WIRE INGESTION BY A RED-BILLED TROPICBIRD *PHAETHON AETHEREUS* CHICK ON SAN PEDRO MÁRTIR ISLAND, MEXICO

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Received 22 July 2024, accepted 30 August 2024

ABSTRACT

Piña-Ortiz, A., Marcuk, V., Gómez-Hernández, S., Castillo-Guerrero, J. A., & Quillfeldt, P. 2025. Wire ingestion by a Red-billed Tropicbird *Phaethon aethereus* chick on San Pedro Mártir Island, Mexico. *Marine Ornithology, 53*(1), 75–82. https://doi.org/10.....

We report the ingestion of wire by a Red-billed Tropicbird *Phaethon aethereus* chick at San Pedro Mártir Island, Gulf of California, Mexico. A scat sample collected from a 4- to 5-week-old chick contained a copper wire ~5.0 mm in length. Biologging revealed the previous foraging trips by one of the parents, and we ascertained the diet of birds in this colony through a molecular approach. From these data, we suggest why this individual was fed wire.

Key words: marine debris, Gulf of California, secondary ingestion, Red-billed Tropicbird, wire ingestion

RESÚMEN

Reportamos la ingestión de un alambre por un polluelo de Rabijunco Pico Rojo *Phaethon aethereus* en la Isla San Pedro Mártir, Golfo de California, México. Una excreta colectada de un polluelo de 4–5 semanas contenía un alambre de cobre de ~5.0 mm de longitud. Se registraron los viajes de forrajeo previos de uno de los progenitores y, mediante un enfoque molecular, determinamos la dieta de las aves de esta colonia. A partir de estos datos sugerimos como este individuo pudo ingerir el alambre.

Palabras clave: detrito marino, Golfo de California, ingestión secundaria, Rabijunco Pico Rojo, ingestión de alambre

INTRODUCTION

Despite significant efforts in recent decades to counteract the increasing pollution and deposition of non-degradable waste in the ocean (Kibria et al., 2023; Schmaltz et al., 2020; Wang et al., 2021; Willis et al., 2022), factors such as high consumption of disposable products, poor regional or local waste disposal control, and weak law enforcement have contributed to the rise of pollutants entering the ocean (Ostle et al., 2019; Sindermann, 1995). The cumulative effects of pollution are a major concern, necessitating an investigation into the impacts on the ecological functionality of marine ecosystems as well as the ecological and physiological consequences for marine wildlife (Cisneros-Montemayor et al., 2019; Sindermann, 1995).

According to the National Oceanic and Atmospheric Administration (NOAA), marine debris is defined as "any persistent solid material that is manufactured or processed directly or indirectly by anthropogenic activity and disposed or entering intentionally or unintentionally into marine or freshwater ecosystems" (NOAA, 2024). Marine debris ingestion has been documented in diverse marine wildlife, including invertebrates, fish, seabirds, sea turtles, and marine mammals (Laist, 1997; Nunes et al., 2021; Provencher et al., 2017; Ryan, 2016; Schuyler et al., 2014). In tropicbirds (family Phaethontidae), the ingestion of marine debris, particularly plastic, has been reported in all three species (Cartraud et al., 2019; Hyrenbach et al., 2013; Madden & Eggermont, 2020; Rapp et al., 2017; Robards, 1993; Sileo et al., 1990; Spear et al., 1995). There are no reports of any other kind of marine debris in tropicbirds.

The factors and pathways contributing to marine debris ingestion and accumulation in marine wildlife are not fully understood (Provencher et al., 2017). However, seabirds can acquire marine debris through direct or indirect ingestion or via the respiratory tract (see Hammer et al., 2016; Navarro et al., 2023; Tokunaga et al., 2023; Wayman et al., 2024). Additionally, environmental factors such as ocean currents, river discharge, wind, precipitation, and sediment processes, along with anthropogenic activities, increase the likelihood of debris ingestion or inhalation (Provencher et al., 2017; Su et al., 2022).

In this study, we report wire ingestion by a Red-billed Tropicbird chick on San Pedro Mártir Island, a protected insular ecosystem in the Gulf of California. This island hosts one of the largest colonies of the species in the region, with 150 pairs (Piña-Ortiz et al., 2018; Tershy & Breese, 1997). In addition to reporting this event, we combined geospatial tracking data and information on the main prey categories—fish and cephalopods—for this colony using DNA metabarcoding analysis. This information suggests a pathway leading to wire ingestion by the chick.

METHODS

Observations

From February to May 2021, we visited San Pedro Mártir Island (28°22′52″N, 112°18′23″W), a 1.9-km² landmass in the Gulf of California located about 50 km east and west from the Mexican states of Baja California and Sonora, respectively (Tershy & Breese, 1997). A seabird monitoring program allowed us to study the foraging ecology of several species breeding on the island (Castillo-Guerrero et al., 2022). Our tasks included measuring and weighing birds, deploying Global Positioning System (GPS) devices, collecting blood samples, and gathering scats (n = 71). Scats were collected opportunistically from both adults and chicks to perform molecular diet analysis (DNA metabarcoding; see Marcuk et al., 2024). All these activities, including wildlife handling and sample collection, were conducted with permission from the Subsecretaria de Gestión para la Protección Ambiental (SGPA, Mexico) and the Dirección General de Vida Silvestre (DGVS, Mexico) under permit SGPA/DGVS/02779/21. We adhered to all applicable institutional (Universidad de Guadalajara, Mexico; Justus Liebig University, Germany) and national (Secretaria de Medio Ambiente y Recursos Naturales, Mexico) guidelines relating to wildlife welfare and conservation.

On 17 March 2021, we focused our field activities on Punta Rabijunco in the northeast of the island, the area with the highest nesting density for Red-billed Tropicbirds. In one nest cavity, we found a single adult and a four- to five-week-old chick. The adult was ringed, and a GPS tag (CatLog-S2; Perthold Engineering LLC; Dallas, USA) was attached using TESA® tape (Norderstedt, Germany) to the tops of four to five central rectrices directly below the uropygial gland. The total weight of the GPS tag, including the tape (12–13 g), was ~1.9% of the adult's body mass (635 g), which is below the recommended threshold (< 3% of body weight; Vandenabeele et al., 2012; Wilson & McMahon, 2006). Five days after deploying the GPS tracker, we revisited the nest and recovered the device. During the handling process, we collected a scat sample from the chick in a 1.5-ml plastic tube and preserved it via suspension in absolute ethanol (99.5% purity; J.T. Baker®). The sample was initially stored in a portable freezer (−2 °C; GoSun®; Cincinnati, USA) in the field and later frozen in the laboratory at −20 °C for further analysis.

Analysis of GPS tracking data

We employed the same methodology used by Piña-Ortiz *et al.* (2024). GPS tracking data obtained from the bird were visually reviewed using the software CatLog_Data-viewer (version 1.0, Catnip Technologies, Ltd.; Hong King, China), with anomalous trajectories and ground-level fixes removed. Fixes indicating an average speed exceeding 80 km/h, the species' flight speed threshold, were discarded. Foraging parameters were determined in R (version 4.3.1; R Core Team, 2023) with RStudio version 2023.06.1 + 524 'Mountain Hydrangea' (RStudio Team, 2023) using the *tripSplit* function of the "track2KBA" package (Beal et al., 2021). This function enabled us to divide the individual's GPS trajectories into multiple foraging trips, separated by the bird's return to the colony. For each foraging trip, we calculated the maximum linear distance from the colony, total trip duration, and total distance travelled. Incomplete foraging trips (those that could not be fully tracked before the individual's return to the colony) were discarded. To ensure accurate partitioning of individual foraging trips and to ensure subsequent trips by burrow-nesting species were not mistakenly grouped as a single trip, we applied a 1.5-km radius filter around the colony to identify arrivals to the colony as the endpoint of a trip (Beal et al., 2021).

DNA analysis of feces

The DNA metabarcoding analysis methods for feces are described in detail by Marcuk *et al.* (2024). Briefly, DNA isolation and library preparation were conducted using the Qiagen Fast DNA Stool Mini Kit (Qiagen; Hilden, Germany). For prey identification at the family level, we employed a metazoan cytochrome oxidase I (COI) primer set during polymerase chain reaction (PCR) amplifications (Leray et al., 2013). Additionally, two specific 16S rDNA primer pairs (Berry et al., 2017; Waap, 2015) were used to identify the main prey categories—fish and cephalopods—based on prior knowledge of the species' diet. The PCR reaction was set up with a 20 µL volume, including 10 µL Qiagen Multiplex PCR Buffer, primers, and a DNA template. A touchdown PCR protocol was used to optimize amplification, with products below 0.5 ng/µL being re-amplified. The resulting amplicons were purified using the illustra™ ExoProStar™ 1-Step kit (Cytiva; Amersham, UK), pooled, and prepared for Illumina sequencing using the Nextera XT DNA Library Preparation Kit (Illumina; San Diego, USA). Final sequencing was performed on an Illumina MiSeq desktop sequencer with 250-basepair (bp) paired-end reads.

To obtain a list of molecular operational taxonomic units (MOTUs), we employed a custom workflow (Masello et al., 2021) in GALAXY (Galaxy Community, 2022). MOTU sequences were matched to reference sequences in the National Center for Biotechnology Information (NCBI) GenBank nucleotide database using the Basic Local Alignment Search Tool algorithm for nucleotides (BLASTn), with a cut-off of 90% minimum sequence identity and a maximum e-value of 0.00001 (Altschul et al., 1990). Taxonomic assignments were made based on the percentage similarity between query and reference sequences, retaining BLASTn assignments with greater than 98% similarity and a minimum sequence length of 190 bp (Deagle et al., 2009; Vesterinen et al., 2013). MOTUs were assigned to the species level only when all retained hits corresponded to the same species. Otherwise, assignments were made to the lowest shared taxonomic level, such as genus or family.

The raw dataset included various unspecific or contaminant DNA sequences, such as human and bacterial DNA, which were excluded from potential prey taxa based on previous literature (Almaguer-Hernández, 2016; Castillo-Guerrero et al., 2011; Diop et al., 2018; Madden et al., 2022, 2023; Nelson, 2006; Stonehouse, 1962). Non-prey MOTUs, including taxa from the orders Insecta, Reptilia, and Aves, were omitted during validation as they were either ecologically irrelevant or had distant distribution ranges. Following the approach of Masello *et al.* (2021), our analysis excluded records with fewer than 10 reads and those in singular MOTUs where the read number accounted for less than 1% of the maximum count.

To analyze the dietary composition of the two main prey groups—fish and cephalopods—we calculated both the frequency of occurrence (FO) and the relative read abundance (RRA). The RRA was used to complement the interpretation of FO (Barrett et al., 2007; McInnes et al., 2017; Young et al., 2020). The FO was determined using the following formula:

$$FO = \left(\frac{n}{t}\right) \times 100$$

where n represents the number of samples in which prey DNA was detected and t is the total number of samples where DNA from the considered prey group was present.

The RRA was calculated with the formula:

$$RRA = \left(\frac{\text{number of reads for a specific prey MOTU}}{\text{total number of reads for all prey MOTUs}}\right) \times 100$$

This represents the percentage ratio of reads in relation to the total number of reads recorded for the respective MOTU. By using both FO and RRA, we aimed to provide a more comprehensive understanding of the prey composition in the diet of the studied species.

RESULTS

Further examination of the feces sample collected from the chicks revealed a copper wire fragment compressed into a circular shape. The extracted wire was 0.3 mm thick and had a maximum deformed diameter of 2.7 mm. When fully extended, it measured 4.9 mm in length (Fig. 1).

The wire was noticed during sample collection, and a routine visual assessment of the chick immediately after confirmed no bleeding or external injuries. Its body mass was consistent with that of other chicks of the same age (620 g vs. 614 ± 37.3 g (mean ± standard error); n = 12), suggesting that the chick was in average body condition. No other plastic debris or wire fragments were present near or inside the nest cavity. Subsequent visits to assess the breeding success of the active nests in this area allowed us to confirm that the chick reached a fledgling age of 89 days (Nelson, 2006; Stonehouse, 1962) and departed the nest around mid-May 2021.

GPS tracking showed that the female parent made six foraging trips during the five days of tag deployment. Foraging trips occurred in two directions, to the northeast and southeast (Table 1, Fig. 2).

Regarding the diet analysis, DNA metabarcoding showed that Red-billed Tropicbirds at this colony prey predominately on fish (FO = 100%), followed by cephalopods (FO = 6.5%). An unidentified mackerel species *Scomber* sp. and Pacific chub mackerel *Scomber japonicus* (Scombridae; FO = 32.3% and FO = 12.9%, respectively), California anchovy *Engraulis mordax* (Engraulidae, FO = 45.2%), and South American pilchard *Sardinops sagax* (Clupeidae, FO = 22.6%) contributed the most frequently in the scat samples (n = 31). Otherwise, the RRA for fish prey families included Atherinopsidae (RRA = 45.3%), Scombridae (RRA = 21.7%), and Engraulidae (RRA = 12.9%; Table 2).

DISCUSSION

This study reports the ingestion of a section of copper wire by a Red-billed Tropicbird chick. A single previous report of marine debris ingestion by a Red-billed Tropicbird had included only plastic, and that was in a 5- to 6-week-old chick at St. Eustatius Island in the Caribbean (Madden & Eggermont, 2020). Plastic ingestion by marine wildlife has been widely reported (Laist, 1997; Ryan, 2016). Ingestion of wire, however, has been rarely documented, though there are some records in such seabirds as Black-browed Albatross *Thalassarche melanophris* (Petry et al., 2007), Kelp Gull *Larus dominicanus* (Yorio et al., 2020), Common Eider *Somateria mollissima* (Holland et al., 2016), Northern Fulmar *Fulmarus glacialis* (van Franeker & Meijboom, 2002); as well as Australian Pelican *Pelecanus conspicillatus*, Fairy Prion *Pachyptila turtur*, Slender-billed Prion *Pachyptila belcheri*, Fluttering Shearwater *Puffinus gavia*, Little Shearwater *Puffinus assimilis*, Short-tailed Shearwater *Ardenna tenuirostris*, Westland Petrel *Procellaria westlandica*, Little Black Cormorant *Phalacrocorax sulcirostris*, and Australian Pied Cormorant *Phalacrocorax varius* (Roman et al., 2016). It should be noted that Roman *et al.* grouped marine debris such as hooks and metal wires into "fishing" and "other" categories, respectively. Therefore, it is impossible to discriminate which species actually ingested this kind of metal debris.

The recorded diet spectrum indicated that fish represent the predominant prey for Red-billed Tropicbirds at San Pedro Mártir Island, which is consistent with prey preferences recorded for this species at other breeding colonies (Almaguer-Hernández, 2016; Castillo-Guerrero et al., 2011; Diop et al., 2018; Madden et al., 2022, 2023; Marcuk et al., 2024; Nelson, 2006). None of the prey previously cited or identified in this study resembles the characteristics of the wire fragment in size or color, which could rule out ingestion based on inappropriate prey recognition. As no traces of any other form of marine debris were found near or inside the nest cavity, direct ingestion by the adult or chick at the nest can be ruled out as a plausible origin.

The likely rational explanation is secondary ingestion of a prey item (probably a fish) swallowed by one of the parents and subsequently fed to the chick. All the main fish prey we detected obtain food by filter-feeding but switch to particulate feeding when prey densities are low (Castro-Hernández & Santana-Ortega, 2000; Hunter & Dorr, 1982; O'Connell & Zweifel, 1972; van der Lingen, 1994).

Fig. 1. Fragment of copper wire (4.9 mm long, 0.3 mm thick) obtained from the scat of a 4- to 5-week-old Red-billed Tropicbird *Phaethon aethereus* chick at San Pedro Mártir Island, Mexico.

TABLE 1
GPS tracking data for Red-billed Tropicbirds *Phaethon aethereus* on San Pedro Mártir Island, Mexico during the 2021 breeding season. Data are for one female parent during six foraging trips over a five-day deployment.

	Median	Maximum	Minimum
Duration	9.1 h	42.7 h	2.4 h
Total distance	126.5 km	399.3 km	57.7 km
Maximum distance from colony	44.5 km	158.4 km	28.4 km

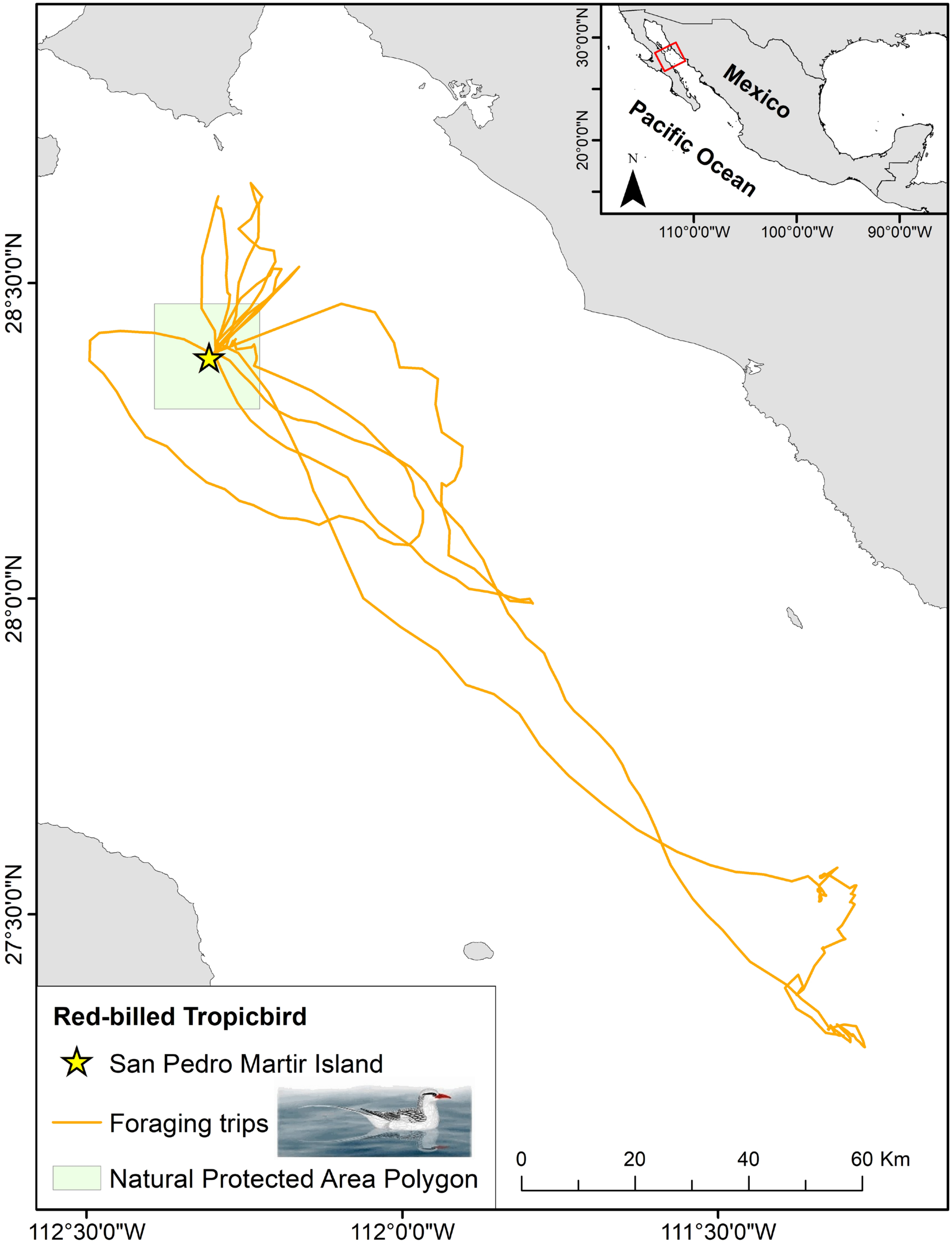

Fig. 2. Foraging trips made by the Red-billed Tropicbird *Phaethon aethereus* female parent from its nest on San Pedro Mártir Island, Mexico, prior to its chick excreting a wire fragment on 17 March 2021. The polygon of the Natural Protected Area is indicated in light green (Diario Oficial de la Federación, 2002).

TABLE 2
Summary of the frequency of occurrence (FO) and relative read abundance (RRA) of the dominant fish families in the prey of Red-billed Tropicbirds *Phaethon aethereus* on San Pedro Mártir Island, Mexico, during the 2021 breeding season.

Taxa	FO (% samples) *n* = 31	RRA (% reads) *n* = 51,679
OSTEICHTHYES		
Scombridae	45.2	21.7
Engraulidae	45.2	12.9
Carangidae	35.5	0.5
Clupeidae	32.3	5.2
Exocoetidae	29.0	7.1
Atherinopsidae	19.4	45.2
Clupeidae	12.9	5.2
Mullidae	9.7	2.1
Batrachoididae	9.7	0.9
Hemiramphidae	6.5	0.3

Food particle size is the prime determinant of the feeding mode (Louw et al., 1998). Based on size and buoyancy, most marine debris is found in the water column and is subject to water mass transport and mixing (Su et al., 2022). Therefore, in the case of copper wire, due to its length and weight, it is difficult to envision how it could enter the individual through filter-feeding. Some element of water mass dynamics could have been involved.

Adult foraging trips of the Red-billed Tropicbirds breeding on the study island overlapped with regional fisheries. All predominant fish prey taxa we found in the diet of the San Pedro Mártir birds are exploited by the fishing industry, like Pacific chub mackerel (Cisneros et al., 1990; Lo et al., 2010), Californian anchovy (Cisneros et al., 1990; Schwartzkopf et al., 2022; Velarde et al., 2013), and South American pilchard (Cisneros-Mata et al., 1995; Nevárez-Martínez et al., 2001; Velarde et al., 2013). Since 0.3-mm-thick copper wire is widely used in electrical wiring and in the operation of ship and small-boat engines, it is possible that a fragment from a vessel could be found floating in the ocean. That would support our hypothesis that the fragment was first ingested by a fish and then later by one of the tropicbird parents during a foraging trip.

As another possibility, ingestion of the wire could have come from baitfish. Sport fishermen often use small pieces of wire to secure bait, such as anchovies, ensuring that the fish remains on the hook while trolling. If bait is discarded with such a wire then consumed by an adult seabird during a foraging trip, the wire could have been incidentally ingested and subsequently fed to the chick. This possibility highlights the potential threats of fishing practices and their unintended impact on marine wildlife.

Considering the size of the wire fragment and the chick's subsequent development and assumed fledging, there appears to have been no consequent damage to the individual. However, in the absence of quantitative records that would allow more detailed interpretations of the direct and indirect risks of marine debris by these tropicbirds, no further assumptions or conclusions can be drawn regarding subsequent effects of ingestion.

The incidence of marine debris in the scat samples was considerably low (*ca*. 1.5%, 1 out of 71 samples). However, other approaches such as necroscopy or data from regurgitates could offer a better approximation of marine debris ingestion by these birds. Therefore, we suggest the establishment of a long-term monitoring scheme that includes the incidence of marine debris ingestion by seabirds in order to assess the level of impact of this threat on the health and breeding parameters of seabird populations in the Gulf of California.

ACKNOWLEDGMENTS

We thank Departamento de Estudios para el Desarrollo Sustentable de Zonas Costeras CUCSUR – Universidad de Guadalajara and Comisión Nacional de Áreas Naturales Protegidas (CONANP – Islas del Golfo de California – Reserva de la Biósfera Isla San Pedro Mártir) for logistical support in the field, specially J. Ventura-Trejo. We also thank Captain E. Ramírez-León for helping us access the island and S. Hernández-Vázquez, M. García-Zamora, and J. Martín-Moreno for their help during fieldwork. Funding was provided by Sonoran Joint Venture (MBJV / F20AP11531-00) and Universidad de Guadalajara. APO was financed by a CONACYT scholarship for doctoral studies abroad (No. 795355) and by a student research grant from the Pacific Seabird Group [PSG SRGA 2021]. VM was granted by the Deutsche Ornithologische Gesellschaft (DOG) with a Forschungsföderung (2021) to cover the costs of the metabarcoding analyses. We also would like to thank Sabine Wagner for the support during the laboratory work. Reviewers and D. Ainley provided valuable comments that helped us to improve the paper. The authors have no competing interests to declare with respect to this work.

AUTHOR CONTRIBUTIONS

AP-O: Conceptualization, fieldwork, formal analysis and investigation, methodology, writing—original draft preparation, writing—review and editing, visualization, funding acquisition, resources. VM: Conceptualization, fieldwork, formal analysis and investigation, writing—original draft preparation, writing—review and editing, visualization, funding acquisition, validation. SG-H: Fieldwork, Formal analysis and investigation, writing—review and editing. JAC-G: Conceptualization, fieldwork, investigation, methodology, writing—review and editing, supervision, project administration, funding acquisition, resources. PQ: Conceptualization, formal analysis and investigation, methodology, writing—review and editing, supervision, funding acquisition, resources.

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