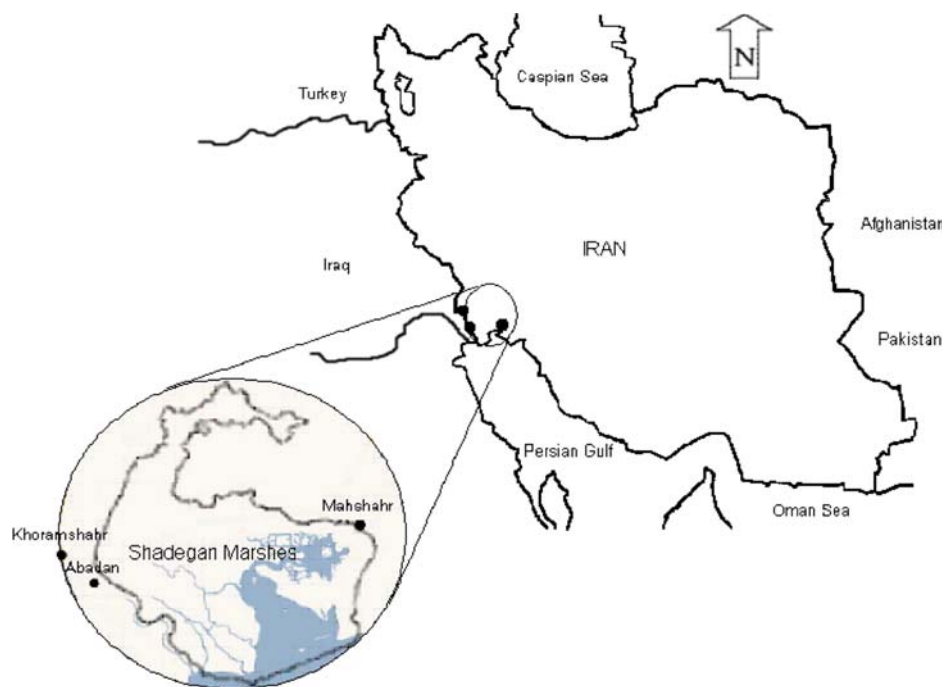
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Fig. 1 Map showing sampling locations in Iran



include degradation due to acid rain falls after 1991 Persian Gulf invasion (Evans 1994) and agricultural runoff from use of fertilizers, herbicides, pesticides, and spills of hazardous substance from various refineries, and Bandar Imam Petrochemical Factory.

Waders are infrequently reported on in the toxicology literature and metal toxicity data is needed for birds from Persian Gulf area. We examined Hg levels in liver, kidney, muscle, and breast feathers of three species of waders [the Black-winged Stilt (*Himantopus himantous*), the Red-wattled Plover (*Hoplopterus indicus*), and White-tailed Plover (*Vanellus leucurus*)] from Shadegan wetlands located in Southwestern Iran at the head of the Persian Gulf. Waders we studied have comparable diets (all three are intermediate consumers and feed mainly on invertebrates and local vegetation). However, White-tailed Plover is more omnivorous than others and frequently preys on invertebrates. Black-winged Stilt is distinct from the other two species in that it has the longest legs, proportional to its body size, allowing it to access deeper waters where it can catch and consume larger prey (Sterry et al. 2001). Consequently we hypothesized that there will be higher Hg concentrations in tissues of Black-winged Stilt when compared to Red-wattled Plover and White-tailed Plover.

Materials and Methods

Table 1 summarizes our data. Thirteen individual birds of three species (Black-winged Stilt $n = 4$; Red-wattled Plover $n = 3$; and White-tailed Plover $n = 6$) were shot and

removed from Shadegan once during February and March of 2006, and then again in October and November of 2007. Birds were weighed and stored in plastic bags that were previously cleaned with acetone and water. Samples were kept at -20°C until dissection and analysis.

Birds were thawed for dissection and sexed. Presence of ovary and oviduct was indicative of a female sex, and appearance of male gonads indicated male sex (Dam et al. 2004). Liver, kidney, pectoral muscle, and breast feathers were carefully removed. Breast feather was selected for analysis because it is believed that exposure to metals is better represented by this type of feather (Burger 1993). Breast feathers were washed vigorously in deionized water, alternated with acetone, to remove loosely adherent external contamination (Burger and Gochfeld 1991). Liver, kidney, and muscle tissue were removed, freeze-dried, homogenized, and ground in a porcelain mortar and pestle. Total tissue Hg was determined by direct combustion atomic absorption spectrometry using advanced mercury analyzer (LECO AMA 254, USA). The advantage of using the thermal decomposition technique for Hg determination is that it does not require sample manipulation. Detection limit of the instrument was $0.001 \text{ mg/kg dry weight (dw)}$. In order to monitor and validate the analytical capability of the method, accuracy of total Hg analysis was checked by running three samples of Standard Reference Materials (SRM), (National Institute of Standards and Technology (NIST), SRM 1633b, SRM 2709, and SRM 2711) in seven replicates (Al-Majid and Preston 2000; Zamani-Ahmadmohammadi et al. 2008).

SPSS (Version 13.0) was used for data analysis. All concentrations are expressed in $\mu\text{g/g}$ of dw and analyses

Table 1 Characteristics of three wader species from Shadegan Wetlands located in Southwestern Iran at the head of the Persian Gulf

Common name	Scientific name	Feeding habit	Sex	Weight (g)
Black-winged Stilt	<i>Himantopus himantopus</i>	Invertebrate predator: consumes mainly invertebrates, frog eggs, tadpoles, and some seeds and vegetation	Male (n = 2)	160 ± 5
			Female (n = 2)	
Red-wattled Plover	<i>Hoplopterus indicus</i>	Invertebrate predator: consumes mainly invertebrates, and some vegetation	Male (n = 1)	178 ± 16
			Female (n = 1)	
			Unknown (n = 1)	
White-tailed Plover	<i>Vanellus leucurus</i>	Omnivorous: consumes mostly insects (i.e. beetles, grasshoppers, caterpillars, fly larvae and locusts). Also, invertebrates, mollusks, crustacean, and some vegetation	Male (n = 3)	134 ± 6
			Female (n = 3)	

were performed on weight of dry tissue because these values are more reliable and consistent than wet weight. Differences amongst means were determined by nonparametric Kruskal–Wallis one-way analyses of variance. A significant ANOVA (p -value < 0.05) was followed by a posteriori pair-wise comparison by Mann–Whitney U and Benferroni procedure (Holm 1979). A nonparametric test was used since it is conservative and best suited for small datasets. Spearman's correlation test was applied to correlation analyses. Values are given in means ± standard errors (SE).

Results and Discussion

Hg concentrations in tissues of waders are shown in Table 2. In Black-winged Stilt Hg concentration was highest in feather, followed by kidney, liver, and muscle, whereas in the Red-wattled Plover and White-tailed Plover Hg was highest in feather and liver, followed by kidney and muscle. According to field and experimental studies, tissue distribution and accumulation of Hg in birds varies widely depending on growth stage, breeding status, molting and migration (Honda et al. 1986). In general, metal exposure and tissue distribution in birds varies based on the route of exposure and duration of exposure (i.e., chronic or acute) (Lee et al. 1989).

In our samples, we found significant correlations between mercury in kidney and breast feather ($r = 0.8$), liver and kidney ($r = 0.91$), muscle and kidney ($r = 0.82$) and muscle and liver ($r = 0.96$, $p < 0.05$, $n = 13$). Waders in this study have very similar diets; they are all intermediate consumers which feed mainly on invertebrates and vegetation. But, White-tailed Plover is a more omnivorous bird compare to the other two species which are more invertebrate predators. Foraging grounds of these waders are also somewhat different which leads to differences in prey size and ultimately Hg intake. Black-winged Stilt, for example, is distinct from the other two species in that proportional to its body size, it has the longest legs which

allows access to deeper waters where it can catch and consume larger prey items (Sterry et al. 2001). Black-wing Stilt also spends more time in shallow waters and coastal lagoons while the two other species spend more time in terrestrial areas where anthropogenic mercury is less widely present. We therefore expected to see dissimilar levels of mercury in tissues of these three wader species. Despite the fact that there were no significant differences in mercury levels between species, Black-wing Stilts with longest legs tended to have the highest mercury body burdens. Similarly Burger and Gochfeld (1993) have reported greater levels of mercury, and other metals, in the taller and larger Great egret compared to smaller and shorter Night heron, Little egret, Pond heron and Cattle egret.

In regards to sex-dependent metal body burden in birds, there is conflicting data. Several studies indicate a difference in metal body burden between male and female birds (Evans and Moon 1981; Hutton 1981; Gochfeld and Burger 1987) while other studies report negligible differences in Hg between sexes (Honda et al. 1986; Furness et al. 1990). Given our small sample size we did not expect to be able to address sex differences in the three species of waders. Nonetheless, with only three males and three females of White-tailed Plover we found that mercury values were larger in liver, kidney and muscle tissue of male of the species than the females (Fig. 2). This finding merits future investigation since a larger sample size will allow a more accurate analysis and detection of significant differences if they indeed exist. Other reports indicate that although female birds can get rid of Hg in their eggs, the amount they shed in this way is usually small compared to the amount put into feathers during molt (Furness 1993). Therefore the small difference that has been reported in Hg body burdens in male and female is consistent with our current data. Evers et al. (2005) argues that this difference in mercury levels between sexes can be attributed to (1) depuration in eggs, (2) sexual dimorphism, and (3) niche partitioning of the forage base. Black-neck Stilt from San Francisco Bay in California had higher levels of Hg in

Table 2 Mercury concentrations ($\mu\text{g/g}$ dry weight) in tissues of three waders

Wader species	Feather	Liver	Kidney	Muscle
<i>Himantopus himantopus</i> (n = 4)	6.6 ± 0.6 (5.3–8.2)	3.5 ± 1 (1.9–6.4)	4.5 ± 0.8 (3–6.7)	1.2 ± 0.2 (0.6–1.8)
<i>Hoplopterus indicus</i> (n = 3)	0.8 ± 0.2 (0.6–1.3)	0.8 ± 0.3 (0.35–1.4)	0.7 ± 0.3 (0.3–1.3)	0.4 ± 0.1 (0.2–0.6)
<i>Vanellus leucurus</i> (n = 6)	1.9 ± 0.5 (0.7–3.8)	1.9 ± 0.5 (0.6–3.9)	1.6 ± 0.4 (0.5–3.5)	0.8 ± 0.2 (0.2–1.4)
p	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$

Values are mean \pm SE followed by range in parenthesis

breast feather ($8.45 \pm 0.8 \mu\text{g/g dw}$), kidney ($6.8 \pm 0.6 \mu\text{g/g dw}$), liver ($7.6 \pm 0.6 \mu\text{g/g dw}$) and muscle ($2.6 \pm 0.2 \mu\text{g/g dw}$) (Eagles-Smith et al. 2008), than those we collected from Shadegan. But, top carnivores from Shadegan marshes, including Common kingfisher (*Alcedo atthis*), Pied Kingfishers (*Ceryle rudis*), and white-breasted kingfisher (*Halcyon smyrnensis*), had lower levels of Hg in breast feather, liver, kidney, and muscle than Black-wing Stilt from the same area (Zamani-Ahmadm Mahmoodi et al. 2008). Over all, Black-wing Stilt in this study had higher feather Hg (mean 6.6 ± 0.6 , range 5.3–8.2 $\mu\text{g/g dw}$) than 5 $\mu\text{g/g dw}$ which is known to cause sub-lethal and reproductive adverse effects (Eisler 1987) and are therefore at risk for Hg toxicity. But, levels of Hg in feathers of Red-wattled Plover and White-tailed Plover were lower than those associated with adverse effects. In contrast Hui et al. (2001) suggests that shorebirds with liver Hg of greater than 3.0 $\mu\text{g/g dw}$ may be vulnerable to sub-lethal adverse effects of this metal.

To our knowledge, this is the first report on Hg levels in Red-wattled Plover and White-tailed Plover. Here we present Hg levels in multiple tissues of three waders from Shadegan marshes in Southwest Iran. Scarcity of data on these birds makes this information an invaluable regional data. Differences in mercury concentrations among the three wader species are likely to have resulted from different feeding habitats and differing locations within Shadegan marshes. As we suspected, although statistically not significant, mercury was higher in tissues of Black-winged Stilt which consumes more invertebrates and has

access to deeper waters. It is conceivable that a larger sample size would have enabled us to reveal significant differences in Hg levels between our species and between the sexes. Regardless, Hg levels in Black-winged Stilt of Shadegan were higher than those associated with adverse effects and even higher than fish-eating birds from the same area which we have previously reported (Zamani-Ahmadm Mahmoodi et al. 2008).

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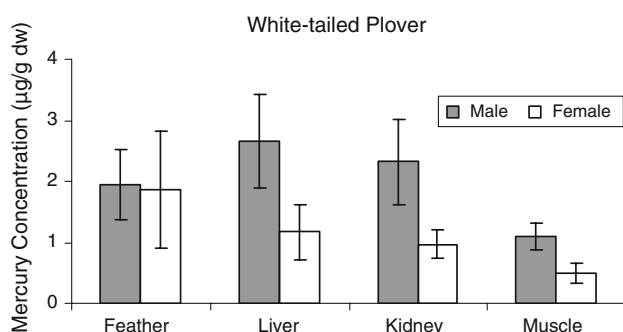


Fig. 2 Mercury concentration in the tissues of male and female White-tailed Plover (Mann–Whitney U , $p > 0.05$)

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