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RESEARCH ARTICLE

Mercury levels in birds and small rodents from Las Orquideas National Natural Park, Colombia

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Abstract

Mercury (Hg) is a heavy metal known as one of the most toxic elements on the planet. The importance of Hg on living organisms resides on its biomagnification ability. Artisanal gold extraction activities release substantial amounts of this metal, polluting the ecosystems. To assess the impact of gold mining in Las Orquideas National Natural Park (Colombia), total Hg (T-Hg) levels were evaluated from 37 bird and 8 small rodent species collected at two sites within the boundaries of the Natural Park (Abriaqui and Frontino municipalities) that have experienced some gold-extraction history. The mean concentration of T-Hg in bird feathers from both sites was 0.84 ± 0.05 μg/g fw. Differences between species were found according to diet. Total Hg levels were greater on insectivorous (1.00 ± 0.08 μg/g fw), followed by nectarivorous (0.73 ± 0.07 μg/g fw) and frugivorus (0.57 ± 0.09 μg/g fw) species. These Hg levels were greater than those found in feathers from a control sample belonging to the species *Penelope perspicax* (0.53 ± 0.03 μg/g fw), a frugivorous species living at the Otun Quimbaya Fauna and Flora Sanctuary, a forest without known gold mining. Mercury concentrations in the livers of small rodents were greater in specimens from Frontino (0.15 ± 0.01 μg/g fw) than those from Abriaqui (0.11 ± 0.01 μg/g fw), but levels were not different between species. These results indicate that Hg in birds depends mainly on their diet, but geographical location may affect Hg concentration in rodents. Moreover, Hg sources in natural parks of Colombia may not rely solely on gold mining, atmospheric deposition, among others factors, could be influencing its accumulation in biota.

Keywords Mining · Mountains · Wild species · Hummingbirds · Mice · Natural parks

Introduction

Mercury (Hg) is a heavy metal widely studied (Jankovská et al. 2014) that pollutes the environment producing adverse effects on ecosystems (Henny et al. 2002; Eisler 2006; Scheuhammer et al. 2007). Its toxicity and accumulation capacity generate a high risk of disease for many organisms (Eisler 2006), in particular because Hg persists in the environment and is prone to biomagnification (Henny et al. 2002; Braune et al. 2005). In Colombia, one of the major sources of Hg contamination in the environment is gold mining, an activity that has been increasing in Latin America (Mancera-Rodríguez and Álvarez-León 2006; Estrada-Guerrero and Soler-Tovar 2014), generating the need to carry out studies that measure the impacts on biota in different affected habitats (Grajewska et al. 2015).

The accumulation of Hg in birds has been used as exposure biomarker (Gómez-Ramírez et al. 2014; Olivero-Verbel et al. 2013) because this metal prevails and accumulates in their

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tissues, mainly through diet or consumption of contaminated water. Birds are vulnerable to Hg pollution because some species are top predators in many aquatic habitats (Scheuhammer et al. 2007; Martin et al. 2018), and this is a chemical with well-known effects on their reproduction (Wiener et al. 2003; Scheuhammer et al. 2007). Moreover, in birds, Hg is slowly eliminated, and some species have extensive lifetimes, which increase the risk of greater Hg bioaccumulation (Frederick et al. 2002).

Mercury levels in birds are usually measured in feathers (Tsipoura et al. 2017; Rumbold et al. 2017; Lucia et al. 2016). These structures provide a useful and non-destructive tissue for Hg analysis because this and other metals are sequestered in the sulphydryl groups of the keratin as feathers grow (Ochoa-acuña et al. 2002). It has been documented that 70% of the total Hg load might be stored in feathers of adult bird species (Becker et al. 2002; Ochoa-acuña et al. 2002). On the other hand, few studies have been developed using native species of Neotropical small rodents as pollution bioindicators. In Colombia, Guerrero-Castilla et al. (2014) evaluated the presence of heavy metals, including Hg in wild rodents present in mining areas, being one of the first reports of metals in organisms present in these areas.

Colombia is considered one of the most biodiverse countries in the world (Díaz and Acero 2003; Bernal et al. 2015), but also it has been named to be the most polluted by Hg in per capita terms (Cordy et al. 2011), therefore, studying Hg concentrations in natural parks should provide information to promote actions that reduce the environmental risks on the biota. The main objective of this study was to report for the first time Hg concentrations in bird feathers and the liver of small rodents from two locations at Las Orquideas National Natural Park, Colombia.

Materials and methods

Study area

This study was carried out in Las Orquideas National Natural Park and its buffering zone, within the municipalities of Abriaqui and Frontino, Department of Antioquia (Colombia) (Fig. 1). The park has an extension of 29118 ha, ranging between 350 and 3400 m above sea level, and includes ecosystems that vary from humid tropical rainforest to paramo. There has been a history of ongoing artisanal mining activities, both within the park and its buffering zone, for the extraction of gold by Hg.

Sample collection

A total of 93 birds and 58 small rodents were captured during June–August 2016 at two localities within the limits of Las Orquideas National Natural Park, requiring 710 net-hours and 904 trap-nights, for birds and rodents, respectively. Birds were collected using mist nets of different lengths (4, 5, and 9 m), whereas small rodents were captured using medium-size Sherman live traps and Victor snap-traps. Birds correspond to 37 species (Fig. 2), each one of them was measured and photographed with the sole exception of *Turdus serranus*, which was captured and included in the analysis, but it could not be photographed. Among these birds, *Henicorhina negreti* is critically endangered, wheras *Arremon castaneiceps* and *Grallaricula flavirostris* are considered near threatened species (IUCN 2017).

Pectoral feathers were carefully removed from each specimen, placed in individual envelopes, and stored at room temperature in a glass desiccator until analysis. Birds were subsequently released. Additionally, samples from the Cauca Guan (*Penelope perspicax*), collected at Otun Quimbaya Fauna and

Fig. 1 Sampling sites at Las Orquideas National Natural Park (green circles). Red circles show the localities with artisanal gold extraction. *OQ* Otun Quimbaya, *LO* Las Orquideas

Fig. 2 Bird species captured for mercury analysis from Las Orquideas National Natural Park

Flora Sanctuary (Risaralda Deparment, Colombia), that given the historic absence of gold mining activities in such locality served as a control reference sample for comparative purposes. Rodents were euthanized with an intracardiac application of 0.1 mL of Eutanex following the guidelines of the American Society of Mammalogy (Sikes and Gannon 2011). Then, the liver was removed and a tissue section was stored and kept at − 20 °C until analysis. A complete list of all captured individuals can be found in Tables 1S and 2S for birds and rodents, respectively.

Mercury analysis in birds and rodents

Approximately 0.7–1.5 mg of bird feathers and 2–3 mg of liver tissue were used for the T-Hg analysis utilizing a 3-cell direct DMA-80 Hg analyzer (Milestone Inc., Shelton, Connecticut, USA), following US EPA Method 7473. In order to homogenize the samples, the feathers were grinded to particles around 2–3 mm with the help of scissors, and the livers were thawed for a few minutes, the excess moisture (blood) was removed with absorbent paper, and finally, a small piece was cut with scissors. The quantification of T-Hg was achieved using calibration curves obtained from Certified Reference Materials (CRMs). These were constructed by linear regression with at least five points, and were considered optimal if the regression coefficient was ≥ 0.99. Analysis of blanks and the use of CRMs IAEA-086 and IAEA-085 (human hair) assessed the accuracy of the method for bird feathers; and DORM-3 (fish protein) from the National Research Council of Canada, for liver tissue. The obtained recovery values for CRMs were 99% and 98% for hair and fish, respectively. Every 15 samples the respective standard was executed for confirmation of the calibration. For all the samples, it was verified that the coefficient of variation was < 15%. The limit of detection (LOD) was calculated on the basis of three times the standard deviation (SD) plus the average of the blanks (Long and Winefordner 1983). Detection limits for feathers and liver were 0.006 μg/g, fw, and 0.002 μg/g, ww, respectively.

Data analysis

The data are presented as mean ± standard errors. Normality and homogeneity of variance were checked by Kolmogorov-Smirnov and Bartlett tests, respectively. Median comparisons between locations were achieved using the non-parametric Mann-Whitney *U* test. Mean differences for T-Hg concentrations between guilds were carried out employing Kruskal-Wallis test followed by a Dunn's post-test. Spearman correlation test was utilized to evaluate the association between variables. Non-parametric tests were employed as most original and transformed data for different groups failed to meet the normality assumptions. The *P* value of < 0.05 was considered statistically significant.

Results

The morphometric data for birds and mice collected in the two sampling sites are found in Tables 1S and 2S (Supplementary Material). Mercury levels in feathers of birds sampled in the park are shown in Fig. 3.

Mercury concentrations according to locality and thropic guild are presented in Fig. 4. The average T-Hg levels for feathers collected from birds at Abriaqui and Frontino were 0.87 ± 0.08 and 0.82 ± 0.06 μg/g fw, respectively; with an overall average level of 0.84 ± 0.05 μg/g fw (Fig. 4a). When the sample was divided based on guilds, the average T-Hg levels for nectarivorous, frugivorous, and insectivorous were 0.73 ± 0.07, 0.57 ± 0.09, and 1.00 ± 0.08 μg/g fw, respectively (Fig. 4b). The bird species with the greatest level of T-Hg in Abriaqui was the nectarivorous *Doryfera ludovicae* (2.26 μg/g fw), and the species with lowest concentration was the frugivorous *Myadestes ralloides* (0.18 μg/g fw). In the case of Frontino, the highest T-Hg concentration was registered in the insectivorous *Campylorhamphus pusillus* (2.65 μg/g fw), whereas the lowest was detected in *Myadestes ralloides* (0.19 μg/g fw).

Total Hg levels were also measured in the feathers of *Penelope perspicax* ($n = 10$), a large bird from Otun Quimbaya Fauna and Flora Sanctuary. This is an endangered bird species with a diet mainly based on fruits, insects, foliage, and flowers (WCS Colombia 2017), and to a lesser extent on

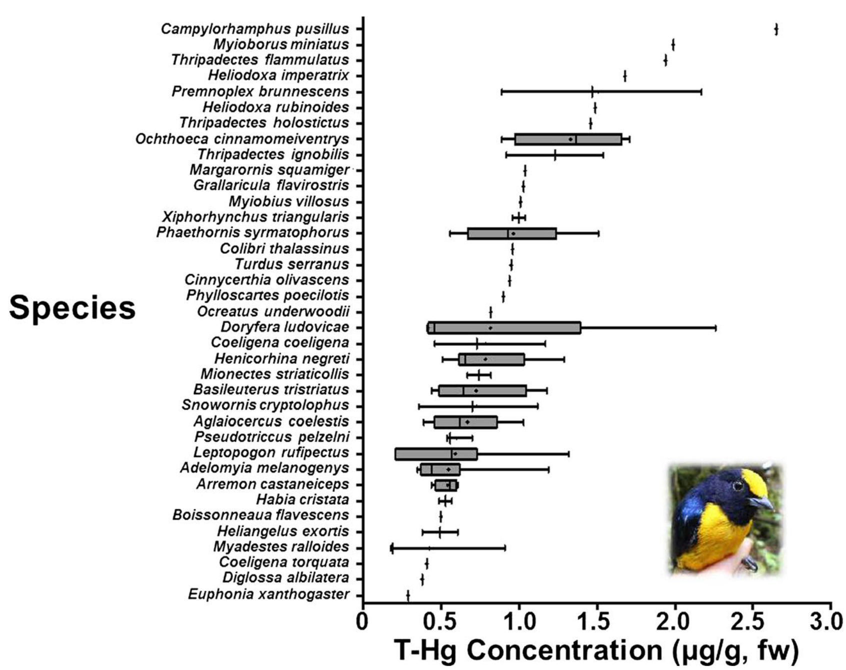

Fig. 3 Total Hg concentrations in feathers obtained from birds collected at Las Orquideas National Natural Park

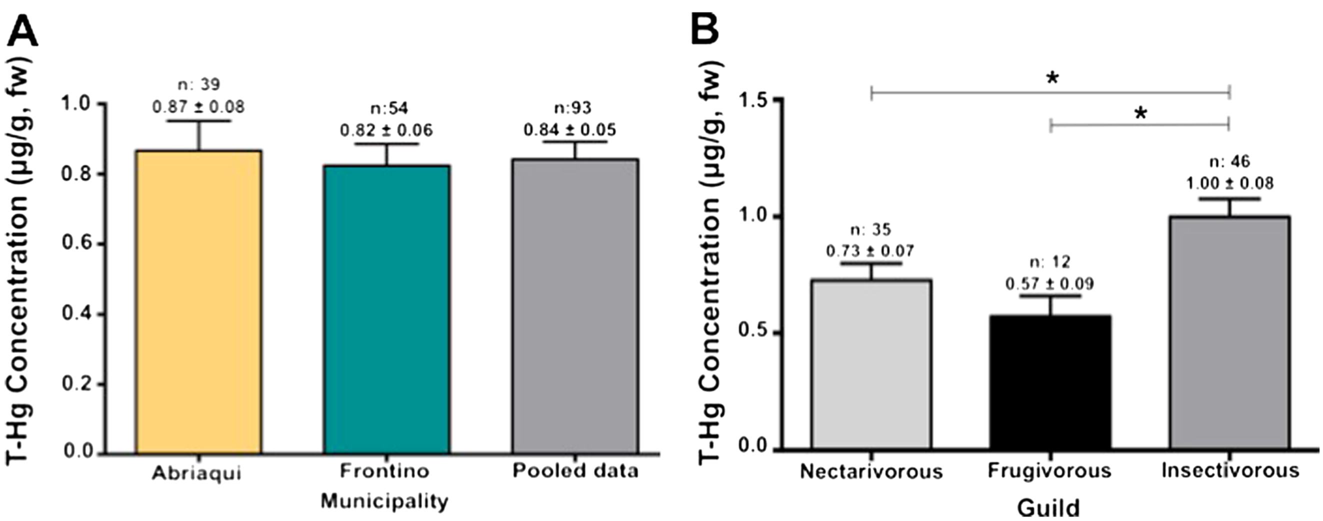

Fig. 4 Total Hg concentrations in bird feathers from Abriaqui and Frontino (**a**) and categorized by specific diet (**b**). *Significance level, $P < 0.05$

leaves, flowers, arthropods, and even molluscs or fungi (Renjifo et al. 2013). Feather samples were obtained directly from the forest floor, and used for comparative purposes, as this location does not have any known nearby gold mining activity. The levels of T-Hg in *P. perspicax* are shown in Fig. 5. The average T-Hg level was 0.53 ± 0.03 μg/g fw, a level one order of magnitude lower than the critical value of 5 μg/g, considered to affect bird health (Burger and Gochfeld 1997).

The correlations between T-Hg concentrations and morphometric variables in birds are presented in Table 1. No statistical association was found between T-Hg and the included variables. However, when the sample was categorized according to their similarities in feeding patterns (trophic guild), interesting results were obtained (Table 2). First, there was a positive but not significant correlation ($\rho = 0.552$; $P = 0.06$) between T-Hg and bird weight for frugivorous birds, suggesting the bioaccumulation process that takes place in these organisms may not only be related to age. Something similar occurs for nectarivorous birds when the metal concentration is correlated to wing length ($\rho = 0.309$, $P = 0.07$) or bird height ($\rho = 0.304$, $P = 0.08$). However, the most interesting finding was the significance obtained for the inverse correlation between T-Hg in feathers and tarsus length in nectarivorous birds ($\rho = -0.412$, $P = 0.01$, $n = 34$).

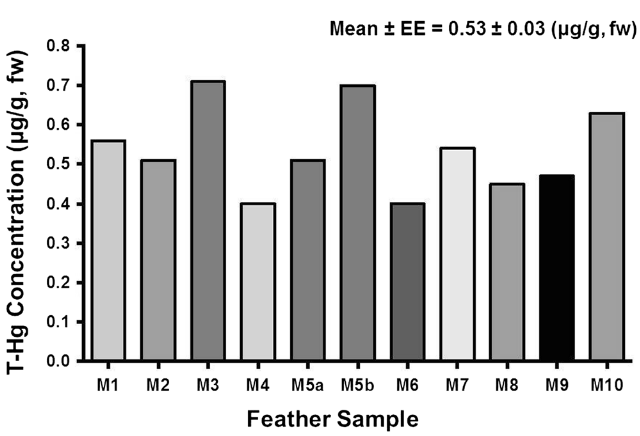

Fig. 5 Total Hg concentrations in *Penelope perspicax* from Otun Quimbaya Fauna and Flora Sanctuary

Mercury concentration in rodents

All the individuals of small rodents captured ($n = 58$) correspond to native species of the subfamily Sigmodontinae (family Cricetidae). Mercury concentrations found in their livers are shown in Fig. 6a. The average T-Hg concentration was 0.12 ± 0.01 μg/g fw, with *Nephelomys pectoralis*, *Nephelomys sp.*, and *Melanomys caliginosus* having the lowest (0.04 μg/g fw), and *Thomasomys bombycinus* the greatest (0.24 μg/g fw) recorded levels. Rodents from Frontino presented greater T-Hg levels than those from Abriaqui (Fig. 6b).

Discussion

This study is the first report assessing T-Hg in bird feathers from a national natural park in Colombia. The results suggest Hg is being deposited in bird feathers, with levels that depend on their trophic guild. Mercury is bioaccumulating in these organisms, eventually reaching toxic concentrations that may affect survival and reproduction in species at several levels of the food chain. In this report, all examined bird species showed Hg accumulation in feathers, but the average registered concentration, 0.84 ± 0.05 μg/g fw, was lower than that suggested to exert detrimental health effects on birds (5.0 μg/g) (Burger and Gochfeld 1997). However, the effect of the concentration of this metal varies among different bird species (Heinz et al. 2009), for instance the Carolina wren, *Thryothorus ludovicianus*, showed negative effects on its reproduction with a Hg concentration of 2.4 μg/g in body

Table 1 Spearman correlations for T-Hg in feathers and morphometric variables for all sampled birds ($n = 92$)

	T-Hg	Weight	Wing length	Height	Tarsus length
T-Hg	1.000				
Weight	0.108 (0.302)	1.000			
Wing length	0.112 (0.287)	0.809 (0.000)	1.000		
Height	0.057 (0.588)	0.815 (0.000)	0.671 (0.000)	1.000	
Tarsus length	0.150 (0.153)	0.652 (0.000)	0.353 (0.000)	0.746 (0.000)	1.000

p values are given in parentheses

feathers (Jackson et al. 2011). On the other hand, there are reports of the existing relationship between Hg content and the trophic position of the species, regardless of the studied matrix studied, even at concentrations below suggested risk thresholds for bird health (Alvárez et al. 2013).

Feathers were chosen as an environmental matrix because it has a high capacity for sequestering Hg (Falandysz et al. 2001; Lewis and Furness 1991; Falandysz et al. 1988; Falandysz and Szefer 1983), and their collection can be carried out without causing pain, suffering, or killing the birds. During the process of feather formation, the heavy metal is stored in this matrix, representing a good evaluation marker for exposure during growth (Monteiro and Furness 1996). Birds are exposed to heavy metals through their food and water, making exposure highly dependent on their diet (Schulwitz et al. 2015). On the other hand, Hg accumulation in feathers may only be used when birds do not have wide migration ranges (Ackerman and Eagles-Smith 2009), as it happens for most birds species studied here, which implies that Hg is circulating within the park boundaries.

Understanding Hg dynamics in birds from natural parks is a fundamental tool to assess the susceptibility risk of the ecosystem to this metal. Results indicate that insectivorous birds were the most exposed to Hg. This is probably due to biomagnification processes lead by the trapped Hg in the organic matter present in leaf litter, trees, and humid areas where birds find insects (Rimmer et al. 2005). The T-Hg levels found in insectivorous species were followed by those detected in nectarivorous birds. This is of great concern since it may indicate atmospheric Hg is being deposited as small microdrops through the rain on the nectar (Aoki et al. 2012).

It has been suggested that adverse reproductive effects in birds may occur when T-Hg levels in feathers reach 5.0 μg/g (Eisler 1987; Burger and Gochfeld 2000), a concentration not registered in this work. Moreover, T-Hg concentrations in feathers between 5 and 40 μg/g fw, not only promote a negative effect on the reproduction outcome, but also induce changes in the population dynamics of some species, although these values cannot be applied as limits or thresholds (Burger and Gochfeld 1997; Evers et al. 2008).

Mercury level in feathers of Cauca Guan living in the Otun Quimbaya Fauna and Flora Sanctuary, a gold mining-free environment, was approximately 0.5 μg/g fw. This value could be interpreted as a fair estimated limit for Hg in birds from low Hg polluted sites. However, this concentration has a striking similarity to the average registered for frugivorous birds in the

Table 2 Spearman correlations for T-Hg in feathers and morphometric variables for sampled birds based on guild categories

	T-Hg	Weight	Wing length	Height	Tarsus length
T-Hg	1.000				
Weight	0.038 (0.82)*[a]	1.000			
	0.552 (0.06)[b]				
	0.143 (0.33)[c]				
Wing length	0.309 (0.07)	0.431 (0.01)			
	0.356 (0.23)	0.608 (0.04)	1.000		
	0.223 (0.13)	0.365 (0.01)			
Height	0.304 (0.08)	0.315 (0.06)	0.178 (0.30)	1.000	
	0.188 (0.53)	0.482 (0.10)	−0.035 (0.90)		
	0.011 (0.93)	0.754 (0.00)	0.546 (0.00)		
Tarsus length	−0.412 (0.01)	−0.154 (0.37)	−0.255 (0.14)	−0.435 (0.01)	1.000
	0.419 (0.16)	0.531 (0.07)	−0.063 (0.83)	0.594 (0.04)	
	0.112 (0.44)	0.729 (0.00)	0.103 (0.48)	0.545 (0.00)	

**P* values are given in parentheses. [a] Nectarivorous ($n = 34$); [b] Frugivorous ($n = 12$); [c] Insectivorous ($n = 46$)

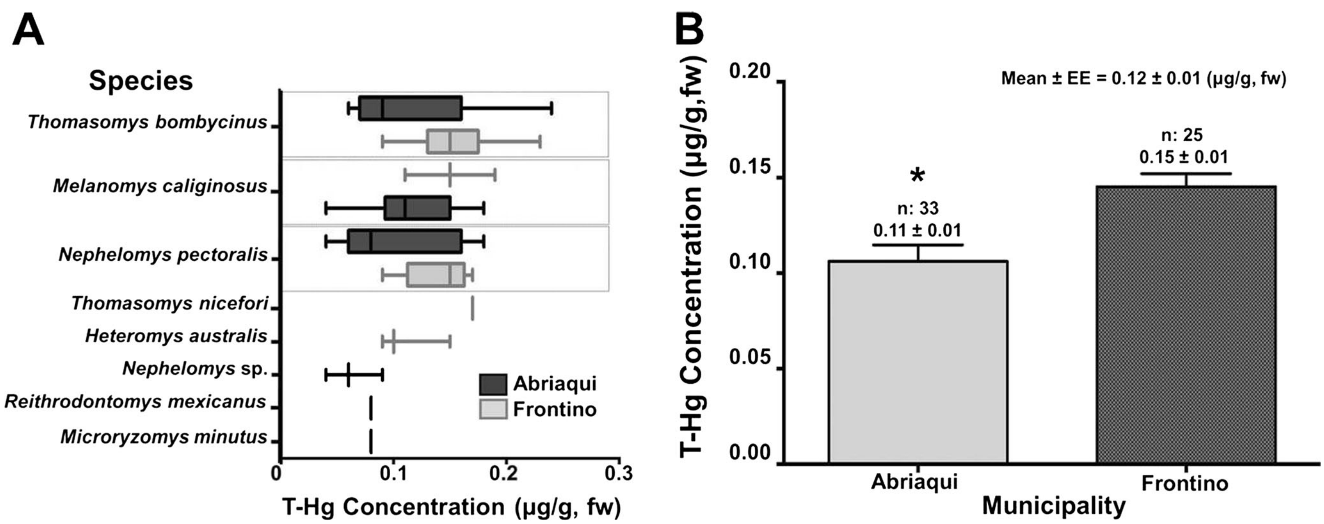

Fig. 6 Total Hg concentrations in liver of small rodents collected in Abriaqui and Frontino. Comparison of T-Hg concentrations between species (**a**) and sampling sites (**b**)

studied natural park, known to receive some influence from nearby gold mining activities (0.57 ± 0.09 µg/g fw). Therefore, it is unlikely that gold mining is the only source of Hg in natural national parks in Colombia.

The overall correlation analysis performed for T-Hg concentrations in bird feathers and morphometric variables did not show statistical relationships. However, grouping birds according to their guilds revealed a different picture. Of special importance was the negative correlation observed between T-Hg and tarsus length ($\rho = -0.412$, $P < 0.01$) in nectarivorous birds. This relationship has also been reported for the northern fulmar (*Fulmarus glacialis*) but for Hg in muscle (SPFO, 2007), and in this marine species, it may indicate Hg content decreases with age, as the tarsus length is usually considered a marker of age in these birds (Riget et al. 2000). However, in hummingbirds, this inverse correlation has not been observed, and it should be further investigated.

In addition to birds, mice were also captured in Las Orquideas National Natural Park, and their livers analized for T-Hg. In average, the small rodents from Frontino have 27% more Hg concentration than those from Abriaqui, probably reflecting a greater number of mining operations in the first location. The overall mean T-Hg concentration was 0.12 ± 0.01 µg/g fw, value a little bit greater than the range reported for hepatic Hg in deer mice (*Peromyscus maniculatus*) from Isle Royale National Park (Michigan, USA) (0.04–0.10 µg/g) (Vucetich et al. 2001). Moreover, total Hg levels in *Clethrionomys glareolus* and *Apodemus sylvaticus*, two mice species collected near a chlor-alkaly plant, displayed T-Hg levels of 0.15 µg/g and 0.23 µg/g, respectively (Bull et al. 1977). Finally, these values were within those registered in the livers of *Mus musculus* specimens obtained from coal mining areas in Colombia (from 0.12 ± 0.03 to 0.33 ± 0.06 µg/g ww) (Guerrero-Castilla et al. 2014).

Conclusions

Taken together, these data suggest birds and wild mice in Las Orquideas National Natural Park (Colombia) are bioacumulating Hg, with concentrations in birds depending on their trophic guild, whereas in rodents the levels were given by differences between sites. The Hg values found in this study should be taken as a reference for future studies, aiming to specially assess long-term adverse effects on bird communities near mining areas. Although clearly Hg in the park is being bioaccumulated by birds and mice, the source may not be related only to gold mining in nearby areas, but perhaps to atmospheric deposition as well. Further research is needed in different bird and mice species to look at the relationship between the food chain and possible negative effects of Hg exposure at relatively low levels.

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Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

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