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ORIGINAL ARTICLE

Total mercury in wild felids occurring in protected areas in the central Brazilian Amazon

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ABSTRACT

This is the first study to investigate mercury (Hg) contamination in felid species in the Brazilian Amazon. We collected 26 fur samples from wild felids of four species (*Puma concolor*, *Panthera onca*, *Leopardus pardalis* and *Leopardus wiedii*) occurring in the Mamirauá and Amanã sustainable development reserves, in the state of Amazonas. Samples were from museum specimens, except for five *P. onca* samples collected from free-living individuals. Total Hg concentrations ranged from 0.12 to 48.1 μg g^{-1}. Concentrations of Hg did not differ significantly between museum specimens and live individuals of *P. onca*, but varied significantly among species, with significantly higher concentrations for *P. onca* and *L. pardalis*, which could be related to factors such as diet and habitat.

KEYWORDS: conservation, mammals, bioaccumulation, fur, heavy metals

Mercúrio total em felinos selvagens em áreas protegidas na Amazônia central brasileira

RESUMO

Este é o primeiro estudo a investigar a contaminação por mercúrio (Hg) em espécies de felinos na Amazônia brasileira. Foram coletadas 26 amostras de pelo de felinos selvagens de quatro espécies (*Puma concolor*, *Panthera onca*, *Leopardus pardalis* e *Leopardus wiedii*) ocorrendo nas reservas de desenvolvimento sustentável Mamirauá e Amanã, no estado do Amazonas. As amostras foram coletadas de espécimes de museu, exceto cinco amostras de *P. onca* obtidas de indivíduos capturados em vida livre. As concentrações de Hg variaram de 0,12 a 48,1 μg g^{-1}. Não houve diferença significativa entre a concentração de Hg no pelo de espécimes de museu e de indivíduos vivos de *P. onca*. Houve variação significativa entre espécies, sendo que *P. onca* e *L. pardalis* tiveram concentrações significativamente mais altas, o que pode estar relacionado a fatores como dieta e hábitat.

PALAVRAS-CHAVE: conservação, mamíferos, bioacumulacão, pelo, metais pesados

INTRODUCTION

Several toxic effects of mercury (Hg), a naturally occurring element, have been described in terrestrial and aquatic species over the last 50 years (e.g., Hoffman 2003; Wren 1986; Borg *et al.* 1969; Burgess *et al.* 2005; Burgess and Meyer 2008; Dietz *et al.* 2006; Krey *et al.* 2015). In vertebrates, hazardous effects of Hg have been described on the central nervous system, DNA molecular structure, reproductive, cardiovascular, endocrine and immune systems (Wolfe *et al.* 1998; Scheuhammer *et al.* 2007; Rice *et al.* 2014). Additionally, mercury undergoes a biomagnification process, which considerably raises the toxicological risks for top predators (Evers 2018).

In the Amazon basin, artisanal and small-scale gold mining has been one of the main anthropogenic sources of Hg in the

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environment (Lacerda and Pfeiffer 1992; Bonotto *et al*. 2018). Beyond gold mining, many other human-driven activities can influence the Hg cycle and increase contamination in organisms. For example, erosion resulting from deforestation and forest burning can influence the Hg input to adjacent aquatic systems (Roulet *et al*. 1998; Farella *et al*. 2006). In addition, in some Amazonian sub-basins, Hg concentrations are naturally high (Silva-Forsberg *et al*. 1999; Lechler *et al*. 2000; Kasper *et al*. 2018).

The majority of studies on Hg contamination has focused on aquatic mammals, due to the known conducive conditions of mercury methylation in some aquatic systems (Ullrich *et al*. 2001). The terrestrial biota has been less studied worldwide, though it can also be impacted by Hg accumulation, mainly through the dietary route (Scheuhammer *et al*. 2007). Amazon felids can be linked to the aquatic environment and reflect levels of aquatic contamination by predating caimans, fishes, large rodents and turtles (Emmons 1987; Silveira *et al*. 2010). Yet, to date no data exists on Hg bioaccumulation in Amazon felids.

The main Hg source for aquatic biota is food intake, and its magnitude of incorporation is related to the trophic level of the organism (Jernelöv and Lann 1971). The Hg that reaches the digestive tract through food ingestion can be absorbed and transferred to the circulatory system, especially for organic mercury molecules such as methylmercury (Boudou and Ribeyre 1985). This Hg is transported via blood to all tissues, distributed in internal compartments (Wiener *et al*. 2003) and, due to its affinity for proteins, accumulated in tissues such as muscles and fur (WHO 1990). Therefore, fur usually has higher concentrations of metals and commonly shows a strong positive correlation with internal tissues (Cumbie 1975; Rashed and Soltan 2005; Brait *et al*. 2009). Fur samples are broadly used as an indicator of Hg bioaccumulation in wild species worldwide (Burton *et al*. 1977; Fonseca *et al*. 2005; Duffy *et al*. 2005; Mora *et al*. 2000). The use of fur samples has the additional advantage of being a non-invasive method for the assessment of Hg levels in wildlife, especially in species that cannot be collected due to ethical and legal reasons.

Felids face serious threats worldwide, especially due to hunting, road traffic accidents and loss of natural habitats (Macdonald and Loveridge 2010). Hg contamination can be an additional threat for felids, as they are top predators and therefore susceptible to biomagnification. Despite the lack of strong evidence for Amazonian felids, there are several studies showing high natural Hg concentrations in abiotic and biotic compartments in the Amazon, even for non-impacted regions (Lechler *et al*. 2000; Kasper 2018). This lack of knowledge for felids is mainly associated to the difficulty in capturing live individuals for sample collection, especially in the Amazon region. Thus, the aim of this study was to investigate the concentration of total mercury (THg) in fur samples from four felid species occurring in two sustainable development reserves in the mid Solimões River basin, in the central Brazilian Amazon, to provide background Hg concentrations for felids living in a non-impacted environment.

MATERIAL AND METHODS

Fur samples were collected from individuals occurring in the Mamirauá and Amanã Sustainable Development Reserves (from here on RDS Mamirauá and RDS Amanã, from the Portuguese *Reserva de Desenvolvimento Sustentável*). Both reserves are located in the middle Solimões River region, in the state of Amazonas, Brazil (Figure 1). Together, the two reserves cover more than 3,400,000 ha of primary forest, with low human disturbance and high wildlife richness (Ayres 1995), mainly floodplain habitats (regionally known as *várzeas*) subject to annual flooding of an extensive network of rivers, lakes and connecting channels (Ramalho *et al*. 2009). Fur samples from five free-living individuals of *Panthera onca* (Linnaeus, 1758) were collected in 2010. We also obtained fur samples of 21 wild felid specimens kept in the museum of the Mamirauá Sustainable Development Institute, in Tefé, Amazonas: *Panthera onca* (n = 9), *Puma concolor* Linnaeus, 1771 (n = 3), *Leopardus pardalis* Linnaeus, 1758 (n = 8) and *Leopardus wiedii* Schinz, 1821 (n = 1) (Supplementary Material, Table S1).

The museum specimens were retrieved from local communities and there are no recorded details of their age or cause of death. It is probable, though, that they were illegally hunted or found dead in the region. The taxidermy process was artisanal through sun drying the skin and, in some cases, with the addition of salt. At the museum, these skins were stored in temperature-controlled cabinets with naphthalene. Free-living individuals of *P. onca* were captured for research projects by the Mamirauá Institute. The animals were sedated with Zoletil and Ketamine and were released after sampling procedures. Fur samples from living individuals and from the museum specimens were cut with aluminum scissors from the dorsal region and stored in polyethylene bags. Capture and handling of the wild individuals of *P. onca* was authorized by IBAMA/SISBIO license no. 11095-3.

The analytical procedures were carried out at the Radioisotope Laboratory Eduardo Penna Franca, at the Institute of Biophysics Carlos Chagas Filho, at the Federal University of Rio de Janeiro (Brazil). All Hg forms present in the sample were quantified. The exogenous contamination from fur samples was previously removed using deionized water. Samples were then kept in an EDTA 0.01% bath for two hours, followed by rinses with deionized water. After that, they were oven-dried at 40 °C. Approximately 0.01 g dry weight, in duplicates, were mineralized with 3 mL of nitric acid (HNO_3 65%, Tedia, Brazil) in a water bath (60 ºC) for 30 minutes. Samples were cooled at room temperature, and the oxidation process was completed by the addition of 5 mL of 5% potassium permanganate ($KMnO_4$ 5%, Tedia, Brazil). Samples were placed in the water bath (60

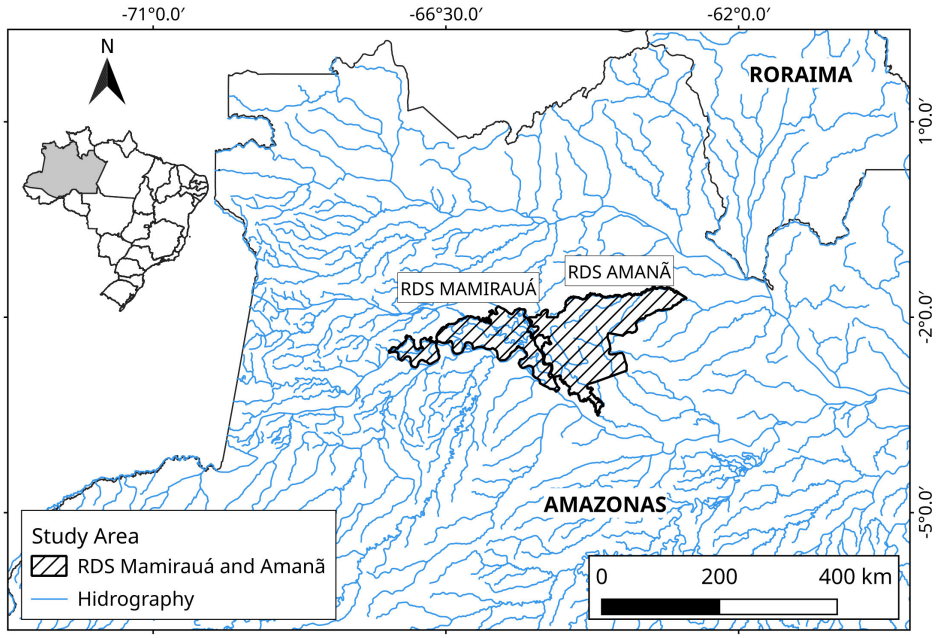

Figure 1. Map showing the location of the study areas: Sustainable Development Reserve (RDS in Portuguese) Mamirauá and Sustainable Development Reserve Amanã, in Amazonas state (highlighted in grey in the small map), Brazil. This figure is in color in the electronic version.

ºC) for 30 minutes and cooled at room temperature overnight. After these procedures, excess of $KMnO_4$ was reduced by adding 1 mL of hydroxylamine hydrochloride ($NH_2OH.HCl$ + 12% NaCl). Finally, the extract was diluted to 12 mL with ultrapure water in falcon tubes.

The determination of THg was made by Cold Vapor Atomic Absorption Spectrometry (CVAAS, FIMS 400, Perkin Elmer) equipped with an autosampler (AS90) flow injection system (FIAS) using the Winlab software (Perkin Elmer) (Bastos *et al.* 1998).

Samples of certified material references from the National Research Council of Canada (DORM-3, fish protein, n = 3) and the Institute of Environmental Medicine of the Karolinska Institutet (human hair, n = 8), were used to test the method accuracy, reaching recovering rates of 96% and 97%, respectively. All samples were analyzed in triplicate for an estimation of analytical variability and precision (coefficient of variation < 10%).

As data were not normally distributed and sample sizes were small, we compared THg concentrations among species using the Kruskal-Wallis test, followed by pairwise comparisons using the Wilcoxon rank sum test. *Leopardus wiedii* was not included in the statistical analysis, as only one sample was available for this species. Differences between museum and free-living *P. onca* samples were tested with the Mann-Whitney-Wilcoxon test. Significance level was 5% (α < 0,05). Data analysis was carried out using the R statistical language, through the RStudio development environment.

RESULTS

THg concentrations ranged from 0.12 to 48.1 μg g^{-1} (Figure 2). *Panthera onca* samples from museum and live individuals did not differ significantly (W = 24, p = 0.90) (Table 1), therefore the samples were pooled for subsequent analyses. THg concentrations differed significantly among species (KW = 7.00; p = 0.03), and the pairwise comparisons indicated that *P. onca* and *L. pardalis* had significantly higher concentrations then *P. concolor* (p = 0.01). The single sample from *L. wiedii* had THg of 0.53 μg g^{-1} (Table 1). Gender of the greatest part of

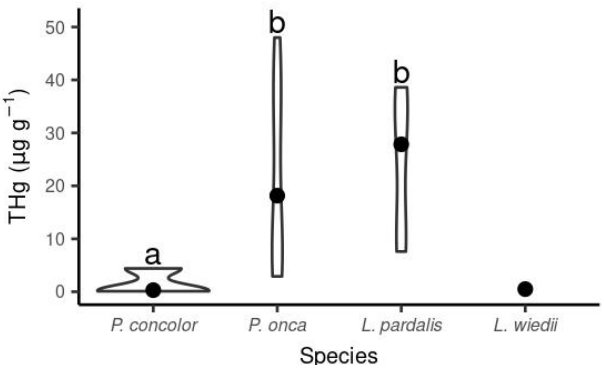

Figure 2. Violin plot of total mercury concentration (THg) grouped by four felid species: *Puma concolor* (n = 3), *Panthera onca* (n = 14), *Leopardus pardalis* (n = 8) and *Leopardus wiedii* (n = 1). Values are the minimum and maximum measured; the width represents data aggregation; the black dot is the median. Species that differed significantly from each other are indicated by different letters (P < 0.05). *Leopardus wiedii* was not included in the comparative analysis, as there is only one sample.

Table 1. Total Hg concentration in fur of wild neotropical felid species. N = number of individuals; Hg values are the mean ± SD (range). Newman's values reported here are monomethyl mercury concentrations because THg reported in the paper are probably contaminated with inorganic Hg (more details in the discussion).

Species	N	Origin	Hg concentration (μg g^{-1})	Reference
Leopardus pardalis	3	Texas	0.70 ± 0.56	Mora *et al.* 2000
Puma concolor coryi [1]	16	Florida, USA	0.60 ± 0.38 (0.03 – 1.52)	Newman *et al.* 2004
Puma concolor coryi [2]	26	Florida, USA	1.62 ± 1.87 (0.11 – 6.68)	Newman *et al.* 2004
Puma concolor [3]	3	Brazilian Amazon, Mamirauá and Amanã RDS	1.64 ± 2.43 (0.12 – 4.40)	this study
Panthera onca [3]	9		22.4 ± 18.8 (3.93 – 48.1)	this study
Panthera onca [4]	5		21.2 ± 15.4 (3.00 – 38.3)	this study
Leopardus pardalis [3]	8		24.5 ± 13.5 (7.65 – 38.6)	this study
Leopardus wiedii [3]	1		0.53	this study

1- museum collection (tanned); 2 – museum collection (untanned); 3 – museum collection; 4 – living individuals

the individuals was not identified, but the only male identified had the highest detected concentration (*P. onca*, 48.1 μg g^{-1}), while the *P. onca* females (n = 6) had a mean concentration of 17.9 (3.0 – 38.7) μg g^{-1}. Two *L. pardalis* individuals were identified as females, with THg concentrations of 10.6 and 24.6 μg g^{-1}.

DISCUSSION

Several abiotic and biotic factors can affect Hg accumulation in wild mammals. The intra- and interspecific variation observed may be related to diet, age, home range, metabolism, gender and even seasonality (Newman *et al.* 2004; Eisler 2006).

Since diet is an important Hg source to biota, understanding food webs is crucial to analyze Hg accumulation. A review on metal accumulation in mammal species established that carnivores inserted in aquatic food webs usually have higher Hg levels compared to terrestrial food webs (Wren 1986). This is probably due to that an expressive part of the Hg methylation occurs in aquatic environments, especially in the sediment–water interface, macrophyte roots and hypolimnion of floodplain lakes (Guimarães *et al.* 2000; Correia *et al.* 2012; Brito *et al.* 2017). In these environments, there are methanogens and iron- and sulfate-reducing bacteria in their anoxic conditions (Kerin *et al.* 2006; Correia *et al.* 2012; Gilmour *et al.* 2013).

The species *P. onca* and *L. pardalis* presented the highest Hg concentrations. The main diet components of *P. onca* are caimans (*Caiman crocodilus* Linnaeus, 1758 and *Melanosuchus niger* Spix, 1825), which are associated with *várzea*-lake environments (Ramalho and Magnusson 2008; Rabelo *et al.* 2019). There is no study concerning dietary habits of *L. pardalis* in the study areas. In other areas, *L. pardalis* was reported as a generalist predator with opportunistic foraging strategy (Emmons 1987; Abreu *et al.* 2008), intensively using all types of habitat, such as forest, river and lake edges (Emmons 1987). In *Araucaria* pine forest in Paraná state (southern Brazil), *L. pardalis* fed on mammals, birds and squamates (Abreu *et al.* 2008). In a tropical rainforest in Peru, *L. pardalis* was reported to attack large birds and a rat (Emmons 1987). The prey biomass of *L. pardalis*, based on scats was mainly composed of mammalian prey (48%) and fish, bird and reptile prey items (52%) (Emmons 1987). Therefore, *L. pardalis* diet in our study area probably includes terrestrial items (e.g. terrestrial rodents) and animals associated with aquatic systems (e.g. reptiles, birds and fish). It is worth mentioning that non-aquatic species (e.g. birds) that have a diet associated with this environment will express the Hg contamination of the aquatic ecosystem (Cristol *et al.* 2008). Piscivorous birds, such as the great egret (*Ardea alba* Linnaeus, 1758), the Neotropical cormorant (*Nannopterum brasilianus* Gmelin, 1789) and the large-billed tern (*Phaetusa simplex* Gmelin, 1789), are abundant in the study areas (Cintra *et al.* 2007) and are likely part of the diet of *L. pardalis*. This opportunistic and wide range of food items may have been reflected in the wide range of Hg values observed for this species.

The species with the lowest Hg concentrations have diets based more on terrestrial prey. While the diet of *L. wiedii* consists mostly of small vertebrates (Rocha-Mendes *et al.* 2010; Bianchi *et al.* 2011), *P. concolor* prefers small and medium-sized mammals (Crawshaw and Quigley 2002). Although no dietary information exists for *P. concolor* and *L. wiedii* in the study areas, we can assume that *L. wiedii,* being a highly skilled arboreal felid, may feed mainly on arboreal small vertebrates. In the same way, despite individuals of *P. concolor* have skills to cross water bodies (Elbroch *et al.* 2010), the species is not as strongly associated with aquatic environments as *P. onca* (Figel *et al.* 2019).

The Amazon region is composed of a mosaic of distinct riverine basins with unique geomorphological and biogeochemical properties (Sioli 1956; Stallard 1985), along with a long ongoing history of gold mining, which can increase Hg concentrations above natural background levels at some basins (Silva-Forsberg *et al.* 1999). Thus, it is not possible to use Hg concentration information from one basin to assess Hg contamination in another basin. We did not find information about Hg concentrations in potential prey species of felids inhabiting our study area in the literature, so that comparisons

of our data within the regional food web are impossible at this moment. This lack of information reinforces the importance of these first Hg results and the need to determine Hg levels in species from other trophic levels.

Data on differences in Hg accumulation between genders are conflicting for mammals. Higher concentrations were found in males of minks and otters (Beck 1977 apud Wren 1986), but in untanned Florida panthers (Table 1) there was no statistically significant difference in methylmercury fur content between genders (Newman *et al*. 2004). Considering that only a few individuals could have their gender identified in our sample (eight females and one male), no statistical analysis was possible.

In Brazil, there are few studies about Hg contamination in fur of wild mammals. Hair of giant otters (*Pteronura brasiliensis* Zimmermann, 1780) from Pantanal rivers contained 2.94 to 3.68 μg g^{-1} of THg (Fonseca *et al*. 2005). There are few studies worldwide on wild felids, yet none in the Amazon region (Table 1). THg concentrations in the fur of *L. pardalis* from the lower Rio Grande Valley, in Texas (USA) (Mora *et al*. (2000) were lower than in our study. A wide variation in monomethyl and inorganic mercury concentration was found in furs of *P. concolor coryi* (Newman *et al*. 2004), reaching values four times higher than our results when considering total Hg maximum values. Many of Newman's *et al*. (2004) samples were contaminated with inorganic Hg, probably due to the museum storage and conservation processes of the *P. concolor coryi* furs, yet the monomethyl Hg concentration, according to the authors, is not susceptible to exogenous contamination, and represents approximately 80% of THg internal fur concentration. Their values for monomethyl Hg concentration ranged from 0.03 to 6.68 μg g^{-1}, which falls within the same range as our values for THg for the same species. This could imply that our values for monomethyl Hg (not measured) would likely be lower than the concentrations for *P. concolor coryi*.

Newman *et al*. (2004) also discussed the effect of exogenous contamination on the assessment of Hg contamination in museum specimens, as mercuric chloride was used in the past for pest control, which could have led to contamination of some specimens. Upon request, the museums where their specimens were housed informed that their tanning processes involved the use of nonmercuric alum salts and tannic acids, among other compounds. Therefore, it is likely that the exogenous Hg contamination of the specimens originated from pest control products. In our study, the museum specimens were tanned by an artisanal process without the use of chemicals. We do not have information about pest control measures in the museum were our samples were stored. Nonetheless, there was no significant difference between our samples from museum skins and live individuals, which indicates that the skins were not contaminated by exogenous Hg.

The identification of safe Hg levels for biota is challenging due to metabolic differences among species and the lack of studies on wild species. Analysis on visons, raccoons and otters suggested a range of normal concentrations in fur from 1 to 5 μg g^{-1} (Sheffy and Amant 1982). In a review, Eisler (2006) recommended that values higher than 2 μg g^{-1} in fur should be interpreted with caution, while Roelke *et al*. (1991) set a conservative "at risk" threshold for total mercury concentrations above 12.6 μg g^{-1} in the fur of Florida panthers (*P. concolor couguar)*. In our study, 89% of the samples had THg values exceeding Eisler's (2006) proposed threshold of 2 μg g^{-1}, and as much as 50% exceeded the Roelke *et al.* (1991) threshold, which might point to harmful contamination levels. However, it is not possible to confirm any negative consequences based on the detected Hg concentrations and more data are necessary in order to understand the relevance of these concentrations for felids in the central Amazon *várzea* regions.

CONCLUSIONS

Our data suggest that the wild felid species in the central Brazilian Amazon floodplain regions of RDS Mamirauá and RDS Amanã, can be exposed to different mercury sources. We detected high concentrations of total mercury, as expected for a top predator, and interspecific differences that may be related to differences in dietary habits. Our limited sample size precluded further analyses to understand the high intraspecific variations found. The concentrations in some specimens exceeded Hg toxicity thresholds for mammals. The high THg concentrations detected in felid species highlight the importance of assessing mercury contamination in the Amazon region, including non-impacted conservation areas.

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SUPPLEMENTARY MATERIAL (only available in the electronic version)

LOPES *et al.* Total mercury in wild felids occurring in protected areas in the central Brazilian Amazon

Table S1. Mercury concentrations (μg g^{-1}, dry weight) in wild felid fur of *Leopardus pardalis, Panthera onca, Puma concolor and Leopardus wiedii* from Mamirauá and Amanã sustainable development reserves (Amazonas, Brazil). NA = data not available.

Species	Location	Date	Sex	Source	Hg (μg g)
Leopardus pardalis	Amanã	NA	NA	museum	9.14
Leopardus pardalis	Amanã	20 Jul 2010	NA	museum	38.1
Leopardus pardalis	Amanã	NA	NA	museum	31.1
Leopardus pardalis	Amanã	14 Jan 2007	female	museum	10.6
Leopardus pardalis	Amanã	28 Mar 2008	female	museum	24.6
Leopardus pardalis	Amanã	14 Jan 2007	NA	museum	36.1
Leopardus pardalis	Amanã	10 Mar 2007	NA	museum	38.6
Leopardus pardalis	Amanã	28 Feb 2008	NA	museum	7.65
Panthera onca	Mamirauá	24 Sep 2009	female	museum	5.63
Panthera onca	Mamirauá	11 May 2010	NA	living	35.1
Panthera onca	Mamirauá	16 Nov 2010	female	living	19.8
Panthera onca	Mamirauá	30 Nov 2010	female	living	3.00
Panthera onca	Mamirauá	3 Jul 2011	male	museum	48.1
Panthera onca	Mamirauá	1 Apr 2010	NA	living	38.3
Panthera onca (juvenile)	Mamirauá	16 Nov 2010	female	living	10.1
Panthera onca	Amanã	2011	female	museum	38.7
Panthera onca	Amanã	1 May 2011	female	museum	30.4
Panthera onca	NA	NA	NA	museum	47.1
Panthera onca	NA	NA	NA	museum	16.5
Panthera onca	NA	NA	NA	museum	5.87
Panthera onca	NA	NA	NA	museum	5.26
Panthera onca	NA	NA	NA	museum	3.93
Puma concolor	Amanã	NA	NA	museum	4.40
Puma concolor	Amanã	NA	NA	museum	0.39
Puma concolor	Amanã	NA	NA	museum	0.12
Leopardus wiedii	Amanã	NA	NA	museum	0.53