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The Use of Blood in *Anas clypeata* as an Efficient and Non-lethal Method for the Biomonitoring of Mercury

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Abstract Hg was analyzed in seven tissues of 52 common shoveler *Anas clypeata* collected from the coast of SE Gulf of California. Mean Hg concentrations were highest in the liver (2,885 ng g^{-1}) and lowest in the gizzard (621 ng g^{-1}); they followed the order: liver, feathers > muscle tissue and tissues of the circulatory system > digestive organs. Hg levels were similar or higher than birds of the same trophic level and feeding habits. Considering the relationships of Hg among tissues and blood we recommend the use of blood as an efficient method to monitor Hg.

Keywords Mercury · Accumulation · *Anas clypeata* · Gulf of California

Although Hg can be found naturally in the environment, anthropogenic activities have increased its global incidence in modern times (Lamborg et al. 2002). In Mexico the most important sources of Hg to the environment are the chlor-alkali process, primary and secondary production of mercury, mobilization of impurities in manufacturing (cement, lime, pulp, paper, nonferrous metals, iron and steel) and impurities from the combustion of coal, oil, natural gas and firewood (PNUMA 2005). Waterfowl can be used as indicators of contamination by heavy metals, including Hg, because these birds are: (1) abundant, (2) distributed over a wide geographical area, (3) may represent various trophic levels and (4) may be sufficiently long-lived to accumulate Hg over a long period of time (Zamani et al. 2009). In addition, the study of Hg contamination in birds could be a tool to assess the potential effects of metal toxicity on organisms of the same trophic level. Furthermore, migratory birds can be used to compare their exposure in different regions of the world across which they fly. *Anas clypeata* is a migratory waterfowl species native to the northern USA and Canada. Like many birds of the family Anatidae, they migrate in autumn via the Pacific route to the east coast of the Gulf of California, Mexico (Sinaloa coast) where it may stay until winter ends or may continue their flight to the south. The economy in Sinaloa state is sustained principally by agriculture, fisheries, shrimp aquaculture and tourism. Agricultural activity is intensive and is directed mainly to vegetables, fruits, grains and pastures and is associated with the use of agrochemicals (fertilizers, pesticides, fungicides). Additionally, industrial activities in Sinaloa state that have some relationship to consumption and disposal of Hg include paper manufacturing, paper products, printing and publishing, production of non-metallic mineral and metal derivates, machinery and equipment. Mining is focused mainly on gold, silver, lead, copper and zinc. The purpose of this paper is to present the Hg concentrations found in the muscle, feathers, blood, gizzard, small and large intestines, liver and

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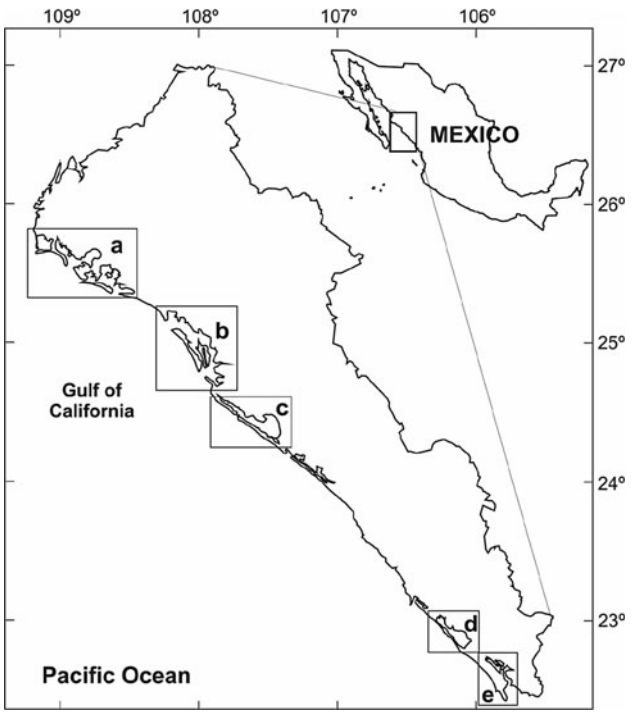


Fig. 1 Map of the SE Gulf of California indicating sampling sites: *a* El Colorado, Topolobampo-Ohuira, Navachiste; *b* Santa María-La Reforma; *c* Altata-Ensenada del Pabellón; *d* Huizache-Caimanero; and *e* Sistema Majahual

heart in the common shoveler *A. clypeata* collected on the coast of Sinaloa during the winter. The results show the advantages of using blood analysis as an alternative method with respect to killing and dissecting of birds for monitoring Hg concentrations.

Materials and Methods

The study area extends across the coastal strip of Sinaloa state, Mexico, between 22°30′40″ and 27°02′42″N in the SE coast of the Gulf of California. The climate along the coast is warm; averages temperatures ranges from 10.5–36°C per year, and the average rainfall of 790 mm per year (INEGI 2011). A total of 52 specimens of common shovelers were collected along the Sinaloa coastal systems (Fig. 1), with the support of local hunting clubs. Thirty specimens were collected between November and December 2001 (arrival period), and 22 between February and March 2002 (return period) (Table 1). Taxonomic identification was made by illustrated keys. The specimens were separated by sex and weighed. Feathers were extracted from different parts of the body. After dissection of the pectoral muscle, underlying tissues and organs were extracted. Samples of muscle, feathers, blood, gizzard, small and large intestines, liver and heart were obtained and the wet weight of each sample was recorded. The samples (except feathers) were lyophilized (for 72 h, −54°C and 133×10^{-3} mbar) then dry weight was recorded. Dried samples were ground in an agate mortar (Retsch). Separately, the feathers were washed with acetone (grade reactive J.T. Baker) and dried in a room free of dust for further analysis.

Prior to use, all materials (plastic and glass) used for the analysis of Hg were washed using baths of HCl 2M, HNO_3 2M and MilliQ water. About 0.25 g of dried and ground sample were digested using 5 mL concentrated HNO_3 (Baker trace metals grade) in Teflon vessels in a CEM microwave (operating conditions: total time 50 min, divided into three stages, 90 % power, pressure from 20 to 90 psi). The digested samples were taken to a volume of 25 mL with MilliQ water and stored in containers of HDPE until analyzed. Hg analysis were carried out by reducing mercury compounds in solution samples using $SnCl_2$ and detection by atomic absorption spectrophotometry coupled to cold vapor generator in a Varian SpectrAA 220 spectrophotometer (Páez-Osuna et al. 2011). To check for contamination, blanks were also analyzed using this procedure including one blank every 10 samples; the lecture of blanks were zero to closely to zero. The accuracy of the Hg method was assessed by analysis of the reference material MA-B-3/TM (IAEA 1987 Monaco) fish muscle and the results (550–580 ng g^{-1}) were within the stated confidence

Table 1 Characteristics of the birds collected in the coast of Sinaloa (SE Gulf of California, Mexico)

Site of capture	Period	Sex	N	Mean weight ± SD (g)
Sinaloa northern coast	24 Nov–7 Dec 2001	F	4	533 ± 6
(a, b, c)		M	3	639 ± 7
	25 Feb–3 Mar 2002	F	7	531 ± 65
		M	8	520 ± 200
Sinaloa southern coast	24 Nov–7 Dec 2001	F	11	451 ± 188
(d, e)		M	7	565 ± 81
	25 Feb–3 Mar 2002	F	6	572 ± 65
		M	6	579 ± 49
			$\sum = 52$	

F female, *M* male, *n* number of individuals, a, b, c, d and e correspond to sampling sites (see Fig. 1)

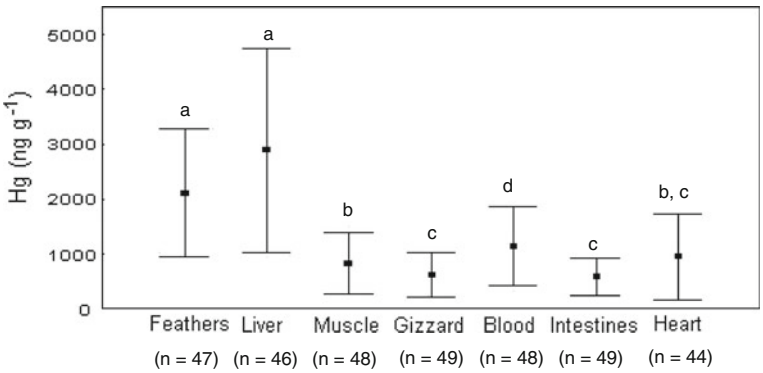


Fig. 2 Hg concentration (dry weight) in tissues of *A. clypeata*. *Error bars* standard deviation. *Different letters* indicate significant ($p < 0.05$) differences in Hg mean concentrations between tissues

Table 2 Hg concentrations (ng g^{-1} dry weight) in birds with similar feeding habits that *A. clypeata*

Species	Tissues	Region of the world	Hg (SD)	References
Anas acuta	Liver	Siberia, Russia	2,800	Kim et al. (1996)
Anas crecca	Liver	Khuzestan, Iran	4,340	Zamani et al. (2009)
Larus argentatus	Liver	Minsener Oldeoog, Germany	4,545	Lewis et al. (1993)
Anas clypeata	Liver	SE Gulf of California	2,885 (1,846)	This study
Anas acuta	Feathers	Siberia, Russia	330	Kim et al. (1996)
Somateria mollissima	Feathers	Alaska, USA	980 (144)	Burger et al. (2008)
Anas crecca	Feathers	Khuzestan, Iran	2,000	Zamani et al. (2009)
A. clypeata	Feathers	SE Gulf of California	2,120 (1,164)	This study
Anas acuta	Muscle	Siberia, Russia	310	Kim et al. (1996)
Anas clypeata	Muscle	SE coast, Gulf of California	470	Ruelas-Inzunza et al. (2009)
Anas crecca	Muscle	Khuzestan, Iran	1,500	Zamani et al. (2009)
Anas clypeata	Muscle	SE Gulf of California	830 (557)	This study

SD standard deviation

Table 3 Correlation coefficients (*r*) obtained from the matrix (simple linear correlation) between Hg concentrations determined in tissues of *A. clypeata*

Tissues	Feathers	Liver	Muscle	Gizzard	Blood	Intestines	Heart
Feathers							
r	1	–	–	–	–	–	–
n							
Liver							
r	–	1	0.82	0.50	0.65	0.74	0.68
n			39	40	37	42	36
Muscle							
r	–	–	1	0.57	0.66	0.80	0.55
n	–			45	42	48	41
Gizzard							
r	–	–	–	1	0.53	0.60	0.40
n					43	48	43
Blood							
r	–	–	–	–	1	0.59	0.50
n						45	39
Intestines							
r	–	–	–	–	–	1	0.60
n							44

Only are given significant ($p < 0.05$) values

–, not significant; *n,* number of data

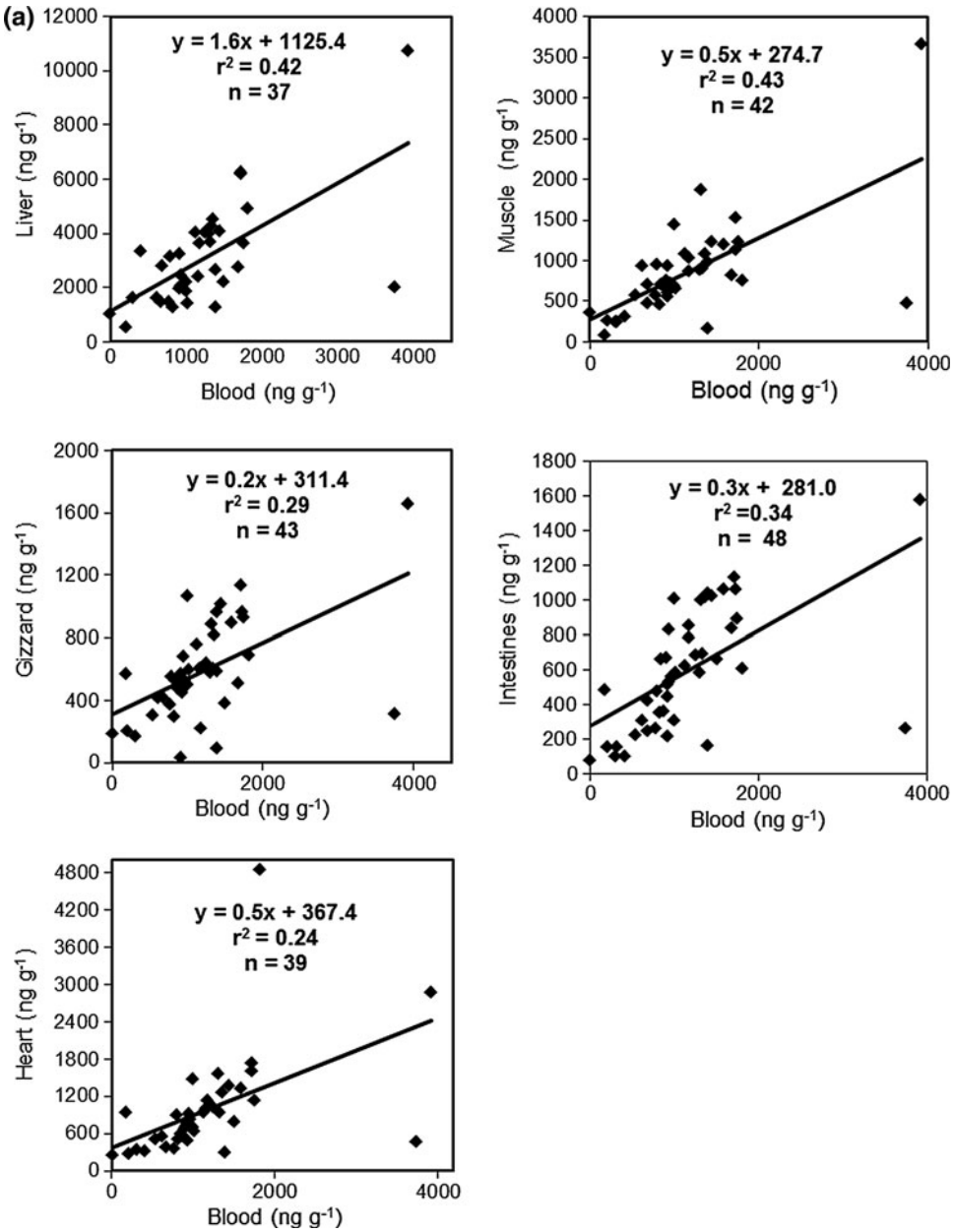


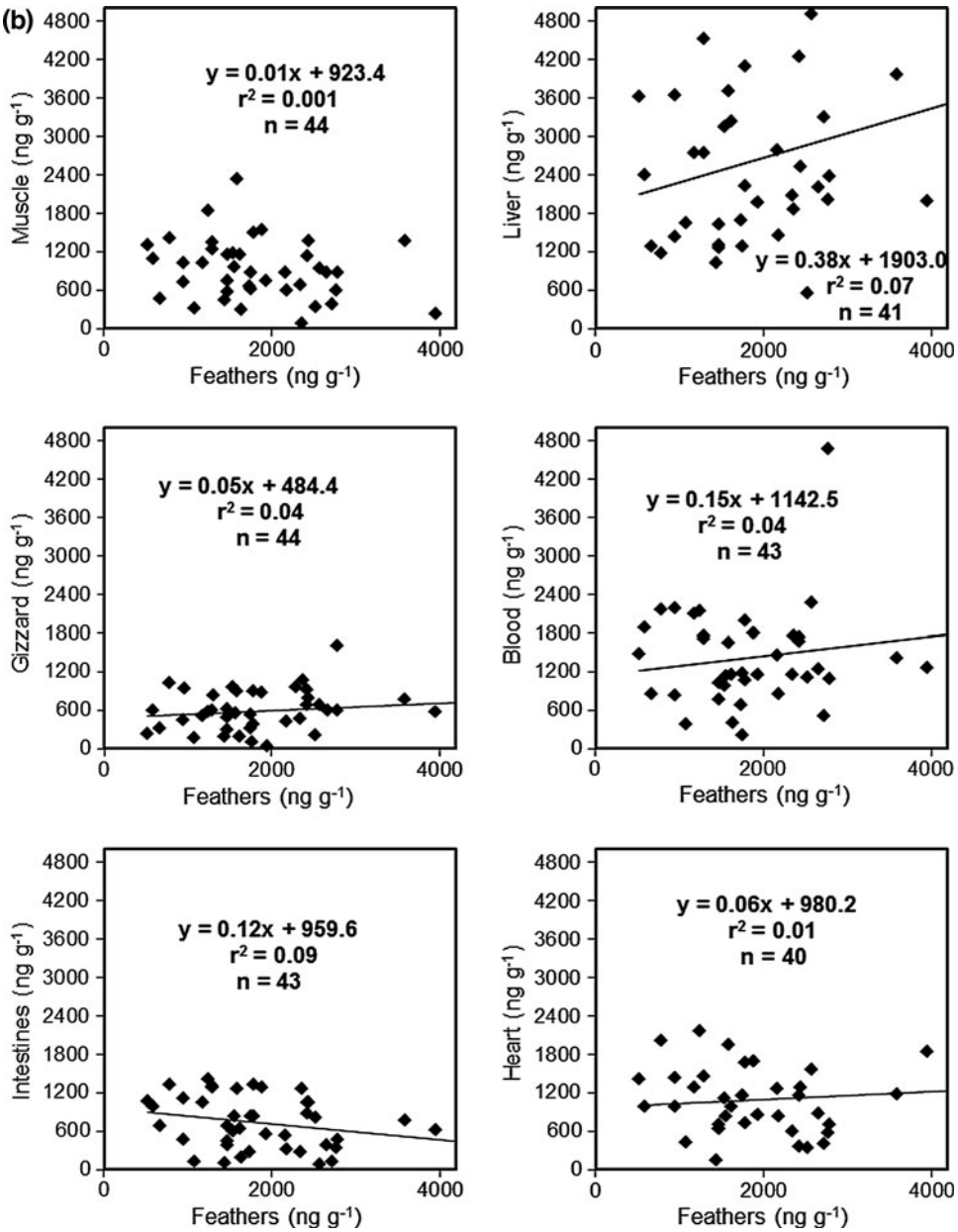
Fig. 3 Linear relationships between the concentration of Hg in the blood (**a**), and feathers (**b**) of *A. clypeata* with other tissues

interval for the reference material (470–610 ng g^{-1}). The coefficient of variation was 5.1 %, obtained with the analysis of 10 replicates of the muscle sample. The limit of detection was 50 ng g^{-1}; which reveal a poorly sensitive method but sufficient for the most of levels registered in the analyzed tissues. Hg concentrations of the seven tissues were compared by one-way ANOVA and with the aim of determining the correlation between Hg concentrations of the different tissues; the pearson correlation coefficient r was calculated. Both statistical tests were performed in Statistica software (Statsoft Inc. 1999). Additionally, data were analyzed by principal component analysis (PCA) using the program XLSTAT 7.5.2 (Addinsoft Company).

Results and Discussion

Hg concentrations in the tissues of *A. clypeata* ranged (in ng g^{-1}) between 478 and 10,727 for liver, 422 and 5,441 for feathers, <50 and 3,926 for blood, 80 and 4,852 for hearth, 77 and 3,658 in muscle, <50 and 1,657 in gizzard, and 104 and 1,735 in intestines. In general such levels showed the following order; liver > feathers > blood > heart > muscle > gizzard > intestines (Fig. 2). A summary of the Hg concentration in three tissues of the common shoveler and other birds with similar feeding habits (including three of Anatidae, with similar migration habits) is shown in Table 2. Here the exception is the species

Fig. 3 continued



Larus argentatus which has a different feeding habit (omnivorous, predominantly carnivorous) and an upper trophic level and presented the highest Hg levels in liver (Lewis et al. 1993). The correlation analysis (Pearson test) among the seven tissues with significant ($p < 0.05$) correlations is shown in Table 3. Plots of linear regressions among blood with various tissues are presented in Fig. 3a. Results from PCA including 7 variables showed three factors that accounted for 77.2 % of the total variation: factor 1 explained 48.3 % of the total variation and included a positive correlation with muscle, hearth, intestines and liver, and a negative correlation with feathers; factor 2 explained 17.2 % of the total variation and included a positive correlation with feathers, gizzard and blood, but a negative correlation with intestines and muscle. Factor 3 explained 11.7 % of variation with a positive correlation with blood. The scatter plot of PCA loadings by variables showed that Hg in feathers is unrelated to Hg concentrations of the rest of tissues; in addition, the plot of PCA loadings by observations showed aggregations with an undefined pattern for the Hg determinations from the two seasons.

In general, the lowest Hg concentrations occurred in organs related to digestive processes (gizzard and intestines). Intermediate mercury concentrations were detected in muscle and the circulatory system (blood and heart); the

highest concentrations corresponded to organs related with the transformation and elimination of Hg (liver and feathers). The liver and feathers had the highest concentrations of Hg as has been found in other studies of birds (e.g. Kim et al. 1996; Burger et al. 2008; Zamani et al. 2009). The removal of Hg in the body may occur through a natural process of hepatic detoxification where the change of organic Hg (mainly methyl mercury) into inorganic Hg occurs. This Hg transformation results in enriched levels of inorganic mercury in the liver (Eagles-Smith et al. 2009). Also, feathers are considered to be a route of elimination of Hg through the moult, so Hg may decrease in other tissues as it accumulates in feathers (Lewis and Furness 1991). Hg concentrations in liver in this study were similar or lower than in the liver of other birds from the northern hemisphere (Table 2). In feathers, concentrations were higher or comparable to those in birds from other studies of the northern hemisphere. The average Hg concentration in the muscle of *A. clypeata* in this study was higher than that reported by Ruelas-Inzunza et al. (2009) who examined total and organic mercury in three individuals for the same species in the same study area. Moreover, the Hg concentration in muscle in this study is higher than that found by Kim et al. (1996) in Siberia, Russia for *Anas acuta* and less than that registered by Zamani et al. (2009) in Khuzestan, Iran in muscle of *Anas crecca*.

Collection of feathers is non-invasive, and allows chemical analysis without killing the birds. Feathers can accumulate metals at concentrations high enough to be detectable in a relatively small sample of tissue (Bianchi et al. 2008). Moreover, in some studies, a positive correlation between the concentrations of Hg in feathers and levels in their internal tissues has been shown (Lewis et al. 1993). However, in the present work no significant correlation was found between the Hg concentrations of feathers and those of internal tissues (Fig. 3b); this may be because feathers reflect the concentrations of Hg that occurred in internal tissues during the time that feathers were formed, which is associated with SH^- groups of keratin (Burger et al. 2008) and is distributed homogeneously (Bianchi et al. 2008). Although the feathers had relatively high Hg levels, we cannot suggest the use of common shoveler feathers in environmental monitoring of Hg. The Hg is typically carried in the blood cells through the circulatory system to various organs. A portion of the Hg that is released into the bile from the liver is reabsorbed by the small intestine. Detoxification may occur in the digestive tract through bacterial action or in the tissues through endogenous enzymatic pathways (Pokras et al. 1998). These biological mechanisms associated with the distribution of Hg may help explain the significant correlation ($p < 0.05$) between the various internal tissues and blood in this study, which can be the basis for proposing blood as an additional poorly non-invasive alternative for monitoring Hg in *A. clypeata* that may reflect more closely than feathers the concentration of Hg levels in other organs (Fig. 3a, b).

The sex of the birds, as well as the body size can be important characteristics that can influence differences in the Hg accumulation (Robinson et al. 2012), although Hg concentrations between female and male in this study were not significantly ($p > 0.05$) different. Weight of birds was not significantly ($p > 0.05$) correlated with Hg concentrations. As of temporary variations, Hg concentration was not significantly ($p > 0.05$) different between periods of sampling, i.e., Hg levels in birds collected during the arrival period versus Hg levels at birds collected during the return period.

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