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RISK ASSESSMENT FOR THE CONSUMPTION OF THE FRESHWATER TURTLE *Podocnemis unifilis* WITH Hg IN AN INDIGENOUS POPULATION LIVING CLOSE TO MINING AREA IN THE BRAZILIAN AMAZON

Evaluación de riesgos para el consumo de la tortuga de agua dulce *Podocnemis unifilis* con Hg en una población indígena que vive cerca de zona minera en la Amazonia brasileña

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Key words: Amazon, tracajá, mercury, Baú River, Kayapó, biomagnification.

ABSTRACT

Podocnemis unifilis is an important source of protein for indigenous communities in the Brazilian Amazon. However, contaminants associated to the mining activities can be affecting this species and, in turn, its consumers. The objective of this study was to evaluate the risk of Hg contamination that the Kayapó indigenous people are exposed to through the consumption of *P. unifilis* in a region affected by mining in the Baú river basin, municipality of Altamira-PA, Brazilian Amazon. Muscle (edible) samples of the collected specimens were digested in a solution of concentrated nitric acid with hydrogen peroxide using a microwave digestion system. Inductively coupled plasma emission spectrometry with hydrogen generation was used to quantify HgT. The health risk assessment was performed according to the methodology proposed by the U.S. Environmental Protection Agency. The muscle of *P. unifilis* presented an average concentration of 0.022 (µg/g wet weight) HgT, a value within the allowed level for the dietary pattern. The risk assessment for human health shows that the consumption of *P. unifilis* presents low risks of intoxication by Hg for the indigenous people. The results show a reduced risk of exposure to Hg due to the consumption of *P. unifilis* by indigenous people. However, if mining activity continues to spread across the region, since Hg biomagnifies in the trophic chain and can represent a serious risk for human health and biota.

Palabras clave: Amazonia, tracajá, mercurio, r*ío Baú, Kayapó, biomagnificación.*

RESUMEN

Podocnemis unifilis es una importante fuente de proteínas para las comunidades indígenas de la Amazonía brasileña. Sin embargo, los contaminantes asociados con la actividad minera pueden estar afectando a esta especie y, a su vez, a sus consumidores. El objetivo de este estudio fue evaluar el riesgo de contaminación por Hg al que están expuestos los indígenas Kayapó por el consumo de *P. unifilis* en una región afectada por la minería en la cuenca del río Baú, municipio de Altamira-PA, Amazonía brasileña. Las muestras de músculo (comestible) de los especímenes recolectados fueron digeridas en una solución de ácido nítrico concentrado con peróxido de hidrógeno utilizando un sistema de digestión por microondas. Se utilizó espectrometría de emisión de plasma de acoplamiento inductivo con generación de hidrógeno para cuantificar el HgT. La evaluación de riesgos para la salud se realizó de acuerdo con la metodología propuesta por la Agencia de Protección Ambiental de los Estados Unidos. El músculo de *P. unifilis* presentó una concentración promedio de 0.022 (µg/g peso húmedo) de HgT, valor dentro de lo permitido por el patrón dietético. La evaluación de riesgos para la salud humana muestra que el consumo de *P. unifilis* presenta riesgo bajo de intoxicación por Hg para los indígenas. Los resultados muestran un riesgo reducido de exposición a Hg debido al consumo de *P. unifilis* por parte de los pueblos indígenas. Sin embargo, si la actividad minera continúa extendiéndose por la región, podría haber un aumento de estos riesgos en el corto plazo, ya que el Hg se biomagnifica en la cadena trófica y puede llegar a representar un serio riesgo para la salud humana y biota.

INTRODUCTION

Natural reservoirs have been depositaries of a variety of by-products from human activities. The presence of potentially toxic elements is responsible for adverse effects on the environment, with repercussions on the economy and public health (Zhou et al. 2020). Mercury (Hg) is a toxic element of great importance due to its capacity for bioaccumulation and biomagnification in the environment, being associated with diseases such as Minamata Syndrome (Balali-Mood et al. 2021). Once released into the aquatic environment, Hg undergoes a series of chemical reactions. Hg exists primarily as elemental (Hg^0) and reactive (Hg^{2+}) Hg, depending on ambient conditions. The emitted Hg can be deposited in aqueous environments by wet and dry depositions, and some can be re-emitted to the atmosphere. The Hg species deposited, mainly the reactive species (Hg^{2+}), can combine with various organic compounds in water and sediments by biotic reactions promoted by sulfur-reducing bacteria and abiotic reactions mediated by solar photolysis, resulting in the conversion to organic Hg, such as methylmercury (MeHg), according to Kim and Zoh (2012). Methylmercury, in turn, is the most concerning form due to its high toxicity and its ability to penetrate biological systems, mainly through the food chain (Gibb and O'Leary 2014).

A factor of great economic and social importance in the Amazon is related to the mining activity; however, this practice generates tons of ore tailings rich in metals with worrying toxicity (Araújo et al. 2022). Contamination by Hg may occur due to the high presence of mining activity in the region (Meneses et al. 2022). In the Amazon, in the last 20 years, there has been a significant increase in the presence of illegal gold mining, establishing socio-environmental problems in the region (Ramos et al. 2020).

The main environmental threats caused by gold mining include Hg intoxication, deforestation, and quantitative and qualitative changes in the composition of flora, fauna, and the entire ecosystem that, consequently, directly and indirectly, affect the nearby population, such as indigenous communities (Adesipo et al. 2020). In the human body, the presence of Hg can lead to lung and kidney damage, as well as neurological disorders that result in sensory alterations. Additionally, Hg can cause constriction of the visual field, hearing loss, ataxia (motor coordination difficulties), and dysarthria (difficulty in articulating words) (Yang et al. 2020).

Podocnemis unifilis (tracajá) is a freshwater turtle that can be found along the rivers of the Amazon, and like fish, it acts as a source of protein for indigenous, riverine, and quilombo communities (Pignati et al. 2018). In the Brazilian Amazon, species of tortoises such as *P. unifilis* are source of food widely consumed by the populations of the region (Frossard et al. 2021). This observation is corroborated by a comprehensive study in the region, which emphasizes the frequent presence of the species *P. unifilis* and other significant species, such as *Podocnemis expansa*, *Chelonoidis carbonaria*, and *Geochelone denticulata*, on local menus.

Estimates indicate that the region annually consumes around 1.7 million chelonians, thus highlighting the vital relevance of these animals in the regional diet and culture (Santelli 2021). The intoxication of indigenous people occurs mainly through the consumption of species of fish and tracajás affected by the phenomenon of bioaccumulation and biomagnification of Hg from mining (Cleary et al. 2021). Conversely, *P. unifilis* can act as a biomarker in monitoring environmental contamination in the region (Borges et al. 2022).

Considering that prolonged consumption of the *P. unifilis* species can expose indigenous communities to Hg intoxication, it is essential and necessary to quantify Hg concentrations and assess the risk factor to understand the potential threat to these individuals (Arcega-Cabrera et al. 2017) when consumed. Under the present scenario, this work was developed to quantify Hg concentrations and evaluate the risk factor to human health resulting from consuming *P. unifilis* species found in an indigenous conservation unit in Altamira-PA, Brazil. The study's hypothesis posits that when turtles inhabit an area influenced by mining activities, Hg levels should be high or moderate compared to those in an untouched location.

MATERIALS AND METHODS

Study area and sampling sites

The study area is part of an indigenous environmental protection reserve that is threatened by the presence of illegal mining. The study included ten sampling points located along the Baú River. This river is located in the municipality of Altamira, in the state of Pará, and indigenous people depend on it as a source of food, including the consumption of *P. unifilis*, a widely consumed species in the region. **Figure 1** shows the collection area, covering an extension of the Báu River of approximately 9 km (GEP 2022).

Collection and treatment of samples

With the help of teams from the Federal Public Ministry in Altamira, indigenous people, the KABU Institute, the Tapajós Special Indigenous Health District, and the National Indian Foundation (FUNAI), ten specimens of the species *P. unifilis* were collected. The collection followed the protocols guided by the National Council for the Control of Animal Experimentation (CONCEA 2016).

The collected specimens presented lengths ranging from 19.0 to 31.5 cm and weights from 675 to 3515 g. The captured individuals were identified and weighed, and measurements of width and length (biometrics) were performed. Among the 10 captured tortoises, seven were males and three were females, all in the stage of juvenile maturity. With the aid of a scalpel, approximately 100 g of material was filleted, removing the upper white portion of the

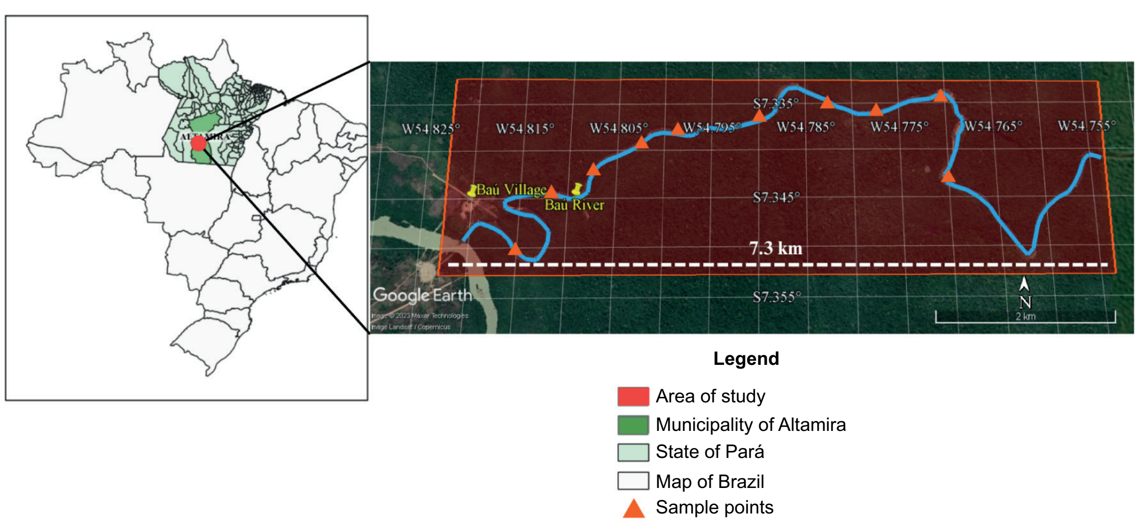


Fig. 1. Study area location map.

muscle. Subsequently, the samples were stored in zip lock bags and packed in with ice for transport. In the laboratory, the samples were ground and homogenized, and then digested. The digestion was made using aliquots of ~500 mg of the sample that was added to the digestion vessel, together with 8 mL of 65% nitric acid (Merck) and 2 mL of 35% hydrogen peroxide (Merck). The mixture was stirred and after 10 minutes, the container was closed and placed in the DAP-60+ microwave oven (Berghof, Germany). The procedure followed the methodology described by Berghof (2013).

Metal analysis and analytical quality

The technique of inductively coupled plasma optical emission spectrometry with hydride generation (VG-ICP OES) was used to quantify (HgT). The instrument used was the iCAP 7000 Series ICP-OES (Thermo Fisher Scientific, Waltham, Massachusetts, USA) and the procedure adopted followed the methodology according to Bartsch (2016). Using a certified standard solution (Merck ICP Multi-element, Darmstadt, Germany). The analytical curves were constructed from 12 blank measurements. Detection limits (LOD) of 0.00001 µg/g of and quantification limits (LOQ) of 0.00003 µg/g were obtained. National Research Council DORM-2 (Certified Reference Material for Dogfish Muscle) was used for trace metal analysis. The precision analysis was expressed in terms of relative standard deviation (RSD), which was equal to 2.1%, and a recovery rate of 99.6%.

Statistical treatment

The results were treated by calculating the mean, standard deviation, box plots, and Pearson's correlation. The programs RStudio (version 1.4.1717) and OriginPro 2022 (SR1 9.9.0.225) performed data analysis on the 64-bit Windows platform. The results were compared with the reference of non-carnivorous fish according to ANVISA (2022) legislation RDC Nº 722 - Ministry of Health of July 01, 2022, given that *P. unifilis* does not have its own legislation.

Risk assessment for consumption of *Podocnemis unifilis*

The hazard quotient (HQ) calculation proposed by the U.S. Environmental Protection Agency (USEPA 1989), according to equation 1, was used to assess the risk to indigenous people from prolonged consumption of muscle of *P. unifilis*. The ratio (HQ) indicates whether there are deleterious risks associated with the human being, but without the risk of presenting mutational or carcinogenic genetic problems (USEPA 1989). There are risks for HQ values > 1 and low risks for HQ ≤ 1.

$$HQ = EDI / RfD \tag{1}$$

Where EDI is the estimated daily intake of contaminated muscle tissue (µg/kg/day); RfD is the reference dose (µg/kg/day). The determination of estimated daily intake (EDI) was determined by equation 2. The formula used to estimate daily intake was adapted according to ATSDR (2016).

$$EDI = C\ x\ \frac{IR\ x\ EF\ x\ ED}{BW\ x\ AT} \tag{2}$$

Where C represents the concentration of the element in muscle tissue (µg/g wet weight); IR is the food intake rate, being 9.1 g/day for adults and 3 g/day for children, according to Targino et al. (2023); EF is the exposure frequency, considering 365 days/year; ED is the average duration of exposure, corresponding to 4 years; BW is the average body weight of the individual during exposure, being 70 kg for 30-year-old men and 24.5 kg for 7-year-old children; AT is the average exposure period, equivalent to 1460 days. In determining the estimated daily intake (EDI), the variables were adapted based on data from the Brazilian population (IBGE 2009, Silva and Santos 2016). The average exposure period considered was from November 2018 to November 2022. For Hg, the reference value (RfD) used was based on the value available in USEPA (2000), which is 0.1 µg k/g day. This value is used as a reference for chronic exposure, specifically for Methyl-Hg.

RESULTS AND DISCUSSION

Mercury results in muscle of *Podocnemis unifilis*

The results of Hg quantification in muscle tissue of *P. unifilis* specimens collected in the Baú River are shown in **figure 2**. It is observed that the concentrations varied from < LOQ (0.00003 µg/g) to 0.152 µg/g between the sampled specimens. In comparison with the legislation adopted for non-carnivorous fish, clearly, the concentrations determined are within the limit allowed by standard RDC Nº 722/2022, which provides for the maximum tolerated limits (LMT) of contaminants in food, the general principles for their establishment and the methods of analysis for conformity assessment purposes.

Although the specimens of *P. unifilis* live in an area influenced by illegal mining, they have average concentrations of HgT 22.73 times below the permitted

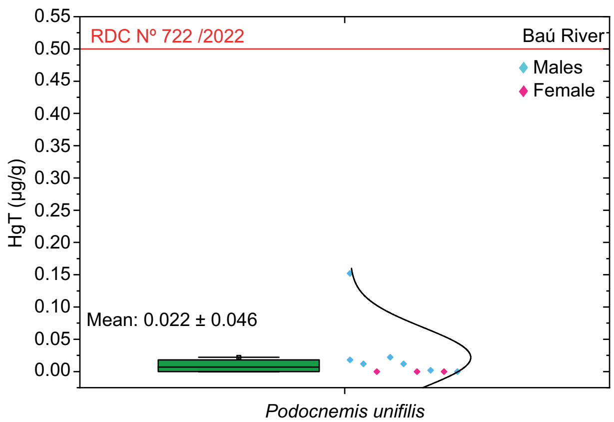
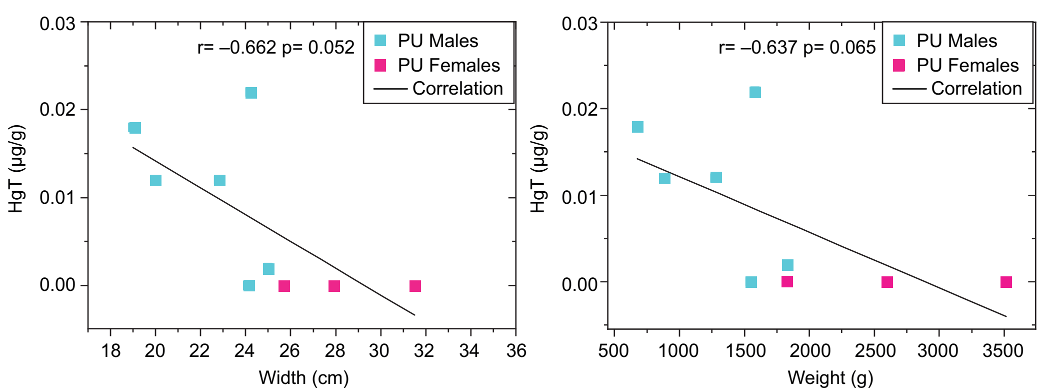


Fig. 2. Box plots of HgT concentration (µg/g wet weight) in the muscle of the turtle *P. unifilis*. ANVISA (2021): 0.5 µg/g of Hg; LOQ (µg/g): 0.00003 for Hg.

limit. These concentrations can be explained by the fact that the species is essentially herbivorous and thus occupies lower trophic levels in the food chain (Bentley and Soebandrio 2017, Silva et al. 2021). Thus, the capture and absorption of the metal is low. However, although the values are low, illegal artisanal mining can, at any given moment, release significant amounts of HgT and trigger the availability of this element in such a form that the turtle accumulates it significantly and, therefore, causes a health problem in the community that consumes this vital source of protein.

The application of descriptive statistics in **figure 3** demonstrates that the variables of length, width, and weight were not factors that contributed to higher levels of Hg in the specimens. This statement is evidenced by the values of Pearson's correlation coefficient (r) being further away from the extremes (1 and –1). Therefore, there is no direct relationship between the variables. However, male individuals show higher levels of Hg in correlation with length, width, and weight variables. This evidence may be related to Hg deposition in the eggs by females, or possible differences in their trophic ecology (Mills et al. 2022).

The Amazon basin is a region where illegal mining has a significant influence. For instance, mining on indigenous lands in the Legal Amazon has seen an impressive growth of 1217% in the last 35 years, with the occupied area by this activity expanding from 7.45 square kilometers (km²) in 1985 to 102.16 km^2 in 2020. It is essential to highlight that gold extraction accounts for 99.5% of this type of mining, and the majority (95%) of these illegal mining areas are concentrated in three indigenous lands: Kayapó, Munduruku, and Yanomami (Mataveli et al. 2022).



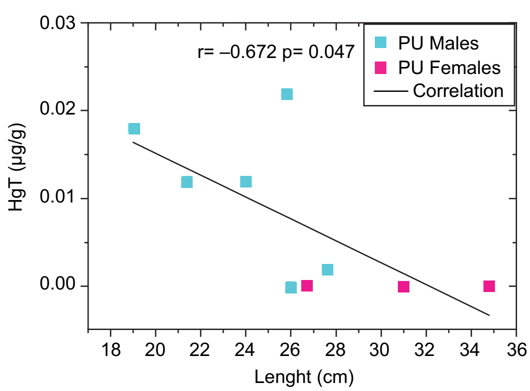


Fig. 3. Pearson's linear correlation between Hg concentrations and length, width and weight values. Legend. PU: *Podocnemis unifilis*; significance at level p = 0.05.

TABLE I. Mean concentration of Hg in the muscle of the specimens *Podocnemis unifilis*, reported in studies in the Amazon region.

Country	HgT (µg/g)	Location	References
Brazil	0.035	Negro River basin	Schneider et al. (2010)
Brazil	0.040	Negro River basin	Schneider et al. (2011)
Brazil	0.013	Purus River basin	Schneider et al. (2015)
Brazil	0.015	Xingu River basin	Souza-Araujo et al. (2015)
Brazil	0.025	Xingu River basin	Pignati et al. (2018)
Brazil	0.011	Purus River basin	Borges et al. (2022)
Brazil	0.022	Baú River basin	Current study

NA: Not available.

However, despite the increase in illegal mining in the region, studies conducted by other researchers have shown that, so far, these activities have had a minimal significant influence in terms of contamination of chelonian (turtle) in the region and elsewhere. These studies demonstrate that these species exhibit low levels of Hg in their bodies, as evidenced in **table I**.

When comparing data on the average concentration of Hg in the muscle of *P. unifilis* reported in different studies in the Amazon region, significant variations in percentage terms are observed. For example, in the Negro River basin, previous studies reported Hg concentrations of 0.035 and 0.040 µg/g, which represent values 59% and 82% higher, respectively, compared to the current study. In the Purus River basin, concentrations of 0.013 and 0.011 µg/g were found, corresponding to values 41% and 50% lower, respectively, compared to the current study. In the Xingu River basin, concentrations of 0.015 and 0.025 µg/g were detected, representing a value 32% lower and 14% higher, respectively, compared to the current study.

These percentage results highlight the differences in Hg concentrations among the various river basins in the Amazon region. It is crucial to emphasize that these variations in HgT concentration are influenced by intense human activities and more pronounced and specific contamination sources in each region (Zhang et al. 2019). The lower levels of Hg found in the Purus River basin indicate a low gold mining activity in that area. On the other hand, the levels observed in the Negro, Xingu, and Baú River basins indicate a continued need for monitoring and the implementation of practical actions to reduce Hg emissions in the environment.

Although some studies with the species *P. unifilis* have shown low concentrations of Hg in their muscle tissue, it is essential to emphasize the need to monitor the levels of this metal in larger samples and on broader spatial and temporal scales since the species exhibits a generalist behavior and phenotypic plasticity that allow it to respond to variable environmental conditions (Borges et al. 2022).

The issue of metal contamination in freshwater turtle species have been studied beyond the Amazon region. Ortiz-Santaliestra et al. (2019) revealed significantly high Hg levels in the blood (8.83 µg/g) of *Mauremys leprosa* specimens, due to the influence of mining activities in the south-central region of Spain. Furthermore, other anthropogenic activities such as industrial processes also contribute to Hg contamination, as evidenced in studies conducted by Slimani et al. (2018) and Van Dyke et al. (2017). In these studies, it was observed that *M. leprosa* and *Chelydra serpentina* specimens present average levels of Hg in their blood of 5 and 0.1 µg/g, respectively.

Toxicological risk assessment for consumption of *Podocnemis unifilis*

The risk quotient (HQ) calculated to assess the potential health risks to indigenous people from Hg exposure is presented in **figure 4**. It was found that for Hg, the HQ has higher values for an adult man. For example, the adult HQ is 1.07 times greater than the value found for a child in relation to Hg. This is explained by the higher consumption of *P. unifilis* by adults compared to children, however, when comparing the determined HQ values, it is noted that all values are below the reference value designated as risk value ($HQ > 1$). In this way, the consumption of *P. unifilis* presents little risk of poisoning the indigenous people, who frequently feed on them.

Finally, the continued mining operation in the area may result in a significant increase in Hg concentrations in the coming years. This can lead to the bioaccumulation of Hg in individuals of the species *Podocnemis unifilis*, making it a source of intoxication

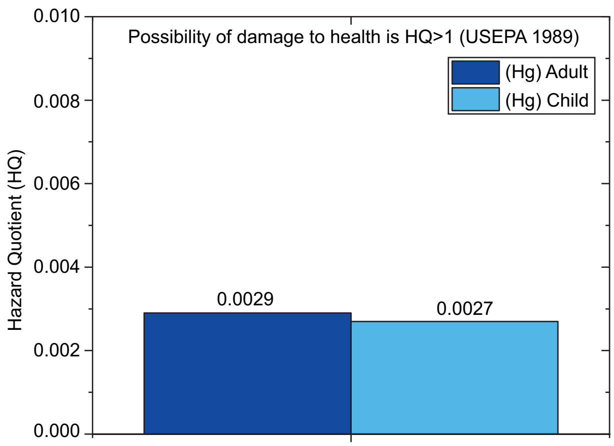


Fig. 4. Hazard quotient (HQ) values for indigenous people.

for indigenous populations in the region. Another factor that may be involved is the fact that the species has a long life (on average 90 years), giving it a unique opportunity to bioaccumulate Hg in its organism (Warwick et al. 2013).

CONCLUSION

The present study revealed that, despite illegal mining in the region, the specimens of *Podocnemis unifilis* collected in the Baú River present Hg concentrations within the permitted range, according to ANVISA (2021). This evidence is attributed to the herbivorous lifestyle of *P. unifilis*, which results in lower capture and accumulation of the contaminant by the species. The risk assessment for the consumption of *P. unifilis* reveals low risks for Hg to cause adverse effects on the health of residents of indigenous communities that ingest the muscle of this turtle. However, the persistence of mining and the recurrence of mining spills in the area linked to the naturally long life of *P. unifilis* may allow the species to accumulate high levels of Hg in their bodies through bioaccumulation and biomagnification processes and, consequently, affect their consumers. Therefore, the study highlights the ongoing need to monitor and assess Hg levels in the region, mainly due to the persistence of gold mining.

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