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ORIGINAL ARTICLE

Mercury bioaccumulation, genotoxic and biochemical biomarkers reveal the health status of yellow-spotted Amazon River turtles (*Podocnemis unifilis*) in an environmental protection area in the Amazon

Ândrocles Oliveira BORGES[1], José ERICKSON[2], Lídia Aguiar da SILVA[3], Cleiton FANTIN[1], Fabíola X. V. DOMINGOS-MOREIRA[3,4,*]

[1] Universidade do Estado do Amazonas (UEA), Programa Multicêntrico de Pós-Graduação em Bioquímica e Biologia Molecular, Manaus, AM, Brazil
[2] Universidade Federal do Espírito Santo (UFES), Programa de Pós-Graduação em Biologia Animal, Laboratório de Genética e Evolução Molecular, Vitória, ES, Brazil
[3] Instituto Nacional de Pesquisas da Amazônia (INPA), Programa de Pós-Graduação em Biologia de Água Doce e Pesca Interior, Manaus, AM, Brazil
[4] Instituto Nacional de Pesquisas da Amazônia (INPA), Coordenação de Dinâmica Ambiental, Manaus, AM, Brazil
* Corresponding author: domingosmoreirafxv@gmail.com; https://orcid.org/0000-0002-1761-4362

ABSTRACT

Chelonians are considered good bioindicators of environmental quality. The assessment of the health status of chelonian populations in the Amazon is important because they are traditionally consumed in large numbers in riverine communities and sustainable use reserves. The present study aimed to evaluate the health of *Podocnemis unifilis* (Testudines, Podocnemididae) in an environmental protection area in the Amazon region in Brazil. We analyzed the biomarkers lipoperoxidation, carbonylation of proteins, occurrence of micronuclei and erythrocytic nuclear abnormalities, quantified metallothioneins, and evaluated mercury bioaccumulation. We generated pioneering data regarding biomarkers in wild Amazonian freshwater turtles. All biomarker responses did not vary significantly between the sexes. The occurrence of oxidative and genotoxic damage, as well as concentrations of metallothioneins was low compared to other studies. In addition, the bioaccumulation of mercury in the muscle of the animals was below the limit recommended for human consumption by the World Health Organization. Our results provide baseline data on mercury bioaccumulation and biomarker responses that can be useful for future comparisons with other freshwater turtles. The data also provide evidence that the sustainable exploitation of these turtles in the study area (Piagaçu-Purus Sustainable Development Reserve) does not pose a risk to local riverine communities, as the detected mercury concentrations are safe for human consumption. In this sense, our results highlight the importance of protected areas for the conservation of healthy turtle populations, at the same time ensuring the health of the human populations that use them as a food resource.

KEYWORDS: freshwater turtle, health parameters, chelonians, Testudinidae, ecotoxicology

Bioacumulação de mercúrio, biomarcadores genotóxicos e bioquímicos revelam o estado de saúde de tracajás (*Podocnemis unifilis*) em uma área de proteção ambiental na Amazônia

RESUMO

Quelônios são considerados bons bioindicadores da qualidade ambiental. A avaliação do estado de saúde de populações de quelônios na Amazônia também é importante porque estes animais são tradicionalmente consumidos em grandes quantidades em comunidades ribeirinhas e em reservas de uso sustentável. O presente estudo avaliou a saúde de *Podocnemis unifilis* (Testudines, Podocnemididae) em uma área de proteção ambiental da Amazônia no Brasil. Analisamos lipoperoxidação, carbonilação de proteínas, ocorrência de micronúcleos e anormalidades nucleares eritrocíticas, quantificamos metalotioneínas, e avaliamos a bioacumulação de mercúrio. Geramos dados pioneiros sobre biomarcadores em quelônios de água doce amazônicos silvestres. Todas as respostas aos biomarcadores não variaram significativamente entre os sexos. A ocorrência de danos oxidativos e genotóxicos, bem como as concentrações de metalotioneínas foram baixas em comparação a outros estudos. Adicionalmente, a bioacumulação de mercúrio no músculo dos animais ficou abaixo dos limites recomendados para consumo humano pela Organização Mundial de Saúde. Nossos resultados constituem um conjunto de dados de referência para bioacumulação de mercúrio e respostas de biomarcadores que podem ser úteis para futuras comparações com outros quelônios de água doce. Os dados também fornecem evidências de que a exploração sustentável desses quelônios na área de estudo (Reserva de Desenvolvimento Sustentável Piagaçu-Purus) não apresenta risco às comunidades ribeirinhas locais, já que as concentrações de mercúrio detectadas são seguras para o consumo humano. Nesse sentido, nossos resultados evidenciam a importância das áreas protegidas para a conservação de populações saudáveis de quelônios, concomitantemente assegurando a saúde das populações humanas que os utilizam como fonte alimentar.

PALAVRAS-CHAVE: tartaruga de água doce, parâmetros de saúde, quelônios, Testudinidae, ecotoxicologia

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INTRODUCTION

Biomarkers are widely used in biomonitoring studies, as they represent useful tools for assessing the health of aquatic organisms, including turtles (Labrada-Martagón *et al.* 2011; Camacho *et al.* 2013; Casini *et al.* 2018). Molecular and cellular biomarkers have been used in studies related to reptile protection and conservation initiatives, especially involving marine and freshwater turtles (Schneider *et al.* 2010; Camacho *et al.* 2012, 2013; Zapata *et al.* 2016).

Oxidative stress is an imbalance in cellular homeostasis between antioxidant defense systems and oxidizing agents, and is commonly known as the route of greatest production of reactive oxygen species (ROS) (Dalle-Donne *et al.* 2003; Yan and Forster 2011). The imbalance generated by excess ROS can be caused by a number of pollutants, such as mercury (Monteiro *et al.* 2010), organochlorine pesticides and other non-essential metals (Labrada-Martagón *et al.* 2011). The process culminates in the oxidation of molecular components essential to life, such as lipids (lipoperoxidation), proteins (carbonylation of proteins) and DNA, and can thus directly affect the health of organisms (Dalle-Donne *et al.* 2003; Yan and Forster 2011). Oxidative damage to cellular lipid components causes severe disturbances to the cell membrane system, which ultimately results in loss of membrane integrity and permeability (Stark 2005, Monteiro *et al.* 2010). In an interconnected way, the final products of lipoperoxidation, malondialdehyde (MDA) and 4-hydroxenonenal (HNE), can oxidize proteins, forming carbonyl groups in their amino acid side chains, and generating partial or total loss of biological activity (Dalle-Donne *et al.* 2003; Yan and Forster 2011).

ROS can establish genotoxic effects, such as DNA breaks, which compromise the expression and conservation of genetic information, which can be evidenced in morphological changes in erythrocyte nuclei (Evans *et al.* 2004; Finlayson *et al.* 2019). Metallothioneins are low molecular weight proteins that act regulating the levels of essential and non-essential metals in tissues. This control indirectly allows reduction of ROS generation, since high concentrations of metals can affect the redox balance in the cellular environment (Schlenk *et al.* 2008).

Environmental protection areas are intended to ensure representative samples of ecologically viable populations and play an important role in the conservation of endangered species (Pantoja-Lima *et al.* 2014). These areas are appropriate to obtain data on health of the biota that can be used as reference parameters for areas where pollution and other environmental impacts can affect the health status of animals (Camacho *et al.* 2013; Casini *et al.* 2018). Yet there are few such data from protection areas.

Podocnemis unifilis (Troschel, 1848, Testudines: Podocnemididae) is one of the most abundant freshwater turtle species in the Amazon region (Vogt 2004) and is listed as vulnerable in the IUCN Red List of Threatened Species (IUCN 2017). It is traditional food resource of human populations in the region and is still widely consumed in riverine communities, including those in sustainable use reserves (Waldez *et al.* 2013). The species was chosen as a bioindicator to generate baseline health data from a protected area and ensure the safe consumption of turtles by the local communities (Kemenes and Pezzuti 2007; Fagundes *et al.* 2016).

In this context, the objectives of this study were: (1) to evaluate the health status of the yellow-spotted Amazon River turtle (*Podocnemis unifilis*) in an environmental protection area through biomarkers that indicate damage in macromolecules, nuclear morphological changes in blood erythrocytes and mercury levels in muscle; and (2) to provide information about food security for human populations in the area regarding mercury concentrations in the turtles.

MATERIAL AND METHODS

Study area and biological material

For this study, 35 specimens of *P. unifilis* (12 females and 23 males) were obtained from the Piagaçu-Purus Sustainable Development Reserve (PP-SDR) located in the municipality of Beruri, state of Amazonas, Brazil. Sampling took place in three locations in PP-SDR: Itapuru-Mirim Lake (4°16'03.6"S; 61°53'45.3"W), Paraná do Itapuru (4°16'57.6"S; 61°54'06.1"W) and Martinho Lake (4°15'21.3"S; 61°57'12.6"W). Taking into account that the three water bodies are interconnected and the mobility of *P. unifilis*, we considered all sampled individuals to belong to one population from the same wider sampling area (Figure 1).

After biometric measurements (maximum straight carapace length – MSCL and total wheight – TW), 2 mL blood samples were obtained via caudal or cervical puncture with heparinized syringes (25 x 7 mm) and stored in cryogenic tubes. The samples were later used to obtain blood smears on microscopy slides. The slides were dried at room temperature and, after 24 hours, were fixed in absolute ethanol for 30 minutes. After blood sampling, the individuals were euthanized at the collection site with a lethal intramuscular injection of propofol (CFMV 2002; CFBio 2012). Once death was confirmed, dissection was performed via removal of the plastron, and samples of the muscle were taken from the pectoral region. The samples were stored in liquid nitrogen and transported to the Laboratório de Ecotoxicologia Aquática na Amazônia at Instituto Nacional de Pesquisas da Amazônia (INPA), where they were stored in an ultra-freezer at -80 °C. Considering that the turtles in PP-SDR can be subjected to unknown upstream pollutant sources, we also sampled six animals (four females and two males) from the Bicho do Rio turtle breeding farm (BRF), located by the AM 70 highway, highway marker 27, Iranduba, Amazonas state,

Figure 1. Sampling points of *Podocnemis unifilis* in the Piagaçu-Purus Sustainable Development Reserve (PP-SDR) in Amazonas state, Brazil.

Brazil (3°11'11.5"S; 60°17'43.8"W). These animals were assumed to be a control for low frequencies of micronuclei and erythrocyte nuclear abnormalities (ENAs), as water quality and sanitary conditions are controlled in the farm. Sampling was authorized by Instituto Chico Mendes de Conservação da Biodiversidade (ICMBIO) under SISBIO license # 50930-1, and all procedures were approved by the Ethics Committee on the Use of Animals in Research – CEUA/INPA, under protocol # 035/2015.

Biochemical biomarkers

Muscle fragments (± 300 mg) were homogenized in sodium phosphate buffer (0.1 M, pH 7.5), at a ratio of 1:10 (weight:volume). The homogenate was centrifuged at 15,000 *g* for 30 minutes at 4 °C. Subsequently, aliquots for the analysis of lipoperoxidation and carbonylation of proteins were separated and stored in a -80 °C ultra-freezer.

Lipoperoxidation (LPO) - The content of malondialdehyde (MDA) in the homogenate was measured by the content of substances that are reactive to thiobarbituric acid (TBARS) (Ohkawa *et al.* 1979). The quantification of MDA was obtained by means of the molar extinction coefficient of 1.56×10^6 m^{-1} cm^{-1} and expressed in nmoles of MDA gram of wet $weight^{-1}$, with spectrophotometry reading at 535 nm (Spectramax Plus, Molecular Devices, USA).

Protein carbonylation (PCA) - The quantification of carbonylated proteins was performed using the reaction of DNPH (2,4-dinitrophenyl-hydrazine) with carbonyl groups in amino acid residues, and generated compounds such as dinitrophenyl-hydrazones that are detected by spectrophotometry at 370 nm (Spectramax Plus, Molecular Devices, USA) (Levine *et al.* 1994). The results were expressed in μmol.mg $protein^{-1}$.

Metallothioneins (MT) - Muscle samples (± 100 mg) were homogenized in Tris-HCl/sucrose buffer (20 mm/500 mm, pH 8.6), phenylmethylsulfonyl fluoride (0.1 m, 0.5 mM) and β-mercaptoethanol (0.01%) at a ratio of 1:5 (weight:volume), and centrifuged at 15,000 *g* for 30 min at 4 °C. The MTs were analyzed using the method proposed by Viarengo *et al.* (1997). A series of precipitations, centrifugations and resuspensions was carried out, resulting in the isolation of the MTs in the pellet, with the addition of Ellman's solution (DTNB 0.4 mM, sodium phosphate buffer NaCl 0.2/2 M, pH 8). The MTs have sulfhydryl groups (SH) in their cysteine residues and, from the comparison with the reduced glutathione curve (GSH) as a reference, the absorbance was determined at 412 nm (Spectramax Plus, Molecular Devices, USA). The MT concentration was expressed in μg mg of $protein^{-1}$.

Protein concentration - Bradford's method was used for determination of the protein concentration at 595 nm (Spectramax Plus, Molecular Devices, USA). Bovine serum albumin was used as the standard (Bradford 1976).

Genotoxicity biomarkers

The blood smears were stained with Giemsa 10% to determine the frequency of micronuclei and ENAs. Two thousand cells per individual were used to identify morphological changes and count frequencies (Carrasco *et al.* 1990).

Total mercury (THg)

THg concentrations in the muscle were determined in six animals (three males and three females) using the methodology of Bastos *et al.* (2015). Approximately 500 mg (wet weight) of muscle was digested in a solution of H_2SO_4 and HNO_3 (1:1) and $KMnO_4$ (5%). After digestion, $KMnO_4$ solution (5%) was added to the samples for 1h 30 min in a digestion block (Tecnal-Mod.007a, Piracicaba, São Paulo, Brazil) at 60 °C. The samples were cooled at room temperature (± 25 °C) and hydroxylamine hydrochloride (12%) was added. The digested samples were diluted with deionized water (Milli-Q

Plus, Millipore, Bedford, MA, USA). The THg reading was obtained using an atomic absorption spectrophotometer (Flow Injection Mercury System - FIMS – 400 - Perkin Elmer, Ueberlingen, Germany). The reference material used was DORM-4 (fish protein certified reference material for trace metals, NRC National Research Council, Canada), with an average recovery of 100% for each battery of samples and a detection limit for THg equal to 0.0007 mg kg^{-1}.

Statistical analysis

The response variables were submitted to the Kolmogorov-Smirnov normality test and the Levene test for homocedasticity. All variables were non-conformant with normality assumptions. The Mann-Whitney U test was used to compare biochemical biomarkers, genotoxicity biomarkers and THg between the sexes, and also between PP-SDR and BRF, and the weight and length of the animals between PP-SDR and BRF. All results were expressed as mean ± standard deviation, and test results were considered significant at $p < 0.05$ (Zar 1996). Since the variables did not conform to normal distribution, we also represented the data as median and interquartile range, and in point dispersion graphs (Weissgerber *et al.* 2015). The basic and agricolae packages of the statistical software R Core Team, version 3.4.1 (R CoreTeam 2017) and Statistics IBM-SPSS 22 were used to perform the analyses.

RESULTS

Mean MSCL of the *P. unifilis* specimens from PP-SDR was 26.0 ± 5.7 cm and mean total weight was 2.71 ± 1.71 kg. Females were larger than males in both length ($p = 0.0004$) and weight ($p = 0.0001$), respectively, 31.0 ± 5.2 cm, 4.29 ± 2.05 kg, n = 12 and 23.5 ± 3.9 cm, 1.89 ± 0.70 kg, n = 23. Mean MSCL of individuals from BRF was 18.7 ± 3.3 cm, and mean weight was 1.05 ± 0.67 kg. Mean MSCL ($p = 0.13$) and weight ($p = 1$) of females (20.0 ± 3.2 cm; 1.20 ± 0.80 kg, n = 4) and males (16.1 ± 2.1 cm; 0.75 ± 0.19 kg, n = 2) did not differ significantly. PP-SDR turtles were significantly larger ($p = 0.001$) and heavier ($p = 0.001$) than those of BRF. By sexes, PP-SDR turtles were also larger (males: $p = 0.01$; females: $p = 0.004$) and heavier (males: $p = 0.007$; females: $p = 0.004$) than those of BRF.

Biochemical biomarkers

Lipoperoxidation (LPO) – Mean lipid peroxide concentration was 14.34 ± 4.32 nanomol g wet weight (w. w.)$^{-1}$ overall (Table 1), 14.66 ± 4.37 (n = 20) for males and 13.77 ± 4.38 nanomol g w. w.$^{-1}$ (n = 11) for females. LPO did not differ significantly between the sexes ($p = 0.64$) (Figure 2a).

Metallothioneins (MT) - Mean MT concentration in samples from PP-SDR was 2.22 ± 1.30 µg mg protein^{-1} (n = 29) (Table 1). There was no significant difference between males (2.52 ± 1.32 µg mg protein^{-1}; n = 19) and females (1.63 ± 1.11 µg mg protein^{-1}; n = 10) ($p = 0.13$) (Figure 2c).

Table 1. Biochemical biomarkers (lipid peroxidation, protein carbonilation, metallothionein) in muscle samples of *Podocnemis unifilis* from the Piagaçu-Purus Sustainable Development Reserve. Values are the mean ± standard deviation followed by the median and interquartile range in brackets.

Biomarker	Parameter values
Lipid peroxidation (nanomol g wet weight^{-1})	14.34 ± 4.32 [14.85; 6.3]
Protein carbonilation (nanomol mg protein^{-1})	1.63 ± 0.40 [1.56; 0.61]
Metallothionein (µg mg protein^{-1})	2.22 ± 1.30 [1.55; 2.18]

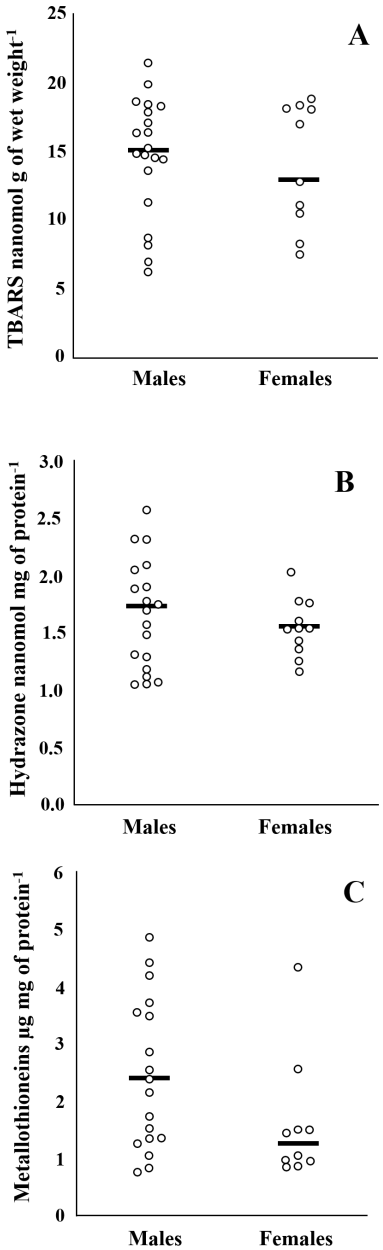

Figure 2. Biochemical biomarkers in the muscle of *Podocnemis unifilis* from the Piagaçu-Purus Sustainable Development Reserve. A – lipoperoxidation; B – carbonylation of proteins; C – metallothioneins. Data were analyzed by the Mann-Whitney U test. Circles indicate each individual and black bars indicate medians in the graph.

Protein carbonylation (PCA) – Mean concentration of hydrazones was 1.63 ± 0.40 nanomol mg protein^{-1} overall (Table 1), 1.67 ± 0.47 (n = 20) for males and 1.54 ± 0.25 nanomol mg protein^{-1} (n = 11) for females. PCA did not vary significantly between the sexes (p = 0.24) (Figure 2b).

Genotoxicity

Erythrocytes of all sampled infividuals presented normal nuclei, as well as micronuclei and nuclei in the following formats: kidney-shaped, lobed, protuberant, blebbed, vacuolate and segmented. There was no significant difference between the sexes in frequency of micronuclei and any nuclei format in PP-SDR (23 males, 11 females) and BRF (2 males, 4 females). Despite the difference in size and weight of animals sampled in PP-SDR and BRF, the frequency of genotoxicity biomarkers did not differ significantly between both sites (Table 2).

Total mercury (THg)

Mean THg concentration in muscle was 0.011± 0.012 μg g^{-1} (n = 6) (Table 3) and did not differ significantly between males (0.004 ± 0.007 μg g^{-1}; n = 3) and females (0.018 ± 0.015 μg g^{-1}; n = 3).

DISCUSSION

The relationship between biomarkers and animal size or sex has been studied for some reptiles (Costantini *et al.* 2009; Amaral *et al.* 2012). In turtles, body size is an important factor associated with responses to biomarkers, as it is related to age and, possibly, to the time of exposure to toxic substances (Schneider *et al.* 2011). Information about the size/age relationship is also important because some chelonians, such as *P. unifilis*, undergo ontogenetic variation in diet, which can make them more vulnerable to pollutants in certain growth stages (Balensiefer and Vogt 2006).

The response of biomarkers can vary between sexes in turtles. For example, females of *Trachemys scripta* (Thunberg & Schoepff, 1972) may bioaccumulate less metals than males as they are able to excrete metals through the eggs (Burger and Gibbons 1998). Higher concentrations of mercury were observed in the muscle of females compared to that of males in *Podocnemis sextuberculata* (Cornalia, 1849) (Schneider *et al.* 2011). Variation between sexes was also observed in the land iguana, *Conolophus subcristatus* (Gray, 1831), with males showing lower oxidative damage than females, indicating that females have lower antioxidant capacity, especially in the reproductive period (Costantini *et al.* 2009). In the present study, the similar response to biomarkers of males and females may be related to the diet of *P. unifilis*, as all sampled individuals were adults, which are predominantly herbivorous in both sexes (Balensiefer and Vogt 2006; Lara *et al.* 2012; Souza-Araujo *et al.* 2015).

Our results on concentration of lipid peroxides are similar to those obtained in studies with other reptiles. Lipid peroxide levels of 18.50 ± 0.7 nanomol g w.w.$^{-1}$ were found in the muscle of subadult *Caiman yacare* (Daudin, 1802) from a

Table 2. Genotoxicity biomarkers (frequency of micronuclei and ENA) in *Podocnemis unifilis* from Bicho do Rio Farm (BRF) and Piagaçu-Purus Sustainable Development Reserve (PP-SDR), Amazonas state, Brazil, and in *Caretta caretta* from the Mediterranean Sea (Casini *et al.* 2018). Values are the mean ± standard deviation followed by the median and the interquartile range in brackets, if available.

Biomarker ($‰_0$)	*Podocnemis unifilis*		*Caretta caretta* (n = 61)
	BRF (n = 6)	PP-SDR (n = 34)	
Micronuclei	1.47 ± 0.74 [1.75; 1.50]	1.44 ± 0.59 [1.50; 1.00]	15.82 ± 15.16
Kidney-shaped	0.75 ± 0.41 [0.50; 0.60]	0.48 ± 0.61 [0.50; 0.60]	1.00 ± 1.91
Lobed	1.83 ± 0.25 [2.00; 0.50]	1.43 ± 0.90 [1.50; 1.50]	207.97 ± 107.42
Segmented	0.00 ± 0.00 [0.00; 0.00]	0.03 ± 0.12 [0.00; 0.00]	0.08 ± 0.28
Vacuolated	0.16 ± 0.25 [0.00; 0.50]	0.13 ± 0.28 [0.00; 0.00]	-
Blebbed	1.75 ± 0.52 [1.75; 0.80]	2.12 ± 1.48 [2.00; 1.16]	-

Table 3. Total mercury concentration in *Podocnemis unifilis* muscle reported in different studies in the Amazon region. Values are the mean ± standard deviation followed by the range in parenthesis and the median; interquartile range in brackets. N = sample size; na = not available.

Location	N	Total mercury (μg g^{-1})	Source
Purus River basin	6	0.011 ± 0.012 (0.000 – 0.034) [0.007; 0.002]	This study
Negro River basin	2	0.034 ± 0.038 (0.059 – 0.011) [na]	Schneider *et al.* (2010)
Negro River basin	2	0.040 ± 0.027 (0.059 – 0.0113) [na]	Schneider *et al.* (2011)
Purus River basin	10	0.013 ± 0.012 (0.004 – 0.043) [na]	Schneider *et al.* (2015)
Xingu River basin	29	~0.015 ± 0.008 (na) [na]	Souza-Araújo *et al.* (2015)
Xingu River basin	50	0.024 ± 0.026 (0.007 – 0.188) [na]	Pignati *et al.* (2018)

natural environment without the influence of contaminants (Furtado-Filho *et al*. 2007). Levels of 9.54 ± 0.9 nanomol g w.w.$^{-1}$ were obtained in red muscle of the negative control group of the freshwater turtle *Trachemys scripta elegans* (Wied, 1839) subjected to hypoxia (Willmore and Storey 1997). Our values were also lower than those recorded in the muscle of a sea turtle *Chelonia mydas agassizii* (Bocourt, 1868) (63.7 ± 7.4 nanomol g w.t.$^{-1}$) caught in a relatively undisturbed feeding area in the Eastern Pacific (Valdivia *et al*. 2007). Thus, our results indicate that there is no evidence of oxidative damage to lipid components that might compromise the health of *P. unifilis* in PP-SDR.

PCA is the most common way to evaluate oxidative damage to the protein components of cells. The blood of healthy adults of *Chelonia mydas* (Linnaeus, 1758) under the influence of non-essential metals from the Atlantic Ocean contained levels of 2.48 ± 0.25 nanomol mg protein^{-1} (da Silva *et al.* 2016). Brain and liver of the negative control group of juvenile *Chrysemys picta* (Schneider, 1783) subjected to hypoxia and cold contained 3.7 ± 0.4 and 2.2 ± 0.2 nanomol mg protein^{-1}, respectively (Baker *et al.* 2007). Average PCA in muscle of Chinese soft-shelled turtles, *Pelodiscus sinensis* (Wiegmann, 1835) was 19 nanomol mg protein^{-1} in a group submitted to infection with two furunculosis pathogens, and 7 nanomol mg protein^{-1} in the control group (Li *et al*. 2021). To our knowledge, there are no published studies on PCA related to metals in turtle muscle, but the comparison with the aforementioned studies indicates that our results for PCA (1.63 ± 0.40 nanomol mg protein^{-1}) represents a low level of oxidative damage.

MT are widely studied in aquatic organisms, but, regarding chelonians, there are only a few studies on marine turtles (Andreani *et al.* 2008; Sinaei 2016). MT in the liver and kidney of *Chelonia mydas* from Costa Rica and *Caretta caretta* (Linnaeus, 1758) from the Mediterranean and Adriatic seas were positively correlated with cadmium and copper, emphasizing the role of these proteins in metal detoxification (Andreani *et al.* 2008). MT concentration in the blood of *C. mydas* was positively correlated with the concentrations of mercury, cadmium, lead, copper and zinc and the turtles were capable of responding quickly and efficiently to environmental contamination (Sinaei 2016). Although these studies have used different methodologies, units of measurement and tissues, they demonstrated a relatioship between the levels of metals and MT content in tissues, especially the induction of these metalloproteins by mercury in aquatic organisms, as already evidenced in *C. mydas* (Sinaei 2016). Our results suggest that the levels of MT are also basal and normal for *P. unifilis* in PP-SDR. Studies that associate MT concentration with the bioaccumulation of other elements in turtle tissues are needed in this region.

ENA are a relevant biomarker as they indicate early stages of damage to genetic material (Shimizu *et al.* 1998; Crott and Fenech 2001) and, as such, can be useful for the establishment of protective measures that simultaneously reverse the damage and prevent the formation of micronuclei, that represent irreversible DNA damage. Casini *et al.* (2018) have generated data regarding these biomarkers of genotoxicity in *C. caretta* from the Mediterranean Sea (see Table 1), which is an environment with high levels of pollution by metals, petroleum products and insecticides. The authors reported high frequencies of biomarkers of genotoxicity in the sampled animals and observed a positive correlation with the concentrations of measured contaminants (polycyclic aromatic hydrocarbons). Based on Casini *et al.* (2018), we can consider that the oxidative damage observed in the genetic material of the species in PP-SDR is basal.

The frequency of micronuclei in *P. unifilis* from BRF (1.47 ± 0.74‰) and PP-SDR (1.44 ± 0.59‰) was lower than that reported for juvenile *P. unifilis* exposed to cadmium in the diet for 60 days (12 ± 5‰, n = 12), and in the respective control group (8 ± 1‰, n = 12) (Frossard *et al.* 2013). It was similar to the frequency reported for the control group of *Trachemys callirostris* (Gray, 1855) exposed to chemical contaminants (0.78 ± 0.58‰; n = 20) (Zapata *et al.* 2016). Since this biomarker represents the final stages of genotoxicity (Shimizu *et al.* 1998; Crott and Fenech 2001; Yasui *et al.* 2015) the low frequency of micronuclei indicates normal cell cycle condition and replication of the genetic material. Thus, in comparison with the aforementioned studies, we reported the occurrence of basal and normal frequencies of micronuclei in erythrocytes of *P. unifilis* in PP-SDR.

Although our *P. unifilis* from PP-SDR were significantly larger than those from BRF, the levels of genotoxicity were similar between the sites and are assumed to be representative of basal frequencies. The results confirm the good environmental conditions maintained at BRF, where even the smallest and youngest animals, which also include items of animal origin in their diet, feed on uncontaminated prey. The low and similar levels of genotoxicity biomarkers indicate the absence of DNA strand break inducers in the environment both at BRF and at PP-SDR.

Mercury is a current concern for human and animal health in the Amazon region (Fadini and Jardim 2001) due to its high toxicity to tissular, neurological and cellular components of organisms (Chan *et al.* 2003; Barcelos *et al.* 2011). Mercury can enter aquatic ecosystems by anthropogenic and natural processes. In the Amazon, mining activity is the main anthropogenic source of mercury, however, there are natural sources in soils that are ultimately incorporated into aquatic systems (Fadini and Jardim 2001; Roulet *et al.* 2001). In the Purus River channel, analyses of water, river bed sediments, suspended solids and fish tissues have revealed that the

lithogenic mercury intake is more significant than that from external sources (Brabo *et al.* 2003; Castro *et al.* 2016). THg levels in the muscle of *P. unifilis* in this study were similar to those found in *P. unifilis* in the Purus River (Schneider *et al.* 2015) and in the lower Xingu River (Souza-Araujo *et al.* 2015), and were lower than the concentrations observed in the Negro River basin (Schneider *et al.* 2010, 2011) and another study from the lower Xingu River (Pignati *et al.* 2018) (see Table 3).

The maximum limit of mercury consumption established for humans is 500 ppb or 0.5 μg g^{-1} (ANVISA 1998; WHO 2008). The consumption of turtles that live in areas contaminated by mercury and other metals can represent a risk to riverine populations. *Chelus fimbriatus* (Schneider, 1783) from the Negro River showed high mercury concentrations of 432 ± 195.5 ppb (Schneider *et al.* 2010). Higher concentrations of mercury tend to be observed in tissues of carnivorous species, such as *C. fimbriatus*, which can be attributed to the biomagnification of mercury in trophic chains. *Podocnemis unifilis* is a predominantly herbivorous species, therefore, low concentrations of mercury were expected in this species (Schneider 2009, 2010). Indeed Pignati *et al.* (2018) and Souza-Araujo *et al.* (2015) observed concentrations twice as high as those observed in our study in *P. unifilis* from the Xingu River basin.

Schneider *et al.* (2011) and Souza-Araujo *et al.* (2015) also found no significant variation of THg between the sexes in *P.unifilis*, which is attributed to both sexes belonging to the same trophic level (predominently herbivorous) (Lara *et al.* 2012; Souza-Araujo *et al.* 2015). A negative and significant correlation between mercury and body size was reported for young individuals of *P. sextuberculata*, and therefore smaller turtles had higher levels of mercury than larger individuals was attributed to ontogenetic alterations in the diet (Schneider *et al.* 2010). The absence of an expressive variation in size within our sample suggests that the animals have similar age and, therefore, similar diets and exposure times to the environment. Additionally, these turtles tend to achieve a rapid balance between the rate of elimination of mercury and the rate of consumption of this metal (Schneider *et al.* 2010, 2011).

The consumption of subadult and adult *P. unifilis* is a very common practice in PP-SDR (Waldez *et al.* 2013), and about 16% of eggs from *P. unifilis* nests are protected by riverine communities through participatory management in the PP-SDR and consumed locally (Erickson *et al.* 2020a). The concentrations of mercury observed in our study are 45 times lower than the threshold for human consumption. These values suggest that the risk of mercury contamination from consumption of *P. unifilis* for the inhabitants of PP-SDR is minimal. However, it is important that mercury levels in *P. unifilis* are monitored in larger samples and wider spatial and temporal scales, as the species has a generalist behavior and phenotypic plasticity to respond to variable environmental conditions (Erickson *et al.* 2020a, 2020b). It would also be relevent to evaluate biomarkers and bioaccumulation of metals on a wider scale in congeneric *Podocnemis*, which generally exhibit more specialized behavior than *P. unifilis* and are restricted to narrower environmental conditions, such as *P. erythrocephala* (Batistella and Vogt 2008), *P. sextuberculata* (Haller and Rodrigues 2006), *P. lewyana* (Páez *et al.* 2009), *P. vogli* (Rueda-Almonacid *et al.* 2007) and *P. expansa* (Vanzolini 2003).

CONCLUSIONS

Our study generated pioneering data regarding biomarkers in Amazonian freshwater turtles collected in the field. We demonstrated low levels of oxidative and genotoxic damage, as well as low concentrations of metallothioneins and mercury in muscle tissue of *P. unifilis* in an environmental protection area. Biomarker response did not differ between sexes. The biomarkers analyzed suggest that there is no evidence of damage to the health of *P. unifilis* in the Piagaçu-Purus Sustainable Development Reserve, and that the reserve seems to be fulfilling its function of preserving the population of this turtle by maintaining a good quality environment. In addition, low concentrations of mercury in the tissues sampled indicate that the sustainable exploitation of these turtles does not pose a risk to local riverine communities, as the detected concentrations are safe for human consumption. Our results provide a set of data on mercury bioaccumulation and biomarker response that can be useful for future comparisons with freshwater turtles. We also provide evidence for the effectiveness and importance of protected areas for the conservation of healthy turtle populations, and also to ensure the health of the human populations that use them as a food resource. The biomarkers evaluated in this study have shown to be adequate tools for biomonitoring the health of Amazonian freshwater turtles and can be applied in biomonitoring of other protected areas. As a future perspective, other categories of biomarkers can be included in the analyses, as well as a larger sample sizes.

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REFERENCES

Amaral, M.J.; Bicho, R.C.; Carretero, M.A.; Sanchez-Hernandez, J.C.; Faustino, A.M.R.; Soares, A.M.V.M.; *et al*. 2012. The use of a lacertid lizard as a model for reptile ecotoxicology studies: Part 2 - Biomarkers of exposure and toxicity among pesticide exposed lizards. *Chemosphere,* 87: 765–774.

Andreani, G.; Santoro, M.; Cottignoli, S.; Fabbri, M.; Carpenè, E.; Isani, G. 2008. Metal distribution and metallothionein in loggerhead (*Caretta caretta*) and green (*Chelonia mydas*) sea turtles. *Science of the Total Environment,* 390: 287–294.

ANVISA. 1998. Agência Nacional de Vigilância Sanitária, Brasil. Portaria 685. Limites máximos de tolerância para contaminantes químicos em alimentos. (https://www.gov.br/agricultura/pt-br/assuntos/inspecao/produtos-vegetal/legislacao-1/biblioteca-de-normas-vinhos-e-bebidas/portaria-no-685-de-27-de-agosto-de-1998.pdf).

Baker, P.J.; Costanzo, J.P.; Lee, R.E. 2007. Oxidative stress and antioxidant capacity of a terrestrially hibernating hatchling turtle. *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology,* 177: 875–883.

Balensiefer, D.C; Vogt, R.C. 2006. Diet of *Podocnemis unifilis* (Testudines, Podocnemididae) during the dry season in the Mamirauá Sustainable Development Reserve, Amazonas, Brazil. *Chelonian Conservation and Biology,* 5: 312-317.

Barcelos, G.R.M.; Angeli, J.P.F.; Serpeloni, J.M.; Grotto, D.; Rocha, B.A.; Bastos, J.K.; *et al*. 2011. Quercetin protects human-derived liver cells against mercury-induced DNA-damage and alterations of the redox status. *Mutation Research - Genetic Toxicology and Environmental Mutagenesis,* 726: 109–115.

Bastos, W.R.; Dórea, J.G.; Bernardi, J.V.E.; Lauthartte, L.C.; Mussy, M.H.; Lacerda, L.D.; *et al*. 2015. Mercury in fish of the Madeira river (temporal and spatial assessment), Brazilian Amazon. *Environmental Research,* 140: 191–197.

Batistella, A.M.; Vogt, R.C. 2008. Nesting ecology of *Podocnemis erythrocephala* (Testudines, Podocnemididae) of the Rio Negro, Amazonas, Brazil. *Chelonian Conservation and Biology,* 7: 12–20.

Brabo, E.S.; Silva, A.P.; Faial, K.R.F.; Mascarenhas, A.F.S.; Santos, E.C.O.; Jesus, I.M.; *et al.* 2003. Assessment of mercury levels in soils, waters, bottom sediments and fishes of Acre State in Brazilian Amazon. *Water, Air, and Soil Pollution*, 147: 61–77.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry,* 72: 248–254.

Burger, J.; Gibbons, J.W. 1998. Trace elements in egg contents and egg shells of slider turtles (*Trachemys scripta*) from the Savannah River Site. *Archives of Environmental Contamination and Toxicology,* 34: 382–386.

Camacho, M.; Boada, L.D.; Orós, J.; Calabuig, P.; Zumbado, M.; Luzardo, O.P. 2012. Comparative study of polycyclic aromatic hydrocarbons (PAHs) in plasma of Eastern Atlantic juvenile and adult nesting loggerhead sea turtles (*Caretta caretta*). *Marine Pollution Bulletin,* 64: 1974–1980.

Camacho, M.; Orós, J.; Boa,da, L.D.; Zaccaroni, A.; Silvi, M.; Formigaro, C.; *et al*. 2013. Potential adverse effects of inorganic pollutants on clinical parameters of loggerhead sea turtles (*Caretta caretta*): Results from a nesting colony from Cape Verde, West Africa. *Marine Environmental Research,* 92: 15–22.

Carrasco, K.R.; Tilbury, K.L.; Myers, M.S. 1990. Assessment of the piscine micronucleus test as an *in situ* biological indicator of chemical contaminant effects. *Canadian Journal of Fisheries and Aquatic Sciences,* 47: 2123–2136.

Casini, S.; Caliani, I.; Giannetti, M.; Marsili, L.; Maltese, S.; Coppola, D.; *et al*. 2018. First ecotoxicological assessment of *Caretta caretta* (Linnaeus, 1758) in the Mediterranean Sea using an integrated nondestructive protocol. *Science of the Total Environment,* 631–632: 1221–1233.

Castro, N.S.S. de; Braga, C.M.; Trindade, P.A. de A.; Giarrizzo, T.; Lima, M. de O. 2016. Mercury in fish and sediment of Purus River, Acre State, Amazon. *Cadernos de Saúde Coletiva,* 24: 294–300.

CFBio. 2012. Conselho Federal de Biologia, Portaria # 148/2012. Procedimentos de captura, contenção, marcação e coleta de animais vertebrados. (http://www.crbio02.gov.br/Noticias.aspx?n=71&t=PORTARIA%20CFBio%20N%C2%BA%20148/2012).

CFMV. 2002. Conselho Federal de Medicina Veterinária, Resolução # 714/2002. Procedimentos e métodos de eutanásia em animais. (www.iz.sp.gov.br/img_editor/docs/res_714.pdf).

Chan, H.M.; Scheuhammer, A.M.; Ferran, A.; Loupelle, C.; Holloway, J.; Weech, S. 2003. Impacts of mercury on freshwater fish-eating wildlife and humans. *Human and Ecological Risk Assessment,* 9: 867–883.

Costantini, D.; Dell'Omo, G.; De Filippis, S.P.; Marquez, C.; Snell, H.L.; Snell, H.M.; *et al*. 2009. Temporal and spatial covariation of gender and oxidative stress in the Galápagos land iguana *Conolophus subcristatus*. *Physiological and Biochemical Zoology,* 82: 430–437.

Crott, J.; Fenech, M. 2001. Preliminary study of the genotoxic potential of homocysteine in human lymphocytes *in vitro*. *Mutagenesis,* 16: 213–217.

Dalle-Donne, I.; Rossi, R.; Giustarini, D.; Milzani, A.; Colombo, R. 2003. Protein carbonyl groups as biomarkers of oxidative stress. *Clinica Chimica Acta,* 329: 23–38.

da Silva, C.C.; Klein, R.D.; Barcarolli, I.F.; Bianchini, A. 2016. Metal contamination as a possible etiology of fibropapillomatosis in juvenile female green sea turtles *Chelonia mydas* from the southern Atlantic Ocean. *Aquatic Toxicology,* 170: 42–51.

Erickson, J.; Farias, I.P.; Zuanon, J. 2020a. The life history of the yellow-spotted amazon river turtle (*Podocnemis unifilis*) as told from the nests. *Salamandra,* 56: 296–308.

Erickson, J.; Fagundes, C.K.; Magalhães, M. dos S.; Dias, L.C.; Vogt, R.C.; Farias, I.P.; *et al*. 2020b. Natural nests incubated in two different soil types lead to an overall balanced sex ratio in *Podocnemis unifilis* hatchlings on the lower Purus river, Brazil. *Salamandra,* 56: 309–316.

Evans, M.D.; Dizdaroglu, M.; Cooke, M.S. 2004. *Oxidative DNA damage and disease: Induction, repair and significance*. *Mutation Research*, 567: 1–61

Fadini, P.S.; Jardim, W.F. 2001. Is the Negro River Basin (Amazon) impacted by naturally occurring mercury? *Science of the Total Environment,* 275: 71–82.

Fagundes, C.K.; Vogt, R.C.; De Marco Júnior, P. 2016. Testing the efficiency of protected areas in the Amazon for conserving freshwater turtles. *Diversity and Distributions,* 22: 123–135.

Finlayson, K.A.; Leusch, F.D.L.; van de Merwe, J.P. 2019. Primary green turtle (*Chelonia mydas*) skin fibroblasts as an in vitro model for assessing genotoxicity and oxidative stress. *Aquatic Toxicology,* 207: 13–18.

Frossard, A.; Ferreira, P.D.; Carneiro, M.T.W.D.; Heringer, O.A.; Endringer, D.C.; Gomes, L.C. 2013. Effect of dietary cadmium on fitness, growth, genotoxicity and accumulation in the Yellow-spotted River Turtle, *Podocnemis unifilis*. *Aquatic Toxicology,* 140–141: 239–241.

Furtado-Filho, O.V.; Polcheira, C.; Machado, D.P.; Mourão, G.; Hermes-Lima, M. 2007. Selected oxidative stress markers in a South American crocodilian species. *Comparative Biochemistry and Physiology - Part C Toxicology and Pharmacology,* 146: 241–254.

Haller, É.C.P.; Rodrigues, M.T. 2006. Reproductive biology of the six-tubercled Amazon River Turtle *Podocnemis sextuberculata* (Testudines: Podocnemididae), in the Biological Reserve of Rio Trombetas, Pará, Brazil. *Chelonian Conservation and Biology,* 5: 280–284.

IUCN. 2017. International Union for the Conservation of Nature. *The IUCN Red List of Threatened Species. Podocnemis unifilis*. (https://www.iucnredlist.org/species/17825/97397562).

Kemenes, A.; Pezzuti, J.C.B. 2007. Estimate of Trade Traffic of *Podocnemis* (Testudines, Podocnemididae) from the Middle Purus River, Amazonas, Brazil. *Chelonian Conservation and Biology,* 6: 259–262.

Labrada-Martagón, V.; Tenorio Rodríguez, P.A.; Méndez-Rodríguez, L.C.; Zenteno-Savín, T. 2011. Oxidative stress indicators and chemical contaminants in East Pacific green turtles (*Chelonia mydas*) inhabiting two foraging coastal lagoons in the Baja California peninsula. *Comparative Biochemistry and Physiology - C Toxicology and Pharmacology,* 154: 65–75.

Lara, N.R.F.; Marques, T.S.; Montelo, K.M.; de Ataídes, A.G.; Verdade, L.M.; Malvásio, A.; *et al*. 2012. A trophic study of the sympatric amazonian freshwater turtles *Podocnemis unifilis* and *Podocnemis expansa* (Testudines, Podocnemidae) using carbon and nitrogen stable isotope analyses. *Canadian Journal of Zoology,* 90: 1394–1401.

Levine, B.R.L.; Williams, J.O.Y.A.; Stadtman, E.R. 1994. Carbonyl assays for determination of oxidatively modified proteins. *Methods in Enzymology,* 233: 346–357.

Li, H.; Bao, L.; Deng, S; Liu, L.; Cheng, J.; Chen, X.; et al. 2021. Invertigation of *Proteus vulgaris* and *Elizabethkingia meningoseptica* invasion on muscle oxidative stress and autophagy in Chinese soft-shelled turtle (*Pelodiscus sinensis*). *Scientific Reports*, 11: 1-12.

Monteiro, D.A.; Rantin, F.T.; Kalinin, A.L. 2010. Inorganic mercury exposure: Toxicological effects, oxidative stress biomarkers and bioaccumulation in the tropical freshwater fish matrinxã, *Brycon amazonicus* (Spix and Agassiz, 1829). *Ecotoxicology,* 19: 105–123.

Ohkawa, H.; Ohishi, N.; Yagi, K. 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry,* 95: 351–358.

Páez, V.P.; Correa, J.C.; Cano, A.M.; Bock, B.C. 2009. A comparison of maternal and temperature effects on sex, size, and growth of hatchlings of the Magdalena River turtle (*Podocnemis lewyana*) incubated under field and controlled laboratory conditions. *Copeia,* 2009: 698–704.

Pantoja-Lima, J.; Aride, P.H.R.; de Oliveira, A.T.; Félix-Silva, D.; Pezzuti, J.C.B.; Rebêlo, G.H. 2014. Chain of commercialization of *Podocnemis* spp. turtles (Testudines: Podocnemididae) in the Purus River, Amazon basin, Brazil: Current status and perspectives. *Journal of Ethnobiology and Ethnomedicine,* 10: 1–10.

Pignati, M.T.; Pezzuti, J.C.B.; Souza, L.C. de; Lima, M. de O.; Pignati, W.A.; Mendes, R. de A. 2018a. Assessment of mercury concentration in turtles (*Podocnemis unifilis*) in the xingu river basin, brazil. *International Journal of Environmental Research and Public Health,* 15: 17–21.

R CoreTeam. 2017. R: *A Language and Environment for Statistical Computing*. Vol. 2.

Roulet, M.; Guimarães, J.R..; Lucotte, M. 2001. Methylmercury production and accumulation in sediments and soils of an Amazonian floodplain: effect of seasonal inundation. *Water, Air & Soil Pollution,* 128: 41–61.

Rueda-Almonacid, J.V.; Carr, J.L.; Mittermeier, R.A.; Rodriguez-Mahecha, J.V.; Mast, R.B.; Vogt, R.C.; *et al.* 2007. *Las Tortugas y Los Cocodrilianos de Los Paises Andinos Del Trópico.* 6th ed. Conservación Internacional/ Editorial Panamericana, Bogotá, 538p.

Schlenk, D.; Handy, R.; Steinert, S.; Depledge, M.H.; Benson, W. 2008. Biomarkers. In: Di Giulio, R.; Hinton, D. (Eds.), *The Toxicology of Fishes*, 2nd ed. CRC Press, United States, p.684–687.

Schneider, L.; Belger, L.; Burger, J.; Vogt, R.C. 2009. Mercury bioacumulation in four tissues of *Podocnemis erythrocephala* (Podocnemididae: Testudines) as a function of water parameters. *Science of the Total Environment,* 407: 1048–1054.

Schneider, L.; Belger, L.; Burger, J.; Vogt, R.C.; Ferrara, C.R. 2010. Mercury levels in muscle of six species of turtles eaten by people along the Rio Negro of the Amazon basin. *Archives of Environmental Contamination and Toxicology,* 58: 444–450.

Schneider, L.; Belger, L.; Burger, J.; Vogt, R.C.; Jeitner, C.; Peleja, J.R.P. 2011. Assessment of non-invasive techniques for

monitoring mercury concentrations in species of Amazon turtles. *Toxicological and Environmental Chemistry,* 93: 238–250.

Schneider, L.; Eggins, S.; Maher, W.; Vogt, R.C.; Krikowa, F.; Kinsley, L.; *et al*. 2015. An evaluation of the use of reptile dermal scutes as a non-invasive method to monitor mercury concentrations in the environment. *Chemosphere,* 119: 163–170.

Shimizu, N.; Itoh, N.; Utiyama, H.; Wahl, G.M. 1998. Selective entrapment of extrachromosomally amplified DNA by Nuclear Budding and Micronucleation during S Phase. *The Journal of Cell Biology,* 140: 1307–1320.

Sinaei, M. 2016. Metallothionein biosynthesis as a detoxification mechanism of heavy metals (Hg, Cd, Pb, Cu, Zn) in green sea turtles (*Chelonia mydas*). *Journal of the Persian Gulf,* 7: 61–70.

Souza-Araujo, J.; Giarrizzo, T.; Lima, M. 2015. Mercury concentration in different tissues of *Podocnemis unifilis* (Troschel, 1848) (Podocnemididae: Testudines) from the lower Xingu River – Amazonian, Brazil. *Brazilian Journal of Biology,* 75: 106–111.

Stark, G. 2005. Functional consequences of oxidative membrane damage. *Journal of Membrane Biology*, 205: 1–16.

Valdivia, P.A.; Zenteno-Savín, T.; Gardner, S.C.; Alonso Aguirre, A. 2007. Basic oxidative stress metabolites in eastern Pacific green turtles (*Chelonia mydas agassizii*). *Comparative Biochemistry and Physiology - Part C Toxicology and Pharmacology,* 146: 111–117.

Vanzolini, P.E. 2003. On clutch size and hatching success of the South American turtles. *Anais da Academia Brasileira de Ciencias,* 75: 415–430.

Viarengo, A.; Ponzano, E.; Dondero, F.; Fabbri, R. 1997. A simple spectrophotometric method for metallothionein evaluation in marine organisms: An application to Mediterranean and Antarctic molluscs. *Marine Environmental Research,* 44: 69–84.

Vogt, R.C. 2004. Tartaruga de manchas-amarelas no Rio Amazonas (*Podocnemis unifilis* Troschel, 1848) (Pelomedusidae). In: Cintra, R. (Ed.). *História Narutal, Ecologia e Conservação de Algumas Espécies de Plantas e Animais da Amazônia*, *v. 2*. Editora INPA, Manaus, p.229–231.

Waldez, F.; Gama e Adário, L.; Marioni, B.; Rossoni, F.; Erickson, J. 2013. Monitoramento participativo da caça de quelônios (Podocnemididae) por comunitários ribeirinhos no baixo Rio Purus e proteção de sítios de desova na RDS Piagaçu-Purus, Brasil. *Revista Colombiana de Ciencia Animal,* 5: 4–23.

Weissgerber, T.L.; Milic, N.M.; Winham, S.J.; Garovic, V.D. 2015. Beyond bar and line graphs: Time for a new data presentation paradigm. *PLoS Biology,* 13: e1002128.

Willmore, W.G.; Storey, K.B. 1997. Antioxidant systems and anoxia tolerance in a freshwater turtle *Trachemys scripta elegans*. *Molecular and Cellular Biochemistry,* 170: 177–185.

WHO. 2008. World Health Organization. Inter-Organization Programme for the Sound Management of Chemicals, # 35, Guidance for Identifying Populations At Risk From Mercury Exposure. (https://www.who.int/publications/m/item/guidance-for-identifying-populations-at-risk-from-mercury-exposure).

Yan, L.J.; Forster, M.J. 2011. Chemical probes for analysis of carbonylated proteins: A review. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences,* 879: 1308–1315.

Yasui, M.; Kamoshita, N.; Nishimura, T.; Honma, M. 2015. Mechanism of induction of binucleated cells by multiwalled carbon nanotubes as revealed by live-cell imaging analysis. *Genes and Environment,* 37: 1–6.

Zapata, L.M.; Bock, B.C.; Orozco, L.Y.; Palacio, J.A. 2016. Application of the micronucleus test and comet assay in *Trachemys callirostris* erythrocytes as a model for in situ genotoxic monitoring. *Ecotoxicology and Environmental Safety,* 127: 108–116.

Zar, J.H. 1996. *Biostatistical Analysis*. 5th ed. Pearson Prentice Hall, New Jersey, 2266p.

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