



Contents lists available at ScienceDirect

## Environmental Pollution

journal homepage: [www.elsevier.com/locate/envpol](http://www.elsevier.com/locate/envpol)

# Presence of artisanal gold mining predicts mercury bioaccumulation in five genera of bats (Chiroptera)<sup>☆</sup>

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## ARTICLE INFO

### Article history:

Received 4 October 2017

Received in revised form

17 January 2018

Accepted 31 January 2018

### Keywords:

Forest disturbance  
Madre de Dios River  
Mammals  
Mercury  
Peruvian Amazon

## ABSTRACT

Mercury, a toxic trace metal, has been used extensively as an inexpensive and readily available method of extracting gold from fine-grained sediment. Worldwide, artisanal mining is responsible for one third of all mercury released into the environment. By testing bat hair from museum specimens and field collected samples from areas both impacted and unimpacted by artisanal gold mining in Perú, we show monomethylmercury (MMHg) has increased in the last 100 years. MMHg concentrations were also greatest in the highest bat trophic level (insectivores), and in areas experiencing extractive artisanal mining. Reproductive female bats had higher MMHg concentrations, and both juvenile and adult bats from mercury contaminated sites had more MMHg than those from uncontaminated sites. Bats have important ecological functions, providing vital ecosystem services such as pollination, seed dispersal, and insect control. Natural populations can act as environmental sentinels and offer the chance to expand our understanding of, and responses to, environmental and human health concerns.

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## 1. Introduction

Mercury (Hg) enters atmospheric and aquatic cycles by both natural and anthropogenic sources, with some human associated examples being coal-fired power plants, mining, and waste combustion (Driscoll et al., 2013; Engstrom et al., 2014; Fitzgerald et al., 1997; UNEP 2013). In the tropics, mercury has been used extensively as an inexpensive and readily available method of extracting gold from fine-grained sediment (Diringer et al., 2015; Swenson et al., 2011), with artisanal mining thought to be responsible for one third of all mercury released into the environment worldwide

<sup>☆</sup> This paper has been recommended for acceptance by Prof. W. Wen-Xiong.

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(Ashe, 2012). In Amazonian Perú, 51% of all mining along the Madre de Dios River and its tributaries is composed of artisanal, illegal camps (Asner et al., 2013). Gold mining activities tripled after 2008, associated with the worldwide economic recession and rising gold prices (Asner et al., 2013; Swenson et al., 2011). In 2016, Perú was listed as the seventh largest producer of gold extracted from river mining in the world (GFMS, 2017). Imports on Hg between 2006 and 2009 rose 42%, with 95% of imports used directly in artisanal gold mining (Swenson et al., 2011). Mercury discharges during the mining process in the Madre de Dios River in Perú have resulted in significantly higher Hg concentrations in sediments downriver from known mining operations (Diringer et al., 2015). At sites closest to active mining activities, Hg transformed by anaerobic microorganisms into toxic monomethylmercury (MMHg) was more than ten times the maximum concentrations than in non-mining areas (Diringer et al., 2015).

Modern MMHg pollution and toxicity has been well studied in aquatic food webs (Haro et al., 2013; Scheuhammer et al., 2007),

but there is a need to investigate the movement and bio-magnification of MMHg through terrestrial food webs (Cristol et al., 2008). Studies of terrestrial insectivores and omnivores (bats, birds, mice, mink and otters) illustrate that MMHg exposure and toxicity could be a source of behavioral, neurological and reproductive problems (Becker et al., 2017a; Cristol et al., 2008; Edmonds et al., 2012; Jackson et al. 2015; Little et al., 2015; Moreno-Brush et al., 2018; Nam et al., 2012; Syaripuddin et al., 2014; Wada et al., 2010; Yates et al., 2014). In bats, individuals with at least 10 ppm total mercury (HgT) in hair exhibited a lack of expected neurochemical responses when compared to bats with low HgT (Nam et al., 2012; Wada et al., 2010; Yates et al., 2014).

Bats (Chiroptera) are generally not used in environmental contamination studies, but are excellent bio-sentinels of MMHg movement from aquatic to terrestrial ecosystems (Becker et al., 2017b; Korstian et al., 2017; Moreno-Brush et al., 2018; Yates et al., 2014). There are several features of bats' life history strategies and biology that make them strong candidates for contaminant studies. Bats are long-lived (typically between seven to ten years), small and mobile, with high site fidelity, and consume between 40% and 100% of their body mass in prey items each night (Cloutier and Thomas, 1992; Handley, 1957; Hickey et al., 2001; Wilson and LaVal, 1974). Some bats are exposed to MMHg via their food source, feeding heavily on emergent insects that spend their larval stages in sediment and water where contaminants readily accumulate (Brigham and Fenton, 1986; Hickey et al., 2001). Aquatic insects, as well as bats, provide a pathway for movement of contaminants from aquatic sediments to terrestrial ecosystems (Currie et al., 1997). Within Chiroptera, there are several distinct feeding guilds, such as frugivores, insectivores, nectarivores, piscivores, carnivores, and hematophores, making comparisons among trophic levels possible.

Curated biological collections can provide insights into changes in contaminant accumulation and concentration while providing baseline data for comparisons over time and space (Campbell and Drevnick, 2015). Museum specimens have been used in very few longitudinal studies of exposure of organisms to mercury (Feathers: Frederick et al. 2004; Vo et al., 2011; Hair: Miura et al., 1978; Newman et al., 2004) and museum and natural history collections are greatly underused overall.

Our study set out to understand: (1) Variation in MMHg concentration among trophic levels of bats and between contaminated and non-contaminated sites in Perú and; (2) changes in MMHg concentrations among different trophic levels of bats before and during recent artisanal gold mining when MMHg deposition in the environment increased. Our predictions were (1) that bats of higher trophic levels should bioaccumulate more MMHg than species at lower trophic levels, as observed in previous comparisons for this taxon (Becker et al., 2017b; Moreno-Brush et al., 2018; Syaripuddin et al., 2014); (2) there should be higher MMHg in bats from present day contaminated sites than historical baseline values, as inferred from bats collected in the early- and mid-1900s; and (3) bats from present day contaminated sites should bioaccumulate more MMHg than bats from present day uncontaminated sites. Following our initial analyses, we were also able to use our dataset to investigate demographic patterns such as MMHg bioaccumulation in reproductive female bats and differences in MMHg from adult and juvenile bats in our field sites.

## 2. Materials and methods

### 2.1. Study design

Our study consisted of four main methods. For fieldwork, we visited three sites in the Peruvian Amazon, two with active gold

mining and one without. We targeted sampling hair from species of the genera *Carollia*, *Myotis*, and *Noctilio* to represent three feeding guilds (frugivore, insectivore, and piscivore), adding the *Macrophyllum* and *Rhynchonycteris* genera while in the field. We then analyzed stable isotope composition of field caught samples to establish trophic structure of the community. Next, we visited natural history museum collections, sampling hair from skins of the species we collected in the field to use for mercury analyses. Lastly, field collected hair was analyzed for HgT, and hair from museum specimens analyzed for MMHg. Museum specimens were analyzed for MMHg because, after initial testing, we determined museum specimens were contaminated at some point during collection or preservation, and HgT measurements did not accurately predict MMHg as it did with field caught specimens.

### 2.2. Field collection: present day hair samples

Bats were sampled between August and November 2013. One site was upriver from all gold mining operations, with no record of direct anthropogenic mercury inputs and was considered an uncontaminated site (UC: Cocha Cashu Biological Station, 11°53'17.58"S, 71°24'27.15"W); and two sites were downriver from several gold mining operations and were considered contaminated sites (C: Centro de Investigación y Capacitación Rio Los Amigos, 12°34'5.43"S, 70°5'58.09"W and Cocha Huitoto, 12°37'26.36"S, 69°58'37.63"W; Asner et al., 2013). All three sites are located in lowland Amazonian forest, have similar environmental conditions and all five focal bat genera (*Carollia*, *Macrophyllum*, *Myotis*, *Noctilio*, and *Rhynchonycteris*) were present at all three sites (Pacheco et al., 1993; Patterson et al., 2006; Pitman, 2007; Reid, 2009). All bat focal species have smaller home ranges than the minimum distance between the contaminated and uncontaminated sites (~170 km), making sites independent and comparable (Bonaccorso et al., 2006; Brooke, 1997; Meyer et al., 2005).

Bats were captured using mist nets and harp traps on forest edges near fruiting shrubs and small trees to target frugivorous bats (*Carollia* spp.), and placed over small streams, river edges and oxbow lakes to target species foraging over water (*M. macrophyllum*, *Myotis* spp., *N. albiventris*, and *R. naso*). The number of bats captured was as follows: *Carollia* spp.: C = 27, UC = 27; *M. macrophyllum*: C = 1, UC = 3; *Myotis* spp.: C = 15, UC = 17; *N. albiventris*: C = 16, UC = 18; *R. naso*: C = 4, UC = 5. Samples for Hg analysis were collected by trimming approximately 4 mg of hair from the dorsal portion of the bat with stainless steel scissors and placing it into a 1.5 mL Eppendorf tube. Scissors were cleaned with ethanol and dried between individuals to reduce cross contamination. Hair is accepted as a valid biomarker of MMHg exposure in bats (Mergler et al., 2007), estimating exposure over the growth period of the hair segment, likely one annual cycle, although the molt cycles of tropical bats has not been well studied (Mergler et al., 2007). Mercury contained in hair is predominantly MMHg (Dietz et al., 2013; Mergler et al., 2007; Newman et al., 2004; Wren, 1986). Reproductive status, age (juvenile or adult), sex and general morphometrics were taken for each individual. All bats were banded with lipped alloy forearm bands labeled with unique identifiers (Porzana, Ltd., East Sussex, UK) and released on site. Reproductive females were considered those lactating, post-lactation or pregnant. Age was determined by bone ossification in the wing bones (Kunz and Anthony, 1982).

### 2.3. Natural history of sampled bat species

To target species that may accumulate MMHg and transfer the contaminant from aquatic to terrestrial food webs, our focal species (trawlers) were those that either consume adult insects with an

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