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Mamíferos acuáticos de Centroamérica
Aquatic Mammals of Central América

Laura J. May-Collado & Ester Quintana-Rizzo • *Editoras científicas* • *Scientific editors*

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Scope

Our journal publishes scientific articles that increase our understanding of biology, conservation, and biomedical life sciences in the tropics. Selection criteria are the quantity and quality of new information and its potential interest to the general audience as well as to specialists. The studied ecosystems, or at least the organisms, must be tropical. We give preference to feature articles that include testable study questions—for example, studies with an experimental design evaluating factors that influence biological variables, or studies that explain the mechanisms underlying biological or biomedical phenomena such as, for example, behavior or physiology. Field studies should present temporal or spatial patterns. We also welcome systematic or phylogenetic studies above the species level, meta-analyses and literature reviews that critically examine what is known and what remains to be done in any field of tropical biology.

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IMPORTANT

We have improved our abstracts to help readers rapidly understand why a paper is important, and what the authors wanted to achieve, how they did it, and what they found. Please conform your abstract to this example*

ABSTRACT

Introduction: Interspecific interactions among tropical mesocarnivorous species and other mammalian trophic guilds have been poorly studied, despite their important implications in the survival, structure, demography, and distribution of these species.

Objective: To analyze if sympatric mesocarnivores coexist or compete in the axis of the temporal and spatial niche.

Methods: From January 2015 to December 2016 we recorded mammals with 26 stations of camera traps (in pairs, facing each other) along roads and animal trails, at Reserva de la Biosfera El Cielo, Tamaulipas, Mexico. We calculated temporal and spatial overlaps with the Czekanowski and Pianka indices.

Results: We obtained 239 margay, 118 ocelot and 22 yaguarundi records. Margay and ocelot were nocturnal (75 % of their records) and had a high temporal overlap (0.85); whereas yaguarundi was fully diurnal, suggesting it may be able to coexist with the other two species. The three species used similar habitats: yaguarundi had 0.81 spatial overlap with margay and 0.72 with ocelot; spatial overlap between margay and ocelot was intermediate (0.53).

Conclusions: There is no interspecific competition among these tropical mesocarnivores, probably due to antagonistic interactions leading to use of different parts of the temporal and spatial axes.

* Sample based on *Interacciones temporales y espaciales de mesocarnívoros simpátricos en una Reserva de la Biosfera: ¿coexistencia o competencia?* By R. Carrera-Treviño, et al. (*Revista de Biología Tropical* 66, 3 (2018): DOI 10.15517/rbt.v66i3.30418

IMPORTANTE

Hemos mejorado nuestros resúmenes para ayudar a los lectores a entender rápidamente por qué un artículo es importante, y lo que los autores querían lograr, cómo lo hicieron, y qué encontraron. Por favor escriba su resumen según este ejemplo*

RESUMEN

Introducción: Las interacciones entre especies de mesocarnívoros tropicales y otros gremios tróficos de mamíferos han sido muy poco estudiadas, a pesar de sus importantes aplicaciones en la supervivencia, estructura, demografía, y distribución de estas especies.

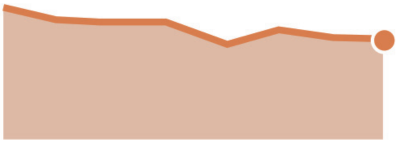
Objetivo: Analizar si los mesocarnívoros simpátricos coexisten o compiten en el eje del nicho temporal y espacial.

Métodos: De enero 2015 a diciembre 2016, registramos mamíferos con 26 estaciones de cámaras trampa (en pares, una frente a la otra) a lo largo de caminos y veredas, en la Reserva de la Biosfera El Cielo, Tamaulipas, México. Calculamos el traslape temporal y espacial con los índices de Czekanowski y Pianka.

Resultados: Obtuvimos 239 registros de margay, 118 de ocelote y 22 de yaguarundi. El caucel y el ocelote son nocturnos (75 % de sus registros) y mostraron un alto traslape temporal (0.85); mientras que el yaguarundí fue totalmente diurno, sugiriendo que puede coexistir con las otras dos especies. Las tres especies usaron hábitats similares: el yaguarundi tuvo un traslape espacial de 0.81 con el caucel y de 0.72 con el ocelote; el traslape espacial entre el caucel y el ocelote fue intermedio (0.53).

Conclusiones: No hay competencia interespecífica entre estas especies de mesocarnívoros tropicales, probablemente debido a interacciones agresivas que conducen al uso de partes diferentes de los ejes temporal y espacial.

* Ejemplo basado en *Interacciones temporales y espaciales de mesocarnívoros simpátricos en una Reserva de la Biosfera: ¿coexistencia o competencia?* Por R. Carrera-Treviño, et al. (*Revista de Biología Tropical* 66, 3 (2018): DOI 10.15517/rbt.v66i3.30418



PRÓLOGO / PROLOGUE

Thirty Years of Aquatic Mammal Research in Central America

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The editors of the Special Issue began their careers as aquatic mammal biologists in the early 90s. At that time, aquatic mammals were not considered essential to Central America's marine or mammalian community (including Panama). Aquatic mammals are charismatic megafauna that can become symbols of conservation, though the significance of their contribution to a particular habitat is often misrepresented. Over the past decade, their importance in this region has become evident as Central America's Pacific and Caribbean waters are home to about 30 % of the world's cetacean diversity (May-Collado, 2009; May-Collado et al., 2018). This knowledge results from a growing community of national and international scientists that, together, reveal aquatic mammals' extraordinary ecology and contribution to the region's economy (i.e., Hoyd & Iñiguez, 2008; May-Collado et al., 2018).

Some of the first studies of aquatic mammals in Central America included assessments of the population status of manatees (e.g., Klein, 1979; Jason, 1980; Reynolds et al., 1995), dolphins from the Eastern Tropical Pacific (i.e., Perrin, 1975; Volkov & Moroz, 1976; Au et al., 1979), and the first report of Guiana dolphins in the Caribbean waters (Bössenecker, 1978). The earliest scientific investigations of Antillean manatees were conducted by visiting scientists, mainly from the United States. During the late 1900s, they worked with local stakeholders to determine the distribution and abundance of manatees in the Wider Caribbean via aerial surveys. By the late 1970s and early 1980s, aerial surveys became a primary tool for counting manatees in remote areas. Since then, aerial surveys have been conducted in Belize (Charnock-Wilson, 1968, 1970; Bengtson & Magor, 1979; O'Shea & Salisbury, 1991; Morales-Vela et al., 2000; Auil, 2004), Guatemala (Quintana-Rizzo, 1993; Quintana-Rizzo et al., 2023), Honduras (Rathbun et al., 1983), Nicaragua (Carr, 1994), Costa Rica (Reynolds et al., 1995), and Panama (Mou Sue et al., 1990). In 2010, several experts from the Central American region provided information that helped to update the Regional Management Plan for the West Indian manatee (UNEP, 2010). This effort was funded by the United Nations Environmental Programme and has served as a conservation and management tool throughout the Wider Caribbean.

From the mid-1980s to 2006, the United States National Oceanic and Atmospheric Administration (NOAA) Southwest Fisheries Science Center, La Jolla, California, started to conduct line transect surveys in the Eastern Tropical Pacific (ETP), which includes the Economic Exclusive Zones of all Central American countries except for Belize. A detailed list of expeditions by year can be found at https://oceanexplorer.noaa.gov/explorations/explorations-by-year.html. The surveys were generally designed to evaluate the status of ETP dolphin stocks caught in tuna nets and surveys of common dolphin stocks. Yet, all cetacean species were recorded during every survey; thus, the data generated represented the most comprehensive data set for many cetacean species in the ETP and Central American waters. NOAA scientists collaborated with researchers from Central America in two country-specific publications based on this large dataset (Costa Rica: May-Collado et al., 2005; Guatemala: Quintana-Rizzo & Gerrodette, 2009).

The first scientific documentation of humpback whales in Central American waters was based on photographs from February 1988 and January 1990 taken near Isla Caños, Costa Rica. These photographs matched whales to individuals identified months earlier in the Gulf of the Farallones and San Luis, California (Steiger et al., 1991). The matches suggested a newly discovered humpback whale migratory route and the breeding area between North America and Central America. The discovery prompted various expeditions between 1995 and the 2000s that identified Central America as an essential seasonal breeding ground from December to March and resulted in the discovery of a second humpback whale breeding population. Some whales that feed in the Antarctic Peninsula migrate to Central America between July and November (Rasmussen et al., 2007). Furthermore, these southern whales undertake the longest reported migration by any mammal: a one-way journey of about 8 677 km (Rasmussen et al., 2007; De Weerdt et al., 2020). In the early 2000s, the humpback whales of Central America became part of the first large-scale international collaborative effort to study the Structure of Populations, Levels of Abundance, and Status of Humpback Whales (SPLASH). SPLASH involved over 50 research groups and over 400 researchers in 10 countries (i.e., Calambokidis et al., 2008; Baker et al., 2008, 2013; Barlow et al., 2011). More recently, in 2023, a second smaller SPLASH project also included humpback whales in Central America (Taylor et al., 2021; Curtis et al., 2022). The different efforts throughout the years resulted in the development of a near-comprehensive North Pacific humpback whale photographic identification dataset (Cheeseman et al., 2023). This is probably one of the most extensive datasets for any marine species.

The research on the biology and conservation of aquatic mammals in Central America has grown exponentially in the last two decades. However, this growth is not represented in the number of scientific publications, restricting collective knowledge and limiting regional management actions that effectively protect these species and their habitat. The region has been referred to as "an emerging region where little research has been published in the peer-reviewed scientific literature" (Kiszka et al., 2021). Yet, for locals, this region includes a dynamic group of researchers who collaborate and communicate actively. In 2020, colleagues in the region organized the first scientific meeting for Central American aquatic mammals, hosted in Panama. Some studies presented in that meeting are published in this Special Issue. In 2022, a significant number of the contributors to this special issue participated in a week-long Important Marine Mammals Areas (IMMA) workshop organized and run by the IMMA Secretariat of the International Conservation of Nature (IUCN) Task Force on Marine Mammal Protected Areas. The group identified over ten IMMAs in Central America that are now officially part of the IMMA e-Atlas (https://www.marinemammalhabitat.org/imma-eatlas).

The limited number of peer-reviewed publications by Central American researchers is due to several reasons, the first of which is language. Most international journals are English-speaking, while the region's language is Spanish (Di Bitetti & Ferreras, 2016; Ramírez-Castañeda, 2020). Even in cases where people publish in English, the publications might not be freely accessible, and publishing in a foreign language limits the local audience because many conservationists, managers, and stakeholders do not speak the language. Further, the typical publishing costs, which vary from several hundred to thousands of dollars (Lourenco et al., 2023), are inaccessible in a region with limited economic resources. The high publication costs are equivalent to the average researcher's monthly or annual salary or the costs of an entire field season. Such limitations significantly impact the ability of researchers in the region to publish. Thus, gray literature has become more common, popular, and accessible. Lastly, a lack of training in scientific writing proficiency and limited publication experience can further discourage local scientists from communicating their work.

Given these difficulties, one of our goals with this Special Issue was to promote local researchers' participation by alleviating these pressures. The authors could choose the language of their publication, and the publication costs were covered. When needed or requested, authors received mentorship and extra time to help them prepare and improve their publications. The publication costs were covered by the international non-profit organization Panacetacea (www.pancetacea.org), which conducts and supports research efforts in Central America. Their contribution made a significant impact on this process. The overall supportive environment of this Special Issue resulted in the participation of researchers from most Central American countries and the publication of exciting data that had not seen the light until now. Still, teams had to make tough choices when deciding whether to make their research accessible to the international community by publishing in English or to the national audience by publishing in Spanish.

For the Special Issue, a general invitation was sent to researchers working in Central America. The invitation was also posted on social media, including Facebook, X (Twitter), and Instagram. Those interested in participating had to submit an abstract highlighting their research contribution. From this original invitation, we received 38 abstracts, of which 33 were asked to submit manuscripts. However, only 21 manuscripts were received. Each manuscript was reviewed by two to three independent international experts worldwide (see list). In total, 15 manuscripts were accepted as full papers (seven) or notes (eight), five were rejected, and one was retracted. Of these 15 manuscripts, 13 involved Central American researchers as primary authors or co-authors, and 12 were lead-authored by women. By country, the number of manuscripts varied: Belize = 1, Guatemala = 4, El Salvador = 1, Nicaragua = 1, Costa Rica = 3, Panama = 4, and one regional paper that includes Central America, Mexico, and the United States.

The Special Issue covers topics on behavior, population structure, communication, genetics, physiology, and conservation. It comprises studies on manatees, humpback whales, Bryde's whales, and different species of dolphins, including bottlenose dolphins, killer whales, pantropical spotted dolphins, and false killer whales. The conservation status of each species varies, but at least one species and two populations described in this Special Issue are considered endangered and vulnerable. The overlapping of their home range with various human activities makes them highly vulnerable to habitat loss and boat collisions (International Union for Conservation of Nature and Natural Resources, 2022). According to the IUCN, the Antillean manatees are categorized as endangered. Humpback whales that use the Central American waters are part of the Central America Distinct Population Segment (DPS) and are classified as Endangered by the United States Endangered Species Act (81 FR 62260, September 8, 2016). The Central America DPS is one of 14 DPS of humpback whales around the world and one of four DPS listed as endangered (Bettridge et al., 2015). A DPS consists of whales sharing the same latitude breeding area and that migrate seasonally to specific mid-to-high latitude feeding grounds, which can differ among individuals (Bettridge, 2019). Finally, the resident population of Bocas del Toro bottlenose dolphins in Panama has been declared a conservation priority due to the reported negative effects of unregulated dolphin-watching activities on their overall health (International Whaling Commission, 2019).

Below, we provide the highlights of each contribution to this issue:

Manatees: Erdsack et al. describe the thermal tolerance of manatees in Belize and show that manatees are at risk of heat stress in tropical waters. Quintana-Rizzo et al. examine the spatial and temporal patterns of manatee occurrence in Guatemala and find that the population has remained relatively stable over 20 years, although a small distributional shift was detected in 2014. In the same country, Machuca-Coronado et al. identified areas of manatee strandings. The study warrants establishing and implementing standard protocols to record stranding events better and more effectively in the future. Mendez et al. identified two genetic lineages in Guatemalan manatees and one new genetic lineage for Central American manatees. The study highlights the importance of studies using nuclear markers to understand manatee population dynamics in the region.

Baleen whales: May-Collado et al. study the impacts of tour boat traffic on humpback whale song detection in Caño Island, Costa Rica, by comparing acoustic data from before, during, and after COVID-19 lockdowns. Their results show that before and after lockdowns, the proportion of whale song presence decreased during hours when more tour boats were present. De Weerdt et al. provide the first song description of Central American humpback whales in Nicaragua and find considerable variability in song structure, suggesting movement between reproductive areas. Ransome et al. describe the migratory destinations of humpback whales between El Salvador and several feeding areas in the North Pacific and find that Salvadorian whales' main migratory destinations are in Southern and Central California. Finally, Rasmussen and Palacios report an important aggregation area for Bryde's whales in the Gulf of Chiriqui, Panama.

Dolphins: Douglas et al. use capture-recapture data from photos of false killer whale dorsal fins to describe their presence in Central America. Their results demonstrate the existence of multiple small populations with large home ranges that include oceanic and coastal waters. Barragan-Barrera et al. contributed to two studies based on a stranding event of rough-toothed dolphins in the Azuero Peninsula, Panama. The first contribution describes total mercury and $\delta^{13}C$ and $\delta^{15}N$ stable isotopes found in their bubbler, revealing high concentrations of mercury that appear to result from bioaccumulation. The second contribution studies their genetic diversity by comparing it with other populations in the Atlantic Ocean and finds high levels of rough-toothed dolphin population structure among ocean basins. Oviedo et al. also used photo-identification to estimate the population dynamics of pantropical spotted dolphins in Golfo Dulce, Costa Rica. Among their findings is that at least some spotted dolphins are long-term residents of the inner part of the gulf. Ortiz-Wolford et al. describe the sighting history of killer whales in Guatemala and an interaction between these dolphins and a Bryde's whale. Duarte-Fajardo et al. study the genetic diversity of bottlenose dolphins in Bocas del Toro. Their results reinforce the previously described genetic isolation of this small population of dolphins and their vulnerability to growing tour boat traffic activities in their critical habitat. Finally, Pacheco-Polanco et al. document the potential presence of Lobomycosis in the Golfo Dulce and Nicoya bottlenose dolphins. Lobomycosis is a chronic mycotic skin disease found in dolphins worldwide.

Together, the contributions of this Special Issue highlight the richness of aquatic mammals in Central America and their importance as members of the marine megafauna community. The contributions show that these species' habitats overlap with human activities, highlighting the need for (1) management and conservation measures and (2) investment in research to ensure such decisions are based on the best available science. Finally, this Special Issue identifies gaps in our understanding of population biology, ecology, behavior, and threats to many species, including those not part of this Special Issue. However, all the papers provide important insights into the opportunities to conserve and protect these valuable species.

Treinta años de investigación sobre mamíferos acuáticos en Centroamérica

Las editoras de este volumen especial comenzaron sus carreras como biólogas de mamíferos acuáticos a principios de los años 90. En ese momento, los mamíferos acuáticos no se consideraban parte de la comunidad marina o de mamíferos de Centroamérica (incluido Panamá). Los mamíferos acuáticos son una megafauna carismática que puede convertirse en símbolo de la conservación, aunque a menudo la importancia de su contribución a un hábitat determinado no es representada. Durante la última década, su importancia en esta región se ha hecho evidente, ya que las aguas del Pacífico y el Caribe de Centroamérica albergan aproximadamente el 30 % de la diversidad de cetáceos del mundo (May-Collado, 2009; May-Collado et al., 2018). Este conocimiento es el resultado de una creciente comunidad de científicos nacionales e internacionales que, juntos, revelan la extraordinaria ecología de los mamíferos acuáticos y su contribución a la economía de la región (p. ej., Hoyd & Iñiguez, 2008; May-Collado et al., 2018).

Algunos de los primeros estudios de mamíferos acuáticos en Centroamérica incluyeron evaluaciones del estado poblacional de manatíes (p. ej., Klein, 1979; Jason, 1980; Reynolds et al., 1995), delfines del Pacífico oriental tropical (p. ej., Perrin, 1975; Volkov & Moroz, 1976; Au et al., 1979), y el primer reporte de delfines de Guyana en aguas del Caribe (Bøssenecker, 1978). Las primeras investigaciones científicas sobre los manatíes de las Antillas fueron realizadas por científicos visitantes, principalmente de Estados Unidos. A finales del siglo XX, trabajaron con partes interesadas locales para determinar la distribución y abundancia de los manatíes en el Gran Caribe mediante reconocimientos aéreos. A finales de los años 1970 y principios de los 1980, los sondeos aéreos se convirtieron en la herramienta principal para contar manatíes en áreas remotas. Desde entonces, se han realizado sondeos aéreos en Belice (Charnock-Wilson, 1968, 1970; Bengtson & Magor, 1979; O'Shea & Salisbury, 1991; Morales-Vela et al., 2000; Auil, 2004), Guatemala (Quintana-Rizzo, 1993; Quintana-Rizzo et al., 2023), Honduras (Rathbun et al., 1983), Nicaragua (Carr, 1994), Costa Rica (Reynolds et al., 1995) y Panamá (Mou Sue et al., 1990). En el 2010, varios expertos de la región centroamericana brindaron información que ayudó a actualizar el Plan de Manejo Regional para el manatí antillano (UNEP, 2010). Este esfuerzo fue financiado por el Programa de las Naciones Unidas para el Medio Ambiente y ha servido como herramienta de conservación y gestión en todo el Gran Caribe.

Desde mediados de los años ochenta hasta el 2006, el Centro de Ciencias Pesqueras del Sudoeste de la Administración Nacional Oceánica y Atmosférica (iniciales en inglés NOAA) de los Estados Unidos, en La Jolla, California, comenzó a realizar estudios de transectos lineales en el Pacífico Tropical Oriental, que incluye las Zonas Económicas Exclusivas de todos los países de Centroamérica excepto Belice. La lista detallada de expediciones por año se encuentran en https://oceanexplorer.noaa.gov/explorations/explorations-by-year.html. Las prospecciones fueron diseñadas generalmente para evaluar el estado de las poblaciones de delfines del Pacífico Oriental Tropical capturados en redes atuneras y prospecciones para el estudio de poblaciones de delfines comunes. Sin embargo, todas las especies de cetáceos fueron registradas durante cada estudio; por lo tanto, los datos generados representaron uno de los estudios más completos de cetáceos en el Pacífico Oriental Tropical y de Centroamérica. Los científicos de la NOAA colaboraron con investigadores centroamericanos en dos publicaciones específicas de dos países basadas en este conjunto de datos (Costa Rica: May-Collado et al., 2005; Guatemala: Quintana-Rizzo & Gerrodette, 2009).

La primera documentación científica de ballenas jorobadas en aguas centroamericanas se basó en fotografías tomadas en febrero de 1988 y enero de 1990 cerca de Isla Caños, Costa Rica. Estas fotografías relacionaron ballenas con individuos identificados meses antes en el Golfo de los Farallones y San Luis, California (Steiger et al., 1991). Las coincidencias sugirieron una ruta migratoria de ballena jorobada recientemente descubierta y el área de reproducción entre América del Norte y América Central. El descubrimiento impulsó varias expediciones entre 1995 y la década de 2000 que identificaron a Centroamérica como un área de crianza importante de diciembre a marzo y también resulto en el descubrimiento de una segunda población reproductora de ballenas jorobadas. Algunas ballenas que se alimentan en la Península Antártica migran a Centroamérica entre julio y noviembre (Rasmussen et al., 2007). Además, estas ballenas australes emprenden la migración más larga registrada por cualquier mamífero: un viaje de ida de aproximadamente 8 677 km (Rasmussen et al., 2007; De Weerdt et al., 2020). A principios de la década de 2000, las ballenas jorobadas de Centroamérica se convirtieron en parte del primer esfuerzo colaborativo internacional a gran escala para estudiar la estructura de las poblaciones, los niveles de abundancia y el estado de las ballenas jorobadas provenientes del hemisferio norte (SPLASH). SPLASH involucró a más de 50 grupos de investigación y más de 400 investigadores en 10 países (Calambokidis et al., 2008; Baker et al., 2008, 2013; Barlow et al., 2011). Más recientemente, en 2023, un segundo proyecto SPLASH más pequeño también incluyó ballenas jorobadas en Centroamérica (Taylor et al., 2021; Curtis et al., 2022). Los diferentes esfuerzos a lo largo de los años dieron como resultado el desarrollo de una base de datos de foto-identificación de ballenas jorobadas casi completo del Pacífico Norte (Cheeseman et al., 2023). Este es probablemente una de las bases de datos más extensos sobre cualquier especie marina.

La investigación sobre la biología y conservación de los mamíferos acuáticos en Centroamérica ha crecido exponencialmente en las últimas dos décadas. Sin embargo, este crecimiento no se ve representado en el número de publicaciones científicas, restringiendo el conocimiento colectivo y limitando acciones de manejo regional que protejan efectivamente a estas especies y su hábitat. Se ha hecho referencia a la región como "una región emergente donde se han publicado pocas investigaciones en la literatura científica revisada" (Kiszka et al., 2021). Sin embargo, para los locales, esta región incluye un grupo dinámico de investigadores que colaboran y se comunican activamente. En el 2020, colegas de la región organizaron la primera reunión científica sobre mamíferos acuáticos centroamericanos, celebrada en Panamá. Algunos estudios presentados en esa reunión se publican en este Volumen Especial. En el 2022, un número significativo de los contribuyentes a este Volumen Especial participaron en un taller de una semana de duración sobre Áreas Importantes para Mamíferos Marinos (IMMA) organizado y dirigido por la Secretaría de IMMA del Grupo de Trabajo sobre Áreas Protegidas de Mamíferos Marinos de la Union Internacional para la Conservación la Naturaleza (UICN). El grupo identificó más de diez IMMA en Centroamérica que ahora son oficialmente parte del e-Atlas de IMMAs (https://www.marinemammalhabitat.org/imma-eatlas).

El número limitado de publicaciones revisadas por parte de investigadores centroamericanos se debe a varias razones, la primera de las cuales es el idioma. La mayoría de las revistas internacionales son de habla inglesa, mientras que el idioma de la región es el español (Di Bitetti & Ferreras, 2016; Ramírez-Castañeda, 2020). Incluso en los casos en que las personas publican en inglés, es posible que las publicaciones no sean de libre acceso, y publicar en un idioma extranjero limita la audiencia local porque muchos conservacionistas, administradores y partes interesadas no hablan el idioma. Además, los costos de publicación típicos, que varían entre varios cientos y miles de dólares (Lourenco et al., 2023), son inaccesibles en una región con recursos económicos limitados. Los elevados costos de publicación equivalen al salario mensual o anual promedio de un investigador o al costo de una temporada de campo completa. Estas limitaciones afectan significativamente la capacidad de publicación de los investigadores de la región. Así, la literatura gris se ha vuelto más común, popular y accesible. Por último, la falta de formación en redacción científica y la limitada experiencia en publicaciones pueden disuadir aún más a los científicos locales de comunicar su trabajo.

Dadas estas dificultades, uno de nuestros objetivos con este Volumen Especial fue promover la participación de los investigadores locales aliviando estas presiones. Los autores podían elegir el idioma de su publicación y los costos de publicación estaban cubiertos. Cuando fue necesario o solicitado, los autores recibieron tutoría y tiempo adicional para ayudarlos a preparar y mejorar sus publicaciones. Los costos de publicación fueron cubiertos por la organización internacional sin fines de lucro Panacetacea (www.pancetacea.org), que realiza y apoya esfuerzos de investigación en Centroamérica. Su contribución tuvo un impacto significativo en este proceso. El ambiente de apoyo general de este Volumen Especial resultó en la participación de investigadores de la mayoría de los países centroamericanos y la publicación de datos interesantes que no habían visto la luz hasta ahora. Aun así, los equipos tuvieron que tomar decisiones difíciles al decidir si hacer que su investigación accesible a la comunidad internacional publicándola en inglés o a la audiencia nacional publicándola en español.

Para el Volumen Especial, se envió una invitación general a investigadores que trabajan en Centroamérica. La invitación también se publicó en las redes sociales, incluidas Facebook, X (Twitter) e Instagram. Los interesados en participar debían enviar un resumen destacando su contribución a la investigación. De esta invitación original, recibimos 38 resúmenes, de los cuales a 33 se les pidió que enviaran manuscritos. Sin embargo, sólo se recibieron 21 manuscritos. Cada manuscrito fue revisado por dos o tres expertos internacionales independientes de todo el mundo (ver lista). En total, 15 manuscritos fueron aceptados como artículos completos (siete) o notas (ocho), cinco fueron rechazados y uno fue retractado. De estos 15 manuscritos, 13 involucraron a investigadores centroamericanos como autores principales o coautores, y 12 fueron escritos por mujeres. Por país, el número de manuscritos varió: Belice = 1, Guatemala = 4, El Salvador = 1, Nicaragua = 1, Costa Rica = 3, Panamá = 4 y un artículo regional que incluye Centroamérica, México y Estados Unidos.

El Volumen Especial cubre temas sobre comportamiento, estructura poblacional, comunicación, genética, fisiología y conservación. Comprende estudios sobre manatíes, ballenas jorobadas, ballenas de Bryde y diferentes especies de delfines, incluidos delfines nariz de botella, orcas, delfines moteados pantropicales y falsas orcas. El estado de conservación de cada especie varía, pero al menos una especie y dos poblaciones descritas en este Volumen Especial se consideran en peligro de extinción y vulnerables. La superposición de sus áreas de distribución con diversas actividades humanas los hace muy vulnerables a la pérdida de hábitat y a las colisiones de embarcaciones (International Union for the Conservation of Natural Resources, 2022). Según La Unión Internacional para la Conservación de los Recursos Naturales, los manatíes antillanos están clasificados como en peligro de extinción. Las ballenas jorobadas que utilizan las aguas centroamericanas son parte del Segmento de Población Distinta de Centroamérica (DPS por sus siglas en inglés) están clasificadas como En Peligro por la Ley de Especies en Peligro de los Estados Unidos (81 FR 62260, 8 de septiembre de 2016). La DPS de Centroamérica es una de las 14 DPS de ballenas jorobadas en todo el mundo y una de las cuatro DPS catalogadas como en peligro de extinción (Bettridge et al., 2015). Un DPS consiste en ballenas que comparten la misma zona de reproducción en latitud y que migran estacionalmente a zonas de alimentación específicas en latitudes medias y altas, que pueden diferir entre individuos (Bettridge, 2019). Finalmente, la población residente de delfines nariz de botella de Bocas del Toro en Panamá ha sido declarada una prioridad de conservación debido a los efectos negativos reportados de las actividades no reguladas de observación de delfines en su salud general (International Whaling Commission, 2019).

A continuación, proporcionamos los aspectos más destacados de cada contribución a este número:

Manatíes: Erdsack et al. describen la tolerancia térmica de los manatíes en Belice y muestran que los manatíes corren el riesgo de sufrir estrés por calor en aguas tropicales. Quintana-Rizzo et al. examinan los patrones espaciales y temporales de la presencia de manatíes en Guatemala y encuentran que la población se ha mantenido relativamente estable durante 20 años, aunque se detectó un cambio mínimo en su distribución en 2014. En el mismo país, Machuca-Coronado et al. identifica áreas de varamiento de manatíes. El estudio justifica el establecimiento e implementación de protocolos estándar para registrar mejor y más eficazmente los eventos de varamiento en el futuro. Méndez et al. identificaron dos linajes genéticos en manatíes guatemaltecos y un nuevo linaje genético para manatíes centroamericanos. El estudio destaca la importancia de los estudios que utilizan marcadores nucleares para comprender la dinámica de la población de manatíes en la región.

Ballenas barbadas: May-Collado et al. estudian el impacto del tráfico de embarcaciones turísticas en la detección del canto de las ballenas jorobadas en la Isla del Caño, Costa Rica, comparando datos acústicos de antes, durante y después de los cierres por COVID-19. Sus resultados muestran que antes y después de los cierres, la proporción de presencia del canto de las ballenas disminuyó durante las horas en que había más barcos turísticos presentes. De Weerdt et al. proporcionan la primera descripción del canto de las ballenas jorobadas centroamericanas en Nicaragua y encuentran una variabilidad considerable en la estructura del canto, lo que sugiere movimiento entre áreas reproductivas. Ransome et al. describen los destinos migratorios de las ballenas jorobadas entre El Salvador y varias áreas de alimentación en el Pacífico Norte y encuentran que los principales destinos migratorios de las ballenas salvadoreñas son el sur y el centro de California. Finalmente, Rasmussen y Palacios reportan una importante área de agregación de ballenas de Bryde en el Golfo de Chiriquí, Panamá.

Delfines: Douglas et al. utilizan datos de captura y recaptura de fotografías de aletas dorsales de falsas orcas para describir su presencia en Centroamérica. Sus resultados demuestran la existencia de múltiples poblaciones pequeñas con grandes ambitos de hogar que incluyen aguas oceánicas y costeras. Barragán-Barrera et al. contribuyeron a dos estudios basados en un evento de varamiento de delfines de dientes rugosos en la Península de Azuero, Panamá. La primera contribución describe el mercurio total y los isótopos estables δ13C y δ15N que se encuentran en la grasa de los delfines, y revelan altas concentraciones de mercurio que parecen ser el resultado de la bioacumulación. La segunda contribución estudia su diversidad genética comparándola con otras poblaciones del Océano Atlántico y encuentran altos niveles de estructura poblacional de delfines de dientes rugosos entre las cuencas oceánicas. Oviedo et al. también utilizó la foto-identificación para estimar la dinámica poblacional de delfines manchados pantropicales en el Golfo Dulce, Costa Rica. Entre sus hallazgos está que al menos algunos delfines manchados tienen patrones de residencia largos sobre todo en la parte interior del golfo. Ortiz-Wolford et al. describe la historia de avistamientos de orcas en Guatemala y una interacción entre estos delfines y una ballena de Bryde. Duarte-Fajardo et al. estudian la diversidad genética de los delfines nariz de botella en Bocas del Toro. Sus resultados refuerzan el aislamiento genético descrito anteriormente de esta pequeña población de delfines y su vulnerabilidad a las crecientes actividades de tráfico de embarcaciones turísticas en su hábitat crítico. Finalmente, Pacheco-Polanco et al. documentan la presencia potencial de lobomicosis en los delfines nariz de botellla del Golfo Dulce y Nicoya. La lobomicosis es una enfermedad micótica crónica de la piel que se encuentra en los delfines en todo el mundo.

En conjunto, las contribuciones de este Volumen Especial resaltan la riqueza de los mamíferos acuáticos en Centroamérica y su importancia como miembros de la comunidad de megafauna marina. Las contribuciones muestran que los hábitats de estas especies se superponen con las actividades humanas, destacando la necesidad de (1) medidas de gestión y conservación e (2) inversión en investigación para garantizar que estas decisiones se basen en la mejor ciencia disponible. Finalmente, este Volumen Especial identifica lagunas en nuestra comprensión de la biología, la ecología, el comportamiento y las amenazas de muchas especies, incluidas aquellas especies que no forman parte de este Volumen Especial. Sin embargo, todos los artículos brindan información importante sobre las oportunidades para conservar y proteger estas valiosas especies.

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SUPPLEMENT • MANATEES

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Heat loss or heat uptake? Skin temperature in Antillean manatees (*Trichechus manatus manatus,* Sirenia: Trichechidae) in Belize

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ABSTRACT

Introduction: The two subspecies of the West Indian manatee (*Trichechus manatus*), Florida manatees (*T. m. latirostris*) and Antillean manatees (*T. m. manatus*), face different environmental challenges. While Florida manatees have to cope with winter water temperatures below their lower critical temperature of ~ 20 °C and air temperatures below freezing, Antillean manatees live in year-round warm Caribbean waters. Sirenians lack effective thermal insulation and have limited capability of controlling peripheral heat loss. Although severe cold related health issues and mortality are primarily known in Florida manatees, it can be assumed that Antillean manatees and other extant sirenians share the cold-sensitivity, but hardly ever experience it. Contrarily, during summer, Antillean manatees may face the opposite form of thermal stress by being exposed to water temperatures close to their body temperature. However, the upper critical temperature of manatees is not known.
Objective: To improve understanding of the impact of high ambient temperatures on manatee physiology.
Methods: We measured skin temperature in six Antillean manatees in two different habitats in Belize, and compared the results to skin temperatures measured in two captive Florida manatees.
Results: We found a similar temperature distribution pattern over the body surface in both subspecies, but significantly higher temperatures and larger temperature ranges among measuring points in Antillean manatees as compared to Florida manatees. In one Antillean manatee, skin temperature was consistently lower than ambient water temperature by up to 2.5 °C. This implies potential heat uptake from the environment, in contrast to the heat loss experienced by Florida manatees at low water temperatures, apparent in skin temperatures above ambient water temperature.
Conclusions: Our findings suggest that heat stress may be a more likely risk for manatees in warm tropical waters. Despite the small sample size, our results present important findings towards understanding thermal tolerance and impact of high ambient temperatures on manatee physiology.

Key words: thermoregulation; peripheral heat loss; heat dissipation; heat retention; blubber lipid composition; thermoregulatory adaptations; surface area to volume ratio SA:V.

RESUMEN

¿Pérdida o absorción de calor? Temperatura de la piel en manatíes antillanos (*Trichechus manatus manatus*, Sirenia: Trichechidae) en Belice

Introducción: Las dos subespecies del manatí antillano (*Trichechus manatus*), los manatíes de Florida (*T. m. latirostris*) y los manatíes antillanos (*T. m. manatus*), enfrentan diferentes desafíos ambientales. Mientras que los manatíes de Florida tienen que hacer frente a temperaturas invernales del agua por debajo de su temperatura crítica ~20 °C y temperaturas del aire por debajo del punto de congelación, los manatíes antillanos viven en aguas cálidas del Caribe durante todo el año. Los sirenios carecen de un aislamiento térmico efectivo y tienen una capacidad limitada para controlar la pérdida de calor periférico. Aunque los problemas de salud y la mortalidad relacionados con el frío se conocen principalmente en los manatíes de Florida, se puede suponer que los manatíes antillanos y otros sirenios existentes comparten la sensibilidad al frío, pero casi nunca la experimentan. Por el contrario, durante el verano, los manatíes antillanos pueden enfrentar la forma opuesta de estrés térmico al estar expuestos a temperaturas del agua cercanas a la temperatura de su cuerpo. Sin embargo, se desconoce la temperatura crítica superior de los manatíes.

Objetivo: Mejorar la comprensión del impacto de las altas temperaturas ambientales en la fisiología del manatí.

Métodos: Medimos la temperatura de la piel en seis manatíes antillanos en dos hábitats diferentes en Belice y comparamos los resultados con las temperaturas de la piel medidas en dos manatíes de Florida en cautiverio.

Resultados: Encontramos un patrón de distribución de temperatura similar sobre la superficie del cuerpo en ambas subespecies, pero temperaturas significativamente más altas y rangos de temperatura más amplios entre los puntos de medición en los manatíes antillanos en comparación con los manatíes de Florida. En un manatí antillano, la temperatura de la piel fue consistentemente más baja que la temperatura ambiente del agua hasta en 2.5 °C. Esto implica una posible absorción de calor del medio ambiente, en contraste con la pérdida de calor que experimentan los manatíes de Florida a bajas temperaturas del agua, lo cual se evidencio con temperaturas de la piel por encima de la temperatura ambiente del agua.

Conclusiones: Nuestros hallazgos sugieren que el estrés por calor puede ser un riesgo más probable para los manatíes en aguas cálidas tropicales. A pesar del pequeño tamaño de la muestra, nuestros resultados presentan hallazgos importantes para comprender la tolerancia térmica y el impacto de las altas temperaturas ambientales en la fisiología de estos mamíferos marinos.

Palabras clave: termorregulación; pérdida de calor periférica; disipación de calor; retención de calor; composición de lípidos de grasa; adaptaciones termorreguladoras; relación superficie-volumen SA:V.

INTRODUCTION

Florida manatees, *Trichechus manatus latirostris* (Harlan, 1824) and Antillean manatees, *Trichechus manatus manatus* (Linnaeus, 1758) are the two subspecies of the West Indian manatee (*T. manatus*). While Antillean manatees are found in the Caribbean and from Mexico down south to the coast of Brazil (Marsh et al., 2011), Florida manatees primarily inhabit Florida coastal and inland waters, with a typical range from Texas to North Carolina in summer with occasional sightings as far north as Massachusetts (Marsh et al., 2011). Although their habitats overlap to a small extent in the Caribbean, each subspecies faces different environmental challenges.

Florida manatees are the northern most Sirenian population and have to face water temperatures (T_{water}) as low as 13 °C in winter over extended periods of time along with air temperatures (T_{air}) below freezing in contrast to summer water temperatures of up to 32 °C and higher (National Oceanic and Atmospheric Administration, 2022). Florida manatees are known to be very sensitive to cold with low metabolic rates (Scholander & Irving, 1941) and a high lower critical temperature of ~ 20 °C (Irvine, 1983) due to poor thermal insulation and limited control of peripheral heat loss. Therefore, they rely on behavioral thermoregulation and migrate to warm water refuges when temperatures drop (Marsh et al., 2011). Still, cold stress is a major threat to Florida manatees, and cold stress syndrome (CSS) affects and kills manatees every winter (Bossart et al., 2002; Hardy et al., 2019). Extant sirenians share low metabolic rates (Gallivan & Best, 1980) and lack of effective thermal insulation (Horgan et al., 2014) and other thermoregulatory adaptations to the aquatic life style (Bryden et al., 1978; Fawcett, 1942; Gallivan et al., 1983), present in other marine mammal species. Therefore, Antillean manatees and other extant sirenians are likely sensitive to cold as well, which is furthermore supported by reports of cold water avoidance behavior (Anderson, 1986; Zeh et al., 2018).

In contrast to Florida manatees, Antillean manatees live in year-round warm Caribbean waters, and knowledge about potential cold sensitivity is only anecdotal. Contrarily, they may experience T_{water} in the range of mammalian body temperature (T_{body}) during summer months (Kaufman & Thompson, 2005), which poses an entirely different thermoregulatory challenge. To investigate and assess the potential risk of heat stress in manatees, knowledge of their upper critical temperature is required. However, manatee upper critical temperature is currently not known.

Due to manatees' poor thermal insulation and limited ability to control peripheral heat loss, their skin temperature (T_{skin}) is ideally suited to indicate the animal's thermal state by assessing heat exchange between body surface and environment (Erdsack et al., 2018; Worthy et al., 2000). Long-term studies of T_{skin} and heat flux in two captive Florida manatees indicated areas of increased heat exchange on the body surface, temperature distribution patterns and potential impact of ambient temperature on manatee T_{skin} (Erdsack et al., 2018; N. Erdsack unpublished).

Besides the unknown upper critical temperature, knowledge about manatee thermoregulation, in particular the impact of high ambient temperature is incomplete. In order to fill some of these gaps, we measured T_{skin} in wild Antillean manatees captured for health assessments in two different habitats in Belize. Here, we present these preliminary data in comparison to T_{skin} measured in captive Florida manatees between 2016 and 2021.

T_{skin} was measured in six adult Antillean manatees captured for health assessments in Belize in May 2019: three females in Southern Lagoon, around Gales Point, and a female and two males in the waters around Placencia. The manatees were captured using a circle net and hefted onto the capture boat for processing. T_{skin} measurements took approximately 10 min and started as soon as the net was removed and the animal was in a safe and stable position on the boat. If necessary, mud was rinsed off with sea water. Animals were shaded by a tarp during processing. T_{skin} was measured using a wireless thermometer with an attached K-Type surface thermocouple (TMD-55W, Amprobe, Everett, WA, USA), which was also used in a long term study of T_{skin} measurements in captive Florida manatees (N. Erdsack unpublished). The 14 defined measuring spots on the manatees' dorsal body surface (Fig. 1A) were selected in accordance with this study (N. Erdsack unpublished). The two trained manatees ("Hugh", "Buffett") are held at Mote Marine Laboratory, Sarasota, FL, U.S. in an outdoor tank at a constant water temperature of 26.3±0.4 °C. For measurements, the manatees were stationing at the water surface and the respective body part was lifted above the water surface. For comparison with Antillean manatees, only measurements at average T_{air} comparable to average T_{air} during measurements in Belize were considered (Hugh: n = 7; Buffett: n = 6). Temperature differences were tested for statistical significance using a two-tailed paired or homoscedastic t-test, respectively, in MS-Excel 2019. Level of significance was $\alpha = 0.5$. Relations between T_{skin} and T_{water}, T_{air} were reported using Pearson correlation coefficient (r).

Average T_{skin} per measuring point in Antillean and Florida manatees are displayed in Fig. 1B. Both subspecies exhibited similar temperature distribution patterns over the body surface. Except for the almost identical temperature on the top of the head, manatees in Southern Lagoon and Placencia exhibited almost congruent temperature distribution patterns. However, average T_{skin} in manatees in Southern Lagoon was significantly higher than in manatees captured around Placencia ($p << 0.0001$), and average T_{skin} in Antillean manatees was significantly higher than in Florida manatees ($p << 0.0001$). Moreover, average temperature range amongst measuring points was significantly larger in Antillean manatees (2.92 ± 1.08 °C, $p = 0.0011$) as compared to Florida manatees (1.28 ± 0.74 °C). One animal captured in Southern Lagoon at $T_{water} = 33$ °C had average

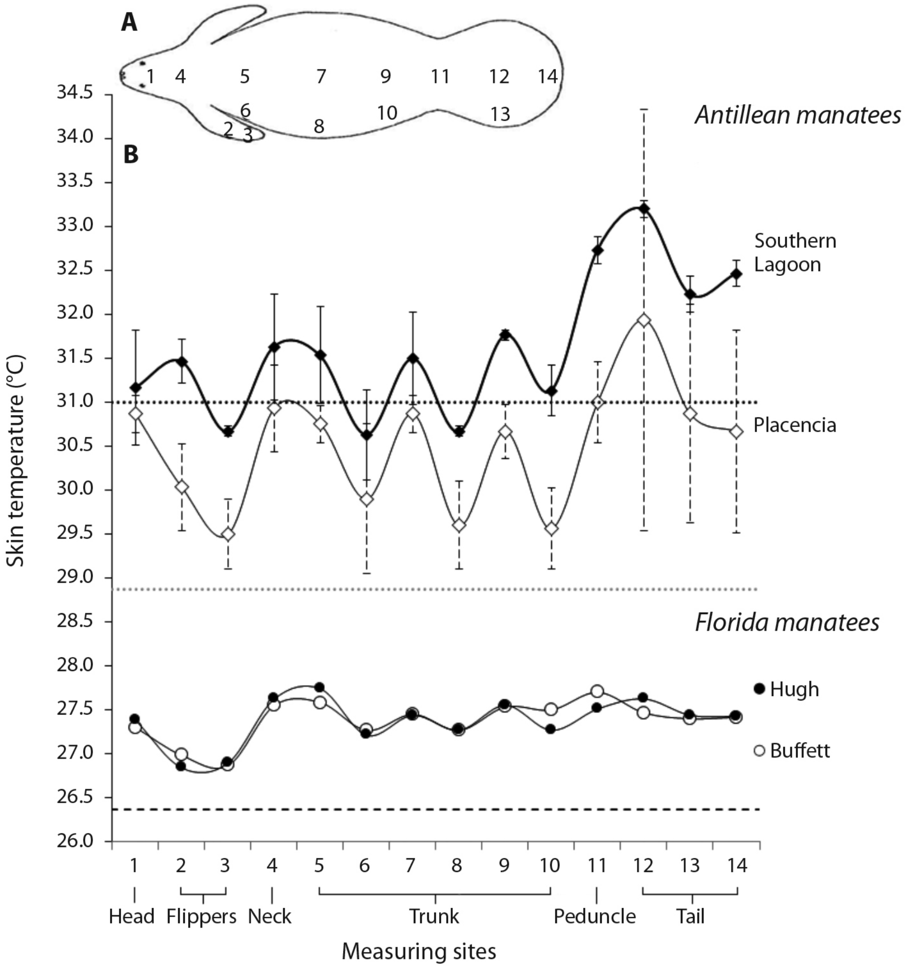

Fig. 1. Location of measuring spots on the manatees' dorsal body surface including ventral (P2) and dorsal (P3) side of the pectoral flipper (A) and average T_{skin}+SD per measuring point (B) in Antillean manatees captured in Southern Lagoon (black diamonds, n = 3), and Placencia (white diamonds, n = 3), and in two captive Florida manatees (Hugh: black dots, n = 7; Buffett: black circles, n = 6). Average T_{water} during measurements was 31±1.7 °C in Southern Lagoon (black dotted line), 28.9±0.6 °C in Placencia (gray dotted line), and 26.3±0.4 °C in the Florida manatee tank (dashed line). Average T_{air} during measurements was 30.2±0.8 °C in Belize, and 30.7±1.3 °C in Florida.

T_{skin} = 31.6 °C and $T_{skin} < T_{water}$ in all but one of the measuring points (center of the tail, P12 = 33.1 °C). In Southern Lagoon, average T_{water} during measurements was 31 ± 1.7 °C, in Placencia 28.9 ± 0.6 °C, and 26.4 ± 0.3 °C in the Florida manatee tank. Average T_{air} during measurements was 30.2 ± 0.8 °C in Belize, and 30.7 ± 1.3 °C in Florida. In Antillean manatees, T_{skin} was weakly correlated to T_{water} (r = 0.60), but no correlation to T_{air} (r = 0.29) was found.

Our measurements revealed similarities but also significant differences in average T_{skin} and T_{skin} distribution between Antillean and Florida manatees. Distribution of T_{skin} over the body surface, indicating body locations with higher and lower heat exchange with the environment, was similar in both subspecies. However, in Antillean manatees, this pattern was much more pronounced, that is, temperature differences between measuring points were significantly larger in Antillean manatees (up to 4.8 °C in individual manatees) than in Florida manatees (up to 2.7 °C). The overall higher T_{skin} in Antillean manatees can likely be attributed to the higher T_{water} in Belizean waters, as indicated by the positive correlation between T_{skin} and T_{water} found in Antillean manatees ($r = 0.6$). A potential impact of netting and handling on metabolic rate and thermal state of individual manatees cannot be excluded. However, T_{water} cannot explain the significantly larger temperature ranges on the body surfaces of Antillean manatees in comparison to Florida manatees.

The most obvious difference between the two subspecies is their body size. Antillean manatees are on average smaller than their Florida conspecifics (Castelblanco-Martínez et al., 2021; Wong et al., 2012), which are the largest extant Sirenian (sub)species. Since heat exchange with the environment occurs primarily via the body surface, a reduced surface-area-to-volume ratio (SA:V) is favorable in terms of heat retention in the cold (Schmidt-Nielsen, 1997). Florida manatees' larger body size along with the small pectoral flippers found in all sirenians result in a reduced SA:V, constituting an adaptation to the colder climate they inhabit in comparison to other extant Sirenians. This was even more pronounced in the extinct Steller's sea cow, *Hydrodamalis gigas* (Zimmermann, 1780), which inhabited the cold waters of the Bering Sea with estimated body lengths and masses up to 10 m and 10 000 kg (Marsh et al., 2011). In comparison to Florida manatees, Antillean manatees have larger SA:Vs due to their smaller body size (Castelblanco-Martínez et al., 2021). Heat retention is likely not essential in this subspecies, considering that they rarely experience $T_{water} < 18$ °C. Contrarily, during summer, Antillean manatees may experience T_{water} in the range of mammalian T_{body}. Data on manatee core T_{body} is scarce. Irvine (1983) reported rectal temperatures of 27-32 °C measured in three captive Florida manatees, but simultaneously measured stomach temperatures of 35-36.8 °C, which, moreover, could be significantly impacted by food intake. Recent measurements of pharyngeal temperature in 20 captive Florida manatees resulted in 35.1-35.9 °C (Martony et al., 2020). Average oral temperature measured in wild manatees during health assessments was significantly higher in Antillean (34.6 ± 0.9 °C) than in Florida (32.6 ± 1.8 °C) manatees (Wong et al., 2012). It is likely that during summer months, T_{water} in shallow Caribbean lagoons can reach and exceed these values, regarding that we measured T_{water} up to 33 °C in Southern Lagoon as early as May. Marsh et al. (2011) even mentioned T_{water} as high as 41 °C in an area frequented by Florida manatees in summer. Without a thermal gradient from the body surface to the surrounding water, an animal is not capable of dissipating excess heat, which will eventually result in heat stress. Average T_{skin} measured in one Antillean manatee in Southern Lagoon with $T_{skin} < T_{water}$ did not differ from average T_{skin} measured in the other manatees in Southern Lagoon; however, T_{water} was higher. We did not observe physiological abnormalities in this manatee, suggesting the presence of physiological and/or behavioral adaptations that help them deal with these extreme thermal conditions, at least temporarily. These findings also leave room for speculations about upper critical temperature and thermal tolerance in Antillean manatees, which may be relatively high in the water.

As the differing body sizes, the observed temperature distribution pattern along with the differences in T_{skin} and T_{skin} ranges over the body surface may indicate anatomical adaptations to differing thermal environments. Sirenians lack arteriovenous anastomoses (AVAs) in the skin (Bryden et al., 1978; Fawcett, 1942), which are essential structures for the regulation of peripheral heat dissipation and retention (Hales, 1985). Thus, the observed temperature distribution pattern on the manatees' body surfaces likely displays underlying anatomical structures and conditions, such as distribution of blood vessels, differences in skin thickness, and variations in blubber distribution. Since manatees, in contrast to other marine mammals, do not have thick insulating blubber layers (Reynolds & Lynch, 2017), potential differences in blubber distribution are more likely in lipid composition rather than thickness. Blubber lipid composition varies between species and individuals (Iverson, 2009), as well as intra-individually between body locations and seasons (Neises et al., 2021). Blubber lipid composition in Antillean manatees may have evolved to facilitate higher heat transfer as opposed to thermal insulation and heat retention. Despite the analogous distribution of blood vessels in the subspecies, apparent in the similar temperature distribution pattern, in Antillean manatees, heat dissipation from underlying blood vessels would be more apparent in increased temperatures in the overlying skin. A further possible cause for the higher T_{skin} range measured in Antillean manatees could be increased heat dissipation at the less insulated body parts at high T_{water}. This is indicated by significantly higher T_{skin} at head, flippers and in particular the tail ($p = 0.0001$) than on the trunk, found in Antillean manatees, but not in the Florida manatee data presented here.

The absence of a correlation between T_{air} and T_{skin} in the presented data can likely be attributed to the preliminary state of the data, with small sample sizes and similar T_{air} during measurements. Heat flux measurements in Florida manatees indicated a potential impact of T_{air} on heat flux (Erdsack et al., 2018). In any case, more temperature measurements in Antillean and in particular wild Florida manatees under varying environmental conditions are required. Comparative analyses of blubber lipid composition will help identify potential differences in thermal properties of blubber and their role in manatee thermoregulation. Despite limitations due to small sample size, our findings provide valuable new information and an important step towards a better understanding of thermal tolerance in manatees and the impact of environmental temperature on manatee physiology, thermoregulation and health. This knowledge is essential for the prevention and treatment of thermal stress in the threatened West Indian manatee.

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 they 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: NE was responsible for data collection, data analysis, and preparation of the initial, revised, and final manuscript, JG assisted in data collection, provided background and environmental information, and JP provided supervision, infrastructure, and technical information for this project. Both JG and JP contributed to various versions of the manuscript.

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Long-term spatiotemporal distribution, abundance, and priority areas for manatees and calves (*Trichechus manatus* Sirenia: Trichechidae) in Guatemala

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ABSTRACT

Introduction: The Antillean manatee (*Trichechus manatus manatus*) is an endangered species found throughout the Caribbean, and the coastal waters of Central and northeastern South America. Their low numbers are the result of a variety of human-related pressures. A small population of manatees has been identified in Guatemala; however, their spatial and temporal dynamics remain unclear.

Objective: To examine long-term trends in the spatiotemporal distribution and abundance of manatees in Guatemala. This included identification of priority areas for manatees including the presence of calves, assessing whether distribution areas are inside protected areas, and studying the relationship between manatee sightings and human activities.

Methods: Nine years of standardized aerial surveys were conducted along the Atlantic coast (1992, 2005-2008, 2010-2011, 2014, 2022). Quantitative approaches to detect priority areas, specifically the Kernel density estimation and the Getis-Ord Gi* statistic, were used in the spatiotemporal analysis. A Spearman rank correlation analysis tested for significant correlations between human activities, coastline topographies, and manatee numbers along coastline segments. Manatee abundance across years, survey sections, and protected areas were also examined.

Results: A total of 293 sightings and 518 manatees were observed including 476 adults (92 %) and 42 calves (8 %). Manatees were most frequently observed as solitary individuals (60 %). Most manatee (61 %) and calf (68 %) sightings occurred inside protected areas where several priority areas were identified. The two priority areas were Refugio de Vida Silvestre Bocas del Polochic (Bocas del Polochic) and Refugio de Vida Silvestre Punta de Manabique, which were identified as important manatee habitats in 1992. Bocas del Polochic had the highest manatee abundance of all protected areas ($p < 0.05$). However, a shift in manatee distribution was recorded in 2014, although the cause is unclear. No annual significant differences in manatee abundance were found over time ($p = 1.0$), but significant differences in abundance were detected between survey sections and protected areas ($p < 0.05$). Manatee numbers had positive significant correlations with ecological and human parameters. The highest correlation was between manatees and rivers ($p < 0.01$), and the weakest correlation was between manatees, motorboats, and fishing nets ($p < 0.01$).

Conclusions: The results indicate that the local manatee population remained relatively stable for over 20 years, although changes in overall distribution were noted. It is unclear if the changes are temporary or permanent. As a sentinel species, manatee distribution shifts can be used as early warnings about the health of the environment and can depict current or potential impacts on individual- and population-level animal health.

Key words: Antillean manatee; mammal; conservation; endangered species; Central America.

RESUMEN

Distribución espacio-temporal a largo plazo, abundancia y áreas prioritarias para manatíes y crías (*Trichechus manatus* Sirenia: Trichechidae) en Guatemala

Introducción: El manatí antillano (*Trichechus manatus manatus*) es una especie en peligro de extinción que se encuentra en todo el Caribe y las aguas costeras de Centro América y noreste de América del Sur. Su bajo número es el resultado de una variedad de presiones relacionadas con los humanos. Se ha identificado una pequeña población de manatíes en Guatemala; sin embargo, su dinámica espacial y temporal sigue sin estar clara.
Objetivo: Examinar las tendencias a largo plazo en la distribución espacio-temporal y la abundancia de los manatíes en Guatemala. Esto incluyó la identificación de áreas prioritarias para los manatíes y las crías, la evaluación de si las áreas de distribución se encuentran dentro de áreas protegidas y el estudio de la relación entre los avistamientos de manatíes y las actividades humanas.
Métodos: Se realizaron nueve años de censos aéreos estandarizados a lo largo de la costa atlántica (1992, 2005-2008, 2010-2011, 2014, y 2022). En el análisis espacio-temporal se utilizaron métodos cuantitativos para detectar áreas prioritarias, específicamente la estimación de la densidad Kernel y el estadístico Getis-Ord Gi*. Un análisis de correlación de rangos de Spearman probó correlaciones significativas entre las actividades humanas, las topografías costeras y el número de manatíes a lo largo de los segmentos de la costa. También se examinó la abundancia de manatíes a lo largo de los años, las secciones de estudio y las áreas protegidas.
Resultados: Se observaron un total de 293 avistamientos y 518 manatíes, incluidos 476 adultos (92 %) y 42 crías (8 %). Los manatíes se observaron con mayor frecuencia como individuos solitarios (61%). La mayoría de los avistamientos de manatíes (61 %) y crías (68 %) ocurrieron dentro de áreas protegidas donde se identificaron varias áreas prioritarias. Las dos áreas prioritarias fueron el Refugio de Vida Silvestre Bocas del Polochic (Bocas del Polochic) y el Refugio de Vida Silvestre Punta de Manabique, que fueron identificados como importantes hábitats para manatíes en 1992. Bocas del Polochic tenía la mayor abundancia de manatíes de todas las áreas protegidas ($p < 0.05$). Sin embargo, se registró un cambio en la distribución del manatí en 2014, aunque la causa no está clara. No se encontraron diferencias significativas anuales en la abundancia de manatíes a lo largo del tiempo ($p = 1.0$), pero se detectaron diferencias significativas en la abundancia entre las secciones de estudio y las áreas protegidas ($p < 0.05$). El número de manatíes tuvo correlaciones positivas significativas con parámetros ecológicos y humanos. La correlación más alta fue entre los manatíes y los ríos ($p < 0.01$), y la correlación más baja fue entre los manatíes y las lanchas motoras y las redes de pesca ($p < 0.01$).
Conclusiones: Los resultados indican que la población local de manatíes se mantuvo relativamente estable durante más de 20 años, aunque se observaron cambios en la distribución general. No está claro si estos cambios son temporales o permanentes. Como especie centinela, el cambio en la distribución del manatí se puede utilizar como advertencia temprana sobre la salud del medio ambiente y puede representar los impactos actuales o potenciales en la salud animal a nivel individual y poblacional.

Palabras clave: manatí Antillano; mamífero; conservación; especies amenazadas; Centroamérica.

INTRODUCTION

Identifying spatiotemporal patterns of distribution and abundance is important for understanding the status of a population. This information can be used to identify areas of importance and to evaluate the effectiveness of established protected areas since animals must utilize or concentrate in those areas if they have conditions that are important for their survival (e.g., food resources, habitat characteristics, etc.; Roberts et al., 2020). Spatiotemporal information is also useful for identifying whether anthropogenic activities impact animals, particularly when their habitat overlaps with these activities as it does in coastal environments (Halpern et al., 2008). Understanding these complex interactions is crucial for the design and implementation of an effective conservation management plan (de Souza et al., 2021; Yoccoz et al., 2001).

The Antillean manatee (*Trichechus manatus manatus*, Linnaeus, 1758) is an endangered species found throughout the Caribbean, and in the coastal waters of Central and northeastern South America (Self-Sullivan & Mignucci-Giannoni, 2008; Quintana-Rizzo & Reynolds, 2010). Their low numbers are the result of human-related pressures including poaching, habitat loss, entanglement in fishing/shrimp nets, chemical contamination, and watercraft collisions (Jiménez, 2002; Lefebvre et al., 1989; Reynolds & Powell 2002; U.S. Fish & Wildlife Service, 2001). Poaching, specifically, has caused an extensive reduction in manatee populations throughout most of the species' range (Lefebvre et al., 1989).

A small population of manatees has been identified in Guatemala. The most recent population estimate is 150 manatees (Quintana-Rizzo & Reynolds, 2010). Poaching resulted in the death of 20 manatees between 2003 and 2016. A more comprehensive update details 48 deaths between 1992 and 2022, although this number includes undetermined causes of mortality (Machuca-Coronado et al., 2023). Different mechanisms exist to protect the species, such as The National Strategy for the Conservation of the Manatee, whose objectives are to monitor and protect manatees, manage and protect their habitat, and promote the cultural and ecological value of the species throughout its range in the country (Herrera et al., 2004). Their recommendations include collecting long-term data on population distribution and identifying the effects of anthropogenic activities on manatees and their habitats. There are also protected areas including the "Biotopo para la Conservación del Manatí Chocón-Machas". This is the first protected area for manatees in Latin America (Lefebvre et al., 1989), although it is mostly terrestrial (Consejo Nacional de Áreas Protegidas [CONAP], 2022).

Marine protected areas (MPAs) can be effective tools for the conservation of species at risk. The protection of manatees could result in the protection of many aquatic organisms. This is because aquatic mammals typically live in large areas where, if effective protection measures are established, numerous other species could be conserved and protected, as well as the ecosystem itself (Hooker & Gerber, 2004; Quintana-Rizzo et al., 2021; Roberts et al., 2020). However, ensuring the effectiveness of MPAs requires a thorough understanding of species distribution, abundance, and habitat relationships (Hunt et al., 2020). In simple terms, it requires knowing where animals are distributed, what areas are important to them and why, how many animals are there, and how they are negatively impacted by human activities.

In the case of aquatic mammals, implementing effective protective measures poses a challenge. They typically have long-range movements, are hard to see at the surface, and have long lives, which bolster the need for multi-year studies. Some field techniques are better suited for the challenge. Aerial surveys can cover large areas in short periods, allowing for the observation of animals from above and the simultaneous examination of human activities. They have been widely used to determine manatee abundance, distribution, and habitat use in the Wider Caribbean, and are one of the most suitable field methods for monitoring sirenian populations in the world (e.g., Edwards et al., 2007; Garrigue et al. 2008; Hagihara et al., 2018; Morales et al., 2000; Olivera-Gomez & Mellink, 2002, 2005).

In Guatemala, a 9.8-hour aerial survey detected nine manatees along the Atlantic coast in 1991 (Ackerman, 1991). A more comprehensive set of monthly aerial surveys were used to generate the first population estimate of 53 manatees in 1992 (Quintana-Rizzo, 1993). In 2005 and 2014, regional surveys were conducted with the objective to examine the manatee numbers in the Belize-Guatemala-Honduras and Mexico-Belize-Guatemala regions (Quintana-Rizzo, 2005a, b). Annual aerial surveys were conducted between regional surveys (Machuca-Coronado & Quintana-Rizzo, 2009; Quintana-Rizzo & Machuca-Coronado, 2008), but none of the studies looked at trends in spatiotemporal patterns of manatee distribution and abundance. They did not examine the relationship between manatee sightings and human activities even though earlier studies recognized that manatees must travel through areas of high boat traffic to move between preferential habitats. Here we present a comprehensive assessment of the data collected during all of the aerial surveys conducted since 1992 and a depiction of long-term trends in the spatio-temporal distribution and abundance of manatees in Guatemala. Priority areas, or areas of particular importance for conservation, for the species were identified including the presence of calves, a determination of whether they are using protected areas, and the co-occurrence of manatee sightings and human activities. The results fill a critical gap in knowledge to ensure that the protection of this endangered species is based on a foundation of the most inclusive data available.

METHODS

Study area: The study area included the entire Atlantic Coast of Guatemala, located along the state of Izabal (Fig. 1.1). It is bordered

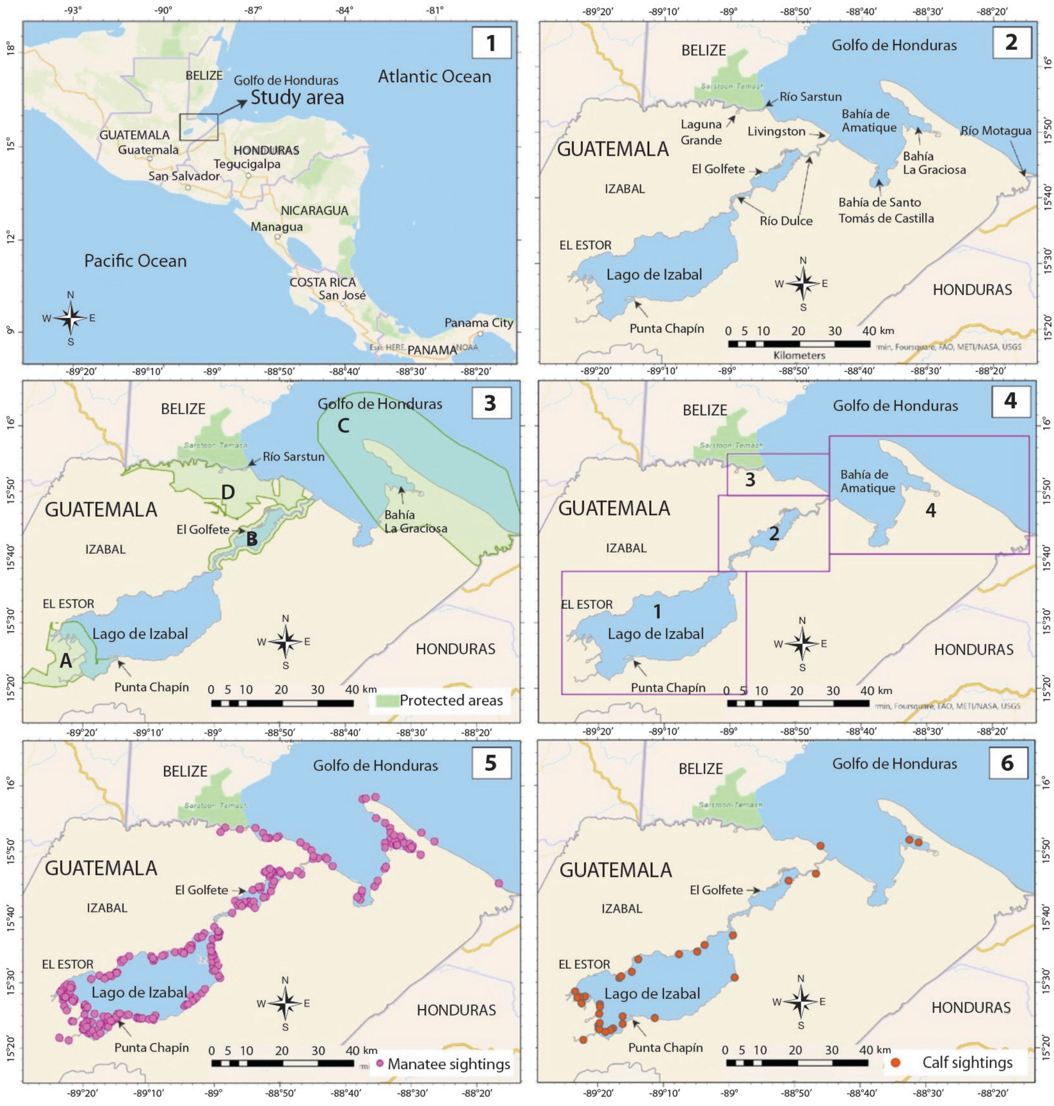

Fig. 1. (1) Location of the study area along the Atlantic Coast of Guatemala including (2) important geographical points, (3) protected areas, (4) survey sections, and location of (5) manatee sightings and (6) calf sightings between 1992 and 2022. Protected areas evaluated: A) Refugio de Vida Silvestre Bocas del Polochic, B) Parque Nacional Río Dulce, C) Refugio de Vida Silvestre Punta de Manabique, and D) Área de Usos Múltiples Río Sarstún.

to the north by the Caribbean Sea and to the east by the Gulf of Honduras. The study area encompasses distinctive aquatic ecosystems. Lago de Izabal is the largest freshwater lake in Guatemala (area = 717 km^2; Fig. 1.2). It connects to the Gulf of Honduras in the Caribbean Sea by Río Dulce. It has an average depth of 12 m and a maximum depth of 18 m (Arrivillaga, 2002). The southwestern part of the lake includes the protected area "Refugio de Vida Silvestre Bocas del Polochic" (Bocas del Polochic, Fig. 1.1). This RAMSAR site is a wetland of great ecological importance, providing food for more than 250 species of birds, both resident and migratory (Fundación Defensores de la Naturaleza, 1997). Midway between the lake and the sea is Río Dulce, a tidal river that broadens into a large and shallow area (4.5 m depth) known as El Golfete (Brinson et al., 1974). Both Río Dulce and El Golfete are part of a protected area called "Parque Nacional Río Dulce" (Fig. 1.3), which has terrestrial and aquatic zones (CONAP, 2005).

The Atlantic Coast forms a semi-enclosed bay called Bahia de Amatique. This bay includes a complex ecosystem of coastal lagoons, marshes, and swamps influenced by tides and the Río Dulce-El Golfete riverine systems. It is part of the protected area "Refugio de Vida Silvestre Punta de Manabique" (Punta de Manabique), a RAMSAR site located in the Gulf of Honduras (Fig. 1.3). Punta de Manabique is one of the most important ecosystems in the country as it is composed of multiple marine, coastal, and terrestrial areas (Yañez-Arancibia et al., 1999). Río Sarstún is another important site, located on the borderline between Guatemala and Belize. Two protected areas are partially located within this basin, the "Área de Usos Múltiples Río Sarstún", a RAMSAR site, located in Guatemala, and the Sarstoon-Temash National Park located in Belize (Fundación para el Ecodesarrollo y la Conservación [FUNDAECO], 2005) (Fig. 1.3).

Survey methods: Standardized aerial surveys were selected as an efficient and cost-effective method to collect data on the distribution and number of manatees (Reynolds et al., 2012). In 1992, April 2005, and 2022, aerial surveys were conducted by two observers who sat on each side of the aircraft and maintained their position throughout the survey. The 1992 surveys covered the center of Lago de Izabal, although since manatees were never sighted there, this portion of the lake was not included in the analysis or future surveys. The May 2005, 2006-2011, and 2014, surveys involved three observers. Two observers sat in the rear seats, while a pilot and another observer sat in the front seats. The front observer (EQR) was the more experienced, participated in all surveys, and was responsible for confirming total manatee numbers. Each observer scanned an area approximately 400 m wide (Olivera-Gomez & Mellink, 2002, 2005). Surveys were conducted parallel to the coast at altitudes of 150 to 200 m and at an average airspeed of 160 km/h from a Cessna 337 or Aero Commander (1992), or a Cessna 335 and Cessna 206 (2005-2022). A GPS continuously recorded the survey path during each survey.

The study area was divided into four sections: Lake Izabal, El Golfete, Livingston-Río Sarstún, and Livingston-Río Motagua (Fig. 1.4). A survey of the entire study area generally took a day, however in cases of unfavorable weather conditions some surveys were conducted over two days. A given section of the survey area was fully surveyed on the same day. Each sighting was considered to be independent because it was impossible to determine whether the manatees moved to different sections of the survey area on different days. When manatees were sighted, the aircraft circled to obtain the most accurate count (number of individuals) possible (Lefebvre & Kochman, 1991). Manatees within close proximity of one another and displaying similar behavior were grouped as one sighting. For each sighting, individuals were classified as adults or calves. Calves were defined as smaller animals up to 1/3 of the adult size (Hartman, 1979) and closely associated with a larger manatee (Irvine, 1982; Reynolds & Wilcox, 1994). One or more manatees were considered a group (Morales-Vela et al., 2000).

Observers recorded the number of manatees (calves and non-calves) and the location of each sighting. For each section of the survey area, environmental conditions such as the percentage of cloud cover and water surface conditions were estimated and recorded by the observers. To ensure that visibility was acceptable for efficient spotting of manatees, surveys were conducted only when sustained winds were < 10 knots and surface conditions scored ≤ 3 on the Beaufort wind scale.

Data analysis: The data were divided into two sets based on the available survey effort (e.g., total kilometers) at the time of the analysis. Data with no survey effort (DNS) included surveys conducted in 1992 and April 2005. Data with survey effort (DWS) included surveys conducted from May 2005 on.

Manatee groups: size, calf presence, and sighting distance from shore. Group size across years were examined using a two-tailed Kruskal–Wallis test, and multiple Mann-Whitney U post hoc tests were used to compare differences between years. The 2-tailed statistical tests were conducted using the SPSS 28.0.1.1 package (2021) at a significance level of 0.05. The percentage of groups with calves inside and outside of protected areas was reported as descriptive statistics (mean ± standard error). Distance of manatee sightings and calf sightings from shore were calculated in ArcGis Pro 3.0.0.

Spatial distribution: two metrics were used to quantify the spatial distribution of manatees and identify priority areas: (1) a hotspot analysis, and (2) Kernel density estimation (Roberts et al., 2020). The first metric allows the use of DWS data as sightings per unit effort, and therefore, the evaluation of sections with uneven coverage. Thus, statistically, a priority area was defined by this study as one that was identified as either a "hot spot" or "cold spot", which are areas of statistically significant spatial clustering (see Hotspot analysis), or an area where the manatee density is more than 85 % of the estimated density in a given year (see Kernel density analysis).

A Hotspot analysis tests for statistically significant spatial clustering using the Getis-Ord Gi* statistic (Getis & Ord, 1992), which determines the spatial clustering of grid cell values that are higher (hot spot) or lower (cold spot) than is expected by random distribution. The study area was divided into 4 km x 4 km grid cells resulting in 246 cells. A 4 km width was chosen because it covered the survey flight path, the meandering sections of the study area, and the mouths of large and medium rivers flowing into the coastline (also referred to as secondary rivers). Medium and large rivers were defined based on the contribution of their streams as >10 to 100 m^3/s and > 100 to 500 m^3/s, respectively (Spillman et al., 2000). Within each grid cell, the total number of manatees and the total length of survey tracks were calculated. If no sightings occurred in a grid cell that was surveyed, the grid cell was attributed a value of zero, but the cell was considered part of the survey effort. A spatial map was created at three levels of confidence (99 %, 95 %, and 90 %), and all clusters that were within the 90 % confidence level were considered hot spots or cold spots.

The second metric used was the Kernel density estimation to identify core activity areas. This nonparametric method estimates density curves, where each observation is weighted by the distance from a central value or core, also named kernel, generating a smoothed surface that describes a likely distribution at a given time (Worton, 1989). The analysis allows the use of data regardless of effort (e.g., both DNS and DWS), and thus, the examination of temporal patterns across the 8 years of surveys. A buffer with a 4 km extension over the coastline was created to include river mouths and delimit the analysis boundaries. The 4 km extension is of a similar size to one side of the grid cells used in the Hotspot analysis. Predicted density rasters were generated using a cell size of 1 km^2. All spatial analyses were conducted using ArcGis Pro 3.0.0.

Relationship between sightings, coastline features, and human activities: A Spearman rank correlation analysis tested for significant correlations between human activities, coastline features such as rivers, and manatee numbers per segment (Alves et al., 2013) in the study area and in each protected area. This correlation analysis was chosen due to the non-linear nature of relationships and the non-normality and heteroscedasticity of most distributions (Alves et al., 2013). The coastline was divided into 4 km segments for a total of 122 segments. For each analysis and segment, the occurrences of five ecological and human parameters were examined: (1) manatee numbers, (2) fishing activity (fishing nets), (3) tourism activity (transiting motorboats, kayaks), (4) commercial traffic (merchant or cargo ships), and (5) rivers. Fishing activity was recorded during the 2006-2008 and 2010 surveys, and tourism activities were recorded during the 2006-2011 surveys by the left rear observer. Since the final sample size of the commercial traffic parameters was small and confined to a specific area, we only reported descriptive statistics. The fifth parameter was based on cartographic and bibliographic analyses and it included the presence of large/medium size rivers in each segment. These are secondary rivers as sections of the coast such as El Golfete and Río Sarstún are part of the main rivers. Thus, for the correlation between manatees and rivers, one protected area was excluded from the analysis (Area de Usos Múltiples Río Sarstún) because no secondary rivers flow into the portion of the main river (Río Sarstún) that was surveyed. All analyses were performed using SPSS 28.0.1.1 package (2021) at a significance level of 0.05.

Manatee abundance: Abundance was measured as density (Krebs, 2014) or the number of manatees per square kilometer to account for incomplete surveys (e.g., the entire study area was not surveyed but individual surveyed sections were fully covered) and an unequal number of surveys per section. Periods of circling were excluded from this and all other analytical calculations. DWS data were used to examine manatee abundance across years, survey sections, and protected areas using a non-parametric Kruskal-Wallis test. Years with incomplete coverage (e.g., 2010 and 2022) of the study area were excluded from this analysis. Multiple Mann-Whitney U post hoc tests were used to compare differences in abundance using the SPSS 28.0.1.1 package (2021) at a significance level of 0.05. Boundaries of the protected areas were extracted from the World Database of Protected Areas (United Nations Environment Programme World Conservation Monitoring Centre & International Union for Conservation of Nature [UNEP-WCMC & IUCN], 2021).

RESULTS

Distribution of sightings: A total of 293 sightings were recorded between 1992 and 2022. This included 518 manatees, 476 adults (92 %) and 42 calves (8 %) (Table 1). Of the 293 sightings, 17 % included groups with calves. Further, 61 sightings were part of the DNS data and 232 sightings were part of the DWS data. Manatees were most frequently observed as solitary individuals (63 %), but a group of 12 individuals was sighted on one occasion. This large group included a calf. Calves were typically sighted in groups of 3 ± 0.30 (standard error) while the mean group size for all manatees, regardless of age class, was 2 ± 0.09 (Fig. 2). Significant differences in group size were found among years (H = 18.02, d.f. = 8, $p = 0.02$). Group size was smaller in 2005 than in 2007, 2014 ($p < 0.05$); it was also smaller in 2006 than in 2007 and 2014 ($p < 0.05$). However, group size was not significantly different between 2005 and 2006, and among all other years ($p > 0.05$).

Analysis of sighting distribution from shore showed that manatees were most frequently sighted at distances ranging from 0.02 km to 2.6 km from the coast. Survey areas are depicted in Figure 1.3. Manatees were most frequently sighted at distances ranging from 0.02 km to 2.6 km from the coast. The mean and median distances from shore were 6.5 km and 5 km, respectively, followed by a continuous

Table 1

Total number of manatees (including adults and calves) sighted per month, protected area, and survey section between 1992 and 2014 along the Atlantic coast of Guatemala.

Year	Month	No. of manatees in study area			No. of manatees in protected areas				No. of manatees in survey sections			
		Total	Adults	Calves	RVSBP	PNRD	AUMRS	RVSPM	1	2	3	4
1992	January	15	13	2	8	1	0	0	14	1	0	0
	March	28	24	4	19	0	0	0	21	0	7	0
	April	15	13	2	7	4	2	1	7	4	2	2
	May	15	15	0	3	2	0	1	3	2	0	10
2005	April	39	36	3	14	6	0	6	22	6	2	9
	May	49	43	6	15	2	0	8	35	2	2	10
2006	July	27	26	1	21	4	0	NS	21	4	2	NS
	October	53	48	5	8	7	0	10	25	7	6	15
2007	January	52	48	4	13	6	0	7	39	6	0	7
	March	38	35	3	5	7	2	20	8	7	2	21
2008	February	40	39	1	11	12	0	NS	28	12	0	NS
2010	May	26	23	3	26	NS	PC	PC	26	NS	PC	PC
2011	March	52	48	4	14	7	1	5	40	3	7	2
2014	May	45	43	2	4	7	0	10	20	14	1	10
2022	August	24	22	2	1	5	NS	NS	19	5	NS	NS
	Total	518	476	42	169	70	5	68	328	76	31	86
	Range	12-53	12-48	0-6	4-26	0-7	0-2	0-10	3-40	0-14	0-7	0-21
	Percentage	100%	92%	8%	54%	22%	2%	22%	63%	14%	6%	17%

PC = poor conditions, NS = no surveyed. RVSBP = Refugio de Vida Silvestre Bocas del Polochic, PNRD = Parque Nacional Río Dulce, RVSPM = Refugio de Vida Silvestre Punta de Manabique, and AUMRS = Área de Usos Múltiples Río Sarstún. Survey sections: (1) Lago de Izabal, (2) El Golfete, (3) Livingston-Río Sarstún, and (4). Livingston-Río Motagua.

decrease in the sighting frequency toward open waters (Fig. 2).

Spatial distribution: Manatees were sighted throughout the study area, but they were unevenly distributed (Fig. 1.3, Fig. 1.4). Most manatee and calf sightings occurred inside protected areas (60 % and 68 %, respectively). Half of the groups with calves were observed in Bocas del Polochic, but in the overall Lago de Izabal, 82 % of groups with calves were sighted. No calves were observed in the coastal waters from Rio Sarstun to Rio Motagua.

A series of priority areas were identified and all occurred inside protected areas. This included three hot spots or areas of high clustering and two cold spots or areas of low clustering (Fig. 3). A hot spot was detected in the protected area Punta de Manabique while a large cold spot and two large hot spots were detected in Lago de Izabal (Fig. 3). In Lago de Izabal, one of the hot spots overlapped by approximately 1.5 km^2 with the protected area Bocas del Polochic. This protected area also overlapped with the cold spot by more than 50 km^2. A small cold sport was identified in the north side of Parque Nacional Río Dulce in El Golfete. No hot or cold spots were identified in other protected areas.

The location, number, and extension of predicted core activity areas for manatees varied across years (Fig. 4). However, some general patterns emerged. Lago de Izabal is a priority area for manatees as one or more core activity areas were detected there in each of the nine years of surveys. Within the lake, the southern corner, which covers the entire aquatic zone of Bocas del Polochic and Punta Chapin, was

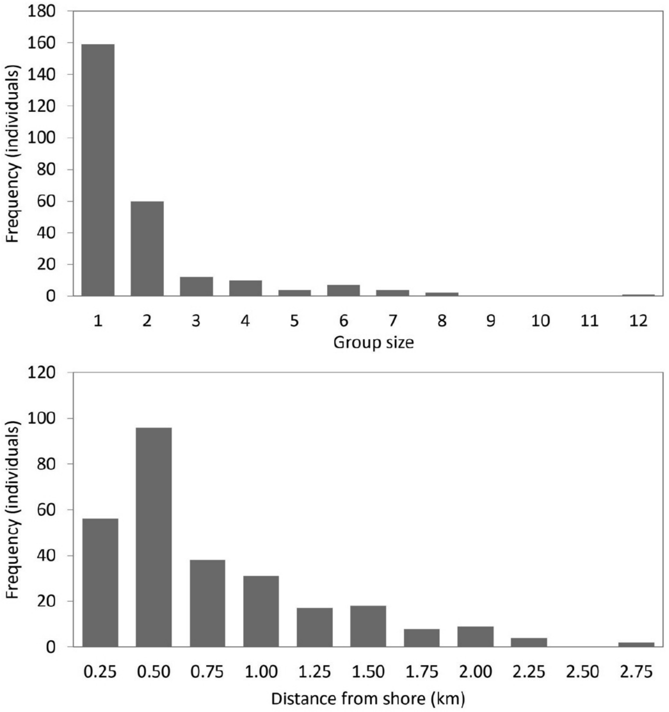

Fig. 2. Frequency distribution of group sizes of manatees and their distance from shore in Guatemala.

consistently identified as a core activity area, although this pattern shifted to the northern shoreline of the lake in 2014 and continued in 2022 (Fig. 4). Another core activity area was detected inside Punta de Manabique, near Bahia La Graciosa. In Parque Nacional Río Dulce, a core activity area was identified but only in half of the nine years of surveys (Fig. 4).

Correlations of coastal features and human activities: For the entire study area, the Spearman rank correlation analysis identified significant correlations between manatees and ecological and human parameters (Table 2). The first and highest positive significant correlation was found between manatees and rivers ($r_s = 0.67$, $p < 0.01$). A similar significant correlation was detected in the inshore protected areas (Bocas del Polochic and Parque Nacional Río Dulce) with the highest correlation ($r_s = 1.00$) being between manatees and rivers of a protected area (Bocas del Polochic). Additionally,

Table 2

Spearman correlation (r_s) between numbers of manatees, selected coastal features, and human activities in the study area and individual protected areas.

Parameters	Study area	Protected Areas			
		RVSBP	PNRD	RVSPM	AUMRS
Coastal features (rivers)	$r_s = 0.67, p < 0.01$*	$r_s = 0.99, p < 0.01$*	$r_s = 0.52, p < 0.01$*	NA	$r_s = -0.07, p = 0.75$
Fishing nets	$r_s = 0.04, p = 0.40$	$r_s = -0.06, p = 0.54$	$r_s = 0.27, p < 0.05$	NA	NA
Tourism activity					
Motorboats	$r_s = 0.07, p < 0.05$*	$r_s = 0.19, p < 0.05$*	$r_s = 0.19, p < 0.05$*	$r_s = -0.15, p = 0.59$	$r_s = 0.14, p = 0.15$
Kayaks	$r_s = -0.40, p = 0.26$	$r_s = 0.06, p = 0.48$	$r_s = -0.15, p = 0.87$	$r_s = -0.31, p = 0.26$	$r_s = -0.06, p = 0.54$

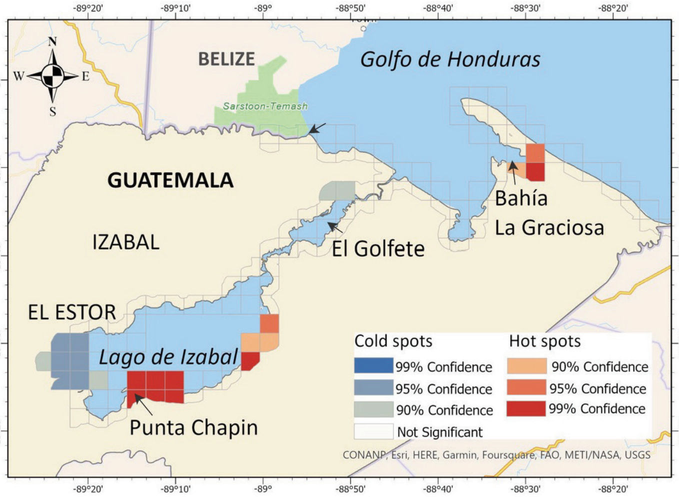

Fig. 3. Hotspots and cold spots of manatees based on sightings per unit effort. Additional details of the study area are shown in Figure 1.

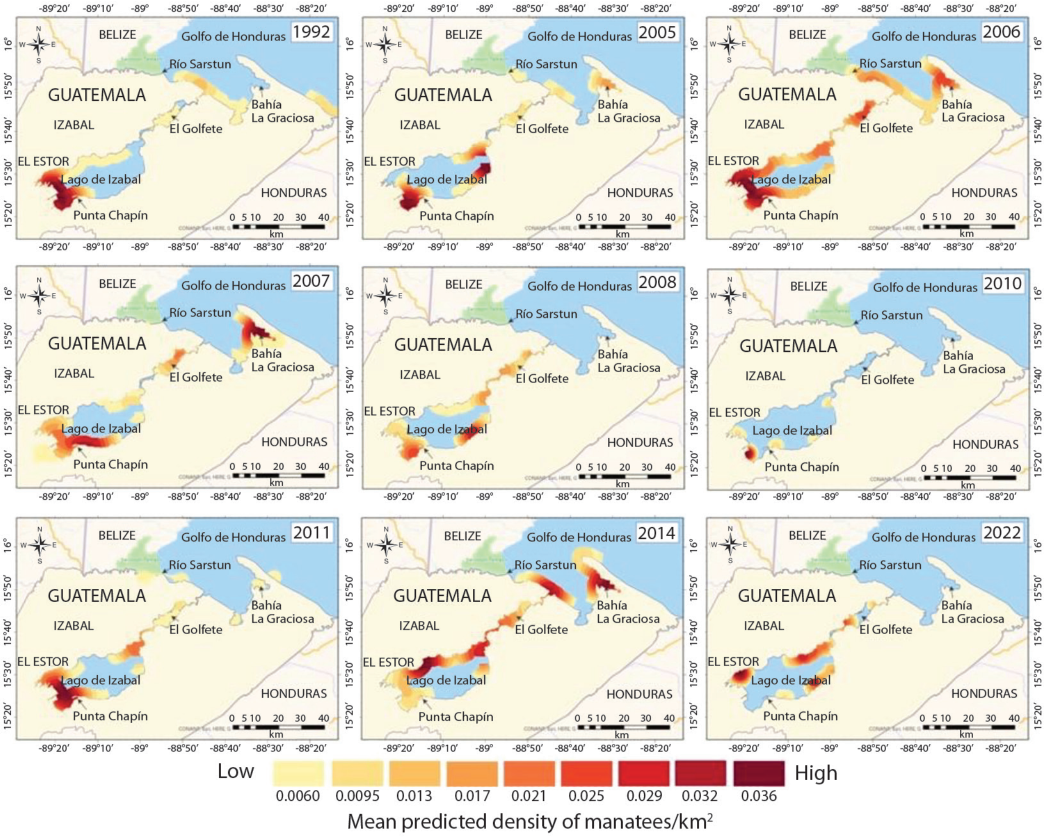

Fig. 4. Interannual variation in core activity areas of manatees in Guatemala between 1992 and 2022 using Kernel estimates. Each section of the study area was surveyed except in 2008 (no survey: Livingston-Río Motagua), 2010 (no survey: El Golfete; poor weather conditions: Livingston-Río Motagua, Livingston-Río Sarstún), and 2022 (no survey: Livingston-Río Motagua, Livingston-Río Sarstún).

manatee sightings were weakly positively correlated with the presence of motorboats ($p < 0.01$) in these two protected areas and with fishing gear ($p < 0.05$) in Parque Nacional Rio Dulce (Table 2). No other correlations were found for the entire study area or in the protected areas.

Commercial traffic in the form of merchant or cargo ships was only observed in the Livingston-Río Motagua section. Within this section, the commercial traffic happened near the Santo Tomas de Castilla port where 10 cargo ships were observed during surveys. A total of eight manatees were sighted in this area.

Manatee abundance: Manatee abundance was estimated for the entire study area, each surveyed section, and each protected area. For a given survey, the mean abundance was 0.10 ± 0.01 manatees/km^2 (range = 0.05 – 0.26 manatees/km^2) and the overall mean calf abundance was 0.01 ± 0.002 manatees/km^2 (range = 0.00 – 0.03 calves/km^2). No annual significant differences in manatee abundance were found among 2015 (May), 2006, 2007, 2008, 2011, and 2014 ($H = 0.82$, d.f. = 6, $p = 0.99$) or the years with complete surveys of the study area.

Manatee abundance was significantly different among sections surveyed and among protected areas ($p < 0.01$). In the first case, no statistical difference in manatee abundance was found between Lago de Izabal and Livingston – Río Motagua ($U = 9.28$, $p = 0.06$). Mean abundance was 0.18 ± 0.02 manatees/km^2 in Lago de Izabal and 0.10 ± 0.02 manatees/km^2 in Livingston – Río Motagua (Fig. 5). However, manatee abundance was significantly different between Lago de Izabal and El Golfete ($U = 12.20$, $p = 0.006$) and between Lago de Izabal and Río Sarstún ($U = 15.20$, $p < 0.001$). Further, manatee abundance was not statistically different among El Golfete and the two more coastal sections (Livingston – Río Sarstún, Livingston – Río Motagua; $p > 0.05$). In these three sections, mean abundance varied between 0.06 and 0.10 manatees/km^2 (Fig. 5).

In the case of the protected areas, Bocas del Polochic (Lago de Izabal) had the highest manatee abundance (1.52 ± 0.03 manatees/km^2) of all protected areas ($p < 0.05$; Fig. 5). Manatee abundance was not statistically different among the other three protected areas ($p > 0.10$).

DISCUSSION

This study comprises the largest effort to quantify and correlate the distribution of manatees along the Atlantic coast of Guatemala, yielding important insights into the local status of the species. Our results show that manatees were nonuniformly distributed along the surveyed area with most sightings being recorded in protected areas (60 %).

Spatiotemporal distribution and priority areas: Our results agree with other studies that found that manatees tend to be located in areas near freshwater sources (Alvarez-Alemán et al., 2017; Castelblanco-Martínez et al., 2018; Favero et al., 2020; Lefebvre et al., 2001; Marsh et al., 2001; Olivera-Gomez et al., 2022; Powell & Rathbun, 1984; Rathbun et al., 1990), with shallow waters (Hartman, 1979; Olivera-Gomez & Mellink, 2005), close to shore (Olivera-Gomez & Mellink, 2005), where aquatic vegetation (Arrivillaga & Baltz, 1999; Poll, 1983; Yañez-Arancibia et al., 1999) known to be part of the species diet (Allen et al., 2018; Alves et al., 2013; Hurst & Beck, 1988) exists, and where there is minimum motorboat traffic and little to no coastal human development. Priority areas were identified in the southern corner of Lago de Izabal, from Punta Chapin to Bocas del Polochic, and the innermost corner of Punta de Manabique (Bahia de Amatique). The southern corner of Lago de Izabal has been an important area for manatees for nearly 30 years (Machuca-Coronado & Quintana-Rizzo, 2011, 2014; Quintana-Rizzo, 1992), although a shift in manatee distribution occurred in 2014. Within the southern corner, manatees clustered in small groups (1-2 manatees) in the northern section and in large groups (up to 8 manatees) in the southern section (Punta Chapin). A high percentage of sightings that included calves was also reported there (50 %) and in coastal waters of the lake (82 %). This distribution may reflect

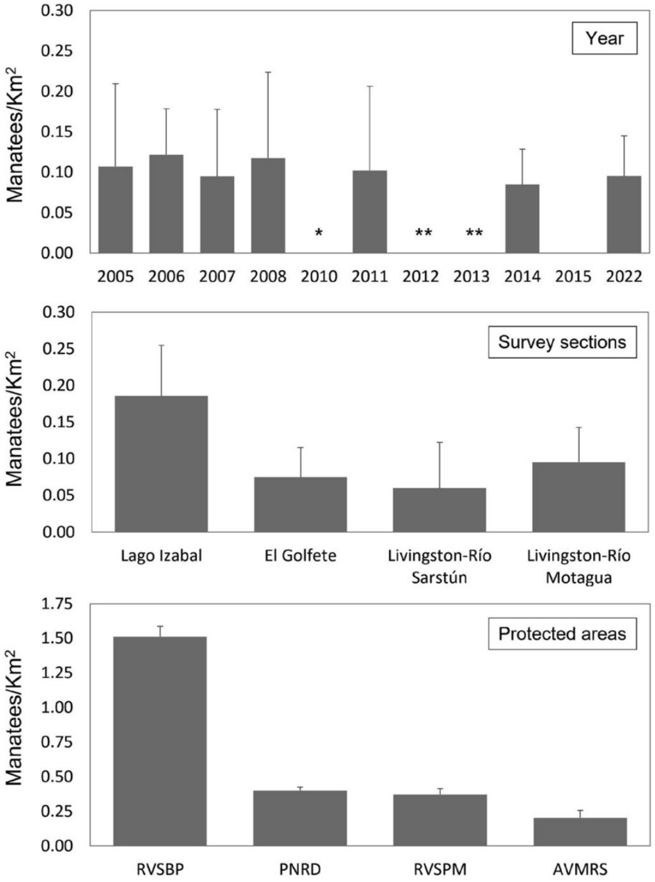

Fig. 5. Relative abundance of manatees for years 2005-2022 with survey effort of the entire study area, each section, and protected areas in Guatemala. Note: * = only one section was completed; thus, mean was not calculated, ** = no aerial surveys were conducted. RVSBP = Refugio de Vida Silvestre Bocas del Polochic, PNRD = Parque Nacional Río Dulce, RVSPM = Refugio de Vida Silvestre Punta de Manabique, and AVMRS = Área de Usos Múltiples Río Sarstún.

habitat requirements specific to this social unit or demographic group (Gannon et al., 2007).

The southern corner of the lake is probably the most protected section for manatees of Lago de Izabal and the entire Atlantic coast, but the area is not free of human activities and interactions. Illegal gillnets of up to 4 km long are commonly sighted there (H. A. Garcia personal communication). An orphaned manatee was entangled in one of those nets in 2014. The calf was rescued from the net but died after a failed rehabilitation attempt (Machuca-Coronado & Quintana-Rizzo, 2014). Further, changes in land use and development have altered anthropogenic nutrient inputs resulting in an increase of raw sewage, strip mining flow, and agricultural fertilizers into the lake (Obrist-Farner et al., 2009). This has negatively impacted the water quality and overall health of the ecosystem. In the southern corner, water quality was classified as medium and good based on the National Sanitation Foundation Water Quality Index (a 100-point scale from excellent, good, medium, bad, to very bad; Aguirre Cordón et al., 2006). The lake has also started to undergo cultural eutrophication, which contributes to frequent harmful algal blooms, the proliferation of invasive species such as *Hydrilla verticillata*, and a decline in fish abundance (Obrist-Farner et al., 2009). Additionally, predictive models indicate that the temperature of the coastal surface waters will increase by 2 – 5 °C by 2100 and that this will be a biological stressor that could alter or harm the aquatic ecosystem (Marchese, 2015).

The 2014 shift in manatee distribution could have been caused by multiple factors. Since manatees are considered sentinel species of the overall health of the ecosystem (Bonde et al., 2004; Bossart, 2011), distributional changes could be used as early warnings about current or potential negative impacts on individual-level and population-level animal health; Bossart, 2011). Those warnings could help identify environmental stressors that could ultimately affect human health associated with the oceans (Bossart, 2011). The fact that a similar pattern was observed in 2022 suggests that the change in manatee distribution is permanent, and future studies will help to determine this. A multispecies approach could be useful in this case since other species like macroinvertebrates are also biological indicators of an aquatic ecosystem in stress (Roldán-Pérez et al., 2016).

Protected areas do not appear to be equally important for manatees as indicated by differences in the number, location, and temporal presence of priority areas. For example, the innermost section of Punta de Manabique is a priority area but its extension and persistence varied over time. No priority areas were identified in Área de Usos Múltiples Río Sarstún while they were only detected in some years in Parque Nacional Río Dulce. There is a high presence of human activities in the former. The demand for land leases in mangrove areas has increased considerably in Parque Nacional Río Dulce, which saw the construction of large tourist complexes (Arrivillaga, 2003). Parque Nacional Río Dulce is one of two coastal areas in the entire country where the transformation of mangrove areas by coastal development and tourist infrastructure is more evident (Arrivillaga, 2003). Notably, we recorded the highest number of motorboats (64 %) and kayaks (68 %) there along the entire Atlantic coast. Manatees that use this area must navigate through a busy intersection of fast-moving vessels, kayaks, and fishing gear. They appear to stay within the coastal waters as suggested by their high proportion of sightings (80 %) within a kilometer from the coast. Females with calves must pay attention in this busy environment for the safety of the calf, and few appear to remain there as suggested by the small percentage of calf sightings (6 %) in this protected area.

Manatee abundance including calf presence: Our manatee abundance estimates are within the lower confidence limit of abundance estimates reported in 1992 (Quintana-Rizzo, 1992). Nevertheless, early abundance estimates were calculated based on Schaeffer et al. (1986) and thus, differences in analytical procedures could account for the observed differences. New population modeling techniques based on aerial survey data could provide more reliable population estimates (e.g., Martin et al., 2015). However, those models require different collection protocols than the ones used in this survey, and observers that are trained in those protocols. A preliminary survey was conducted in this way, and there are plans to apply one of those models to the results.

Mean manatee abundance was comparable to that reported in other parts of Mesoamerica including Bahia de Chetumal, although the study was conducted at a different time of the year (Morales-Vela & Olivera-Gómez, 1994). The overall percentage of calf sightings (12 %) and number of calves sighted (8 %) were also comparable to other parts of Mesoamerica (Callejas-Jiménez, 2021; Edwards et al., 2014; Morales-Vela et al., 2000) and the Caribbean (Alvarez-Alemán et al., 2017).

Mean manatee abundance was not significantly different from 2005 to 2014. This represents approximately 10 years of relative stability, which is significant for a small population of an endangered species. Yet at least 20 manatees died around the same time (2003-2016; Machuca-Coronado & Corona Figueroa, 2019) representing 13 % of a minimum population of 150 manatees (Quintana-Rizzo & Reynolds, 2010). This begs the question of how the population could appear to remain stable at this mortality rate, which is likely underestimated. Possible explanations include that the population is higher than 150 manatees or that the survey methodology does not account for the re-sighting of individual animals (i.e., one individual could be sighted twice in the same survey). Alternatively, or in conjunction with this, there could be an influx of manatees moving to and from neighboring countries such as Belize, which has the largest manatee population of Antillean manatees in the Caribbean (Morales-Vela et al., 2000; O'Shea & Salisbury, 1991), and Honduras. This influx could counterbalance the effects of mortality. However, this assumes that mortality remains at a sustainable rate, which is unclear. Better population estimates and record keeping of mortality events are needed to understand the dynamics of the Guatemalan manatee population.

Conservation and management: The identification of priority areas for manatees should be used to better focus management efforts in the future. This level of information is needed to design and evaluate the effectiveness of protected areas for the conservation of the species. Manatee protection needs to be strengthened by ensuring that priority areas and critical resources (e.g., freshwater sources, feeding areas) are given special management consideration. It is urgent that the impact of human activities, such as those caused by the cultural eutrophication of Lago de Izabal, are minimized, and controlled. Similar actions are needed to minimize the use of fishing nets in protected areas. Motorboat speed restrictions need to be implemented to increase safety for manatees traveling through and between protected areas, considering the movement of animals between the inshore waters of the lake and the marine environment. This 150 km (round-trip) movement is well within the species capabilities since manatees can conduct 600-km$^+$ round-trip migrations (Deutsch & Barlas, 2016). Manatee movements among different parts of the coastline highlight the importance of connectivity between protected areas. The most effective conservation and management actions require a comprehensive understanding of animal movement patterns and habitat selection as depicted by this study.-

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: E.Q.R conceived the original idea for this manuscript. E.Q.R, O.H.M.C, and H.A.G. expanded and agreed on the details of the publication. All authors collected data and helped secure funding for aerial surveys and related field work. E.Q.R. (lead) and O.H.M.C. processed and analyzed the data. E.Q.R. lead the manuscript writing with contributions to drafting, critical review, and editorial input from O.H.M.C. and H.A.G.

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SUPPLEMENT • MANATEES

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Characteristics and spatial identification of Antillean manatee (*Trichechus manatus manatus* Sirenia: Trichechidae) strandings in Guatemala

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ABSTRACT

Introduction: Marine mammal strandings can be used to determine the impacts of anthropogenic activity on the species survival, population health, and levels of environmental pollution in ecosystems. In addition, these data can help design conservation and management strategies as well as identify priority areas for the species. The West Indian manatee (*Trichechus manatus*) is listed as endangered throughout its range. In Guatemala, it is distributed along the Caribbean coast. Anthropogenic activities appear to be having a direct pressure on the species, affecting the status of the population.

Objective: The general purpose of this study was to analyze the 30 years of documented manatee stranding reports available for Guatemala. Two specific objectives were defined to: 1) conduct a spatial analysis of manatee strandings to identify the areas of high stranding concentration, and 2) determine whether there were sex differences in the number of strandings.

Methods: Characteristics and areas of concentration of manatee strandings were described along the Caribbean coast between 1992 to 2022. Sites with the highest probable density of strandings were identified using a Kernel density analysis. We compared the number of stranding events that occurred in each protected area that included manatees in their management plans and each municipality of Izabal. We determined whether there were sex differences in the number of stranded manatees. We use the potential biological removal to estimate the maximum number of manatees that can be removed from the population due to anthropogenic causes without negatively impacting species survival.

Results: Forty-three manatee stranding events were recorded throughout the species' range in Guatemala (48 total individuals). The sites with the highest predicted density of strandings were Santo Tomás de Castilla bay, followed by Lake Izabal (including the limits of the Refugio de Vida Silvestre Bocas del Polochic) and Parque Nacional Río Dulce. The majority of the cases were registered in protected areas (60 %). Estimates of potential biological removal were equivalent to less than one manatee per year, which was exceeded by the annual average of stranded manatees recorded (three manatees).

Conclusions: Anthropogenic activities cause mortality of manatees in this region, despite the implementation of protected areas. Poaching was identified as the main cause of manatee mortality in Guatemala. The implementation of management strategies focused on minimizing threats to the species, based on the application of environmental legislation and environmental awareness, is essential. The creation and implementation of a protocol for the stranding of marine mammals on the Caribbean coast of Guatemala is necessary, in order to obtain standardized records of these events and conduct rescue efforts and releases when possible.

Key words: marine mammals; mortality; conservation; poaching; protected areas.

RESUMEN

Características e identificación espacial de los varamientos del manatí antillano (*Trichechus manatus* Sirenia: Trichechidae) en Guatemala

Introducción. Los registros sobre varamientos de mamíferos marinos pueden utilizarse para determinar los impactos de la actividad antropogénica en la sobrevivencia de la especie, la salud de las poblaciones y los niveles de contaminación ambiental en los ecosistemas. Además, estos datos pueden utilizarse para diseñar nuevas estrategias de conservación y manejo en las áreas prioritarias para la especie. El manatí antillano (*Trichechus manatus manatus*) está catalogado en peligro de extinción en todo su rango de distribución. En Guatemala, se distribuye a lo largo de toda la costa caribeña. Las actividades antropogénicas están ejerciendo una presión directa sobre la especie, afectando el estado de las poblaciones.

Objetivos. El objetivo general de este estudio fue analizar 30 años de información sobre los eventos de varamiento de manatíes en Guatemala. Dos objetivos específicos fueron definidos: 1) realizar un análisis espacial sobre las áreas con mayor concentración y densidad de varamientos de manatíes, y 2) determinar diferencias en el número de manatíes varados con base en datos sobre el sexo.

Métodos. Se describieron las características y áreas de concentración de varamientos de manatíes registrados de 1992 a 2022, en la costa caribeña. Se identificaron los sitios con mayor densidad probable de varamientos utilizando el análisis sobre densidad de Kernel. Se comparó el número de eventos de varamientos ocurridos en cada área protegida y municipio de Izabal donde se distribuye la especie. Se determinaron diferencias en la edad, sexo, y causa de muerte en los varamientos. Además, se utilizó la eliminación biológica potencial para estimar el número máximo de manatíes que pueden ser removidos de la población debido a causas antropogénicas, sin afectar negativamente la supervivencia de la especie.

Resultados. Se registraron 43 eventos de varamiento de manatíes en todo el rango de distribución de la especie en Guatemala (48 individuos en total). Los sitios con mayor densidad prevista de varamientos fueron en la Bahía Santo Tomás de Castilla, seguido del Lago de Izabal (incluyendo los límites del Refugio de Vida Silvestre Bocas del Polochic) y el Parque Nacional Río Dulce. La mayoría de los eventos se registraron en áreas protegidas (60 %). Las estimaciones sobre la eliminación biológica potencial fueron equivalentes a menos de un manatí por año, sobrepasando la media anual de manatíes varados (tres manatíes).

Conclusiones. Las actividades antropogénicas causan mortalidad en los manatíes distribuidos en la región, a pesar de la creación de áreas protegidas. La cacería ilegal fue identificada como la principal causa de muerte de los manatíes en Guatemala. Es fundamental la implementación de estrategias de manejo enfocadas en minimizar las amenazas hacia la especie, teniendo como base la aplicación de la legislación y conciencia ambiental. Es necesario crear e implementar un protocolo de varamiento de mamíferos marinos en la costa caribeña de Guatemala, para obtener registros estandarizados sobre este tipo de eventos y realizar rescates y liberaciones cuando sea posible.

Palabras clave: mamíferos marinos; mortalidad; conservación; cacería ilegal; áreas protegidas.

INTRODUCTION

Understanding animal strandings helps attain information about endangered aquatic species that are hard to see on the surface. Stranding data provides critical information on the species' life history, population health, environmental contamination levels, and general impacts of anthropogenic activities on marine ecosystems (National Oceanographic and Atmospheric Administration [NOAA], 2019). It is one of the primary sources of evidence regarding the effect of human activities such as vessel strikes and fishery interactions on aquatic mammals (Van der Hoop et al., 2013). The data is also extremely valuable for informing management and policy decisions (Geraci et al, 2005).

Spatial analysis of stranding data can be helpful for recognizing patterns. For example, identifying areas with a high concentration of strandings can depict the interaction between a species and human activities if strandings occur in areas of high vessel traffic (Bedriñana-Romano et al., 2021). Spatial patterns can also help enhance surveillance and monitoring programs and predict carcass deposition during mortality events (Norman, 2008). This is extremely important in the case of endangered species, where the death of one individual could significantly impact the population's health (Onens et al., 2022).

The Antillean manatee (*Trichechus manatus manatus*) is one of the subspecies of the West Indian manatee, and it is classified as endangered throughout its range (Diario de Centro América [DCA], 2021; Quintana-Rizzo & Reynolds, 2010; Self-Sullivan & Mignucci-Giannoni, 2008). Manatee hunting is prohibited by the Presidential Agreement of 1981 (DCA, 1981), which is still in effect. Further, Article 80 of the General Law on Fisheries and Aquaculture prohibits the intentional capture or hunting of threatened or endangered marine mammals (Ministerio de Agricultura, Ganadería y Alimentación, 2002).

Poaching has significantly reduced different manatee populations throughout their distribution, including those in Central America (Machuca-Coronado & Corona-Figueroa, 2019; Ruiz Valladares et al., 2008). Interestingly, in this region, Belize has the largest population of Antillean manatees, estimated to be around 1 000 individuals (O'Shea & Salisbury, 1991; Quintana-Rizzo & Reynolds, 2010), while the neighboring country of Guatemala has only approximately 150 manatees (Quintana-Rizzo & Reynolds, 2010). Although Guatemala has a small coastline, an average of 50 manatees were sighted per survey of the entire Atlantic coast during aerial surveys conducted between 1992 and 2014 (Quintana-Rizzo, 1993, Quintana-Rizzo, 2005; Quintana-Rizzo & Machuca-Coronado, 2008; Quintana-Rizzo et al., 2023).

There are anthropogenic activities that have put direct pressure on manatees. In Guatemala, the recognized threats are poaching for meat consumption, bycatch, entrapment and hooking in fishing gear, vessel collisions, and habitat deterioration and contamination (Herrera et al., 2004; Machuca-Coronado & Corona-Figueroa, 2019). Despite efforts to address cases of local manatee strandings, quantified information and an analysis of how each threat affects the manatee population are scarce or nonexistent. Thus, the general purpose of this study was to analyze the 30 years of documented manatee stranding reports available for Guatemala. The specific objectives were to: 1) conduct a spatial analysis of manatee strandings to identify the areas of high stranding concentration and 2) determine whether there were sex differences in the number of strandings. The information was used to evaluate the role of protected areas in conserving the species. We also used the potential biological removal (PBR) approach to determine if the level of human-caused mortality in the manatee population could be sustained while allowing the population to recover. The PBR approach is suited to data-poor situations (Punt et al., 2020). The study results are expected to strengthen the management of protected areas and bring attention to cases of stranded manatees. The evidence can be used to promote the creation of an aquatic mammal stranding network and to help regulate anthropogenic activities in the Caribbean of Guatemala.

MATERIALS AND METHODS

Study area: The study area included the state of Izabal, which encompasses the distribution area of the Antillean manatee in Guatemala. The state is bordered on the north by the Caribbean Sea, and on the east by the Gulf of Honduras (Arrivillaga & Baltz, 1999; Ixquiac et al., 2008). It includes five municipalities: El

Estor, Livingston, Morales, Los Amates, and Puerto Barrios (Fig. 1). Izabal has remnants of warm subtropical and very humid tropical forest, wetland areas, mangrove forests, estuaries with patches of submerged vegetation, secondary forests, areas of intensive agricultural cultivation (e.g., oil palm, cattle pastures, banana, pineapple, cardamom, etc.), urban centers, beaches, and port areas (Suárez, 2011; Yáñez-Arancibia et al., 1999).

There are four designated protected areas within the study area, which include manatees as a conservation element of their management plans (Machuca-Coronado & Corona-Figueroa, 2019). Those protected areas are: (1) Refugio de Vida Silvestre Bocas del Polochic (RVSBP; total area 207.60 km^2: 143.60 km^2 terrestrial and 64.00 km^2 aquatic zones; Fundación Defensores de la Naturaleza [FDN], 2003), (2) Parque Nacional Río Dulce (PNRD; total area 165.90 km^2: 93.92 km^2 terrestrial and 71.98 km^2 aquatic zones; Consejo Nacional de Áreas Protegidas [CONAP], 2019), (3) Área de Uso Múltiple Río Sarstún (AUMRS; total area 475.82 km^2: 437.93 km^2 terrestrial and 37.89 km^2 aquatic zones; Fundación para el Ecodesarrollo y la Conservación [FUNDAECO], 2009), and (4) Refugio de Vida Silvestre Punta de Manabique (RVSPM; total area 1,519 km^2: 492.89 km^2 terrestrial and 1,025.89 km^2 marine zones; Fundación Mario Dary Rivera [FUNDARY] et al., 2006) (Fig. 1).

Manatee stranding records: Information on live and dead manatee stranding events was compiled in two ways. First, we obtained records documented by the government (CONAP) and non-government (FDN and FUNDAECO) organizations in Izabal. Second, we obtained data on manatee strandings in national museum collections, and grey literature (e.g., unpublished reports, newspaper articles, theses, and social media). Stranding records were stored in a database that included the following data points: year of the stranding, stranding location, geographical coordinates (when available), name of the organization or person who made the report, manatee sex, manatee age class, cause of death if known (poaching, bycatch, boat impact), and whether the stranding occurred within a protected area. In some cases, it was impossible to record all of these data points, due to the degree of decomposition of the carcasses or due to lack of record by the stranding response personnel. Data compiled in technical reports generated by CONAP and non-government organizations were considered "verified." The data was classified as "unverifiable" when obtained from unofficial sources or non-technical reports (e.g., communication with fishermen or tour operators). Boundaries of the protected areas were extracted from the World Database of Protected Areas (United Nations Environment Programme World Conservation Monitoring Centre & International Union for Conservation of Nature [UNEP-WCMC & IUCN], 2021).

Data analysis: Descriptive statistics (average and standard deviation) were used for stranding events that occurred over the years inside protected areas and municipalities. Percentages of manatee strandings based on sex, age class, and cause of death were calculated. To determine whether there were sex differences in the number of stranded manatees, we used a chi-square test in R statistical software (R Core Team, 2021). The spatial analysis included two components. First, to assess whether there are concentrated areas where strandings occur, a Kernel density plot was created, which estimated the probability density function of a stranding along the coast. The Kernel density analysis was performed using a raster with cells of 1 km^2 size on the minimum convex polygon (MCP) using ArcGIS Pro 3.0.0. The MCP is the smallest possible convex polygon encompassing all the known locations of manatee strandings (Hayne, 1949). Spatial Kernel density is a non-parametric method that measures the local spatial clustering of a point pattern, visualized by a continuous estimated surface density layer distributed through and between all points (Worton, 1989). Second, we compared the concentration of stranded manatees and the number of stranding events between

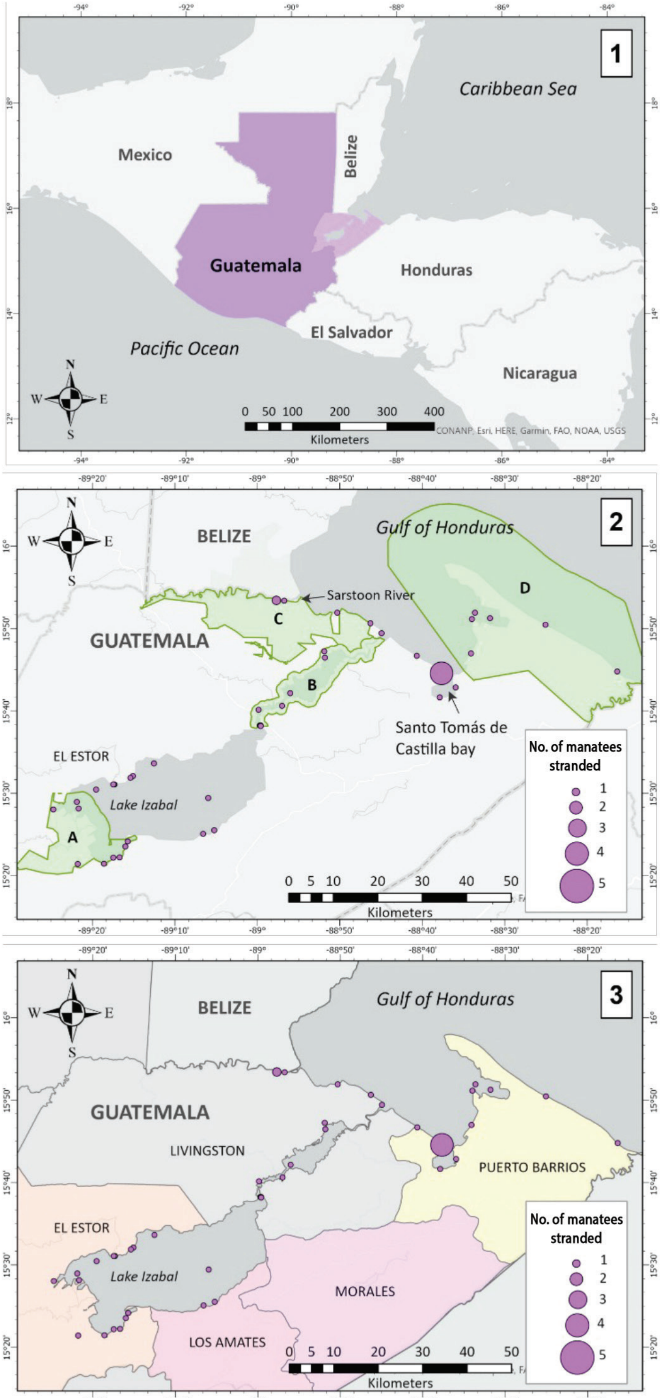

Fig. 1. (1) Study area and general overview of the region. Location of *Trichechus manatus manatus* stranding events between 1992 and 2022 along the Caribbean coast of Guatemala, including (2) protected areas and (3) municipalities. Protected areas are A) Refugio de Vida Silvestre Bocas del Polochic (RVSBP), B) Parque Nacional Río Dulce (PNRD), C) Área de Uso Múltiple Río Sarstún (AUMRS); and D) Refugio de Vida Silvestre Punta de Manabique (RVSPM).

protected areas and between municipalities, by using a chi-square test in R statistical software (R Core Team, 2021).

We used the potential biological removal (PBR) to estimate the maximum number of animals that could be removed from the Guatemalan manatee population (due to anthropogenic mortality) without affecting the optimal population size (Wade, 1998). The U.S. Marine Mammal Protection Act developed the PBR statistic for managing the impacts of fishing on marine mammal populations. PBR is calculated as:

$$Nmin \times 1/2Rmax \times Fr$$

Where Nmin is the estimate of the minimum population size of the stock, 1/2Rmax is one-half the maximum theoretical or estimated rate of population growth, and Fr is the recovery factor which is selected depending on the species conservation status (lower values for poorer conservation status) and varies from 0.1 to 1 (Wade, 1998). To calculate the PBR for the Guatemalan population, we used the most recent population estimate of 150 manatees (Quintana-Rizzo & Reynolds, 2010). Additionally, a PBR for 70 % and 40 % of this population were calculated since those percentages are within the range of older population estimates (Quintana-Rizzo, 1993). We used the minimum (0.04) and maximum (0.08) growth rates previously described or suggested for West Indian manatees (Marsh et al., 2011; National Marine Fisheries Service [NMFS], 2016; Runge et al., 2004) after Galves et al. (2022). A Fr equal to 0.1 was used because this is the value expected for endangered species or stocks that are declining (Taylor et al., 2000; Wade, 1998).

RESULTS

A total of 48 manatee individuals were recorded in 43 stranding events from 1992 to July 2022 (Table 1; $\bar{x}$ = 1.07, SD = 0.33). Of the 48 manatees, 96 % (N = 46) were dead animals and only two were live animals (4 %, all female orphan calves). The age group was determined for 46 manatees; 61 % were adult manatees (n = 28), 26 % calves (N = 12), and 13 % juveniles (N = 6). Sex was determined for 24 manatees (50 %). No significant difference was found in the percentage of male and female manatee strandings (χ^2 = 0.050, d.f. = 1, p = 0.818). Cause of death was determined in 65 % of the stranded manatees (N = 30), due to the degree of decomposition of the carcasses or lack of record in some cases. Poaching was the main cause of mortality (70 %, N = 21), followed by entanglement in fishing gear (hereafter referred to as bycatch) (17 %, N = 5) and boat collisions (13 %, N = 4; Fig. 2). Entanglement in fishing nets was recorded in six separate events. Of these events, three were recorded in the RVSBP (two dead adult manatees and one live calf), two in the PNRD (two dead manatee calves), and one in Lake Izabal (one dead adult manatee). The highest number of stranding events were reported in 2015 and 2020, with a total of 6 stranded manatees each year (Fig. 3). Since Guatemala has not implemented a protocol for marine mammal stranding, there were no standardized records of stranding. Consequently, there were no recorded reports of manatee stranding events during the following years; 1993-2002, 2006, 2010, 2012, and 2017 (Fig. 3).

Stranding events were recorded throughout the distribution range of manatees in Guatemala (Fig. 1). The Kernel analysis showed that the highest predicted density of strandings occurred in the Santo Tomás de Castilla bay and surrounding waters. The second highest predicted stranding density was the southern corner of Lake Izabal including the limits of the protected area RVSBP, and a third, less dense area, was in the southern section of the protected area PNRD (Fig. 4).

Of the 43 stranding events, 65 % (N = 28) occurred inside protected areas (AUMRS: $\bar{x}$ = 1.33, SD = 0.58; PNRD: $\bar{x}$ = 1.00; SD = 0.00; RVSBP: $\bar{x}$ = 1.00; SD = 0.00; RVSPM: $\bar{x}$ = 1.00; SD = 0.00) involving 29 individual manatees. In protected areas, no statistically significant differences were found among the number of stranding events (χ^2 = 3.43, d.f. = 3, p = 0.330) and the number of manatees stranded

Table 1

Chronological records of *Trichechus manatus manatus* strandings in Guatemala from 1992 to 2022. Date is reported as year, month, and day when available.

Date	No. manatees	Age class /sex	Municipality	Protected area*	Cause of stranding
1992/03/01	1	NA/NA	Livingston	PNRD	Boat impact
2003	1	Adult/NA	Livingston	PNRD	Unverifiable
2004	1	Adult/NA	El Estor		Bycatch
2004	1	Juvenile/NA	El Estor		Poaching
2005/05/25	1	Adult/NA	Puerto Barrios	RVSPM	Poaching
2005/06/18	1	Juvenile/male	Livingston	PNRD	Unverifiable
2005/09/20	1	Adult/NA	El Estor		Poaching
2005/10/20	1	Juvenile/NA	El Estor	RVSBP	Poaching
2007/05/10	1	Adult/female	El Estor	RVSBP	Bycatch
2007/12/05	1	Calf /male	El Estor	RVSBP	Unverifiable
2008	2	Adults/NA	Livingston	AUMRS	Poaching
2008/07/12	1	Calf/female	El Estor	RVSBP	Orphan
2008/08/11	1	Adult/NA	Livingston		Poaching
2009/06/08	1	Calf/NA	El Estor	RVSBP	Poaching
2009/06/10	1	Adult/female	El Estor	RVSBP	Poaching
2009/07/20	1	Adult/NA	El Estor		Poaching
2009/10/01	1	Adult/NA	El Estor	RVSBP	Unverifiable
2011/07/01	1	Calf /male	Livingston	PNRD	Boat impact
2013	1	NA/NA	El Estor		Poaching
2013/01/23	1	Adult/NA	El Estor	RVSBP	Bycatch
2013/08/09	1	Juvenile/female	Puerto Barrios	RVSPM	Unverifiable
2014/05/05	1	Calf/NA	Los Amates		Unverifiable
2015/04/06	1	Adult/female	Livingston	AUMRS	Poaching
2015/07/01	5	1 adult/female 3 adults/NA 1 calf/NA	Puerto Barrios		Poaching
2016/06/01	1	Adult/NA	El Estor		Unverifiable
2016/07/24	1	Adult/NA	Puerto Barrios	RVSPM	Poaching
2018/07/19	1	Adult/female	Livingston	AUMRS	Poaching
2018/07/31	1	Adult/female	Puerto Barrios	RVSPM	Poaching
2019/10/13	1	Adult/male	El Estor		Unverifiable
2020	1	Calf/NA	Puerto Barrios	RVSPM	Unverifiable
2020/02/15	1	Adult/male	Livingston	PNRD	Boat impact
2020/02/27	1	Juvenile/male	El Estor		Unverifiable
2020/05/06	1	Adult/male	Puerto Barrios		Unverifiable
2020/05/13	1	Juvenile/male	El Estor	RVSBP	Unverifiable
2020/06/14	1	Calf/NA	Livingston	PNRD	Bycatch
2021/07/21	1	Calf/female	Livingston		Orphan
2021/11/13	1	Calf/male	Puerto Barrios	RVSPM	Unverifiable
2021/12/09	1	Adult/NA	Livingston	PNRD	Poaching
2022/02/04	1	Adult/male	Los Amates		Unverifiable
2022/04/29	1	Calf/male	Livingston	PNRD	Unverifiable
2022/05/05	1	Calf/male	Livingston	PNRD	Bycatch
2022/07/12	1	Adult/male	Puerto Barrios		Boat impact
2022/07/14	1	Adult/male	Puerto Barrios	RVSPM	Unverifiable

NA = Not available. * PNRD (Parque Nacional Río Dulce); RVSPM (Refugio de Vida Silvestre Punta de Manabique); RVSBP (Refugio de Vida Silvestre Bocas del Polochic); AUMRS (Área de Uso Múltiple Río Sarstún).

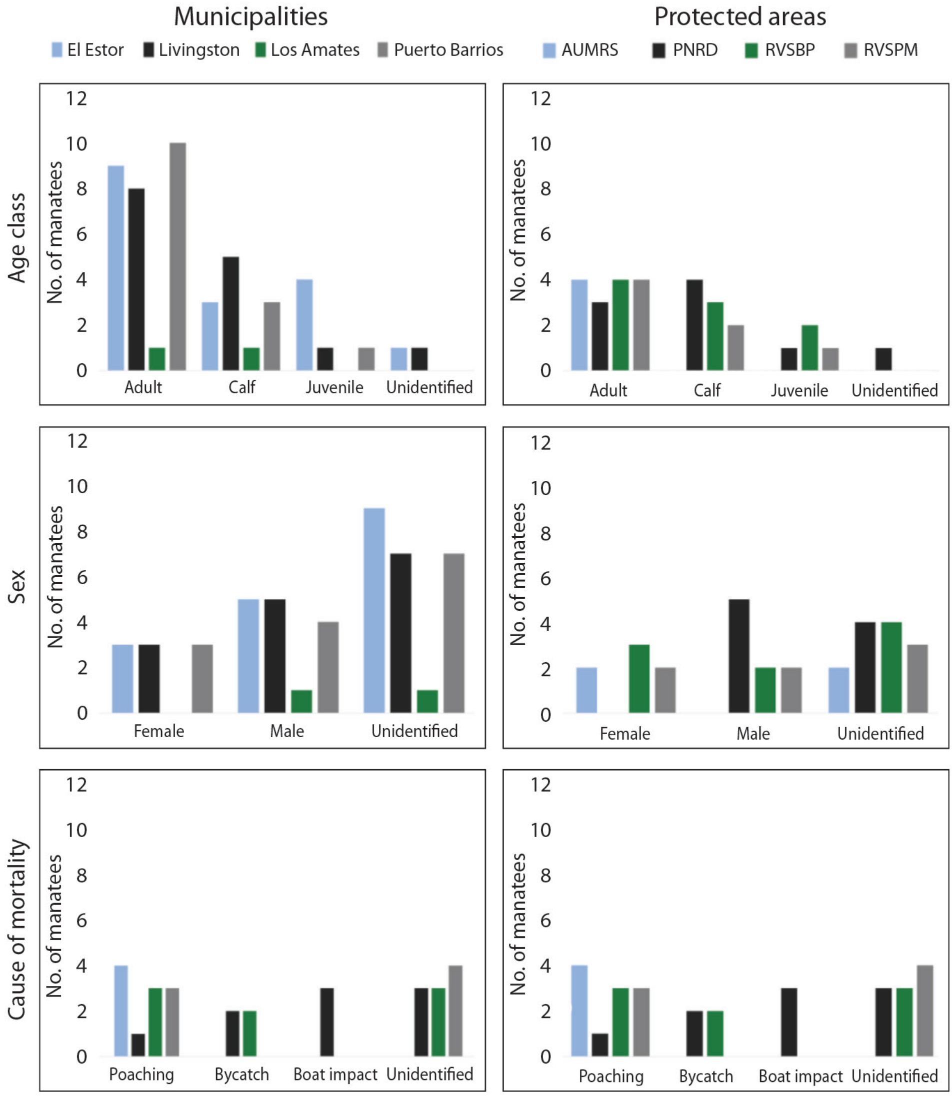

Fig. 2. *Trichechus manatus manatus* strandings based on age class, sex, and cause of mortality for each municipality and protected area on the Caribbean coast of Guatemala.

(χ^2 = 0.231, d.f. = 3, p = 0.510). Regardless of location, the stranding events of two protected areas combined included the highest proportion of the strandings in the study area (42 %). Those protected areas were RVSBP and PNRD (stranding events in each: 21 %, N = 9; stranded manatees N = 9).

In municipalities, significant differences were found in the number of stranding events (X^2 = 11.7910, d.f. = 3, p = 0.0081) and the number of manatees stranded (χ^2 = 11.50, d.f. = 3, p = 0.009) among them. The highest number of stranding events were recorded in El Estor (39 %, N = 17; $\bar{x}$ = 1.00; SD = 0.00), followed by Livingston (33 %, N = 14; $\bar{x}$ = 1.07; SD = 0.27), Puerto Barrios (23 %, N = 10; $\bar{x}$ = 1.17, SD = 0.58), and Los Amates (5 %, N = 2; $\bar{x}$ = 1.00, SD = 0.00). The percentage of stranded manatees in each municipality was 35 % (N = 17) in El Estor, 31 % (N = 15) in Livingston, 29 %

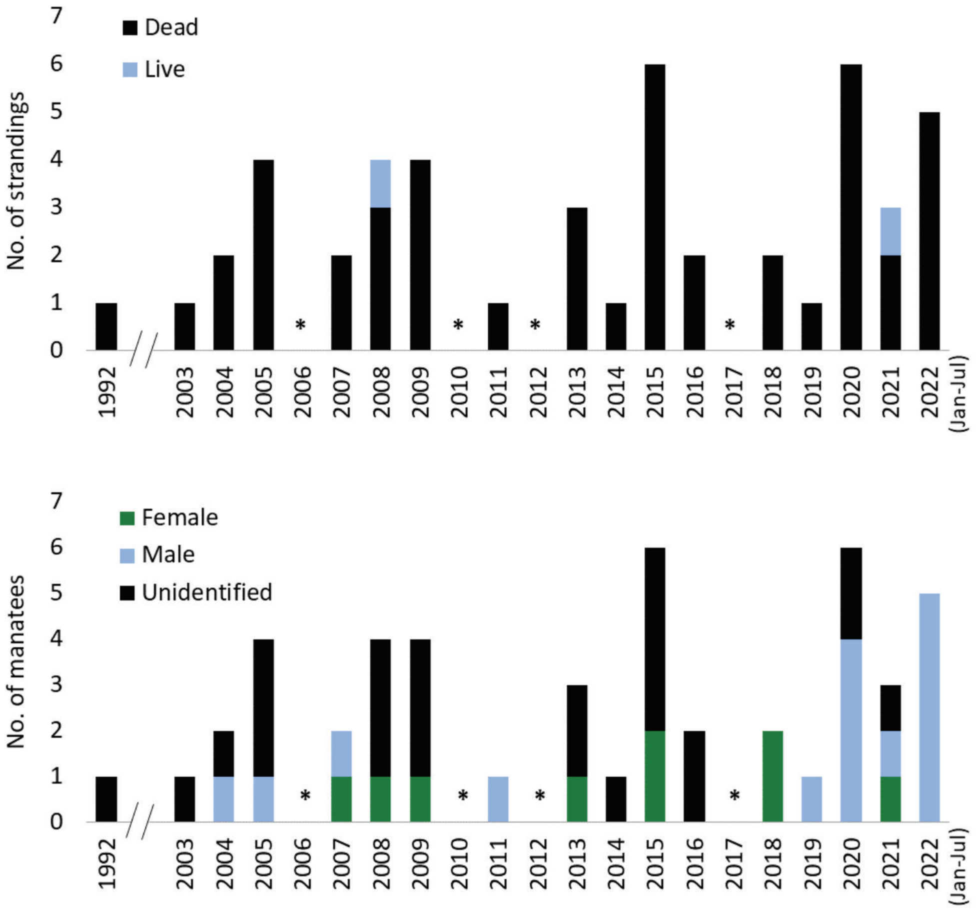

Fig. 3. Number of *Trichechus manatus manatus* strandings (live and dead) and number of individuals per sex identified from 1992 to July 2022. * = years with no stranding reports.

(N = 14) in Puerto Barrios and 4 % (N = 2) in Los Amates (Fig. 2).

The mean annual verified mortality for years with reported sightings was three manatees, with three yearly events directly resulting from anthropogenic causes. The estimated PBR for a population of 150 manatees is 0.6 if the growth rate is 0.08 and 0.3 if the growth rate is 0.04. The PBR decreases for smaller populations of manatees (Table 2). Therefore, in all cases, the PBR was equivalent to one manatee. This PBR was exceeded in each reported year.

DISCUSSION

This study compiles data on manatee strandings over 30 years throughout the species distribution range in Guatemala. It represents the country's most extensive and complete summary of manatee strandings. The results show that a significant number of strandings occurred in particular areas of the coast, including protected areas, and that a large percentage of the strandings involved the poaching of adult males.

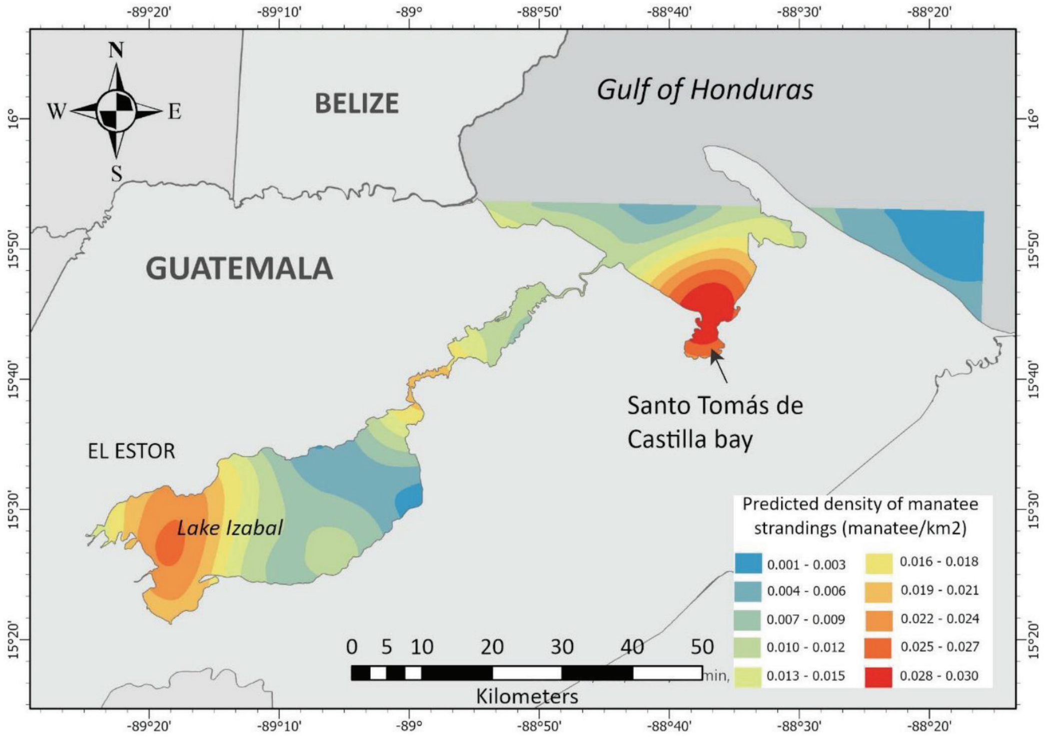

Fig. 4. Predicted Kernel density of stranded *Trichechus manatus manatus* along the Caribbean coast of Guatemala.

Table 2

Potential biological removal for the population of *Trichechus manatus manatus* in Guatemala using both maximum (0.08) and minimum (0.04) estimated growth rates based on Marsh et al. (2011), NMFS (2016), and Runge et al. (2004).

Population size estimates*	Potential Biological Removal	
	Growth rate = 0.08	Growth rate = 0.04
60	0.24	0.12
105	0.40	0.21
150	0.60	0.42

* Based on (Quintana-Rizzo, 1993; Quintana-Rizzo & Reynolds, 2010).

Predicted density of stranded manatees. The predicted density of stranded manatees was highest in Santo Tomás de Castilla bay and surrounding waters, as well as in the southern corner of Lake Izabal. In both locations, manatee carcasses with harpoons or wounds on the body consistent with hunting processes were recorded. Poaching was the leading cause of death in these places (> 55 % of stranded manatees). These areas are in close proximity to municipal markets where there is evidence of an illegal market for manatee meat (Ruiz Valladares et al., 2008). In Santo Tomás de Castilla bay, the Kernel density area of high concentration of stranded manatees (0.019 to 0.030 manatees/km^2) included the clustering of nine manatees that were part of five stranding events. Two events involved the poaching of six manatees, including a calf. The other causes of mortality were unverifiable. In this bay, groups of manatees are commonly observed (T. Sandoval, pers. obs.), possibly due to the presence of aquatic vegetation and seagrass patches (Arrivillaga & Baltz, 1999; Ixquiac et al., 2008; MacDonald, 2011; Yáñez-Arancibia et al., 1999). However, Santo Tomás de Castilla bay is a vulnerable area for manatees due to the commercial vessel traffic of port Santo Tomás de Castilla. This medium-sized commercial port is located in the southwestern part of the bay. The vessel traffic in this area includes oil/chemical tankers, container ships, bulk carriers, general cargo, and crude oil tankers. Vessels as large as 244 m have entered the bay and port (Marine Traffic, 2022).

The other area with a high predicted stranding density of manatees was the southern corner of Lake Izabal, including the area within the limits of a protected area (RVSBP). Kernel density analysis projected strandings in the center of Lake Izabal. However, only one stranding was recorded at approximately 6.5 km from the coast, and it could be a case of a carcass transported by currents to the deep waters of the lake. All other stranding events were within 1.5 km from shore. In the southern corner of Lake Izabal, 37% (N = 16) of the total stranding events were recorded, representing the highest concentration of strandings in the entire country. This is worrisome as RVSBP has been identified as a priority area for the manatees in Guatemala (Quintana-Rizzo, 1993, Quintana-Rizzo, 2005; Quintana-Rizzo & Machuca-Coronado, 2008; Quintana-Rizzo et al., 2023), and poaching was the leading cause of death within this protected area (60 %, N = 3), followed by bycatch (40 %, N = 5). Since the sex of most of the manatees poached is unknown (N = 11, 52 %), there is no clear evidence of a sex-bias in manatee poaching. However, in another study, some hunters recognized that female manatee meat is softer and has a better flavor (Del Valle, 2002).

The highest mortality cases involved adults (eight out of 15 identified by age-class) and males (five out of eight identified by sex). Similar sex- and age-biased poaching mortality has been observed in other wildlife populations (Archie & Chiyo, 2012; Ginsberg & Milner-Gulland, 1994; Jones et al., 2018). From a demographic standpoint, age- and sex-biased poaching mortality is expected to bear fitness consequences in a population (Ginsberg & Milner-Gulland, 1994). The PBR suggests that this mortality is indeed having an effect on the Guatemalan manatee population. Furthermore, removing adult individuals from a population could lead to a more significant reproductive investment by young adults, with potentially detrimental consequences on their overall survival (Ginsberg & Milner-Gulland, 1994). In Guatemala, poaching continues to be a significant challenge in spite of the fact that manatees are protected by the Fisheries and Aquaculture law, Decreto 80-2002, which declares that it is illegal to hunt any endangered marine mammal and that the fine for breaking this law is high for the socio-economic status of the average person living in Izabal (approximately USD $1 055.00 – USD $10 555.00). However, effective law enforcement is problematic and generates conflict between the authorities and residents as there are no alternatives for the sustainable use of natural resources.

Protected areas, management, and conservation.

Although protected areas have management plans and the legal basis to protect manatees and natural resources, more than half of the cases of strandings were reported within those areas (N = 28; 65 %). All types of mortality were identified, but poaching was again the leading cause of mortality. Eleven cases of poaching were registered in all four protected areas combined. Further, bycatch was identified in RVSBP and PNRD, but mortality due to motorboat collisions was only reported in PNRD. This protected area is one of the sites with the highest tourism records in the country (Instituto Guatemalteco de Turismo [INGUAT], 2015). Watercraft collisions are among the leading causes of anthropogenic mortality for manatees in other habitats in Florida (Lightsey et al., 2006), Puerto Rico (Mignucci-Giannoni et al., 2000), and Belize (Galves et al., 2022). In Guatemala, studies have shown that manatee sightings are (weakly) positively correlated with the presence of motorboats (Quintana et al., 2023) in PNRD, suggesting the probability of vessel collisions exists.

The variety and number of stranding cases inside different protected areas demonstrate that management strategies and actions have not been sufficient for species protection. There is no surveillance within the protected areas to prevent poaching. Protected areas managers have not proven effective at preventing poaching or other threats to biological diversity. In addition, it is necessary to regulate the speed of boats within protected areas to avoid collisions with manatees. Although there is no national legislation that supports the implementation of navigational regulations, in each municipality, the authorities must disclose to motorboat users the current navigation guidelines. For example, the PNRD Management Plan stipulates navigation at speeds between 9 and 18 km/h (CONAP, 2019). This aspect is important, because in this protected area, mortality related to boats was recorded. Indeed, the shallow, narrow waters and interconnected channels of PNRD require high regulation (Corona-Figueroa, 2012). Evidence shows that speed restrictions reduce manatee deaths (Calleson & Frohlich, 2007; Laist & Shaw, 2006).

Municipalities, management, and conservation.

Poaching was the leading cause of manatee death in the municipalities of Puerto Barrios, El Estor, and Livingston. In these municipalities, it is known that manatee poaching has occurred for at least 20 years (Del Valle, 2001; Machuca-Coronado & Corona-Figueroa, 2019). Groups of hunters have been identified moving along the coast to areas with manatee presence (including inside protected areas). Hunters from Livingston and Puerto Barrios have even been reported to travel to Belizean territory, where hunting is also illegal. In the 1990s, there were reports of manatee poaching in Belize that appeared to be the result of poaching from Guatemala and possibly Honduras (E. Quintana-Rizzo pers. obs.). In August-September 1995, at least 35 manatee carcasses were found in the Port Honduras area, Belize, in what appeared to be a butchering site. Manatee skulls had deep cut marks, and evidence suggests they were shot, harpooned, or chased until exhausted (Bonde & Potter, 1995). A year later, a second report of poaching was documented south of Port Honduras, where another nine manatees were found dead (Morales-Vela et al., 2000). In Guatemala, manatee meat is illegally sold in the main municipal markets at a lower price than other types of meat (Quintana-Rizzo & Reynolds, 2010; Ruiz Valladares et al., 2008).

In this study, reports of manatee entanglement in fishing gear occurred inside and outside protected areas. The use of trawl nets in large-scale commercial fishing (nets > 4 km in length) is common in the municipalities of El Estor and Livingston, even though this type of fishing is prohibited in inner or continental waters (Ministerio de Agricultura, Ganadería y Alimentación, 2002). Large fishing nets interrupt the movement of manatees, mainly at the mouth of rivers; they also reduce the available habitat for the species (Machuca-Coronado & Corona-Figueroa, 2019; Ruiz Valladares et al., 2008).

Differences in the number of reported stranding events in each municipality are likely related to the presence of government (CONAP) and non-governmental organizations co-managing protected areas in the different municipalities. Those organizations have taken the lead in reporting and documenting manatee strandings. In the case of El Estor, since 2007, FDN established a protocol to record manatee stranding data in collaboration with community members, fishers, and boat captains. This collaboration was fundamental in implementing the procedures, mainly in the notification of cases and location of manatee carcasses (Quintana-Rizzo & Machuca-Coronado, 2008), some of which are part of this study. These actions represent the first efforts toward the establishment and implementation of a stranding protocol for marine mammals in Izabal (Quintana-Rizzo & Machuca-Coronado, 2008); however, implementation has slowed down in recent years. In Livingston and Puerto Barrios, the presence of CONAP is fundamental to registering and confirming manatee stranding cases. Since 2010, systematic reports of each manatee stranding have been produced. In Los Amates, the number of stranding records is lower than in other municipalities;

this could be because the government authorities of this municipality have not implemented a stranding protocol in their work plans.

Sex and age class.

Most stranding records include dead manatees, and only two cases of live manatees were recorded, both corresponding to female orphan calves (Machuca-Coronado & Quintana-Rizzo, 2014; Quintana-Rizzo et al., 2008; T. Sandoval personal communication July 21, 2021). Calf strandings could be related to the poaching of the corresponding mothers. Manatee hunters are known to focus on adults (Ruiz Valladares et al., 2008); however, they take the opportunity to hunt calves when they are in the company of their mother (Ruiz Valladares et al., 2008). Poaching targeted to this demographic group can directly affect the population's stability. Females have low reproductive rates since they do not reach sexual maturity until 3-years of age and produce an average of one calf every two to four years, which means that the growth of manatee populations is significantly slow (Hartman, 1979; Powell, 2002; Quintana-Rizzo & Reynolds, 2010).

Stranding records were biased towards adult males; 63 % of the stranding events corresponded to this demographic group. Male manatees are vulnerable because they spend more time moving around than females and calves (Quintana-Rizzo & Reynolds, 2010). This behavior pattern may increase their exposure to different threats. In fact, 75 % (N = 3) of the registered cases of boat collisions correspond to male manatees. However, the relationship between strandings and demographics is likely underestimated because sex and cause of death determination were only possible in 50 % (N = 24) and 36 % (N = 16) of events, respectively.

Potential biological removal.

PBR has been used to calculate the species-specific level below which human-induced mortality must be reduced for species survival (Marsh et al., 2004). If the anthropogenic mortalities are less than the PBR, then a depleted population should recover given sufficient time (Williams et al., 2016). Our PBR estimates were equivalent to less than one manatee per year, which is lower than the annual average number of stranded manatees recorded in Guatemala over the past thirty years. As such, anthropogenic activities affect the local manatee population, although long-term studies on population dynamics are needed to understand the gravity of their impact.

Conservation and future recommendations.

The success of conservation relies on key factors, including the collection of scientific data to understand species threats, the development of stakeholder partnerships to address potential threats, consistent enforcement of regulations to protect the species, and equally on public adherence to protection programs. The lack of any one of these factors makes conservation efforts more challenging and less efficient. In Guatemala, there is a great need to increase awareness of the threats facing manatees and their habitat at all levels, and the enforcement of laws is critical. Poaching was identified as one of the main threats affecting the local manatee population more than 30 years ago (Lefebvre et al., 1989; Quintana-Rizzo, 1993), and this study shows that the threat continues to be significant. If manatees are to survive in Guatemala, developing a strategic plan to significantly reduce human-related mortalities is crucial to their conservation and proper management.

There is also a great need to develop and implement a protocol for manatee strandings. This includes performing necropsies and taking samples of biological material. The existing marine mammal stranding protocol for the Guatemalan Pacific coast (WWF, 2018) could serve as a starting point, but a protocol specifically focused on manatees must be included. In addition, a marine mammal stranding network needs to be established in the Caribbean area, together with training sessions for technicians, park rangers, and key local people. Finally, a reduction in anthropogenic mortality of manatees can be accomplished through education and proactive management and training of the park rangers and other stakeholders, together with a conservation plan that includes law enforcement, mortality assessment, scientific research, and stakeholder involvement and cooperation.

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SUPPLEMENT • MANATEES

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Initial characterization of mitochondrial DNA control region haplotypes of the Antillean manatee (*Trichechus manatus manatus*, Sirenia:Trichechidae) in Guatemala

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ABSTRACT

Introduction: Small populations are at risk of losing genetic variability much faster than large populations; this subsequently decreases their ability to adapt when facing environmental changes. A small population of the endangered Antillean manatee (*Trichechus manatus manatus*) has been identified in Guatemala.

Objective: This study explored the genetic diversity of the Antillean manatee in Guatemala by analysing mitochondrial DNA control region haplotypes in the two most important habitats for the species, Bahía La Graciosa, a coastal bay and Bocas del Polochic, a coastal wetland, both located in the Izabal State.

Methods: Genetic samples were collected using non or minimally invasive sampling techniques: scraping of epidermal tissue, collection of floating feces, and collection of tissue from carcasses. DNA extractions, DNA amplification using polymerase chain reaction (PCR), and sequencing of the control D-loop region were used to process and analyse the samples.

Results: Seven mitochondrial DNA sequences were obtained from 36 samples collected (minimum of four and maximum of seven individuals). Four haplotypes were identified, A01, A03, A04, and J01. No other Central American country has reported this number of haplotypes in a manatee population, and it is the first time that haplotype A01 has been reported for the region. The Guatemalan manatee population comprises at least two genetic lineages, the Florida/Greater Antilles lineage (haplotypes A01, A03, and A04) and the Mesoamerican lineage (J01).

Conclusion: Further studies, with the use of nuclear markers, are necessary to understand the population dynamics between Bahia La Graciosa and Bocas del Polochic to identify the number of management units present in the country; also, the degree of relatedness with the Belizean population needs to be established to better coordinate conservation efforts.

Key words: Non-invasive genetic sampling; endangered species; control D-loop region; Lago de Izabal; Atlantic coast of Guatemala; conservation management plans.

RESUMEN

Caracterización inicial de haplotipos de la región control de ADN mitocondrial del Manatí Antillano (*Trichechus manatus manatus* Sirenia:Trichechidae) en Guatemala

Introducción: Las poblaciones pequeñas corren el riesgo de perder variabilidad genética mucho más rápido que una población de mayor tamaño; disminuyendo, así mismo, su capacidad de adaptarse ante cambios ambientales.

Una pequeña población de la especie en peligro de extinción el manatí antillano (*Trichechus manatus manatus*) ha sido identificada en Guatemala.

Objetivo: Este estudio explora la diversidad genética del manatí antillano en Guatemala mediante el análisis de haplotipos de la región de control del ADN mitocondrial en los dos hábitats más importantes identificados para la especie, Bahía La Graciosa, un bahía costera y Bocas del Polochic, un humedal costero, ambos localizados en el departamento de Izabal.

Métodos: Las muestras genéticas se colectaron utilizando técnicas de muestreo no invasivas o mínimamente invasivas: raspado de tejido epidérmico, recolección de heces y recolección de tejido extraído de cadáveres. Se usaron extracciones de ADN, amplificación de ADN médiate la reacción en cadena de la polimerasa (PCR) y secuenciación de la región control D-loop.

Resultados: Se obtuvieron un total de siete secuencias de ADN mitocondrial de 36 muestras recolectadas. Cuatro haplotipos fueron identificados, A01, A03, A04 y J01. Ningún otro país centroamericano ha reportado esta cantidad de haplotipos en una población de manatíes y es la primera vez que se reporta el haplotipo A01 para la región. La población de manatíes guatemaltecos comprende al menos dos linajes genéticos, el linaje Florida/Antillas Mayores (haplotipos A01, A03 y A04) y el linaje mesoamericano (J01).

Conclusión: Son necesarios más estudios, con el uso de marcadores nucleares, para comprender la dinámica poblacional entre Bahía La Graciosa y Bocas del Polochic y para poder identificar el número de unidades de manejo presentes en el país: además, se debe establecer el grado de relación con la población de Belice para coordinar mejor los esfuerzos de conservación.

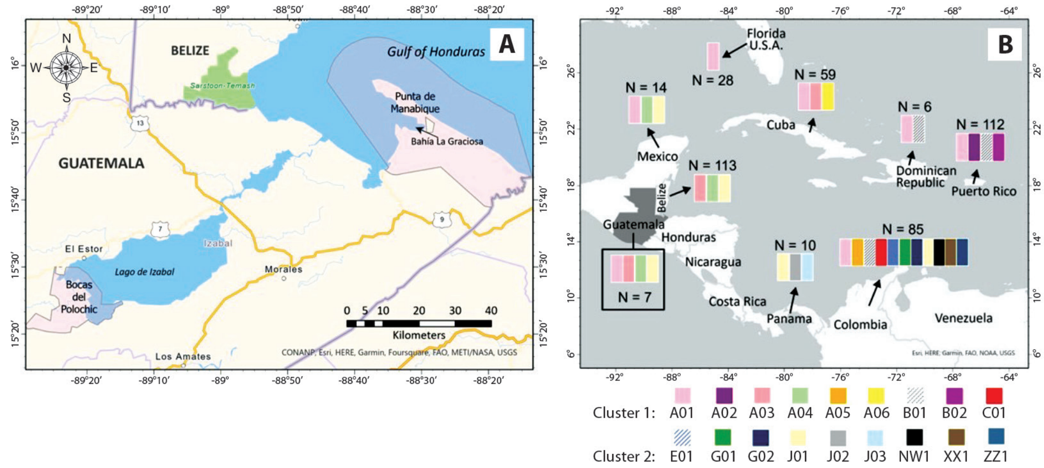
Palabras clave: Muestro genético no invasivo; especie en peligro de extinción; región control D-loop; Lago de Izabal; Costa Atlántica de Guatemala; planes de manejo de conservación.

INTRODUCTION

Genetic studies on the West Indian manatee, *Trichechus manatus* Linnaeus, 1758 have shown low haplotype and nucleotide diversity, with 29 haplotypes identified to date, distributed in three distinct lineages: Cluster I, Cluster II, and Cluster III (Alvarez-Aleman et al., 2022; Caballero et al., 2021; Díaz-Ferguson et al., 2017; Garcia-Rodriguez et al., 1998; Satizabal et al., 2012; Vianna et al., 2006). Cluster I includes haplotypes found in Florida and the Greater Antilles, Cluster II is distributed from the Gulf of Mexico to the Caribbean coast of South America, and Cluster III is exclusive to Brazil and Guyana (Vianna et al., 2006). The Antillean manatee (*T. m. manatus*), classified as endangered on the IUCN Red List (Self-Sullivan & Mignucci-Giannoni, 2008), is one of the two subspecies of the West Indian manatee and is found in small population pockets throughout most of its range.

In Central America, the genetic variability of the Antillean manatee has been studied in Belize (Hunter et al., 2010) and Panama (Díaz-Ferguson et al., 2017); these countries share the Cluster II lineage (Díaz-Ferguson et al., 2017). Belize has the largest manatee population in the western hemisphere with around 1 000 animals (Hunter et al., 2010) and, through conservation efforts, could assist in the recovery of the adjacent Guatemalan population (Quintana-Rizzo & Reynolds III, 2010). The most recent estimate is 150 individuals in Guatemala (Quintana-Rizzo & Reynolds III, 2010), and threats like illegal hunting limit the survival of the population (Machuca-Coronado & Corona, 2019). The objective of this study was to explore the genetic diversity of the Antillean manatee in Guatemala in two areas that are ecologically distinct but important for the species, Bocas del Polochic, an inshore wetland and Bahia La Graciosa, a coastal bay (Fig. 1A).

Manatee epidermal tissue, feces, and carcass skin cuts were collected at the two study sites between July 2012 and February 2011. To obtain epidermal tissue, a modified method, initially developed by Carney et al. (2007) was employed. A scraper with increased perforations (from 2 mm to 5 mm wide) and a lighter pole (4 m of aluminum instead of 2 m of PVC) were used to scrape the dorsal part of the manatee without hurting the animal and subsequently storing the tissue with 75 % ethanol and



C

Location	N	HT	List of haplotypes	Reference
Belize	113	3	A03, A04, J01	Hunter et al., 2010
Guatemala	4≤N≥7	4	A01, A03, A04, J01	This study
Panama	10	3	J01, J02, J03	Díaz-Ferguson et al., 2017

Fig. 1. (A) Location of the two sampling sites and protected areas: Bocas del Polochic Wildlife Refuge in Lago de Izabal and Bahía La Graciosa, part of the Punta de Manabique Wildlife Refuge, both located on the Atlantic coast of Guatemala. (B) Manatee mitochondrial control region D-loop haplotypes found in Guatemala and other parts of the Americas and the number of samples analyzed per country. Each haplotype is depicted in distinct colors and grouped into its corresponding cluster, previously defined by Vianna et al. (2006). Data sources: Mexico, Florida and the Dominican Republic (Vianna et al., 2006), Cuba (Alvarez-Alemán et al., 2022), Puerto Rico (Hunter et al., 2012), Colombia (Caballero et al., 2021), Panama (Díaz-Ferguson et al., 2017), and Belize (Hunter et al., 2010). (C) Number of genetic haplotypes (HT) and sample size (N) for Antillean manatee in Guatemala and other Central American countries (Belize and Panama). Since floating fecal samples cannot be assigned to different manatees, the sample size of Guatemalan manatees in this study could be less than seven, which is the number of sequences reported but not less than four because four different haplotypes were identified.

1 X TE (pH 8). After a manatee sighting, fecal samples were collected within a 100 m radius of the sighting. These were collected using new disposable plastic bags and latex gloves (Muschett et al., 2009) and were stored at room temperature in sterile containers with 95 % ethanol using a proportion of 1:3 (Muschett et al., 2009). Lastly, skin cuts from dead carcasses were collected opportunistically and stored at -20 °C. Two extraction protocols were used to treat tissue samples with different degradation states: the phenol-chloroform method (Muschett et al., 2009) was used for samples with high DNA concentration and a silica method (Höss & Pääbo, 1993) was used for low DNA concentrations. Two extraction protocols were also used to assay fecal samples: Zhang et al. (2006) and Marrero et al. (2009). In the Zhang et al. (2006) protocol, instead of binding the DNA to a spin column with guanidine thiocyanate, the samples were resuspended in 500 µl of isopropanol at -20 °C. A modification was used to increase the purity index of the extractions to 1.6–2.0. This modification consisted of applying the silica method (Marrero, 2009) to the resuspended samples.

Mitochondrial control region D-loop DNA was amplified by PCR using 1X Buffer (Promega), 4 mM of $MgCl_2$, 150 µM of each dNTPs, 0.3 µM of each primer (CR-4 and CR-5), 1.5 U of Taq DNA polymerase (Promega) and 1 µl of extracted DNA with a concentration of 100 ng/µl in a total reaction volume of 25 µl. The PCR cycling conditions were: 94 °C for 3 min, followed by 35 cycles of 94 °C for 1 min, 47 °C for 1 min, 72 °C for 1 min, and a final extension of 72 °C for 1 hr (García-Rodríguez et al., 1998). The optimum annealing temperature for fecal samples was 54 °C instead of 47 °C. PCR products were run on a 1 % agarose gel and visualized by electrophoresis to confirm amplification of the expected ~ 410 bp size. The dNTPs and primers were removed using the Novagen purification kit. The PCR products were bi-directionally sequenced (Macrogen Inc., Korea) to ensure a high confidence level in each nucleotide.

ChromasPro (2.1.10.1) was used to assemble the forward and reverse strands into contigs. A BLAST search of each contig was used to identify the top match haplotype, previously registered and defined by Vianna et al. (2006). A Clustal Omega alignment was generated between the contig and the haplotype, where shared polymorphisms and percentage alignment were used to define each sample as its respective haplotype.

A total of 36 manatee samples were collected. Of these, 29 samples (27 fecal samples and two tissue samples from carcasses) were obtained in Bahía La Graciosa, and seven samples (two fecal samples and five tissue samples from carcasses) were obtained in Bocas del Polochic. No epidermal tissue samples were collected during the study. Noise reduction was a primary challenge for approaching manatees since another research was simultaneously being carried out in the same boat. This field experience limited the application of this specific method in the region. However, approaching manatees is delicate due to their elusive behavior.

DNA was amplified from 22 of the samples collected; all came from Bahía La Graciosa. Seven samples were successfully sequenced; one came from tissue samples and six from fecal samples. The main reason for a lack of sequence results from tissue samples was contamination; the preservation, storage, and management of samples was not optimal. On the other hand, the limiting factor for processing fecal samples was degradation; fecal samples degrade very quickly when exposed to UV radiation.

Double coverage sequences ranged in length from 390-444 bp. Two sequences had read lengths of less than 410 bp, the length of characterized haplotypes in Genbank. However, these sequences of 390 and 392 bp in length were 100 % matches to the J01 haplotype, the most variable of the haplotype sequences identified here. To confirm each haplotype, forward and reverse chromatograms for each manatee sequence were aligned with the reference haplotype from Genbank (Supplementary Material 1). In all cases, there was a 100 % match between the reference sequence and the chromatograms. Only one sequence can be conclusively traced back to an individual since it consisted of a tissue sample from a carcass. The remaining samples consisted of feces collected exclusively in the bay area; therefore, there is a risk that a single individual was sampled multiple times and/or that these sequences could only be representative of a few bay area resident individuals.

Four haplotypes were identified, A01, A04, A03 and J01 (Fig. 1B and Fig. 1C), with respective frequencies of 43 %, 14 %, 14 %, and 29 %. These haplotypes have all been found in Central America, except for haplotype A01 (Fig. 1B). All manatee sequences have been registered in GenBank (OQ587957-OQ587965). Genetic diversity parameters like haplotype frequency, haplotype diversity, and nucleotide diversity could not be determined due to the aforementioned risk.

This study reports four manatee mitochondrial control region D-loop haplotypes for Guatemala, in a minimum of four individuals and a maximum of seven manatees. This is the highest number of haplotypes reported for Mesoamerica; all other genetically studied populations of the region, namely Belize, Panama and the Caribbean coast of Mexico, have reported three haplotypes (Díaz-Ferguson et al., 2017; Hunter et al., 2010; Nourisson et al., 2011) (Fig. 1B). This significant finding in a relatively small sample size strongly indicates that a more comprehensive population genetic analysis of the Guatemalan manatees is warranted. Belize, the adjacent manatee population to Guatemala, has reported the haplotypes A03, A04, and J01 in 113 individuals; (Fig. 1C) (Hunter et al., 2010). These three haplotypes have all been identified in Guatemala, along with the A01 haplotype that was reported in three out of seven sequences. Panama has also reported a different set of three manatee haplotypes: J01, J02, and J03 (Díaz-Ferguson et al., 2017).

Each haplotype identified in Guatemala was matched with the corresponding cluster previously named and classified by Vianna et al. (2006) (Supplementary Material 2). These clusters are lineages inferred using nucleotides and sequential divergence parameters to construct phylogenetic relationships. Haplotypes A01, A03, and A04 belong to Cluster I (Florida and Greater Antilles) and haplotype J01 belongs to Cluster II (Mesoamerica) (Vianna et al., 2006). The presence of Cluster I and Cluster II gives a bimodal character to the manatee population of Guatemala. This pattern has also been detected in manatees sampled from Mexico, Belize, Colombia, and Venezuela (Vianna et al., 2006), indicating that the manatees in Guatemala likely share a common ancestry with the rest of the Mesoamerican manatee population.

The presence of the A01 and the A03 haplotypes in the study suggests that the Guatemalan manatees have a genetic relationship with the North American and/or Greater Antilles population (Fig. 1B). Haplotype A03 has been reported in Cuba (Alvarez-Alemán et al., 2022) and Belize (Hunter et al., 2010). A01 is the only haplotype reported for the Florida population and has a broad distribution across the wider Antilles (Hunter et al., 2012; Vianna et al., 2006). Florida manatees are known to travel outside their normal range [e.g., from Florida to Cuba, Alvarez-Aleman et al. (2010); from Florida to Mexico, Castelblanco et al. (2021)]; interestingly, the haplotype A01 has not been reported in the Belizean manatee population, where 16 times as many samples have been analyzed to date (Hunter et al., 2010).

A complete mitochondrial diversity study and an analysis of nuclear DNA markers would provide a further understanding of the origin and genetic status of the Guatemalan manatee population. Nuclear DNA markers would be fundamental in determining the number of management units present in the country. They would aid in delineating population genetic relationships with neighboring countries, like Belize, and across the region. Conservation efforts should focus on determining the distribution of each genetic lineage within the country as well as the degree of interbreeding and, prioritize the preservation of each ancestral lineage and promote habitat/population connectivity where possible.

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.

See supplementary material
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Southern Hemisphere humpback whales (*Megaptera novaeangliae*) (Artiodactyla: Balaenopteridae) singing activity at Caño Island Biological Reserve, Costa Rica before, during, and after COVID-19 lockdowns

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ABSTRACT

Introduction: Boat traffic is recognized as a major contributor of underwater noise. Increasing presence of boats in coastal habitats is predicted to have important repercussions on the communication of marine mammals. In Costa Rica, the waters of the Caño Island Biological Reserve are an important breeding area for humpback whales from the Breeding-Stock G (BSG). Their predicted and abundant presence has fueled the development of whale watching activities as an important component of the local economy, and while the country has norms of conduct for this activity, whales often interact with multiple boats at the same time. The lockdowns associated with the COVID-19 pandemic provided a unique opportunity to study the potential impacts of noise associated with boat traffic on the singing activity of humpback whales.

Objective: Determine whether noise levels and boat acoustic presence around Caño Island Biological Reserve changed during the COVID-19 lockdowns, and if it did, what is the impact on song detection of BSG humpback whales.

Methods: Acoustic recordings were made using a bottom-mounted autonomous underwater recorder for 30 days in September 2019, 2020, and 2021, resulting in a total recording effort of 480 hours.

Results: Our results show that broadband underwater noise levels (dB_{RMS}) during pre-lockdown were significantly higher, particularly at frequencies below 1kHz, than during and post-lockdown. This is likely due to a decrease in the proportion of boat acoustic presence during the lockdown. Although the proportion of whale songs detected did not vary among years, whale songs were detected similarly throughout the day during the lockdown, compared to pre-and-post lockdown where the proportion of whale song presence decreased during hours when more boats were present.

Conclusions: This study shows a clear change in underwater noise levels during the COVID-19 lockdown, likely due to a decrease in boat presence. The study also highlights the potential impact of noise associated with boat traffic on humpback whale singing activity. The results of this study can inform the Conservation Areas of Osa

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(ACOSA) in charge of managing Caño Island Biological Reserve, to develop and implement mitigation measures to regulate underwater anthropogenic noise associated with tour boats.

Key words: ambient noise levels; boat traffic; whale-watching; tourism; bioacoustics.

RESUMEN

Actividad de canto de las ballenas jorobadas (*Megaptera novaeangliae*) (Artiodactyla: Balaenopteridae) del hemisferio sur en la Reserva Biológica de la Isla del Caño, Costa Rica, antes, durante y después de los cierres asociados a la pandemia de COVID-19

Introducción: Se reconoce que el tráfico de embarcaciones es uno de los principales contribuyentes al ruido marino. Se predice que la creciente presencia de barcos en los hábitats costeros tendrá importantes repercusiones en la comunicación de los mamíferos marinos. En Costa Rica, las aguas de la Reserva Biológica Isla del Caño son un hábitat reproductivo importante para las ballenas jorobadas de la población reproductiva G (BSG). Su presencia ha impulsado el desarrollo de las actividades de observación comercial de ballenas, el cual es un componente importante de la economía local. Aunque el país tiene normas de conducta para esta actividad, las ballenas a menudo interactúan con múltiples barcos turísticos al mismo tiempo. Los cierres y limitaciones de movilización de botes asociados a la pandemia de COVID-19 brindan una oportunidad para estudiar el impacto potencial del ruido asociado al tráfico de embarcaciones en la actividad de canto de las ballenas jorobadas.
Objetivo: Determinar si los niveles de ruido ambiental bajo el agua y la presencia de botes cambiaron antes, durante y después de los cierres y cuarentena por COVID-19, y si estos cambios influyen en la detección de cantos de machos de ballenas jorobadas.
Métodos: las grabaciones acústicas se realizaron con una grabadora autónoma montada en el fondo marino durante 30 días en septiembre de 2019, 2020 y 2021, resultando en un esfuerzo de grabación de 480 horas.
Resultados: Nuestros resultados muestran que los niveles de ruido ambiental (dB_{RMS}) antes del cierre (2019) fueron significativamente más altos, particularmente a frecuencias bajas (<1kHz), que durante y después de los cierres asociados a la pandemia de COVID-19. Es probable que esto se deba a una reducción en la presencia de embarcaciones durante el cierre. Aunque la detección de cantos de ballenas jorobadas no varió entre años, durante la cuarentena se detectaron los cantos de ballenas de forma uniforme durante el día, mientras que antes y después de la cuarentena se notó un decrecimiento en la proporción de cantos a horas donde hay más detección de botes presentes.
Conclusiones: Este estudio muestra un cambio claro en los niveles de ruido ambiental durante el cierre de COVID-19, probablemente debido a una disminución en la presencia de embarcaciones. El estudio también muestra el potencial impacto del ruido asociado al tráfico de embarcaciones en la actividad del canto de las ballenas jorobadas. Los resultados de este estudio pueden informar al Área de Conservación de Osa (ACOSA), la cual está a cargo de la Reserva Biológica Isla del Caño, a desarrollar e implementar medidas que regulen el ruido antropogénico bajo el agua asociado a tráfico de botes turísticos.

Palabras clave: ruido ambiental; tráfico de botes; observación de ballenas; turismo; bioacoustica.

INTRODUCTION

Boat traffic (i.e., ecotourism, personal use, fishing, water-taxis) is increasing rapidly in many coastal environments contributing to an increasingly noisier ocean (Erbe et al., 2019). Concerns about the potential repercussions of underwater noise impacts on whale communication are increasing among scientists worldwide (e.g., Cholewiak et al., 2018, Erbe et al., 2019). High noise levels can reduce the distance at which these animals can communicate (e.g., Cholewiak et al., 2018, Rey-Barquero et al., Laude et al., 2022) and can interrupt biologically important behaviors (e.g., Amrein et al., 2020; Sprogis et al., 2020). For example, Rey-Barquero et al., (2021) developed a model based on empirical data that showed that even in the presence of a single whale-watching boat, humpback whale, *Megaptera novaeangliae* (Borowski, 1781) song communication could be reduced by as much as 63 %. Another study found whale-watching activities to be one of the factors contributing to a decrease in communication space, with humpback whales experiencing masking levels of 80% or more

(Cholewiak et al., 2018). These studies show the potential negative impact of noise associated with boat traffic, particularly that of activities that directly target these animals, such as whale watching activities.

Whale-watching is a major catalyst of the local economy in Latin America (Tambutti & Gómez, 2020). The most recent review of the state of whale watching in Latin America is by Hoyt and Iñiguez (2008). The authors find that between 1996 and 2006, boat-based whale watching activities in the region grew at a rate three times higher than the rate of world tourism and five times higher than the rate of all Latin American tourism over the same period. Although many countries have adopted whale watching guidelines, compliance and enforcement remains limited (Gagne et al., 2022). As a result, whales might be exposed to noisy soundscapes.

In March 2020, the COVID-19 pandemic led to a global and national lockdown in Costa Rica to limit the spread of the virus. Widespread mandatory lockdowns and stay-at-home orders severely limited human mobility and activities in land and in the ocean. Costa Rica's economy shrank by 4.1% in 2020 as a consequence of these measures, and among the factors driving this trend was the sharp drop in tourism (Economic Survey of Latin America and the Caribbean, ECLAC, 2021). According to the CEIC Global Economy Database (2022), the number of air transported passengers dropped from 2,033,146 to 455,830 passengers. Worldwide, this drop in human mobility resulted in an increase in animal communication ranges; for urban songbirds, their communication range doubled in some places (Derryberry et al., 2020) and for dolphin and fish it increased to up to 65 % (Pine et al., 2021).

Male humpback whales are highly vocal animals that perform acoustic displays primarily during the breeding season (Herman et.al., 2013). The South Pacific waters off the coast of Costa Rica, and particularly the north and east side of Caño Island, are an important breeding area for Southeastern Pacific humpback whales, also known as the International Whaling Commission, designated Breeding Stock G (BSG) (Palacios-Alfaro et al., 2012; Rasmussen et al., 2007) as well as for whales from the "Central America" distinct population segment (DPS) (Bettridge et al., 2015). The BSG whales observed in Costa Rica migrate from feeding areas off the Antarctic Peninsula and the Fuegian Archipelago in Chile (Acevedo et al., 2017; Rasmussen et al., 2007). Multiyear boat surveys in this area indicate that BSG whales are particularly abundant in September (Palacios-Alfaro et al., 2012), and for this reason coastal communities in the area celebrate a whale festival during this month (Palacios-Alfaro, personal communication, 1 september 2022).

A previous study using passive acoustic monitoring data near Caño island found that male song activity occurred throughout the day, with a drop in singing activity during the morning hours, suggesting a potential response to whale watching tour boats presence (Chereskin et al., 2019). In other breeding areas in Brazil, increased boat traffic was shown to have a negative effect on humpback whale singing activity, with whales singing less when the number of boats increased (Sousa-Lima & Clark, 2008). In a recent study, Laute et al. (2022) found that on Icelandic foraging areas during the COVID-19 pandemic, whale watching trips were reduced by 68.6 %, and humpback whale call detection increased by nearly twofold. In this study we examine whether noise levels and boat acoustic presence around Caño Island Biological Reserve changed during the COVID-19 lockdowns, and if it did, how the detection of BSG humpback whale song was impacted.

MATERIALS AND METHODS

Study Area: This study took place at Caño Island Biological Reserve, 16 km northeast off the Osa Peninsula in the south Pacific coast of Costa Rica. This reserve protects approximately 58 km^2 of marine habitat around the island (Executive Decree 20790-MIRENEM, 1991). An autonomous underwater recorder was deployed in a location at the northeast side of the island called El Jardin (8.719 N/-83.863W, Fig. 1) at a depth of 25 m. This location is approximately 0.8 km from the island and is characterized by a sandy bottom (Chereskin et al., 2019). This area of the island has been highlighted by previous studies as an important reproductive habitat for BSG humpback whales (e.g., Rasmussen et al., 2007; Palacios-Alfaro et al., 2012). The island is also a tourist attraction with tour boats arriving to the island throughout the day to observe whales and dolphins, snorkel, and scuba dive.

Data Collection: An autonomous underwater recorder, a Soundtrap model 300 SD (Ocean Instruments; frequency range 20 Hz-150 kHz ±3dB; self-noise of less than sea-state in the bandwidth 100 Hz-2 kHz, and sensitivity of -203 dB re V/µPa) was deployed in El Jardin for the entire month of September 2019, 2020, and 2021. The recorder was programmed to record the soundscape at a sampling rate of 48 kHz for five minutes every 30 minutes in 2019, and for 15 minutes every 60 minutes in 2020 and 2021, resulting in a total of 480 hours recording effort. For this study, we only analyzed the first five minutes of every hour for each year, resulting in a total of 180 hours analyzed. These 5-min files were uploaded to RFCx ARBIMON (Rainforest Connection, 2020) for cataloguing and spectral inspection for presence/absence of whale songs and boats. The proportion of acoustic files with song and boat presence was calculated by dividing the number of 5-min files with whale songs and boats by the total number of 5-min files per year and time of day.

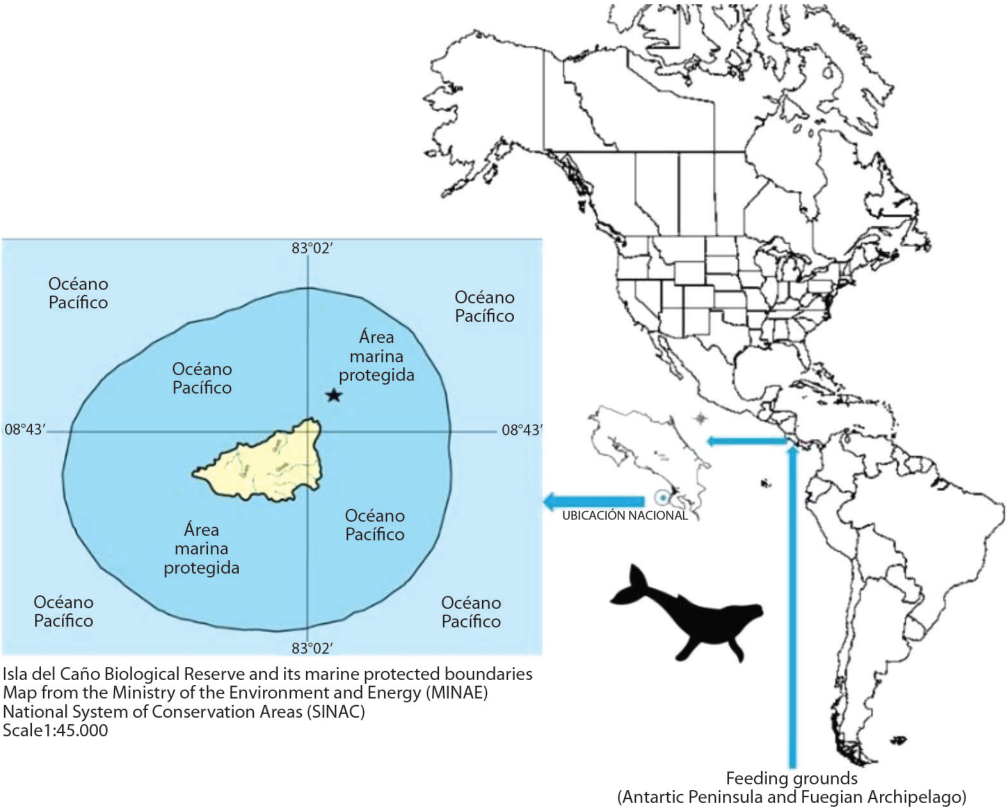

Fig. 1. Location of Caño Island Biological Reserve off the Osa Peninsula in the South Pacific of Costa Rica. The figure also shows a cartoon of a straight-line from Costa Rica towards the feeding areas off the Antarctica Peninsula and the Fuegian Archipelago in Chile to Caño Island, Costa Rica. The black star represents the deployment site of the autonomous underwater recorder.

Ambient Noise Levels: To measure broadband ambient noise levels we used the software dBWav from Marshall-Day Acoustics (https://www.marshallday.com) which is tailored for Ocean Instrument Soundtrap hydrophones. Upon calibration following Ocean Instruments instructions, the first five minutes of every hour were selected manually. Manual selection was done because dBWav does not automatically select the region of interest. We selected a subsample of days for this part of the data analysis including the following days for each year: 4, 7, 10, 13, 16, 19, 22, 25, and 28. The average root-mean-square (RMS_{dB}) was then generated for each 1/3 octave bands ranging from 12.5 Hz to 10 kHz.

Statistical analysis: The statistical software SPSS 25 (IBM, 2017) was used for summary statistics and statistical analyses. An ANOVA test for multiple independent variables (n-way) was used to study the effect of year (pre, during, and post lockdown), time of day (hour), and 1/3 octave frequency bands on noise levels (RMS_{dB}). Broadband noise levels were compared among years using a non-parametric Kruskal-Wallis' test. The 1/3 octave bands were grouped into two categories: below and above 1kHz frequency categories. Below 1 kHz included bands from 12.5 to 800 Hz and above 1 kHz bands from 1 to 10 kHz. A Kruskal-Wallis test was also used to assess if there are differences in these two frequency categories among years. A Chi-Square one sample test for goodness of fit was used to compare the proportion of recordings with whale song and boat presence per year. Finally, the proportion of noise levels, whale song, and boat presence were plotted against time of day using a smoothing spline with a bootstrap confidence of fit using a lambda of 0.05 to look for diel patterns in ambient noise levels and whale song and boat presence within each year.

RESULTS

Ambient Noise Levels: The results from the ANOVA analysis suggest that year (F =1610.5, df=2, p<0.001), time of day (hour) (F=4.8, df=23, p<0.001), 1/3 octave frequency bands (F=369.8, df=27, p <0.001), and the interactions between year*time of day (F=9.6, df=46, p <0.001) and year*1/3 octave frequency bands (F=176.7, df=52, p <0.001) influenced the variation in ambient noise levels. Broadband ambient noise levels varied significantly among years (Kruskal-Wallis=876.06; df=2, p <0.001, Table 1, Fig. 2A, Fig. 2B). In general, ambient noise levels were higher in pre-lockdown than during and post-lockdown (Table 1, Fig. 2A) and these differences among years were maintained throughout the day (Fig. 2B). When accounting for frequency categories

Table 1
Summary statistics of ambient noise levels (in dB) and the proportion of whale song and boat presence in pre-lockdown, during lockdown, and post-lockdown at Caño Island Biological Reserve.

	Pre-lockdown (2019)	During lockdown (2020)	Post-lockdown (2021)
Ambient noise levels (dB)			
Mean±SD	99.8 ± 15.3	93.0 ± 11.6	92.0 ± 11.0
Coefficient of Variation (CV)	15.3	12.5	11.8
Range (minimum-maximum)	63.9 -- 134	63.7 -- 127.4	62.1 -- 126.8
Acoustic boat presence			
Mean±SD	0.08 ± 0.13	0.03 ± 0.06	0.04 ± 0.09
Coefficient of Variation (CV)	152.8	169.6	193.9
Range (minimum-maximum)	0 – 0.44	0 – 0.22	0 – 0.30
Acoustic whale song presence			
Mean±SD	0.98 ± 0.02	0.99 ± 0.01	0.97 ± 0.04
Coefficient of Variation (CV)	2.0	0.8	4.2
Range (minimum-maximum)	0.93 – 1	0.96 – 1	0.86 – 1

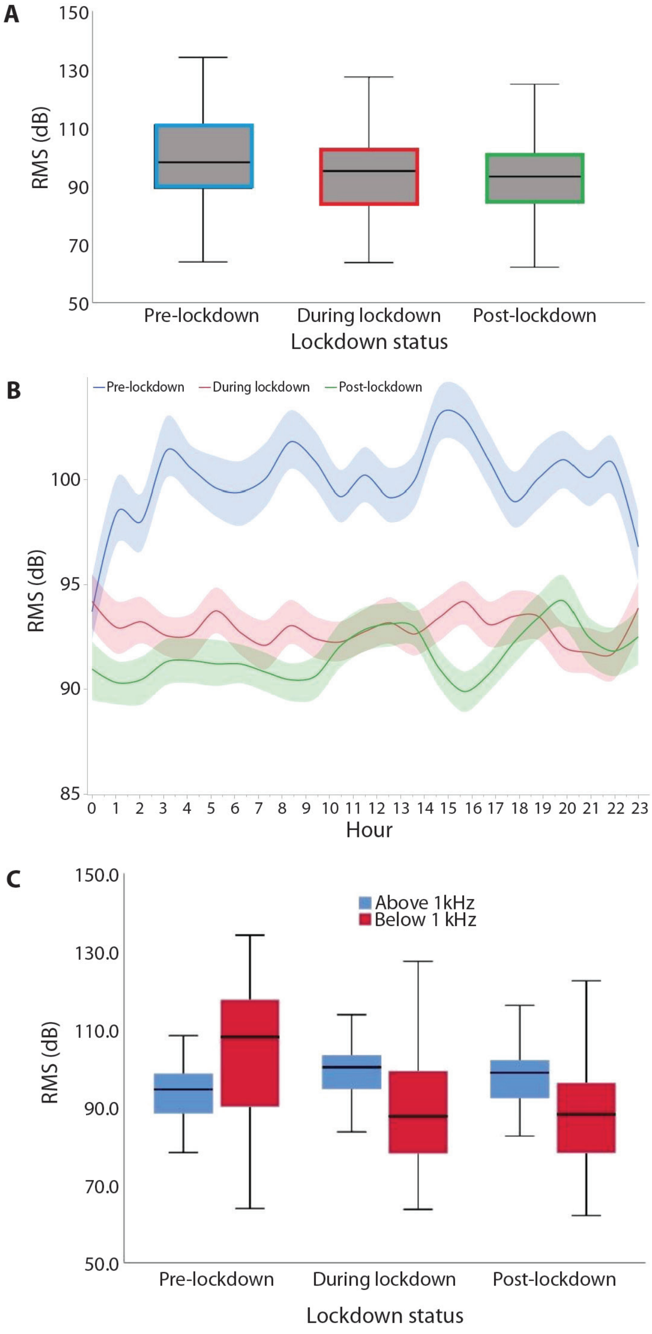

Fig. 2. A. Broadband ambient noise (RMS_{dB}) levels by year. **B.** Broadband ambient noise levels (RMS_{dB}) by time of day. **C.** Broadband ambient noise levels (RMS_{dB}) by year and frequency category. Broadband noise levels measured as the average root-mean-square (RMS_{dB}) (a) by year and (b) time of day, in blue pre-lockdown (2019), in red during lockdown (2020) and in green post-lockdown (2021), and by (c) 1/3 octave frequency bands grouped into two categories below 1 kHz in red (12.5 to 800 Hz) and above 1 kHz in blue (1-10 kHz) and year, at Caño Island Biological Reserve, Costa Rica. The box plots display median, first and third quartiles, and maximum and lower values (excluding outliers) and the line plot uses a smoothing spline and bootstrap confidences of fit with a lambda of 0.05.

above and below 1 kHz we find significant differences among years. Ambient noise levels below 1 kHz were significantly higher in pre-lockdown (Kruskal-Wallis=1138.5; df=2, $p < 0.001$, Fig. 2C) while ambient noise levels above 1 kHz were significantly higher during and in post-lockdown (Kruskal-Wallis=465.5; df=2, $p < 0.001$, Fig. 2C).

Acoustic presence of whale songs and boats: A total of 2 058 5-min per hour files were used for this analysis (2019 = 695 files, 2020 = 643 files, 2021 = 720 files). Of these, a total of 2 028 5-min files had an acoustic presence of whale song (2019 = 686 files, 2020 = 642 files, 2021 = 700 files) and 117 5-min files contained sounds of passing boats (2019 = 60 files, 2020 = 22 files, 2021 = 35 files). The proportion of 5-min files with boats passing by was significantly lower during lockdown than in pre-lockdown and post-lockdown (χ^2=61, df=2, p =0.003, Table 1). Interestingly, the diel acoustic presence of boats across years followed a similar pattern, with a peak in presence between 9 a.m. and noon for all years (Fig. 3A). No significant differences ($p > 0.05$) were found in the proportion of 5-min files with whale songs across years, but pre-lockdown and post-lockdown years showed the greatest reduction in the proportion of files with whale songs compared to during the lockdown (Table 1, Fig. 3B). In addition, we found differences in diel patterns of the proportion of whale song presence among years. During pre-lockdown, the highest proportion of recordings with whale songs occurred at midnight and around 2 p.m. During the lockdown, the presence of whale songs was almost constant throughout the day, with a slight decrease between 9 a.m. and 2 p.m., and in post-lockdown a sharper decrease in whale song presence occurs approximately between 5 a.m. and 6 p.m., with the lowest proportion of whale song presence occurring between 10 a.m. and noon (Fig. 3B).

DISCUSSION

This study shows that underwater ambient noise levels and boat presence changed between pre-lockdown, during lockdown, and post-lockdown periods around Caño Island Biological Reserve. The year before the COVID-19 pandemic had the highest mean underwater noise levels, particularly at low frequency below 1 kHz, and a higher proportion of boat presence. Although the proportion of whale song presence did not vary significantly among years, but we did observe a decrease in the proportion of acoustic files with whale songs during pre-and-post-lockdown. We also found differences in the diel pattern of song presence by time of day among years, suggesting a potential impact of tour-boat traffic in the area.

Boat traffic and shipping activities are the most dominant sources of underwater noise at low frequency (Southall et al., 2017). During the COVID-19 pandemic these activities were significantly reduced due to a slowdown of the global trade activity in order to mitigate and minimize the spread of the virus (Ryan et al., 2021; Thompson & Barclay 2020). This decrease in low-frequency underwater noise levels resulted in a potential increase in animal communication ranges during the COVID-19 lockdowns. For example, in New Zealand, underwater ambient noise levels dropped nearly threefold, resulting in an increased in dolphin and fish communication ranges to up to 65 % (Pine et al., 2021), while in Iceland a reduction of whale watching trips resulted in a twofold increase in humpback whale call (Laute et al., 2022), and a similar increase in dolphin whistle detection rates were found in Panama (Gagne et al., 2022).

Caño Island Biological Reserve is an important breeding area for humpback whales (Rasmussen et al., 2007). Therefore, it is not surprising that their overall presence did not change between periods. However, Caño Island

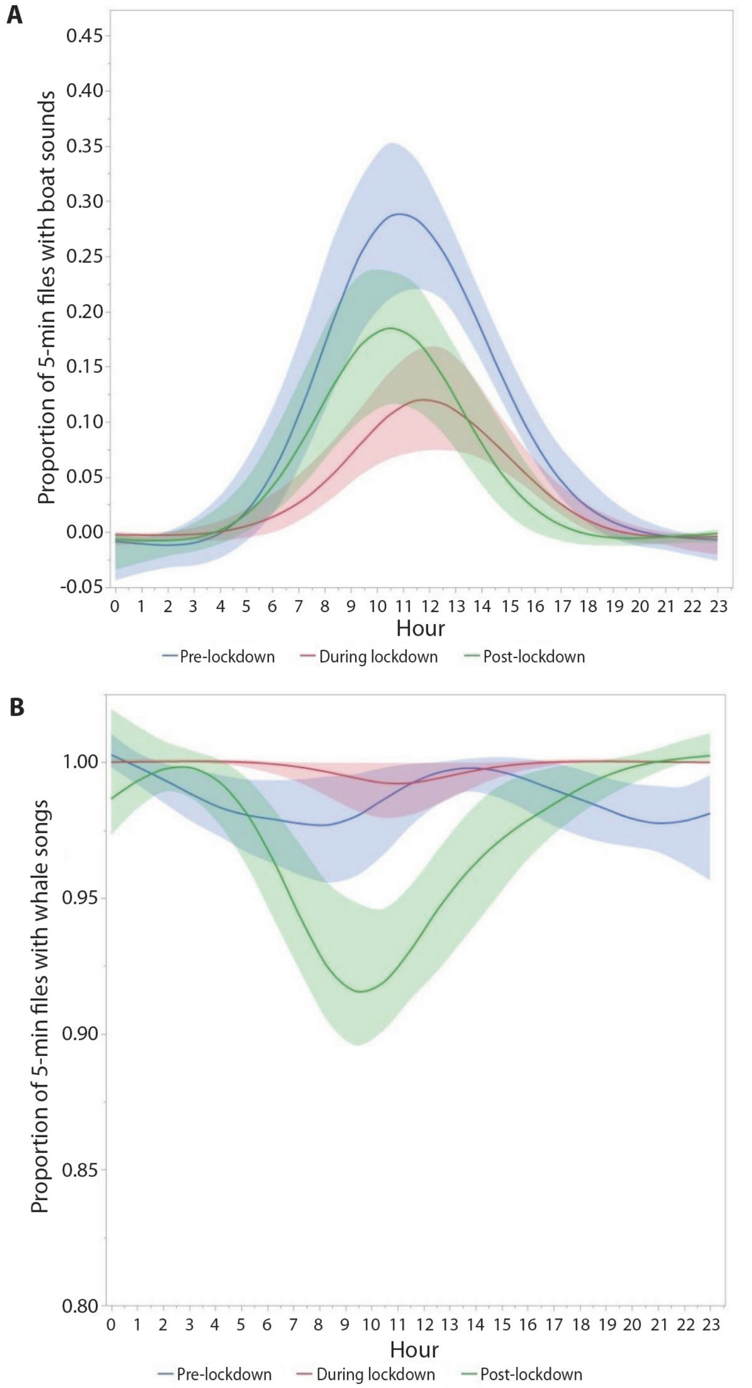

Fig. 3. Diel song detection of BSG humpback whale songs (a) and boat sounds (b) measured as the proportion of 5-min files per hour with songs and boat sounds during pre-lockdown (2019 in blue), during lockdown, (2020 in red) and post-lockdown (2021 in green) at Caño Island Biological Reserve, Costa Rica. These figures were done using a smoothing spline and bootstrap confidences of fit (light read and blue colors) with a lambda of 0.05. To facilitate visualization of the data, notice that scales for the proportion of boats and whale song detections are different.

Biological Reserve is also an important tourist destination, and while whales are not the primary target for all tour activities, boat presence is high in the area (Chereskin et al., 2019). A previous study showed that in September at Caño Island, BSG humpback whale singing activity happens primarily at nighttime hours (Chereskin et al., 2019). Similar diel singing activity patterns have been described in other humpback whale populations and appears to be widespread (e.g., Cholewiak 2008; Homfeldt et al., 2022). However, in this study we found that during the COVID-19 lockdown, the proportion of whale song presence was similar throughout the day (i.e., Fig. 3B). This suggests that in 'normal' times tour-boats might be reducing whale singing activity during the day, leading to higher rates during nighttime hours. For example, we observed a decrease in the proportion of whale song detections when boat presence increased during the day, particularly in pre-and-post-lockdown years. A high presence of tour-boats could lead to signal masking, which can lower the proportion of time that whale songs are detected by passive acoustic monitoring sensors, and similarly impacting detection among whales. Alternatively, whales may invest less in singing when there is a high presence of boats (and associated noise) and invest more time in singing at nighttime hours when boat presence is low (e.g., Parks et al., 2014). Such decision-making tradeoffs have been shown in humpback whales off the Ogasawara Islands in south Tokyo, where whales stopped singing when boats were nearby (Tsujii et al., 2018), and in humpback whales from the Abrolhos National Marine Park in Brazil, where humpback whales sang less when the number of boats increased (Sousa-Lima & Clark 2008).

The function of humpback whale song continues to be a subject of debate. Songs have been proposed to function as a reproductive display, by which males compete among themselves and attract potential mates (Herman 2007, Garland & McGregor 2020; Whitten 2019). That these songs can be masked by the underwater noise from human activities or that whales stop their acoustic displays when there is a high presence of boats is of concern. The high proportion of acoustic files with whale songs during the COVID-19 lockdown suggests that whales have the potential to sing similarly throughout the day, but nighttime is likely the most efficient time to sing when boat traffic is high during the day, and highlights the potential impact boat traffic can have in their reproductive displays (i.e., males singing less) (Sousa-Lima & Clark 2008; Tsujii et al., 2018,) and habitat use (i.e., changing behavioral activities) (Sprogis et al., 2020).

As lockdown restrictions are lifted, tour boat presence is expected to return to normal at Caño Island. This study shows the potential impacts of unregulated tour boat presence on this important reproductive environment. These impacts are an important consideration for mitigation efforts and government investment in the enforcement of whale-watching regulations, as well as in the design and implementation of measures to regulate anthropogenic noise levels in this protected area.

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: LJMC conceptualized the study, collected data in the field and the lab, coordinated data collection, performed data analysis, interpreted the data, and drafted and revised the manuscript. SB generated noise level data, and contributed to the drafting of the manuscript. GD, generated presence-absence data, and contributed to the drafting of the manuscript. JDPA and JJA collected data in the field, recovered and deployed soundtraps, and contributed to the drafting of the manuscript.

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First song description of the humpback whales, *Megaptera novaeangliae* (Balaenopteridae: Artiodactyla), breeding off Nicaragua

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ABSTRACT

Introduction: Humpback whales belonging to the Central American (CA) Distinct Population Segment breed off the Pacific coast of Nicaragua. Knowledge on this endangered population and its conservation status is limited.
Objective: The aim of this study is to provide the first description of the CA humpback whale song off Nicaragua, which helps further understanding on the population's dynamics.
Methods: Acoustic recordings of songs were obtained during dedicated boat-based surveys at two locations on the Pacific coast of Nicaragua in 2018. Recordings were made from the boat using a portable system for a total of 23 hours and 56 minutes over 32 days from January to April 2018. A total of nine recordings of high enough quality for the song analysis were identified during this period from three different days at Padre Ramos (PR) (northern site) and four different days at San Juan del Sur (southern site). Song structure was described using standard humpback whale song elements, i.e. themes, phrases, and units.
Results: A total of seven themes, seven phrases, and 19 unit types were identified. Three of the themes were common and frequently repeated in a song cycle while the others were less common in the repertoire and were recorded only during the middle of the season. Song theme order was variable, both within and across song sessions.
Conclusions: This study provides the first song description of humpback whales along the Pacific coast of Nicaragua. Comparison of these songs with other datasets from the CA population and other breeding areas in the Northern Hemisphere could help understand the migratory patterns of these animals and the level of connectivity among populations since song can be socially learnt. Future data collection of humpback whale song recordings in Nicaragua is necessary to gain further understanding on the song structure variation within this population and the mechanisms of song transmission and dynamics across populations in the region.

Key words: Acoustics; Central America; Song structure; North Pacific; Reproduction.

RESUMEN

Primera descripción del canto de las ballenas jorobadas, *Megaptera novaeangliae* (Balaenopteridae: Artiodactyla) que se reproducen en Nicaragua

Introducción: La ballena jorobada del segmento poblacional Centroamericano se reproduce en la costa Pacífica de Nicaragua. El conocimiento sobre la biología y estado de conservación de esta población amenazada y su estado de conservación es limitado.
Objetivo: El objetivo de este estudio es generar la primera descripción del canto de las ballenas jorobadas de la población de Centroamérica observadas en aguas nicaragüenses.
Métodos: Se obtuvieron grabaciones acústicas durante salidas de investigación en dos localidades de la costa Pacífica de Nicaragua en el 2018. Las grabaciones se realizaron desde el bote utilizando un sistema portátil durante un total de 23 horas y 56 minutos en 32 días de enero hasta abril del 2018. Un total de nueve grabaciones de suficiente calidad para el análisis de los cantos fueron identificados durante este periodo en tres días en Padre Ramos (Norte) y cuatro días en San Juan del Sur (Sur). La estructura del canto se describió de acuerdo con los elementos estándares de canciones de ballenas jorobadas: temas, frases y unidades.
Resultados: Se identificaron siete temas, siete frases y 19 unidades. Tres temas eran comunes y se repetían varias veces dentro de una canción mientras que otras eran menos comunes en el repertorio de canto y se grabaron solo en la mitad de la temporada. El orden de los temas en la canción fue variable a dentro de la canción y entre las sesiones de canto.
Conclusiones: Este estudio representa la primera descripción del canto de las ballenas jorobadas en el Pacifico nicaragüense. La comparación de estos cantos con los de otras localidades de la población de Centroamérica y con otras áreas de reproducción de poblaciones en el hemisferio norte contribuirá a comprender mejor los patrones migratorios de estos animales y el nivel de conectividad entre poblaciones desde que la canción fue aprendida. La recolección futura de datos de cantos de ballenas jorobadas en Nicaragua es necesaria para mejorar la comprensión de la variación en la estructura del canto de esta población y el mecanismo de transmisión y dinámica de canto entre poblaciones de la región.

Palabras clave: Acústica; Centroamérica; Estructura de canción; Pacifico Norte; Reproducción.

INTRODUCTION

The humpback whale, *Megaptera novaeangliae* (Borowski, 1781) is known for its seasonal migration between breeding and feeding areas. There are 14 recognized Distinct Population Segments (DPS), of which seven located in the North Pacific, and amongst which the Central America DPS (Bettridge et al., 2015), the focus of this study. The Central America whales (hereafter CA whales) migrate to Central America between January and April, from their feeding areas on the West coast of the United States (Calambokidis et al., 1997; Calambokidis et al., 2000; De Weerdt et al., 2022). The waters off Nicaragua are considered important breeding habitats for CA whales with Padre Ramos being used mainly by mother-calf pairs, and San Juan del Sur mainly by adult whales (De Weerdt et al., 2022). CA whales are listed as endangered according to the National Marine Fisheries Service (NMFS) and little information is available on their ecology (Bettridge et al., 2015).

Humpback whale males produce structured and complex songs (Payne & McVay, 1971), and while singing activity occurs extensively during the breeding season, it has also been described along migration corridors and on feeding areas (Kowarski et al., 2022; McSweeney et al., 1989; Schall et al., 2022). Humpback whale songs are used as a parameter to study individual movements and population dynamics (Cerchio et al., 2001; Cholewiak & Cerchio, 2022; Darling, Goodwin, et al., 2019; Darling & Sousa-Lima, 2005). Males from the same population conform to similar songs, which evolve through the season. This evolution partially happens by the error accumulation of singers. Accumulation of changes, the sharing of such changes and culture are determined by the social interactions between singers (Garland et al., 2022).

Because only males are known to sing (Cholewiak & Cerchio, 2022; Darling & Bérubé, 2001; Darling et al., 1983), their songs are thought to be a male precopulatory display

(Dines et al., 2015) used to attract females and/or mediate male-male interactions (Herman, 2017; Smith et al., 2008; Tyack & Clark, 2000). Humpback whale songs are traditionally classified into themes, phrases, and units, allowing comparisons of song elements across geographical regions (Darling & Sousa-Lima, 2005; Payne & Payne, 1985; Payne et al., 1983) and between populations (Cholewiak & Cerchio, 2022; Garland et al., 2011; Schall et al., 2022). Within a breeding season, the song can vary, however, males eventually conform to the same song for their population (Herman, 2017). Songs sung by humpback whales undergo both an evolutionary and a revolutionary process (Owen et al., 2019). The evolutionary process consists of song modification over time by accumulation of small changes resulting in a progressive change (Cerchio et al., 2001; Garland et al., 2011), while revolution, or cultural diffusion, is a rapid change whereby a novel song appears and is adopted by the population (Noad et al., 2000). A song can therefore change within and between seasons in any given population.

Humpback whale song studies can provide valuable insights on population connectivity and function (Garland et al., 2011; Garland et al., 2022; Garland, Gedamke, et al., 2013). Understanding changes in humpback whale songs within and between seasons can help understand migratory routes, by comparing songs between regions. Furthermore, studying song composition within and between seasons can provide insights on the cultural transmission that occurs in different humpback whale populations (Darling, Acebes, et al., 2019; Garland et al., 2015; Zandberg et al., 2021). Presently, there is only one study describing songs of CA humpbacks at their breeding ground in 2016-2017 off Caño Island, Costa Rica (Chereskin et al., 2019). This present study provides additional information on the song of CA whales breeding in Central America; until now no description of song is available for animals breeding in Nicaraguan waters. Recent work comparing phrase compositions of humpback whale songs sung between 2011 and 2013 in Mexico, Hawaii, the Philippines and Japan showed that mixing of population throughout the North Pacific occurs (Darling, Acebes, et al., 2019).

Boat based surveys took place between January and April 2018 on two study sites along the Nicaraguan Pacific coast: Padre Ramos, northern Nicaragua and San Juan del Sur, southern Nicaragua. During the surveys, an omni-directional hydrophone (H2a Aquarian) connected to a recorder (Tascam DR-05V2) was deployed to determine presence/absence of singers every 30 minutes. Whenever a singer was detected, and if their song was audible above the background noise, a recording was made at a sampling rate of 92 kHz and 24 bits resolution for as long as the song was detectable.

A total of 108 recordings (San Juan del Sur, n = 66; Padre Ramos, n = 42) were made in 32 days of boat survey effort during the 2018 breeding season. Any recordings with multiple audible singers and/or with low signal-to-noise ratio (SNR) were excluded from further analyses. SNR was estimated by measuring NIST Quick Signal-to-Noise ratio (Sostres Alonso & Nuuttila, 2015) for each labeled unit in every recording (RAVEN 1.5; (K. Lisa Yang Center for Conservation Bioacoustics at the Cornell Lab of Ornithology, 2022)). Recordings that captured at least 10 minutes of a song were selected for the analysis as humpback whale songs are known to last on average 10-15 minutes (Cerchio et al., 2001). An additional criterion was that the SNR of the recordings had to be 14 dB or above. We considered a humpback whale song as a continuous recording containing at least one performance of each theme in that moment of the season (as it happens that themes are added or lost within the season) (Cholewiak et al., 2013). Songs recorded on different days were considered as samples of distinct individuals (Warren et al., 2020), while recordings from the same day were assumed to be from the same individual. Each analyzed recording contained either a song or a song fragment (i.e., part of a song composed of a stereotyped series of vocalizations, repeated at least once), as described in

Kowarski et al. (2019). Whether songs were incomplete because the animal moved away, stopped singing or did not perform the full song, could not be determined due to the logistical constraints of the setup (e.g. boat drifting substantially from original location). Only nine recordings were above 10 minutes in length and met our SNR criterion. The selected recordings totaled 3 hours and 30 minutes for the analysis. These recordings were collected on three different days at PR and four different days at SJDS; therefore, based on the assumption that each day represents a new singer, an estimated number of seven singers were recorded (Fig. 1).

Songs were manually analyzed in RAVEN Pro by generating spectrograms, with a Fast Fourier Transform (FTT) of 1 024 points resolution, and a Hanning window (50 % overlap), corresponding to frequency resolution of 43.1 Hz. Finer frequency resolution (2 Hz) was used for the graphical representations presented in this study. Following the method of Cholewiak et al. (2013), songs were classified into themes, phrases, and units. For the unit classification, the following acoustic variables were considered: fundamental frequency contour, tonal quality type (e.g. tonal or pulsed), duration (delta time), peak, low, high, and delta frequency (Malige et al., 2020). Each unit type was assigned a unique letter (Green et al., 2011). To test the consistency of the manual unit classification, a Random Forest Analysis (RFA) was applied on the measured variables of the units: low, high, Delta and peak frequencies and the duration (Delta time). A Random Forest model is a machine learning algorithm that combines multiple decision trees using "bagging" to create a final classification that averages all the decisions. The Out Of Bag (OOB) score is a way to validate a Random Forest model (Hastie et al., 2009).

Phrases were assigned only after reviewing multiple recordings to ensure consistency. The guidelines described in Cholewiak et al. (2013) were followed for the identification of phrases within the recordings. Transitional phrases were also identified and marked as such. However, these are not included in the results to clearly indicate the song sequence. Finally, themes were identified and assigned a unique color (Cholewiak et al., 2013), based on the phrase delimitations and sequence. A total of 19 unit types were identified based on the manual classification. The RFA of 1500 trees tested the manual classification of units and gave an OOB error rate of 31 %, showing duration and peak frequency as the most important measurable variables for unit classification. This OOB value indicates that manual classification was robust and reliable throughout the dataset. All unit types were shared on both Nicaraguan sites.

The CA male whales in Nicaragua sang songs made of seven themes represented by different colors in Fig. 1. The themes represented by the colors light and dark green are constituted of the repetition of phrases 4 or 3, respectively. These phrases differed by just one unit (j vs. n). However, since they were sung consistently separately, they were considered as unique themes (Fig. 1). The red and purple themes are made up of the repetition of phrases 1 and 2 respectively. These phrases share a common subphrase made of units a and b at the beginning of the phrase. At the start of the season, almost all themes were recorded on both sites. However, the sequence of themes varied between study sites (and within the season). In January, all themes were present in San Juan del Sur, except for the light green theme. However, the sample size at this location is limited to a single day of recording for January. The same themes observed in January in San Juan del Sur were present in Padre Ramos in February, except for the light green theme that appeared in Padre Ramos. Finally, the yellow and orange themes were not encountered in March at both sites and in April in San Juan del Sur only. There was only one common month of recording between sites (March), but both sites presented the same overall number of themes. Due to the lack of common recordings in the same months, it is challenging to compare the two sites.

The results of this study show a lot of variability in theme sequences. This indicates the 2018 Nicaragua CA song did not follow a

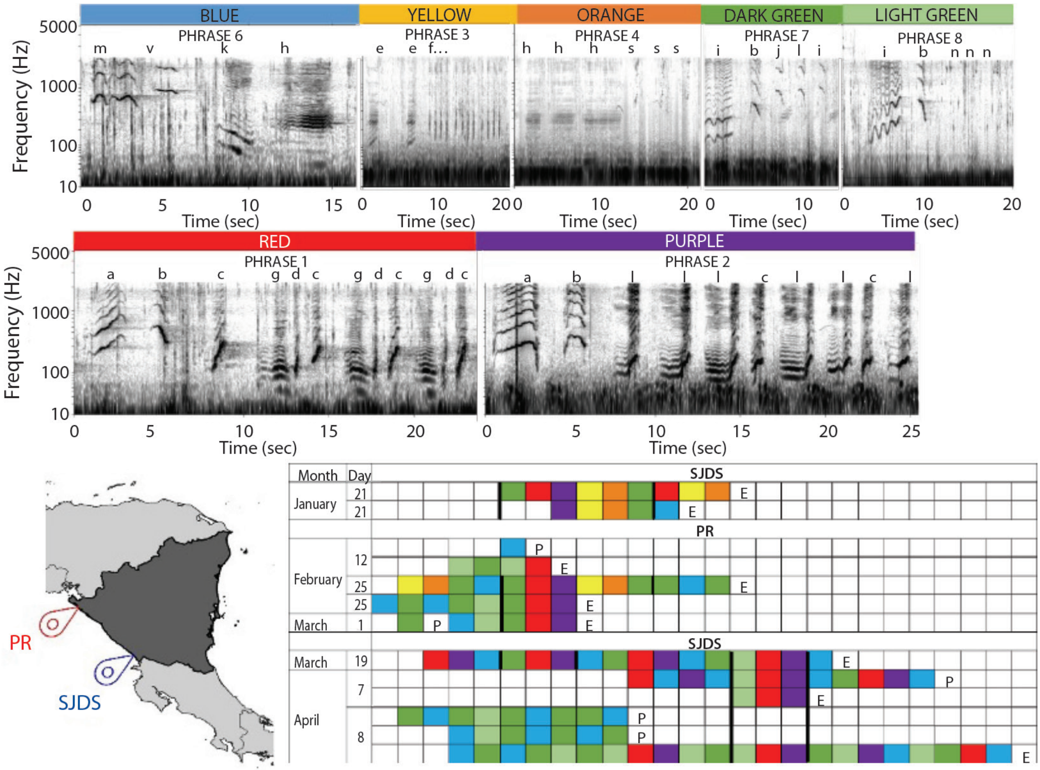

Fig. 1. Spectrograms of phrases identified in CA humpback whales singing off Nicaragua (hamming window, frequency resolution: 2 Hz, frame length: 0.128 s, time step: 0.032 s); the repetition of such phrases results in the theme identified at the top of each spectrogram. Distinct themes are represented by the colors red, yellow, orange, dark green, light green, purple and blue. The sequence of themes per recording are presented per site at Padre Ramos: PR (north) and at San Juan del Sur: SJDS (south) at the bottom of the figure (table) (P = Pause in whale singing; E = End of recording). A new line was started when there was a pause in the singing in the recording that was longer than the silence between phrases. The theme sequence was aligned (thick vertical black lines) arbitrarily to try to identify matching sequences across recordings.

strict theme order, as described for some other Southern hemisphere populations (Garland, Noad, et al., 2013). The presence of common themes at both sites suggests that a certain level of movement of males takes place; this is in contrast with the low level of photographic recapture observed between sites (De Weerdt et al., 2022). This inconsistency highlights the need for further research to understand whale movements in this area. To confirm if the same song is sung on both sites at the same time, synchronous recordings during the entire season would be needed. Gradual change of song along the season was observed in studies on other breeding grounds, such as Mexico and Hawaii (Cerchio et al., 2001; Darling, Acebes, et al., 2019). Compared to other Northern hemisphere songs, this study showed a more 'elaborate' and less organized song structure than previously reported for the 2016-2017 breeding season in Caño Island, Costa Rica (Chereskin et al., 2019). No further comparison with the song from Costa Rica could be achieved due to the limited information presented about individual unit characteristics and the resolution of the spectrograms. Within season comparison between breeding grounds would allow to gain a better insight on the dynamics and potential exchanges between animals in these breeding grounds.

Although our dataset was rather small, a poorly structured theme sequence could be observed in the second half of the season; this could be due to the mixing with other populations such as the Mexican DPS; furthermore, error accumulation over the entire season could be an alternative or concurrent explanation for the observed lack of structured sequence in the song (Mcloughlin et al., 2018; Mercado III et al., 2005) and/or the evolution of the song as the season progresses. In other words, whales may be rearranging themes and introducing or removing elements until the end of the season. A broader dataset would be required to determine if the pattern observed in this study is representative of the CA population in Nicaragua or if these are idiosyncrasies from individual singers. Interestingly, an extensive variability in the order in which themes are sung was also observed in some recordings from Socorro, Mexico, in unpublished work from Smith-Aguilar, as reported in Cholewiak et al. (2013). The gradual replacement of the yellow and orange themes by the more common blue theme as the season progressed, suggests that gradual song changes took place in the songs of Nicaraguan whales. This process is described as song evolution and it has been widely reported for other humpback whale populations in both hemispheres (Fournet et al., 2018; Garland et al., 2011; Mercado, 2021; Payne & Payne, 1985). The findings of this paper can be used for future regional song comparisons with whales from the North Pacific to assess population connectivity. Future work should explore song evolution and the seasonal dynamics within the Central America population.

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Author Contributions: JDW, conceptualized the study, acquired the data, and contributed to analysis interpretation, and drafted the initial and revised versions of this manuscript. DD, RSL, and FP contributed to data analysis and interpretation of results, and contributed to the drafting of the manuscript at all stages.

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SUPPLEMENT • WHALES

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Migratory destinations of endangered humpback whales, *Megaptera novaeangliae* (Cetartiodactyla: Balaenopteridae), from El Salvador

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ABSTRACT

Introduction: The study of many aspects of cetacean ecology is made possible by identifying individuals through space and time. Humpback whales (*Megaptera novaeangliae*) can be easily identified by photographing their ventral tail flukes' unique shape and pigmentation patterns. The small and endangered distinct population segment (DPS) of Central America humpback whales visit El Salvador seasonally each winter; however, dedicated research has been extremely limited there. Before 2018, only 11 individual whales had been photo-identified, and the migratory destinations of Salvadoran humpback whales were unknown. In recent years, photo-identification efforts have increased, and today there are 92 individually identified humpback whales from El Salvador.

Objective: To identify the main high-latitude feeding areas of Salvadoran humpback whales.

Methods: Using the online matching platform Happywhale, Salvadoran whales were matched via automated image recognition to a global humpback whale fluke photo-identification catalog of 66 043 individuals.

Results: In total, 80 (87.0 %) of the whales photographed in El Salvador were matched to individuals seen in North Pacific feeding areas. Sighting histories of Salvadoran whales resighted in feeding areas ranged from two to 29 years (average = 12.1, SD = 5.8). While we note that survey effort was likely very different between regions, the main feeding area of Salvadoran humpback whales on Happywhale was Central California (n = 70, 76.1 %). Of these whales, 21 (22.8 %) had also been sighted in Southern California, while just three (3.3 %) individual whales were registered only in Southern California. Additionally, two whales (2.2 %) were sighted in Southern British Columbia, Canada, and one whale was matched to a humpback whale from Southeast Alaska. This whale (of unknown sex) has a sighting history of 27 years but no prior documentation in a breeding area and is the first published sighting of a Southeast Alaskan humpback whale in the breeding area of the endangered Central America DPS.

Conclusions: Our study shows that while Salvadoran humpback whales were matched to various feeding areas in the Eastern North Pacific, their primary migratory destinations are in Southern and Central California.

Key words: large whale conservation; endangered populations; migratory species; critical habitat protection; Central America distinct population segment.

RESUMEN

Destinos migratorios de ballenas jorobadas en peligro de extinción, *Megaptera novaeangliae* (Cetartiodactyla: Balaenopteridae), del El Salvador

Introducción: El estudio de muchos aspectos de la ecología de los cetáceos es posible gracias a la identificación de individuos a través del espacio y el tiempo. Las ballenas jorobadas (*Megaptera novaeangliae*) se pueden identificar fácilmente al fotografiar la forma única de la aleta caudal y el patrón de pigmentación de su lado ventral. Una parte del segmento de población distinta (DPS), pequeña y en peligro de extinción de las ballenas jorobadas de América Central visita estacionalmente cada invierno El Salvador, sin embargo, la investigación ha sido extremadamente limitada. Antes del 2018, solo existían imágenes de identificación de 11 ballenas individuales, y se desconocía el destino migratorio de las ballenas jorobadas salvadoreñas. En los últimos años, los esfuerzos de foto-identificación han aumentado, y hoy en día hay 92 ballenas jorobadas identificadas individualmente en El Salvador.
Objetivo: Identificar las principales áreas de alimentación en latitudes altas de las ballenas jorobadas salvadoreñas.
Métodos: Utilizando la plataforma en línea Happywhale, las aletas caudales de las ballenas de El Salvador fueron comparadas a través del reconocimiento automático de imágenes con un catálogo global de foto-identificación de 66 043 individuos.
Resultados: En total, 80 (87.0 %) de las ballenas fotografiadas en El Salvador se compararon con individuos vistos en las áreas de alimentación del Pacífico Norte. El historial de re-avistamientos de ballenas salvadoreñas en áreas de alimentación fluctó de dos a 29 años (promedio = 12.1, d.e. = 5.8). La principal zona de alimentación para las ballenas jorobadas salvadoreñas fue el centro de California (n = 70, 76.1 %). De estas ballenas, 21 (22.8 %) también se habían avistado en el sur de California. Solo tres ballenas se habían visto (3.3 %) únicamente en el sur de California. Dos ballenas jorobadas (2.2 %) fueron avistadas en el sur de la Columbia Británica, Canadá, y lo más importante, una fue identificada como una ballena jorobada del sureste de Alaska. Esta ballena de sexo desconocido tiene un historial de avistamientos de 27 años, pero sin documentación previa en un área de reproducción. Este es el primer avistamiento publicado de una ballena jorobada del sudeste de Alaska en el DPS en peligro de extinción de América Central, y tal vez sea particularmente importante por razones de manejo.
Conclusiones: Este estudio encuentra que aunque las ballenas jorobadas que llegan a El Salvador se observan en varias localidades del Pacifico norte, su migración tiene como destino principal las aguas del Pacifico sur y central de California.

Palabras clave: conservación de ballenas; poblaciones en peligro; especies migratorias; protección de hábitat crítico; Segmento Poblacional Distinto de Central America.

INTRODUCTION

The humpback whale, *Megaptera novaeangliae* (Borowski, 1781) is a large migratory baleen whale with a cosmopolitan distribution, that typically migrates long distances between high-latitude summer feeding areas and low-latitude winter breeding areas (Dawbin, 1966). Their extensive annual migrations can be over 16 000 km round trips (Rasmussen et al., 2007; Stone et al., 1990), and therefore humpback whales of many worldwide populations will travel through the territorial waters of multiple countries each year. This creates challenges for developing management strategies for endangered and recovering populations and means understanding migratory patterns and connections is critical for conservation. To aid with management, in 2016 the National Oceanic and Atmospheric Administration (NOAA) of the US government identified 14 main breeding regions of humpback whales for a worldwide status review, which they defined as having distinct population segments (DPSs) that visit to breed and calve each year; nine of these were classified as "Not at Risk", one as "Threatened", and only four as "Endangered" (Bettridge, 2015). The list of endangered DPSs included the Central America DPS of the North Pacific humpback whale, which was described as small and genetically distinct, and considered to be at moderate risk of extinction (Bettridge, 2015).

The study of many aspects of cetacean ecology is made possible by identifying individuals through space and time. Humpback whales can be easily identified by their ventral tail flukes' unique shape and pigmentation patterns (Katona & Whitehead, 1981). For the last five decades, photographic identification (e.g. photo-identification) of humpback whales and collaboration between international researchers has allowed for a growing understanding of the movements and migrations of humpback whale populations globally (Calambokidis et al., 2000, Calambokidis et al., 2001, Calambokidis et al., 2008; Cheeseman et al., 2021) However, worldwide there remain a few areas of seasonal habitat of the species where research has not been initiated. One such place was the Central American country of El Salvador, considered part of the breeding area of the Central America DPS. Whilst cetacean research was growing worldwide, El Salvador was in the midst of civil war, and dedicated cetacean surveying was extremely limited. Prior to 2018, only 11 individual humpback whales' fluke identification images existed, and therefore the migratory destinations of Salvadoran humpback whales were unknown. Increased photo-identification efforts began in the Pacific waters in 2018, and dedicated cetacean surveying was initiated in 2020. Here we present, for the first time, migratory connections of a significant number of Salvadoran humpback whales to North Pacific feeding areas, to aid in international management decisions and planning for the endangered Central America DPS.

Between 1999 and 2006, 11 photo-identification images of humpback whales were collected in the Pacific waters of El Salvador as part of Cascadia Research Collective research efforts throughout Central America (Calambokidis et al., 2008, Rasmussen et al., 2012). In 2006, a small whale-watching industry began in Los Cóbanos, Sonsonate, El Salvador (Castaneda et al., 2021). In 2018, these tourism trips were then used as a research platform. Data collected included photo-identification images of humpback whales, Global Positioning System (GPS) tracks, and basic environmental data (sea state, water temperature, cloud cover and visibility). In total, over 33 whale watch trips were conducted which resulted in over 109 hours of surveying, and 10 new photo-identification images. In 2020, 10 dedicated cetacean surveys were completed, involving 64 hours of surveying, encountering five groups of humpback whales and identifying seven individuals. In 2021, 41 research surveys were completed, of a total of 129 hours of surveys, 54 humpback whale groups were encountered, and 49 unique photo-identification images were collected. Since 2019, an additional 15 photo-identification images have also been collected on whale watch trips in the region.

All unique photo-identification images of individual humpback whales were uploaded to the research collaboration and citizen science web platform Happywhale (www.happywhale.com). Individual whales were matched via automated image recognition to a global humpback whale fluke photo-identification catalog of 66043 individuals, of which 27 536 were identified in the North Pacific Ocean (Cheeseman et al., 2021). Images were matched with an expected accuracy of 97–99 % of potential matches found, match results were manually confirmed, and unmatched individuals with fluke identification photos of sufficient quality were considered new to the dataset and given an ID number. Great-circle distances between sighting locations of matched whales were then calculated using the methodology of Bowditch (1994).

Overall, 92 individual humpback whales have been identified in El Salvador between 1999–2021 during the boreal winter months, December through March. Via Happywhale, 80 (87.0 %) of the humpback whales photographed in El Salvador were matched to individuals seen in North Pacific feeding areas. No matches were made to areas outside of the North Pacific. Sighting histories of Salvadoran humpback whales resighted in feeding areas ranged from two to 29 years (average = 12.1, sd = 5.8).

The main feeding area where whales were resighted was Central California (n = 70, 76.1 %). Of these whales, 21 (22.8 %) had also been sighted in Southern California. While only three (3.3 %) individually identified humpback whales were documented in just Southern California, and in no other feeding area. In total, 16 whales (17.4 %) had been sighted in Northern California, of which seven (8.8 %) had also been sighted in the centre of the state and five (6.3 %) in both Central California and Southern California. Two humpback whales (2.2 %) were sighted in Southern British Columbia, Canada, and most significantly, one individual photographed in El Salvador in 2021, was identified as a humpback whale that has been documented feeding in Southeast Alaska (SEAK) in multiple years (Fig. 1). In fact, this whale of unknown sex and named SEAK-5011, has been sighted five times over 27 years in SEAK, spanning four decades, being photographed in 1994, 2004 and 2021. Prior to 2021, SEAK–5011 had not been documented in a breeding area. On 5 February 2021, SEAK–5011 was seen in a group of two adult whales, near Los Cóbanos, Sonsonate, El Salvador. That same year but after 196 days, SEAK– 5011 was photographed in SEAK on the 20 August 2021, followed by another nearby sighting on 26 August 2021. Both feeding area observations were made in Frederick Sound, SEAK, and a minimum great-circle distance between the sightings from El Salvador and SEAK was calculated as approximately 6 100 km.

Previous studies have shown that humpback whales from the Central America DPS predominantly feed in the coastal waters of California (Calambokidis et al., 2000, Calambokidis et al., 2008; Rasmussen et al., 2012; Steiger & Calambokidis, 1991). Our results support this finding with 83.7 % (77/92 whales) of whales photographed in El Salvador being matched to images taken in the waters of California, and

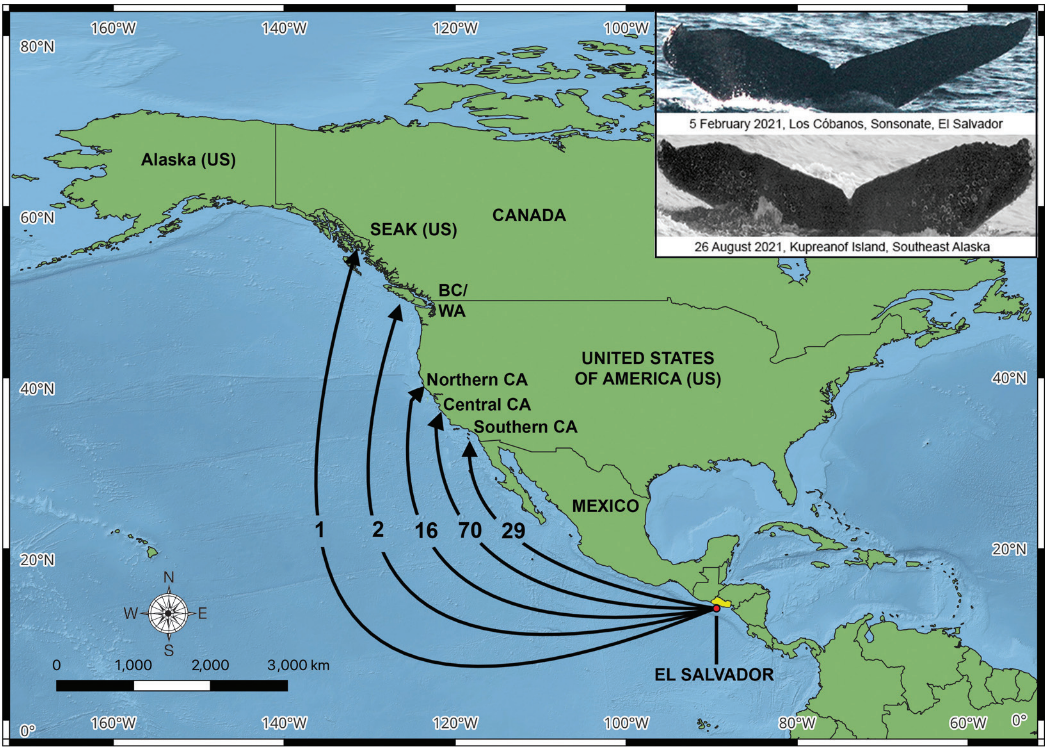

Fig. 1. Migratory connections of humpback whales from El Salvador. Numbers in arrows represent the number of individual whales matched between locations. Photo inset is of humpback whale SEAK–5011. CA= California, WA= Washington state (US), BC= British Columbia (Canada), SEAK = Southeast Alaska.

suggest that Southern and Central California are likely the most important feeding areas of humpback whales that winter in El Salvador. Our analysis found that nearly all Salvadorian humpback whales registered in Southern California (87.5 %, 21/24 whales) were also seen feeding in Central California. These results could be interpreted as implying the Southern California region is a transitory area to preferred feeding areas further north, rather than the terminal end of migration. However, we note that Happywhale effort is far greater in Central California (Author, Ted Cheeseman, Unpublished data), and also the possible source of these results.

The presence of humpback whales of the Central America DPS throughout the California feeding areas has particular significance to regional fishing regulations and management. Entanglement of humpback whales in gear of the California Dungeness crab pot fishery has increased at an alarming rate since 2014 (Lebon & Kelly, 2019), and has been demonstrated to reduce survivorship, particularly for juvenile humpback whales (Tackaberry et al., 2022). Large whales may travel thousands of kilometers carrying fishing gear and be entangled for many months, even years (Cassoff et al., 2011), and mulitple entangled humpback whales have been encountered in El Salvador (Castaneda et al., 2022). In the US, the species is protected under the Marine Mammal Protection Act (MMPA) and Endangered Species Act (ESA). The continued documentation of the seasonal presence of the endangered Central America DPS in coastal California, an area where entanglement rates are high and increasing (Lebon & Kelly, 2019), should be a critical consideration for the future management of regional fisheries.

Our results also support previous findings (Calambokidis et al., 2000, Calambokidis et al., 2008), that a small number of humpback whales from the Central America DPS may be present in the more northern North Pacific feeding areas of Washington State (US) and British Columbia (Canada). Although matching attempts of humpback whale catalogs between North Pacific feeding areas and the Central America DPS has been occurring since the 1990s (Calambokidis et al., 2000, Calambokidis et al., 2008; Rasmussen et al., 2012; Steiger & Calambokidis, 1991), this is the first published report of a humpback whale photographed between Central America and SEAK. This has relevance for the management and protection of the SEAK feeding region of humpback whales. As part of the final rule of the Endangered Species Act published in April 2021, the United States' National Marine Fisheries Service (NMFS) recently delisted SEAK for humpback whales, stating that it was not 'Critical Habitat' for any of the endangered or threatened North Pacific DPSs. SEAK humpback whales are most commonly matched to the Hawaiian Archipelago (Calambokidis et al., 2008), a breeding region of a DPS classified as "Not at Risk" (Bettridge et al., 2015). The recapture of just one whale between SEAK to the endangered Central America DPS does not negate NMFS's "Not Critical" designation. However, the documentation of a whale in SEAK from a previously unsurveyed breeding region, combined with the fact that ~38 % of humpback whales that feed in SEAK are yet to be matched to breeding areas (Happywhale, unpublished data), lends to the possibility of more, as-of-yet undiscovered migratory connections between the two regions. If more matches are found between Central America and SEAK, it could have repercussions for future regional conservation and management planning in Alaska.

Lastly, our study shows the importance of initiating research efforts in unsurveyed areas of humpback whale habitat. This is especially true in breeding areas of the at risk (threatened and endangered) DPSs. Although SEAK–5011 has a sighting history of 27 years in the SEAK feeding areas, this adult whale had never before been documented in a breeding area. This is despite several decades of intensive photo-identification and matching efforts in the neighbouring Mexico DPS (Calambokidis et al., 2008, Cheeseman et al., 2021, Urbán et al., 2000) and predominantly the southern region of the Central America DPS (Calambokidis, 1991; Calambokidis et al., 2000, Calambokidis et al., 2008; Rasmussen et al. 2012; Steiger &). Humpback whales have been shown to exhibit strong site-fidelity to seasonal breeding habitat in the North Pacific (Acebes et al., 2021; Herman et al., 2011). Therefore, this sighting of a SEAK humpback whale in a previously unsurveyed region of the breeding area of the endangered Central America DPS, highlights the need for expansion of research efforts to include all known humpback whale habitat. It also exemplifies the merit of the new research efforts that have been initiated in El Salvador, and the value of continued surveying and cetacean studies in the Pacific waters of this country.

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: NR conceptualized this study, collected field data, analyzed the data, and drafted early and revised versions of this manuscript. MGC collected field data and edited the manuscript, TC curated photographic data and edited the manuscript, JC and FS collected and curated photographic data, and edited various versions of this manuscript.

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SUPPLEMENT • WHALES

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Bryde's Whale (*Balaenoptera edeni brydei,* Artiodactyla: Balaenopteridae) aggregation area in the Gulf of Chiriqui, Panama

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ABSTRACT

Introduction: Even though they occur worldwide in tropical and subtropical waters, Bryde's whales (*Balaenoptera edeni*) are one of the most enigmatic of all the balaenopterid species. Previous research has reported the presence of Bryde's whales off the Pacific coast of Panama, but little is known about their behaviors, abundance, and distribution in these waters.

Objective: We report on an area in the Gulf of Chiriqui, Panama, where Bryde's whales have regularly been sighted.

Methods: We conducted small-boat surveys for marine mammals in the Gulf of Chiriqui between 2001 and 2022, during both the austral (26 300 km) and boreal (4 700 km) winter seasons.

Results: We made 25 sightings of 33 Bryde's whales. Sightings were made in nine of the years surveyed, and most were in the austral winter season (92 %). Most sightings were of one animal (80 %), and one sighting was of a mother-calf-escort trio. The aggregation area appears near the Contreras Islands, where most sightings (84 %) were made. We have photo-identified 14 individual whales using the dorsal fin. One individual has been sighted over two years (2014 and 2015), while the remaining have been documented only once. No photographic matches have been made to other areas.

Conclusions: The persistent use of this aggregation area could indicate it is used for foraging, and the presence of a calf may indicate the area is also used for reproduction. The one inter-annual resight suggests this area may be used repeatedly by some of the same animals. Further research is needed off Panama to better understand this population of Bryde's whales, its conservation status, and its relationship to the broader eastern tropical Pacific populations. Expanding the marine protected areas in the Gulf of Chiriqui may be warranted in order to protect this aggregation area.

Key words: Central America; baleen whales; eastern tropical Pacific; occurrence; photo-identification.

RESUMEN

Área de Agregación de la Ballena de Bryde (*Balaenoptera edeni brydei)* en el Golfo de Chiriquí, Panamá

Introducción: Aunque la ballena de Bryde (*Balaenoptera edeni*) tiene una distribución global en aguas tropicales y subtropicales, la especie es una de las más enigmaticas entre los balaenoptéridos. Observaciones previas han reportado la presencia de ballenas de Bryde en la costa del Pacífico de Panamá, pero sabemos poco acerca de su comportamiento, abundancia, y distribución en estas aguas.

Objetivo: Reportamos un área en el golfo de Chiriquí, Panamá, donde la ballena de Bryde ha sido avistada frecuentemente.

Métodos: Realizamos muestreos para mamíferos marinos desde embarcaciones pequeñas en el golfo de Chiriquí entre el 2001 y el 2022, tanto durante el invierno austral (26 300 km) como el boreal (4 700 km).
Resultados: Registramos 25 avistamientos de 33 ballenas de Bryde. Los avistamientos ocurrieron en nueve de los años muestreados, la mayoría en el invierno austral (92 %). La mayoría de avistamientos fueron de un animal (80 %), y un avistamiento fue de un trío madre-cría-escolta. El área de agregación ocurre cerca de las islas Contreras, donde la mayoría de los avistamientos (84 %) fueron registrados. Foto-identificamos 14 individuos usando la aleta dorsal. Un individuo fué avistado en dos años (2014 y 2015), mientras que el resto solamente ha sido documentado una vez. No se han registrado recapturas fotográficas con otras áreas.
Conclusiones: El uso repetido de ésta área de agregación podría indicar que es usada para alimentación, y la presencia de un trío madre-cría-escolta también sugiere actividad reproductiva. El único re-avistamiento interannual sugiere que el área es usada por algunos de los mismos animales. Se necesita más investigación en aguas panameñas para entender mejor esta población de ballena de Bryde, su estatus de conservación, y su relación con otras poblaciones del Pacífico tropical oriental. Una expansión de las áreas marinas protegidas en el golfo de Chiriquí puede ser necesaria para proteger esta población.

Palabras clave: Centroamérica; ballenas de barbas; Pacífico tropical oriental; ocurrencia; foto-identificación.

INTRODUCTION

Bryde's whales (*Balaenoptera edeni* Anderson, 1878) are one of the most enigmatic of all the balaentopterid species, despite having a worldwide distribution in tropical and subtropical waters, including in the Pacific, Atlantic, and Indian Oceans (Kato & Perrin, 2018). Much remains unclear about this species, including its taxonomy, distribution, and migration. Currently there are two subspecies recognized; *B. edeni edeni* is smaller and typically found in coastal waters, and *B. edeni brydei* is larger and globally distributed (Kato & Perrin, 2018; Kershaw et al., 2013,). Both subspecies have been found in the Indian and Pacific oceans, and *B. edeni brydei* also has been found in the Caribbean (Luksenburg et al., 2015). Unlike other baleen whale species, Bryde's whales do not make long annual migrations between cold-water feeding areas and warm-water breeding areas, but remain in tropical and subtropical waters year-round. However, some populations have shown evidence of large-scale movement within this range, while others appear to be resident (Best, 2001; Kato & Perrin, 2018; Lagerquist et al., 2017; Murase et al., 2015). Bryde's whales are considered opportunistic feeders and can feed on small pelagic schooling fish or zooplankton. Different populations tend to show a preference for one type of prey depending on geographic location (Constantine et al., 2018; Kato & Perrin, 2018).

Between 1986 and 2005, large-scale visual surveys in offshore waters of the eastern tropical Pacific (ETP) documented Bryde's whales between 30°N and 15°S (Hamilton et al., 2009). Additionally, an acoustic study in the ETP between 1999 and 2001 detected Bryde's whale calls at seven different offshore stations between 12° N and 8° S (Heimlich et al., 2005). Across the broader eastern Pacific, other reports of Bryde's whales include off southern California (Kerosky et al., 2012; Smultea et al., 2012), Mexico (Salvadeo et al., 2011; Tershy, 1992; Viloria- Gómora et al., 2021), Nicaragua (De Weerdt et al., 2021), Costa Rica (May-Collado et al., 2018), Colombia (Palacios et al., 2012), Ecuador, including the Galapagos Islands (Alava et al., 2013; Biggs et al., 2017; Castro et al., 2017; Palacios & Forney, 2008), Peru (Clarke & Aguayo, 1965; Ramirez, 1986; Valdivia et al., 1981), and Chile (Clarke & Aguayo, 1965; Gallardo et al., 1983; Pastene et al., 2015). Bryde's whales have been previously reported in Panama (May-Collado et al., 2018; Rasmussen & Palacios, 2013), but little information is available from the Gulf of Chiriqui.

This study reports on Bryde's whales sighted in the Gulf of Chiriqui from long-term, small-boat surveys between 2001-2022.

MATERIALS AND METHODS

Study site: The Gulf of Chiriqui lies in the western part of Panama and is bordered by the Azuero Peninsula to the east, and Punta Burica to the west (7°18' - 8°18' N & 82°54' - 81°36' W; Fig. 1). This gulf is characterized by generally shallow waters (< 300 m) and many island groups. Monthly sea surface temperature ranges between 25 – 29 °C (Randall et al., 2020). However, like the rest of the ETP, the gulf is subject to the anomalous effects from the regional El Nino-Southern Oscillation (ENSO) phenomenon, which results in warmer sea surface temperatures (Podestá & Glynn 2001; Randall et al., 2020; Wang & Fiedler, 2006;). Two protected areas have been established by the government of Panama (Fig 1a): Coiba National Park and its Special Zone of Marine Protection, which is also a UNESCO World Heritage Site and includes Coiba Island and the Contreras Islands (https://whc.unesco.org/en/list/1138/), and the Gulf of Chiriqui National Marine Park, which includes the Paridas Islands. The gulf is also well-known for its use as a breeding area by two different populations of humpback whale, *Megaptera novaeangliae* (Borowski, 1781), one migrating from feeding areas in the Southern

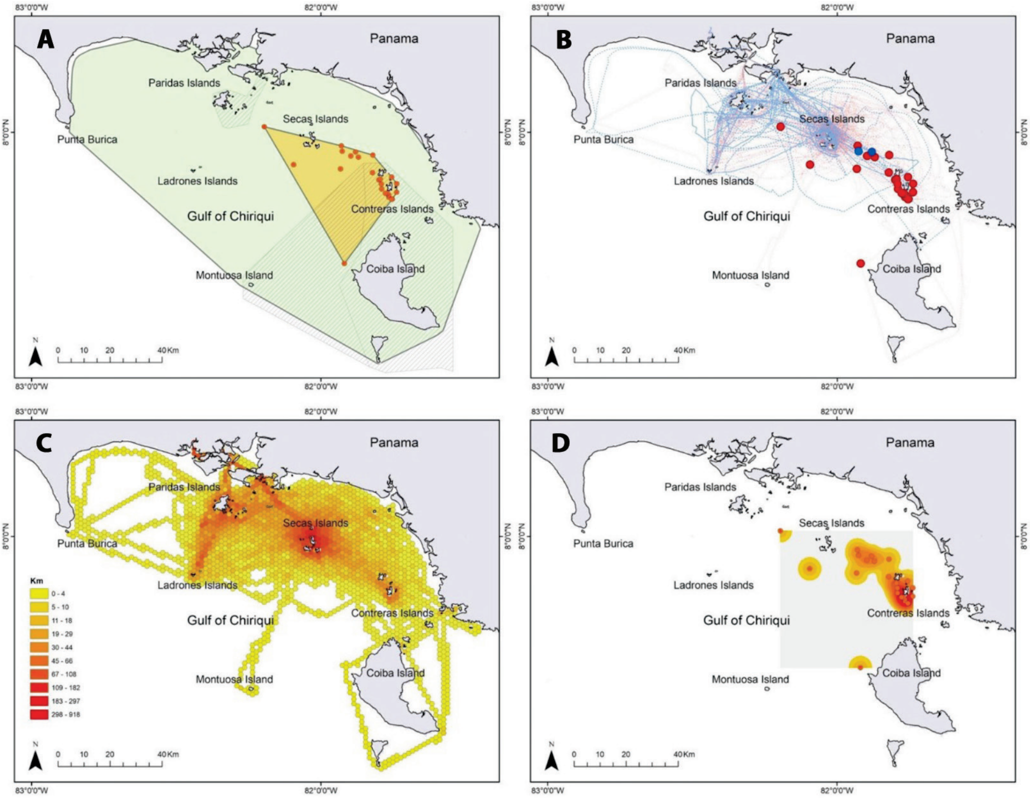

Fig. 1. A. Minimum convex polygons (MCP) for both survey area (green) and sighting locations (orange), red circles indicate sighting locations for all Bryde's whale sightings. The hatched overlays represent marine protected areas, including Gulf of Chiriqui National Marine Park at the Paridas Islands, and Coiba National Park (right side of Coiba hatched overlay) and its Special Zone of Marine Protection (left side of overlay). **B.** Survey effort tracklines and Bryde's whale sighting locations for two seasons (blue for boreal winter and red for austral winter). **C.** Gridded representation of the survey effort in the Gulf of Chiriqui using a honeycomb grid with 1-km edges. **D.** Kernel density distribution for Bryde's whale sightings.

Hemisphere, and one migrating from feeding areas in the Northern Hemisphere (Rasmussen et al., 2007).

Boat surveys: Visual surveys were conducted from a small boat in the Gulf of Chiriqui between 2001 and 2022, in both the boreal winter (January-March) and austral winter (July-September), to correspond with the breeding seasons of the two humpback whale populations that use this area (Rasmussen et al., 2007). Humpback whales were the primary species targeted, but all marine mammal species encountered were documented. Data collected included time, GPS location, number of animals in the sighting, and behaviors. Surveys were non-systematic in design and conducted in waters less than 200 m depth, near coastlines, island groups, and rocky outcroppings, which are the preferred humpback whale breeding habitat (Clapham & Mead, 1999; Rasmussen & Palacios, 2013). When Bryde's whales were encountered, identification photographs of the right and left sides of the dorsal fin were taken with a Nikon SLR camera equipped with a telephoto lens.

Data analyses: Given the extensive and non-uniform survey effort, the survey tracklines were gridded for visual interpretation using honeycomb cells with 1-km edges with the XtoolsPro software (https://xtools.pro/) for ArcMap v. 10.8.2 (Esri, Redlands, CA). Kernel density analysis of the sighting locations was conducted with ArcMap to characterize the concentration of Bryde's whale sightings. The total survey area and the area used by the Bryde's whales were estimated using minimum convex polygon analysis (MCP) in ArcMap.

RESULTS

We surveyed a total of 31 000 km over 334 days. Most effort (26 300 km and 294 days) was in the austral winter between 2002 and 2019 (with the exception of 2005 and 2016, when no effort took place) (Fig. 1b). The remaining effort (4 700 km and 40 days) was in the boreal winters of 2001-2003, 2018, and 2022 (Fig. 1b). Surveys covered most of the gulf, with the greatest concentration near the island groups of Secas, Paridas, Ladrones, and Contreras (Fig. 1b and c).

Between 2004 and 2022 we made 25 sightings of 33 Bryde's whales. Most sightings (n = 23, 92 %) were in August and September, and two sightings (8 %) were in February (Fig. 1b). Whales were sighted in nine separate years (2004, 2007, 2009, 2013-2015, 2018, 2019, 2022), with the most sightings in one year (nine) occurring in 2014. Most sightings were of single whales (n = 20, 80 %), three sightings were of two whales (12 %), and one sighting was of four whales (4 %). One sighting (4 %) in September 2004 included a mother-calf-escort trio.

Encounter rates (whales seen per 100 km surveyed) were calculated for both the austral and boreal seasons to give an index of relative abundance. Bryde's whales were seen at a rate of 0.09 whales/100 km in the austral winter, and 0.04 whales/100 km in the boreal winter. For comparison, humpback whales were documented during these same surveys at a rate of 13.79 whales/100 km in the austral winter and 0.41 whales/100 km in the boreal winter.

All sightings were near the Contreras and Secas island groups, and one near Coiba Island (Fig. 1b). All sightings were made in depths of less than 200 m. Distances to shore ranged between 1 and 16.1 km (mean = 5.0 km, median = 2.5 km, SD = 4.3 km).

The kernel density analysis of the sighting locations revealed the highest concentration of sightings around the southern Contreras Islands, and extending to the northwest towards the Secas Islands (Fig. 1d). The total survey area, as delineated by a MCP, was estimated at 11 800 km^2, while the MCP of the area of Bryde's whale sightings was estimated at 1 200 km^2, or 10 % of the survey area (Fig. 1a).

Of the 25 sightings of Bryde's whales, most (n = 17, 68 %) were under 20 minutes in duration, either because the whales were difficult to follow or due to bad weather. The longest period we followed a single whale was 1 hour 25 minutes. Most whales exhibited an erratic surfacing pattern, in which the direction of travel changed between each surface series, and sometimes within a surface series. Five sightings included close approaches by the whales to the boat.

We photographed dorsal fins of 19 whales, of which 14 were of good quality for identification purposes. Of the 14, six had distinctive nicks or shape, and eight were nondescript. One individual (identification #009) was sighted over two years on 6 September 2014 and again on 5 September 2015. The estimated distance between the two sighting locations was 12 km. The remaining 13 whales were identified only once.

DISCUSSION

In the Gulf of Chiriqui, Bryde's whales are seen at a much lower rate (0.09 whales/100 km in the austral winter and 0.04/100 km in the boreal winter) than humpback whales (13.79/100 km in the austral winter and 0.41/100 km in the boreal winter). The results of the kernel density estimate as well as the MCP of the aggregation area both also indicate that despite the extensive survey effort, Bryde's whales are more likely to be found near the Contreras and Secas island groups than anywhere else in the Gulf of Chiriqui, regardless of the season or year. Such a persistent aggregation area suggests this may be a localized area of elevated productivity where Bryde's whales are foraging. The Contreras Islands are historically known by local fishers to be one of the most productive areas in the Gulf of Chiriqui, including small schooling fish (L. Bernal, personal communication, 6 May 2022), although this area is currently protected and no fishing is allowed within the National Park boundaries (see Fig. 1a). Examples of Bryde's whale feeding areas described less than 10 km to shore include in the Galapagos Islands, Ecuador (Biggs et al., 2017), the Beibu Gulf, China (Chen et al., 2019), and the Gulf of California, Mexico (Tershy, 1992). Further study is needed specifically in the Contreras and Secas Islands to assess the oceanographic and bathymetric conditions that may lead to a localized area of high productivity (Wingfield et al., 2011).

The September sighting of a mother-calf-escort trio in Chiriqui could indicate that in addition to this being a feeding area, it may also be an area used for reproduction. Mother-calf pairs have been seen in an area associated with foraging in the Galapagos Islands (Biggs et al., 2017), suggesting it is an area where both foraging and breeding behaviors both occur. Further study would also address the extent to which Bryde's whales are using Chiriqui for reproductive purposes in addition to foraging.

The seasonality of Bryde's whales in this area is somewhat unclear. This study reports sightings in February, August and September, but there are also anecdotal reports from residents and naturalists in Chiriqui from November and December (L. Klein, V. Wilson, personal communication, 19 April 2022). Between 2013-2021 seven strandings of Bryde's whales were reported off Panama in February, March, April, August and December, although none of these were in Chiriqui (L. Trejos-Lasso, personal communication, 29 April 2022). A current study in Chiriqui using continuously sampling bottom-mounted hydrophones could add information on the seasonality of Bryde's whales in the area (L. May-Collado, personal communication, 4 May, 2022).

While the re-encounter of one of our photo-identified whales to the same area a year later could suggest that the same animals may be using this area year after year, further study is necessary to determine the extent of site fidelity. Other studies have reported varying degrees of site fidelity for this species (Athayde et al. 2020; Figueiredo et al. 2014, Lodi et al. 2015, Tezanos-Pinto et al. 2017,). There is no information on whether this population is strictly coastal or may also move to offshore areas. Different populations of Bryde's whales have shown a variety of seasonal movement patterns. Two distinct populations of Bryde's whales in South Africa are an example of this, with one, the Southeast Atlantic population, migrating between the equator and 34° S, while the South

African coastal population is resident year-round (Best, 2001; Constantine et al., 2018).

A previous study compared our photo-identification catalog to 51 individually identified Bryde's whales from Ecuador (both the continent and the Galapagos) and Peru, and found no matches (Castro et al., 2017). Further comparisons among regional photo-ID catalogs as well as satellite tagging could yield more information about the movements of these whales.

The taxonomy of this population and how it relates to other Bryde's whale populations in the ETP is also worthy of investigation. Genetic sampling will help determine the taxonomic status as well as help to resolve some of the uncertainty of this species on a global level (Constantine et al., 2018). A previous genetic study determined that Bryde's whales off Brazil, Chile, and Peru were the *B. brydei* form (Pastene et al., 2015). Further genetic sampling is needed not only off Panama, but in other areas of the ETP as well.

While the Gulf of Chiriqui does contain some marine protected areas (MPAs), extending some of these MPAs to better include the Bryde's whale aggregation area we have identified here may be warranted. The gulf is an important breeding area for humpback whales from the southeast Pacific (Rasmussen & Palacios, 2013) and is also used by humpback whales from the Central America Distinct Population Segment, which is currently listed as endangered by the USA government (Rasmussen et al., 2011, Rasmussen & Palacios, 2020; National and Oceanic Atmospheric Administration, 2016). In comparison with humpback whales, Bryde's whales are seen far less frequently in the Gulf of Chiriqui, yet they have been consistently sighted since 2004 and are concentrated in a relatively small area of the gulf. Globally, the conservation status of Bryde's whales is considered of "Least Concern" by the IUCN (Cooke & Brownell, 2018). However, the restricted distribution and low encounter rates in the Gulf of Chiriqui suggests that either this is a small and localized population, or that this habitat supports a small number of visiting individuals. This population could be particularly vulnerable due to its restricted distribution within the gulf, although this restricted distribution is well-suited for conservation purposes as it lends itself to a clear designation of a protected area. While some of the Bryde's whale aggregation area (as determined by the MCP analysis) is within the Coiba National Park MPA, some if it is outside the protected zone (Fig. 1a). Therefore, it may be advisable to expand this MPA to include all of the area where Bryde's whales are sighted. Connecting it with the Gulf of Chiriqui Marine Park in the Paridas Islands would create a continuous protected corridor which could benefit not only Bryde's whales, but other marine species as well.

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Author Contribution: KR collected data in the field, conceptualized and conducted data analysis, and drafted the manuscript. DMP collected data in the field, conceptualized and assisted with data analysis, and contributed to the manuscript.

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False killer whales (*Pseudorca crassidens* Cetacea: Delphinidae) along the Pacific coast of Central America and Mexico: Long-term movements, association patterns and assessment of fishery interactions

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ABSTRACT

Introduction: Worldwide, false killer whales (*Pseudorca crassidens)* are infrequently encountered, yet long-term studies have shown strong site fidelity as well as long-term associations among individuals in several locations. Detailed studies of this species have primarily been conducted around tropical oceanic islands or in the subtropical southern hemisphere.

Objectives: We assess movements and association patterns among false killer whales along the Pacific coasts of the USA, Mexico, Guatemala, Nicaragua, Costa Rica including Isla del Coco, and Panama, representing one of the longest-running (albeit non-continuous) studies of this species. We also examine photos for evidence of interactions with fisheries, a known source of mortality to false killer whales.

Methods: From Central America, we selected 212 individuals (50 encounter groups) out of 244 individuals (56 encounters) for inclusion in analyses based on photo quality and distinctiveness. Photos were collected on dedicated surveys from 1991-1994 and dedicated and opportunistic surveys from 1998-2022. Other than the effort off the oceanic Isla del Coco (1993-1994), surveys were undertaken in continental shelf waters. Additionally, we selected by photo quality and distinctiveness 124 (33 encounter groups) out of 189 individuals from southern California and Mexico for inclusion in these analyses. Association patterns were analyzed in SOCPROG and movements were analyzed in R.

Results: Of the 328 total individuals, 158 (48.2 %) were encountered more than once, and 114 (34.8 %) were re-sighted after a year or more. The longest individual sighting history spanned 26.2 years with six re-sightings over that period between southern Costa Rica and Panama. Association and movement analyses revealed that individuals identified off southern Costa Rica and Panama linked into a single social network, with extensive movements between the two countries. Three individuals encountered off northern Costa Rica were re-sighted off northern Nicaragua, and individuals encountered off Nicaragua were encountered off Guatemala and central mainland Mexico. Nine matches were found among false killer whales between central mainland Mexico and Central America. There were no matches between the mainland coastal waters and the 33 individuals encountered around Isla del Coco. Dorsal fin disfigurements consistent with interactions with line fisheries ranged from 0 to 21 % for individuals within social clusters identified by community division.

Conclusions: The infrequency of sightings combined with a high re-sighting rate of individuals and groups from the same area, suggests multiple small populations with large home ranges that include coastal waters. Small populations are sensitive to environmental changes, and as the human population grows, so do the demands on fisheries and ecotourism, which could directly impact the different populations. Additional effort in offshore areas is needed to determine the population status of false killer whales in pelagic waters, how often false killer whales using coastal waters move into pelagic waters, and the relationship between whales in the two habitats.

Key words: small cetacean; photo-identification; fisheries; dorsal fin disfigurement; social network; social organization.

RESUMEN

Falsas orcas (*Pseudorca crassidens* Cetacea: Delphinidae) de la costa del Pacífico de Centroamérica y México: movimientos, patrones de asociación y evaluación de interacciones pesqueras

Introducción: Alrededor del mundo, las falsas orcas (*Pseudorca crassidens*) son encontradas con poca frecuencia, aunque estudios a largo plazo han demostrado una fuerte fidelidad al sitio, así como asociaciones a largo plazo entre individuos. Estudios detallados de esta especie se han realizado principalmente alrededor de islas oceánicas tropicales o en la región subtropical del hemisferio sur.

Objetivo: Se evaluaron los movimientos y patrones de asociaciones a largo plazo entre falsas orcas, a lo largo de las costas del Pacífico de E. U., México, Guatemala, Nicaragua, Costa Rica (incluyendo a la Isla del Coco) y Panamá, lo que representa uno de los más extensos (aunque no continuos) estudios de esta especie. Además, se analizaron las fotos de aletas dorsales en busca de evidencia de interacciones con la pesca, una fuente de mortalidad conocida para las falsas orcas.

Métodos: Seleccionamos a 212 (50 encuentro grupos) de 244 individuos (56 encuentros) de Centroamérica para incluirlos en los análisis basados en la calidad y el carácter distintivo de las fotografías. Utilizamos fotos recopiladas en muestreos dedicados de 1991 a 1994 y encuentros dedicados y oportunistas de 1998 a 2022. Aparte del esfuerzo alrededor de la isla oceánica, Isla del Coco (1993-1994), se realizaron estudios en aguas de la plataforma continental. Además, seleccionamos basados en la calidad y el carácter distintivo de las fotografías 124 (33 encuentro grupos) de 189 individuos del sur de California y México para incluirlos en los análisis. Patrones de asociaciones fueron analizados en SOCPROG y los movimientos fueron analizados con el programa R.

Resultados: Del total de 328 individuos encontrados en Centroamérica, 158 (48.2 %) fueron observados más de una vez y 114 (34.8 %) se volvieron a avistar después de un año o más. El historial de avistamientos individuales más largo abarcó 26.2 años con seis re-avistamientos durante ese período entre el sur de Costa Rica y Panamá. Los análisis de asociación revelaron que todos los individuos identificados en el sur de Costa Rica y Panamá, se vincularon a una sola red social, con amplios movimientos entre los dos países. Tres individuos encontrados frente al norte de Costa Rica fueron avistados frente al norte de Nicaragua, y los individuos encontrados frente a Nicaragua fueron encontrados frente a la región central continental de México. Hubo traslape de nueve individuos entre México y Centroamérica. No hubo traslape entre los individuos avistados en el continente y los 33 individuos identificados alrededor de la Isla del Coco. Las desfiguraciones de la aleta dorsal, consistentes con interacciones con artes de pesca que usan líneas variaron de 0 a 21 % para los individuos dentro de los grupos identificados por división de la comunidad.

Conclusiones: La poca frecuencia de avistamientos combinada con muchos re-avistamientos de individuos y grupos en la misma área, sugiere que las falsa orcas representan muchas poblaciones pequeñas con áreas de distribución grandes que incluyen aguas costeras. Las poblaciones pequeñas son sensibles a los cambios ambientales y, a medida que crece la población humana, también lo hacen las demandas sobre la pesca y el ecoturismo, lo que podría afectar directamente a estas poblaciones. Se necesitan más estudios en las áreas alejadas de la costa para determinar el estado de conservación de las falsas orcas en regiones pelágicas, la frecuencia con la que las falsas orcas que usan aguas costeras se trasladan a aguas pelágicas y la relación entre ellas en los dos hábitats.

Palabras clave: pequeños cetáceos; foto-identificación; pesca; desfiguración de la aleta dorsal; red social; organización social.

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

INTRODUCTION

False killer whales, *Pseudorca crassidens* (Owen, 1846) primarily inhabit pelagic tropical and warm temperate waters worldwide, with the highest density in the tropics (Ferguson & Barlow, 2003). There are a few well-documented populations that are island-associated or encountered in nearshore waters (Baird et al., 2008; Palmer et al., 2017; Zaeschmar, 2014). Although this species is highly surface-active and tends to travel in large groups (20–100 individuals), false killer whale encounters are infrequent even in areas where they are resident year-round (e.g., Hawaiʻi; Baird, 2016) or present seasonally (e.g., New Zealand; Zaeschmar, 2014). In the eastern tropical Pacific (ETP), extensive large and small vessel surveys conducted in the Exclusive Economic Zone of Costa Rica from 1979 to 2001 documented only nine encounters of false killer whales (May-Collado et al., 2005) and sightings were relatively few and sparsely distributed throughout the ETP (Martínez-Fernández et al., 2011; Quintana-Rizzo & Gerrodette, 2009; Quintana-Rizzo, 2012; Wade & Gerrodette, 1993). Based on extensive survey work of the eastern Pacific Ocean from 1985-2005, no false killer whales were encountered north of Mexico (Hamilton et al., 2009), although there have been occasional documented encounters off California and even farther north into British Columbia (Baird et al., 1989; Norris & Prescott, 1961).

The first photographic identification (photo-ID) study of this species was conducted from 1991-1995 by Acevedo-Gutiérrez et al. (1997) in the coastal waters of Golfo Dulce, southern Costa Rica, and off Isla del Coco, an island approximately 500 km southwest of Costa Rica. Acevedo-Gutiérrez et al. (1997) found that individuals were re-sighted over two years in Golfo Dulce and three years off Isla del Coco, and stable associations between some individuals were evident. Based in the same area of southern Costa Rica (not including Isla del Coco) but using a separate photo-ID catalog and dataset, Sánchez Roblado et al. (2020) estimated that 92 false killer whales used this area. Although these earlier studies were spatially limited, they indicated that there is a small population encountered occasionally off southern Costa Rica, with no documented interchange with Isla del Coco (Fig. 1). An entirely separate photo-ID study identified 14 individuals but documented no re-sightings in Guatemala (Quintana-Rizzo, 2012).

The question of how far these individuals are ranging along Mexico, Central America, and into offshore waters is unclear. Genetic and photo-ID studies in the eastern Hawaiian Islands show a distinct insular/island-associated population of false killer whales with high site fidelity (Baird et al., 2008, Baird et al., 2012), and a genetically differentiated broadly ranging offshore population (Anderson et al., 2020; Chivers et al., 2007; Fader et al., 2021; Martien et al., 2014). Home ranges for groups and individuals from the main Hawaiian Islands' insular population tend to be extensive, but predictable (Baird et al., 2012), while individuals from the pelagic population appear to be much wider ranging (Anderson et al., 2020; Fader et al., 2021). The maximum travel distance for a satellite tagged insular main Hawaiian Islands false killer whale was 421 km (Baird et al., 2010), while it was 2 263 km for an individual from the pelagic population (E. Oleson personal communication, 27 July, 2022). Although far less is known about populations elsewhere, Palmer et al. (2017) reported that the maximum travel distance from a satellite-tagged false killer whale in the Arafura and Timor Seas off Australia was about 880 km from 104 days of tag transmission. Off New Zealand, Zaeschmar (2014) reported a maximum travel distance of 647 km based on photo-ID. From photo recapture studies in the Hawaiian Islands, the greatest span of years for an individual was 33 years (S. Mahaffy 6 May, 2022, personal communication).

The ongoing studies on false killer whales in the Hawaiian Islands have shown that there are three discrete yet partially overlapping populations (Chivers et al., 2007, Chivers et al. 2010; Baird et al., 2008, Baird et al., 2010, Baird et al., 2013; Martien et al., 2014). Individuals within a population maintain strong bonds over decades, hunt cooperatively, and share prey with hunting partners (Baird, 2016; Martien et al., 2019). Martien et al. (2019) found that both male and female main Hawaiian Islands insular false killer whales remain in their natal social groups throughout their lives and that between 34 to 64 % of matings occurred in the same

Fig. 1. Map of sighting locations (red circles) of false killer whales (*Pseudorca crassidens*) with acceptable quality photographs. Line width between sightings reflects the number of re-sightings of individuals between areas. Map created using R Statistical Software (R Core Team, 2021).

social group. This differs from other highly social species that may practice natal dispersal or exogamy to avoid inbreeding. As with other top predators, false killer whale population numbers are fairly low, they are slow to mature, have low birth rates, and females remain active in their family groups past their reproductive years (Baird, 2018a). False killer whales are active during the day and night and have been observed feeding on various species of fish including neritic, demersal, bathydemersal, reef, and pelagic game fish (Baird, 2016; Herzing & Elliser, 2016) and an analysis of stomach contents of stranded animals has shown that oceanic and neritic-oceanic squids make up a large part of their diet as well (Alonso et al., 1999). False killer whales' cooperative and adaptable hunting styles as well as their propensity to share prey within their group and occasionally with divers and boaters (Baird, 2018b) tend to put this species in conflict with small and large fishing boat operators where human and false killer whale fishing areas overlap.

Globally, false killer whales and other dolphin species are known to take the bait and catch off hook and line fisheries, and in the Hawaiʻi-based deep-set longline fishery, false killer whales are the cetacean species most frequently recorded as hooked as bycatch (Bradford et al., 2014; Forney & Kobayashi, 2005). One study of depredation in the Hawaiʻi-based deep-set longline fishery with 21 % observer coverage reported that ~6 % of hauls from 2004-2018 experienced odontocete depredation, most of it thought to be from false killer whales (Fader et al., 2021). The Inter-American Tropical Tuna Commission [IATTC] manages large longline vessels (>24 m) in the area from 50^0 N to 50^0 S from the coast of the Americas to the 150^0 W meridian of the eastern Pacific Ocean, with 1 123 longline vessels currently authorized to fish in this area (IATTC, 2022). Importantly, from the perspective of documenting fishery interactions, vessels less than 24 m are not managed by the IATTC and are not required to carry observers (IATTC, 2011). The Organization of the Fisheries and Aquaculture Sector of the Central America Isthmus [OSPESCA] reports more than 5 000 vessels in the coastal and Pacific longline fleet (OSPESCA, 2012). The IATTC has repeatedly recommended at least 20 % observer coverage on longline vessels in this area, yet coverage has remained at only 5% (IATTC, 2019), and since April 2020, due to the COVID pandemic, the requirement of any observer coverage may be waived upon request (National Oceanic and Atmospheric Administration [NOAA], 2022). Thus, indirect methods of assessing whether individual false killer whales have survived fishery interactions may be the only way to determine the magnitude of such interactions on groups or populations.

In the case of false killer whales, when an individual ingests a hook or is hooked in the mouth and is able to break free from the line, scars around the mouthline and dorsal fin are often the only external evidence that an animal has been hooked and survived (Baird & Gorgone, 2005; Baird et al., 2014, Baird et al., 2017). In the absence of observer coverage, Baird et al. (2014) conducted a photo review of dorsal fin disfigurements and scarring of individual false killer whales encountered in Hawaiian waters. They found that 7.5 % of the individuals from the main Hawaiian Islands population bore scars consistent with fisheries interactions and had higher rates of fishery-related injuries than the offshore or northwestern Hawaiian Islands populations (Baird et al., 2014).

The purpose of the current study was to examine the movements of false killer whales along the Pacific coast of North and Central America, ranging from southern California to Panama. We combined the results of independent photo-ID efforts from six different countries, providing an assessment of association patterns and site fidelity, as well as examining individuals for evidence of prior fishery interactions. We hope that our findings will add to the current body of knowledge on false killer whales, as well as inspire future collaborative research with this species in these regions.

MATERIALS AND METHODS

Study area: The ETP is characterized by a strong shallow thermocline, relatively high sea surface temperatures and strong winds (Heileman, 2008). The southern part of Mexico and northern part of Central America form one side of the eastern Pacific warm pool, which constitutes an open-ocean biogeographic province with a distinct biological community (Fiedler & Talley 2006). The study area is part of a marine mega-ecosystem characterized by gulfs, bays, coastal lagoons, and extensive intertidal areas and barriers (Gocke et al., 2001; Lizano & Alfaro, 2004). It includes part of the Costa Rica Dome (CRD), an open-ocean upwelling region caused by a seasonally changing combination of interconnected features including the Intertropical Convergence Zone, coastal jets and eddies, and geostrophic balance at the eastern extreme of the 10° N thermocline ridge (Mora-Escalante et al., 2020). The CRD supports a higher density of marine fauna including cetaceans than other parts of the Central American marine ecosystem (Fiedler & Talley, 2006; Lavín et al., 2006), and likely influences the high productivity of the Pacific Central American coast (Heileman, 2008). For the purpose of examining movements, the study area was broken down into a number of regions reflecting discrete study sites: southern California, southern Baja California, the central and southern Mexican mainland coasts, Guatemala, Nicaragua, northern and southern Costa Rica, Panama and Isla del Coco.

Data collection: Research efforts varied by year, season and among regions (Table 1; Fig. 1). False killer whale photos were collected from 1991-1992 on directed surveys for cetaceans along southern Costa Rica and 1993-1994 off Isla del Coco (Acevedo-Gutiérrez et al., 1997). From 1998 to 2009 false killer whale encounters were documented during directed humpback whale surveys conducted during the dry season (December-March) along the Pacific coast of southern Costa Rica and northern Nicaragua (Table 1) by Cascadia Research Collective (CRC) as well as independent researchers. Other efforts included systematic cetacean surveys conducted in Guatemala year-round 2008-2009 and between December-April from 2018 onward, and Costa Rica 2005-2006. In the majority of cases, effort was conducted from "pangas" or tour boats with an outboard motor, and were restricted to returning to the launch location at the end of the survey day. Photos and encounter details from 2010 to 2022 were collected and shared with CRC from directed surveys and whale and dolphin watch operations working along Nicaragua, Costa Rica, and Panama expressly for this project. The majority of CRC surveys were nonsystematic and attempted to cover a large coastal area with the primary objective of discovering humpback whales. Non-humpback whale cetacean encounters were approached for sighting position, species identification, group size estimation, and photo documentation depending on species and time of day. Sighting positions were based on an onboard GPS (directed surveys), an estimated position based on the photographers' description, or a "general" position based on where the vessel launched and returned. During false killer whale encounters from directed surveys, efforts were made to photograph all individuals from a group, regardless of age class, or distinctiveness. As with the Central America collection, the southern California-Mexico photo-ID catalog and sighting data were collected by both directed research efforts and opportunistic sightings from ecotourism businesses. Mexico data were collected from 2004, 2007 to 2008, and 2011 to 2020 (Lazcano-Pacheco et al., 2023). Survey effort and collection methods are described by Ortega-Ortiz et al. (2014).

Data analysis: Groups were defined as all individuals encountered on the same day within a region, and IDs obtained during that day were pooled as part of a single encounter. This is a broader definition for a group than is typically used for association analysis of odontocetes, however, as with Baird et al. (2008), we view that the choice is justified for this species based on the infrequency of false killer whale encounters and the small groups encountered,

Table 1

Number of groups encountered (number of identifications) of false killer whales (*Pseudorca crassidens*) by year and region, and total number of individuals by region, restricted to identifications with fair to excellent quality photographs and slightly to very distinct dorsal fins. Group was defined as all individuals encountered on the same day in the same general area. Number of groups in which group size estimates with acceptable photos are available are also included.

Year	S. California	Baja California	Cent. Mainland Mexico	S. Mainland Mexico	Guatemala	Nicaragua	N. Costa Rica	S. Costa Rica	Panama	Isla del Coco	Total
1991								1 (10)			1 (10)
1992								1 (7)			1 (7)
1993										2 (11)	2 (11)
1994										5 (40)	5 (40)
1998								2 (5)			2 (5)
2000								1 (2)			1 (2)
2004			1 (7)								1 (7)
2005						1 (4)		4 (24)			5 (28)
2006								3 (36)			3 (36)
2007								1 (1)			1 (1)
2008			1 (5)		2 (9)	1 (7)		1 (1)			5 (22)
2009					1 (6)			6 (102)			7 (108)
2010								3 (12)			3 (12)
2011			1 (8)								1 (8)
2012			3 (17)	1 (1)				3 (4)			7 (22)
2013		2 (11)	1 (13)	2 (20)							5 (44)
2014	4 (36)										4 (36)
2015	1 (1)		2 (18)						2 (9)		5 (28)
2016	1 (10)		2 (6)			2 (13)					5 (29)
2017			1 (1)			1 (9)					2 (10)
2018			4 (7)		1 (1)				2 (20)		7 (28)
2019	1 (9)		1 (4)						1 (9)		3 (22)
2020			2 (13)				1 (15)				3 (28)
2021	1 (3)					2 (23)					3 (26)
2022	1 (7)										1 (7)
Sum of groups	9	2	19	3	4	7	1	26	5	7	83 (577)
Sum of groups that included group size estimates	5	2	17	3	4	7	1	24	5	7	75
Mean group size (SD)	34.5 (16.2)	42.7 (52.7)	54.5 (60.1)	17.7 (6.8)	12.5 (7.7)	13.6 (8.1)		17 (13.0)	41 (15.6)	18.1 (10.9)	28.2 (34.7)
Median (range)	32.5 (15-60)	42.7 (5-80)	35 (5-200)	20 (10-23)	14 (2-20)	10 (6-25)		13 (1-50)	25 (25-60)	14 (4-34)	18 (1-200)
Sum identifications	66	11	99	21	16	56	15	204	38	51	577
Sum individuals	37	10	77	16	16	45	15	95	31	33	375

often traveling in the same direction and spread out over many kilometers (see e.g., Bradford et al., 2014). This choice is also justified based on the many positive re-sightings of individuals between subgroups encountered on the same day. To report the mean and median group size when only a range (min/max) of group size was available, we chose the median of the two numbers. When multiple encounters occurred on the same day of the same group of animals, we chose the largest group size number, with the assumption that smaller estimates were derived from counts of subgroups encountered. Following the photo-ID protocol described in Baird et al. (2008), a Central America catalog was constructed from photographs of individuals taken off Guatemala, Nicaragua, Costa Rica and Panama. Photos from each encounter were sorted by individual into folders, assigned temporary IDs and given separate scores for photo quality (1 – poor, 2 – fair, 3 – good, 4 – excellent) and distinctiveness (1 – indistinct, 2 – slightly distinct, 3 – distinct, 4 – very distinct). Photo quality was based on focus, angle, and proportion of the dorsal fin visible, and distinctiveness was based on the absence or presence of notches on the leading and trailing edge of the fin, and/or the dorsal fin shape. Poor quality or indistinct individuals were only compared to individuals encountered on the same day, within region, while all fair to excellent quality photos of slightly distinct to very distinct IDs were compared to all IDs in the catalog. When possible, photos of unusual scars on the body and along the mouthline were noted for each individual, and scored with the likelihood that the injury could have been associated with a fishery interaction (Baird et al., 2014, Baird et al., 2017). Once the temporary ID had been compared to the catalog, the best left and/or right dorsal fin of each individual was assigned a unique ID number, or if it was found in the historical catalog the ID was collapsed into the existing record of that individual. Every positive match found between sightings was confirmed by at least two experienced matchers. The southern California-Mexico catalog was created with similar practices as described above with quality and distinctiveness scores for each individual. Once completed, the Central America catalog was compared with the southern California-Mexico false killer whale catalog, with two experienced matchers confirming all positive matches between catalogs. In an effort to avoid false-negative matches between these two catalogs, a second experienced matcher (SDM) compared 18 % of the IDs in the southern California-Mexico catalog that had not been found by the initial matcher (ABD) (Elliser et al., 2022), and no additional matches were found. For all catalogs, only those IDs with photo quality and distinctiveness categories of two (fair) or better were compared between catalogs – these are defined as “acceptable quality” identifications. Our decision to include “fair” quality photos and slightly-distinctive IDs was made to retain a reasonable sample size for analysis and interpretation, and while it is more permissive than many studies with larger catalogs or for less remote locations, it is not without precedent (Baird et al., 2021; Elliser et al., 2022).

Linear-geographical distances between all possible pairs of encounter locations both within regions and among all regions were calculated for all encounters where acceptable identification photos and latitude and longitude were available using R Statistical Software (R Core Team, 2021). To control for pseudoreplication, when more than one individual was identified from a particular encounter, that encounter location was only used once in the calculations. If there was more than one encounter in an area on the same day (that were pooled as a single encounter), the first location was used. Combinations of encounters were generated using the *combinations* function within the *gtools* package (Warnes et al., 2020). Straight line geographical distances were calculated using the *st_distance* function within the *sf* package (Pebesma, 2018). Distances between all encounter combinations for each individual sighted on two or more occasions were also calculated. Because of the sociality of false killer whales, there were several instances where multiple individuals were re-sighted together more than once. Hence, when summarizing distances across individual re-sightings, we only used a single set of calculated distances between pairs of individual sightings to avoid pseudoreplication.

Association analyses of photo-identified individuals were undertaken in SOCPROG 2.9 with MATLAB 9.5 (Whitehead, 2009), and social network metrics were calculated and illustrated in Netdraw 2.176 (Borgatti, 2002). To provide a quantitative measure of the frequency of co-occurrence of individuals, while controlling for effort (Whitehead, 2008), we used the half-weight index of association (HWI). Whitehead (2008) and Cairns & Schwager (1987) recommend the use of HWI in situations where it is likely that not all individuals within a sampling period are identified or when individuals of a pair are more likely to be observed separately than when together.

We used SOCPROG to assess whether the false killer whales in our study could be divided into meaningful social clusters based on levels of association between individuals using community-based modularity (Newman, 2004; Whitehead, 2009). This method divides the population into clusters in a way that maximizes associations within clusters rather than between them. A modularity value greater than 0.3 is considered to indicate the useful division of a population (Newman, 2004). We checked all cluster assignments to make sure that they made logical sense based on our knowledge of these data and the eigenvector or final bifurcation involving the individual. The eigenvector value corresponds to the certainty in the assignment of an individual in the cluster in which the individual was placed, with values near zero indicating uncertainty. We tested whether individuals showed preferential associations with companions, using the preferred/avoided association test in SOCPROG (Bejder et al., 1998; Whitehead, 2009). The null hypothesis of this test is that individuals will associate with the same probability with all other individuals in the population without individual preference. Based on similar studies (Baird et al., 2008), we tested our data against 20 000 randomly permuted variations, so that the resultant P value was determined by the proportion of 20 000 permutations that had higher Standard Deviation (SD) values of the association indices than the SD of the association indices found in our data. For these analyses, we restricted our data to all individuals seen two or more times. We refer to groups of three or more individuals linked by association in the social network as separate components, and these components could be comprised of one or more social clusters based on the association analyses.

To evaluate possible fishery interactions, the primary catalog curator (ABD) reviewed the best left and/or right dorsal fin photo of each individual in both the Central America and southern California-Mexico catalogs for evidence of fishery interactions. Based on the presence of linear cuts, dorsal fin disfigurement (e.g., deep cut on the leading edge, missing dorsal fin, bent dorsal fin) or scarring of the area immediately in front or behind the dorsal fin (Baird et al., 2014), ABD chose photos of individuals for further evaluation. Each individual was assigned a score of one (not consistent), two (possibly consistent), or three (consistent) with a fishery interaction by ABD and two additional reviewers (SDM, RWB) with experience in reviewing dorsal fin injuries in relation to fishery interactions. The reviews were conducted independently and sent to ABD who averaged the scores for each individual whale. To assess differences in fishery interactions among areas or social clusters, we considered individuals with an average score of >2.6 as having injuries consistent with a fishery interaction (i.e., at least two reviewers would have to have scored it 3 (consistent) and the third reviewer would have to score 2 (possibly consistent)), following Baird et al. (2014).

RESULTS

Acceptable quality photos were obtained from 83 encountered groups, resulting in 577 identifications and 328 individuals (Table 1; Fig. 1). Group sizes were available for 75 of the 83 encounters, with a mean group size of 28.2

(SD = 34.7, range = 1-200, median = 18) (Table 1; SMT1). The smallest groups encountered were in Guatemala with a mean of 12.5 (SD = 7.7, range = 2 –20, n = 4 groups), and the largest groups were encountered in central mainland Mexico, with a mean of 54.5 (SD = 60.1, range = 5-200, n = 17 groups) (Table 1). The number of individuals identified in each encountered group (n = 83) ranged from one to 40 (mean = 7.6, SD = 10.0, median = 5). Of the 328 individuals identified, 158 (48.2 %) were encountered more than once, with the span of years between first and last sightings ranging from one to 9 577 days (26.2 years). One hundred and fourteen individuals (34.8 %) were seen over periods of a year or greater, and the maximum times an individual was documented was 10.

Association analyses revealed nine separate components containing three or more individuals in the social network, as well as five individuals that were not linked by association to any others (Fig. 2A). Matches were found between individuals encountered off southern California and Baja California (Fig. 1; Table 2), but no individuals from either of those regions were encountered farther south, and all but two of the 39 individuals encountered off southern California and Baja California were linked by association in the same component of the social network (Fig. 2A). Of the 328 individuals in the social network, the largest component included 183 identifications of 119 individuals (37.4 %) from 29 encounters, with individuals documented from mainland Mexico to northern Costa Rica. Within this component, individuals encountered off central mainland Mexico were also sighted in Guatemala, and as far south as southern Nicaragua. Nicaragua encounters occurred either close to the northern or southern borders of the country, with four individuals encountered in both the north and south. Nine of 45 individuals encountered off Nicaragua were also seen off northern Costa Rica (Table 2). Four smaller groups of individuals and two lone individuals photographed along the coast from mainland Mexico to Nicaragua were not linked to the largest component of the social network (Fig. 2A). The second largest

Table 2

Re-sightings of false killer whales (*Pseudorca crassidens*) within and among regions. Individuals that are re-sighted in multiple regions appear in the total count for each region

Region	S. California	Baja California	Central Mainland Mexico	S. Mainland Mexico	Guatemala	Nicaragua	N. Costa Rica	S. Costa Rica	Panama	Isla del Coco
S. California	12									
Baja California	7	1								
Cent. Mainland MX	0	0	21							
S. Mainland MX	0	0	9	5						
Guatemala	0	0	1	2	0					
Nicaragua	0	0	5	5	4	9				
N. Costa Rica	0	0	0	0	0	9	0			
S. Costa Rica	0	0	0	0	0	0	0	59		
Panama	0	0	0	0	0	0	0	10	6	
Isla del Coco	0	0	0	0	0	0	0	0	0	16

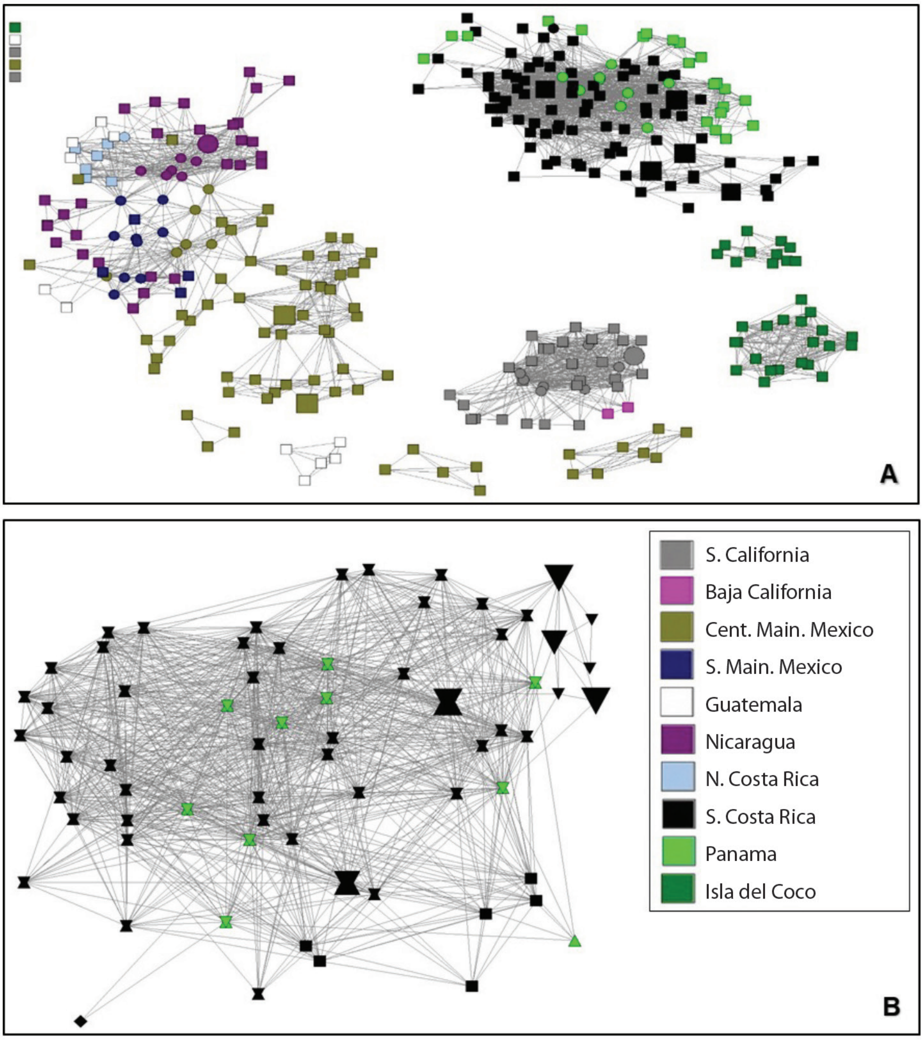

Fig. 2. Social network of false killer whales (*Pseudorca crassidens*), with individuals presented as nodes and lines between nodes indicating individuals encountered in the same area on the same day, restricted to individuals with acceptable quality and distinctiveness scores. Node color indicates the region where it was first encountered. Number of sightings by region: Southern California (n = 9); Baja California (n = 2); central mainland Mexico (n = 19); southern mainland Mexico (n = 3); Guatemala (n = 4); Nicaragua (n = 7); northern Costa Rica (n = 1); southern Costa Rica (n = 26); Panama (n = 5); Isla del Coco (n = 7). Large nodes represent individuals with injuries consistent with fishery interactions. **A**. All individuals from California to Panama and Isla del Coco (1991 to 2022), (n = 328). The greatest number of individuals linked by association (upper left – n = 119) are from mainland Mexico to northern Costa Rica. The second largest number linked by association (upper right – n = 116) represents individuals from southern Costa Rica and Panama. Node shape indicates individuals encountered in multiple regions (circle) or single region (square). **B.** False killer whales from southern Costa Rica to Panama (1991 to 2022), restricted to individuals seen 2 or more times. Node shape represents clusters: hourglass – Cluster 3; up triangle – Cluster 4; diamond – Cluster 9; square – Cluster 13; down triangle – Cluster 17 (Clusters with evidence of fishery interaction are listed in Table 4). Location first seen is indicated by color as per A. Social network metrics were calculated and illustrated in Netdraw 2.176.

number of individuals linked by association (242 identifications of 116 individuals over 31 encounters) was from southern Costa Rica and Panama (Fig. 2B). All individuals documented in those two areas were linked in the same component of the social network, with ten individuals encountered in both regions (Table 2). Out of the 116 southern Costa Rica and Panama individuals, 52.6 % (61) individuals have been re-sighted on more than one day, and 40.5 % (47) individuals have been re-sighted for more than one year.

No matches were found between the 33 individuals documented (from seven encounters) around Isla del Coco and individuals encountered in coastal waters, although re-sightings of individuals were reported within the region (Table 2). The majority (21 of 33, 63.6 %) of individuals from Isla del Coco were linked by association in one component of the social network, and 11 (33.3 %) were linked in another (Fig. 2A).

The average straight-line distance among re-sightings of individuals (mean = 303.3 km, SD = 505.4, median = 51.2) was far less than the average distance among encounters (mean = 1 796.7 km, SD = 1 551.2, median = 1 594.1). Greatest straight-line distances between re-sightings of individuals were between Mexico and Central America (mean = 794.5 km, SD = 626, median = 639.5), with 2 265 km being the maximum straight-line travel distance for an individual (CRC_CA_Pc234_MX_Pc176) sighted off central mainland Mexico on 1 February 2020 and re-sighted off southern Nicaragua 6 August 2021 (Table 3, Fig. 1). Average re-sighting distances of individuals between southern Costa Rica and Panama were far less than between the other regions (mean = 79.9 km, SD = 88.5).

Social network cluster analysis and community structure: Using community division by modularity and social network analysis, we found that the 328 distinct individuals identified could be assigned into 22 clusters by association (modularity = 0.77, maximum modularity type 1 controlling for gregariousness). Tests for preferred/avoided associations among individuals were significant (P = 0.999), so that we could reject the null hypothesis that associations were random. Repeated associations were documented most consistently in the southern Costa Rica-Panama social network, with average and maximum mean association HWI values among individuals of 0.09 (SD = 0.04) and 0.79 (SD = 0.18), respectively (Fig. 2B). Individuals within the Costa Rica-Panama social network from Cluster 3 (average and maximum mean association HWI values 0.11 (SD = 0.03) and 0.80 (SD = 0.17)) had the longest association between individuals (CRC_CA_Pc027 and CRC_CA_Pc028) spanning 12.1 years, with five sightings of these whales encountered together. Except for the first and last encounters of CRC_CA_Pc027, in 2005 and

Table 3

Straight-line distances between all possible pairs of encounters where individual false killer whales (*Pseudorca crassidens*) were photo-identified, and distances among re-sightings of individuals. For the comparison of distances of re-sighted individuals, the grand mean/median values are shown.

	Mean distance (SD) (km)	Median distance (km)	Maximum distance (km)
All countries – all possible pairs	1 796.7 (1 551.2)	1 594.1	5 096.3
Re-sighted individuals	303.3 (504.4)	51.2	2 265.1
S. California & Baja California – all possible pairs	628.7 (784.0)	50.9	1 655.8
Re-sighted individuals	543.5 (745.7)	34.4	1 655. 8
Cent. Mainland Mexico-N. Costa Rica – all possible pairs	1 162.5 (874.0)	1 095.0	2 710.1
Re-sighted individuals	794.5 (626.5)	639.5	2 265.1
S. Costa Rica & Panama – all possible pairs	79.8 (83.3)	48.6	246.1
Re-sighted individuals	79.8 (88.5)	42.1	238.6

2019, CRC_CA_Pc027 and CRC_CA_Pc028 have been encountered together every time that one has been sighted. Long term associations have been observed in the southern California and Baja California region as well, with two individuals (IDs MX_085 and MX_90) encountered together five times between 2013-2021 (9.0 years). Additionally, individuals MX_085 and MX_166 have been encountered together five times between 2014-2022 (8.0 years).

Insufficient information on survey effort is available to quantify seasonal sighting rates. However, it is worth noting that thirty-eight (76.0 %) of Central America encounters occurred during the Boreal winter (November-March), while 90.0 % of southern California-Mexico encounters occurred during the same period. All of the southern California and one of the two Baja California encounters occurred in March, the second Baja California encounter occurred in May.

Fishery interactions: Seventeen individuals were initially selected for review of injuries consistent with fisheries interactions, 12 from the Central America catalog and five from the southern California-Mexico catalog. Of these, our three reviewers agreed that nine had injuries consistent with fisheries interaction (average score > 2.6): one off southern California, two off central mainland Mexico, one individual seen off Guatemala, Nicaragua, and northern Costa Rica, and five from southern Costa Rica (see Fig. 3 for examples). A single individual encountered off Isla del Coco bore injuries that may have been related to fisheries, but received an average score of 2.3. Individuals with fishery-related injuries were found in five of the clusters identified through community division, with the greatest percentage of individuals with fisheries-related injuries (21.4 %) found in southern Costa Rica (Cluster 17) (Table 4), and the second greatest percentage from Cluster 12 encountered off central mainland Mexico, with 7.1 % of individuals having fishery related injuries. One of the individuals from Cluster 21 (Fig. 3G) had injuries consistent with fishery interactions, although it is also possible the wound was caused by a propeller injury.

DISCUSSION

Our analyses of false killer whales individually identified from 1991 to 2022 from southern California to Panama show high levels of site fidelity, particularly off southern Costa Rica and Panama, and strong associations among individuals, with maximum HWI association values among individuals exceeding 0.50. Gero et al., (2008) and Durrell et al., (2004) note associations are considered strong when the HWI between associates was at least twice the mean index of all the dyads in the unit or cluster being considered, which was the case in Cluster 3 from southern Costa Rica and Panama. From re-sightings of individuals photographed off central mainland Mexico and Nicaragua, we documented travel distances greater than those observed from satellite-tagged pelagic false killer whales in Hawaiian waters (Anderson et al. 2020; E. Oleson personal communication, 27 July, 2022) especially considering that we

Table 4
Number and percentage of individual false killer whales (*Pseudorca crassidens*) by cluster (determined through community division) with injuries consistent with fishery interactions.

Cluster	Regions documented	Number of individuals in cluster	Number (%) with injuries consistent with fishery interactions
6	S. California & Baja California	37	1 (2.7 %)
12	Cent. Mainland Mexico	28	2 (7.1 %)
10	Cent. Mainland Mexico - N. Costa Rica	33	1 (3.0 %)
17	S. Costa Rica	14	3 (21.4 %)
3	S. Costa Rica & Panama	69	2 (2.9 %)
Total		182	9 (4.9 %)

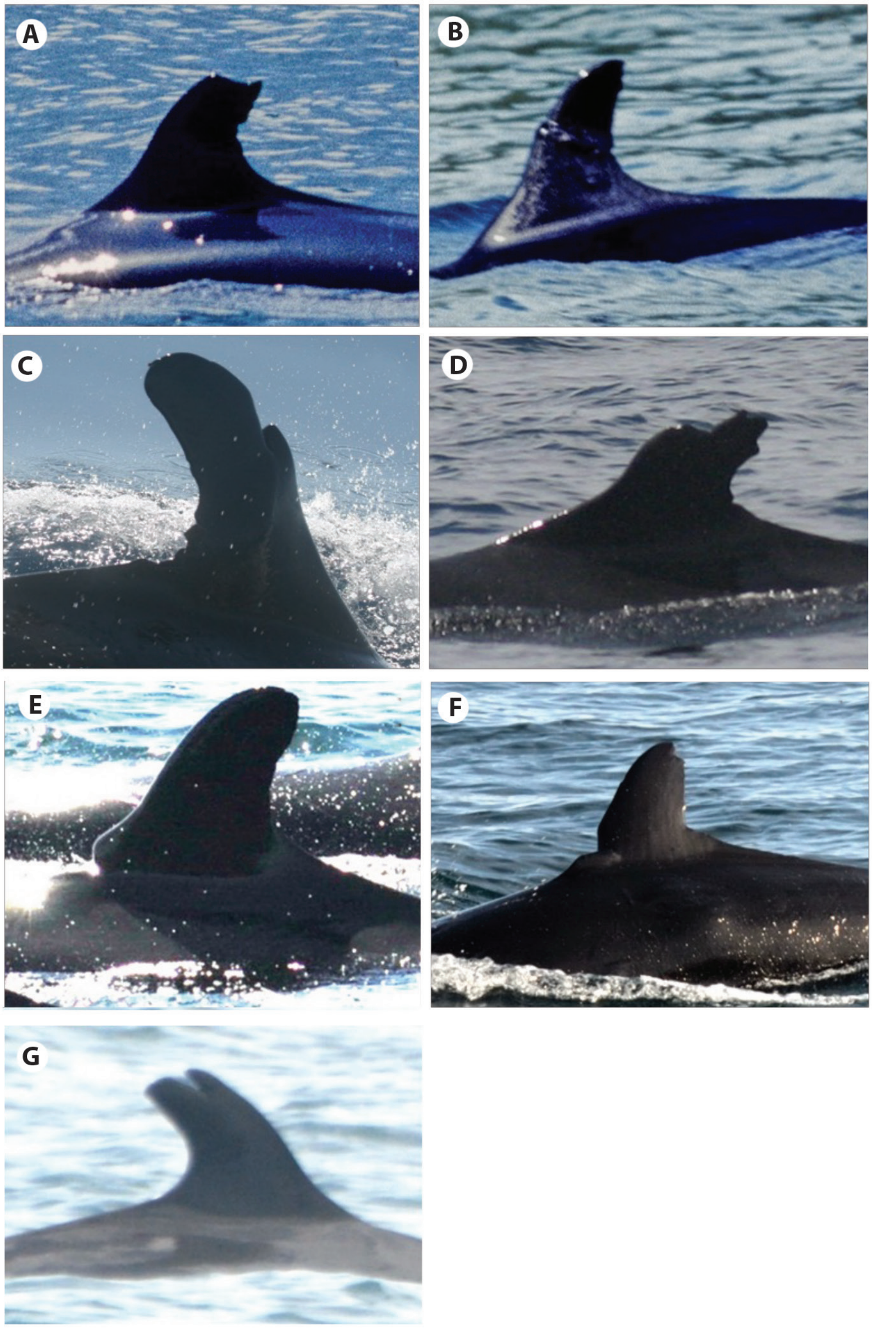

Fig. 3. Examples of scarring and injury of dorsal fins determined to be consistent with fisheries interaction (FI) from southern Costa Rica, (A) CRC_CA_Pc23, 1992, (B) CRC_CA_Pc102, 1991, (C) CRC_CA_Pc057, 2009, (D) CRC_CA_Pc032, 2006, and Central Mainland Mexico (E) MX_074, 2013, (F) MX_084, 2013. (G) CRC_CA_Pc188 from northern Costa Rica is an example of an injury that we determined to be consistent with fisheries interaction, although whether the injuries were caused by a line or propeller is unknown.

calculated straight-line distances, and did not account for non-linear movements and intervening land masses. False killer whales encountered off southern Costa Rica and Panama appear to have a much smaller range of travel, smaller even than the insular false killer whales tagged in the Hawaiian Islands (Baird et al., 2012). It is important to note, however, that there was very limited effort off the continental shelf, so we are unable to assess offshore movements. The infrequency of sightings of false killer whales on the continental shelf could be an indication that individuals documented in our study spend a considerable proportion of their time in offshore waters, that local abundance is low, or a combination of both factors. There were no matches between individuals documented off Isla del Coco in 1993 or 1994 and the mainland. While the mainland photographic sample was largely obtained from six to more than twenty years later, there were matches from the Acevedo-Gutiérrez et al. (1997) effort off mainland Costa Rica from prior to the Isla del Coco effort.

We found that the proportion of individual false killer whales with evidence of surviving prior fishery interactions for at least one cluster was higher than those found in the endangered main Hawaiian Islands insular population (Baird et al., 2014). Individuals from Cluster 17, which had the greatest percentage of fisheries interactions (21.4 %), have only been encountered off southern Costa Rica. They were encountered by Acevedo-Gutiérrez et al. (1997) in Golfo Dulce in 1991 and 1992 and the most recent sightings of any of these animals occurred off Drake Bay in 2006. In a similar study of fishery-related injuries in Hawai'i, the evaluators found significant differences in fishery interaction rates by population and cluster, with 12.8 % of individuals from Cluster 3 from the main Hawaiian island population determined to have fishery-related scarring. Baird et al. (2014) found a significant bias towards females with injuries consistent with fisheries interactions, which, the authors note, could reduce the potential population growth rate to a greater extent than if fishery interaction was unbiased by sex. Although we identified the sex of some individuals based on close attendance of small calves, performing a comparable test with our data was beyond the scope of this study. It is important to note that false killer whales can incur severe injuries that may appear to be fishery related from sources other than fisheries interaction. For example, Ortega-Ortiz et al. (2014) describe at least one individual with injuries obtained from interactions with a billfish or sailfish.

There are a number of assumptions and biases in this study that we would like to note. Many of the encounters in this study came from community scientists or were collected opportunistically, and thus not all individuals within larger groups were necessarily photographed. Thus, there are a lot of isolated individuals or small clusters in the social network that in reality should be linked to other individuals. For example, the 2018 encounter of the only individual not linked to a cluster from Guatemala had an estimated group size of two. Therefore, we can safely assume that there is at least one and likely a number of clusters that use our larger study area that are unaccounted for in these data. Group size estimates were collected with different methodologies, and we acknowledge that false killer whale group size is difficult to assess, as individuals are generally fast moving in a spread-out group (Baird et al., 2008; Bradford et al., 2014). Sighting positions are estimated in most non-directed survey efforts, and this can impact the calculated distance between sightings, although this should not influence the large difference between encounter locations and re-sighting locations documented. Distinct individuals are more likely to be photographed and recognized over time, even with lesser quality photographs, so there are likely some missed matches within our catalog, particularly given the long duration of our study. The two individuals with the longest sighting history (CRC_CA_Pc063 seen over 26 years, and CRC_CA_Pc009 seen over 19 years) were both very distinct. Although we are not estimating abundance here nor survival, we would like to note that our stated method of including "fair" quality photos as well as only considering recaptures with high certainty, could result in higher abundance estimates and lower re-sighting rates (Ashe & Hammond, 2022).

We recommend additional effort in offshore areas as well as satellite tagging to clarify population structure and relationship to animals in coastal areas, especially in light of the lack of connection between Central America and Isla del Coco. Additional acceptable ID photos from any region would be welcome, especially offshore waters, the Galapagos Islands, Isla del Coco, and South America. One of our authors (DMP) recalls false killer whale encounters a handful of times in the Galapagos during the warm-water season. From records collected from 1923 to 2003 there is a single record of a mass stranding of six false killer whales in the Galapagos Islands (Palacios et al., 2004). False killer whales are also encountered farther south, with an encounter noted off central Ecuador in 2003 (Baird, 2010; Castro, 2004). Photos of two individuals from the Galapagos were not included in this study due to poor quality; however, these photos are available to compare to any future identifications that we receive.

Field effort in most of the study sites was seasonally biased. However, southern California has whale and dolphin watch excursions year-round, therefore the apparent seasonal presence of false killers along southern California in March is likely not random. This consistency of presence could allow for planning a successful photo-ID and tagging effort on this group of false killer whales, which could shed light on these animals' whereabouts over time. Throughout the study area, individuals whose livelihoods depend on whale and dolphin watch tourism would benefit if these animals' patterns were better understood, and most importantly, we hope that future studies could help inform fisheries so that they could avoid encounters that are detrimental to false killer whales as well as the humans whose livelihoods depend on the same food source. Currently, to learn more about how and why false killer whales are interacting with longline vessels, the fisheries have options of either increasing observer coverage and/or the installment of electronic monitoring systems (EMS) on fishing vessels. Due to the stated difficulty of finding observers willing to take part in the observer program, as well as the difficulties of dealing with the COVID pandemic, Costa Rica and other countries have appealed to the IATTC to provide funds for EMS (Villanueva, 2018).

In addition to recommending future survey work, we urge greater cooperation among researchers and community scientists in sharing historical catalogs/photo collections. Our data collection has been a slow process, spanning 31 years with sometimes a few photos and a general location from a tour vessel representing the only encounter for a year or a region. Additional catalogs/photo collections exist throughout this study area, which we were not able to access, and we hope that with careful planning and cooperation these data will become available in the near future. As with other odontocetes, false killer whales are viewed as sentinel species in their environment, and studies in the Hawaiian Islands have shown they have high levels of lipophilic contaminants (Kratofil et al., 2020). Greater cooperation among researchers with these data will lead to a greater understanding of how false killer whales use these waters and the depth of long-term associations with individuals and their habitat.

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: ABD conceptualized the manuscript, coordinated data acquisition, performed analysis, interpreted the data results, and drafted the initial and revised versions of this manuscript. FGA, AAG, SDM, KR, EQR, JDW, DMP, DMF, CLP, CDOO, JC, and RWB contributed with data, and interpretation of the results for each study site, reviewed and approved the various drafts and final version of the manuscript. NR, AFJ and FV contributed data and reviewed the final version of the manuscript.

See supplementary material
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More pieces for the puzzle: novel information on the genetic diversity and population structure of *Steno bredanensis* (Artiodactyla: Delphinidae) in Central America and the Caribbean Sea

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ABSTRACT

Introduction: The rough-toothed dolphin (*Steno bredanensis*) inhabits oceanic waters of tropical latitudes and exhibits philopatry in some oceanic islands. However, the species has been observed in shallow coastal waters in a few areas. Particularly in Central America, the rough-toothed dolphin has been reported by occasional records and strandings. For instance, the first confirmed record of this species in the Panama's Caribbean was on July 17, 2012, in a coastal region of the Chiriquí Lagoon during a bottlenose dolphin monitoring survey. Similarly, the first rough-toothed dolphin mass stranding reported for the Pacific of Panama was on April 20, 2016, at the Ostional Beach, where 60 dolphins stranded and ten died. These sightings and events offered a valuable opportunity to obtain samples to conduct genetic studies, which are scarce in the region.

Objective: In this study, we present the first assessment of genetic diversity for rough-toothed dolphins based on mitochondrial DNA Control Region (mtDNA-*CR*) in the Panamanian Pacific and the Wider Caribbean.

Methods: Samples were collected in Colombia (N=5), Panama (N-Caribbean=1, N-Pacific=9), and Puerto Rico (N=3) from free-ranging and stranded individuals. DNA was extracted from each sample, and a mtDNA segment of around 534 to 748 bp was amplified through the PCR reaction. The obtained sequences were compared with rough-toothed dolphin haplotypes previously published in NCBI (N=70), from the Atlantic, Indian, and the Pacific Oceans.

Results: Our findings showed significant population structure among ocean basins (strong differentiation with Φ_{ST} data), and high genetic diversity within each phylogroup. Only the Atlantic Ocean showed high genetic differentiation within the basin, detecting three phylogroups: the Caribbean, northern, and southern Atlantic.

Conclusions: These findings support previous genetic studies that indicate high levels of population structure among ocean basins, although this species seems to be widely dispersed. However, samples from Panama and the Caribbean appear to show connectivity between highly differentiated Atlantic and Pacific Oceans. Therefore, our results highlight the need for more research to assess the rough-toothed dolphin genetic and population status in Central America, as the piece of the puzzle needed to clarify its taxonomy and genetic differentiation worldwide. This information is needed due to the rough-toothed dolphin IUCN categorization as "Least Concern" and its classification into appendix II according to CITES. While individuals are potentially threatened by incidental fishing, no management units are currently used to conserve this species despite its high genetic differentiation.

Key words: Delphinids; cetaceans; Control Region; mtDNA; Caribbean; Pacific Ocean; conservation.

RESUMEN

Más piezas del rompecabezas: información preliminar sobre la diversidad genética y estructura poblacional de *Steno bredanensis* (Artiodactyla: Delphinidae) en Centroamérica y el Mar Caribe

Introducción: El delfín de dientes rugosos (*Steno bredanensis*) habita aguas oceánicas de latitudes tropicales y muestra filopatría en algunas islas oceánicas. Sin embargo, la especie ha sido observada en algunas áreas costeras de aguas poco profundas. Particularmente en Centroamérica, los delfines de dientes rugosos han sido reportados por registros ocasionales y varamientos. Por ejemplo, el primer registro confirmado de la especie en el Caribe Panameño ocurrió el 17 de julio de 2012 en una región costera de la Laguna de Chiriquí, durante un monitoreo de delfín nariz de botella. De manera similar, el primer reporte de un varamiento masivo de delfines de dientes rugosos en el Pacífico Panameño ocurrió el 20 de abril de 2016, en la Playa Ostional, donde 60 delfines vararon y diez murieron. Estos avistamientos y eventos ofrecen una valiosa oportunidad para obtener muestras con el fin de realizar estudios genéticos, los cuales son escasos en la región.

Objetivo: En este estudio, presentamos la primera evaluación de la diversidad genética de los delfines de dientes rugosos basado en la Región Control de ADN mitocondrial (*CR*-ADNmt) en el Pacífico Panameño y la región Caribe.

Métodos: Las muestras fueron colectadas en Colombia (N=5), Panamá (N-Caribe=1, N-Pacífico=9), y Puerto Rico (N=3) de individuos vivos y varados. El ADN fue extraído para cada muestra, y un segmento de ADNmt de aproximadamente 534 a 748 pb fue amplificado mediante la reacción en cadena de la polimerasa PCR. Las secuencias obtenidas fueron comparadas con haplotipos de delfines de dientes rugosos de los Océanos Atlántico, Índico y Pacífico, publicados previamente en NCBI (N=70).

Resultados: Nuestros resultados mostraron una estructura poblacional significativa entre las cuencas oceánicas (una alta diferenciación con base en datos de Φ_{ST}), y una alta diversidad genética dentro de cada filogrupo. Solo el Océano Atlántico mostró una alta diferenciación dentro de la cuenca, detectando tres filogrupos: el Caribe, Atlántico norte y sur.

Conclusiones: Estos resultados soportan los estudios genéticos previos que indican altos niveles de estructura poblacional entre las cuencas oceánicas, aunque esta especie parece estar ampliamente distribuida. Sin embargo, las muestras de Panamá y el Caribe parecen mostrar conectividad entre las cuencas altamente diferenciadas del Océano Atlántico y Pacífico. Por lo tanto, nuestros resultados destacan la necesidad de realizar más investigación para determinar el estado genético y poblacional de los delfines de dientes rugosos en Centroamérica, como la pieza del rompecabezas que falta para esclarecer su taxonomía y diferenciación genética a nivel mundial. Esta información es necesaria debido a que el delfín de dientes rugosos está categorizado ante la UICN como "Preocupación Menor" y está clasificado en el apéndice II de CITES. Aunque los individuos pueden estar potencialmente amenazados por captura incidental, no existen actualmente unidades de manejo para conservar esta especie a pesar de su alta diferenciación genética.

Palabras clave: Delfínidos; cetáceos; Región Control; ADNmt; Caribe; Océano Pacífico; conservación.

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

INTRODUCTION

The rough-toothed dolphin, *Steno bredanensis,* (G. Cuvier in Lesson, 1828) is distributed worldwide in tropical, sub-tropical, and warm-temperate latitudes (Jefferson, 2018). In general, the species has oceanic habits, but shows some preference for volcanic islands where deep waters are close to the coast, such as the Canary Islands, French Polynesia, and Hawaii (Kerem et al., 2016). Particularly in these Indo-Pacific and Pacific islands, where the species has been extensively studied, the rough toothed-dolphins show some degree of philopatry (Baird, 2016; Oremus et al., 2012). In the Eastern Mediterranean, the rough-toothed dolphin is reported as an oceanic species with a seasonal migration pattern between neritic and oceanic habitats (Kerem et al., 2016). Consequently, the species may occupy both habitats, despite the apparent tendency to be distributed in oceanic areas. Indeed, in countries along the Western Atlantic and the Caribbean including Brazil, Colombia, Honduras, and recently in Panama, rough toothed dolphins have been reported mainly in neritic zones (Barragán-Barrera et al., 2015; Farías-Curtidor & Ayala, 2015; Farías-Curtidor & Barragán-Barrera, 2017, Farías-Curtidor & Barragán-Barrera, 2019; Kuczaj & Yeater, 2017; Ott & Danilewicz, 1996; Santos et al., 2019).

It is clear the need to study the rough-toothed dolphins worldwide, in order to understand their distributional patterns as a first step to assess adequately its conservation threats, since marine mammal coastal populations are more exposed to threats than the oceanic ones (Avila et al., 2018). Central America deserves special attention as a big gap of information about rough-toothed dolphins, despite that the entire region may potentially be occupied by this species (Kiszka et al., 2019). For instance, only in 2012 (July 17 at 9:50 a.m.), the rough-toothed dolphin was reported and confirmed genetically for the first time in the Caribbean of Panama, when a group of about six adults was observed jumping and traveling close to shore within the Chiriquí Lagoon during a common bottlenose dolphin (*Tursiops truncatus*) (Montagu, 1821) survey (Barragán-Barrera et al., 2015). Similarly, in 2016, a rare mass-stranding event of 60 rough-toothed dolphins, of which ten died on the beach despite local efforts to rescue them, was reported for the first time at the Ostional Beach, on the Pacific coast of Panama (May-Collado et al., 2017). In general, the few sightings in Central American Caribbean have taken place in coastal areas (May-Collado et al., 2017), and even some individuals appear to show residency patterns like off the coast of Utila in Honduras (Kuczaj & Yeater, 2017).

Definition of neritic and/or oceanic habits of rough-toothed dolphins, as well their population status, is needed to assess adequate management plans. The main threat reported for the species is bycatch in oceanic waters of Brazil (Donato et al., 2019; Monteiro-Neto et al., 2000). However, neritic individuals also may be affected by contamination and fishery interactions, particularly in the Atlantic coast of the USA and in Brazil, where mass strandings have been reported (Baptista et al., 2016;

Donato et al., 2019; Ewing et al., 2020; Lailson-Brito et al., 2012; Lemos et al., 2013; Meirelles & Barros, 2007; Struntz et al., 2004). In light of this, genetic studies based on samples collected opportunistically from stranding events in areas where non-monitoring programs are established, for example in Central America, could be useful to provide an initial status of rough-toothed dolphins' population structure. A recent work aimed to assess this was conducted using samples collected worldwide, and showed clear genetic differentiation among ocean basins based on both nuclear and mitochondrial markers (Albertson et al., 2022). Particularly, a strong distinction was detected between Atlantic and Pacific oceans, which suggested a potential incipient speciation to at least subspecies level (Albertson et al., 2022; da Silva et al., 2015). However, the authors recognized the need for including more samples that represent a larger area in the Pacific and especially in the Indian Ocean to confirm this assumption (Albertson et al., 2022). Additionally, this work only included six samples from the Caribbean and one from Brazil, which could imply no divergence detection between these two areas, despite a strong differentiation previously described based on mitochondrial DNA (mtDNA) data (da Silva et al., 2015).

Following the Albertson et al. (2022) recommendation, herein we provide new insights into genetic diversity and population structure of rough-toothed dolphins based on mtDNA Control Region (mtDNA-*CR*) using new samples collected from the Central American Pacific coast, specifically from Panama, as well as new samples from the Caribbean. Additionally, this study aimed to corroborate or not the population differentiation reported by Albertson et al. (2022), emphasizing on dolphins from Central America, which potentially may provide the resolution needed to clarify the potential subspeciation process among ocean basins. This study provides relevant baseline data about the genetic status of the rough-toothed dolphin in the region, as a first step to understand its population status, and thus propose future adequate management plans for this enigmatic species in Central America.

METHODS

Study area: The study area in Central America, where rough-toothed dolphin samples were collected, encompasses the Azuero Peninsula on the Pacific coast of Panama and the Chiriquí Lagoon on the Caribbean coast of Panama (Fig. 1). The Azuero Peninsula, where the Ostional beach is situated, is located at the central portion of Panama, whose coast is dominated by small portions of mangrove and beach vegetation (Friedman & Grandmont, 2019). In the Ostional beach, a rare event of a mass stranding was reported on April 19th, 2016 (Fig. 2A). The Chiriquí Lagoon, where one sample of one individual from a group of six free-ranging adult dolphins was collected on July 17th, 2012 (Fig. 2B), is a semi-enclosed lagoon located in the Bocas del Toro Province at Western Caribbean of Panama, an area highly influenced by precipitation (Guzmán & Guevara, 1998).

Additionally, samples from the Caribbean basin, coming from Colombia and Puerto Rico, were included in this study (Fig. 1). Samples from Colombia were collected from two locations: 1) in waters of Dibulla, located in La Guajira Peninsula on the northern portion of Colombia, where four samples of free-ranging adult individuals from a group of around 15 dolphins were collected on May 19th, 2015 (Fig. 2C) (Farías-Curtidor & Ayala, 2015; Farías-Curtidor & Barragán-Barrera, 2017, Farías-Curtidor & Barragán-Barrera, 2019), and 2) in Gaira, located in the Magdalena department, where one sample from a stranded individual was collected. These two areas are in the Eastern Colombian Caribbean and are influenced by upwelling events (Arévalo-Martínez & Franco-Herrera, 2008; Fajardo, 1979; Gutiérrez et al., 2015), so cetaceans have been usually reported there (e.g., Barragán-Barrera, do Amaral, et al., 2019, Barragán-Barrera, Luna-Acosta, et al., 2019; Farías-Curtidor et al., 2017; Fraija et al., 2009; Pardo & Palacios, 2006). Regarding

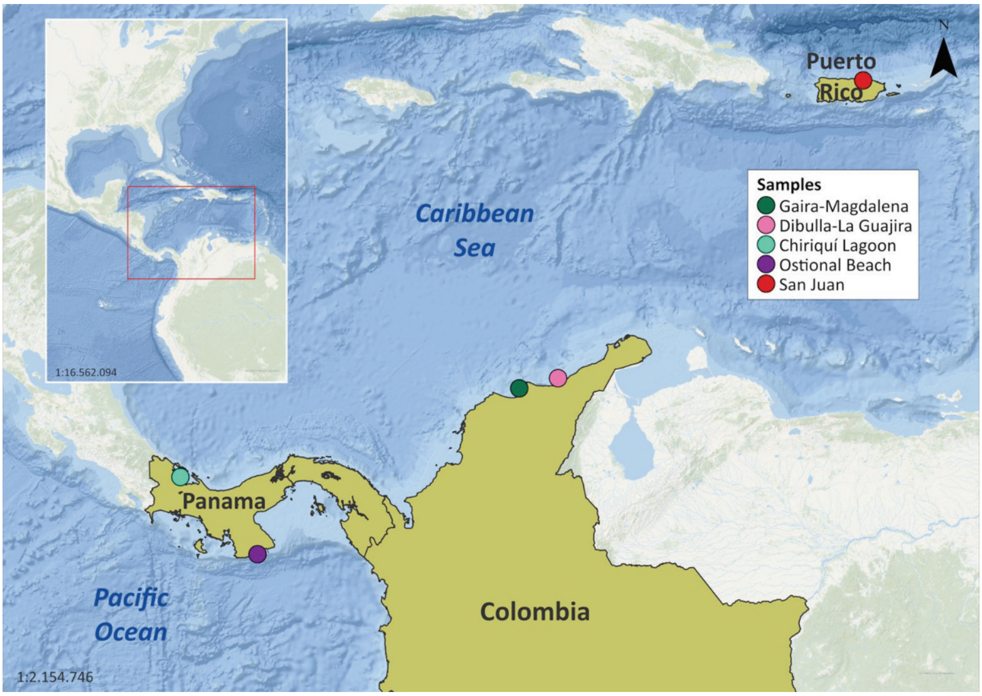

Fig. 1. Location of the samples of Rough-toothed dolphins (*Steno bredanensis*) in the Caribbean coast of Colombia (Gaira-Magdalena and Dibulla-La Guajira), Panama (Chiriquí Lagoon), and Puerto Rico (San Juan), as well as the Pacific coast of Panama (Ostional Beach).

Puerto Rico, it is a Caribbean archipelago that is part of the Greater Antilles (Fig. 1), and due its localization between the Caribbean Sea and the Atlantic Ocean, holds a high marine mammals' diversity in the Wider Caribbean (Mignucci-Giannoni, 1989).

Sample collection: Following Barragán-Barrera et al. (2017), rough-toothed dolphins tissue samples in the Chiriquí Lagoon (N = 1) and Dibulla-La Guajira Peninsula (N = 4) were collected from free-ranging animals using the PAXARMS system (Krützen et al., 2002). Samples from the Azuero Peninsula (N = 9), Gaira-Magdalena (N = 1), and Puerto Rico (N = 3) were collected from stranded animals. Samples were preserved in 70 % ethanol and at −20 °C (Amos & Hoelzel, 1991) for subsequent laboratory analysis.

DNA extraction, PCR, sequencing, and sexing: DNA was extracted from tissue samples using the DNeasy kit (QIAGEN) following the manufacturer's instructions. A portion of mtDNA-*CR* of around 534 to 748 bp was amplified by the polymerase chain reaction (PCR), using the primers Dlp5G (5'- GGAGTACTATGTCCTGTAACCA-3') or Dlp8G (5'-CCATCGWAGATGTCTTATTTAAGRARTTCTA-3') and Dlp1.5 (5'-TCACCCAAAGCTGRARTTCTA-3'), following the protocol described in Baker et al. (1998). PCR products were purified following a Polietilenglicol protocol (PEG 20 %), and DNA was sequenced using the Sanger sequencing method (Sanger & Coulson, 1975). Following Gilson et al. (1998), the sex of each individual sampled was identified using the male-specific SRY gene and ZFY/ZFX genes of males and females.

Data analysis: The software Geneious v. 4.8.5 (Drummond et al., 2009) was used to edit manually all obtained sequences. These haplotypes were compared to 70 GenBank

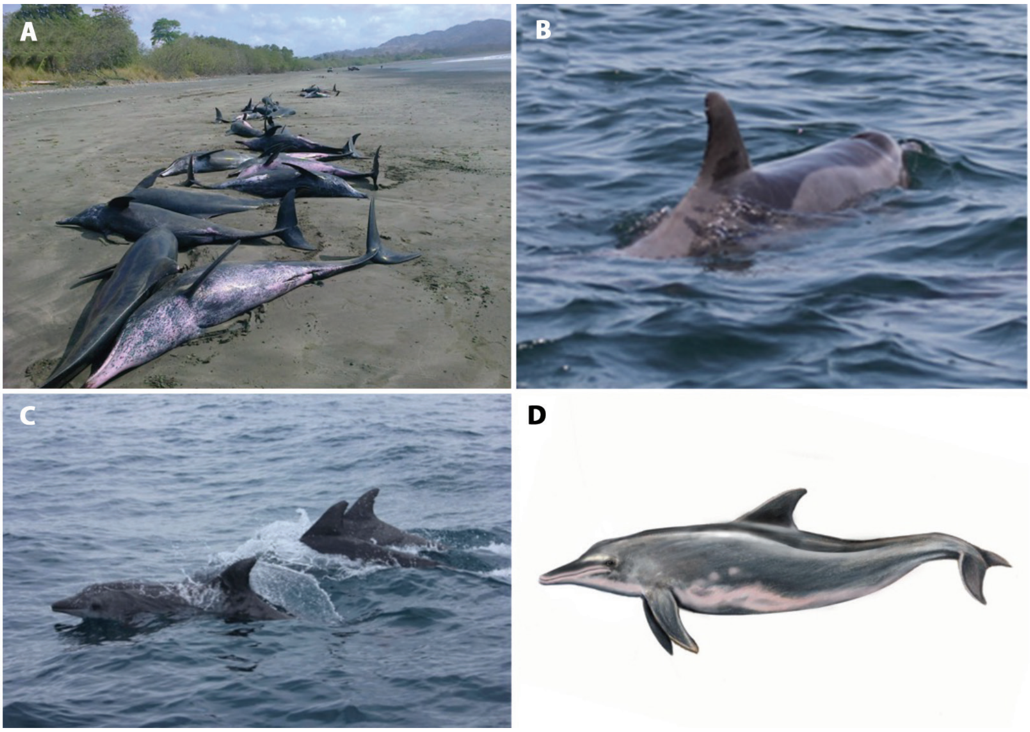

Fig. 2. Sightings of rough-toothed dolphins (*Steno bredanensis*) in which tissue samples for this study were collected. A) A rare mass-stranding occurred in April 2016 at Ostional Beach in the Azuero Peninsula, Pacific coast of Panama; photo courtesy Lissette Trejos. B) The first confirmed sighting of rough-toothed dolphins in the Caribbean coast of Panama, at Chiriquí Lagoon, reported in July 2012; photo by Mónica Acosta. C) A group recorded in May 2015 consisting of 14 adults and one calf in La Guajira Peninsula, Caribbean coast of Colombia; photo by Nohelia Farías-Curtidor. D) A species description illustrated by Emmanuel Laverde © www.arteyconservacion.com

published sequences from Brazil, China, Eastern Pacific coast of Central and North America, India, Indonesia, Japan, Mediterranean Sea, New Zealand, Puerto Rico, Samoa Island, Society Islands, South Africa, South Korea, Spain, Taiwan, Thailand, and USA (Western Atlantic coast and Hawaii) (SMT1). The R script RemoveRedundantTaxa was used to characterize the haplotypes. All sequences were aligned obtaining an alignment of 383 bp. In order to understand the relationships among all sequences, the CIPRES Science Gateway V. 3.3 software was used (Miller et al., 2010) to build a maximum likelihood phylogenetic tree using the evolutionary model of Generalized-Time-Reversible γ + Invariant (GTR γ + I) substitution and 1 000 bootstrap replicates.

Because no information about haplotype frequencies from Indonesia and Thailand was available, these were not included in the subsequent analyses. To determine the number of phylogrups for all haplotypes obtained worldwide, the software Arlequin v. 3.5 (Excoffier & Lischer, 2010) was used to assess the best F_{CT} value by running several possible combinations of geographic areas (two to eight groups) based on ocean basin and/or previous studies (Albertson et al., 2017, Albertson et al., 2022; da Silva et al., 2015; Oremus et al., 2012). The same software was used to assess the fixation indices (F_{IS} and F_{IT}). Once the number of phylogrups was identified, the PopART software (Clement et al., 2000) was used with the TCS method to build a haplotype network to visualize to what population the haplotypes used in this study

Table 1
Estimates of mitochondrial differentiation among the five phylogrups identified for rough-toothed dolphins (*Steno bredanensis*) in the Atlantic, Indian, and Pacific Oceans. F_{ST} value is shown above diagonal and Φ_{ST} below diagonal. P-value is indicated under each value in parentheses (significant values are considered as $P < 0.05$). Haplotype (h) and nucleotide diversity (π) are shown on the diagonal for each phylogrup.

F_{ST} Φ_{ST}	Brazil-South Atlantic Ocean	Caribbean	North Atlantic Ocean	Indian Ocean	Pacific Ocean
Brazil- South Atlantic Ocean	$h = 0.628$ $\pi = 0.121$	0.233 ($P = 0.009$)	0.268 ($P < 0.001$)	0.262 ($P < 0.001$)	0.189 ($P < 0.001$)
Caribbean	0.644 ($P < 0.001$)	$h = 0.978$ $\pi = 0.109$	0.100 ($P < 0.001$)	0.046 ($P < 0.001$)	0.043 ($P < 0.001$)
North Atlantic Ocean	0.746 ($P < 0.001$)	0.904 ($P < 0.001$)	$h = 0.840$ $\pi = 0.060$	0.124 ($P = 0.009$)	0.104 ($P = 0.009$)
Indian Ocean	0.562 ($P < 0.001$)	0.768 ($P < 0.001$)	0.340 ($P < 0.001$)	$h = 0.929$ $\pi = 0.167$	0.057 ($P = 0.027$)
Pacific Ocean	0.490 ($P < 0.001$)	0.780 ($P < 0.001$)	0.570 ($P < 0.001$)	0.269 ($P < 0.001$)	$h = 0.942$ $\pi = 0.083$

belonged and their frequency. To assess the genetic subdivision among phylogrups identified, the software Arlequin v. 3.5. (Excoffier & Lischer, 2010) was used to conduct an analysis of molecular variance (AMOVA) (Excoffier et al., 1992), so the pairwise comparison of population differentiation indices F_{ST} and Φ_{ST} between all phylogrups were assessed. The same software was used to assess diversity indices as nucleotide (π) and haplotype diversity (h), as well as the neutrality defining *Tajima's D* index for each phylogrup.

RESULTS

A total of 18 samples were collected, and 17 were successfully amplified (94,44 %) except one from the Ostional Beach. In total, the 17 sequences obtained represent 13 new haplotypes ranging between 534 and 748 bp. For Panama, one haplotype was identified in the Caribbean while five were identified for the Pacific. Among these five haplotypes, sequences SbEPP1 (748 bp) and SbEPP1.1 (691 bp), which were identical, were reported as different haplotypes due the first one was longer, and we do not have any evidence of polymorphism present in the non-amplified region. For Colombia (Caribbean basin), three haplotypes were identified in Dibulla-La Guajira and one in Gaira-Magdalena. For Puerto Rico, three haplotypes were identified (SMT1). All these new haplotypes were deposited and are available in GenBank (https://www.ncbi.nlm.nih.gov/genbank/) under accession numbers OR436925-OR436936.

Comparison among these new haplotypes with previously 70 GenBank published sequences from Atlantic, Indian, and Pacific Oceans, showed strong differentiation among ocean basins. Despite of this, four Caribbean haplotypes, including the ones from Colombia (Dibulla-La Guajira), Panama, and Puerto Rico (SbGCC2, SbGCC3, SbPC1, and SBPRC3) nested within the Pacific Ocean clade (the maximum likelihood phylogenetic tree illustrates in green color the Caribbean haplotypes; Fig. 3). Consequently, reciprocal monophyly was not detected between the Atlantic and Pacific Oceans, and this last one appears to be a paraphyletic group (boostrap support > 95 %; Fig. 3). The remaining four Caribbean haplotypes (SbMCC1, SbPRC1, SBPRC2, and SbGCC1) exhibited connectivity to the entire Atlantic Ocean. The SbMCC1 haplotype from Gaira-Magdalena (Colombian Caribbean) nested with haplotypes from the Atlantic coast of the USA, as well as with the haplotypes SbPRC1 and SBPRC2 from Puerto Rico. The Colombian haplotype SbGCC1 from Dibulla-La Guajira

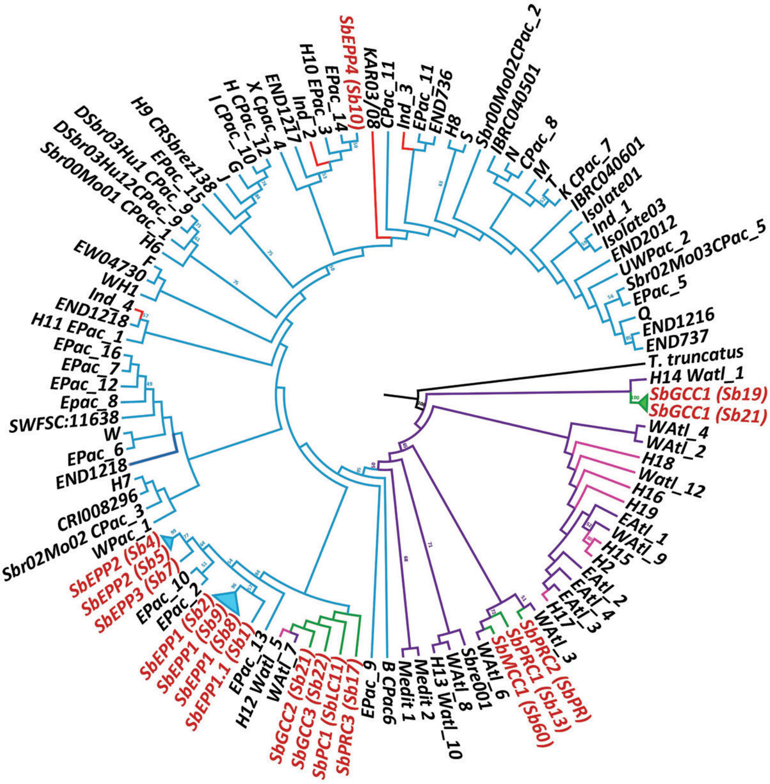

Fig. 3. Phylogenetic reconstruction by maximum likelihood of rough-toothed dolphins (*Steno bredanensis*) Control Region (752 bp) haplotypes in the Atlantic (purple and pink clades), Indian (red), and Pacific (blue) Oceans. Caribbean clades are colored green. Phylogeny in a circular polar form shows bootstrap support in branches with percentages of 100 % or > 50 %. Red letters represent the samples used for this study. Outgroup: Common bottlenose dolphin, *Tursiops truncatus*.

showed a higher connectivity to the Atlantic basin since it nested with haplotypes from the Atlantic coast of the USA and Canary Islands (Spain) (Fig. 3). Despite this, high population structure was detected between the Caribbean and the North Atlantic phylogrups. Regarding the haplotypes from the Ostional Beach in the Panamanian Pacific (SbEPP1, SbEPP1.1, SbEPP2, SbEPP3, and SbEPP4), these exhibited connectivity with haplotypes from the entire Pacific basin, since they nested with haplotypes from Central Pacific (mainly Hawaii and Society Island), Indo-Pacific (India), Eastern Pacific (Pacific coast of America), and Thailand (Fig. 3).

In the analysis of population structure, we identified five phylogrups based on the best F_{CT} value ($F_{CT} = 0.47$, $P = 0.005$): 1) Brazil-South Atlantic Ocean, 2) Caribbean, 3) North Atlantic Ocean, 4) Indian Ocean, and 5) Pacific Ocean. The Caribbean phylogrup was formed by haplotypes from the Caribbean coast of Colombia, Panama, and Puerto Rico. The North Atlantic Ocean phylogrup consisted of haplotypes from the Eastern Mediterranean Sea, Canary Islands (Spain), and the Atlantic coast of the USA. The Indian Ocean phylogrup was formed by haplotypes from India and South Africa. The Pacific Ocean phylogrup included haplotypes from the Central Pacific, China, Eastern Pacific, Hawaii, Japan, Pacific waters of Panama, Samoa Islands, Society Island, South Korea, Taiwan, and Western Pacific. Pairwise comparisons showed strong population structure among phylogrups,

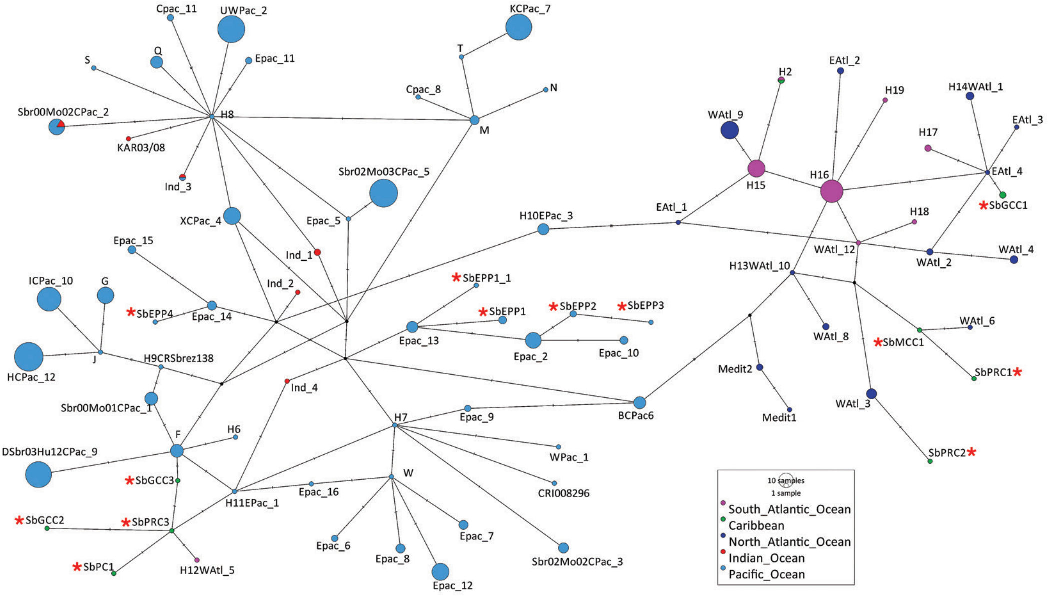

Fig. 4. Haplotype network reconstruction for rough-toothed dolphins (*Steno bredanensis*) in the Caribbean, North Atlantic Ocean, South Atlantic Ocean (Brazil), Indian Ocean, and Pacific Ocean, under parsimony criteria with the TCS algorithm (N = 83, 383 bp). The circle's size indicates the frequency of each haplotype. Black dot indicates the hypothetical ancestral haplotype, and the perpendicular lines between the haplotypes refer to the number of nucleotide substitutions between them. New haplotypes reported in this study are indicated with a red asterisk (*).

mainly at nucleotide level (Φ_{ST}) (Table 1). The haplotype network (Fig. 4) showed the relationship among haplotypes and their frequencies from the five phylogrups.

The genetic diversity indexes showed high haplotype and nucleotide diversity for each phylogrup, with the highest haplotype diversity in the Caribbean, followed by the Pacific Ocean and the Indian Ocean. Brazil-South Atlantic Ocean exhibited the lowest haplotype diversity (Table 1). Regarding *Tajima's D* index, it was significant only for the Brazil-South Atlantic Ocean phylogrup and showed a negative value (*Tajima's D* = -1.792, P = 0.013). In general, the fixation indices for all phylogrups showed a significant value only for the endogamy index (F_{IS} = 0.501, P < 0.001).

DISCUSSION

This is the first study on the genetic population structure of rough-toothed dolphins using samples from Central America (specifically from Panama) based on mtDNA-*CR* data. Although we included individuals from new geographic regions that had not been sampled before (Panama and the Caribbean), our data supports strong population structure reported previously for the species among the Atlantic, Indian, and Pacific Oceans (Albertson et al., 2022; da Silva et al., 2015), and provide new insights about genetic differentiation within the Atlantic basin (da Silva et al., 2015; Donato et al., 2019). In general, our findings highlight that individuals in Central America may contain crucial genetic information to elucidate population status of rough-toothed dolphins in both Atlantic and Pacific Oceans, where a process of incipient subspeciation has been suggested (Albertson et al., 2022).

Genetic structure of Panamanian and Caribbean dolphins in the Atlantic Ocean

Our findings suggest the Atlantic Ocean is represented by three phylogrups: one in the Caribbean, a second one in the North

Atlantic Ocean, and a third one in Brazil-South Atlantic Ocean. Although two samples (haplotype SbGCC1) from Dibulla-La Guajira (Colombian Caribbean) nested with haplotypes from the Atlantic coast of the USA and Canary Islands (Spain), as well the Chiriquí Lagoon sample (SbPC1, Panamanian Caribbean) nested with haplotypes from the Atlantic coast of the USA (Fig. 3), a high population structure exists between the Caribbean and North Atlantic areas (Table 1). These findings may be explained by the oligotrophic condition of Caribbean waters (Aguirre, 2014; Corredor, 1979; Müller-Karger & Aparicio, 1994), which promote high dolphin dispersion toward productive Caribbean areas located mainly in coastal areas (Barragán-Barrera, do Amaral, et al., 2019). Consequently, despite oceanic habits of species such as pantropical spotted dolphins (*Stenella attenuata*) (Gray, 1846) and rough-toothed dolphins, individuals have been registered in coastal areas along the Caribbean Sea (Barragán-Barrera et al., 2015, Barragán-Barrera, do Amaral, et al., 2019; Farías-Curtidor & Ayala, 2015; Farías-Curtidor & Barragán-Barrera, 2017, Farías-Curtidor & Barragán-Barrera, 2019; Kuczaj & Yeater, 2017). For instance, rough-toothed dolphins in Dibulla-La Guajira have been observed in waters between 2 to 6.8 nautical miles from shore (Farías-Curtidor & Ayala, 2015; Farías-Curtidor & Barragán-Barrera, 2017, Farías-Curtidor & Barragán-Barrera, 2019). Additionally, this species has been reported as philopatric in coastal Caribbean waters of Honduras (Kuczaj & Yeater, 2017). These philopatric habits towards coastal areas in Central America, likely because of lack of resources in oceanic waters, may result in the genetic differentiation observed here. However, the high haplotype and nucleotide diversity also suggests a high connectivity among individuals within the Caribbean phylogrup. Therefore, more research focused on distribution, habitat use, local genetic patterns, and satellite tagging is needed to confirm if rough-toothed dolphins exhibit philopatric habits along specific Central American Caribbean areas, or if dolphins migrate between coastal and oceanic areas as has been reported in the Mediterranean Sea (Kerem et al., 2016).

The genetic differentiation observed between rough-toothed dolphins from the Caribbean and North Atlantic Ocean with Brazil-South Atlantic Ocean had been previously described (Donato et al., 2019; Silva et al., 2015), and agrees with other genetic studies with delphinids such as the Atlantic spotted dolphin *Stenella frontalis* (Cuvier, 1829) (do Amaral et al., 2021), bottlenose dolphin (Fruet et al., 2014), clymene dolphin *Stenella clymene* (Gray, 1850) (Nara et al., 2017), and common dolphin (Amaral et al., 2012), which reported a similar isolation pattern. Oceanographical features like the Amazon River mouth and the North Brazilian Current appear to act as a barrier that is segregating fauna from the northern and southern Brazil (Costa et al., 2017). Consequently, and due the isolation pattern for delphinid species in southern Brazil, some populations may be at risk (Fruet et al., 2014). Particularly rough-toothed dolphins from Brazil are threatened due to bycatch (Donato et al., 2019; Monteiro-Neto et al., 2000), which may imply a reduction of this population. Indeed, the significant and negative *D'Tajima* value found for this phylogrup suggests it is in expansion after a historical bottleneck (Weber et al., 2004). The population reduction resulted in unique haplotypes emerging, so the expansion observed here. Therefore, although we don't have information about the causes of historical bottleneck, our findings support the da Silva et al. (2015) recommendation in considering the Brazil-South Atlantic Ocean phylogrup as a distinct management unit due to the restricted gene flow.

Genetic diversity of Panamanian dolphins in the Pacific Ocean

New samples from the Pacific coast of Panama exhibited a genetic connectivity across the Pacific Ocean. These findings are not in agreement with the Albertson et al. (2022) previous study, in which they reported population structure between the Central, Eastern, and Western

Pacific. Particularly in Central Pacific, where oceanic islands are present including Hawaii, Samoa, and Society Islands, rough-toothed dolphins show genetic structure and high philopatry, with restricted movements even between closer islands (Albertson et al., 2017, Albertson et al., 2022; Baird, 2016; Oremus et al., 2012). However, this kind of residency pattern has not been reported in the Pacific basin of Central America. The few rough-toothed dolphin records in this region have been done in oceanic waters (May-Collado et al., 2017). Consequently, it is possible this species reflects oceanic habits, and conducts long migrations along the Pacific Ocean (Learmonth et al., 2007), as is reflected by several shared haplotypes among individuals, as well as by their high haplotype diversity.

Long and multiple migrations patterns across Pacific islands have been reported in green turtle, *Chelonia mydas* (Linnaeus, 1758) (e.g., Dutton et al., 2014; Jensen et al., 2016), which are able to travel distances larger than 2 000 km influenced mainly by sea currents (Read et al., 2014). Additionally, oceanographic events like El Niño Southern Oscillation (ENSO), may change the ocean current direction in relation to the warm (North-Equatorial Current) and cold currents as Humboldt Current (Richmond, 1990). As a result, temporary migration routes have been reported for many species, like fishes (Victor et al., 2004), green turtles (Seminoff et al., 2008), and marine mammals (Learmonth et al., 2007), which allows genetic connectivity along the Pacific Ocean.

One rough-toothed dolphin haplotype from Panama (SbEPP4) was nested with two haplotypes from India and Thailand. Despite this, significant population structure was detected between Indian and Pacific Oceans although F_{ST} and Φ_{ST} values were lower (Table 1). Similarly, pairwise comparisons between the Pacific and Caribbean basins showed lower F_{ST} and Φ_{ST} values (Table 1). These findings may be the result of some shared ancestral haplotypes between these three oceans basins (Fig. 3; SMT1), which may imply potential highly conserved haplotypes or genetic connectivity. Indeed, Albertson et al. (2022) found few shared haplotypes between the Atlantic and Pacific Oceans, as well as did not detect population structure between the Indian and Pacific Ocean based on nuclear marker data. Likely, individuals from Central America may be maintaining genetic connectivity among the three ocean basins, but until no more samples will be obtained, or satellite tagging research is conducted, this assumption can not be tested.

Conservation and research implications for dolphins in Central America

Although this is the first genetic assessment of rough-toothed dolphins in Central America, this study highlights the need for conducting monitoring and sample collection as well as more genetic assessments along the region. Particularly individuals from Central America are very useful to understand whether genetic connectivity between Atlantic and Pacific Oceans exist or are ancestral. Furthermore, the role of Panama Isthmus closure in the divergence of these phylogrups has not been completely understood (Albertson et al., 2022). Despite this, a potential subspeciation has been reported between these two oceans basin (Albertson et al., 2022), and our findings based on new Central America and Caribbean samples support the population structure despite their grouping with Pacific haplotypes.

The high and significant endogamy index value detected here was intriguing since it suggests the species has a low intrinsic genetic diversity due to considerable inbreeding. (Weber et al., 2004). Likely, variable sites within the species are not significant in relation to DNA analyzed here, despite mtDNA-*CR* having a high mutational rate because it is not under natural selection. Consequently, if the rough-toothed dolphin is an endogamic species, its status in the IUCN Red List as “Least Concern” (Kiszka et al., 2019) should be changed to some threatened level. However, our findings must be considered with caution, as we only provide information from a short portion of the maternal lineage. Analyzing bi-parental genes, and specifically microsatellite data, are needed to confirm this endogamy hypothesis. However, previous research with rough-toothed dolphins from French Polynesia using 14 microsatellite loci did not detect endogamy (Oremus et al., 2012). Consequently, samples from Centro America could be useful to assess if endogamy exists for this species.

Understanding the population or sub-speciation status of rough-toothed dolphins will be essential for their conservation, and sample collection in Central America is critical to achieving this goal. The Brazil-South Atlantic Ocean phylogrup is an example of effectiveness of large sampling to assess its conservation status, since the increasing number of samples collected has allowed the genetic isolation detection (da Silva et al., 2015; Donato et al., 2019).

The Central American rough-toothed dolphins, which are distributed in both Atlantic and Pacific Oceans, may be exposed to different threats, but no information about risks has been obtained yet in this region. The closest risk case for this species has been reported in the Colombian Caribbean, where few isolated bycatch cases were registered (Avila et al., 2018; Avila & Giraldo, 2022). However, both Caribbean and Pacific waters along the Central American region, mainly in Costa Rica, present high bycatch risk for oceanic delphinids as the pantropical spotted dolphin (Pino, 2021). Considering this, rough-toothed dolphins may be at risk in Central America, but this information is virtually unknown. We hope this study provides the baseline data needed to assess the rough-toothed dolphin genetic and population status in Central America, which will be essential for its management in the region. Herein, we invite to Marine Mammalogists working in Central America from Belize, Costa Rica, El Salvador, Honduras, Guatemala, and Nicaragua, to create transnational conservation efforts to increase sampling of rough-toothed dolphins in both the Eastern Pacific and Caribbean Sea in order to clarify the intraspecific genetic dynamics of this species. Furthermore, Panama must promote continuous genetic surveillance of its populations.

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.

See supplementary material
a10v71s4-MS1

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SUPPLEMENT • SMALL CETACEANS

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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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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 δ^{13}C and δ^{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), δ^{13}C values ranged between −16.8 and −15.2 ‰ (mean = −16.2; SD = 0.6 ‰), and δ^{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 δ^{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 δ^{13}C y δ^{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 δ^{13}C entre −16.8 y −15.2 ‰ (promedio= −16.2; DE= 0.6 ‰), y los de δ^{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 δ^{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

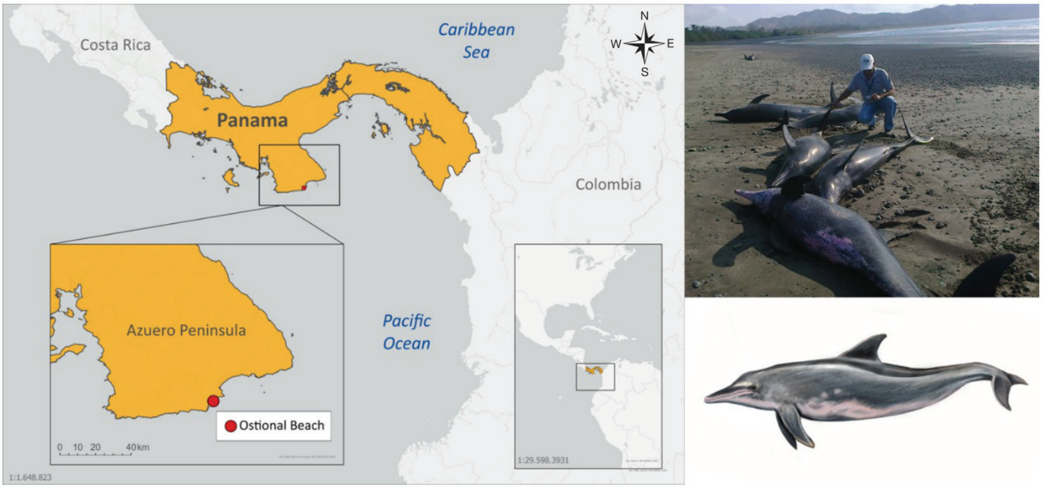

Fig. 1. Location of Ostional Beach in the Azuero Peninsula, Pacific coast of Panama, where the mass stranding of rough-toothed dolphins (*Steno bredanensis*) occurred in April 2016. On right above, a photo of the mass stranding. On right below a species description illustrated by Emmanuel Laverde © www.arteyconservacion.com

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 δ^{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 δ^{13}C composition results, lipids should be removed, since they are depleted in δ^{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 δ^{13}C and atmospheric N_2 for δ^{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 δ^{13}C and 0.09 for δ^{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
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Population size and demographic parameters of pantropical spotted dolphin (*Stenella attenuata graffmani*) (Cetartiodactyla: Delphinidae) in Golfo Dulce, Costa Rica

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ABSTRACT

Introduction: The coastal form of pantropical spotted dolphins (*Stenella attenuata graffmani*) is commonly found along the Pacific coast of Costa Rica. Within Golfo Dulce, a fiord-like-embayment bordering the Osa Peninsula, pantropical spotted dolphins are sympatric with inshore bottlenose dolphins (*Tursiops truncatus*) and these marine predators provide an important source of revenue for local communities through boat-based tours.

Objective: Here we estimated the population size and demographic parameters of the coastal pantropical spotted dolphins in Golfo Dulce.

Methods: The study area was surveyed using non-random boat surveys. Upon encounter, dolphins were individually photo-identified using natural marks in their dorsal fins to estimate population abundance and survival using three emigration scenarios.

Results: A total of 280 dolphins were photoidentified, 65 % of which were observed only once. A total of 30 models were produced, and only two were considered to be parsimonious. Both models explain seasonal apparent survival and its variation due to heterogeneity in capture-recapture probability, one under no emigration (ΔQAICc = 0.00) and the other under random emigration (ΔQAICc = 1.72). We deemed the latter to be a more realistic model as it better reflects our in-situ observations. Under this preferred model the population size of pantropical spotted dolphins in Golfo Dulce varied from 187.30 individuals (CI: 168.67 – 208.02, CV: 0.11) to 367.88 individuals (CI: 341.51 – 396.31, CV: 0.07), with no significant differences in abundance due to seasonality and very high apparent survival (S = 0.98, CI: 0.68 – 0.99, SE: 0.02).

Conclusions: The number of identified dolphins and the proportion of individuals seen only once suggest the fluid movement of the population in and out of the gulf. However, the population size and demographic estimates are characterized by several identified individuals regularly recaptured inside the gulf. This group of dolphins appears to favor the inner basin as a critical foraging habitat. Given the increase of anthropogenic impacts within Golfo Dulce, future management and conservation efforts will require the recognition of an ecologically discrete population unit of coastal pantropical spotted dolphins within the gulf.

Key words: Golfo Dulce; *Stenella attenuatta graffmani*; population size; survival; robust design; Costa Rica; photoidentification.

RESUMEN

Tamaño poblacional y parámetros demográficos del delfín manchado pantropical (*Stenella attenuata graffmani*) (Cetartiodactyla: Delphinidae) en el Golfo Dulce, Costa Rica.

Introducción: La forma costera del delfín manchado pantropical (*Stenella attenuata graffmani*) se encuentra comúnmente a lo largo de la costa Pacífica de Costa Rica. En el Golfo Dulce, una bahía similar a un fiordo en la Península de Osa, los delfines manchados pantropicales son simpátricos con los delfines nariz de botella costeros (*Tursiops truncatus*) y estos depredadores marinos proporcionan una importante fuente de ingresos para las comunidades locales a través de actividades de avistamientos ecoturísticos.

Objetivo: Se estimó el tamaño de la población y los parámetros demográficos de los delfines manchados pantropicales costeros para el Golfo Dulce.

Métodos: El área de estudio se estudió mediante muestreos no aleatorios desde embarcaciones. Tras el encuentro, los delfines fueron foto-identificados individualmente usando marcas naturales en sus aletas dorsales, para estimar la abundancia y supervivencia de la población usando tres escenarios posibles de emigración.

Resultados: Un total de 280 delfines fueron foto-identificados, 65 % de los cuales fueron observados una sola vez. Se elaboraron un total de 30 modelos, y sólo dos se consideraron parsimoniosos. Ambos modelos explican la supervivencia estacional aparente y su variación debido a la heterogeneidad en la probabilidad de captura-recaptura, uno bajo emigración nula (ΔQAICc = 0.00) y el otro bajo emigración aleatoria (ΔQAICc = 1.72). Consideramos que este último es un modelo más realista, ya que refleja mejor nuestras observaciones in situ. Bajo este modelo seleccionado, el tamaño de la población de delfines manchados pantropicales en el Golfo Dulce varió de 187.30 individuos (CI: 168.67 – 208.02, CV: 0.11) a 367.88 individuos (CI: 341.51 – 396.31, CV: 0.07), sin diferencias significativas en la abundancia debido a la estacionalidad y una supervivencia aparente muy alta (S = 0.98, CI: 0.68 – 0.99, SE: 0.02).

Conclusiones: El número de delfines identificados y la proporción de individuos vistos una sola vez sugieren un movimiento fluido de la población dentro y fuera del golfo. Sin embargo, el tamaño de la población y las estimaciones demográficas se caracterizan por varios individuos identificados y re-capturados regularmente dentro del golfo. Este grupo de delfines parece favorecer la cuenca interior como hábitat crítico de alimentación. Dado el incremento de los impactos antropogénicos dentro del Golfo Dulce, los futuros esfuerzos de manejo y conservación requerirán el reconocimiento de una unidad poblacional ecológicamente discreta del delfín manchado pantropical costero dentro del golfo.

Palabras clave: Golfo Dulce; *Stenella attenuatta graffmani*; tamaño poblacional; sobrevivencia; diseño robusto; Costa Rica; fotoidentificación.

INTRODUCTION

The pantropical spotted dolphin, *Stenella attenuata* (Gray, 1846) is distributed throughout the Eastern Tropical Pacific (ETP) where two genetically and morphologically distinct populations or ecotypes are recognized, oceanic and coastal (Perrin 1975, Perrin 2001, Perrin 2018). The oceanic ecotype lives in pelagic waters where, along with spinner dolphins, *Stenella longirostris*(Gray, 1828), it tends to associate with yellowfin tuna, resulting in high mortality due to the tuna purse seine fishery (Ballance et al., 2021; Cramer et al., 2008; Scott et al., 2012). The coastal ecotype is distributed at < 200 km from the coast of Central America where it is exposed to various human activities including commercial whale watching (Montero-Cordero & Lobo 2010; Pacheco-Polanco 2016); water quality and coastal habitat modification (Pacheco-Polanco 2016); and marina development projects near critical coastal cetacean habitats (Herra-Miranda et al., 2016).

Of the two ecotypes, the oceanic ecotype's population and demographic status are best known. Since the late 1970s, the U.S. government agency National Oceanographic Atmospheric Administration (NOAA) has generated management recommendations based on estimates of abundance, population size, survival, migration, and recruitment of oceanic pantropical spotted dolphins in the ETP (e.g., Gerrodette & Forcada 2005; Gerrodette et al., 2008; Wade et al., 2007). These efforts are the responsibility of NOAA, which is one of three U.S. federal agencies responsible for implementing the mandate of the Marine Mammal Protection Act. This mandate establishes that marine mammal populations that are endangered by the impact of human activities must be recovered, and to this end, periodic quantitative assessments of their population dynamics must be made (Marine Mammal Protection Act, 1972). However, such efforts have not been made for the coastal ecotype. Presently, there are few estimates of the population size of coastal spotted dolphins at the Central American region level (Gerrodette & Palacios, 1996; Palacios & Gerrodette 1996) or even by country, and they do not include demographic characterizations of the populations studied.

The coastal pantropical spotted dolphin (*S. attenuata graffmani*) is probably the most representative cetacean of the Pacific coast of Costa Rica. Several aspects regarding its ecology have been documented since the late 1990s, in particular, its presence and distribution (Acevedo & Buckhart, 1998; Cubero-Pardo, 1998; Cubero-Pardo, 2007a; Holst et al., 2017; Martinez-Fernandez et al., 2011, Martinez-Fernandez et al., 2014; May-Collado et al., 2005;; Oviedo, 2007, Oviedo, 2008; Oviedo et al., 2009, Oviedo et al, 2015), fine-scale relative abundance estimation (May Collado & Forcada, 2012), genetic identity (Escorza-Treviño et al., 2005; Leslie et al., 2019; Leslie & Morin, 2018), behavior (Cubero-Pardo, 2007b; May-Collado & Morales, 2005; Oviedo, 2007, Oviedo, 2008; Oviedo et al., 2018), interaction with fisheries (Palacios-Alfaro, 2006) and tourism activities (Montero-Cordero & Lobo, 2010). However, the size of the population in the Pacific of Costa Rican and their demography have not yet been assessed.

This study focuses on estimating the population size, and some demographic parameters (apparent survival, capture-recapture probability, and emigration) for the coastal pantropical spotted dolphins in Golfo Dulce. This gulf is a coastal marine habitat with tropical fiord characteristics, where the species has semi-pelagic habits and is sympatric with the inshore ecotype of the bottlenose dolphin (*Tursiops truncatus*). In Golfo Dulce, the spotted dolphins show habitat partitioning, facilitating the coexistence of both marine predators (Oviedo, 2018; Oviedo et al., 2018). Previous demographic assessments have shown that dolphins of coastal habits show seasonal variability in abundance, affecting demographic characteristics and possibly reproductive strategies in males and females (Bolaños-Jiménez et al., 2022). For these reasons, this study will consider the possible effects of dry (November-May, as reported in Oviedo et al., 2018) and rainy (June-October, as reported in Oviedo et al., 2018) seasons on the demography and population size of this delphinid in Golfo Dulce. There remains a need for a regional quantitative assessment of population dynamics and demographic information for this dolphin ecotype.

MATERIALS AND METHODS

Study Area: Golfo Dulce is a stratified estuary of tectonic origin, located in the South Pacific region of Costa Rica, centered at 8°33’N and 83°14’W (Svendsen et al., 2006). It has a 215 m deep internal basin with a 60 m sill that restricts ocean circulation (Morales-Ramírez et al., 2015; Svendsen et al., 2006); a length of 50 km; a width of 10-15 km; and a total area of approximately 750 km^2 (Von Wangelin & Wolff, 1996; Wolff et al.,1996). The climate is tropical and humid with a rainy season from June to early November, generating an average monthly rainfall of 100–700 mm. The main freshwater supply comes from the Coto Colorado, Tigre, Esquinas, and Rincón rivers, forming estuaries and mangrove zones in their areas of influence. They also affect the gulf’s circulation pattern, resulting in a stratified current structure (Spongberg & Davis, 1998). Due to its physiographic and hydrological characteristics, Gulf Dulce can be divided into three sub-areas: 1) a deep inner basin with a maximum depth of about 215 m, an anoxic layer below 100 meters (Brenes & León, 1988) and restricted surface circulation; 2) a flat outer basin with an average depth of 70 m (Hebbeln et al., 1996), which begins 20 km from the mouth of the Gulf; and 3) a third area corresponding to the transitional-oceanic zone at the mouth of the Gulf (Oviedo et al., 2009, Oviedo et al., 2015), where this gulf communicates with the Pacific Ocean. In this external oceanic portion, depths close to 1 000 m are reached at a relative distance of 6 km (Fig. 1).

Field data collection: Pantropical spotted dolphin surveys were conducted from June 2011 to April 2014 in the three sub-areas described above. The surveys were conducted from a 7 m long boat with a 115 HP four-stroke outboard motor, zig-zagging from the point of origin (Bahía Rincón or Puerto Jiménez) to spatially cover as much of each sub-area as possible. The surveys were conducted between 7:00 am and 4:00 pm. During each survey, four observers were on board, one of which served as the main photographer, generally supported by another secondary photographer, they photographed as many dolphins as possible in each observed group using digital DSLR cameras (Canon 7D/70D) equipped with 400 mm telephoto lenses.

The definition of a group used in this study is that of Karczmarski et al. (2005): a spatial aggregation of animals engaged in similar (often the same) activities and interacting with each other on short enough time scales that there is little (or no) change in group membership. During each sighting, the boat approached within approximately 100 m of the group, and then the geographic position of the boat was recorded, as was its position relative to the group, the time of the encounter, group size, and composition, and the behavior records (initial and 10-minute) for the encounter. Specifically, group size was recorded, while group composition was classified according to the presence of adults, juveniles, and calves.

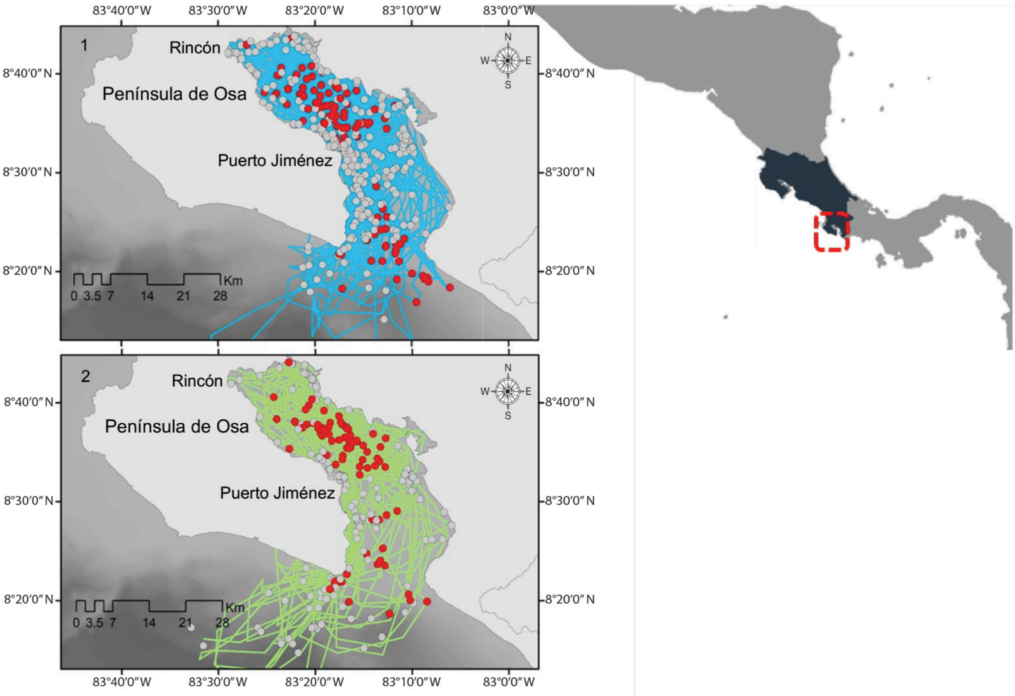

Fig. 1. The study area in Golfo Dulce: 1) Photoidentification sampling during the rainy seasons of 2011, 2012, and 2013. Blue lines correspond to the routes traveled during data collection. 2) Photoidentification sampling during the dry seasons of 2011–2012, 2012–2013, and 2013–2014. Green lines correspond to the routes traveled during data collection. Red circles: encounters and photographic sampling locations with the pantropical spotted dolphin (*S. a graffmani)*. Gray circles: encounters with other cetaceans.

The identification of the different age classes was based on Perrin (1975), Perrin (2001), and Perrin (2018), where the increase of spots on the body determines the maturity of the individuals. Accordingly, juveniles and calves with a length of 1/3 of the length of the mother have a coloration pattern composed of two shades of gray and a few ventral spots. In contrast, adults are more pigmented with white spots that merge dorsally.

The stable association of an adult with a small calf or medium size juvenile, though not always spotted in the infant position (Mann & Smut, 1998, Mann & Smut, 1999), was used as a cue to assign the female gender. On several occasions clear photographs of the genital slits supported sex identification. Highly acrobatic displays or bouts of social behavior such as copulation facilitated photographic evidence to differentiate male and female individuals. Behavior was described as feeding, resting, socializing, traveling, and milling, following the definitions of Marfurt et al., (2022) and Machado et al., (2019). Once the initial data collection was completed, the group-follow protocol for photoidentification was initiated.

Photoidentification: The traditional method of individual identification in dolphins uses photographs of the dorsal fin, which allows the modeling of presence/absence data by mark-recapture analysis (Bolaños-Jimenez et al. 2022; Brooks et al., 2017; Hupman et al., 2018; Parra et al., 2006; Wilson et al., 1999; Wursig & Jefferson, 1990; Zanardo et al. 2016). In one photography session, an individual may end up being photographed several times, depending on group size and behavior. After the photographs were collected, they were categorized based on their quality and distinctiveness and then used to build an identification catalog with images of each individual dolphin in Golfo Dulce. The selection process ensured that each sampling occasion was backed by a type-photo of every individual, if available. The type-photo was then associated with the presence record on a specific date and location. Photographic quality was scaled as 1-100, with a minimum use criterion of ≥ 70. A quality of more than 70 meant that the fin occupied no less than a quarter of the photo, was focused and defined, and was as perpendicular as possible to avoid distortion by angles (parallax) (Karczmarski et al., 2005). Distinctiveness refers to the presence of individual marks. Each high-quality photograph of the dorsal fin was assigned a distinctiveness (D) value between 0-4. Zero is when a dorsal fin is smooth, with no apparent markings, and four is a dorsal fin that is conspicuously distinctive, e.g., a malformed or partially mutilated fin. Individual dolphin identification in this study was based on the presence and distribution of marks (nicks and notches) on the dorsal fin profile. Other natural markings such as discolorations, scars, and spotting patterns were not ruled out but played a secondary role. Only dorsal fin photographs with quality ≥ 70 and distinctiveness from 1 to 4 were considered in this analysis. The construction of the catalog was carried out in the Discovery program (Galey & Karczmarski, 2012), which allows the processing and selection of the photographs based on the criteria described above. It also associated the sighting data with the identified dolphin and facilitated the construction of a matrix with the capture history of each individual (the basic input for the mark-recapture analysis).

Analysis of population size and demography: The capture-recapture matrices were used to estimate the population size and characterize the demography of pantropical spotted dolphins in Golfo Dulce, using a “robust design” model (Pollock et al., 1990). This model involves primary and secondary levels of sampling. The robust design integrated a series of short-time closed models at the secondary sampling level, where the effect of sampling time and individual heterogeneity on the probability of capture was controlled. The demographic closure models were connected by a frame model at the primary sampling level, which released the demographic closure, assuming a population where births, deaths, as well as the entry of immigrants and the exit of emigrants were considered temporally. This allowed us to infer dynamics associated with metapopulations (migratory movements in the absence of physical geographic barriers), following Kendall & Bjorkland (2001). For the secondary level, the sampling occasions were unified by month within the seasons, while the primary occasions were focused explicitly on the seasons (Rainy: June-October, Dry: November-May).

The above demographic aspects were met, pursuant with the validation of several basic assumptions of the mark-recapture analysis, under the robust design approach: (a) marks are not lost; although they can be modified, they are sufficiently distinctive to guarantee individual identification; b) samples are instantaneous at secondary levels; c) given a sampling occasion, all individuals in the population have the same probability of capture; d) survival of marked individuals does not vary from one capture occasion to the next at primary levels (seasons) of demographic openness; e) capture occasions at secondary levels (months) are with demographic closure within each primary level: and f) captures are independent among individuals without aggregation and overdispersion effect.

All models considered were analyzed using the Mark software interface in the “R” environment (R-Mark Laake et al., 2013). The following demographic parameters of pantropical spotted dolphins in Golfo Dulce were estimated:

(Sj) apparent survival (does not refer to biological survival, but demographic survival by presence) at the first season j for j>1.
(γ”) probability of emigrating before season j, being present in j-1, for j>1
(γ’) probability of emigrating before season j, being absent in j-1, for j>2
(pij) probability of the first capture in sample i of season j for i ≥ 1
(cij) probability of recapture in sample i of season j for i > 1
(Nj) the population size of marked individuals.

For this study, we set pij = cij, since photoidentification, being non-invasive and not promoting adverse reaction, did not directly affect the probability that a previously identified dolphin is recaptured (Parra et al., 2006). Three classes of temporal emigration models were evaluated: 1) Markovian emigration (γ’ γ”), where the probability of a dolphin being present at Golfo Dulce would be conditional on presence or absence on the previous sampling occasion (Kendall & Nichols, 2002; Kendall et al., 1997); 2) random emigration (γ’= γ”), where the probability of a dolphin being present in the gulf is independent of presence or absence on the previous sampling occasion; and 3) no emigration (γ’ = γ” = 0), where there is no movement out of the gulf.

Before the construction and evaluation of models by robust design, the fitting of the capture history matrix was corroborated, using a goodness-of-fit test in the Release GOF program. The lack of fit to temporality led to the estimation of a variance inflation factor (ĉ), which levelled the dispersion of the data. Thirty models were constructed and selected using the Akaike selection criterion (AICc). Considering the dispersion implied by the variance inflation factor (ĉ) the QAICc was used under the criterion that models with a difference expressed by Delta-QAICc greater than 10 lacked support (Burham & Anderson, 2004), and those less than two should not be discarded. To obtain the total population size, the number of marked individuals ($\hat{N}$) was related to the proportion of marked individuals in the sample ($\hat{\theta}$) (Wilson et al., 1999):

$$\widehat{N}_{\text{total}} = \widehat{N} / \widehat{\theta}$$

The variance was estimated as follows:

$$\text{Var}\,(\widehat{N}_{\text{total}}) = \widehat{N}^2_{\text{total}}\,[\ \text{Var}(\widehat{N})\ /\widehat{N}^2 + 1-\widehat{\theta}) / \,n\widehat{\theta}^2\ ]$$

Where n is the total number of individual dorsal fins for which was estimated. The coefficient of variation for the total population CV ($\hat{N}_{\text{total}}$) could be defined as the sum of the coefficients of variation of $\hat{N}$ and $\hat{\theta}$:

$$\text{CV}\,(\widehat{N}_{\text{total}}) = \sqrt{((\text{CV}(\widehat{N}))^2 + (\text{CV}(\widehat{\theta}))^2}$$

The standard confidence interval could unrealistically set the lower bound to zero.

Therefore, we followed Burnham et al., (1987) using a log-normalized confidence interval, such that the lower limit is given by $\hat{N}_L = \hat{N}/r$, and the upper limit by $\hat{N}_U = \hat{N}\,r$.

For a 95% confidence interval, r would be given by:

$$r = \exp\{1.96 \sqrt{\ln(1+(CV(\hat{N}_{total}))^2 + (CV(\hat{\theta}))^2\ )}$$

Where $(1+(CV(\hat{N}_{total}))^2$ is an approximation of $var(\ln \hat{N}_{total})$.

The variation of the total population size as a function of the seasons was explored using the non-parametric test Kruskal Wallis at a significance level (alpha) of 0.05.

RESULTS

There were 201 surveys for photographic capture of pantropical spotted dolphins in Golfo Dulce from 2011 to 2014, equating to 1 102 hours over 25 months and a travel effort of 18 730 km (Fig. 1). Details are shown in Table 1.

A total of 26 352 photographs were taken and analyzed, resulting in the identification of 280 individuals, 65 % of which were recorded only once. The rest of the dolphins cataloged showed an important level of recapture (98 individuals). The discovery curve, or the cumulative curve of entries into the catalog by sampling occasion, shows three trends (Fig. 2) that could explain the dynamics above: a non-asymptotic curve that incorporates all identifications, including those with individuals only captured once, and distinctiveness equal to one. Another curve becomes asymptotic only with distinctiveness greater than one, and finally, a nearly constant curve of individuals cataloged with very distinctive fins (D > 3).

For pantropical spotted dolphins the goodness-of-fit test showed significantly high dispersion ($\chi^2 = 22.81$, df = 8, p = 0.001). This is mainly due to the significant effects of the probability that capture-recapture is affected by individual heterogeneity, which is duly addressed by the robust design models. Due to the degree of dispersion, a variance inflation factor was estimated ($\hat{c} = 22.81/8 = 2.85$). Thirty models were generated from the capture history of individuals cataloged under the criteria of quality > 70 and distinctiveness ≥ 1.

The best-fitted models were those of apparent survival by sex group and the variation in the probability of capture due to heterogeneity during both seasons; among these, the best fit was that of no emigration (ΔQAICc = 0.00), followed by the random migration model (ΔQAICc = 1.72). Based on these two scenarios the population size differed in coefficient of variation (Table 2). For the no-emigration models, the coefficient of variation remained between 7 – 10 % (Table 3), and for the random-emigration models, the coefficient of variation was between 7 – 12 % (Table 4). If the spotted dolphin population in Golfo Dulce is considered under a pattern of no movement out of the study area, the population size of the

Table 1

Details of the search effort and the identification photographs included in the pantropical spotted dolphin catalog, including level of distinctiveness: D= 1, D≥ 2, D> 3.

Season	No. Months	No. Surveys	No. Hours	No. Cataloged IDs (New)		
				D = 1	D ≥ 2	D ≥ 3
Rainy 2011	3	28	143	17	22	24
Dry 2011_2012	5	18	94	74 (57)	59 (37)	25 (1)
Rainy 2012	4	44	263	89 (15)	65 (6)	25 (0)
Dry 2012_2013	4	29	155	111 (22)	71 (6)	25 (0)
Rainy 2013	5	56	291	134 (23)	72 (1)	25 (0)
Dry 2013_2014	4	26	156	182 (48)	73 (1)	25 (0)

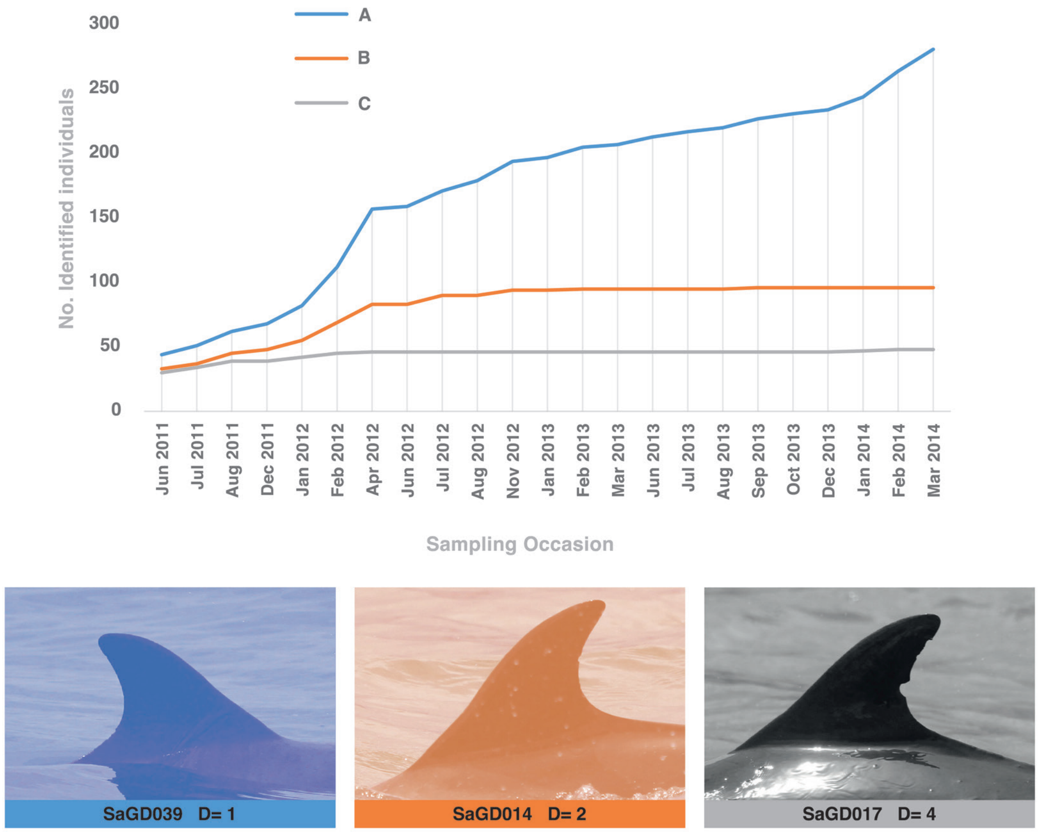

Fig. 2. Discovery curves for the coastal pantropical spotted dolphin in Golfo Dulce: A) The blue curve represents all cataloged individuals, including those recorded only once. B) The orange curve contains only the individuals with dorsal fins with D>1. C) The gray curve contains the individuals with dorsal fins classified under a distinctiveness level D>3. The bottom panel shows representative photographs of distinctiveness levels, from left: one (blue), two (orange), and 4 (gray).

Table 2

Selected best-fitted models generated from the capture history of pantropical spotted dolphins in Golfo Dulce under the Robust Design. S= apparent survival, P= capture probability, Het= heterogeneity, S♀ (CI) = apparent survival (confidence interval) for females, S♂ (CI)= apparent survival (confidence interval) for males, p-hat = capture probability, ψ ’ = ψ ” emigration probability. The notation ‘(.)’ implies that a given parameter was kept constant.

Models	Classification Criteria				Demographic Parameters			
	No. Parameters	QAICc	ΔQAICc	QAICc Weighted	S♀ (CI)	S♂(CI)	p-hat	ψ’=ψ”
S (. Sex) P(Het) No-Emigration	11	266.94	0.00	0.64	0.98 (CI: 0.81-0.99)	≈ 1 (CI: 0.99-1)	0.10	0.00
S (. Sex) P(Het) Random Emigration	12	268.67	1.72	0.27	0.98 (CI: 0.68-0.99)	≈ 1 (CI: 0.99-1)	0.12	0.16

Table 3

Population size of the pantropical spotted dolphin in Golfo Dulce under the no-emigration robust design model. ⊖ = proportion of individuals tagged per season, N-Marked= number of marked individuals, N-Total= Total population size, CI = Confidence Interval, CV Coefficient of Variation, ♀ = dolphins identified as females, ♂ = dolphins identified as male.

Seasons	Cluster	⊖	N-Marked	N-Total	CI	CV
Rainy 2011	♀		80.51	203.82	189.95-218.71	0.07
	♂	0.79	58.51	148.13	136.30-160.99	0.08
	Total			**351.95**	**326.24-379.70**	**0.07**
Dry 2011-2012	♀		81.38	235.88	220.95-251.82	0.07
	♂	0.69	64.38	181.61	173.35-200.88	0.07
	Total			**422.49**	**394.30-452.71**	**0.07**
Rainy 2012	♀		52.21	132.18	121.07-144.30	0.09
	♂	0.79	38.21	96.73	87.22-107.28	0.10
	Total			**228.91**	**208.29-251.59**	**0.09**
Dry 2012-2013	♀		47.48	137.62	126.30-149.96	0.09
	♂	0.71	55.16	99.94	90.30-110.62	0.10
	Total			**237.57**	**415.42-461.15**	**0.09**
Rainy 2013	♀		55.16	139.65	128.26-152.04	0.09
	♂	0.80	42.16	106.73	96.80-117.69	0.10
	Total			**246.38**	**225.06-269.73**	**0.09**
Dry 2013-2014	♀		42.42	122.96	112.27-134.66	0.09
	♂	0.68	32.42	93.97	**84.63-104.34**	**0.10**
	Total			**216.93**	196.90-239	0.09

species varies between 217 to 422 dolphins. For the random emigration model, the population size ranges from 187 to 368 individuals. However, these differences are not significant (Kruskal Wallis: $\chi^2 = 2.08$, df = 1, p = 0.150), and similarly, there is no statistical difference in the population size of pantropical spotted dolphins in Golfo Dulce between seasons (Kruskal Wallis: $\chi^2 = 0.05$, df = 1, p =0.827).

DISCUSSION

The demographic analysis of coastal pantropical spotted dolphins in Golfo Dulce through capture-recapture modeling resulted in the selection of two models, one where emigration was null and another one that suggested fluid dynamics of entries and exits to the population of pantropical spotted dolphins in Golfo Dulce, independent of the season evaluated. No matter the model, we found the population size of spotted dolphins in this gulf does not exceed 400 individuals. These results indicated that at least a portion of the population, equating to more than 90 individuals, remains for extended periods as demonstrated by a high level of recaptures and suggesting site fidelity throughout the year, particularly in the inner basin (Acevedo & Buckhart, 1998; Cubero-Pardo, 1998; Cubero-Pardo, 2007a; Oviedo, 2007; Oviedo et al., 2015, Oviedo et al., 2018). The study further indicates that the conditions associated with seasonality do not affect the population size and demographic patterns of spotted dolphins in Golfo Dulce. The discovery curves (Fig. 2) suggest that dolphins with low dorsal fin distinctiveness may not be readily recaptured. Hupman et al. (2018) found low

Table 4

Population size of the pantropical spotted dolphin in Golfo Dulce, under the robust design random emigration model. ⊖ = proportion of individuals tagged per season, N-Marked= number of marked individuals, N-Total= Total population size, CI = Confidence Interval, CV Coefficient of Variation, ♀ = dolphins identified as females, ♂ = dolphins identified as male.

Season	Cluster	⊖	N-Marked	N-Total	CI	CV
Rain 2011	♀		70.67	178.91	165.88-192.97	0.08
	♂	0.79	48.67	123.22	112.35-135.14	0.09
	Total			**302.13**	**278.23-328.10**	**0.08**
Dry 2011-2012	♀		71.96	208.58	194.51-223.67	0.07
	♂	0.69	54.96	159.30	147.00-172.64	0.08
	Total			**367.88**	**341.51-396.31**	**0.07**
Rain 2012	♀		45.8	115.95	105.43-127.40	0.09
	♂	0.79	31.8	80.51	71.78-90.29	0.12
	Total			**196.46**	**177.31-217.69**	**0.11**
Dry 2012-2013	♀		41.89	121.42	110.77-133.09	0.09
	♂	0.71	28.89	83.74	74.87-93.66	0.11
	Total			**205.16**	**185.64-226.75**	**0.10**
Rain 2013	♀		48.85	123.67	112.95-135.41	0.09
	♂	0.80	35.86	90.76	81.57-100.98	0.10
	Total			**214.43**	**194.52-236.40**	**0.09**
Dry 2013-2014	♀		37.31	108.14	98.11-119.20	0.10
	♂	0.68	27.31	79.16	70.55-88.81	0.12
	Total			**187.30**	**168.67-208.02**	**0.11**

dorsal fin distinctiveness in oceanic dolphins, such as *Delphinus* sp., to be associated with a low level of recaptures of identified individuals.

For the study population, apparent survival derived from the best-fitted models was relatively high. It only decreased slightly for females. When considering the no-immigration model, the survival of female pantropical spotted dolphins in Golfo Dulce would be above reported biological survival levels (Brooks et al., 2017; Taylor et al., 2007) and is supported by the low relative width of the confidence interval. However, it should be considered that the model based on non-immigration only describes a partial reality, focusing only on those individuals that develop long-term site fidelity. The model that considered random-type migration seemed closer to what was recorded in the capture histories of photo-identified individuals. Under this approach, the survival of females did not change in magnitude, but it did change in precision, as the width of the confidence interval was larger. Additionally, under this model, a comparatively low probability of emigration was established, which would support the dynamics of visiting individuals and resident individuals sharing the same seascape, as has been documented in coastal dolphin populations in Australia (Brooks et al., 2017), though with no apparent effect of seasonality.

The physiography of Golfo Dulce as a basin, or semi-enclosed internal sea, supported expectations of some demographic closure in the spotted dolphin population. Indeed, even during in situ sampling, several individuals evidenced a notable degree of recaptures, although there was no physical barrier to effectively prevent their departure from Golfo Dulce. In addition to the differences in individual behavior affecting the probability of capture (heterogeneity), there is nevertheless the possibility of fluid transience, which rules out the non-immigration that framed the best-fitted model (probability of capture as a function of individual heterogeneity, constant survival, and no emigration). Consequently, the model where the probability of capture was fitted as a function of individual heterogeneity with constant survival, under random emigration was considered the most parsimonious (Delta QAICc ≤ 2).

The seasonal survival estimate defined the true survival of pantropical spotted dolphins in Golfo Dulce, as well as the respective trend of random emigration, based on the high probability that the survival of an adult remains constant over relatively short periods when compared with its life span (≈ 45 years is the oldest age of a reproductive female) (Taylor et al., 2007). Notwithstanding a catastrophic event affecting pantropical spotted dolphin survival over the study period, emigration patterns are expected to be associated with individual heterogeneity and apparent survival estimates. The seasonal survival estimate is relatively high (0.99 +/- 0.01), but similar to that expected for other adult and sub-adult (non-calf) dolphins. For example, spinner dolphins (*S. longirostris*) from Hawaii have a survival of 0.97 +/- 0.05 (Tyne et al., 2014), and Mediterranean Sea common and striped dolphins, *Delphinus delphis, Linneaeus, 1758-Stenella coeruleoalba (Meyen, 1833)* of 0.94 +/- 0.05 (Santostasi et al., 2016).

Pantropical spotted dolphin population size apparently fluctuated between seasons, but this pattern was not significant. The population size of pantropical spotted dolphins in Golfo Dulce is a relatively discrete number considering that groups larger than 1 000 individuals have been documented outside the gulf (Authors' unpublished data 2005-2022). Fluctuations in population size are expected to be primarily influenced by the availability of resources in Golfo Dulce, especially given the presence of another delphinid, the inshore bottlenose dolphin (*T. truncatus*), which, as another marine predator, exerts additional pressure on available prey resources (Cubero Pardo, 2007a; Oviedo, 2007; Oviedo et al., 2018).

Based on the above, potential prey availability in Golfo Dulce would be the primary determinant for the aggregation pattern, site fidelity, and movement of this species in the study area, as proposed by Gowans et al. (2007) for several dolphin species with an intermediate home range pattern. Those authors argue that variations in home range patterns reflect a gradient of resource availability from predictable to extremely variable. Golfo Dulce may be a relatively predictable resource locality for pantropical spotted dolphins in terms of prey availability, and considering the availability of females for breeding, and refuge from predation. Relatedly, a study by Marin-Alpizar (2011) found small pelagic fish of the family Hemiramphidae to be present in this gulf year-round. If this fish is an important source in the diet of spotted dolphins, it may support not only the resident dolphin population but also be available to transient dolphins. To expand our understanding of the habitat-use patterns of these dolphins, future studies should incorporate spatial and temporal variations of their prey into their models.

It is important to recognize that individual differences in behavior, as well as the possibility of each dolphin leaving the Golfo Dulce, influenced variation in survival and probability of capture. To minimize the associated bias during field sampling, efforts were made to provide an equal distribution of individual photographic capture probability within each spotted dolphin encounter. Still, the grouping pattern of the dolphins in this gulf, especially in group sizes of ≥ 100 individuals, may have affected photographic coverage. Additionally, the sample size could also have been affected by our strict selection and analysis protocol. Given the increased human impacts in the study area (Herra-Miranda et al., 2016; Pacheco et al., 2016), effective conservation and management of pantropical spotted dolphins and other coastal/inshore cetaceans within Golfo Dulce require the recognition of discrete populations as management units. Therefore, to facilitate the detection of population-level changes over time, it is important to effectively determine the demographic parameters, as estimated here.

Ethical statement: the authors declare that we 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 contributions: LOC conceptualized the study. LOC, DHM, and JDPP designed boat surveys, carried out field data collection and data analysis, coordinated data management, and wrote this manuscript.

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Killer whales, *Orcinus orca* (Artiodactyla: Delphinidae) in Guatemala: sighting records, photographic match, and interaction with Bryde's whales, *Balaenoptera edeni* (Artiodactyla: Balaenoptera)

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ABSTRACT

Introduction: Killer whales (*Orcinus orca*) have a global distribution, but many low-latitude populations such as the Eastern Tropical Pacific are not well studied.

Objective: Provide a review of killer whale sightings in the Exclusive Economic Zone of the Pacific of Guatemala, for which there has previously been little information.

Methods: We reviewed national and regional publications, as well as anecdotal records of killer whale sightings in Guatemala.

Results: We document five sightings spanning three decades (1990-2020). Four sightings were recorded between 2010 and 2020 and occurred within the San José Canyon or over the continental shelf. Group size varied from 1-15 individuals ($\bar{x}$: 6 ± 4.3). We report a photographic match of three killer whale individuals from a single pod previously sighted in Cabo Corrientes, México, 11 years and 1778 km apart. We also describe an interaction between a killer whale pod and two adult Bryde's whales (*Balaenoptera edeni*), in which the Bryde's whales followed a female killer whale and its calf.

Conclusions: This study represents a contribution to the knowledge of killer whales in the region. It highlights the importance of data collection from both scientific and anecdotal records and supports the need of establishing national and regional monitoring and conservation programs for cetaceans.

Key words: Central America; cetaceans; Eastern Tropical Pacific; orca; photo-ID; resighting.

RESUMEN

Orcas, *Orcinus orca* (Artiodactyla: Delphinidae) en Guatemala: registros de avistamientos, fotoidentificación e interacciones con ballenas de Bryde, *Balaenoptera edeni* (Artiodactyla: Balaenoptera)

Introducción: Las orcas (*Orcinus orca*) tienen una distribución global, pero muchas poblaciones de bajas latitudes, como el Pacífico Tropical Oriental, no han sido bien estudiadas.

Objetivo: Proporcionar una revisión de los avistamientos de orcas en la Zona Económica Exclusiva del Pacífico de Guatemala.

Métodos: Revisamos publicaciones nacionales y regionales, así como registros anecdóticos de avistamientos de orcas en Guatemala.
Resultados: Documentamos cinco avistamientos que abarcan tres décadas (1990-2020). Registramos cuatro avistamientos entre 2010 y 2020 que ocurrieron en el Cañón de San José o sobre la plataforma continental. El tamaño de grupo varió de 1 a 15 individuos ($\bar{x}$: 6 ± 4.3). Reportamos una coincidencia fotográfica de tres individuos de orcas de un solo grupo previamente avistado en Cabo Corrientes, México, con 11 años y ~1 778 km de diferencia. También describimos una interacción entre un grupo de orcas y dos ballenas de Bryde adultas (*Balaenoptera edeni*), en la que las ballenas de Bryde siguieron a una orca hembra y su cría.
Conclusión: Este estudio contribuye al conocimiento de las orcas en la región. Destaca la importancia de recopilar datos tanto de registros científicos como anecdóticos y apoya la necesidad de establecer programas nacionales y regionales de monitoreo y conservación de cetáceos.

Palabras clave: Centro América; cetáceos; foto-ID; orca; Pacífico Tropical Oriental; recaptura.

INTRODUCTION

The killer whale, *Orcinus orca* (Linnaeus, 1758) is a widely distributed cetacean inhabiting all oceans (Forney & Wade, 2006). It is most frequently found in coastal temperate and polar waters of high productivity (Forney & Wade, 2006), where some populations have been well documented (e.g., Best et al., 2010; Dahlheim et al., 2008; Guinet et al., 2015; Jourdain et al., 2019; Pitman & Ensor, 2003). Despite recent advances in killer whale research in the Eastern Tropical Pacific (ETP; e.g., Denkinger et al., 2020; Guerrero-Ruiz et al., 2005; Pacheco et al., 2019; Vargas-Bravo et al., 2021), the distribution, ecology and behavior of killer whales in the region remains poorly understood. Records of killer whales are sporadic in Guatemala (Cabrera et al., 2012, Cabrera et al., 2014; Hill et al., 1991), and reports of sightings have mainly been anecdotal. To better understand killer whales in the region and establish a baseline of research, we review five killer whale sightings in Guatemala from 1990 till 2020, including three previously unreported. We also document photographic matches of a killer whale pod sighted 11 years and 1 778 km apart. Finally, we describe a pair of Bryde's whales, *Balaenoptera edeni* (Anderson, 1878) following a killer whale pod.

Killer whale sighting records from Guatemala were obtained by reviewing national and regional publications and anecdotal records from third parties. We also provide additional details of a sighting previously reported by the authors (Cabrera et al., 2012). The original observers from anecdotal records were contacted to obtain detailed information of the sighting, including date, coordinates or approximate location, estimated number of individuals, as well as photographic and/or video evidence.

Using photographs and videos, we identified age class and sex according to the following categories: adult male, individual showing relatively large erect dorsal fin; adult female, only differentiated if the individual was accompanied by a calf; calves, small size and marked association with another adult individual; juveniles, they can be females without associated calf, or young males whose dorsal fin is not fully developed (Vargas-Bravo et al., 2021).

To gain insights into possible movements of killer whale pods and individuals, good quality photographs were compared with the following regional photo-identification catalogs of killer whales: Black et al., (1997); Ellis et al., (2008); Olson & Gerrodette (2008). Experts in the area were also contacted to compare the photographs with unpublished catalogs from the Galapagos Islands (J. Denkinger & Alarcon, n.d.) and from the Mexican central Pacific (C. Ortega-Ortiz, personal communication, October 27, 2020).

Killer whale sighting records: We documented five killer whale sightings off Guatemala between the years 1990 and 2020 (Fig. 1A). Two sightings were recorded during separate research surveys, two during whale watching activities, and one during a sport fishing trip. Four of the sightings were recorded during 2010 and 2020 and occurred within the San José Canyon or over the continental shelf off the coast of Escuintla and Santa Rosa. Four sightings occurred during the Guatemalan dry season equivalent to the Northern Hemisphere winter season (November to February). Below we describe the five killer whale sightings:

#1 On September 1, 1990, a pod of five individuals was recorded 278 km from the coastline between Escuintla and Santa Rosa (10°56' N 92°28' W) during a cetacean research survey of the ETP conducted by the United States National Oceanic and Atmospheric Administration (Hill et al., 1991).

#2 On February 14, 2010, a solitary adult male was observed traveling ~10 km from Monterrico coastline, Santa Rosa (13°47'12.1056"N 90°33'9.7812"W). The sighting occurred during a whale watching trip by the company Extremo a Extremo (P. Cabrera, personal communication, October 21, 2020).

#3 On December 17, 2011, a pod of four killer whales consisting of one male, two females, and one calf was recorded by the authors during a research survey at 13°33'22.8"N 91°29'15.9"W, ~48 km off Escuintla (Cabrera et al., 2012).

#4 On November 24, 2017, a pod of five to ten killer whales was recorded during a sport fishing trip off Guatemala. The exact location was not recorded but was approximately 13°22'N 90°51'W. The pod consisting of at least one male and several females and/or juvenile males was traveling and did not interact with the boat (C. Monteros, personal communication, October 16, 2020).

#5 On January 25, 2020, a pod consisting of a minimum of ten (range 10-15) killer whales, was reported by the whale watching company Serviturismo Las Lisas. The pod consisted of at least two males and several females/young males and calves. The sighting occurred in the San José Canyon, ~74 km off the coast of Santa Rosa (13°15'01.4"N 90°40'0.1"W). Some members of the pod, mainly the calves, swam under and around the boat (O. Marroquín, personal communication, January 26, 2020).

Photographic match of a killer whale pod: Through photo-identification, three adult individuals from the same pod photographed in 2011, were matched with a sighting photographed 11 years earlier, on August 15, 2000, as part of group No. 25.00 off Cabo Corrientes, Mexico (20°16'13.2"N, 105°45'24.6"W) consisted of seven individuals (Olson & Gerrodette, 2008). The shortest distance between sightings is ~1 778 km. The female in Fig. 1B matches E060 (Olson & Gerrodette, 2008) and was identified by two notches on the back of her dorsal fin. The female with a calf (E061, Fig. 1C) was identified by the unique pattern on its saddle patch, although a modification had occurred on the tip of its dorsal fin (red arrow). The male (E059, Fig. 1D) was identified by the beveled tip.

Interaction between killer whales and Bryde's whales: We observed a peculiar interaction between a killer whale pod and two adult Bryde's whales (Fig. 1E). The sighting occurred on December 17, 2011, and began at 11:20h when we recorded a "splash" at ~1 km from our vessel (65-foot frigate). Once in the area, at 11:33h, a female killer whale and a calf approached the vessel for a few minutes, swimming slowly, sideways, and near the water surface. A few minutes later, two Bryde's whales were observed swimming slowly at ~100 m from the killer whales. At 11:44h, when the killer whales started moving away from the boat, the Bryde's whales swam behind the killer whales and accelerated, a behavior that we consider as chasing or pursuit. During the observation, the Bryde's whales remained within ~100 m of the killer whales while traveling at high speed. One of the Bryde's whales breached

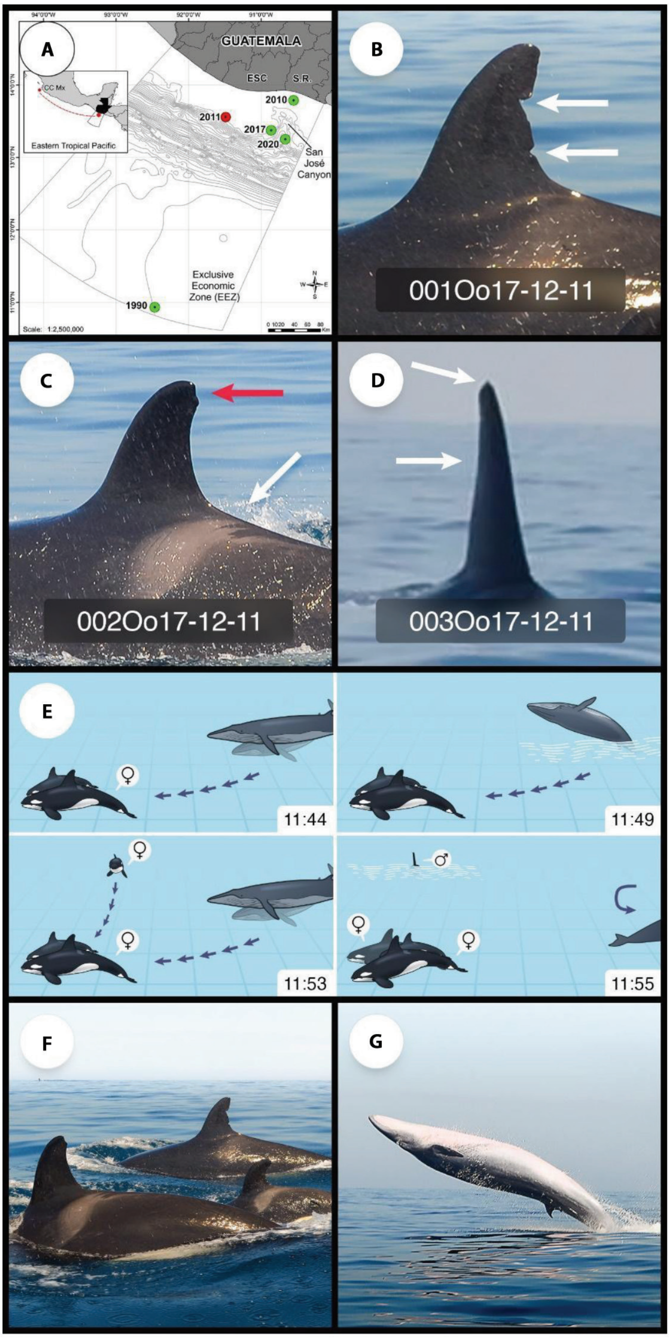

Fig. 1. A. Killer whale sightings off Guatemala. Location of Escuintla (ESC) and Santa Rosa (S.R.). Insert shows the location of the photographic match between our sighting #3 and group 25.00 reported by Olson & Gerrodette (2008); B-D. Killer whales from sighting #3 that match individuals E-060 (B), E-061 (C) and E-059 (D) from group 25.00. Arrows point to the original (white) and new (red) distinctive marks. Inserted numbers indicate the catalog number based on Cabrera et al. (2012); E. Schematic representation of killer whale-Bryde's whale encounter in 2011; including two Bryde's whale following killer whales, Bryde's whale breaching, two killer whales accompanying calf and retreat of Bryde's whales; F. Female killer whales positioning the calf between them; G. Bryde's whale breaching while following the killer whales.

twice, at 11:49h and 11:51h (Fig. 1G). The event lasted about 11 minutes, ending when a second female killer whale approached and positioned herself on the opposite side of the first female, positioning the calf in the middle (Fig. 1F). At 11:55h, the two Bryde's whales turned away from the killer whales. At 11:58h, we spotted the fin of a male killer whale at ~500 m away. The male slapped his tail at least three times, and then the two females and the calf dove and swam to join the male. The four killer whales continued to swim very close until they were out of sight. At no time did we observe a direct response or attack by the killer whales towards the Bryde's whales.

Killer whales are rare visitors in Guatemala: Five opportunistic sightings collected over a span of 30 years suggests that killer whales are rare in Guatemala, and there are no indications of resident pods. Although no predatory behavior was observed in any of the reports, it is possible that the killer whales that approach the Guatemalan coast between November and February do so in search of humpback whale calves (Pacheco et al., 2019), or other cetaceans as it has been observed in the ETP (e.g., Flórez-González et al., 2007; Pitman et al., 2007; Testino et al., 2019). Killer whales from low latitude regions such as the ETP and Hawaii have been considered generalists feeding not only on marine mammals, but also on fish and turtles (e.g., Baird et al., 2006; Vargas-Bravo et al., 2021).

A photographic match represents the longest time interval and distance recorded for a killer whale pod in the ETP: The photographic match between three killer whale individuals sighted in Guatemala and previously in Mexico provides insights into killer whale's movements and behavior in the ETP. Although the minimum distance (1 778 km) between the two sights is well below previous reports (5 535 km (Guerrero-Ruiz et al., 2005) and 3 839 km (Pacheco et al., 2019)) for a single individual in the region, it represents the longest distance recorded for multiple individuals from the same pod in the ETP. The time interval of 11 years between the sightings provides information on family association or cohesion. At least three of the seven killer whales originally recorded by Olson & Gerrodette (2008) as part of their group number 25.00 have potentially remained together for at least 11 years. In addition, the new photographs of the individuals can be used to update the original photo-identification catalog from Olson & Gerrodette (2008) particularly for those individuals with fin modifications, such as female E061.

Interaction between Bryde's whales and killer whales. To our knowledge, the described interaction between these two species has not been reported before. Previous report of Bryde's whales and killer whales interactions have involved either an attack of the killer whales (Alava et al., 2013; Silber et al.,1990), fleeing of the Bryde's whales (Ford & Reeves, 2008) or indifference in which both species moved away from one another (Villegas-Zurita et al., 2016). Although we did not observe any direct attack from the killer whales towards the Bryde's whales or any other species, the observed Bryde's whales behavior could be associated with an antipredatory response such as the "fight strategy" (Ford & Reeves, 2008), or an altruistic behavior such as "mobbing" (Pitman et al., 2017). However, these strategies have not been reported for Bryde's whales and other rorquals of the genus *Balaenoptera,* but only for whales with robust body shapes such as humpback whales (*Megaptera novaeangliae*) (Ford & Reevs, 2008; Pitman et al., 2017). Additionally, it is known that breaching represents an activity of high energy cost (Segre et al., 2020). The Bryde's whale breaches observed during the interaction could be associated with a demonstration of strength against potential predators or as a means of communication (Whitehead, 1985). Other behaviors such as social interaction including playing or curiosity could also be considered as an explanation. Given the novelty of the behavior, more research needs to be done in the future before drawing any conclusion.

Implications for research and conservation: Although the number of killer whale sighting records in Guatemala is limited, this information contributes to current efforts to increase knowledge on distribution, movements, and intra- and interspecific behaviors of killer whales in the ETP. The potential increase in killer whale sighting frequency over the past decade may be attributed to an increased sighting effort, which includes citizen science, whale watching, and research programs. This highlights the importance of establishing long-term monitoring programs of cetaceans in the country. The cross-border movements observed in some killer whales emphasize the need for international conservation efforts, as killer whales are not bound by political borders.

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: Conceptualization, AAC, JOW; Investigation, JOW, AAC, JCR; Formal analysis, JOW; Writing – Original Draft JOW, AAC; Writing – Review & Editing, All authors; Supervision, AAC; Funding Acquisition, AAC, JOW.

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Mitochondrial DNA supports the low genetic diversity of *Tursiops truncatus* (Artiodactyla: Delphinidae) in Bocas del Toro, Panama and exhibits new Caribbean haplotypes

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ABSTRACT

Introduction: The common bottlenose dolphin (*Tursiops truncatus*) is one of the most studied cetaceans worldwide; however, information about the genetic structure of wild populations is scarce in some regions like Central America and the Caribbean. There are two known genetic forms identified in the Caribbean based on mitochondrial DNA Control Region (mtDNA-*CR*) data: the 'inshore (or coastal) form' and the 'Worldwide distributed form'. In general, the inshore form refers to coastal and highly philopatric populations that show low genetic diversity. Worldwide distributed form refers to highly mobile populations with coastal and oceanic individuals that do not show philopatry and usually display high genetic diversity.

Objective: To determine the preliminary genetic status of common bottlenose dolphins in La Guajira, Colombian Caribbean, using a hypervariable portion of mtDNA-*CR*. The obtained haplotypes were compared with samples collected in Panama (likely 'inshore form') and with haplotypes previously found in other areas of the Caribbean.

Methods: In 2016, a total of 26 skin samples were obtained by remote biopsy system (PAXARMS) in two locations, La Guajira (Colombia, N=7) and Bocas del Toro (Panama, N=19). DNA was extracted, samples sexed, and a segment of mtDNA-*CR* (~550-750 bp) was amplified by PCR. The successfully amplified DNA sequences were manually reviewed and cleaned, and subsequently compared with 44 haplotypes previously reported for the Caribbean.

Results: The mtDNA-*CR* sequences from Bocas del Toro shared the same unique inshore haplotype previously reported for this population, while the samples from La Guajira represented six novel haplotypes, five belonging to the Worldwide distributed form and one to the 'inshore form.' Population structure analysis revealed two phylogroups for the Caribbean (F_{ST}=0.1353, Φ_{ST}=0.3330) with high haplotype diversity: Panama(Bocas del Toro)-Bahamas-Cuba-Mexico (*h*=0.8489, π=4.2536 %) and Colombia-Costa Rica-Honduras-Puerto Rico (*h*=0.8837, π=4.2423 %).

Conclusions: These findings support the results previously reported for common bottlenose dolphins in Bocas del Toro-Panama and reinforce the need to protect this vulnerable 'inshore' population by treating it as a unique population management unit. Mitochondrial DNA analysis of samples collected from La Guajira dolphins provide the first insight into the genetic diversity of common bottlenose dolphins in this region, indicating the presence of both inshore and Worldwide distributed genetic forms. The potential connectivity of this last form among La Guajira-Colombia, Costa Rica, and Honduras in Central America highlights the need for more genetic and ecological studies to determine the appropriate management units for this species in Central America and the Caribbean.

Key words: bottlenose dolphin; cetaceans; mtDNA; genetic diversity; La Guajira, Colombia, Central America.

RESUMEN

ADN mitocondrial soporta la baja diversidad genética de *Tursiops truncatus (*Artiodactyla: Delphinidae) en Bocas del Toro, Panamá y detecta nuevos haplotipos en el Caribe

Introducción: El delfín nariz de botella común (*Tursiops truncatus*) es uno de los cetáceos más estudiados a nivel mundial. Sin embargo, la información sobre la estructura genética de sus poblaciones silvestres es escasa en algunas regiones, como Centroamérica y el Caribe. Con base en datos de la Región de Control del ADN mitocondrial (ADNmt-*CR*), dos formas genéticas han sido identificadas en el Caribe: la 'forma *inshore* (o costera)' y la 'forma mundialmente distribuida'. En general, la forma costera se refiere a poblaciones costeras y altamente filopátricas que muestran baja diversidad genética. La forma mundialmente distribuida se refiere a poblaciones altamente móviles con individuos costeros y oceánicos que no muestran filopatría y generalmente muestran alta diversidad genética.

Objetivo: Para determinar el estado genético preliminar de los delfines nariz de botella comunes en La Guajira, Caribe colombiano, se realizó un análisis genético utilizando una porción hipervariable de ADNmt-*CR*. Los haplotipos obtenidos se compararon con muestras recolectadas en Panamá (probablemente la forma costera) y con haplotipos encontrados previamente en otras áreas del Caribe.

Métodos: En 2016 se obtuvo un total de 26 muestras de piel colectadas con el sistema de biopsia remota (PAXARMS) en dos localidades, La Guajira (Colombia, N=7) y Bocas del Toro (Panamá, N=19). Se extrajo el ADN, se sexaron las muestras, y un segmento de ADNmt-*CR* (~550-750 pb) se amplificó mediante PCR. Las secuencias de ADN amplificadas con éxito se revisaron y limpiaron manualmente; posteriormente, se compararon con 44 haplotipos reportados previamente en el Caribe.

Resultados: Las secuencias de ADNmt-*CR* de Bocas del Toro compartieron el mismo haplotipo costero único reportado previamente para esta población, mientras que las muestras de La Guajira representaron seis haplotipos nuevos, cinco pertenecientes a la forma mundialmente distribuida y uno a la forma costera. El análisis de la estructura de la población reveló dos filogrupos para el Caribe (F_{ST}=0.1353, Φ_{ST}=0.3330) con alta diversidad haplotípica: Panamá(Bocas del Toro)-Bahamas-Cuba-México (*h*=0.8489, π=4.2536 %) y Colombia-Costa Rica-Honduras-Puerto Rico (*h*=0.8837, π=4.2423 %).

Conclusiones: Estos hallazgos respaldan los resultados previamente reportados para los delfines nariz de botella comunes en Bocas del Toro-Panamá y refuerzan la necesidad de proteger a esta vulnerable población costera tratándola como una unidad de manejo poblacional única. Para La Guajira, estos resultados de ADNmt resultan en el primer esfuerzo por determinar la diversidad y estructura genética del delfín nariz de botella común en esta región, los cuales sugieren que ambas formas genéticas están presentes en el área, siendo la forma mundialmente distribuida la predominante. La conectividad potencial de esta última forma entre La Guajira-Colombia, Costa Rica y Honduras en Centroamérica destaca la necesidad de realizar más estudios genéticos y ecológicos para determinar las unidades de manejo apropiadas para esta especie en Centroamérica y el Caribe.

Palabras clave: delfín nariz de botella; cetáceos; ADNmt; diversidad genética; La Guajira, Colombia, Centroamérica.

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

INTRODUCTION

The common bottlenose dolphin, *Tursiops truncatus* (Montagu, 1821) is a cosmopolitan species inhabiting a variety of nearshore and oceanic environments in tropical and temperate waters. It is currently listed as Least Concern by the International Union for the Conservation of Nature (IUCN) (Wells et al., 2019). This broadly distributed species is one of the most studied cetaceans worldwide (Reynolds et al., 2000). However, genetic information is still scarce for populations in some regions, particularly in coastal areas of Central America and the Caribbean, where few studies have been conducted. The lack of information regarding population structure may result in poor management practices, since coastal populations show a high degree of genetic differentiation and face more anthropogenic threats due to the proximity to human populations (e.g., Barragán-Barrera et al., 2017; Caballero et al., 2012; Fruet et al., 2014; Natoli et al., 2004; Parsons et al., 2006; Tezanos-Pinto et al., 2009). Therefore, targeted studies like this one, address critical data gaps for geographic regions of interest and are key for ensuring effective conservation and management programs.

Common bottlenose dolphins are highly adaptable to different environmental conditions, where habitat selection and resource specialisation likely shape patterns of movement, gene flow, and population structure (Hoelzel et al., 1998; Wiszniewski et al., 2010). For example, ‘inshore’ and ‘offshore’ ecotypes have been described in the Gulf of Mexico and the Northwest Atlantic based on a variety of factors such as genetics, haemoglobin profile, and morphometry (Duffield et al., 1983; Hersh & Duffield, 1990; Hoelzel et al., 1998; Natoli et al., 2004; Sellas et al., 2005). However, Tezanos-Pinto et al. (2009) provided a worldwide phylogeographic perspective highlighting that the ‘offshore’ of the Northwest Atlantic is genetically interconnected to several mitochondrial DNA (mtDNA) haplotypes distributed worldwide regardless of the habitat where samples were collected (coastal or pelagic) or ocean basin. Therefore, although the ‘inshore form’ is present in the Wider Caribbean, only the populations located in Florida-western North Atlantic, the Bahamas (Caballero et al., 2012; Natoli et al., 2004; Tezanos-Pinto et al., 2009), and Bocas del Toro Archipelago, Panama (Barragán-Barrera et al., 2017) have been successfully classified as entire ‘inshore form’.

The inshore populations found in the western North Atlantic, which are highly structured among bays (e.g., Sellas et al., 2005), have recently been proposed as a new species based on an integrative approach comparing mitochondrial and nuclear DNA with morphometrics (Costa et al., 2022). These findings indicate that inshore bottlenose dolphins are likely under-characterized and deserve additional attention. Given this, it is imperative to conduct studies on dolphin populations in the Caribbean and Central America regions. This research should take into consideration that the inshore form is primarily found in the Wider Caribbean, specifically in Bocas del Toro (Barragán-Barrera et al., 2017; Caballero et al., 2012; Tezanos-Pinto et al., 2009).

The ‘inshore’ population found in Bocas del Toro exhibits a strong population structure and low genetic diversity (all individuals possess a single mitochondrial DNA Control Region (mtDNA-*CR*) haplotype) compared to other populations in the Caribbean (Barragán-Barrera et al., 2017). The population is small, shows high site fidelity, and restricted coastal feeding habits; all characteristics usually attributed to the inshore ecotype (Barragán-Barrera, Luna-Acosta et al., 2019; May-Collado et al., 2015, May-Collado et al., 2017). However, recent observations in the field suggest that oceanic individuals may be entering within the north-west region, which may result in potential genetic flow (B. Pérez, personal communication, 2022). Additional analyses are needed to better understand the genetic isolation and the ‘inshore form’ genetic connectivity between the Bocas del Toro dolphins and other dolphin populations in the Caribbean.

Little is known about the population found in La Guajira (Colombian Caribbean). To date, most of the studies have focused on habitat use and occurrence (Combatt & González, 2007; Palacios et al., 2013). In the absence of data on genetic diversity and population structure, it is unknown if bottlenose dolphins in La Guajira belong to the inshore form or 'Worldwide distributed form', or if both forms coexist. This information gap prevents adequate management plans for this population, despite bottlenose dolphins in Colombia are facing threats related to bycatch, interactions with fishing gear, direct capture, vessel traffic/transit, pollution, and pathogens (Avila & Giraldo, 2022).

Here, we evaluate the genetic diversity and population structure of the common bottlenose dolphin in Bocas del Toro and La Guajira by comparing the mtDNA-*CR* marker to 44 haplotypes previously published from various locations in the Caribbean Sea (Barragán-Barrera et al., 2017; Caballero et al., 2012). Results from this work will contribute to the understanding of the genetic structure of bottlenose dolphins in the Central American Caribbean and represent a critical first step to local conservation efforts.

METHODS

Study area: The Bocas del Toro Archipelago is located on the western Caribbean coast of Panama (Fig. 1), and has great marine biodiversity associated with coral reefs, seagrasses, and mangroves (Coates et al., 2005; Guzmán et al., 2005). La Guajira, in the western coast of Colombia, is the northernmost area of South America (Fig. 1). It is one of the most biodiverse regions in Colombia; it holds several coastal and marine ecosystems such as mangroves, coral reefs, coastal wetland, sandy beaches, and seagrasses (Corporación Autónoma Regional de La Guajira & Instituto de Investigaciones Marinas y Costeras, 2012).

Sample collection: Tissue samples were collected from wild common bottlenose dolphins using remote biopsy darts fired from a

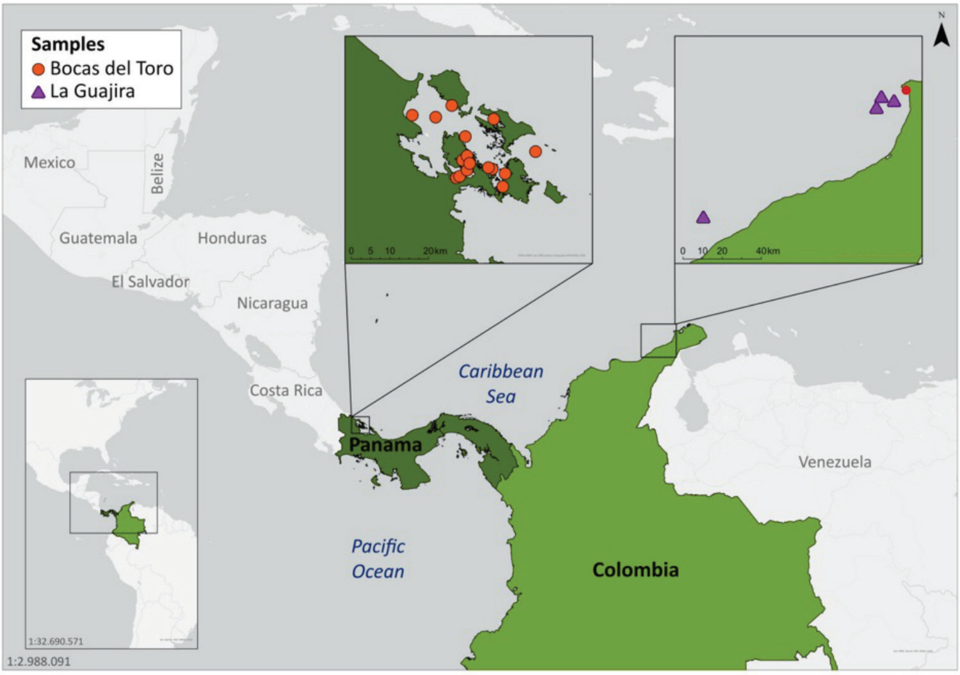

Fig. 1. Sample locations in Bocas del Toro Archipelago, Panama (orange dots) and La Guajira, Colombia (purple triangles) in the Caribbean Sea. Red dot indicates the location of Cabo de la Vela in La Guajira.

modified rifle (PAXARMS) (Krützen et al., 2002). This is a standardised methodology for collecting small skin biopsies from small cetaceans (Tezanos-Pinto & Baker, 2012). Samples were collected in February, March, and October of 2016 in Bocas del Toro, and in May and June of 2016 in La Guajira. GPS data and photographs of the dolphin biopsied were also collected. The latter allowed individual identification avoiding re-sampling (Fruet et al., 2014; Krützen et al., 2002). Samples were preserved in 70 % ethanol and stored at −20 °C (Amos & Hoelzel, 1991) for further laboratory analysis.

DNA extraction, PCR, sequencing, and sexing: DNA was extracted from skin samples using the DNeasy kit (QIAGEN). Fragments of approximately 535 - 684 bp of mtDNA-*CR* were amplified through the polymerase chain reaction (PCR), using the primers Dlp8G (5'-CCATCGWAGATGTCTTATTTAAGRARTTCTA-3') or Dlp5G (5'-GGAGTACTATGTCCTGTAACCA-3') and Dlp1.5 (5'-TCACCCAAAGCTGRARTTCTA-3') (Baker et al., 1998). Amplification followed this protocol: an initial pre-denaturation step of 94 ºC as denaturation for 2 min, with 34 cycles of 2–4 repeat times of 30 s at 94 ºC, followed by 45 s at 55 ºC as annealing and an extension at 72 ºC, with a final elongation after the last cycle of 10 min at 72 ºC (Baker et al., 1998). PCR products were visualized on agarose gel before sequencing. DNA was cleaned and sequenced in both directions with the Sanger sequencing method (Sanger & Coulson, 1975) on an ABI 3500 DNA automated sequencer using the Big Dye^{TM} Terminator v.3.1 Cycle Sequencing kit. In order to identify the sex of the samples collected, we used male-specific SRY gene and ZFY/ZFX genes of males and females (Gilson et al., 1998). Fragments were amplified by PCR using the protocol proposed by Gilson et al. (1998) and visualised on agarose gel to determine sex.

Data analysis: All mitochondrial sequences were checked, cleaned manually and aligned using the software Geneious v. 2022.1.1 (Drummond et al., 2009). To confirm that the samples collected were from common bottlenose dolphins and not from other delphinid species of the delphinid complex *Delphinus-Stenella-Tursiops*, we used BLAST in NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to compare our sequences to publicly available reference sequences and confirm species of origin. Unique haplotypes were assigned using the R script RemoveRedundantTaxa in RStudio (v. 2022.02.3+492). Haplotypes obtained were compared to 44 previously published sequences on NCBI GenBank from the Bahamas, Colombia, Costa Rica, Cuba, Honduras, Jamaica, Mexico, Puerto Rico, and Virgins Islands (Accession numbers: JN596281-JN596321) (Caballero et al., 2012), Costa Rica (Accession numbers: KY817220-KY817221), and Panama (Bocas del Toro) (Accession number: KX833116) (Barragán-Barrera et al., 2017). The complete alignment was 386 bp.

To identify which of the two genetic forms of common bottlenose dolphins the samples analysed belonged to, a Maximum Likelihood phylogenetic tree was constructed with the evolutionary model of Generalised-Time-Reversible γ + Invariant (general reversible time substitution model GTR γ + I) substitution and 1 000 bootstrap replicates using RAxML-HPC BlackBox tool in CIPRES Science Gateway V. 3.3 (Miller et al., 2010). As outgroup we defined a sample from an Atlantic spotted dolphin, *Stenella frontalis* (Cuvier, 1829) collected in La Guajira in 2016, since bootstrap value indicates 100 support segregating *T. truncatus* from *S. frontalis,* and this species has been used before as outgroup for mtDNA analysis on bottlenose dolphins (e.g., Wang et al., 1999). Also, a haplotype network was constructed using the TCS method in PopART software (Clement et al., 2000) to visualise to what population the haplotypes used in this study belonged and their frequency. Finally, we defined the number of phylogroups for the Caribbean by calculating F_{CT} using Arlequin v. 3.5 (Excoffier & Lischer, 2010). We used the same software to assess estimates of genetic structure (F_{ST} and Φ_{ST}), nucleotide (π), and haplotype diversity (h).

Here, samples from Jamaica and the US Virgin Islands were excluded due to the low number of samples (N = 1).

RESULTS

A total of 26 tissue samples from common bottlenose dolphins were collected; seven in La Guajira, Colombia, and 19 in Bocas del Toro, Panama. All samples from Panama were successfully amplified, sequenced and included in genetic structure and diversity analyses. Among these samples, only a single haplotype was found. Six out of the seven samples from La Guajira were successfully amplified, each representing a unique haplotype. Overall, ten males and nine females were identified in Bocas del Toro samples, and four males and three females were identified in La Guajira samples.

When the sequences obtained from this study were compared with 44 previously Caribbean haplotypes (Barragán-Barrera et al., 2017; Caballero et al., 2012; SMT1), all samples from La Guajira resulted in new haplotypes not previously reported for the Caribbean, while the only haplotype found in the samples from Bocas del Toro was identified as the same unique inshore haplotype reported previously (Barragán-Barrera et al., 2017). New *T. truncatus* haplotypes were submitted to GenBank (https://www.ncbi.nlm.nih.gov/genbank/) as accession numbers OR090913-OR090918 and the new *S. frontalis* (outgroup) haplotype was submitted to GenBank as accession number OR090919.

Further comparisons conducted through phylogenetic analyses, showed samples from Bocas del Toro nested with the previously reported haplotype BOC (Accession number: KX833116), which had been reported by Barragán-Barrera et al. (2017) as 'inshore.' Five out of six samples from La Guajira nested with the Worldwide distributed form, and only one sample nested with the inshore form, grouping with the inshore Q haplotype from Cuba (Fig. 2). The haplotype network (Fig. 3) shows the relationships among haplotypes, frequency, and locations.

In the genetic structure analysis using the new sequences found here with the previous ones reported in the Caribbean, we evaluated from two to five phylogroups. Based on the most probable phylogroups, we defined two (F_{CT} = 0.2655, P = 0.0352). One phylogroup formed by Bocas del Toro (Panama)-Bahamas-Cuba-Mexico (mostly formed by inshore haplotypes) and the other one by Colombia-Costa Rica-Honduras-Puerto Rico (mostly formed by Worldwide distributed form haplotypes). We reported similar values of haplotype and nucleotide diversity between both phylogroups (Table 1), but values of F_{ST} and Φ_{ST} showed strong population structure between them, especially at the nucleotide level (F_{ST} = 0.1353, Φ_{ST} = 0.3330, P < 0.0001, Table 1). We found relatively high haplotype and nucleotide diversity in both phylogroups considered in this analysis, with the highest haplotype diversity found in the Colombia-Costa Rica-Honduras-Puerto Rico phylogroup (Table 1).

Table 1

Estimates of mitochondrial differentiation among the two forms ('inshore form' and 'Worldwide distributed form') of common bottlenose dolphin (*Tursiops truncatus*) located in the Caribbean Sea. F_{ST} value is shown above diagonal and Φ_{ST} below diagonal. P-value is indicated under each value. Haplotype (h) and nucleotide diversity (π) are shown on the diagonal for each phylogroup.

F_{ST} / Φ_{ST}	Bocas del Toro (Panama)-Bahamas-Cuba-México	Colombia-Costa Rica-Honduras-Puerto Rico
Bocas del Toro (Panama)-Bahamas-Cuba-México	h = 0.8489 +/- 0.0179 π = 4.2536% +/- 0.021	0.1353*
Colombia-Costa Rica-Honduras-Puerto Rico	0.3330*	h = 0.8837 +/- 0.0337 π = 4.2423% +/- 0.0214

Significant values (p < 0.05) are indicated with an asterisk (*).

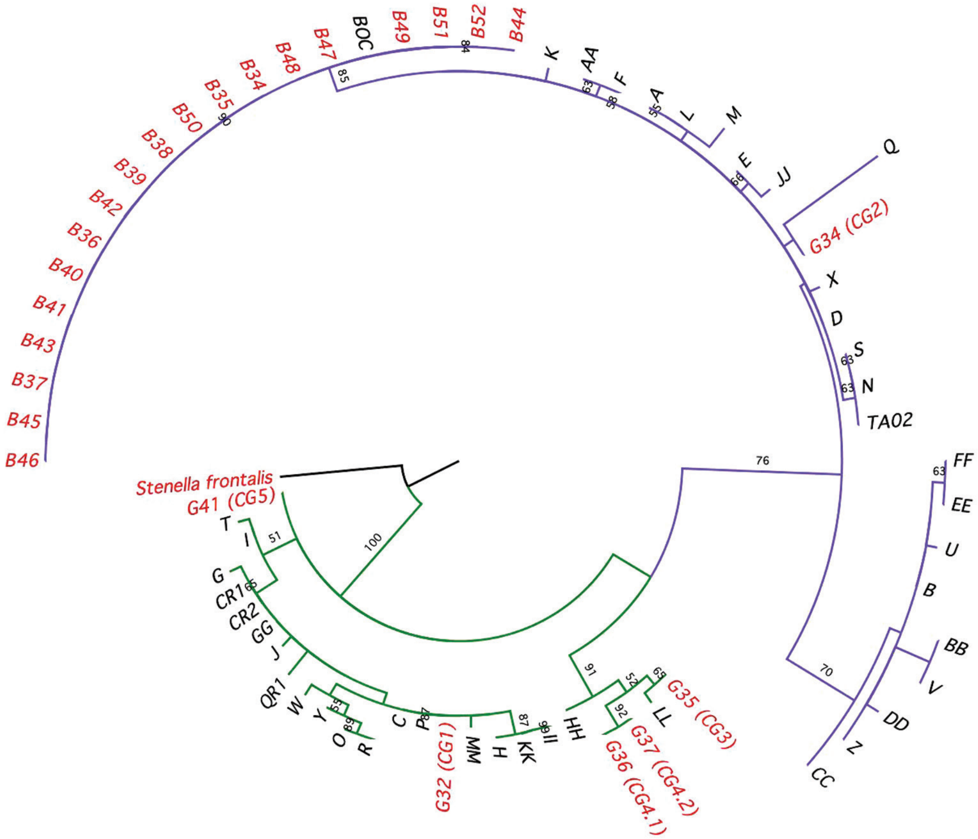

Fig. 2. Phylogenetic reconstruction by Maximum Likelihood of Control Region haplotypes from common bottlenose dolphins (*Tursiops truncatus*) in the Caribbean Sea (N = 70, 386 bp). Phylogeny in a circular polar form shows bootstrap support in branches with percentages > 50 %. Purple line groups the 'inshore form' and the green line groups the 'Worldwide distributed form'. Red letters represent the samples used for this study and the braces indicate the new haplotypes. Outgroup: Atlantic spotted dolphin (*Stenella frontalis*).

DISCUSSION

This study provides new insights into the population structure and genetic diversity of common bottlenose dolphins from La Guajira (Colombia) and Bocas del Toro (Panama) in the western Caribbean using mtDNA-*CR* data. The results confirm that a single unique haplotype is present among the individuals sampled in Bocas del Toro, and it is unique to the Caribbean Sea (Barragán-Barrera et al., 2017). This population has low mitochondrial diversity and both sexes appear to be highly philopatric (Barragán-Barrera et al., 2017). Previous photo-ID data supports high site fidelity (e.g., May-Collado et al. 2012, 2015, 2019), and genetic data shows a strong population structure based on nuclear data (nine microsatellite loci), as well as that both sexes share the same unique inshore haplotype not reported in other Caribbean area (Barragán-Barrera et al., 2017). This site fidelity may be explained by the prey availability for dolphins within the Archipelago (Barragán-Barrera, Luna-Acosta et al., 2019), as well as for the shallow areas suitable as nursery (May-Collado et al., 2019). Even though most studies have demonstrated male-biased dispersal (Baker et al. 1993; Escorza-Trevino & Dizon, 2000; Möller et al. 2004; Rosel et al. 1999), philopatry in both sexes has been documented

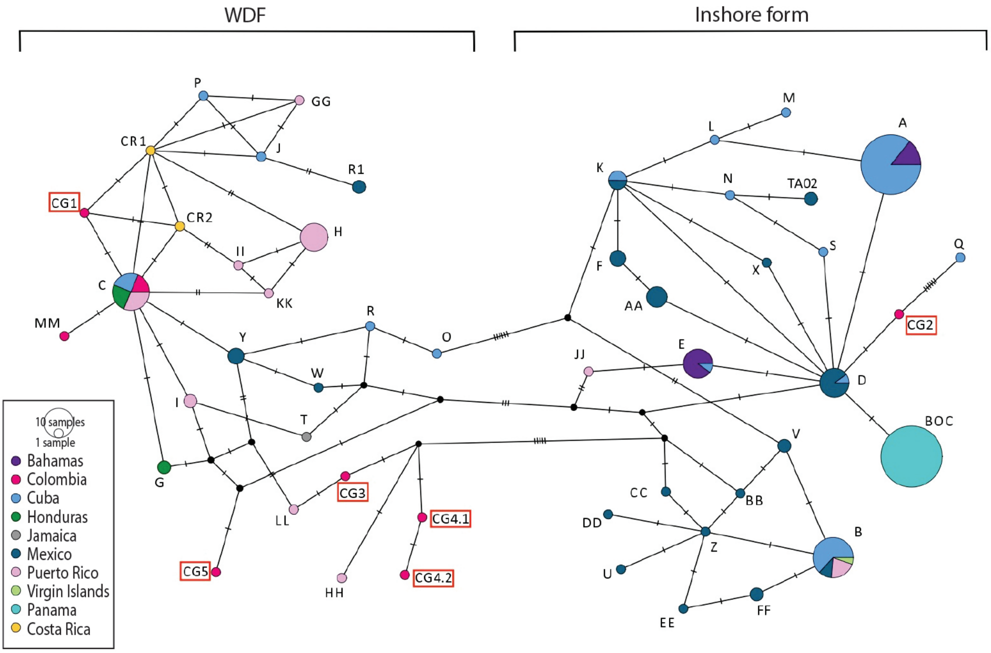

Fig. 3. Haplotype network reconstruction for common bottlenose dolphins (*Tursiops truncatus*) in the Caribbean Sea under parsimony criteria with the TCS algorithm (N = 50, 386 bp). The size of the circles indicates the frequency of each haplotype. Black dots indicate the hypothetical ancestral haplotype, and the perpendicular lines between the haplotypes refer to the number of nucleotide substitutions between them. New haplotypes are highlighted within a red square.

before in odontocetes. For example, in resident killer whales located in British Columbia and Washington State (Bigg et al., 1987) and pilot whales located in the Faeroe Islands (Amos et al., 1993). In some bottlenose dolphin populations, males are more mobile than females even in resident populations (Möller & Beheregaray, 2004).

Conversely, the genetic pattern of the population of La Guajira is very different. Here, we infer that dolphins are mainly of the Worldwide distributed form, in addition to a few inshore dolphins. A previous study (Combatt & González, 2007) suggested that dolphins in La Guajira probably belonged to the inshore form (based on observations and occurrence data). However, in general, La Guajira Peninsula does not have closed and protected bays as does the Bocas del Toro Archipelago. This geography and oceanic foraging may promote admixture among different forms. Nonetheless, the area is influenced by upwelling conditions, mainly in the low (south) and high (north) Guajira, offering nutrients to dolphin prey species (Barragán-Barrera, Luna-Acosta et al., 2019; Gutiérrez Leones et al., 2015; Paramo et al., 2011). Although this upwelling is variable throughout the year and between years (Andrade & Barton, 2005), it has been related to presence of dolphins in La Guajira (Farías-Curtidor et al., 2017). The area appears to be important for transit, and consequently, a very mobile common bottlenose dolphin population may visit La Guajira when oceanographic conditions are favourable. Nevertheless, the individual nested with 'inshore form' haplotypes could suggest the potential presence of inshore populations in the high Guajira (where the sample was collected), or admixture between forms. The sampled inshore individual was a male in a group of 15 individuals that were feeding close to a fishing boat in the Cabo de la

Vela (12.113333º N & -72.294533º W) (Fig. 1). In this area, mainly in the north, some enclosed bays may offer adequate habitats for dolphins, but sufficient monitoring data to confirm this assumption does not exist.

La Guajira appears to be an important transit area for common bottlenose dolphins in the Wider Caribbean, where the presence of the Worldwide distributed form individuals formed by dolphins with both coastal and oceanic habits, may allow for genetic flow among inshore populations. In the Wider Caribbean, two differentiated phylogroups were detected based on the high F_{ST} and Φ_{ST} values: the phylogroup formed by Bocas del Toro (Panama)-Bahamas-Cuba-Mexico that is mostly represented by the inshore form, and the one formed by Colombia-Costa Rica-Honduras-Puerto Rico which is mostly represented by Worldwide distributed form individuals. Despite this differentiation, similar haplotype and nucleotide values were found between the two phylogroups, likely because some countries such as Colombia (La Guajira), Cuba, Mexico, and Puerto Rico shelter both genetic forms (Caballero et al., 2012). Furthermore, three haplotypes (CG3, CG4.1, and CG4.2) from three samples obtained in La Guajira seem to be intermediate haplotypes between inshore and Worldwide distributed forms (Fig. 2 and Fig. 3). Bootstrap support values segregating both forms are high (Fig. 2), but more extensive sampling for genetic and genomic analyses are needed to elucidate the origin of these intermediate haplotypes. Here, we hypothesize these haplotypes could be either conserved haplotypes or the result of the genetic exchange between inshore and Worldwide distributed forms. It is interesting to note that the HH and LL haplotypes form Puerto Rico also nest in this intermediate zone. A previous study using mitochondrial DNA suggested possible ancestral connectivity between Puerto Rico and the Mediterranean Sea (Tezanos-Pinto et al., 2009); therefore, this may support our second hypothesis.

The common bottlenose dolphin is likely at risk in Colombia and worldwide (Avila et al., 2018; Avila & Giraldo, 2022), particularly coastal populations that are more vulnerable to human activities (Avila et al., 2018). In the Caribbean, a recent review documented at least 68 threats for common bottlenose dolphins: in Aruba (2 threats), Bahamas (2), Bermuda (1), Colombia (2), Cuba (42), Dominican Republic (5), Guyana (3), Haiti (2), Honduras (1), Mexico (1), Puerto Rico (2), and Venezuela (3) (Avila et al., 2018). These threats are related to anthropogenic activities that include fisheries (reported as interaction with fishing activities in six cases), hunting (25), tourism (23), scientific research (7), unreported direct human activities (1), urban development (1), and unidentified sources (5) (Avila et al., 2018). However, common bottlenose dolphins in the Caribbean could be exposed to more threats in other countries that were not included in the review. These threats include contamination by mercury exposure, reported in Bocas del Toro, Panama (Barragán-Barrera, Luna-Acosta et al., 2019), and in Colombia and Belize for this species and other small delphinids (Barragán-Barrera, Farías-Curtidor et al., 2019; González-Velásquez et al., 2020), as well as boat traffic, reported extensively in Bocas del Toro-Panama (e.g., Kassamali-Fox et al., 2020; May-Collado et al., 2012, 2017; May-Collado & Quiñones-Lebrón, 2014; Pérez-Ortega et al., 2021). The general lack of monitoring in the Central American Caribbean may further threaten inshore populations of common bottlenose dolphins.

Considering the threats that bottlenose dolphins face in the Wider Caribbean, identifying genetically distinct dolphin populations is critical to designing and implementing management plans. The case of common bottlenose dolphins in Bocas del Toro exemplifies this situation, where a small (seemingly isolated) population that is vulnerable to contaminant exposure and boat traffic from dolphin-watching activities (Barragán-Barrera, Luna-Acosta et al., 2019; May-Collado et al., 2017), has been proposed to be categorised as endangered at the local level based on ecological and genetic studies (Barragán-Barrera et al., 2017, Barragán-Barrera, Luna-Acosta et al. 2019; May-Collado et al., 2017).

The definition of population structure is required for effective conservation planning. Considering the threatened level for the common bottlenose dolphins in the Central American Caribbean, further genetic studies are needed. The 'inshore form,' previously reported in the Caribbean (Barragán-Barrera et al., 2017; Caballero et al., 2012; Tezanos-Pinto et al., 2009), needs to be studied in detail (particularly in Central America) to detect the inshore form level of isolation from the western North Atlantic (Costa et al., 2022). The results of population structure studies would provide critical information for local management plans. Conversely, in the case of Worldwide distributed form dolphins, a regional plan at the Caribbean level is urgent to maintain the genetic flow between populations. At the national level, Colombia and Panama must strengthen research and develop effective management plans to accomplish better boat and fishing practices, and reduce anthropogenic stressors for the inshore populations. La Guajira deserves special attention as a potentially important transit area for both common bottlenose dolphins' forms in the Caribbean Sea.

Here, we provide further evidence that dolphins in Bocas del Toro belonged to the 'inshore form,' and for the first-time provide genetic evidence that the dolphins sampled in La Guajira include both genetic forms, mainly the Worldwide distributed form. The single inshore haplotype in La Guajira may indicate that the northern portion of the Colombian Caribbean may be occupied by 'inshore' individuals. However, there is insufficient data to confirm this assumption or establish the common bottlenose dolphin conservation status, unlike in Bocas del Toro.

This study constitutes the first step to decreasing the knowledge gaps related to the genetic status of common bottlenose dolphins in the western Caribbean and highlights the urgent need of conducting more studies in Colombia and in the Central American Caribbean. Confirming the real conservation status of common bottlenose dolphins and the genetic connectivity between populations is key to determine at which scale management must be implemented. Under this context, further research is critical for conservation management of inshore populations that could be isolated and at high risk, but their status is currently unknown.

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See supplementary material
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Lobomycosis Like Disease in Bottlenose Dolphins *Tursiops truncatus* (Artiodactyla: Delphinidae) from Costa Rica Pacific Waters

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ABSTRACT

Introduction: Lobomycosis, is a chronic mycotic disease of the skin and sub-dermal tissue caused by *Paracoccidioides ceti*, which affects dolphins worldwide. In Costa Rica, the incidence of lobomycosis Like Disease (LLD) has been documented in inshore common bottlenose dolphins (*Tursiops truncatus*) from the waters of Golfo Dulce, South Pacific.

Objective: Examine the prevalence pattern of LLD in inshore *T. truncatus* from Costa Rica Pacific waters, specifically in Golfo Dulce and Golfo de Nicoya.

Methods: We examined over 20 000 photographs of *T. truncatus*, collected during long-term research on cetaceans in 2005-2020 (n = 476 surveys), in Golfo Dulce, as well of nine sightings *T. truncatus* from the Golfo de Nicoya , obtained during 17 samplings events in 2014-2022, these records for the presence of LLD allowed to calculate the prevalence rate with a confidence interval.

Results: The prevalence rate of LLD in adult photo-identified individuals was 13.1 % (CI: 12 -14.2) in Golfo Dulce, and 100 % in Golfo de Nicoya, considering the size of the population for the period of 2022.

Conclusion: The persistence and high prevalence levels of LLD in small, localized, communities of *T. truncatus* are a cause for serious concern.

Key words: bottlenose dolphin; lobomycosis Like Disease; Golfo Dulce; Golfo de Nicoya; inshore ecotype.

RESUMEN

Enfermedad similar a la generada por *Lobomicosis* en delfines nariz de botella *Tursiops truncatus* (Artiodactyla: Delphinidae) de las aguas del Pacífico de Costa Rica

Introducción: La enfermedad similar a Lobomicosis (LLD), asociada al hongo *Paracoccidioides cetii*, es una condición dérmica frecuentemente reportada en pequeños cetáceos alrededor del mundo. En Costa Rica se ha documentado la presencia de LLD en *Tursiops truncatus* del ecotipo costero en aguas del Golfo Dulce, Pacífico Sur.

Objetivo: Examinar el patrón de prevalencia de la enfermedad en el ecotipo costero de *T. truncatus* en la costa Pacífica de Costa Rica, específicamente en Golfo Dulce y Golfo de Nicoya.

Métodos: Se examinaron cerca de 20 000 registros fotográficos de *T. truncatus* provenientes de un monitoreo de cetáceos a largo plazo entre el 2005-2020 (n = 476 muestreos) en Golfo Dulce, así como nueve encuentros de *T. truncatus* en Golfo de Nicoya derivados de 17 muestreos realizados entre 2014-2022, estos registros fueron analizados, para identificar y estimar el índice promedio de prevalencia de LLD mediante un intervalo de confianza.

Resultados: El índice promedio de prevalencia de LLD en individuos adultos marcados es de 13.1 % (CI: 12–14.2) en Golfo Dulce, y de 100 % en Golfo de Nicoya considerando el tamaño de la población para el periodo del 2022.

Conclusión: La alta incidencia de LLD y el tamaño poblacional notablemente reducido de *T. truncatus* en aguas del Golfo Dulce y Golfo de Nicoya podría ser indicativo de una baja calidad de agua y degradación ambiental, por la presión de las actividades humanas que se realizan en las zonas costeras.

Palabras clave: Delfín Nariz de Botella; enfermedad similar a Lobomicosis; Golfo Dulce; Golfo de Nicoya; Costa Pacífica; ecotipo costero.

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

INTRODUCTION

Skin lesions have been described in odontocetes and mysticetes worldwide (Burdett Hart et al., 2012; Van Bressem and Van Waerebeek., 1996; Wilson et al., 1999). They may be caused by viruses, bacteria, fungi, and protozoa and appear to have been increasing in frequency during the last two decades (Daura-Jorge & Simões-Lopes, 2011; Van Bressem et al., 2009). Their occurrence and severity seem to be related to natural and anthropogenic factors such as water quality, inland water discharges, climate change, and habitat degradation (Burdett Hart et al., 2012; Daura-Jorge & Simões-Lopes, 2011; Gómez-Salazar et al., 2012; Harzen & Brunnick, 1997; Kiszka et al., 2009; Moore, 2008; Van Bressem et al., 2009; Wilson et al., 1999).

Caused by the yeast-like organism *Paracoccidioides ceti*, (Vilela, et al., 2016), lobomycosis (lacaziosis) is a chronic fungal disease of the skin and subdermal tissues that affects dolphins. It is characterized by raised, sometimes ulcerated or plaque-like, dermal lesions of grayish, whitish, or slightly pinkish color that can spread to disfigure large areas of the dorsal, dorsolateral, and pedunculated regions of the body (Bermudez et al, 2009; Bessensen et al. 2014; Daura-Jorge & Simões-Lopes, 2011; Félix, Van Bressem et al. 2019; Kiszka et al. 2009; Murdoch et al. 2010; Paniz-Mondolfi et al. 2012; Ramos et al. 2018; Rotstein et al. 2009, Van Bressem et al. 2009, Van Bressem et al. 2015).

Lobomycosis-like disease (LLD) is a condition whose clinical presentation is reminiscent of lobomycosis but lacks confirmation based on histologic and molecular diagnosis (Kiszka et al., 2009; Ramos et al., 2018; Van Bressem et al., 2009, 2015; Vilela et al., 2021). Nevertheless, there is a very good correlation between at-sea observations and corresponding photographic documentation and laboratory diagnosis (Murdoch et al. 2008; Sacristan et al. 2015; Van Bressem et al., 2007), even up to 100 %, as reported by Murdoch et al. (2008). In the common bottlenose dolphin, cases of lobomycosis appear to be significantly associated with disorders of the adaptive immune system, which may be related to chronic exposure to chemicals and biological pollutants (Reif et al., 2009).

In Costa Rica, the prevalence of LLD has been documented in inshore bottlenose dolphins in the waters of Golfo Dulce in the period 1991-1992, 2010-2011 (Bessesen et al., 2014). Here, we examine the prevalence of LLD in inshore bottlenose dolphins, *Tursiops truncatus* (Montagu, 1821)) from Costa Rican Pacific waters, update previous observations of the incidence in the Golfo Dulce bottlenose dolphin population ten years after the first observation, and report new cases of the disease in a small, localized dolphin population inhabiting Golfo de Nicoya.

Golfo Dulce is a 50 km long and 10–15 km wide tectonic embayment located in Costa Rica between 8°33’N and 83°14’W (Acevedo & Burkhart, 1998; Cubero, 1998; Oviedo et al., 2015; Rincón & Ballestero, 2015; Svendsen et al., 2006). The main freshwater inputs come from the Coto-Colorado, Tigre, Esquinas and Rincón rivers. This embayment has been divided into three sub-areas defined by topography and oceanographic conditions: the inner basin, the sill area, and the transitional oceanic area at the mouth of Golfo Dulce (Dalsgaard et al., 2003, Dalsgaard et al., 2005; Ferdelman et al., 2006; Morales et al., 2015; Oviedo et al., 2009, Oviedo et al., 2015; Quirós, 2003; Thamdrup et al., 1996).

Golfo de Nicoya is located on the Pacific coast of Costa Rica (10”N, 85°W) and is one of the largest estuaries (1 530 km²) in Central America. It extends about 80 km from its narrowest point at the mouth of the Tempisque River to its widest point (about 55 km) where it borders the open ocean (Maurer & Vargas 1984; Brenes et al., 2001; Wolf et al., 1998).

We conducted 476 photo-identification surveys in Golfo Dulce and 17 in Golfo de Nicoya, using a 7-m-long boat powered by a 115-horsepower four-stroke engine as a research platform during two seasons, wet (June - October) and dry (November - May), from 2005 to 2022. Each survey was conducted between 7:00 am and 4:00 pm on a given day. There were four observers on board the research vessel, with one observer acting as the primary photographer, usually assisted by another secondary surveyor, to photograph as many dolphins in the group as possible. Photographs were taken with an SRL digital camera (Canon 7D/70D) equipped with a 400 mm telephoto lens. In the presence of a group of dolphins, we photographed the dorsal fins of all individuals within the group, as perpendicular to the body axis as possible. Photographs were classified according to the classes described in Sanchez-Robledo et al. (2020). A quality criterion was prioritized in photographic data processing (Karczmarki et al., 2005). Therefore, only high-quality images (80 % and above on a quality scale from 1 to 100 %) were selected and used in the analysis. The photo processing was designed to eliminate any sampling bias, thereby strengthening one of the key assumptions of the capture-mark-recapture models, the likelihood of homogeneous capture of all individuals in the group. Once all LLD photographic records were organized and selected, we validated the diagnosis after consultation with an expert (Marie-Françoise Van Bressem, personal communication, 6 October, 2022). After analysis and classification of all folders, we counted the number of individuals with LLD.

We estimated an LLD prevalence rate, taking into account the individual history of encounters, which allowed us to calculate annual prevalence indices from the rainy season of 2011 to the rainy season of 2020. This was done by considering the annual presence records of non-calf individuals with photographic evidence of LLD in relation to all non-calf individuals recorded and photographed in that year. The latter resulted in a prevalence rate for Golfo Dulce, presented with a confidence interval. In the case of Golfo de Nicoya, the number of individuals with LLD in all surveys where coastal bottlenose dolphins were recorded was also equal to the number of individuals recorded and photographed per year, due to the low number of dolphins in this population. Data consisted of 590 *T. truncatus* sighting records collected during long-term cetacean research (2005 - 2020) in Golfo Dulce. In Golfo de Nicoya, the data collected consists of nine sightings of *T. truncatus* from 17 boat sampling events (2014: n = 6; 2015: n = 8, 2019: n = 1, 2022: n = 2).

In the period 2005-2020, the average percentage of dolphins identified with LLD in Golfo Dulce was 13.1 (CI: 12 – 14.2) in 105 dolphins photo-identified (Fig. 1; Supplementary Table 1). The highest prevalence estimated in 2014 was 16.2 %, whereas the lowest prevalence was estimated at 9.1 % in 2019 (SMT1), within this range most of the prevalence values varied around 12 to 14 %.

These prevalence levels are comparable with those reported for other coastal populations of *T. truncatus* from South America (5.4 % - 44.4 %), North America (12.4 %), and *Tursiops aduncus* (Ehrenberg, 1832 [1833]) from Africa (8.4 %), the percentage of individuals with skin lesions in Golfo Dulce is consistent with those reported in these locations (Kiszka et al., 2009; Moreno et al., 2008; Murdoch et al., 2008; Félix, Van Bressem et al., 2019).

The estimated prevalence of LLD in *T. truncatus* from the Golfo de Nicoya was 100 % (LLD dolphin; n = 6 from identified individuals, all of them adults; n = 6) (Fig. 1) in 2019 and 2022. Such a high prevalence rate in an inshore dolphin community is unprecedented. The highest prevalence rate reported until now was 44.4 % in a small community (n= 9) of

T. truncatus from Salinas, Ecuador (Félix, Van Bressem et al., 2019). In Golfo Dulce the majority of LLD cases were documented in males (n=5), with only two females affected by the disease and two unidentified sex individuals. Similarly, in Golfo de Nicoya three males were identified as LLD dolphins, along with two females and one unidentified sex individual. The lesions of the dorsal fin progressed over years in a dolphin TtGN005 from Golfo de Nicoya and individual TtGD003 from Golfo Dulce (Fig. 1).

There are intrinsic demographic characteristics that differ between these two populations, while the coastal bottlenose dolphin population in Golfo Dulce would be considered discrete at just over 100 individuals, with a portion of it showing important site fidelity (Oviedo, 2018), the Golfo de Nicoya population is smaller than ten individuals. In the course of our assessment, we captured the same adult individuals on each sampling occasion, suggesting not only site fidelity but also an apparent lack of immigration into this population. There are other similarly small populations of the coastal bottlenose dolphin ecotype elsewhere in Latin America, such as the Santa Elena Peninsula, Ecuador, where these dolphins were observed intermittently from 2005-2018, with a mean group size of 5.31 dolphins/group (SD = 1. 97, range 1–10) (Félix, Zavala et al., 2019), and in the Tramandai estuary in southern Brazil, where they have been observed since the early 90s, with an average of nine dolphins (including calves) commonly recorded over the years (Di Giacomo & Otts, 2016), with at least two dolphins affected by LLD (study period 1991- 2008) (Moreno et al., 2008). Félix, Zavala et al. (2019) suggested that such small dolphin populations may be a remnant of larger communities, possibly affected by external environmental stressors, likely of anthropogenic origin. In the case of Golfo de Nicoya, the high prevalence of LLD in such a small community is a cause for serious concern.

Demography and social behavior influence the incidence and transmission of the disease (Félix, Van Bressem et al., 2019). Even though the analysis of social behavior and characteristics of these populations is in progress. There are important similarities in terms of sex classes occurrence, distribution, and social behavior profiles of both populations, with that reported by Félix, Van Bressem et al (2019). In the case of Golfo de Nicoya, as mentioned above, the high prevalence could be explained primarily by the demography of such a small, localized community, where most of the individuals affected are males, basically half of the adults observed in the field, with at least a high ranked individual (TtGN001; Fig.1). Whereas, in Golfo Dulce, LLD is equally observed mostly in males (five out of nine cases). The medium to low prevalence rate (13.14 %) could be affected by the incidence of the disease in one out of two male alliances. This lower-ranked pair (TtGD015 and TtGD016; Fig.1) had a wider home range than the dominant alliance. All of this supports the notion of horizontal disease transmission and geographic spread by non-resident individuals (Félix, Van Bressem et al., 2019; Van Bressem et al., 2015).

Although differences in sample size may explain some of this variation, environmental and individual factors are also likely to play an important role. Burdett Hart et al. (2011) suggested that differences in freshwater input could influence the development and persistence of lobomycosis between *T. truncatus* communities of west and east coast estuaries in Florida; this could be the case for Golfo Dulce and Nicoya.

In Golfo Dulce and Golfo de Nicoya, the presence of polychlorinated biphenyl compounds (PCBs, DDT, DDD, DDE, dieldrin, and BHC) is associated with agriculture practices (Spongberg & Davis, 1998; Spongberg, 2004a; Spongberg, 2004b; Umaña., 1998. Along the riverbanks draining into the Golfo Dulce, a number of agricultural practices have been developed, such as cattle ranching and monocultures of African palm and banana (Umaña, 1998). The increase in these activities has introduced heavy metals (Fe 5.8 ug/g, Zn 96 ug/g, Cu 87 ug/g, Pb 6 ug/g) and organochlorine pesticides into the water column (5000 ug/g) and sediments (15.7 ug/g) (Acuña-González et

Fig. 1. A. Cases of lobomycosis-like disease in inshore bottlenose dolphins from Golfo Dulce (framed by dark blue rectangle) and Golfo de Nicoya (framed by light blue rectangle) in the Pacific waters of Costa Rica. **1.b** Lesion progression in individual TtGD003 (Golfo Dulce) and TtGN005 (Golfo de Nicoya).

al., 2004; Spongberg & Davis, 1998; Spongberg, 2004a; Spongberg, 2004b).

Many other anthropogenic activities, including agriculture, forestry, tourism, aquaculture, salt-mining, mining and industry have been carried out in the Golfo de Nicoya, making the area vulnerable to contamination by petroleum hydrocarbons, wastewater, pesticides, heavy metals and organic compounds (León Coto et al., 1998; Marín-Alpizar, 2000;

Morera-Gonzalez et al., 2019;). These can be associated with other particles and become available to biota and bioaccumulate in the lipid tissues of organisms (León Coto et al., 1998; Marín-Alpizar, 2000; Morera-Gonzalez et al., 2019).

Very low concentrations of PCBs and DDT can bioaccumulate and suppress the immune system of these animals, thereby facilitating the onset of disease (Jepson et al., 2005; Reif et al., 2009; Ross., 2002), which could be the case in the Golfo Dulce and Golfo de Nicoya bottlenose dolphin population.

The anthropogenic impacts that the coastal populations of *T. truncatus* have been exposed to may become critical and even cause a decline in the largest and smallest populations of these coastal dolphins in the country. Although there is no documented mortality caused by the disease in Golfo Dulce and Golfo de Nicoya, a high rate of disease could lead to septicemia, considering the severity and sometimes open lesions, these can be exposed to bacteria (Van Bressem et al. 2015). Additionally, the spread to key anatomical areas, such as the rostrum, could impede prey consumption and result in emaciated individuals. If we put more pressure on these areas through coastal development, such as marinas and luxury hotels, the cetacean populations and the tourism industry that depends on them would be at risk. The restricted geographic range used by these populations of *T. truncatus* and the strong dependence of these dolphins on specific environmental characteristics (structure and available resources) makes them vulnerable to habitat loss and degradation. The persistence, and high prevalence levels of LLD, in small, localized, communities of *T. truncatus* in the Pacific Ocean of Costa Rica is a cause for serious concern.

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.

See supplementary material
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