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ORIGINAL ARTICLE

Cascading impacts of anthropogenically driven habitat loss: deforestation, flooding, and possible lead poisoning in howler monkeys (*Alouatta pigra*)

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Abstract To construct informed conservation plans, researchers must go beyond understanding readily apparent threats such as habitat loss and bush-meat hunting. They must predict subtle and cascading effects of anthropogenic environmental modifications. This study considered a potential cascading effect of deforestation on the howler monkeys (*Alouatta pigra*) of Balancán, Mexico. Deforestation intensifies flooding. Thus, we predicted that increased flooding of the Usumacinta River, which creates large bodies of water that slowly evaporate, would produce increased lead content in the soils and plants, resulting in lead exposure in the howler monkeys. The average lead levels were 18.18 ± 6.76 ppm in the soils and 5.85 ± 4.37 ppm in the plants. However, the average lead content of the hair of 13 captured howler monkeys was 24.12 ± 5.84 ppm. The lead levels in the animals were correlated with 2 of 15 blood traits (lactate dehydrogenase and total bilirubin) previously documented to be associated with exposure to lead. Our research illustrates the urgent need to set reference values indicating when adverse impacts of high environmental lead levels occur, whether anthropogenic or natural, and the need to evaluate possible cascading effects of deforestation on primates.

Keywords Climate change · Lead exposure · Plumbism · Ecotoxicology · Conservation

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Introduction

The rate of deforestation is showing signs of decreasing in a number of countries, but it continues at an extremely high rate in others (Wright and Muller-Landau 2006; Jacob et al. 2008). Between 2000 and 2012, it is estimated that 2.3 million km^2 of forest was lost globally, with only 0.8 million km^2 gained (Hansen et al. 2013). Tropical forest cover, the habitat of most primates, decreased by 2101 km^2 per year during this time (Hansen et al. 2013). To be most effective in conservation efforts, managers must understand not only the obvious (e.g., decreasing hunting and logging aids in primate conservation), but also the subtle and cascading effects of anthropogenic environmental modification. In the past, conservation biologists have typically responded to change and attempted to take corrective action after negative situations have occurred (Caughley 1994; Chapman and Peres 2001; Wright et al. 2007). However, it would be more effective if researchers could predict negative change before it occurred and take action to prevent population declines.

This study considered the potential cascading effects of deforestation on the howler monkeys (*Alouatta pigra*) of Balancán, Tabasco, Mexico. Floods in the Usumacinta River basin have increased dramatically in magnitude, extent, and intensity in recent years, primarily due to deforestation at the headwaters and along the river's banks

(Gama et al. 2010). This results in slow evaporation of waters up to 4 m deep (Rivera-Trejo et al. 2009) during the dry season, potentially leading to the accumulation of heavy metals in the soils and plants. It is well documented that reduced water velocity leads to heavy metals settling out of flowing water (Rothwell et al. 2007; Poot et al. 2007; Liu et al. 2009).

Because lead settles as water flow declines, and assuming that the water of the Usumacinta River contains lead, we first sought to examine lead concentrations in the soils and plants in the environment used by the howler monkey population. Increased environmental lead levels could result in higher lead content in animal tissues, potentially higher than previously documented for primates. Consequently, we tested for evidence of exposure to lead (plumbism) in the population, as indicated by changes in blood chemistry.

Lead is a naturally occurring heavy metal; however, it is also a byproduct of anthropogenic processes (e.g., welding, plumbing, construction, and the use of lead shot) and results from inappropriate disposal of plastics, batteries, and electronics (Tong et al. 2000; Rattner 2009; Engel et al. 2010). Lead toxicity is a significant public health issue worldwide (Tong et al. 2000; Mitra et al. 2009), and lead is normally acquired from ingesting contaminated water, soil, or food, or by breathing contaminated air. Exposure to high lead levels damages vertebrate, nervous, renal, circulatory, hepatic, and reproductive systems (Goyer 1990; Monsu et al. 2001; Infantas 2005). Clinical evidence of lead toxicity includes fatigue, irritability, nausea, abdominal pain, headache, anemia, weight loss, and motor weakness (Infantas 2005). The effects from lower levels of lead exposure are more subtle but may be significant, and a great deal of research on children has documented impacts on intellectual function (Lanphear et al. 2005; Jusko et al. 2008) and loss of motor control (Bhattacharya et al. 2007). High lead levels have been documented for wildlife (Swarup et al. 2000; D'Have et al. 2005; Balagangatharathilagar et al. 2006), but the impact of lead exposure on wildlife is poorly understood because clinical signs of illness are difficult to detect, lead toxicity is often identified only post-mortem (Engel et al. 2010), and reference values for adverse impacts of exposure to high environmental lead levels are generally lacking (Iyengar and Wolttlez 1988; Allard et al. 2010). For primates, a number of recent publications on wild populations or newly captured animals brought into captivity provide a starting point for understanding naturally occurring lead levels or levels in situations expected to bring about acute lead exposure (Engel et al. 2010; Schillaci et al. 2011; Lee et al. 2012). Research on captive animals experimentally subjected to lead exposure allows us to understand the effects of chronic high (Hindle and Stevenson 1930; Fisher 1954; Zook et al. 1974) or low (Gilbert and Rice 1987; Rice and Karpinski 1988; Rice 1997) levels of lead contact.

Methods

Study site

We have studied the howler monkeys in Balancán, Tabasco (latitude: 17° 44′05″N, longitude: 91° 30′17″W, altitude: 25 m) since 2006 (Pozo-Montuy and Serio-Silva 2006; Serio-Silva et al. 2006; Pozo-Montuy et al. 2009; Díaz-López 2010; Bonilla-Sanchez et al. 2012). The original vegetation was a mix of rain forest, evergreen tropical forest, and spiny evergreen bloodwood (*Haematoxylum campechianum*) forest (Mendoza and Dirzo 1999). However, the government encouraged colonization and land conversion for grazing cattle and growing crops in the 1960s (Reyes-Castillo 1978; Pozo-Montuy et al. 2008), and the deforestation rate for the middle Usumacinta watershed is estimated to be as high as 1.6 % annually (Mendoza and Dirzo 1999). This forest conversion has resulted in moisture loss, creating a climate with 8-month droughts lasting from February to September, when historically, the dry season was only 3 months, from March to May (Moguel and Molina-Enríquez 2000). Climate change and land conversion have resulted in very dry years alternating with extensive flooding and increased silting in rivers, streams, and lakes, which occurs in the absence of forests that would normally retain the soil around bodies of water (Chapman and Chapman 2003; Comision Nacional del Agua (CNA) 2010; Gama et al. 2010; Pozo-Montuy et al. 2011). For example, heavy rainfall in 2007 resulted in the flooding of 850,000 ha of grassland and 115,959 ha of cropland (Rivera-Trejo et al. 2009).

Sample collection

We captured 13 black howler monkeys (6 adult males; 6 adult females; 1 juvenile female) in 2008 and 2009 as part of a regional survey of primate diseases (Améndola-Pimenta et al. 2009). In total, these fragments covered an area of 5.26 km^2. Each captured individual was a member of 1 of 13 different groups. The animals were darted by a veterinarian (L.G-F.) trained in wildlife capture and immobilized with 10 mg/kg ketamine (Glander et al. 1991). The darting was performed with a CO_2-pressurized rifle that used 3-ml plastic darts with 1.5 × 30-mm collared needles. To reduce risk to the animals, a team member climbed the trees to dislodge the tranquilized animal and dropped it to a four-member team on the ground that caught the animal and swiftly took it to a shaded area where samples were obtained. The tranquilized monkeys were placed on a clean, dry, smooth surface, and heart rate, rectal temperature, and respiratory frequencies were monitored. Ophthalmic ointment (Duralagrima) was applied to prevent desiccation of the corneas. The eyes were covered and the ears plugged. The animals were checked for ectoparasites and botflies, and morphometric measurements were collected. During anesthesia, 2 to 4 g of hair samples were collected from the back and both sides of the animal. Hair was cut at the base with scissors by a team member wearing gloves, placed in sealed bags, and weighed. No animals were injured during the darting, capture, or release, and all wee returned safely, apparently unharmed, to their original group.

We collected 6 to 10 ml of blood by venipuncture of the femoral vein and immediately divided it among three microtainers respectively containing ethylenediaminetetraacetic acid (EDTA), heparin, or no anticoagulant. The EDTA microtainer was used for hematology, performed in a local laboratory in Balancán within 12 h after collection. Blood samples were centrifuged at the field station, and plasma and serum were frozen at −20 °C. We received official permission for darting from the Secretaria de Medio Ambiente y Recursos Naturales (SEMARNAT) of the Federal Government, Mexico (no. SGPA/DGVS/05938). The research complied with protocols approved by the Animal Care Committee of INECOL and adhered to the legal requirements of Mexico.

Exposure to lead can be detected through changes in blood traits. Blood counts for animals with lead poisoning usually reflect a slight anemia with the presence of nucleated erythrocytes, dotted basophil, and increased leukocytes. Blood biochemistry analysis may show an increase in liver enzyme activity and altered renal activity, and sometimes, increases in cholesterol and blood glucose are documented. Infantas (2005) presents a detailed description of changes in blood chemistry associated with lead toxicity. Due to the possibility of using blood tests to detect possible dangerous levels of lead exposure, we quantified a series of blood traits (Table 1) and related each trait to the lead level detected from the hair analysis.

We collected 500 g of soil and terrestrial vegetation from each of the 13 sites, packed these in plastic bags, and sent them to the Environmental Biotechnology Group at the Institute of Ecology in Xalapa, Veracruz, México.

Laboratory analysis of hair, plants, and soil

The samples were digested following the general procedures outlined by Olguin et al. (2005). For hair, we selected 0.5 g from each individual. All plants were washed to remove surface soil and dust, dried to 60 °C, ground (0.5 particle size), and packed in a new plastic bag. Equal amounts of all plants from a site were mixed, and a 0.5-g composite sample from each site was selected for digestion. For soils, all samples were mixed and sieved to remove stones and plant material, and a 0.5 g composite sample was taken for digestion. At each site, more than 15 species of plants often were growing in the areas where the monkeys could come to the ground to walk between fragments or eat ground vegetation. Given the large number of species, the need to sample soils from many locations, the exploratory nature of our study, and the high cost of analysis, we analyzed a composite for the plants. A next step in our research program will be to determine the lead levels of particular items, specifically those eaten by different howler monkey groups, and to sample water lead levels and evaluate what amount of lead could be left after the evaporation of up to 4 m of water.

After digestion, all the samples were analyzed using atomic absorption spectroscopy and assessed using a previously derived lead calibration curve (Buck, Scientific, 210 VGP). The detection limit was 0.08 mg/L until 20 mg/L, and the reference material was Stock Lead Standardized Solution (Aldrich TM Atomic Adsorption Standards) of 1,000 mg of lead. Our controls were performed with undyed, clean, and dry human hair. A control hair unleaded and one sample with a known concentration of lead both were processed in same way (same time and weight) as monkey samples. All the samples had detectable lead concentrations, and all were analyzed in triplicate.

A Student's t test was used to test for differences in the lead content of hair between the males and females, whereas to test for relationships between lead levels and blood characteristics, a parametric Pearson correlation was used, and because the sample was small and normality could not be validated accurately, we also report Spearman rank correlations. Because we had only one juvenile animal, we report the values with and without that animal's values. Note that if a correction were to be done for multiple comparisons (e.g., Bonferroni adjustments), none of the correlations of the hair lead and blood chemicals would be significant, and we did not want to inflate type 2 errors by doing this correction: in this case, stating that a conservation issue does not exist when in reality it does. Clearly, more research on this topic is needed (see later) with appropriate control conditions. Here, as elsewhere, two-tailed tests were used, with a significant difference indicated by a p value lower than 0.05. All statistics were run with SPSS version 21.

Results

The average lead content of the hair of these animals was 24.12 ± 5.84 ppm (range 16.5–34). There were no significant differences between the sexes (male = 23.50 ± 5.84;

Table 1 Lead content (ppm) of howler monkey hair (*Alouatta pigra*), their food, and the soils in Balancán, Mexico and blood traits

Sex	Wt	Hair	Plant	Soil	Blood characteristics						Hepatic function								Renal
					ERI	LEU	HB	HTC	MCHC	MCV	ALB	AP	ALT	AST	BIL	COL	GLU	LDH	CRE
Male	6	34	5.36	21.9	3.22	7.4	9.2	27	34.3	84	2.2	303	33	79	0.17	86	112	218	1.1
Female	9	33.5	3.39	10.4	4.38	4.3	12.1	36.7	33.1	84	2.6	231	24	79	0.18	85	107	166	0.7
Male	5	28	4.76	32.5							2.8	443	26	64	0.17	71	93	134	0.7
Female	9	28	6.2	22.4	3.83	7.3	10.7	33.3	32.3	87	2.3	289	31	86	0.07	66	87	190	0.8
Female	3	26	4.14	22.8	3.05	3.6	8.3	25.5	32.5	84	2.5	749	36	105	0.23	81	122	279	0.6
Female	8	25	6.04	11.6	3.57	4.9	9.4	28.5	33.2	80		254	28	71	0.13	99			
Female	9	24	19.5	21.7	4.69	6.1	12.3	37.6	32.8	80	2.8	297	33	158	0.25	108	75	308	1.6
Male	5	22	5.27	7.99	3.27	7.7	9.4	28	33.6	86	3.0	669	27	80	0.22	91	91	220	0.7
Male	6	21.5	3.5	21.5							2.7	90	23	62	0.20	82	91	198	0.8
Female	7	21	7.38	14.3	3.8	5.1	9.1	28.5	31.8	75	2.3	222	22	70	0.24	109	130	200	1
Male	5	19	5.61	11.9	2.91	5.8	8.1	24.7	33	85		372	29	96	0.26	66	3		
Male	6	16.5	1.56	17.3							2.7	218			0.21	95	37	400	
Female	8	15	3.42	20	3.26	3.3	9.0	26.9	33.4	82	2.4	124	27	94	0.28	167	97	325	1.4
Median	6	24	5.27	19.98	3.42	5.45	9.3	25.25	33.05	84	2.6	289	28	79.5	0.21	86	92	218	0.8

[a] Weight is in kilograms. Blood characteristics include traits reflecting blood function [*ERI* erythrocytes (10^6/mm^3), *LEU* leucocytes (10^3/mm^3), *HB* hemoglobin (g/dL), *HTC* hematocrit (%), *MCHC* mean corpuscular hemoglobin concentration (g/dL), *MCV* medium corpuscular volume (μm^3)] and hepatic function [*ALB* albumin (g/L), *AP* alkaline phosphatase (U/L), *ALT* alanine aminotransferase (U/L), *AST* aspartate aminotransferase (U/L), *BIL* total bilirubin (mg/dL), *COL* cholesterol (mg/dL), *GLU* glucose (mg/dL), *LDH* lactate dehydrogenase (U/L)], and renal function (*CRE* creatinine (mg/dL)<PUB1>

female 24.42 ± 5.84; $t = 0.250$; $p = 0.807$; Table 1). Unlike previous studies documenting high lead levels in juveniles (Engel et al. 2010), the juvenile howler monkey had lead levels similar to those of the adults (26.0 ppm).

The level of lead in the soil was 18.18 ppm, but this did not correspond to high levels of lead in the plants (5.85 ppm; Table 1).

The lead levels in the animals' hair samples were correlated with lactate dehydrogenase (LDH: Pearson $r = -0.650$, $p = 0.030$; Spearman's rho $r_{sp} = 0.62$, $p = 0.042$; juvenile-excluded BIL, Pearson $r = -0.68$, $p = 0.032$; Spearman's rho $r_{sp} = 0.63$, $p = 0.053$, Table 1) and total bilirubin levels of their blood (BIL, Pearson $r = -0.597$, $p = 0.031$; Spearman's rho $r_{sp} = 0.71$, $p = 0.006$; juvenile-excluded BIL, Pearson $r = -0.62$, $p = 0.031$; Spearman's rho $r_{sp} = 0.76$, $p = 0.004$), but these two variables were not correlated with each other if normality is assumed. However, they were correlated using a nonparametric approach (Pearson $r = 0.52$, $p = 0.102$; Spearman's rho $r_{sp} = 0.697$, $p = 0.017$; juvenile-excluded BIL, Pearson $r = -0.51$, $p = 0.137$; Spearman's rho $r_{sp} = 0.69$, $p = 0.026$).

Discussion

What is the biologic significance of these howler monkey lead levels?

A number of recent studies investigating wild or recently captured primates have provided similar data on lead concentration in hair. This allows us to evaluate the potential biologic significance of the lead levels we documented for the howler monkeys in Balancán, but the real need is for reference values associated with the adverse health or behavioral impacts of such high environment lead levels (Allard et al. 2010). Engel et al. (2010) quantified the average lead content in hair from free-ranging rhesus monkeys (*Macaca mulatta*) only 3 km from the densely populated city of Kathmandu, Nepal to be 4.5 ppm of hair, and the maximum concentration in their sample was 10.2 ppm. All our samples had higher lead levels (average 24.12 ppm) than those observed in these rhesus monkeys that had frequent contact with people and their waste. Similarly, long-tailed macaques (*Macaca fascicularis*) from highly urbanized areas of Singapore did not have high levels of lead in their hair ($n = 27$, mean $= 2.51$ ppm, maximum $= 6.45$ ppm [Schillaci et al. 2011]), and the lead levels found in hair from rhesus macaques (*Macaca mulatta*) from Southwest China was only 0.66 ppm (Lee et al. 2012).

A rich comparative data set exists for people exposed to different levels of lead contamination (Fergusson et al. 1981; Sannal et al. 2007), and the study of Sannal et al. (2007) suggests that the ratio of blood to hair lead concentrations is approximately 1:1 (log µg of Pb/g of hair vs log µg of Pb/dl of blood). The Centers for Disease Control and Prevention suggest that in children, levels of concern in blood are 0.05 ppm (http://www.cdc.gov/nceh/lead/ACCLPP/blood_lead_levels.htm; accessed 22 March 2104) and that blood levels below 10 µg/dL are associated with decreased intellectual functioning in children (Jusko et al. 2008; Engel et al. 2010). We did not observe any of the clinical signs described to be associated with lead toxicity in humans, such as fatigue, irritability, nausea, abdominal pain, headache, weight loss, or motor weakness (Infantas 2005). However, it is not clear what sort of field observations would be indicative of these clinical signs.

Ways forward

This research represents a first step to understanding the cascading effects of anthropogenic change on primates in this region of Mexico and in regions experiencing similar changes worldwide. With respect to flooding and lead concentration, research is needed to understand whether lead is being concentrated and then transferred to the primates and under what conditions. Changes in the flooding regimen represent a likely mechanism leading to high lead levels, but other potential mechanisms for this region, such as lead associated with the development of the petroleum industry, should be investigated.

It would be extremely valuable to identify behavioral changes, if any, associated with increased lead levels in primates because attaining behavioral indices indicative of lead exposure is logistically much easier than capturing and conducting detailed laboratory analysis. In addition, such indices could potentially be used as an early warning sign identifying populations exposed to dangerous lead levels. If the altered flooding regimen is the mechanism leading to the increased exposure to lead, a comparison of the behavior of groups in flooded and nonflooded areas, controlling for ecologic differences between the habitats, may show behavioral indices of lead exposure. Early exposure to environmental lead impedes balance (Bhattacharya et al. 2007). Thus, if a population has been exposed to increased lead levels, the animals would be predicted to make more falls, to take less risk in leaps across gaps, and to use larger branches in travel. Comparing lead levels in howler monkeys with those in local human populations may show a public health issue and help to establish whether primates are suitable sentinels for lead exposure in humans (Engel et al. 2010; Schillaci et al. 2011).

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