Revista de Biología Tropical, ISSN: 2215-2075, Vol. 71(S4): e57188, diciembre 2023 (Publicado Nov. 01, 2023)

REVISTA DE Biología Tropical

https://doi.org/10.15517/rev.biol.trop..v71iS4.57188

First mercury and stable isotope assessment from an unusual mass stranding of rough-toothed dolphins (*Steno bredanensis*) (Artiodactyla: Delphinidae) in Azuero peninsula, Pacific coast of Panama

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Received 11-VIII-2022. Corrected 09-XII-2022. Accepted 26-V-2023.

ABSTRACT

Introduction: Small cetaceans are good bioindicators of environmental contamination; however, knowledge about their ecotoxicological status in Central America is scarce. In Panama, access to samples from wild populations to determine the ecotoxicological status of oceanic dolphins is limited; therefore, stranding events provide an alternative for obtaining samples. In April 2016, a rare mass stranding event occurred in the Azuero Peninsula (Pacific coast of Panama), where 60 rough-toothed dolphins (*Steno bredanensis*) stranded, including ten which died on the beach.

Objective: To assess total mercury (THg) concentrations, and $\delta^{13}C$ and $\delta^{15}N$ stable isotope values in rough-toothed dolphins for the first time in this region.

Methods: Nine skin samples were collected from adults, stored in 70 % ethanol, and posteriorly analyzed to determine THg concentrations and stable isotope values.

Results: THg concentrations ranged from 4 764 to 18 689 ng g^{-1} dry weight (dw) (mean = 12 841; SD = 5 083 ng g^{-1} dw), $\delta^{13}C$ values ranged between −16.8 and −15.2 ‰ (mean = −16.2; SD = 0.6 ‰), and $\delta^{15}N$ values ranged between 14.3 and 15.9 ‰ (mean = 15.0; SD = 0.5 ‰).

Conclusions: High THg concentrations reported for this species in the Azuero Peninsula are consistent with values reported for rough-toothed dolphins in other areas worldwide, such as the central-northern Rio de Janeiro State in Brazil and La Guajira in the Colombian Caribbean. Elevated mercury (Hg) concentrations may be related to the rough-toothed dolphin diet, which according to $\delta^{15}N$ values found here, appears to be based mainly on high trophic level prey that bioaccumulate more Hg in their tissues compared to lower trophic level organisms. However, additional dietary studies would be required to support these findings. Continuing monitoring of traditional dietary analysis, as well as contamination levels in fish and dolphins, is necessary to understand the dolphins' ecotoxicology in Panama.

Key words: *Steno bredanensis*; dolphins; cetaceans; contamination; heavy metals; ecotoxicology; Panama.

RESUMEN

Primera evaluación de mercurio e isótopos estables de delfines de dientes rugosos (*Steno bredanensis*) provenientes de un varamiento masivo inusual en la península de Azuero, Costa Pacífica de Panamá

Introducción: Los pequeños cetáceos son buenos bioindicadores de la contaminación ambiental; sin embargo, el conocimiento acerca de su estado ecotoxicológico en Centroamérica es escaso. En Panamá, el acceso a muestras para determinar el estado ecotoxicológico de delfines oceánicos es limitado; por lo tanto, los varamientos proveen una alternativa para obtener muestras. En abril de 2016, un raro evento de varamiento masivo ocurrió en la Península de Azuero (Pacífico panameño), en el cual 60 delfines de dientes rugosos (*Steno bredanensis*) vararon incluyendo diez que murieron en la playa.
Objetivo: Determinar los niveles de mercurio total (THg), e isótopos estables de $\delta^{13}C$ y $\delta^{15}N$ en los delfines de dientes rugosos por primera vez en la región.
Métodos: Nueve muestras de piel de adultos fueron colectadas, almacenadas en etanol al 70 %, y analizadas posteriormente para determinar THg e isótopos estables.
Resultados: Las concentraciones de THg variaron entre 4 764 y 18 689 ng g^{-1} de peso seco (dw) (promedio= 12 841; DE= 5 083 ng g^{-1} dw), los valores de $\delta^{13}C$ entre −16.8 y −15.2 ‰ (promedio= −16.2; DE= 0.6 ‰), y los de $\delta^{15}N$ entre 14.3 y 15.9 ‰ (promedio= 15.0; DE= 0.5 ‰).
Conclusiones: Los altos niveles de THg reportados para esta especie en la Península de Azuero son consistentes con los reportados en la piel de los delfines de dientes rugosos en otras áreas del mundo, como en el estado de Río de Janeiro en Brasil y La Guajira en el Caribe colombiano. Las altas concentraciones de mercurio (Hg) pueden estar relacionadas con la dieta de los delfines de dientes rugosos, la cual, de acuerdo a los valores de $\delta^{15}N$ encontrados aquí, parece estar basada en presas de alto nivel trófico que acumulan más Hg en sus tejidos. Sin embargo, estudios dietarios adicionales son requeridos para confirmar estos resultados. Un monitoreo continuo de la dieta usando análisis tradicionales, así como de los niveles de contaminación en peces y delfines, es necesario para entender la ecotoxicología de los delfines en Panamá.

Palabras clave: *Steno bredanensis;* delfines; cetáceos; contaminación; metales pesados; ecotoxicología; Panamá.

Nomenclature: SMT1: Supplementary material Table 1; SMF1: Supplementary material Figure 1.

INTRODUCTION

Mercury (Hg) is a global contaminant that is biomethylated in aquatic sediments by microorganisms (Alcala-Orozco et al., 2019; Wiener & Suchanek, 2008) from the inorganic form (Hg^{2+}) to the organic methylmercury (CH_3Hg^+; MeHg) form. The organic MeHg form is more toxic and bioaccumulates in aquatic organisms through an individual's life, causing deleterious effects (Bossart, 2011; Correa et al., 2014; Reif et al., 2015; Schwacke et al., 2002; Wiener et al., 2003). Top predators are particularly vulnerable to the harmful effects because Hg biomagnifies through aquatic trophic chains (Bosch et al., 2016).

Because of their role as top predators, dolphins tend to bioaccumulate high Hg levels in their tissues, so they can be bioindicators of contamination worldwide (e.g., Aubail et al., 2013; Barragán-Barrera, Luna-Acosta et al., 2019; Cáceres-Saez et al., 2015). However, in Central America little information is available on the toxicological status of dolphins. The few Hg assessments that have been conducted in this region have focused mainly on fish from the Pacific coast of Costa Rica and Nicaragua, examining Hg concentrations in four elasmobranchs, as well as 23 other freshwater and marine fishes (Elliot et al., 2015; Sandoval et al., 2015). Likewise, for feeding ecology studies based on stable isotopic data, one study has been conducted with demersal elasmobranchs in the Costa Rican Pacific basin (Espinoza et al., 2015). To date, the only published cetacean study in Central America which determined both total Hg (THg) and stable isotope measurements was conducted on common bottlenose dolphins *Tursiops truncatus* (Montagu, 1821) from the Bocas del Toro Archipelago in the Panamanian Caribbean (Barragán-Barrera, Luna-Acosta et al., 2019).

The lack of ecotoxicological studies focused on cetaceans in Panama is mainly due to the difficulty in obtaining tissue samples from wild populations, particularly for species with oceanic habits like the rough-toothed dolphin, *Steno bredanensis* (G. Cuvier in Lesson, 1828). This species is found mainly in oceanic waters in tropical latitudes (Jefferson, 2018), with some occurrences in nearshore waters of oceanic islands (Baird, 2016; Oremus et al., 2012). Access to tissue samples of oceanic species is challenging, particularly where no long-term marine mammal monitoring programs have been established. Therefore, stranding events provide a good alternative to obtaining samples from oceanic dolphins.

On the night of April 19th, 2016, a group of 60 rough-toothed dolphins was reported in a rare mass stranding event on the Pacific coast of Panama. This event occurred on Ostional beach (“Playa Ostional”), in the Tonosí district of the Azuero Peninsula, 340 kilometers southwest of Panama City (Fig. 1). The event was unusual because this species strands less frequently than other marine mammal species (Mackey et al., 2003), and most strandings in Panama have been reported as isolated individuals (May-Collado et al., 2017). In the early morning of April 20th, 2016, experienced marine biologists and veterinarians from the Environmental Ministry of Panama (MiAmbiente), the International Maritime University of Panama (UMIP by its acronym in Spanish), the Universidad de Panamá, the Aquatic Resources Authority (ARAP by its acronym in Spanish), Fundación Panacetacea (non-governmental organization), and local fishermen attended to the stranded animals. Ten of the 60 individuals died on the beach (Fig. 1), and the remaining animals were rescued and moved to deeper waters. According to their total lengths, nine of the ten deceased individuals were classified as adults (> 255 cm) and one as a calf (< 1m) (Mackey et al., 2003; Reeves et al., 2008). Necropsies were conducted on the adults *in situ*, and basic morphometric data and tissue

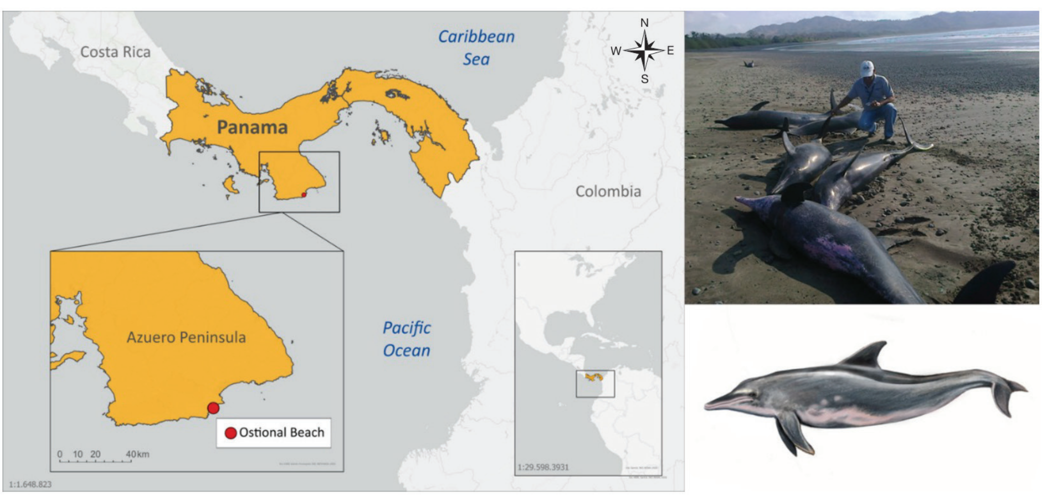

Fig. 1. Location of Ostional Beach in the Azuero Peninsula, Pacific coast of Panama, where the mass stranding of rough-toothed dolphins (*Steno bredanensis*) occurred in April 2016. On right above, a photo of the mass stranding. On right below a species description illustrated by Emmanuel Laverde © www.arteyconservacion.com

samples were collected. Sex was determined by external examination, identifying five females and four males. The calf was transported to the laboratory to conduct the necropsy and posterior analyses. To prevent sampling biases related with tissue decomposition, fresh or moderate carcasses with body condition code 2 or 3 (Kuiken & Hartmann, 1991; Geraci & Lounsbury, 1993) should be used (Méndez-Fernández et al., 2020). Therefore, because all animals were freshly dead (body condition code 2; Geraci & Lounsbury, 1993), samples were used for this ecotoxicological assessment.

Although the liver is considered the main storage organ for Hg (e.g., Mackey et al., 2003), recent analyses in small cetaceans have shown that skin also reflects the concentration of Hg in the internal organs (e.g., Aubail et al., 2013; Cáceres-Saez et al., 2015; Fontaine et al., 2015). Therefore, nine skin samples collected from the adults (stored in 70 % ethanol at -20 °C) were used to conduct the Hg and stable isotope assessments. Because the remoteness of the stranding area, ethanol was the preferable storage method; however, in cetaceans' skin, ethanol could affect the composition of the $\delta^{13}C$ stable isotope concentration by showing depletion (e.g., Hidalgo-Reza et al., 2019; Kiszka et al., 2014). Nevertheless, the magnitude of ethanol effects on this isotope in dolphins' skin has not been fully confirmed like a linear relationship as has been assessed in other taxa (Kiszka et al., 2014). To address the potential issues related to ethanol preservation on the $\delta^{13}C$ composition results, lipids should be removed, since they are depleted in $\delta^{13}C$ (De Niro & Epstein, 1978; Tieszen et al., 1983). Previously to this, samples were covered with aluminum foil and left on a bench to let the ethanol evaporate. Posteriorly, samples were washed ten times with distilled water, which was evaporated at 45 °C over 48 h, and samples were then ground and freeze-dried.

To extract lipids for isotopic analyses, the whole sample (up 50 mg each) was delipidated as follows: 4 ml of cyclohexane was added, next, the sample was agitated constantly for 10 min, centrifuged at 4 500 rpm for 5 min, and the lipid supernatant was discarded. This process was repeated three times, and then the sample was dried at 45 °C in an oven for 48 h. Finally, around 0.02 – 0.04 mg lipid-free sample was weighed in a tin cup to perform posterior stable isotope analyses in a continuous flow mass spectrometer (Delta V Plus with a Conflo IV Interface, Thermo Scientific, Bremen, Germany) coupled to an elemental analyzer (Flash 2000 or EA Isolink, Thermo Scientific, Milan, Italy). The usual δ notation relative to Vienna PeeDee Belemnite Standard for $\delta^{13}C$ and atmospheric N_2 for $\delta^{15}N$, in parts per thousand (‰), was used to express the results (Méndez-Fernández et al., 2020). Measurements in duplicates of internal laboratory standards (acetanilide) during each autorun indicated an experimental precision (SD) of 0.03 for $\delta^{13}C$ and 0.09 for $\delta^{15}N$. To determine if lipid extraction was efficient, the C:N ratio was assessed using the percent C and N elemental composition, in which values lower than four indicate good lipid removal (Lesage et al., 2010).

As described in Vélez et al. (2021), THg concentrations were measured using an atomic absorption spectrometer AMA-254 (Altec © Advanced Mercury Analyzer-254). To control the analytical quality of THg measurements, these were repeated at least two times until there were analytical differences below 10%. Additionally, blanks were run at the beginning of the analytical session, and certified reference material (CRM) TORT-2 (Reference Material of lobster hepatopancreas marine certified by the National Research Council of Canada) were used after blanks and every four analyses. The CRM measured concentration was 251 ng g^{-1} (n = 2) and showed good precision with a percentage of recovery of 93 %. THg measurements are presented in ng g^{-1} on a dry weight basis (dw) and the detection limit was 0.05 ng.

The results found all nine samples (females: n = 5; males: n = 4) collected from the rough-toothed dolphins showed detectable concentrations of THg, with a mean of 12 841; SD = 5 083 ng g^{-1} dry weight (dw) ranging between 4 764 to 18 689 ng g^{-1} dw (SMT1). Regarding the stable isotopes, the C:N ratios reflected an efficient lipids removal (SMT1), so the results showed that $\delta^{13}C$ values ranged between –16.8 and –15.2 ‰ (mean = –16.2; SD = 0.6 ‰), and $\delta^{15}N$ values ranged between 14.3 and 15.9 ‰ (mean = 15.0; SD = 0.5 ‰) (SMT1).

This study presents the first assessment of THg and stable isotope measurements in rough-toothed dolphins from Central America, which is considered to be a species of "Least Concern" but with an "unknown population trend" by the IUCN Red List (Kiska et al., 2019). Bycatch and direct harvest have been considered threats to this species (Avila et al., 2018; Kiska et al., 2019), and metal exposure may be considered as a potential hazard. Several negative effects have been associated to Hg in marine mammals, such as immunotoxicity (Desforges et al., 2016), neurotoxicity (Krey et al., 2015), reproductive, endocrine, heart, and kidney damage (Bossart, 2011; Correa et al., 2014; Kershaw & Hall, 2019; Schwacke et al., 2002), as well as cancer (Béland et al., 1993; Martineau et al., 1994). The high concentrations that rough-toothed dolphins bioaccumulate may warrant special attention and qualifies them as a bioindicator species in oceanic waters. However, the rough-toothed dolphin is a highly migratory species, so THg levels reported in their tissues do not necessarily reflect the local Hg levels.

Studies in several areas worldwide have found rough-toothed dolphins to have high THg levels (SMT2). For instance, in La Guajira (Colombian Caribbean), an assessment of Hg concentrations in the skin of five dolphin species (Atlantic spotted dolphin *Stenella frontalis* Cuvier, 1829; common bottlenose dolphin; common dolphin *Delphinus* sp.; rough-toothed dolphin; and spinner dolphin *Stenella longirostris* (Gray, 1828)) showed the highest values for rough-toothed dolphins (THg-skin-mean = 16 817; SD = 3 815 ng g^{-1} dw; n = 3; Barragán-Barrera, Farías-Curtidor, Luna-Acosta et al. 2019, Barragán-Barrera, Farías-Curtidor, Chávez-Carreño et al., 2019). Similarly in Brazil, specifically in the central-northern Rio de Janeiro State, rough-toothed dolphins showed the highest values in their muscle (THg-mean = 10 150; SD = 6 230 ng g^{-1} dw; n = 9), in comparison to muscle of coastal species like the Franciscana (*Pontoporia blainvillei* (Gervais & d'Orbigny, 1844); THg-mean = 1 920; SD = 960 ng g^{-1} dw; n = 16) and Guiana dolphin (*Sotalia guianensis* (Van Bénéden, 1864); THg-mean = 3 910; SD = 2 160 ng g^{-1} dw; n = 28) (Baptista et al., 2016). The same pattern was observed in the southern Rio de Janeiro State in Brazil, where rough-toothed dolphins showed the highest THg values in their liver (THg-mean = 594 800; SD = 200 300 ng g^{-1} dw; n = 3) in comparison to the liver of the coastal form of common bottlenose dolphin (THg-mean = 4 380; SD = 2 470 ng g^{-1} dw; n = 10) and the offshore Atlantic spotted dolphin (THg-mean = 8 130; SD = 10 470 ng g^{-1} dw; n = 3) (Lemos et al., 2013).

The high THg levels likely reflect the rough-toothed dolphins high-trophic prey preferences, which appear to be indicated by the isotopic values enriched in $\delta^{15}N$ that have been reported in dolphins' skin here and in other areas worldwide (SMT3). Examples include the southern Rio de Janeiro State in Brazil ($\delta^{15}N$-mean = 14.5; SD = 0.1 ‰; n = 3; $\delta^{15}N$-mean-autumn = 18.1; SD = 0.5 ‰; N = 4; $\delta^{15}N$-mean = 18.6; SD = 0.2 ‰; n = 5; Paschoalini et al., 2021; Troina et al., 2020, 2021), La Guajira in the Colombian Caribbean ($\delta^{15}N$-mean = 12.8; SD = 0.1 ‰; n = 3; Barragán-Barrera, Farías-Curtidor, Chávez-Carreño et al., 2019), and Moorea Island in the Society Archipelago ($\delta^{15}N$-mean = ~ 14.7 ‰; N = 35; Kiszka et al., 2010). The rough-toothed dolphin diet consists of cephalopods and fish of various sizes, including large and carnivorous fish with high trophic levels like black skipjack (*Euthynnus lineatus* Kishinouye, 1920), mahimahi (*Coryphaena hippurus* Linnaeus, 1758), and ribbonfish (*Trichiurus lepturus* Linnaeus, 1758) (Ortega-Ortiz et al., 2014; Pitman & Stinchcomb, 2002; West et al., 2011). Unfortunately, we didn't find any content in the carcasses' stomachs, so insights about dolphins' diet in Panamanian Pacific waters is still unknown.

The $\delta^{13}C$ values reported here are depleted in $\delta^{13}C$, which suggests oceanic habits. These isotopic measurements are similar to those found in skin samples stored in ethanol of rough-toothed dolphins from La Guajira in the Colombian Caribbean (δ^{13}C-mean = −14.7; SD = 0.2 ‰; n = 3; Barragán-Barrera, Farías-Curtidor, Chávez-Carreño et al., 2019), and the Society Archipelago (δ^{13}C-mean = ~ −14.9 ‰; n = 35; Kiskza et al., 2010) where the species has neritic habits (Farías-Curtidor & Barragán-Barrera, 2017, Farías-Curtidor & Barragán-Barrera, 2019; Oremus et al., 2012). Indeed, the species has been reported in coastal waters along the Caribbean of Honduras and Panama (Barragán-Barrera et al., 2015; Kuczaj & Yeater, 2017). However, some δ^{13}C values reported for rough-toothed dolphins in the Panamanian Pacific basin are similar to those reported for frozen skin samples of common dolphin oceanic form (*Delphinus delphis* Linnaeus, 1758) in the Gulf of California, Mexican Pacific (δ^{13}C-mean = −18.3; SD = 0.2 ‰) (Elorriaga-Verplancken et al., 2020). Nevertheless, this interspecific comparison should be interpreted with caution due potential bias derived from ethanol preservation on our samples (Kiskza et al., 2014). Thus, until more information about the effect of ethanol on rough-toothed dolphin skin samples, as well as their potential prey and the isoscapes is provided, it is not possible to assess the ecological habitats of rough-toothed dolphins in the Pacific basin of Panama.

Further monitoring is needed to assess the rough-toothed dolphins' feeding ecology in the Panamanian Pacific basin, including the assessment of their diet through stomach content analysis or direct feeding behavior. For isotopic analysis, it is highly recommended to collect samples and storage frozen. Additionally, complementary analyses that include the characterization of isotopic content of organic material content at the base of local food webs, in order to determine local carbon sources as well nitrogen reference levels, are necessary. This study provides the first contribution of ecotoxicological knowledge on a little-known cetacean predator found in Central America, providing important baseline data to understand the feeding ecology as well as the contamination of dolphins in the region.

Ethical statement: the authors declare that they all agree with this publication and made significant contributions; that there is no conflict of interest of any kind; and that we followed all pertinent ethical and legal procedures and requirements. All financial sources are fully and clearly stated in the acknowledgments section. A signed document has been filed in the journal archives.

Author Contribution: DCBB, LTL, BPO conceptualized the study. DCBB, LTL acquired funding. LTL, BPO, RSV collected samples. JJ provided logistic support. DCBB performed lab and statistical analysis, and wrote the draft and revisions of this manuscript. BPO participated in editing some versions of this manuscript. All authors approved the final version.

See supplementary material
a11v71s4-MS1

ACKNOWLEDGMENTS

We especially thank the Ministry of Environment (MiAmbiente) of the Republic of Panama, the National Aeronaval Service of Panama (SENAN), and the International Maritime University of Panama (UMIP) for their logistic support. Special thanks to the community of the Ostional Beach in Tonosí for their rapid response and help in the rescue effort. We thank P. Bustamante, C. Churlaud, and M. Brault-Favrou for facilitating Hg analyses, and G. Guillou for conducting the mass spectrometer analyses. This study was carried out under the scientific permit SE/AO-1-16 from (MiAmbiente) granted to Fundación Panacetacea Panamá. The Vicerrectoría de Investigaciones from Pontificia Universidad Javeriana is acknowledged by providing a Postdoctoral Grant (Call 2021-2) to D. Barragán (2022), who also thanks to the Instituto Javeriano del Agua for its support during the Postdoctoral stay. This study was supported partially by the Small Grant in Aid of Research from the Society for

Marine Mammalogy (D. Barragán, 2019). We thank Kristin Rasmussen for her English revision to the manuscript, Alejandra Duarte for her support preparing the map, and Emmanuel Laverde from Arte y Conservación (www.arteyconservacion.com) for making the dolphin illustration. Finally, we thank the anonymous reviewers whose comments improved the final version of this manuscript.

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