Justus Liebig University Giessen

The Breeding Ecology of the Red-Billed Tropicbird (*Phaethon aethereus*) within a Productivity Gradient: Evaluating the Impact of Local Conditions on Body Size and Foraging Ecology

---

Alberto Piña Ortiz

Dissertation presented for the degree of
Doctor rerum naturalium

Faculty 08 Biology and Chemistry
Giessen | June 2024

JUSTUS-LIEBIG-
UNIVERSITÄT
GIESSEN

JUSTUS LIEBIG UNIVERSITY GIESSEN

The Breeding Ecology of the Red-Billed Tropicbird (*Phaethon aethereus*) within a Productivity Gradient: Evaluating the Impact of Local Conditions on Body Size and Foraging Ecology

---

SUPERVISORS / BETREUER

Prof. Dr. Petra Quillfeldt

Justus Liebig University Giessen | Germany

DEAN / DEKAN

Prof. Dr. Holger Zorn

FB08 - Biologie und Chemie Justus Liebig University Giessen | Germany

REVIEWERS / GUTACHTER

Prof. Dr. Emily Poppenborg Martin

Justus Liebig University Giessen | Germany

Prof. Dr. Nikola-Michael Prpic-Schäper

Justus Liebig University Giessen | Germany

Prof. Dr. Simon Thorn

Philipps Universität Marburg | Germany

Giessen | 2024

DECLARATION / SELBSTSTÄNDIGKEITSERKLÄRUNG

I declare that I have completed this dissertation single-handedly without the unauthorized help of a second party and only with the assistance acknowledged therein. I have appropriately acknowledged and cited all text passages that are derived verbatim from or are based on the content of published work of others, and all information relating to verbal communications. I consent to the use of anti-plagiarism software to check my thesis. I have abided by the principles of good scientific conduct laid down in the charter of the Justus Liebig University Giessen „Satzung der Justus-Liebig-Universität Gießen zur Sicherung guter wissenschaftlicher Praxis“ in carrying out the investigations described in the dissertation.

_____

Alberto Piña Ortiz

CONTENTS

ABSTRACT

The environment plays a central role in shaping the biology of marine life at different scales and ecosystems. Factors such as wind, temperature, salinity, pH levels, topography and nutrient availability affect the behaviour, distribution and adaptation of marine organisms, influencing their physiology, behavioural patterns and life-history strategies. Therefore, this thesis investigates how local environmental conditions (e.g. air temperature, sea surface temperature, chlorophyll-a, bathymetry) affect the body size and foraging ecology (behaviour and diet) of the Red-billed Tropicbird (*Phaethon aethereus*) along a productivity gradient in the Mexican Pacific, in order to understand how the environment influences the biology of this seabird and how it adjusts its phenotype, behaviour and diet in response to local conditions. The body size variation and sexual size dimorphism (SSD) in colonies of red-billed tropicbirds along a productivity gradient in the Mexican Pacific are evaluated (Chapter 1). The species shows phenotypic plasticity with an increase in body size from south to north (1-9%), correlated with environmental productivity. SSD is only present in two northern colonies, where males are larger than females. The SSD detected in colonies with larger body sizes, together with high chlorophyll-a values and low sea surface temperature values, suggests that environment-mediated body size variation is a crucial factor in SSD. Tracking data combined with stable isotope values (δ^{15}N and δ^{13}C), and observations of parental nest presence, meal size and chick feeding events, show that breeding adults employ a bimodal foraging strategy as soon as the chick hatches, and that the parental duties of offspring care and provisioning are clearly linked to the foraging behaviour of the species during this stage (Chapter 2). A comparative assessment of the feeding ecology of the species, through analysis of faecal (DNA metabarcoding) and blood (stable isotopes) samples, between sites located in systems of contrasting productivity, revealed that the species exhibits trophic plasticity based mainly on the consumption of mesopelagic and epipelagic offshore fish (Chapter 3). Furthermore, variation in diet between sites is influenced by the abundance and composition of prey present in each system, and fluctuations throughout the breeding season are linked to prey availability due to changes in local oceanographic conditions, as well as to the energetic demands that adults must satisfy according to their breeding stage. Overall, this research highlights the phenotypic and trophic plasticity of red-billed tropicbirds, as well as their foraging behaviour. These aspects are influenced by environmental factors, demonstrating that this interaction is fundamental to the species' ecology. The findings contribute to a better understanding of the species' ecology, specifically its foraging behaviour, feeding habits, and evolution. The information from this dissertation is expected to be useful for developing current or future marine management plans that promote sustainable use of marine resources, while considering habitat protection and the conservation of biological components.

ZUSAMMENFASSUNG

Umweltgegebenheiten spielen auf verschiedenen Ebenen eine zentrale Rolle für die Ausprägung biologischer Eigenschaften mariner Lebewesen in diversen Ökosystemen. Umweltfaktoren wie Wind, Temperatur, Salzgehalt, pH-Wert, Topografie und Nährstoffverfügbarkeit wirken sich auf das Verhalten, die räumliche Verteilung und die Adaptionsmechanismen von Meeresorganismen aus und beeinflussen ihre Physiologie, ihre Verhaltenaweisen und ihre life-history Strategien. In der vorliegenden Doktorarbeit wird daher untersucht, wie sich lokale Umweltbedingungen (z.B. Lufttemperatur, Meeresoberflächentemperatur, Chlorophyll-a, Bathymetrie) auf die Körpergröße und die Nahrungsökologie (Nahrungssuchverhalten und Nahrungszusammensetzung) von Rotschnabel-Tropikvögeln (*Phaethon aethereus*) in einem Produktivitätsgradienten im mexikanischen Pazifik auswirken, um zu verstehen, wie die Umwelt die Biologie dieses Seevogels beeinflusst und wie er seinen Phänotyp, sein Verhalten und seine Ernährung als Reaktion auf lokale Umweltbedingungen anpasst. Die Variation der Körpergröße und der Sexualdimorphismus in der Körpergröße (SSD) in Kolonien von Rotschnabel-Tropikvögeln entlang eines Produktivitätsgradienten im mexikanischen Pazifik werden bewertet (Kapitel 1). Die untersuchte Art zeigt phänotypische Plastizität mit einer Zunahme der Körpergröße von Süden nach Norden (1-9 %), die mit der Umweltproduktivität korreliert. SSD ist nur in zwei nördlichen Kolonien vorhanden, wobei die Männchen größer sind als die Weibchen. Die in Kolonien mit höheren Körpergrößen festgestellte SSD in Verbindung mit hohen Chlorophyll-a-Werten und niedrigen Werten der Meeresoberflächentemperatur deutet darauf hin, dass die durch die Umwelt vermittelte Variation der Körpergröße ein maßgebender Faktor für SSD ist. Telemetriedaten in Verbindung mit stabilen Isotopenwerten (δ15N und δ13C) sowie Beobachtungen der elterlichen Nestpräsenz, der Größe der Mahlzeiten und der Fütterungshäufigkeit der Küken zeigen, dass brütende Adulte eine bimodale Futterstrategie anwenden, sobald die Küken geschlüpft sind, und dass die elterlichen Aufgaben der Nachkommenbetreuung und -versorgung eindeutig mit dem Ernährungsverhalten der Art in dieser Brutphase verbunden sind (Kapitel 2). Eine vergleichende Bewertung der Ernährungsökologie der Art durch Analyse von Kot- (DNA-Metabarcoding) und Blutproben (stabile Isotope) zwischen Brutstandorten mit unterschiedlicher Produktivität ergab, dass die Art eine trophische Plastizität aufweist, die hauptsächlich durch den Verzehr von meso- und epipelagischen Hochseefischen bedingt ist (Kapitel 3). Die Schwankungen während der Brutsaison hängen mit der Verfügbarkeit der Beutetiere aufgrund von Veränderungen der lokalen ozeanografischen Bedingungen sowie mit den energetischen Anforderungen der Elterntiere zusammen, die die brütenden Individuen je nach ihrem Brutphase erfüllen müssen. Zusammenfassend unterstreichen diese Untersuchungen die phänotypische und

trophische Plastizität von Rotschnabel-Tropikvögeln sowie ihr Verhalten bei der Nahrungssuche. Diese Aspekte werden durch Umweltfaktoren beeinflusst, was zeigt, dass diese Interaktion für die Ökologie der Art von grundlegender Bedeutung ist. Die Ergebnisse tragen zu einem besseren Verständnis der Ökologie dieser Seevogelart bei, insbesondere ihres Nahrungssuchverhaltens, ihr Ernährungsökologie und ihrer Evolution. Die Informationen aus dieser Dissertation können für die Entwicklung aktueller oder künftiger Mangementpläne, die den Schutz mariner Lebensräume und den Erhalt der damit verbundenen biologischen Komponenten berücksichtigen und somit eine nachhaltige Nutzung mariner Ressorcen fördern, nützlich sein.

SYNTHESIS

1| General Introduction

Environment and its relation to the biology of wildlife

Firstly, I found it necessary to introduce the term biodiversity, which refers to all the variety and variability of life that exists on earth, including variation at the genetic, species, and ecosystem levels (Walker 1992; Harper and Hawksworth 1994; Benn 2010). The environment naturally has a close relationship with all the species that exist on the planet (biodiversity), and with the biology of these species. Basically, species within the ecosystem context depend on interactions with other organisms and the environment in which these exist (Vegiopoulos 2019). Biodiversity is the outcome of macro- and micro-evolutionary processes over millions of years that enable species to survive and adapt to the diverse environmental conditions Earth has experienced and continues to undergo (Harper and Hawksworth 1994).

Now, in the context of the ocean, biotic and abiotic factors act as the major drivers of marine wildlife biology, shaping the structure and function in marine ecosystems (e.g., coral reefs, estuaries, open ocean), and influencing the behaviour, distribution, and adaptation of marine organisms across spatial and temporal scales (Elliot and Whitfield 2011; Costello and Chaudhary 2017; Wagner et al. 2020). The environment influences marine wildlife to adapt and evolve in response to changing conditions (Bindoff et al. 2019). Factors such as temperature, salinity, pH levels, and nutrient availability dictate the suitability of habitats for different species. Adapting to these conditions ensures survival and successful reproduction within specific ecological niches (Palumbi and Palumbi 2014; Morrissey et al. 2018; Howell 2023). These environmental factors directly affect the physiology, behaviour, and life history strategies of marine wildlife. For instance, changes in water temperature can impact metabolic rates, breeding cycles, and migration patterns of marine organisms (see Boyd 2004; Weimerskirch 2007; Andrews and Ernstipp 2016). Variations in ocean currents, tides, and wave action also influence feeding behaviours, foraging ranges, and habitat selection (e.g., Gaspar et al. 2006; Yoda et al. 2014). The range of marine ecosystems present unique environmental conditions and resources that determine the distribution and abundance of species. For instance, coastal zones, coral reefs, estuaries, and polar regions offer distinct environments, all of which provide unique assemblages for marine life. Environmental changes affect marine ecosystems at various time scales (e.g., seasonal or annual) determining the biological cycles and migratory patterns of marine fauna. In addition, long-term trends such as climate change and human activities are altering the marine environment at an unprecedented rate (Orgeret et al. 2022). These changes lead to shifts in species

distributions, phenology and ecosystem dynamics over time, impacting on the resilience and adaptability of marine species.

Seabirds and the marine environment

The seabirds, which include albatrosses, petrels, penguins, gulls and others, have a fundamental role in the dynamics of the marine ecosystem. These birds have evolved several physiological adaptations, such as salt glands, and unique feeding strategies that have enabled them to live at sea (Schreiber and Burger 2001; Hamer et al. 2001). Their wide diversity and ability to thrive at sea make seabirds key indicators of ocean health and its dynamics.

Marine and coastal ecosystem changes are particularly noticeable in seabirds. Their foraging patterns and distribution are closely tied to the availability and abundance of prey species, making them sensitive to variations in ocean conditions, such as temperature, currents, and prey availability (Shealer 2001; Hamer et al. 2001). Seabirds also play a role in nutrient cycling in the environment, as they are considered to be fertilisers of coastal areas by transferring marine nutrients to terrestrial ecosystems through their guano (Zmudczyńska-Skarbek and Balazy 2017; Schnug et al. 2018). This process has a cascading effect on local flora and fauna, influencing the overall biodiversity of these ecosystems (Schnug et al. 2018; Signa et al. 2021). Monitoring seabird populations provides scientists with insights into the health of marine ecosystems and helps identify potential threats, such as overfishing or changes in climate (Boersma et al. 2001; Montevecchi 2001).

Furthermore, the conservation of seabirds is crucial for maintaining the balance of marine food webs. As both predators and scavengers, they regulate prey populations and contribute to the overall stability of marine ecosystems. Human activities, including pollution, habitat destruction, and climate change, pose significant threats to seabird populations, highlighting the importance of conservation efforts to protect these birds and the marine environments they inhabit (Boersma et al. 2001; Burger and Gochfeld 2004).

Research on seabird ecology for marine environmental conservation and management

Research on seabird ecology is of great importance for the conservation and management of marine resources (Ronconi et al. 2023; Young and VanderWerf 2023). Due to their high sensitivity, seabirds are often excellent indicators of ocean health and dynamics, providing relevant information about the conditions of the marine environments in which they inhabit (Parsons et al. 2008). Aspects such as feeding, distribution, and population trends are closely related to prey availability, ocean conditions, and environmental variations (Young and

Ballance 2023). Constant monitoring of seabirds serves as an early warning system, helping to detect early changes and disturbances in marine ecosystems (Diamond and Devlin 2003).

The use of seabirds as ecological indicators provides insights into the dynamics of climatic and/or oceanic processes (e.g., ENSO phenomenon, oceanic currents), especially when such research is focused at the species level with a wide distribution, providing the opportunity to assess morphological, physiological, genetic, and behavioural aspects across multiple colonies (Yamamoto et al. 2016; Nunes and Bugoni 2018; Petalas et al. 2021). For instance, seabirds exhibit a broad range of body sizes, ranging from smaller species, such as storm petrels, to larger ones, like albatrosses (Brooke 2001). However, assessing the body size variation within a single species could enable to explore the ecological implications of size differences (Blackburn et al. 1999). Variations in body size could influence several aspects in the ecology of the species, as foraging ranges, energy requirements and breeding strategies. Therefore, the assessment of intra-specific body size variation can provide information on how species adapt to local environmental conditions, contributing to a more accurate understanding of their ecological roles (e.g., Yamamoto et al. 2016; Nunes et al. 2017).

Moreover, seabirds display a diverse range of foraging behaviours, including plunge-diving, surface-feeding, and other specialized techniques (Shealer 2002). Then, the evaluation of the foraging behaviour on a species-specific basis enhances our understanding of the foraging strategies employed by each colony or even by indivuduals therein (Mott et al. 2016; Horswill et al. 2023). Furthermore, due to their ecological diversity, seabirds exhibit species-specific dietary preferences. Diet ranges from fish to squid and other invertebrates, highlighting the adaptability of each species to the distinct prey resources available in their respective foraging areas (Shealer 2002; Barrett et al. 2007). Diet determination by species can provide information on the composition and utilisation of items in marine food webs. Intra- and inter-population analysis of the long-term diet of a species can be a turning point to elucidate whether alterations in diet composition are occurring, serving as an accurate bioindicator of changes in oceanic conditions (Iverson et al. 2007; Jacoby et al. 2023; Querejeta et al. 2023). In this respect, the evaluation of the food composition of seabird species is fundamental to understanding the dynamics of marine ecosystems and the ecological functions of seabirds in the ocean. Accordingly, on this research I aimed to address certain aspects of Red-billed Tropicbird breeding ecology related to body size, foraging behaviour and diet, and how these are adjusted based on local environmental conditions in different colonies along the Mexican Pacific.

Evolutionary ecology - Patterns of geographic variation in body size as adaptations to local environmental gradients

Phenotypic plasticity can be defined as the ability of a genotype to generate different phenotypes depending on specific environmental conditions (Pigliucci 2001; West-Eberhard 2003; deWitt and Scheiner 2004; Moczek et al. 2011; Fig. 1). Essentially, this term encompasses all types of phenotypic variation induced by the environment, which can influence morphological, physiological and behavioural aspects of the phenotype of an organism, as well as its life history (Sommer et al. 2020). Seabirds have a number of attributes for life in the ocean, such as highly developed flight skills, physiological adaptations and opportunistic feeding strategies that have allowed them to be widely distributed and inhabit different regions throughout the sea (Goldstein 2001; Shealer 2001; Paiva et al. 2010; Elliott et al. 2013; Putman et al. 2020; Wynn et al. 2020; Sutton et al. 2023). This wide distribution has allowed several species to inhabit regions with different climates (e.g., tropical-temperate or temperate-polar), so that populations have made a number of adaptations based on the climate they inhabit (Friesen et al. 2007; Nunes and Bugoni 2018). For example, intraspecific variations in body size or physical traits have been observed in seabirds distributed along geographic gradients (Moen 1991; Barrett et al. 1997; Wojczulanis-Jakubas et al. 2011; Jakubas et al. 2014; Yamamoto et al. 2016). These geographic variations in body size have been attributed to abiotic (e.g., sea surface temperature, air temperature, latitude, longitude, and wind speed) and biotic (e.g., competition, predation, genetic differentiation, sexual selection, and prey availability) factors. However, research on the influence of abiotic factors has received the most attention (Jakubas et al. 2014; Bandeira et al. 2016; Seeholzer and Brumfeld 2017).

Under this approach, research on intraspecific geographic variation in seabirds with temperate or polar distribution has indicated that variations in body size are mainly attributed to environmental temperature (Moen 1991; Barrett et al. 1997; Wojczulanis-Jakubas et al. 2011; Jakubas et al. 2014; Yamamoto et al. 2016). These results are in agreement with the rules of Bergmann and James, based on the heat conservation hypothesis, which assumes that the heat loss of an endothermic organism is proportional to its surface area:volume ratio (Bergmann 1847; Blackburn et al. 1999). Consequently, species distributed along a latitudinal gradient are expected to exhibit a cline in body size, with small and large sizes in warmer and colder areas, respectively (Bergmann 1847; Blackburn et al. 1999). However, this pattern does not fully align with that found in seabirds with tropical distributions, as no direct relationship has been found between body size and air temperature in these areas (Le Corre and Jouventin 1999; Nunes et al. 2017). Therefore, it has been established that, for species whose populations are distributed over large tropical and subtropical areas, not only temperature plays a central role in

body size variations, but also other environmental (e.g. chlorophyll-a and wind speed), genetic (e.g. population structure) and/or ecological (e.g. foraging behaviour) factors have a significant influence on this phenomenon (Jakubas et al. 2014; Yamamoto et al. 2016; Nunes et al. 2017).

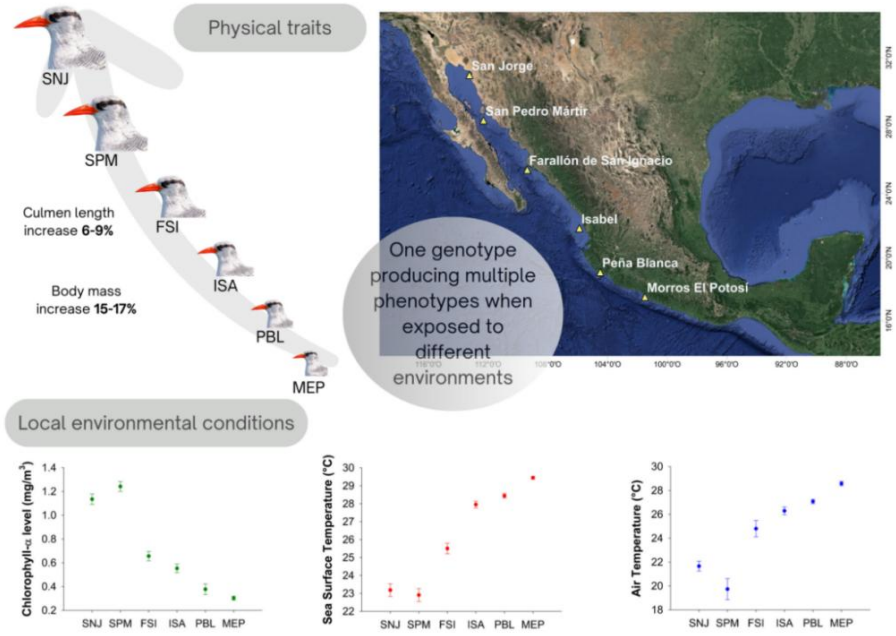


Figure 1. A graphical illustration of phenotypic plasticity. This particular case shows the variation of body size and culmen length in the Red-billed Tropicbird across its distribution range in the Mexican Pacific. Six breeding sites with local environmental parameters and a productivity gradient. SNJ = San Jorge, SPM = San Pedro Mártir, FSI = Farallón de Sam Ignacio, ISA = Isabel, PBL = Peña Blanca, MEP = Morros El Potosí.

Movement Ecology - Foraging behaviour of a central-place forager

Movement ecology is an interdisciplinary field that focuses on the study of animal movement habits and their ecological implications. It covers a wide range of species, from insects over birds and mammals, and aims to understand the mechanisms that regulate their movements (González-Solís and Shaffer 2009; McGlynn 2012; Courbin et al. 2022; Reyna-Hurtado et al. 2023). Charles Sutherland Elton, a pioneer of Animal Ecology, highlighted the differential features of animal systems, emphasising their inherent mobility (Elton 1933). This perspective gave rise to Movement Ecology, which relates individual behaviour to spatial processes at various ecological levels, from populations to communities. This is why recent efforts to consolidate movement studies are united under the framework of Movement Ecology, providing conceptual unity to the field (Börger 2016). Research within Movement Ecology covers a wide range of issues, exemplifying its broad applications. In particular, research on the effects of the environment on the movement decisions of wildlife stands out, especially in the current scenario of global change (Allen and Singh 2016). For instance, the response of migratory birds to extreme weather events sheds light on how animals adapt to environmental fluctuations (Senner et al. 2015). In diving seabirds, it has been shown that behavioural flexibility may not be sufficient when

constraints on movement capacity interact with changing environmental conditions (Orben et al. 2015). Foraging movements of surface-diving seabirds show the importance of oceanographic processes in determining prey accessibility, with implications for marine reserve design (Boyd et al. 2015).

Central-place foragers, as seabirds, represent an opportunity to elucidate the mechanisms by which these animals navigate and exploit their environment from a central location (Bell 1990). Hence, in seabirds, movement ecology can approach the study of the interaction between ecological functions and the dynamic of foraging strategies during the breeding season (González-Solís and Shaffer 2009). Breeding seabirds must cope with the demands of their parental duties, as well as the constraints of central-place foraging in the marine environment (Young and Ballance 2023). Studying the dynamics of their movements provides insight into how they optimise resource acquisition, select feeding habitats and adapt to spatio-temporal fluctuations in prey availability (Chimienti et al. 2017). Parental duties play a key role in shaping foraging behaviour in breeding seabirds (e.g., Wojczulanis-Jakubas et al. 2018; Piña-Ortiz et al. 2024: Fig. 2). The foraging behaviour of adults can be distinguished as the breeding season progresses. For instance, during courtship, foraging may be influenced by the need to exhibit fitness, which contributes to mate attraction. Once eggs have been laid, adults requires energy-efficient foraging to sustain long periods in the nest. As chicks hatch and progress through their growth, foraging dynamics in adults seabirds undergo significant adjustments. To meet the growing nutritional needs of chicks, parents may modify their foraging behaviour, including the selection of prey that offer greater nutritional value or are more energy efficient to capture, while still meeting their own nutritional requirements (Lerma et al. 2022; Phillips et al. 2023b; Piña-Ortiz et al. 2024).

Understanding the foraging behaviour of central-place seabirds requires a integrated research approach. Tools such as GPS tracking and direct observation provide direct information on movement patterns and behaviours. These technologies deliver an immediate and qualitative insight into the daily activities of seabirds (Browning et al. 2018; Bernard et al. 2021). In addition, the use of molecular tools and stable isotope analysis deepens our understanding of foraging ecology, revealing details about trophic levels, prey preferences and dietary variation (Deagle et al. 2007; Inger and Bearhop 2008; Carreiro et al. 2020; Ceia et al. 2022). While GPS tracking and direct observation capture immediate behaviours, stable isotopes provide a detailed understanding of the underlying ecological dynamics. This combined approach ensures a comprehensive exploration of how seabirds forage across their marine environment, make foraging decisions and also contribute to the ecosystem (e.g., Votier et al. 2010; Mendes et al. 2018).

Investigating the movement ecology and foraging behaviour of breeding seabirds holds profound significance for marine ecology, conservation efforts and resource management. These studies contribute crucial insights into the health and dynamics of marine ecosystems, serving as indicators of environmental marine conditions (Dunphy et al. 2020). Understanding how seabirds navigate, optimize resource acquisition, and adapt to varying conditions during the breeding season, researchers gain valuable information about the availability and distribution of marine resources (Weimerskirch et al. 1994). This knowledge is crucial in shaping effective conservation strategies for marine species and in managing marine resources sustainably. Moreover, breeding seabirds play a vital role in regulating local marine populations by controlling prey abundance, thereby influencing the overall balance within ecosystems (Elliott et al. 2008; de la Cruz et al. 2022; Montevecchi 2023). Conservation initiatives driven by insights from movement ecology studies not only protect biodiversity but also help secure fisheries, ensuring a sustainable supply of marine resources for human populations (Lescroël et al. 2016). Ultimately, the investigation of movement ecology in breeding seabirds contributes to a comprehensive understanding of marine ecosystems, fostering their resilience and benefiting both wildlife and human communities.

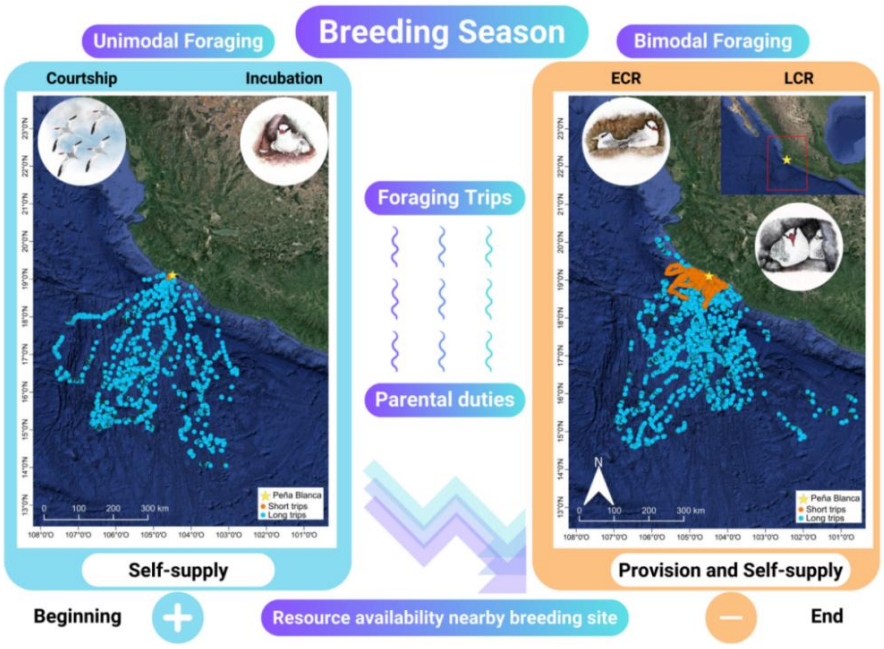


Figure 2. Graphical representation of the foraging strategies adopted by The Red-billed Tropicbird (*Phaethon aethereus*) during its breeding season. This case, the species initially undertakes long foraging trips - Unimodal foraging strategy - at the start of the breeding season. However, once the chick hatches, parents adopt a bimodal foraging strategy, alternating between short trips for chick provisioning and long trips for self-supply. This suggests that the bimodal foraging strategy is linked to parental duties and the availability of resources close to the breeding site. Bird illustrations elaborated by Vladislav Marcuk.

Foraging ecology - Dietary variation and foraging areas based on local environmental conditions

Foraging ecology plays a fundamental part in marine community dynamics, revealing how species manage foraging and food acquisition in their respective habitats (Croll et al. 1998; Shealer 2001; Lerma et al. 2020; Don Bowen and Jonsen 2022). This field of study focuses on understanding feeding behaviour patterns and prey selection strategies in response to highly variable environmental conditions (Stephens et al. 2007; Danchin et al. 2008). This variability is evidenced in seabirds, where colonies of the same species can exhibit marked differences in their diets and foraging areas (Paiva et al. 2010; Diop et al. 2018; Jacoby et al. 2023). These variations are intrinsically linked to local marine environmental conditions (e.g., sea surface temperature, chlorophyll-a, bathymetry), prey availability, oceanographic currents and other ecological (e.g., competition, chick provisioning, sex-specific foraging behaviour) and physiological (e.g., nutritional requirements) factors (Paiva et al. 2010; Reyes-González et al. 2021; Jacoby et al. 2023; Querejeta et al. 2023). This highlights the adaptability of populations to their specific environments, revealing the complexity of interactions between seabirds and their habitat.

Trophic plasticity refers to the ability of species to adjust their feeding habits in response to variations in resource availability (Paiva et al. 2010; Dehnhard et al. 2016; Gaglio et al. 2018; Fig. 3). This adjustment is fundamental for seabirds and other organisms, allowing them to optimise their diet and foraging strategies according to changes in the marine environment (Barrett et al., 2007; Masello et al. 2010, Dehnhard et al. 2016). For seabirds, this adaptability has been explored through studies examining trophic plasticity as a crucial mechanism in the dynamics of foraging ecology, playing a prominent role in the survival and breeding success in changing marine environments (Cherel et al. 2014; Gaglio et al. 2018). Research in this area has revealed variations in diet and foraging areas in seabird populations sharing a common habitat (Reisinger et al. 2020; Petalas et al. 2021; Fromant et al. 2021). Traditionally, conventional methods, such as analysis of stomach contents and regurgitates, have been fundamental to understanding the feeding preferences of seabirds (Chiaradia et al. 2003; Barrett et al. 2007). However, limitations of these methods, such as their invasiveness and lack of taxonomic resolution in some cases, have driven the development of advanced approaches. Novel methods, such as DNA metabarcoding and stable isotope analysis, offer significant advantages in providing a more complete and accurate insight into seabird diets (Deagle et al. 2007; Inger and Bearhop 2008). The ability of DNA metabarcoding to identify prey species in more detail and stable isotope analysis to provide information on trophic levels consumed represent valuable advances. Combining these techniques not only overcomes the individual limitations of each method, but also provides a more integrative understanding of seabird trophic

ecology, allowing for more comprehensive and accurate investigations in this dynamic field (Carreiro et al. 2020; Ceia et al. 2022).

The identification of diets and their variability in seabird populations enhances the understanding of trophic ecology, and also plays a crucial role from a number of perspectives that are fundamental for environmental management and marine conservation (Phillips et al 2023a; Kim et al. 2023; Ronconi et al. 2023; Young and VanderWerf 2023). These studies provide valuable information on the health and dynamics of marine ecosystems by revealing key trophic interactions between seabirds and their prey. From an ecological perspective, understanding how these species respond to fluctuations in food availability sheds light on the resilience and adaptability of marine ecosystems in the face of environmental change (Young and Ballance 2023; Ronconi et al. 2023; Pistorius et al. 2023). Furthermore, such research is essential for conservation, as it allows the identification of potential threats, such as overexploitation of resources or habitat degradation, and the design of effective management strategies (e.g., Vilchis et al. 2015; Kim et al. 2023). Variability in the diets of seabirds also has direct implications for humans, as many communities depend on fisheries and marine resources for their livelihoods (Tasker and Sydeman 2023). Understanding how seabird populations relate to marine resources contributes to the sustainable management of these areas, promoting ecosystem health and ensuring resource availability for future generations (Montevecchi 2023). The importance of these studies lies ultimately in the intricate connection between the health of seabird populations, marine ecology and human well-being, highlighting the need to continue and deepen this crucial research.

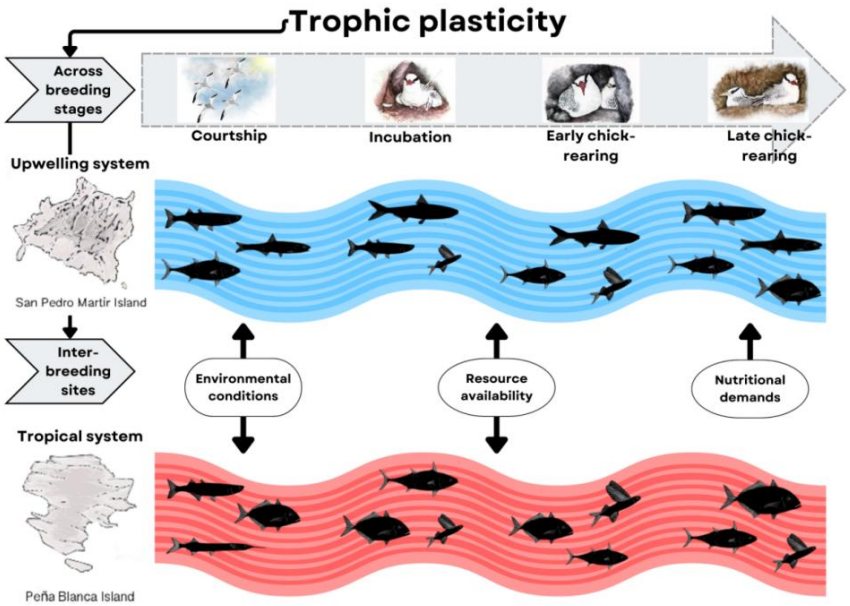


Figure 3. Graphical illustration of the trophic plasticity adopted by red-billed tropicbirds during the breeding season. The species shows dietary plasticity throughout its breeding season and between breeding sites.

Specifically, this case illustrates how dietary profile between breeding sites (Upwelling vs. Tropical) is different and how fish prey change between breeding stages. Bird illustrations elaborated by Vladislav Marcuk.

The family Phaethontidae (Tropicbirds)

The tropicbirds are a family of tropical pelagic seabirds currently classified in their own order (Phaethontiformes; Chesser et al. 2010). Previously, tropicbirds were grouped in the order Pelecaniformes, but molecular research has revealed that Phaethontiformes are distantly related to Procellariiformes, and they are currently classified in the clade Eurypygimorphae (Kennedy and Spencer 2004; Hackett et al. 2008; Mayr et al. 2023).

The genus Phaethon consists of three species: the Red-billed Tropicbird (*Phaethon aethereus*), the Red-tailed Tropicbird (*P. rubricauda*), and the White-tailed Tropicbird (*P. lepturus*). They are characterised by predominantly white plumage with elongated central tail feathers, and short, weak legs that are of limited use for propulsion (Nelson 2006). In general, individuals are medium-sized and robust but aerodynamic, measuring up to 50 cm in length excluding the elongated tail rectrices. *P. rubricauda* is the largest species in the genus, weighing between 590–1,095 g (n = 38; Nelson, 2006), followed by *P. aethereus* (weight: 450–720 g, n = 170; Piña-Ortiz et al. 2023) and *P. lepturus* (220–410 g, n = 90; Nelson, 2006).

All three species of tropicbirds are peripatric for the western Indian Ocean. However, *P. aethereus* and *P. lepturus* are sympatric for the Atlantic Ocean, and *P. rubricauda* and *P. lepturus* are sympatric for the Pacific. Red-billed and White-tailed tropicbirds have a pantropical distribution, but Red-tailed Tropicbird is absent from the Atlantic Ocean (Nelson 2006; Fig. 4). Population size estimations indicate that the White-tailed tropicbird has the largest (400,000 mature individuals) and most widely distributed population (Nelson 2006; BirdLife International 2020).

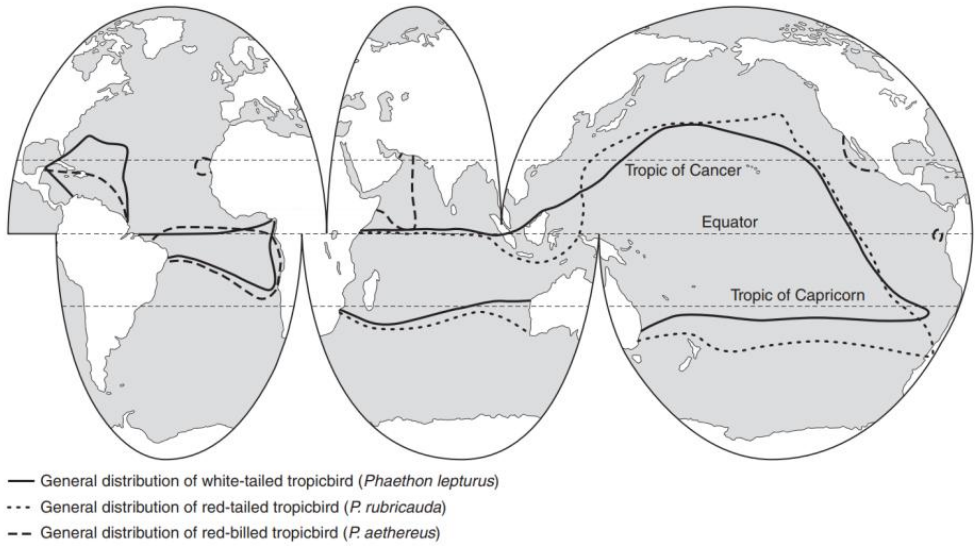


Figure 4. Global distribution of the three species of Tropicbirds (From Nelson 2006).

The feeding habits of tropicbirds are characterised by a diverse diet, consisting mainly of flying fish (Exocoetidae), other small fish, squid (Ommastrephidae) and crustaceans (Nelson 2006). This diversity of diet is evidence of their adaptation to a pelagic lifestyle, as they spend most of their time flying over the ocean in pursuit of prey. Tropicbirds are known for their monogamous behaviour, often breeding together for several years and returning to the same nest site repeatedly. Courtship displays are characterized by aerial and noisy performances, including synchronized aerial displays featuring zigzag flying and undulated downward gliding of their distinctive tail streamers. Nest sites are typically found in remote colonies on islands, ranging from crags or ledges on cliffs to scrapes on the ground or hollows of trees. Incubation lasts 40-46 days, with both parents taking turns caring for the chicks (Nelson 2006). Tropicbirds are solitary or found in pairs while foraging at sea, where they employ their remarkable plunge-diving technique to catch prey, often emitting distinctive vocalizations during different behavioural contexts (Spear and Ainley 2005).

The Red List of Threatened Species of the International Union for Conservation of Nature (IUCN) lists each of these species globally as "Least Concern" (BirdLife International 2019, 2020a, 2020b), yet the population trend for all three species is decreasing, due to various threats including predation by invasive species, road construction and housing development, and oil pollution at sea (Lee and Walsh-McGehe 2000).

The study species: The Red-billed Tropicbird (*Phaethon aethereus*)

The Red-billed Tropicbird (*Phaethon aethereus*) is a medium-sized (550–750 g) pelagic seabird with its predominant white plumage, elongated central tail feathers, and distinctive features such as short, weak legs (Fig. 5). These birds exhibit limited use of their legs for propulsion, emphasizing their adaptation to an aerial lifestyle (Nelson, 2006). Breeding occurs annually for this seabird, which lays a single egg within crevices or caves. During the breeding season, it becomes a central-place forager, conducting foraging trips covering distances of up to 600 km from its breeding sites (Nelson 2006; Diop et al. 2018). Parental care is shared between pair members, involving an incubation period of around 43 days and a fledgling stage lasting about 85 days (Castillo-Guerrero et al. 2011). Noteworthy is the slight male-biased SSD reported for some breeding sites in the Pacific and Atlantic oceans (Nunes et al. 2013; Piña-Ortiz et al. 2023).

The Red-billed Tropicbird breeds on islands in the Atlantic, Indian, and Pacific Oceans, encompassing a wide geographical distribution (Orta 1992; Lee and Walsh-McGehee 2000). According to the Catalogue of Life (Roskov et al. 2015), three subspecies are considered for P. a. aethereus: *P. a. aethereus*, with records for the Fernando de Noronha, Abrolhos, Ascension and St. Helena islands in the south Atlantic; *P. a. mesonauta* which

is distributed in tropical and subtropical waters of the Caribbean, eastern Pacific and eastern Atlantic; and *P. a. indicus* which occurs in the Red Sea, Persian Gulf and Gulf of Aden (Blake et al. 1977; del Hoyo et al. 1992; Nellis 2001; GBIF 2013).

Its presence in the eastern Pacific extends across various locations, including the Gulf of California, the Revillagigedo Islands, Hawaii, the Galapagos Islands, Chañaral, and possibly the Plata Islands in Ecuador and San Lorenzo in Peru (Howell and Webb, 1990; Everett and Anderson, 1991; Vilina et al. 1994; Nelson 2006; Spear and Ainley 2005; Vanderwerf and Young 2007; BirdLife International 2013).

In Mexico, the Red-billed Tropicbird is distributed from the Consag and San Jorge islands in the northern Gulf of California to the Morros El Potosí islands in the southern Mexican Tropical Pacific (Piña-Ortiz et al. 2018). The breeding season of the species in the some colonies within the Mexican Pacific are distinctly seasonal, beginning in late October and ending in early June (Castillo-Guerrero et al. 2011). Contrary, in the South Atlantic (Ascension Island) and the southern eastern Pacific (Galapagos Archipelago), individuals breed throughout the year (Stonehouse 1962; Snow 1965; Harris 1969).

Figure 5. Adult individual (*Phaethon aethereus*) observed during monitoring at Peña Blanca islet during the 2020 breeding season. **Picture:** Sandy Azucena Castañeda.

The life span in the genus *Phaethon* ranges from 10 to 23 years (Klimkiewicz and Futcher, 1989; Nelson 2005). Although *P. aethereus* does not carry out traditional migration, both adults and juveniles disperse widely during the non-breeding season (del Hoyo et al. 1992; Nellis 2001), with dispersal records reaching up to approximately 1,500 km away from their nesting areas (Harris, 1969). Despite its wide distribution, it is considered the least abundant species of the genus, with an estimated 16,000-30,000 mature individuals. Although it maintains relatively stable populations in places such as the Galapagos and Cape Verde, the overall population appears to

be decreasing (BirdLife International 2019). In Mexico, the breeding population has been estimated at 1,901–2,725 breeding pairs, constituting around 50% of the eastern Pacific breeding pairs and 25% of the global population, respectively (Piña-Ortiz et al. 2018).

Study area and general fieldwork procedure

The Mexican Pacific (the Pacific Economic Exclusive Zone of Mexico, 32° 43′ and 14° 32′ N, 118° 27′ and 92° 13′ W; Fig. 1) consists of more than 2.3 million km^2 of ocean, with a coastline of 7,146 km. It extends from Baja California in the north to Chiapas in the south, including the oceanic islands of Guadalupe and the Revillagigedo archipelago (Espinosa 2004). This area is influenced by the California Current and the Costa Rica Coastal Current, which form a transition zone at the mouth of the Gulf of California. The location of the transition zone varies depending on the relative intensity of the currents and the pattern of prevailing winds. In addition, surface circulation of the Mexican Pacific is dominated by the seasonal movement of this transition zone (Fernández et al. 1992).

The Gulf of California is a mainly subtropical system, although the northern Gulf of California resembles a temperate system during winter and has exceptionally high rates of primary productivity due to its topography, warm climate, and upwelling systems (Lluch-Cota et al. 2007). This high primary productivity supports large populations of small pelagic fish, which constitute the primary food source for many piscivorous animals, including squid, fish, seabirds, and marine mammals (e.g., the California sea lion [*Zalophus californianus*]; Mercado-Santana et al. 2017). Upwelling generally occurs of the mainland coast with north-westerly winds during winter (December–May) and on the Baja California coast with south-easterly winds during summer (July–October). June and November constitute transition periods (Lluch-Cota et al. 2007). The Mexican Tropical Pacific is a highly productive region influenced by the southernmost portion of the California Current during the winter, which seasonally transforms the conditions of this region from tropical to subtropical. The northernmost limit of the Mexican Tropical Pacific extends to the area where the California Current turns westward during summer, leaving behind a region under the influence of the warm Costa Rica Coastal Current. This complex region also includes a narrow shelf that steeply drops of to great oceanic depths (Wilkinson et al. 2009).

The research comprising this dissertation was carried out on six islands along a productivity gradient in the Mexican Pacific: Isla San Jorge, Isla San Pedro Mártir, Farallón de San Ignacio, Isla Isabel, Islote Peña Blanca and Morros El Potosí. The first three sites are within the Gulf of California, whilst the remaining sites are in the

Mexican Tropical Pacific (Fig. 6). In the first chapter, body traits of a total of 187 breeding individuals were measured between 2012 and 2021 at the aforementioned sites, in order to assess intraspecific body size variation among colonies and its relationship with local environmental variables at each site. In the second chapter, the study focused on the Peña Blanca islet. During six consecutive breeding seasons (2017-2022), a total of 161 breeding adults in incubation or chick rearing (≤ 4 weeks of age) were tagged with GPS devices. GPS loggers were mounted with TESA® tape (Norderstedt, Germany) to the top of four or five central rectrices directly below the uropygial gland. Loggers and tape weighed between 8-16 g, which corresponded to ~ 2.4% (1.5-2.9%) of adult body mass (536.85 ± 50.56 g; range: 432.9-664.6 g, n = 54; Piña-Ortiz et al. 2023), and below the recommended weight threshold of 3% for avian-attached devices (Wilson and McMahon 2006; Vandenabeele et al. 2012). In addition, blood samples were collected from adults and chicks for stable isotope analysis (δ^{15}N and δ^{13}C), and parental presence at the nest, meal size and parent-chick feeding events were recorded during 2020-2022. All this with the aim of assessing the foraging behaviour of the species and its relationship to parental duties. Fieldwork for the study of the third chapter focused on the San Pedro Mártir island and the Peña Blanca Islet during the 2021 breeding season. Faecal and blood samples were collected for DNA-metabarcoding and stable isotope analysis, respectively, to assess the diet of the species at each site and throughout the breeding season. All applicable institutional and/or national guidelines for wildlife welfare and conservation were followed in all research that is part of this dissertation. The smallest possible amount of blood was taken from each animal, and they were not handled beyond the time set out in the guidelines (see the Ethics approval section of each chapter). During adult sample collection, eggs and chicks were cared for by staff until the parents returned to the nest. No adults left the nest after capture. Subsequent monitoring during the breeding seasons confirmed that chicks were not abandoned by their parents after being handled for the purposes of this dissertation.

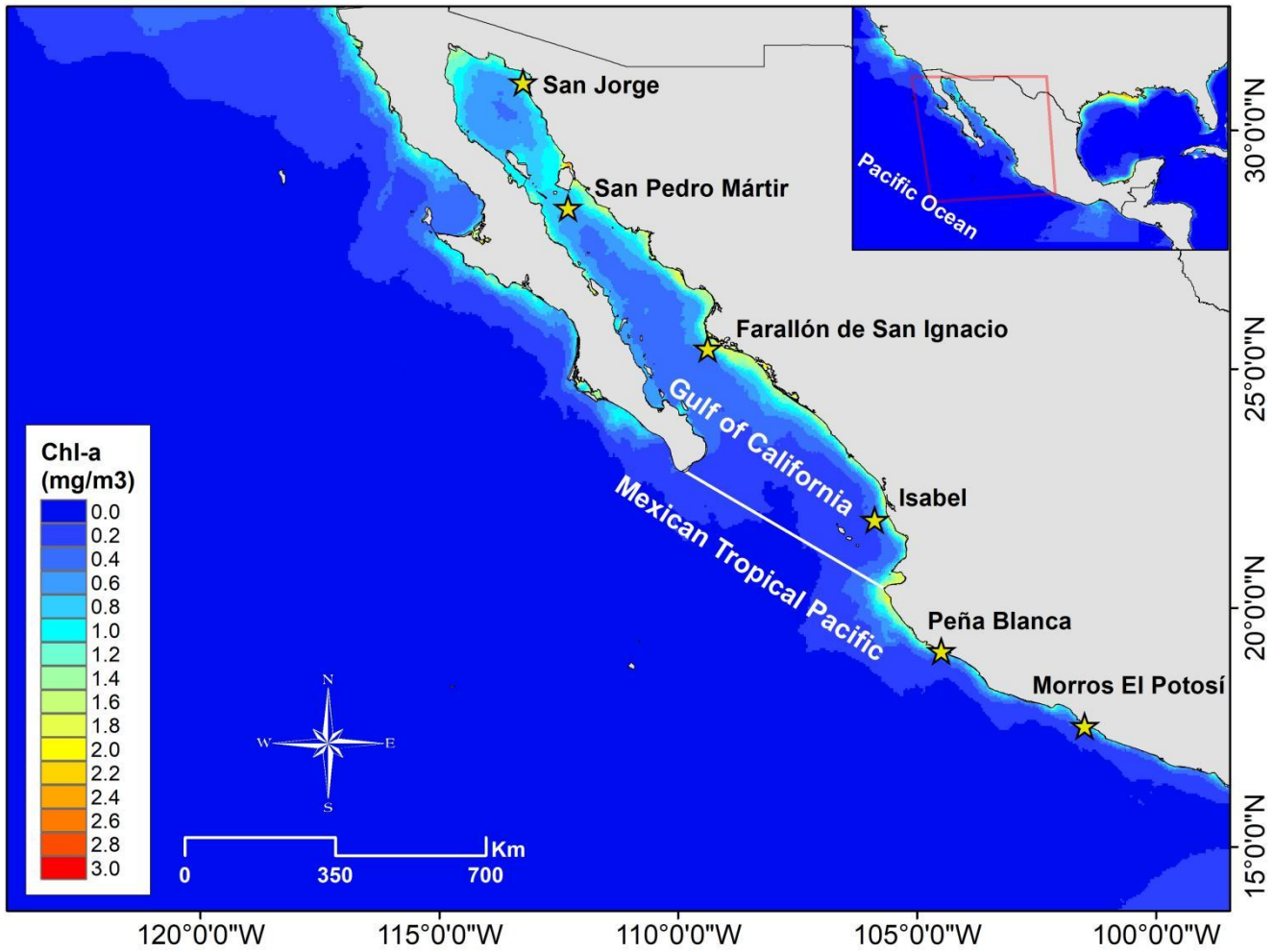


Figure 6. The distribution of Red-billed Tropicbird sampling sites in the Mexican Pacific and Chlorophyll-a concentrations ($mg \cdot m^{-3}$). The sampling sites are indicated by yellow stars.

2| Objectives and Structure of the Thesis

This PhD thesis aimed to evaluate the effect of local oceanographic conditions (e.g., sea surface temperature, chlorophyll-a, bathymetry) on body size and foraging ecology (i.e., foraging behaviour and diet) in the Red-billed Tropicbird (*Phaethon aethereus*) along a productivity gradient in the Mexican Pacific. This was done in order to determine how the environment shapes the biology of a widely distributed pelagic seabird, and the latter must adjust its foraging behaviour, diet and phenotype over time to cope with the local conditions of the site it inhabits. The included aspects can be classified into three broad research topics:

- Evolutionary Ecology: focusing on how local environmental conditions (e.g., chlorophyll-a, air temperature, and sea surface temperature) influence the physical traits of red-billed tropicbirds.
- Movement Ecology: focusing on foraging behaviour subject to parental duties and the availability of resources surrounding breeding sites
- Feeding Ecology: focusing on the variation in dietary composition resulting from the availability of prey subject to changing oceanographic conditions

For **Chapter I**, a dataset was utilized, collected from a total of 6 colonies covering the breeding distribution of the species in the Mexican Pacific. **Chapter II** focused on a dataset collected over a period of 6 years (2017-2022) in the largest colony (Peña Blanca Islet) for the species in the region. **Chapter III** concentrated on data collection in the two most significant colonies for the species in the region (San Pedro Martir and Peña Blanca), located in areas with contrasting oceanographic conditions (upwelling vs. oligotrophic, respectively). In addition, **Appendix II** addresses a subject closely tied to this thesis, utilizing samples gathered during its development. Nevertheless, given it was not primarily written by me, it was not included in the main body of the thesis. Likewise, during the development of this thesis, I had the opportunity to analyse a dataset consisting of concentrations of organochlorine pesticides in the blood of blue-footed boobies (*Sula nebouxii*) from two coastal colonies in Northwest Mexico. However, as this work is not directly related to the main research of the thesis, it was not included as a substantial chapter but rather incorporated into **Appendix III**. In **Appendix IV**, a work conducted during the research period of this thesis is presented, consisting to opportunistic sampling during the data collection that constitutes **Chapter III**. However, this work significantly deviates from the objectives of the thesis, leading me to include it as an appendix rather than a main chapter.

Specific objectives

Chapter I: Assess body size variation among red-billed tropicbirds across their breeding range in the Mexican Pacific.

- **Relationship with environmental variables:** Investigate the relationships between body traits of red-billed tropicbirds and environmental variables such as air temperature, sea surface temperature, and chlorophyll-*a* characterizing each breeding area.
- **Inter-colony variation and sexual size dimorphism (SSD):** Examine the patterns of inter-colony variation in body size and how SSD, with males being larger than females, influences these patterns.
- **Positive Relationship Between SSD and Body Size:** Test the hypothesis that there is a positive relationship between SSD and body size, expecting that colonies with larger individuals exhibit greater SSD compared to colonies composed of smaller individuals.
- **Latitudinal Variation in Body Size:** Predict and assess a latitudinal south-to-north increase in body size among red-billed tropicbirds, based on the expectation that individuals breeding at higher latitudes utilize oceanic areas with specific environmental conditions differing from those breeding at lower latitudes.

Chapter II: Assess the foraging ecology and parental care patterns of breeding red-billed tropicbirds on Peña Blanca Islet, Mexico.

- **Characterization of foraging areas:** To characterize the foraging areas surrounding Peña Blanca Islet, including their oceanographic features such as sea surface temperature, chlorophyll-*a*, and bathymetry.
- **Behavioural monitoring:** Using GPS data loggers, monitor the at-sea behaviour of red-billed tropicbirds during both the egg incubation and chick-rearing stages.
- **Isotopic analysis:** Measure the isotopic values of carbon (δ^{13}C) and nitrogen (δ^{15}N) in whole blood samples from both adults and chicks to evaluate the assimilated foods and potential variations.
- **Parental care assessment:** Assess parental presence at nests, feeding frequency, and the amount of food given to chicks, taking into account the age of the chicks.

Chapter III: Investigate the diet of the Red-billed Tropicbird in the Mexican Pacific using a DNA metabarcoding and stable isotope (δ^{15}N and δ^{13}C) approach.

- **Inter-Colony Diet Comparison:** Compare the diet between two populations of red-billed tropicbirds in the Mexican Pacific (Peña Blanca and San Pedro Mártir).
- **Temporal Diet Variation:** Compare the diet between different breeding stages within each colony.
- **Impact of Environmental Conditions:** Investigate the impact of contrasting environmental conditions on the diet, expecting that the colony located in the upwelling waters (San Pedro Mártir) will be more affected by temporal changes due to being reliant on predictable food resources, as opposed to the colony in tropical waters (Peña Blanca), which is expected to show less or no variability during the breeding stages

3| Chapter Outline

The outline of each of the chapters that make up this thesis is presented as follows.

Chapter I | Body size variation in a tropical seabird along a latitude-productivity gradient

Outline: The first chapter of this thesis focuses on assessing the variation in body size among red-billed tropicbirds across six breeding sites located along a productivity gradient in the Pacific Ocean, spanning latitudes from 17°N to 31°N. The main aim was to understand how environmental conditions influence the physical traits of these birds. The study considered variables such as chlorophyll-a (indicative of marine productivity), air temperature, and sea surface temperature at each breeding site. Additionally, the investigation delved into the presence of sexual size dimorphism (SSD) and its potential impact on the observed body size differences between colonies. From 2012 to 2021, measurements and weights of 187 adult tropicbirds were taken. Several body structures were analysed to assess SSD, and an environmental index was created by combining the values of chlorophyll-a, air temperature, and sea surface temperature within an 80-kilometer radius around each breeding site, along with the latitude of the colonies. The relationships between body traits and this environmental index were explored using regression models. The findings revealed a positive body-size cline from south to north (1–9%), indicating that the birds exhibited larger sizes in the northern colonies. This size gradient was attributed to environmental conditions, but the study faced challenges in isolating the specific contribution of each environmental factor due to high correlations among them. The research highlights the importance of considering a more comprehensive set of environmental variables in future studies. Moreover, SSD was observed in two northern colonies, where males were larger than females. This suggests that environmental factors, particularly in colonies with larger body sizes, high chlorophyll-a, and low sea surface temperatures, play a crucial role in shaping both body size variation and the observed sexual dimorphism in red-billed tropicbirds. The findings emphasize the complexity of the interplay between environmental conditions and the phenotypic plasticity of the species.

Contributions: Lead author, originally participated in the formulation of the idea around which this work was developed, collected material and participated in the fieldwork (measuring and weighing birds), developed methodology, contributed to the laboratory work (molecular analysis), data analysis, writing—original draft preparation and review and editing, and resources.

Chapter II | Parental duties and foraging strategies of a tropical pelagic seabird (*Phaethon aethereus*, Aves: Phaethontidae) during the breeding season

Outline: This chapter presents an integrative study on the foraging behaviour and parental care strategies of the Red-billed Tropicbird during their breeding season. The research focuses on understanding how these seabirds find a balance between self-feeding and provisioning for their chicks. This balance is crucial for the survival of both adults and their offspring, especially in their constantly fluctuating marine environment. The methodology of the study incorporates the use of GPS tracking to monitor the movement patterns of the tropicbirds, coupled with stable isotope analysis to investigate the diet composition of both the adult birds and their chicks. This dual approach allows for a detailed examination of the foraging strategies employed by the Red-billed Tropicbird, providing insights into the distances travelled for food, and the differentiation in dietary intake between the adults and their offspring. One of the key findings of the research is the identification of a bimodal foraging strategy employed by the Red-billed Tropicbird. This strategy involves a combination of short foraging trips close to the breeding site, aimed at frequent feeding of chicks, and long trips with higher energy consumption, probably aimed at self-feeding and obtaining higher quality prey. This bimodal pattern is indicative of adaptive strategies that seabirds deploy to optimise energy expenditure against the nutritional needs of their chicks, ensuring maximisation of breeding success. In addition, the study explores the nest attendance patterns of adult tropicbirds, revealing a collaborative effort among mates to ensure the ongoing care and protection of the offspring. The dietary analysis provided by the stable isotope offers a window into the trophic level and feeding zones that constitute the foraging ecology of the Red-billed Tropicbird and its chicks. This information is crucial for understanding the ecological niche occupied by these birds and the potential impacts of environmental changes on their food sources. In conclusion, this chapter contributes significantly to the field of marine science and seabird ecology by shedding light on the foraging and parental care strategies of the Red-billed Tropicbird. The findings underscore the adaptability of these seabirds to their environment and the complexities inherent in their life histories. This research not only advances our understanding of tropicbird behaviour but also emphasizes the importance of such studies in informing conservation strategies for seabirds and the marine environments they inhabit.

Contributions: Lead author and corresponding author, initially contributed to shaping the concept upon which this project was built, collected material (blood samples) and participated in the fieldwork (GPS deployment, birds handling, field notes), developed methodology, formal analysis and investigation, writing—original draft preparation and review and editing, funding acquisition and resources.

Chapter III | Trophic plasticity of a tropical seabird revealed through DNA metabarcoding and stable isotope analyses

Outline: This chapter focused on provides a detailed analysis of the dietary habits of the Red-billed Tropicbird at two breeding sites located in the Mexican Pacific but with contrasting local oceanographic conditions. The research employs an innovative approach, including DNA metabarcoding of scat samples and stable isotope analysis of blood samples, to assess the diet of this seabird. This dual approach allows an accurate understanding of the trophic ecology of the species, highlighting its food preferences and its ability to adapt in response to environmental conditions. The findings reveal a significant reliance on epipelagic fish, supplemented by occasional consumption of cephalopods and small crustaceans. This dietary composition underscores the Red-billed Tropicbird's feeding flexibility and its ability to exploit a range of prey types across different marine habitats. Moreover, the study compares dietary variations between two colonies, uncovering differences that reflect local oceanographic influences and prey availability. This aspect of the research illustrates the importance of spatial heterogeneity in shaping the foraging behaviour and dietary intake of seabirds. Further, the investigation extends to assessing dietary shifts across different breeding stages of the tropicbirds, offering insights into how breeding demands influence foraging strategies and nutritional choices. Such an analysis is pivotal in understanding the energy requirements and constraints faced by breeding seabirds, and how these factors drive their foraging decisions. this chapter significantly advances our understanding of the trophic ecology of the Red-billed Tropicbird highlighting the bird's trophic plasticity and its ability to adapt to varying environmental conditions through flexible foraging strategies. This adaptability is crucial for the species' survival in the dynamic and often unpredictable marine ecosystem. The study not only contributes to the ecological knowledge of seabirds but also underscores the importance of employing holistic and advanced analytical techniques to unravel the complex interactions within marine food webs.

Contributions: Collaborative lead author (with V. Marcuk), involved in manuscript composition, editing, and served as corresponding author. Contributed to conceptualizing the project, gathered materials (scat and blood samples), actively participated in fieldwork (coordinating field activities and handling birds), developed methodology, conducted formal analysis and investigation, contributed to drafting the original manuscript, review, and editing, contributed to project administration, funding, and managed resources.

4| General Conclusions and Future Outlook

General conclusions

This cumulative thesis comprises three chapters that primarily explore ecological and behavioural aspects, specifically in the areas of evolutionary ecology, movement ecology and feeding ecology, in a tropical pelagic seabird. This research comprehensively addressed how local environmental conditions shape several aspects in the breeding ecology of the Red-billed Tropicbird (*Phaethon aethereus*) along a productivity gradient in the Mexican Pacific, highlighting the plasticity, and changes in the foraging strategies (i.e., foraging behaviour) of the species in response to the challenges imposed by the environment during the breeding season. In each of the chapters, I have evaluated specific aspects of the biology of the species, ranging from geographic variation in body size (**Chapter I**), foraging strategies during the breeding season (**Chapter II**), to dietary diversity (**Chapter III**), all in response to specific marine environmental conditions.

Geographical body size variation can provide insights into evolutionary processes such adaptation and natural selection (Zink 1989; Stillwell and Fox 2009; Stillwell 2010). Variations in body size across different environments may reflect local adaptations to specific ecological conditions, including temperature, food availability, and predation pressure (Valenzuela-Sánchez et al. 2015; Yamamoto et al. 2016; Wei et al. 2018; Romano et al. 2021; Henry et al. 2022). By studying these variations, researchers can obtain a better understanding of how species evolve and adapt to their habitats over time. Additionally, geographical variation in body size can support conservation strategies by identifying populations that may be more vulnerable to environmental changes or anthropogenic impacts (Diniz-Filho et al. 2009; Zheng et al. 2023). For instance, populations with smaller body sizes may be less resilient to habitat loss, climate change, or overexploitation. Recognising these patterns allows conservation efforts to be targeted to protect and manage at-risk populations effectively. Similarly, assessing body size variations in wildlife can provide valuable information for predicting species responses to climate change (Gardner et al. 2011; Zheng et al. 2023). As environmental conditions are changing rapidly, species may undergo phenotypic changes, such as alterations in body size, to adapt to new ecological conditions. Understanding these variations helps to anticipate the responses of species to climate change and to assess potential impacts on wild populations. Following the earlier explanation, the main results obtained in the study on the geographical variation of body size in red-billed tropicbirds were:

– The Red-billed tropicbird demonstrates significant phenotypic plasticity, showcasing a positive, south-to-north body-size cline ranging between 1 and 9%, which corresponds to environmental productivity. This

variability in body size across geographical locations underscores the adaptability of the species to environmental conditions, with larger body sizes observed in areas of higher productivity. This adaptation suggests that local conditions, such as air temperature, sea surface temperature and marine productivity (indicated by chlorophyll-a levels), play a crucial role in the growth and development of these birds (Yamamoto et al. 2016; Nunes et al. 2017). The greater body mass observed in more productive areas could be an evolutionary response to maximise energy efficiency in foraging, allowing more energy reserves to be stored for breeding and survival during periods of food scarcity.

– The assess on sexual size dimorphism (SSD) in red-billed tropicbirds revealed unexpected results, with evident SSD observed only in two northern colonies (males > females), contradicting previous findings (Nunes et al. 2013). While hypotheses such as sexual selection and fecundity selection have been proposed to explain SSD, no definitive consensus has been reached. The results of this study suggest a complex interplay of environmental factors influencing body size and SSD, with implications for understanding ecological and evolutionary processes.

– The relationship between body size and environmental conditions in red-billed tropicbirds provides a perspective on how seabirds may respond to climate change and other environmental perturbations. The phenotypic plasticity observed in this species suggests a potential mechanism for coping with variations in their environment, but also raises questions about the limits of this adaptability in the face of rapid and extreme changes in oceanic conditions.

Research on the foraging behaviour of marine wildlife, such as seabirds, is fundamental to understanding movement patterns, dispersal / migration routes, and habitat utilisation areas within the framework of movement ecology (González-Solís and Shaffer 2009; Courbin et al. 2022). By monitoring seabirds during their trips, relevant information on navigation, resource distribution and habitat preferences is obtained, thereby contributing to the targeting and protection of key foraging grounds and utilisation areas (e.g., migratory corridors; Camphuysen et al. 2012; Soanes et al. 2016; Amélineau et al. 2021). Further, assessment of foraging behaviour provides insights into the ecological dynamics of marine ecosystems by unveiling the availability, distribution and abundance of prey, which are key components of the marine food web (Elliott et al. 2008; Goyert et al. 2014; de la Cruz et al. 2021). This information facilitates the assessment of ecosystem health, the identification of main prey species and the prediction of environmental change responses. Consequently, this knowledge provides the baseline for conservation efforts aimed at the mitigation of human impacts such as overfishing, habitat degradation and pollution. In addition, assessing foraging behaviour sheds light on the

parental duties and foraging strategies of seabirds, as they often display specific strategies to provision their chicks (Phillips et al. 2023). Understanding how parental duties influence foraging behaviour is crucial for understanding reproductive success and population dynamics, especially in such a complex environment as the ocean (e.g., tropical waters; Piña-Ortiz et al. 2024). Thus improving conservation strategies focused on protection of breeding and foraging habitats. Drawing from the insights obtained through tracking data, stable isotopes, and parental nest attendance, the foraging behaviour and parental duties of red-billed tropicbirds yielded the following conclusions:

– Breeding red-billed tropicbirds switch from a unimodal to a bimodal foraging strategy as soon as the chicks hatch. Similar patterns in other colonies suggest an intrinsic behaviour rather than a response to specific conditions (Madden et al. 2022, et al. 2023). This strategy allows parents to balance chick provisioning with self-maintenance by alternating short trips close to the colony and longer trips to pelagic areas, repectively (Phillips et al. 2023). However, differences in environmental conditions between trips indicate a mixed approach to resource acquisition. Overall, these findings advance our understanding of seabird foraging strategies, highlighting the importance of considering parental and environmental factors in seabird ecology.

– Red-billed tropicbirds use different core utilisation areas on short and long foraging trips. The bimodal foraging strategy observed in seabirds, including red-billed tropicbirds, is a response to the challenges of central-place foraging and the need to meet the energetic demands of both parents and chicks. This strategy allows for efficient use of resources while minimising competition for prey. The fluctuating presence of parents in the nest during the chick rearing period reflects the dynamic nature of parental care, focused on maximising food supply to the chicks during key stages of their growth. The use of coastal and pelagic areas by red-billed tropicbirds during chick rearing suggests a complex interplay between energetic demands, resource availability and competition. While foraging in near-shore areas offers proximity and predictability, longer trips to pelagic areas may provide access to alternative prey sources. This highlights the flexibility of seabirds in navigating variable environmental conditions to ensure breeding success without sacrificing their own fitness.

– The parental presence pattern in red-billed tropicbirds during chick growth appears to be intricately linked to the energetic demands of both parents and chicks. During early chick rearing, adults prioritise frequent food supply to their chicks, employing a combination of short and long foraging trips. This strategy ensures regular provisioning when chicks are lacking the reserves to withstand long periods of fasting. As chicks

become older and build up larger lipid reserves, parental presence in the nest gradually decreases and parents spend more time foraging to meet their own energy needs. This change in parental behaviour is consistent with what has been observed in other seabird species, where coordination between partners on foraging trips decreases as the chick rearing period progresses (Tyson et al. 2017; Wojczulanis-Jakubas et al. 2018). However, the factors influencing the decision to undertake short or long foraging trips in red-billed tropicbirds have not yet been fully elucidated. Future research focusing on factors such as parental body condition and its impact on foraging decisions could provide valuable information on the mechanisms driving parental investment in offspring of this species.

– The distinct isotopic signatures (δ^{15}N and δ^{13}C) observed in the blood samples of adult and chick red-billed tropicbirds suggest differential prey consumption between parents and offspring, with parents likely provisioning their chicks with prey enriched in ^{15}N during both early and late chick-rearing periods. This isotopic difference corresponds to the shift in foraging grounds utilized by adult tropicbirds during the chick-rearing phase, indicating a specialized function of parental foraging in meeting the nutritional needs of their young. The decision of parents to undertake long foraging trips to less productive pelagic areas, despite the availability of more proximate, resource-rich areas near the colony, may be driven by a complex interplay of factors including the energetic demands of offspring, resource distribution, and competition for prey. While foraging far from the colony may incur greater travel costs, it offers potential benefits such as reduced competition, increased capture success, and access to higher-quality prey. Understanding the factors that influence parental foraging decisions and foraging trip directions in red-billed tropicbirds deserves further research to elucidate the mechanics of their foraging ecology and breeding strategies.

Seabird diet studies provide crucial information on the feeding ecology of this group, revealing their habits, preferences and foraging behaviour (Furness and Monaghan 1987; Croxall 2009). This knowledge is essential for assessing how seabird populations use resources in their habitats, including prey availability, seasonal variations and spatial distribution, thus predicting their response to environmental changes (Boyd et al. 2016; Serratosa et al. 2020). Furthermore, analysis of seabird diets contributes to the understanding of wider trophic interactions within marine ecosystems, highlighting their role as indicators of ecosystem health and dynamics (Iverson et al. 2007; Mallory et al. 2010; Rajpar et al. 2018). Diet studies also contribute to conservation efforts by identifying key prey sources and assessing the impact of environmental factors on prey availability, targeting measures to address feeding hotspots and mitigate threats such as overfishing and pollution (Paiva et al. 2008; Velarde et al. 2013). In addition, tracking changes in seabird diet serves as an early alert system for ecosystem

disturbance, facilitating the prioritisation of conservation actions and providing information on sustainable management practices for marine resources and habitats.

– Unlike previous methods based on regurgitates or stomach contents (e.g., North 1946; Castillo-Guerrero et al. 2011; Madden et al. 2022, et al. 2023), DNA metabarcoding of faecal samples allowed higher taxonomic resolution and accurate identification of prey species, offering advantages in dietary analysis. Integration of metabarcoding and stable isotope data revealed the trophic role of red-billed tropicbirds as top predators in marine pelagic systems, feeding primarily on offshore mesopelagic and epipelagic fish species. In addition, the study identified key prey species for the different populations, such as Californian anchovy and Pacific chub mackerel for the San Pedro Mártir individuals and several species of flying fish for the Peña Blanca birds. While previous research indicated a prominent role of cephalopods and crustaceans in the diet of red-billed tropicbirds, this study suggests a lower importance of cephalopods, possibly influenced by methodological biases. Overall, these results contribute to our knowledge of seabird feeding habits and highlight the importance of employing molecular techniques in dietary studies to obtain a complete view of marine food webs and trophic interactions.

– Inter-colony variation in the diet of red-billed tropicbirds, highlighting the influence of regional marine systems on prey composition and abundance. The observed differences in prey diversity and reliance on specific species between breeding sites reflect variations in foraging behaviour driven by local environmental conditions, such as upwelling versus oligotrophic oceanic waters. Despite differences in prey availability, individuals from both sites exhibit trophic plasticity, adjusting their foraging behaviour to utilize fluctuating food resources in their respective regions. While competition for finite resources is expected to increase with larger colony sizes, surprisingly, similar niche breadth and prey range were observed between sites, suggesting factors beyond direct competition may contribute to individual specialization. Isotopic analysis further supports the ecological connections within the seabird community, with consistent trophic positions observed across study sites and alignment with $\delta^{15}N$ values in other prey species. Overall, these findings underscore the complex interplay between resource availability, competition, and ecological connectivity in shaping the dietary patterns of red-billed tropicbirds across different breeding colonies.

– The temporal variation in prey composition observed in red-billed tropicbirds highlights their trophic plasticity and adjustable foraging behaviour throughout the breeding season. This study reveals that fluctuations in diet are influenced by breeding stage, collection date and local oceanographic conditions

such as sea surface temperature and primary productivity. Differences in prey availability and breeding constraints determine different feeding patterns during each breeding stage, with individuals adjusting their diet to meet changing energetic demands. In particular, the prevalence of certain prey species varies according to breeding stage, reflecting the nutritional requirements of adults and chicks at different stages. Furthermore, the influence of regional climatic events, such as the El Niño-Southern Oscillation cycle, on prey availability underscores the dynamic interplay between environmental factors and trophic dynamics in marine ecosystems. The observed dietary adjustments in response to temporal variations in prey availability highlight the importance of conducting research under variable conditions to fully understand the constraints and ecological dynamics shaping the foraging behaviour of red-billed tropicbirds. Additionally, the potential impact of fisheries on prey availability merits further investigation to elucidate the long-term implications for the diet and foraging patterns of this species in the face of anthropogenic pressures.

Future Outlook

After completing my doctoral research work, I consider that significant progress was made, but also new questions arose about how the environment shapes the breeding ecology of the Red-billed Tropicbird in the Mexican Pacific. Specifically, progress was made in understanding how local environmental variables influence the diet, behaviour and foraging decisions of the species, as well as its phenotype. However, this study has generated new questions that need to be addressed in future research, either using the same study species or examining other species. Regarding the first chapter, which focuses on geographical variation in body size, it is important to note that, while supporting the idea that environmental temperature is not the only variable determining phenotypic plasticity in tropical seabirds, the exact role of other environmental, genetic and ecological variables in determining body size remains to be elucidated. Incorporation of additional variables could provide a more complete understanding of phenotypic plasticity and its interaction with genetic variability.

In relation to the second chapter, future research on foraging ecology should consider the coordination between partners, as well as the specific role of each member in care and provisioning duties. It is important to determine how parents coordinate chick caring and what factors influence the decision to undertake long foraging trips. Furthermore, it would be relevant to assess variation in foraging behaviour throughout the breeding season and its relationship to resource availability and roles within the pair. Regarding the determination of diet and trophic ecology, it would be beneficial to explore the use of different DNA fragments (e.g., COI, 16s, 12s) and the incorporation of multiple primer pairs to obtain a more complete picture of the diet of the species.

In addition, as a result of the study conducted using the Red-billed Tropicbird as a study species, including this thesis, there are a number of future research projects that I would like to continue working on in the short to medium term. These include the non-breeding distribution of individuals from Peña Blanca colony, foraging ecology in other colonies in the Mexican Pacific, assessment of pollutants (e.g. trace metals, perfluoroalkyl substances [PFAS]), honest signalling of individual quality, the presence of blood parasites, and host-parasite interactions.

Finally, this research highlights the importance of long-term monitoring of seabird populations as indicators of the health and quality of marine ecosystems. This approach can provide valuable information for decision-making in the conservation and management of marine resources, as well as for those who benefit from their use. Continuous monitoring will identify trends and changes in the availability and abundance of marine resources, which is crucial for the long-term sustainability of these ecosystems.

5| References

Allen AM, Singh NJ (2016) Linking Movement Ecology with Wildlife Management and Conservation (Rectified). Front Ecol Evol 3:155. https://doi.org/10.3389/fevo.2015.00155

Amélineau F, Merkel B, Tarroux A, Descamps S and others (2021) Six pelagic seabird species of the North Atlantic engage in a fly-and-forage strategy during their migratory movements. Mar Ecol Prog Ser 676:127–144. https://doi.org/10.3354/meps13872

Andrews RD, Enstipp MR (2016) Diving physiology of seabirds and marine mammals: Relevance, challenges and some solutions for field studies. Comp Biochem Physiol Part A Mol Integr Physiol 202:38–52. https://doi.org/10.1016/j.cbpa.2016.07.004

Barrett RT, Anker-Nilssen T, Krasnov YV (1997) Can Norwegian and Russian razorbills *Alca torda* be identifed by their measurements? Mar Ornithol 25:5–8

Barrett RT, Camphuysen K, Anker-Nilssen T, Chardine JW, Furness RW, Garthe S, Veit R (2007) Diet studies of seabirds: a review and recommendations. ICES J Mar Sci 64(9):1675–1691. https://doi.org/10.1093/icesjms/fsm152

Bell WJ (1990). Central place foraging. In: Searching Behaviour. Chapman and Hall Animal Behaviour Series. Springer Dordrecht. Pp 171–187. https://doi.org/10.1007/978-94-011-3098-1_12

Benn J (2010) What is Biodiversity? United Nations Environment Programme (UNEP). [https://www.unesco.pl/fileadmin/user_upload/pdf/BIODIVERSITY_FACTSHEET.pdf. Accessed on 13 March 2024]

Bergmann K (1847) Ueber die Verhältnisse der Wärmeökonomie der Thiere zu ihrer Grösse. Gött Stud 3:595–708

Bernard A, Rodrigues ASL, Cazalis V, Grémillet D (2021) Toward a global strategy for seabird tracking. Conserv Lett 14(3):e12804. https://doi.org/10.1111/conl.12804

Bindoff NL, Cheung WWL, Kairo JG, Arístegui J, Guinder VA, Hallberg R, Hilmi N, Jiao N, Karim MS, Levin L, O'Donoghue S, Cuicapusa SRP, Rinkevich B, Suga T, Tagliabue A, Williamson P, 2019 In: Pörtner H-O, Roberts DC, Masson-Delmotte V, Zhai P, Tignor M, Poloczanska E, Mintenbeck K, Alegría A, Nicolai M, Okem A, Petzold J, Rama B, Weyer NM (Eds) Changing Ocean, Marine Ecosystems, and Dependent Communities. IPCC Special Report on the Ocean and Cryosphere in a Changing Climate. Cambridge University Press, Cambridge, UK and New York, USA, pp 447–587. https://doi.org/10.1017/9781009157964.007

BirdLife International. 2020. Phaethon lepturus. The IUCN Red List of Threatened Species 2020: e.T22696645A163887639. https://dx.doi.org/10.2305/IUCN.UK.2020-3.RLTS.T22696645A163887639.en. Accessed on 15 February 2024.

BirdLife International. 2020. Phaethon rubricauda. The IUCN Red List of Threatened Species 2020: e.T22696641A163889221. https://dx.doi.org/10.2305/IUCN.UK.2020-3.RLTS.T22696641A163889221.en. Accessed on 15 February 2024.

BirdLife International. 2019. Phaethon aethereus. The IUCN Red List of Threatened Species 2019: e.T22696637A155736224. https://dx.doi.org/10.2305/IUCN.UK.2019-3.RLTS.T22696637A155736224.en. Accessed on 15 February 2024.

Blackburn TM, Gaston KJ, Loder N (1999) Geographic gradients in body size: a clarifcation of Bergmann's rule. Divers Distrib 5:165–174. https://doi.org/10.1046/j.1472-4642.1999.00046.x

Boersma PD, Clark JA, Hillgarth N (2002) Seabird Conservation In: Schreiber EA, Burger J (Eds) Biology of Marine Birds. CRC Press LLC, Boca Raton, FL USA, pp 559–580.

Börger L (2016) EDITORIAL: Stuck in motion? Reconnecting questions and tools in movement ecology. J Anim Eco 85(1):5–10. https://doi.org/10.1111/1365-2656.12464

Boyd IL (2004) Migration of Marine Mammals. In: Werner D (Eds) Biological Resources and Migration. Springer, Berlin, Heidelberg, pp 203–210. https://doi.org/10.1007/978-3-662-06083-4_20

Boyd C, Castillo R, Hunt GLJ, Punt AE, VanBlaricom GR, Weimerskirch H, Bertrand S (2015) Predictive modelling of habitat selection by marine predators with respect to the abundance and depth distribution of pelagic prey. J Anim Ecol 84(6):1575–1588. https://doi.org/10.1111/1365-2656.12409

Boyd C, Grünbaum D, Hunt GLJ, Punt AE, Weimerskirch H, Bertrand S (2017) Effects of variation in the abundance and distribution of prey on the foraging success of central place foragers. J Appl Ecol 54(5):1362–1372. https://doi.org/10.1111/1365-2664.12832

Browning E, Bolton M, Owen E, Shoji A, Guilford T, Freeman R (2017) Predicting animal behaviour using deep learning: GPS data alone accurately predict diving in seabirds. Methods Ecol Evol 9(3):681–692. https://doi.org/10.1111/2041-210X.12926

Brooke ML (2002) Seabird Systematics and Distribution: A Review of Current Knowledge In: Schreiber EA, Burger J (Eds) Biology of Marine Birds. CRC Press LLC, Boca Raton, FL USA, pp 57–85.

Burger J, Gochfeld M (2004) Marine Birds as Sentinels of Environmental Pollution. EcoHealth 1:263–274. https://doi.org/10.1007/s10393-004-0096-4

Camphuysen KCJ, Shamoun-Baranes J, Bouten W, Garthe S (2012) Identifying ecologically important marine areas for seabirds using behavioural information in combination with distribution patterns. Biol Conserv 156:22–29. https://doi.org/10.1016/j.biocon.2011.12.024

Carreiro AR, Paiva VH, Medeiros R, Franklin KA, Oliveira N, Fagundes AI, Ramos JA (2020) Metabarcoding, stable isotopes, and tracking: unraveling the trophic ecology of a winter-breeding storm petrel (*Hydrobates castro*) with a multimethod approach. Mar Bio 167:14. https://doi.org/10.1007/s00227-019-3626-x

Castillo-Guerrero JA, Guevara-Medina MA, Mellink E (2011) Breeding ecology of the Red-billed Tropicbird *Phaethon aethereus* under contrasting environmental conditions in the Gulf of California. Ardea 99:61–71. https://doi.org/10.5253/078.099.0108

Ceia FR, Xavier JC, Carreiro AR, Dos Santos I, Cherel Y (2022) Conventional and modern approaches to study seabird trophic ecology and diet. In: Ramos JA, Pereira L (Eds) Seabird Biodiversity and Human Activities Vol I. CRC Press. Florida. pp. 19–35.

Cherel Y, Connan M, Jaeger A, Richard P (2014) Seabird year-round and historical feeding ecology: blood and feather δ^{13}C and δ^{15}N values document foraging plasticity of small sympatric petrels. Mar Eco Pro Ser 505:267–280. https://doi.org/10.3354/meps10795

Chesser RT, Banks RC, Barker FK, Cicero C, Dunn JL, Kratter AW, Lovette IJ, Rasmussen PC, Remsen JV, Rising JD, Stotz DF, Winker K (2010) Fifty-first supplement to the American Ornithologists' Union Check-list of North American Birds. The Auk 127(3):726–744. https://doi.org/10.1525/auk.2010.127.3.726

Chiaradia A, Costalunga A, Kerry K (2003) The diet of Little Penguins (*Eudyptula minor*) at Phillip Island, Victoria, in the absence of a major prey–Pilchard (*Sardinops sagax*). Emu 103(1):43–48. https://doi.org/10.1071/MU02020

Chimienti M, Cornulier T, Owen E, Bolton M, Davies IM, Travis JMJ, Scott BE (2017) Taking movement data to new depths: Inferring prey availability and patch profitability from seabird foraging behavior. Ecology and Evolution 7(23):10252–10265. https://doi.org/10.1002/ece3.3551

Costello MJ, Chaudhary C (2017) Marine Biodiversity, Biogeography, Deep-Sea Gradients, and Conservation. Curr Biol 27(11):R511–R527. https://doi.org/10.1016/j.cub.2017.04.060

Courbin N, Pichegru L, Seakamela M, Makhado A, Meyër M, Kotze PGH, Mc Cue SA, Péron C, Grémillet D (2022) Seascapes of fear and competition shape regional seabird movement ecology. Commun Biol 5:208. https://doi.org/10.1038/s42003-022-03151-z

Croll DA, Tershy BR, Hewitt RP, Demer DA, Fiedler PC, Smith SE, Armstrong W, Popp JM, Kiekhefer T, Lopez VR, Urban J, Gendron D (1998) An integrated approch to the foraging ecology of marine birds and mammals. Deep Sea Res Part II Top Stud Oceanogr 45(7):1353–1371. https://doi.org/10.1016/S0967-0645(98)00031-9

Croxall JP (2009) Seabirds: Feeding Ecology and Role in Marine Ecosystems. Cambridge University Press

Danchin E, Giraldeau L-A, Cézilly F (2008) Behavioural Ecology: An Evolutionary Perspective on Behaviour. Oxford University Press, Oxford.

Deagle BE, Gales NJ, Evans K, Jarman SN, Robinson S, Trebilco R, Hindell MA (2007) Studying seabird diet through genetic analysis of faeces: a case study on macaroni penguins (*Eudyptes chrysolophus*). PLoS One 2(9):e831. https://doi.org/10.1371/journal.pone.0000831

Dehnhard N, Ludynia K, Masello JF, Voigt CC, McGill RA, Quillfeldt P (2016) Plasticity in foraging behaviour and diet buffers effects of inter-annual environmental differences on chick growth and survival in southern

rockhopper penguins *Eudyptes chrysocome chrysocome*. Polar Biology 39(9):1627–1641. https://doi.org/10.1007/s00300-015-1887-5

de la Cruz A, Ramos F, Tornero J, Rincón MM, Jiménez MP, Arroyo GM (2022) Seabird distribution is better predicted by abundance of prey than oceanography. A case study in the Gulf of Cadiz (SW, Iberian Peninsula). ICES J Mar Sci 79(1):204-217. https://doi.org/10.1093/icesjms/fsab256

deWitt TJ, Scheiner SM (2004) Phenotypic Plasticity. Oxford University Press, Oxford.

Diamond A, Devlin C (2003) Seabirds as Indicators of Changes in Marine Ecosystems: Ecological Monitoring on Machias Seal Island. Environ Monit Assess 88:153–181. https://doi.org/10.1023/A:1025560805788

Diop N, Zango L, Beard A, Ba CT, Ndiaye PI, Henry L, González-Solís J (2018). Foraging ecology of tropicbirds breeding in two contrasting marine environments in the tropical Atlantic. Mar Eco Pro Ser 607:221–236. https://doi.org/10.3354/meps12774

Diniz-Filho JAF, Rodríguez MA, Bini LM, Olalla-Tarraga MA, Cardillo M, Nabout JC, Hortal J, Hawkins BA (2009) Climate history, human impacts and global body size of Carnivora (Mammalia: Eutheria) at multiple evolutionary scales. J Biogeogr 36(12):2222–2236. https://doi.org/10.1111/j.1365-2699.2009.02163.x

Don Bowen W, Jonsen ID (2022) Foraging Ecology and Behavior. In: Costa, DP, McHuron EA (Eds) Ethology and Behavioral Ecology of Phocids. Ethology and Behavioral Ecology of Marine Mammals. Springer, Cham. https://doi.org/10.1007/978-3-030-88923-4_6

Dunphy BJ, Vickers SI, Zhang J, Sagar RL, Landers TJ, Bury SJ, Hickey AJR, Rayner MJ (2020) Seabirds as environmental indicators: foraging behaviour and ecophysiology of common diving petrels (*Pelecanoides urinatrix*) reflect local-scale differences in prey availability. Mar Biol 167:53. https://doi.org/10.1007/s00227-020-3672-4

Elliott KH, Le Vaillant M, Kato A, Speakman JR, Ropert-Coudert Y (2013) Accelerometry predicts daily energy expenditure in a bird with high activity levels. Biol Lett 9:20120919. https://doi.org/10.1098/rsbl.2012.0919

Elliott KH, Woo K, Gaston AJ, Benvenuti S, Dall' Antonia L, Davoren GK (2008) Seabird foraging behaviour indicates prey type. Mar Ecol Pro Ser 354:289–303. https://doi.org/10.3354/meps07221

Elliot M, Whitfield AK (2011) Challenging paradigms in estuarine ecology and management. Estuar Coast Shelf Sci 94(4):306–314. https://doi.org/10.1016/j.ecss.2011.06.016

Elton CS (1933) Exploring the animal world. Allen and Unwin, London EN.

Friesen VL, Burg TM, McCoy KD (2007) Mechanisms of population differentiation in seabirds. Mol Ecol 16(9):1765–1785. https://doi.org/10.1111/j.1365-294X.2006.03197.x

Fromant A, Delord K, Bost C-A, Eizenberg YH, Botha JA, Cherel Y, Bustamante P, Gardner BR, Brault-Favrou M, Lec'hvien A, Arnould JPY (2021) Impact of extreme environmental conditions: Foraging behaviour and trophic ecology responses of a diving seabird, the common diving petrel. Prog Oceano 198:102676. https://doi.org/10.1016/j.pocean.2021.102676

Furness RW, Monaghan P (1987) Seabird Feeding Ecology. In: Seabird Ecology. Tertiary Level Biology. Springer, Boston, MA. https://doi.org/10.1007/978-1-4613-2093-7_3

Gaglio D, Cook TR, McInnes A, Sherley RB, Ryan PG (2018) Foraging plasticity in seabirds: A non-invasive study of the diet of greater crested terns breeding in the Benguela region. PLoS One 13:e0190444. https://doi.org/10.1371/journal.pone.0190444

Gardner JL, Peters A, Kearney MR, Joseph L, Heinsohn R (2011) Declining body size: a third universal response to warming? Trends Ecol Evol 26(6):285–291. https://doi.org/10.1016/j.tree.2011.03.005

Gaspar P, Georges J-Y, Fossette S, Lenoble A, Ferraroli S, Le Maho Y (2006) Marine animal behaviour: neglecting ocean currents can lead us up the wrong track. Proc Biol Sci 273(1602):2697-2702. https://doi.org/10.1098/rspb.2006.3623

Goldstein DL (2002) Water and Salt Balance in Seabirds. In: Schreiber EA, Burger J (Eds) Biology of Marine Birds. CRC Press LLC, Boca Raton, FL USA, pp 467–483.

González-Solís J, Shaffer SA (2009) Introduction and synthesis: spatial ecology of seabirds at sea. Mar Ecol Pro Ser 391:117–120. https://doi.org/10.3354/meps08282

Goyert HF (2014) Relationship among prey availability, habitat, and the foraging behavior, distribution, and abundance of common terns Sterna hirundo and roseate terns S. dougallii. Mar Ecol Pro Ser 506:291–302. https://doi.org/10.3354/meps10834

Hackett SJ, Kimball RT, Reddy S, Bowie RCK, Braun EL, Braun MJ, Choinowski JL, Cox WA, Han K-L, Harshman J, Huddleston CJ, Marks BD, Miglia KJ, Moore WS, Sheldon FH, Steadman DW, Witt CC, Yuri T (2008) A Phylogenomic Study of Birds Reveals Their Evolutionary History. Science 320(5884) 1763–1768. https://doi.org/10.1126/science.1157704

Hamer KC, Schreiber EA, Burger J (2002) Breeding Biology, Life Histories, and Life History–Environment Interactions in Seabirds In: Schreiber EA, Burger J (Eds) Biology of Marine Birds. CRC Press LLC, Boca Raton, FL USA, pp 217–262.

Harper JL, Hawksworth DL (1994) Biodiversity: measurement and estimation Preface. Philos Trans R Soc Lond B Biol Sci 345(1311):5-12.

Henry E, Santini L, Huijbregts MAJ, Benítez-López A (2022) Unveiling the environmental drivers of intraspecific body size variation in terrestrial vertebrates. Glob Ecol Biogeogr 32(2):267–280. https://doi.org/10.1111/geb.13621

Horswill C, Warwick-Evans V, Esmonde NPG, Reid N, Kirk H, Siddiqi-Davies KR, Josey SA, Wood MJ (2023) Interpopulation differences and temporal synchrony in rates of adult survival between two seabird colonies that differ in population size and distance to foraging grounds. Ecology and Evolution 13(10):e10455. https://doi.org/10.1002/ece3.10455

Howell AB (2023) Aquatic Mammals: Their Adaptations to Life in the Water. Legare Street Press

Inger R, Bearhop S (2008) Applications of stable isotope analyses to avian ecology. Ibis 150:447–461. https://doi.org/10.1111/j.1474-919X.2008.00839.x

Iverson SJ, Springer AM, Kitaysky AS (2007) Seabirds as indicators of food web structure and ecosystem variability: qualitative and quantitative diet analyses using fatty acids. Mar Ecol Prog Ser 352:235-244. https://doi.org/10.3354/meps07073

Jacoby J, Mancini PL, Bertrand SL, Efe MA, Bugoni L, Nunes GT (2023) Biogeographic variation on dietary aspects of a widely distributed seabird. Mar Bio 170:21. https://doi.org/10.1007/s00227-022-04171-3

Jakubas D, Wojczulanis-Jakubas K, Jensen J-K (2014) Body size variation of European storm petrels *Hydrobates pelagicus* in relation to environmental variables. Acta Ornithol 49:71–82. https://doi.org/10.3161/000164514X682904

Kennedy M, Spencer HG (2004) Phylogenies of the Frigatebirds (Fregatidae) and Tropicbirds (Phaethonidae), two divergent groups of the traditional order Pelecaniformes, inferred from mitochondrial DNA sequences. Molecular Phylogenetics and Evolution 31(1):31–38. https://doi.org/10.1016/j.ympev.2003.07.007

Kim MA, Harriosn CS, Tasker ML (2023) Legal and cooperative mechanisms for conserving marine birds. In: Young L, VanderWerf E (Eds) Conservation of Marine Birds. Elsevier Academic Press. pp 297–319. https://doi.org/10.1016/B978-0-323-88539-3.00011-X

Le Corre M, Jouventin P (1999) Geographical variation in the Whitetailed tropicbird *Phaethon lepturus*, with the description of a new subspecies endemic to Europa Island, southern Mozambique Channel. Ibis 141:233–239. https://doi.org/10.1111/j.1474-919X.1999.tb07546.x

Lerma M, Castillo-Guerrero JA, Hernández-Vázquez S, Garthe S (2020) Foraging ecology of a marine top predator in the Eastern Tropical Pacific over 3 years with different ENSO phases. Mar Biol 167:88. https://doi.org/10.1007/s00227-020-03699-6

Lerma M, Dehnhard N, Castillo-Guerrero JA, Fernández G (2022) Nutritional state variations in a tropical seabird throughout its breeding season. J Comp Physiol B 192:775–787. https://doi.org/10.1007/s00360-022-01456-3

Lescroël A, Mathevet R, Péron C, Authier M, Provost P, Takahashi A, Grémillet D (2016) Seeing the ocean through the eyes of seabirds: A new path for marine conservation? Mar Policy 68:212–220. https://doi.org/10.1016/j.marpol.2016.02.015

Madden H, Satgé Y, Wilkinson B, Jodice PGR (2022) Foraging ecology of Red-billed Tropicbird Phaethon aethereus in the Caribbean during early chick rearing revealed by GPS tracking. Mar Ornithol 50:165–175

Madden H, Boehm H, Mielke L (2023) Foraging Ecology of Red-Billed Tropicbirds on Saba, Caribbean Netherlands, During Early Chick-Rearing. Ardea 111:1–18. https://doi.org/10.5253/arde.2022.a14

Mallory ML, Robinson SA, Hebert CE, Forbes MR (2010) Seabirds as indicators of aquatic ecosystem conditions: A case for gathering multiple proxies of seabird health. Mar Pollut Bull 60(1): 7–12. https://doi.org/10.1016/j.marpolbul.2009.08.024

Masello JF, Mundry R, Poisbleau M, Demongin L, Voigt CC, Wikelski M, Quillfeldt P (2010) Diving seabirds share foraging space and time within and among species. Ecosphere 1:1–28. https://doi.org/10.1890/ES10-00103.1

Mayr G, De Pietri VL, Love L, Mannering A, Crouch E, Reid C, Scofield RP (2023) Partial skeleton from the Paleocene of New Zealand illuminates the early evolutionary history of the Phaethontiformes (tropicbirds). Alcheringa: An Australasian Journal of Palaeontology 47(3):315–326. https://doi.org/10.1080/03115518.2023.2246528

McGlynn TP (2012) The Ecology of Nest Movement in Social Insects. Annu Rev Entomol 57:291–308. https://doi.org/10.1146/annurev-ento-120710-100708

Mendes RF, Ramos JA, Paiva VH, Calado JG, Matos DM, Ceia FR (2018) Foraging strategies of a generalist seabird species, the yellow-legged gull, from GPS tracking and stable isotope analyses. Mar Biol 165:168. https://doi.org/10.1007/s00227-018-3421-0

Moczek AP, Sultan S, Foster S, Ledon-Rettig C, Dworkin I, Nijhout HF, Abouheif E, Pfennig DW (2011) The role of developmental plasticity in evolutionary innovation. Proc Biol Sci 278:2705–2713. https://doi.org/10.1098/rspb.2011.0971

Moen SM (1991) Morphologic and genetic variation among breeding colonies of the Atlantic pufn (Fratercula arctica). Auk 108:755–763. https://doi.org/10.1093/auk/108.4.755

Montevecchi WA (2002) Interactions between Fisheries and Seabirds: In: Schreiber EA, Burger J (Eds) Biology of Marine Birds. CRC Press LLC, Boca Raton, FL USA, pp 527–558.

Montevecchi WA (2023) Interactions between fisheries and seabirds: Prey modification, discards, and bycatch. In: Young L, VanderWerf E (Eds) Conservation of Marine Birds. Elsevier Academic Press. pp 57–95. https://doi.org/10.1016/B978-0-323-88539-3.00013-3

Morrissey JF, Sumich JL, Pinkard-Meier DR (2018) Introduction to the Biology of Marine Life. Jones and Bartlett Learning Burlington, MA, USA.

Mott R, Herrod A, Clarke RH (2016) Interpopulation resource partitioning of Lesser Frigatebirds and the influence of environmental context. Ecology and Evolution 6(23):8583–8594. https://doi.org/10.1002/ece3.2565

Nelson JB (2006) Pelicans, cormorants, and their relatives, the Pelecaniformes. Oxford University Press, New York, USA

North MEW (1946) Malt Island-A Bird-rock in the Gulf of Aden. Ibis 88:478–501. https://doi.org/10.1111/j.1474-919X.1946.tb03501.x

Nunes GT, Bugoni L (2018) Local adaptation drives population isolation in a tropical seabird. J Biogeogr 45(2): 332–341. https://doi.org/10.1111/jbi.13142

Nunes GT, Leal GDR, Campolina C, de Freitas TRO, Efe MA, Bugoni L (2013) Sex determination and sexual size dimorphism in the Red-billed tropicbird (*Phaethon aethereus*) and white-tailed tropicbird (*P. lepturus*). Waterbirds 36:348–352. https://doi.org/10.1675/063.036.0312

Nunes GT, Mancini PL, Bugoni L (2017) When Bergmann's rule fails: evidences of environmental selection pressures shaping phenotypic diversifcation in a widespread seabird. Ecography 40:365–375. https://doi.org/10.1111/ecog.02209

Orben RA, Paredes R, Roby DD, Irons DB, Shaffer SA (2015) Body size affects individual winter foraging strategies of thick-billed murres in the Bering Sea. J Anim Ecol 84(6):1589–1599. https://doi.org/10.1111/1365-2656.12410

Orgeret F, Thiebault A, Kovacs KM, Lydersen C, Hindell MA, Thompson SA, Sydeman WJ, Pistorius PA (2022) Climate change impacts on seabirds and marine mammals: The importance of study duration, thermal tolerance and generation time. Ecol Lett 25(1):218–239. https://doi.org/10.1111/ele.13920

Paiva VH, Geraldes P, Ramírez I, Meirinho A, Garthe S, Ramos JA (2010) Foraging plasticity in a pelagic seabird species along a marine productivity gradient. Mar Ecol Prog Ser 398:259–274. https://doi.org/10.3354/meps08319

Paiva VH, Tavares PC, Ramos JA, Pereira E, Antunes S, Duarte AC (2008) The Influence of Diet on Mercury Intake by Little Tern Chicks. Arch Environ Contam Toxicol 55:317–328. https://doi.org/10.1007/s00244-007-9118-x

Palumbi SR, Palumbi AR (2021) The Extreme Life of the Sea. Princeton University Press, New Jersey, USA.

Parsons M, Mitchell I, Butler A, Ratcliffe N, Frederiksen M, Foster S, Reid JB (2008) Seabirds as indicators of the marine environment. ICES J Mar Sci 65:1520–1526. https://doi.org/10.1093/icesjms/fsn155

Petalas C, Lazarus T, Lavoie RA, Elliott KH, Guigueno MF (2021) Foraging niche partitioning in sympatric seabird populations. Sci Rep 11:2443. https://doi.org/10.1038/s41598-021-81583-z

Phillips RA, Fort J, Dias MP (2023a) Conservation status and overview of threats to seabirds. In: Young L, VanderWerf E (Eds) Conservation of Marine Birds. Elsevier Academic Press. pp 33–56. https://doi.org/10.1016/B978-0-323-88539-3.00015-7

Phillips JA, Guilford T, Fayet AL (2023b) How do resource distribution and taxonomy affect the use of dual foraging in seabirds? A review. Behav Ecol 34:769–779. https://doi.org/10.1093/beheco/arad052

Pigliucci M (2001) Phenotypic Plasticity: Beyond Nature and Nurture: Syntheses in Ecology and Evolution. Johns Hopkins University Press, Baltimore.

Piña-Ortiz A, Castillo-Guerrero JA, Enríquez-Paredes LM, Fernández G, Hernández-Vázquez S, Quillfeldt P (2023) Body size variation in a tropical seabird along a latitude-productivity gradient. J Ornithol 164:327–339. https://doi.org/10.1007/s10336-022-02037-5

Piña-Ortiz A, González-Zamora DA, Paz JA, Hernández-Vázquez S, Mellink E, Bustamante P, Quillfeldt P, Castillo-Guerrero JA (2024) Parental duties and foraging strategies of a tropical pelagic seabird (*Phaethon aethereus*, Aves: Phaethontidae) during the breeding season. Mar Biol 171:64. https://doi.org/10.1007/s00227-023-04375-1

Pistorius PA, Sydeman WJ, Watanuki Y, Thompson SA, Orgeret F (2023) Climate change: The ecological backdrop of seabird conservation. In: Young L, VanderWerf E (Eds) Conservation of Marine Birds. Elsevier Academic Press. pp 245–276. https://doi.org/10.1016/B978-0-323-88539-3.00020-0

Putman NF (2020) Animal Navigation: Seabirds Home to a Moving Magnetic Target. Curr Biol 30(14):R802–R804. https://doi.org/10.1016/j.cub.2020.05.061

Querejeta M, Lefort M, Bretagnolle V, Boyer S (2023). Metabarcoding fecal samples to investigate spatiotemporal variation in the diet of the endangered Westland Petrel (*Procellaria westlandica*). Avian Conservation and Ecology 18:17. https://doi.org/10.5751/ACE-02410-180117

Rajpar MN, Ozdemir I, Zakaria M, Sheryar S, Rab A (2018) Seabirds as Bioindicators of Marine Ecosystems. In: Mikkola H (Ed) Seabirds. InTech [Online]. http://dx.doi.org/10.5772/intechopen.75458

Reisinger RR, Carpenter-Kling T, Connan M, Cherel y, Pistorius PA (2020) Foraging behaviour and habitat-use drives niche segregation in sibling seabird species. R Soc Open Sci 7:200649. http://dx.doi.org/10.1098/rsos.200649

Reyes-González JM, De Felipe F, Morera-Pujol V, Soriano-Redondo A, Navarro-Herrero L, Zango L, García-Barcelona S, Ramos R, González-Solís J (2021) Sexual segregation in the foraging behaviour of a slightly dimorphic seabird: Influence of the environment and fishery activity. J Anim Eco 90(5):1109–1121. https://doi.org/10.1111/1365-2656.13437

Reyna-Hurtado R, Chapman CA, Melletti M (2023) Movement Ecology of Afrotropical Forest Mammals. Springer Cham: Springer Nature Switzerland AG. https://doi.org/10.1007/978-3-031-27030-7

Romano A, Séchaud R, Roulin A (2021) Geographic variation in body size and plumage colour according to diet composition in a nocturnal raptor. J Avian Biol 52(6):e02716. https://doi.org/10.1111/jav.02716

Ronconi RA, Smith JL, Allard KA (2023) Protecting marine habitats: Spatial conservation measures for seabirds at sea. In: Young L, VanderWerf E (Eds) Conservation of Marine Birds. Elsevier Academic Press. pp 497–544. https://doi.org/10.1016/B978-0-323-88539-3.00007-8

Schreiber EA, Burger J (2002) Seabirds in the Marine Environment In: Schreiber EA, Burger J (Eds) Biology of Marine Birds. CRC Press LLC, Boca Raton, FL USA, pp 1–17.

Schnug E, Jacobs F, Stöven K (2018) Guano: The White Gold of the Seabirds In: Mikkkola H (ed) Seabirds. IntechOpen Limited, London, UK. Pp 79–100. http://dx.doi.org/10.5772/intechopen.79501

Seeholzer GF, Brumfeld RT (2017) Isolation by distance, not incipient ecological speciation, explains genetic diferentiation in an andean songbird (Aves: Furnariidae: *Cranioleuca antisiensis*, line-cheeked spinetail) despite near threefold body size change across an environmental gradient. Mol Ecol 27:279–296. https://doi.org/10.1111/mec.14429

Senner NR, Verhoeven MA, abad-Gómez JM, Gutiérrez JS, Hooijmeijer JCEW, Kentie R, Masero JA, Tibbitts TL, Piersma T (2016) When Siberia came to the Netherlands: the response of continental black-tailed godwits to a rare spring weather event. J Anim Ecol 84(5):1164–1176. https://doi.org/10.1111/1365-2656.12381

Serratosa J, Hyrenbach KD, Miranda-Urbina D, Portflitt-Toro M, Luna N, Luna-Jorquera G (2020) Environmental Drivers of Seabird At-Sea Distribution in the Eastern South Pacific Ocean: Assemblage Composition Across a Longitudinal Productivity Gradient. Front Mar Sci 6:838. https://doi.org/10.3389/fmars.2019.00838

Shealer DA (2002) Foraging Behavior and Food of Seabirds. In: Schreiber EA, Burger J (Eds) Biology of Marine Birds. CRC Press LLC, Boca Raton, FL USA, pp 137–177.

Signa G, Mazzola A, Vizzini S (2021) Seabird influence on ecological processes in coastal marine ecosystems: An overlooked role? A critical review. Coast Shelf Sci 250:107164. https://doi.org/10.1016/j.ecss.2020.107164

Soanes LM, Bright JA, Carter D, Dias MP, Fleming T, Gumbs K, Hughes G, Mukhida F, Green JA (2016) Important foraging areas of seabirds from Anguilla, Caribbean: Implications for marine spatial planning. Mar Policy 70;85–92. https://doi.org/10.1016/j.marpol.2016.04.019

Sommer RJ (2020) Phenotypic Plasticity: From Theory and Genetics to Current and Future Challenges. Genetics 215(1):1–13. https://doi.org/10.1534/genetics.120.303163

Stephens DW, Brown JS, Ydenberg RC (2007) Foraging: Behavior and Ecology. University of Chicago Press. https://doi.org/10.7208/chicago/9780226772653.001.0001

Stillwell RC (2010) Are latitudinal clines in body size adaptive? Oikos 119:1387–1390. https://doi.org/10.1111/j.1600-0706.2010.18670.x

Stillwell RC, Fox CW (2007) Environmental effects on sexual size dimorphism of a seed-feeding beetle. Oecologia 153:273–280. https://doi.org/10.1007/s00442-007-0724-0

Sutton GJ, Angel LP, Speakman JR, Arnould JPY (2023) Determining energy expenditure in a large seabird using accelerometry. J Exp Biol 226(23):jeb246922. https://doi.org/10.1242/jeb.246922

Tasker ML, Sydeman WJ (2023) Fisheries regulation and conserving prey bases. In: Young L, VanderWerf E (Eds) Conservation of Marine Birds. Elsevier Academic Press. pp 439–455. https://doi.org/10.1016/B978-0-323-88539-3.00005-4

Tyson C, Kirk H, Fayet A, Van Loon EE, Shoji A, Dean B, Perrins C, Freeman R, Guilford T (2017) Coordinated provisioning in a dualforaging pelagic seabird. Anim Behav 132:73–79. https://doi.org/10.1016/j.anbehav.2017.07.022

Valenzuela-Sánchez A, Cunningham AA, Soto-Azat C (2015) Geographic body size variation in ectotherms: effects of seasonality on an anuran from the southern temperate forest. Front Zool 12:37. https://doi.org/10.1186/s12983-015-0132-y

Vandenabeele SP, Shepard EL, Grogan A, Wilson RP (2012) When three per cent may not be three per cent; device-equipped seabirds experience variable fight constraints. Mar Biol 159:1–14. https://doi.org/10.1007/s00227-011-1784-6

Vegiopoulos N (2019) How does the environment play a role in biodiversity? The European Molecular Biology Laboratory (EMBL) [https://www.embl.org/about/info/course-and-conference-office/2019/09/how-does-the-environment-play-a-role-in-biodiversity/ Accessed on 13 March 2024]

Velarde E, Ezcurra E, Anderson DW (2013) Seabird diets provide early warning of sardine fishery declines in the Gulf of California. Sci Rep 3:1332. https://doi.org/10.1038/srep01332

Vilchis LI, Johnson CK, Evenson JR, Pearson SF, Barry KL, Davidson P, Raphael MG, Gaydos JK (2015) Assessing ecological correlates of marine bird declines to inform marine conservation. Conserv Biol 29(1):154–163. https://doi.org/10.1111/cobi.12378

Votier SC, Bearhop S, Witt MJ, Inger R, Thompson D, Newton J (2010) Individual responses of seabirds to commercial fisheries revealed using GPS tracking, stable isotopes and vessel monitoring systems. J Appl Ecol 47(2):487–497. https://doi.org/10.1111/j.1365-2664.2010.01790.x

Wagner D, Friedlander AM, Pyle RL, Brooks CM, Gjerde K, Wilhelm T´A (2020) Coral Reefs of the High Seas: Hidden Biodiversity Hotspots in Need of Protection. Front Mar Sci 7:567428. https://doi.org/10.3389/fmars.2020.567428

Walker BH (1992) Biodiversity and Ecological Redundancy. Conserv Biol 6(1):18–23. https://doi.org/10.1046/j.1523-1739.1992.610018.x

Wei X, Yan L, Zhao C, Zhang Y, Xu Y, Cai B, Jiang N, Huang Y (2018) Geographic variation in body size and its relationship with environmental gradients in the Oriental Garden Lizard, *Calotes versicolor*. Ecol Evol 8(9):4443–4454. https://doi.org/10.1002/ece3.4007

Weimerskirch H (2007) Are seabirds foraging for unpredictable resources? Deep Sea Res Part II Top Stud Oceanogr 54(3–4):211–223. https://doi.org/10.1016/j.dsr2.2006.11.013

Weimerskirch H, Doncaster CP, Cuenot-Chaillet F (1994) Pelagic seabirds and the marine environment: foraging patterns of wandering albatrosses in relation to prey availability and distribution. Proc R Soc B: Biol Sci 255(1343):91–97. https://doi.org/10.1098/rspb.1994.0013

West-Eberhard MJ (2003) Developmental Plasticity and Evolution. Oxford University Press, New York.

Wilson RP, McMahon CR (2006) Measuring devices on wild animals: what constitutes acceptable practice? Front Ecol Environ 4:147–154. https://doi.org/10.1890/1540-9295(2006)004[0147:MDOWAW]2.0.CO;2

Wojczulanis-Jakubas K, Jakubas D, Welcker J, Harding AMA, Karnovsky NJ, Kidawa D, Steen H, Stempniewickz L, Camphuysen CJ (2011) Body size variation of a high-arctic seabird: the dovekie (*Alle alle*). Polar Biol 34:847–854. https://doi.org/10.1007/s00300-010-0941-6

Wojczulanis-Jakubas K, Araya-Salas M, Jakubas D (2018) Seabird parents provision their chick in a coordinated manner. PLoS ONE 13:e0189969. https://doi.org/10.1371/journal.pone.0189969

Wynn J, Padget O, Mouritsen H, Perrins C, Guilford T (2020) Natal imprinting to the Earth's magnetic field in a pelagic seabird. Current Biology 30(14):2869-2873.e2. https://doi.org/10.1016/j.cub.2020.05.039

Yamamoto T, Kohno H, Mizutani A, Yoda K, Matsumoto S, Kawabe R, Watanabe S, Oka N, Sato K, Yamamoto M, Sugawa H, Karino K, Shiomi K, Yonehara Y, Takahashi A (2016) Geographical variation in body size of a pelagic seabird, the streaked shearwater *Calonectris leucomelas*. J Biogeogr 43:801–808. https://doi.org/10.1111/jbi.12654

Yoda K, Shiomi K, Sato K (2014) Foraging spots of streaked shearwaters in relation to ocean surface currents as identified using their drift movements. Pro Oceano 122:54-64. https://doi.org/10.1016/j.pocean.2013.12.002

Young LC, Ballance LT (2023) Ecology of marine birds. In: Young L, VanderWerf E (Eds) Conservation of Marine Birds. Elsevier Academic Press. pp 3–32. https://doi.org/10.1016/B978-0-323-88539-3.00016-9

Young LC, VanderWerf EA (2023) Conclusions and the future of seabird conservation. In: Young L, VanderWerf E (Eds) Conservation of Marine Birds. Elsevier Academic Press. pp 579–586. https://doi.org/10.1016/B978-0-323-88539-3.00006-6

Zheng S, Hu J, Ma Z, Lindenmayer D, Liu J (2023) Increases in intraspecific body size variation are common among North American mammals and birds between 1880 and 2020. Nat Ecol Evol 7:347–354. https://doi.org/10.1038/s41559-022-01967-w

Zink RM (1989) The Study of Geographic Variation. The Auk 106:157–160. https://doi.org/10.2307/4087776

Zmudczyńska-Skarbek K, Balazy P (2017) Following the flow of ornithogenic nutrients through the Arctic marine coastal food webs. J Mar Syst 168:31–37. https://doi.org/10.1016/j.jmarsys.2016.12.006

Chapter I

BODY SIZE VARIATION IN A TROPICAL SEABIRD ALONG A LATITUDE-PRODUCTIVITY GRADIENT

Piña-Ortiz Alberto, Castillo-Guerrero José Alfredo, Enríquez-Paredes Luis Manuel, Fernández Guillermo, Hernández-Vázquez Salvador, Quillfeldt Petra

Journal of Ornithology Volume 164, pages 327–339, (2023)
https://doi.org/10.1007/s10336-022-02037-5

Journal of Ornithology (2023) 164:327–339
https://doi.org/10.1007/s10336-022-02037-5

ORIGINAL ARTICLE

Body size variation in a tropical seabird along a latitude-productivity gradient

Alberto Piña-Ortiz[1] · José Alfredo Castillo-Guerrero[2] · Luis Manuel Enríquez-Paredes[3] · Guillermo Fernández[4] · Salvador Hernández-Vázquez[2] · Petra Quillfeldt[1]

Received: 6 May 2022 / Revised: 3 November 2022 / Accepted: 15 November 2022 / Published online: 21 November 2022

Abstract
Body size can vary across geographical gradients, and these clines have been mainly attributed to temperature (i.e., the heat conservation hypothesis). However, in tropical areas, this pattern is not necessarily fulfilled. Furthermore, it is not known whether a body size cline is sex-biased in dimorphic species. Therefore, we aimed to evaluate the intra-specific variation in body size in a tropical seabird, the Red-billed Tropicbird (*Phaethon aethereus*), at six breeding colonies in the Pacific Ocean (17–31° N) and to relate body traits to environmental variables in each colony. Further, we examined sexual size dimorphism (SSD) in the species and its possible influence on the pattern of inter-colony variation in body size. We measured body traits in 187 adults. SSD was evaluated using culmen, ulna, and tarsus lengths and body mass. Chlorophyll-a, air temperature, and sea surface temperature (SST) values within an 80-km radius of each breeding site and the latitude of each island were used to create an environmental index. The relationships between body traits and the environmental index were assessed using regression models. Red-billed tropicbirds exhibited a positive, south-to-north body-size cline (between 1 and 9%) related to environmental conditions, and SSD was evident at only two northern colonies (males > females). The body size cline in the species could be influenced by a set of abiotic and biotic factors, which has likely led to phenotypic plasticity. The sexual dimorphism detected in colonies with larger body sizes along with high chlorophyll-a values and low SST values suggest that environmental-mediated variation in body size is a crucial mediator of SSD.

Keywords Body-size cline · Local environmental conditions · *Phaethon aethereus* · Phenotypic plasticity · Sexual size dimorphism

Communicated by C. Barbraud.

✉ José Alfredo Castillo-Guerrero
alfredo.castillo@academicos.udg.mx

[1] Department of Animal Ecology and Systematics, Justus Liebig University (JLU) Giessen, Heinrich-Buff-Ring 26, 35392 Giessen, Germany

[2] Departamento de Estudios para el Desarrollo Sustentable de la Zona Costera, Centro Universitario de la Costa Sur, Universidad de Guadalajara, Gómez Farías 82, San Patricio-Melaque, Municipio de Cihuatlán, Jalisco C.P. 48980, México

[3] Facultad de Ciencias Marinas, Universidad Autónoma de Baja California, Carretera Transpeninsular Ensenada-Tijuana 3917, Colonia Playitas, Ensenada, Baja California C.P. 22860, México

[4] Unidad Académica Mazatlán, Instituto de Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México, Av. Joel Montes Camarena S/N, Mazatlán, Sinaloa C.P. 82040, México

Zusammenfassung

Variation der Körpergröße bei einem tropischen Seevogel entlang eines Breitengrad-Produktivitäts-Gradienten. Die Körpergröße von Tieren kann über geografische Gradienten hinweg variieren, und diese Unterschiede wurden hauptsächlich auf die Temperatur zurückgeführt (Hypothese der Wärmeerhaltung). In tropischen Gebieten trifft dieses Muster jedoch nicht unbedingt zu. Darüber hinaus ist nicht bekannt, ob die Körpergröße bei dimorphen Arten geschlechtsabhängig ist. Aus diesem Grund haben wir uns zum Ziel gesetzt, bei einem tropischen Seevogel, dem Rotschnabel-Tropikvogel (*Phaethon aethereus*), in sechs Brutkolonien im Pazifischen Ozean (17–31° N) die intra-spezifische Variation der Körpergröße zu untersuchen und Körpermerkmale mit Umweltvariablen in der Kolonie in Beziehung zu setzen. Darüber hinaus untersuchten wir den sexuellen Größendimorphismus (SSD) bei dieser Art und seinen möglichen Einfluss auf die Variation der Körpergröße zwischen den Kolonien. Wir haben die Körpermerkmale von 187 erwachsenen Tieren gemessen. Die SSD wurde anhand der Länge von Schnabellänge, Ulna und Tarsus sowie der Körpermasse bewertet. Chlorophyll-a, Lufttemperatur und Meeresoberflächentemperatur (SST) in einem 80-km-Radius um jeden Brutplatz sowie der Breitengrad jeder Insel wurden zur Erstellung eines Umweltindex verwendet. Die Beziehungen zwischen den Körpermerkmalen und dem Umweltindex wurden mithilfe von Regressionsmodellen bewertet. Rotschnabel-Tropikvögel von Süden nach Norden verlaufende Zunahme der Körpergröße (zwischen 1–9%) auf, die mit den Umweltbedingungen zusammenhing. SSD (Männchen > Weibchen) war nur in zwei nördlichen Kolonien zu beobachten. Das Körpergrößengefälle bei dieser Art könnte durch eine Reihe von abiotischen und biotischen Faktoren beeinflusst werden, die zu phänotypischer Plastizität führen. Der sexuelle Dimorphismus, der in Kolonien mit größeren Körpergrößen zusammen mit hohen Chlorophyll-a-Werten und niedrigen SST-Werten festgestellt wurde, legt nahe, dass umweltbedingte Variationen der Körpergröße ein entscheidender Vermittler von SSD sind.

Introduction

Physical traits in terrestrial and aquatic vertebrates (e.g., fish, mammals, and birds) often show substantial variation across geographical gradients (Ashton 2002; Rodríguez et al. 2006; Emmrich et al. 2014; Bandeira et al. 2016). These geographical body-size clines have been attributed to abiotic (e.g., sea surface temperature, air temperature, latitude, longitude, and wind speed) and biotic factors (e.g., competition, predation, genetic differentiation, sexual selection, and prey availability). However, the influence of abiotic factors has been investigated with greater intensity (Jakubas et al. 2014; Bandeira et al. 2016; Seeholzer and Brumfield 2017). Research on geographical body size variation in multiple animal taxa has focused on Bergmann's rule at interspecific scales (Bergmann 1847) or James' rule (proposed by Blackburn et al. 1999) at intraspecific scales (Rensch 1938; James 1970). These rules assume that heat loss from an endothermic organism is proportional to its surface area:volume ratio (heat conservation hypothesis). Hence, species distributed along a latitudinal gradient are expected to show a geographical body-size cline, with small and large body sizes in warmer and colder areas, respectively (Bergmann 1847; Blackburn et al. 1999).

Intraspecific variation in seabird body size along large-scale climatic gradients has been evaluated over extensive distribution ranges or even the entire distribution ranges of species that inhabit temperate or polar regions, and the resulting body size clines have been found to follow either the rules of Bergmann or James (Moen 1991; Barrett et al. 1997; Wojczulanis-Jakubas et al. 2011; Jakubas et al. 2014; Yamamoto et al. 2016). However, when evaluating the body-size clines of seabirds distributed in tropical regions, the resulting patterns have not entirely fit the established assumptions of either rule (Le Corre and Jouventin 1999; Nunes et al. 2017). Furthermore, no direct relationship between body size and air temperature has been found in tropical areas, suggesting that temperature is only a key determinant of body size below a certain threshold (Geist 1987; Rodríguez et al. 2006). Thus, it has been suggested that while temperature plays a vital role in determining variations in body size in temperate and cold areas, other environmental (e.g., chlorophyll-a and wind speed), genetic (e.g., population structure), and/or ecological (e.g., foraging behavior) factors may also influence this trait among populations distributed over large tropical and subtropical areas (Jakubas et al. 2014; Yamamoto et al. 2016; Nunes et al. 2017).

Seasonal and large-scale marine primary productivity is not necessarily directly related to latitude (Longhurst et al. 1995; Ma et al. 2014), although small-scale marine provinces show latitudinal gradients in primary productivity (e.g., Gulf of California and Mexican Tropical Pacific; Wilkinson et al. 2009). Thus, primary productivity values around seabird colonies could result in local divergences in body size among neighboring populations due to the different environmental pressures that each population might face at each site (Barbraud et al. 1999; Friesen 2015; Nunes et al. 2017).

Populations subject to different environmental conditions are also subject to different selective pressures, and thus local adaptation can act as a barrier to gene flow

among wild populations under these circumstances (Sexton et al. 2014). Individuals that remain within population-specific non-breeding grounds or near the colony boundaries throughout the year could avoid exposure to atypical environmental conditions, which would in turn result in the positive selection of individuals that are best suited to the local conditions, with the resulting diversity due to phenotype and genotype shaping (Friesen 2015). Hence, it is reasonable that body size traits can be shaped by environmental variables operating on both large (e.g., air temperature) and small (e.g., local levels of phytoplankton biomass or chlorophyll-a) scales, with larger and heavier individuals occurring in productive and high-latitude areas (Nunes et al. 2017).

In addition to the geographical variation in body size, sexual size dimorphism (SSD) is relatively common in seabirds (Fairbairn and Shine 1993; Serrano-Meneses and Székely 2006; Manciniet al. 2013), with sexual selection notably influencing seabird SSD (Barbraud and Jouventin 1998; Serrano-Meneses and Székely 2006; Dale et al. 2007). Multiple abiotic factors have been proposed to explain the variation in SSD in vertebrate and invertebrate taxa (Blanckenhorn et al. 2006; Roitberg 2007; Stillwell and Fox 2007; Lengkeek et al. 2008; Ulian and Rossi 2016). For instance, temperature may help drive selection by affecting males and females differently, with substantial differences in the extent of SSD among populations within a given range (Blanckenhorn et al. 2006; Stillwell and Fox 2007). Furthermore, comparative analyses of SSD within and across species have found a greater extent of SSD at the intraspecific level than at the interspecific level (Ulian and Rossi 2016). In addition, some studies have shown that male body size within a given species varies with latitude to a greater extent than female body size. Thus, some factor that varies systematically with latitude is responsible for producing this pattern (Blanckenhorn et al. 2006). However, in seabirds, the question of whether adults of sexually dimorphic species exhibit similar or sex-biased geographical variation patterns in body size has never been evaluated, even though body size variation has been observed in both males and females of sexually dimorphic seabird species (Jakubas et al. 2014; Yamamoto et al. 2016; Nunes et al. 2017).

The Red-billed Tropicbird (*Phaethon aethereus*) is a pelagic seabird with a pantropical oceanic distribution (Orta et al. 2020). This medium-sized (550–750 g) long-lived seabird breeds annually, laying a single egg inside crevices or caves, and becomes a central-place forager during the breeding season, conducting foraging trips of up to 600 km from its breeding sites (Nelson 2006; Diop et al. 2018). Parental care is shared between pair members, with the incubation and fledgling stages lasting approximately 43 and 85 days, respectively (Castillo-Guerrero et al. 2011). In the Atlantic Ocean, a slight male-biased SSD has been reported (SSD index of 1.01–1.03 for all significant body traits; Nunes et al. 2013).

Red-billed Tropicbird breeding colonies in the Pacific Ocean are distributed from the Gulf of California and Revillagigedo Archipelago in Mexico to Chañaral Island in Chile and the Galapagos Islands (Nelson 2006). Red-billed tropicbirds breed on a total of 14 islands along the Mexican Pacific, including those of the Gulf of California and the Mexican Tropical Pacific, where latitudinal gradients in primary productivity (north-to-south) and sea surface temperature (SST; south-to-north) are present (Wilkinson et al. 2009; Piña-Ortiz et al. 2018). In addition, low genetic differentiation and a high degree of connectivity between populations have been reported for the species in this region (Castillo-Guerrero et al. 2020).

This study aimed to evaluate the variation in body size between breeding red-billed tropicbirds across their distribution range in the Mexican Pacific (18–31° N, 104–114° W) by examining the relationships between body traits and the environmental variables (i.e., air temperature, SST, and chlorophyll-a) that characterize each breeding area. We weighed and measured the body traits of red-billed tropicbirds at six breeding colonies found on the islands in the study area. As males are larger than females (Nunes et al. 2013), we also evaluated if sexual dimorphism influenced any patterns of inter-colony variation in body size. We predicted that males would be larger than females and that a positive relationship between SSD and body size would be present. Thus, SSD would be greater in colonies where larger individuals were present compared to the SSD values of colonies composed of smaller individuals. We also predicted that a latitudinal south-to-north increase in body size would be present that reflected the variability in local environmental conditions given that individuals breeding at higher latitudes utilize oceanic areas with higher chlorophyll-a and lower air temperature and SST values than those breeding at lower latitudes.

Materials and methods

Study area and fieldwork

We collected body size data in six colonies during the breeding season in the incubation and chick-rearing stages from October to May 2012–2013, 2015–2016, and 2020–2021 (Table 1). The study colonies were located on islands across the breeding range of the Red-billed Tropicbird in the Mexican Pacific from 17–31° N. From north to south, the study colonies were San Jorge (SNJ), which is one of the northernmost colonies for the species; San Pedro Mártir (SPM); Farallón de San Ignacio (FSI); Isabel (ISA); Peña Blanca (PBL); and Morros El Potosí (MEP; Fig. 1). The selected colonies

Table 1 Sampling dates, location, island area and number of breeding pairs in six colonies of the red-billed tropicbird (*Phaethon aethereus*) sampled throughout the Gulf of California and Mexican Tropical Pacific between 2012 and 2021

Study island[a] and sample size (*n*)	Population size[b] (breeding pairs)	Geographic location	Island area (ha)	Sampling collection dates
SNJ (23)	5–17	31° 00′ 45′′N, 113° 14′ 38′′W	14	Feb 2015
SPM (43)	150–190	28° 22′ 52″N, 112° 18′ 23″W	267	May 2012, Jan 2015, Feb 2020
FSI (20)	150–228	25° 26′ 15′′N, 109° 22′ 39′′W	17	Mar 2012, May 2013
ISA (11)	87–155	21° 50′ 40′′N, 105° 53′ 02′′W	194	Apr 2015
PBL (68)	1,200–1,650	19° 06′ 11′′N, 104° 29′ 12′′W	10	Oct–Dec 2015, Feb 2016, Feb-Mar 2020
MEP (22)	100	17° 31′ 57′′ N, 101° 29′ 18′′ W	15	Jan 2021

[a]Study islands: *SNJ* San Jorge; *SPM* San Pedro Mártir; *FSI* Farallón de San Ignacio; *ISA* Isabel; *PBL* Peña Blanca; *MEP* Morros El Potosí

[b]Population sizes were reviewed in Piña-Ortiz et al. 2018. For details of the original source, see publication

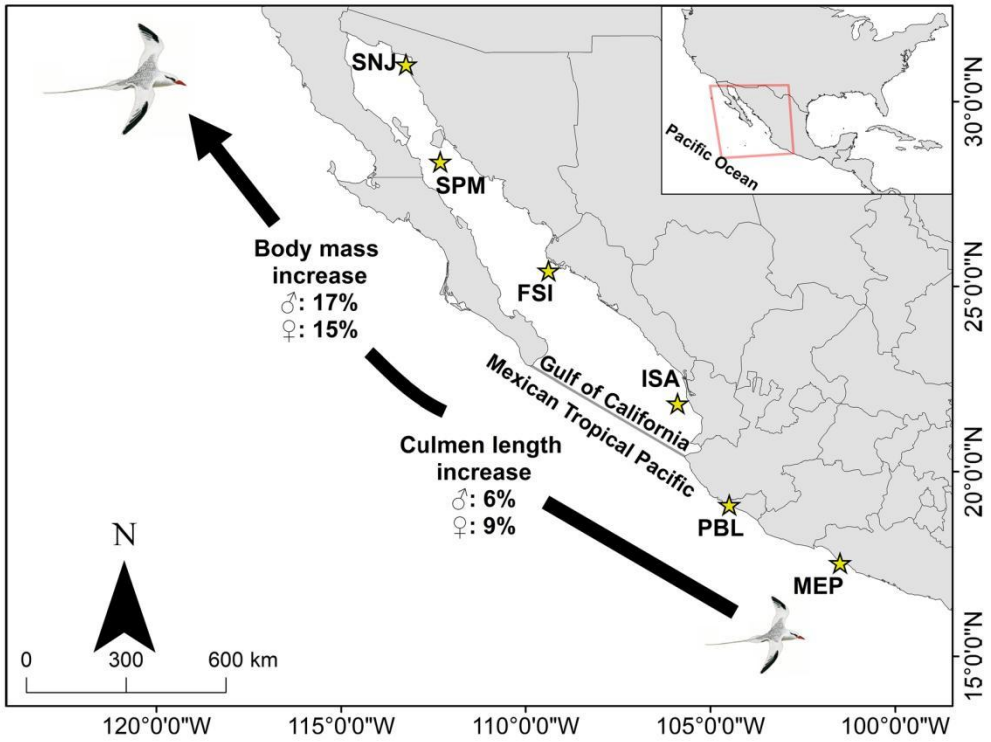


Fig. 1 Geographic locations of red-billed tropicbird colonies in the Gulf of California and Mexican Tropical Pacific sampled in this study. The solid line shows the boundary between the two ecological regions where the breeding sites are distributed. Black arrows indicate the clinal increase in body mass and culmen length (%) shown by males (♂) and females (♀) from south to north. Study locations from north to south: *SNJ* = San Jorge; *SPM* = San Pedro Mártir; *FSI* = Farallón de San Ignacio; *ISA* = Isabel; *PBL* = Peña Blanca; *MEP* = Morros El Potosí

varied in population size from a few pairs in SNJ to 1600 breeding pairs in PBL (Piña-Ortiz et al. 2018; Table 1). The distances between neighboring islands ranged from ~ 300 km (SNJ to SPM) to ~ 550 km (FSI to ISA). Four study colonies were found on islands in the Gulf of California, while the other two were found on islands in the Mexican Tropical Pacific (Wilkinson et al. 2009; Fig. 1).

The Gulf of California is a mainly subtropical system, although the northern Gulf of California resembles a temperate system during winter and has exceptionally high rates of

primary productivity due to its topography, warm climate, and upwelling systems (Lluch-Cota et al. 2007). This high primary productivity supports large populations of small pelagic fish, which constitute the primary food source for many piscivorous animals, including squid, fish, seabirds, and marine mammals (e.g., the California sea lion [*Zalophus californianus*]; Mercado-Santana et al. 2017). Upwelling generally occurs off the mainland coast with northwesterly winds during winter (December–May) and on the Baja California coast with southeasterly winds during summer (July–October). June and November constitute transition periods (Lluch-Cota et al. 2007). The Mexican Tropical Pacific is a highly productive region influenced by the southernmost portion of the California Current during the winter, which seasonally transforms the conditions of this region from tropical to subtropical. The northernmost limit of the Mexican Tropical Pacific extends to the area where the California Current turns westward during summer, leaving behind a region under the influence of the warm Costa Rica Coastal Current. This complex region also includes a narrow shelf that steeply drops off to great oceanic depths (Wilkinson et al. 2009).

In general, latitudinal gradients of primary productivity and SST throughout the year have been documented for both regions, with the highest and lowest values of primary productivity observed in the north and south, respectively, and SST showing the opposite pattern (Pennington et al. 2006; Lluch-Cota et al. 2007). However, this observed gradient is not related per se to latitude but is influenced by topography, sea currents, and upwellings. In the Gulf of California, for example, it has been pointed out that the northernmost regions maintain high levels of productivity throughout the year because of strong tidal currents that lead to constant water-column mixing (Simpson et al. 1994). In the Midriff Islands region, strong tidal mixing influences water-column conditions up to 500 m depth and brings nutrient-rich waters toward the surface and subsurface layers, creating conditions of constant upwelling (Álvarez-Borrego 2002). In contrast, the southern region is the deepest within the Gulf of California and has been described as complex given that its thermohaline structure is related to the mixing of the North Equatorial Current, California Current, and the waters of the Gulf of California, which is reflected in the relatively low productivity values observed in this portion of the gulf (Álvarez-Borrego 2012; Lavín et al. 2013; Mercado-Santana et al. 2017).

We hand-captured 187 breeding adults in nest burrows during incubation or chick-rearing stages and measured their body mass and culmen, ulna, and tarsus lengths (Figure S1, Table S1). We measured body mass using a portable electronic scale to the nearest 5 g, and the remaining measurements were taken using vernier calipers (±0.01 mm; Table S1). To avoid resampling, we marked the measured birds with alphanumeric bands on the tarsus or non-toxic paint on the culmen. Measurements were collected for both adults if both were in the nest, and measurements were only taken once per nest. A. Piña-Ortiz and J. A. Castillo-Guerrero collected the measurements. They measured the same 22 individuals (4 in SPM and 18 in PBL) included in this study. Pearson correlation tests were performed for ulna, culmen, and tarsus lengths to evaluate the relationships among the body traits measured by both authors. All body lengths collected by both researchers were significantly correlated with each other ($r = 0.63–0.77$; p values < 0.002 for all pairwise comparisons).

Sex determination

We took a blood sample of each specimen from the brachial vein with a 16-mm 25G needle and stored the sample in a buffer (100 mM tetrasodium EDTA, 100 mM Tris, 10 mM NaCl, and 1% sodium dodecyl sulfate). Afterward, we extracted the genomic DNA from the blood samples in the laboratory following a proteinase K and salt-extraction protocol (Aljanabi and Martinez 1997). DNA quantification and quality were assessed by electrophoresis on 1.5% agarose gels stained with ethidium bromide (0.5 µg/ml). A 1000-bp molecular weight marker (DNA size standard Invitrogen™) was used to determine the relative concentration of the extracted genetic material. This process allowed us to identify low-yielding samples likely to result in low amplification (allelic dropout or shorter fragment dominance). These samples were re-extracted or re-precipitated to obtain a concentration between 15 and 25 ng/µl. Molecular sexing was performed using 2550/2718 primers (Fridolfsson and Ellegren 1999). The Polymerase Chain Reactions (PCR) contained 1xPCR buffer (20 mM Tris–HCl, 50 mM KCl, 1.5 mM $MgCl_2$, and 0.2 mM dNTPs), 0.7 µM of each primer, 0.8 U of Taq DNA polymerase (Invitrogen, Waltham, USA), and 15–25 ng of template DNA in a total volume of 25 µl. Thermal cycling consisted of 4 min at 94 °C followed by 30 cycles of 30 s at 94 °C, 30 s at 52 °C, and 30 s at 72 °C, with a final extension of 5 min at 70 °C. We separated the PCR products by electrophoresis in 2.0% agarose gels stained with ethidium bromide (0.5 µg/ml). The direct observation of gels under UV transillumination revealed that individuals identified as males were characterized by only one band on the gel, while two bands characterized females (Fig. S2).

Blind sample replicates (15%) and positive and negative controls were systematically performed for each set of amplifications to avoid incorrect assignments or false positives. Samples from known blue-footed booby (*Sula nebouxii*) female and male specimens were used as positive controls. Negative controls included two no template reactions: one from a no-tissue sample extraction and another using ultrapure water. Of the 187 individuals, 171 birds

were successfully sexed (91.44%). However, samples from 3 individuals (1.66%), 1 individual (0.55%), 12 individuals (6.6%), and 1 (0.55%) individual in SPM, FSI, PBL, and MEP, respectively, failed to amplify, and thus their sex could not be confirmed. This result may have been due to the low amounts of DNA in these samples.

Environmental data

For the study area, we obtained environmental data of SST (°C; 11 µm SST algorithm) and chlorophyll-a concentrations (mg/m^3; chlorophyll OCx algorithm) between August 2002 and January 2021 from the Aqua-Modis Sensor (NASA/GSFC-OBPG 2014). First, we downloaded a monthly data series of each oceanographic variable with 4 km per pixel resolution. Then, the year-round average SST and chlorophyll-a values were calculated within an 80-km radius surrounding each colony, according to the average foraging distance used by breeding red-billed tropicbirds from Peña Blanca Island to feed their chicks (González-Zamora 2019). Likewise, air temperature data for each study island was obtained from the nearest weather station (Puerto Peñasco station 26072 for SNJ, Bahía de Kino station 26179 for SPM, Topolobampo [DGE] station 25098 for FSI, San Blas station 18029 for ISA, Manzanillo [OBS] station 6018 for PBL, and Zihuatanejo [DGE] station 12127 for MEP; data available from www.smn.conagua.gob.mx/es/climatologia/informacion-climatologica/informacion-estadistica-climatologica). We used the average monthly air temperature data available between January 1975 and December 2015 recorded by each weather station.

Statistical analyses

We used culmen, ulna, and tarsus lengths and body mass data to perform range-wide statistical analyses (Table S1). First, we plotted the data set for each variable per island to look for outliers and identify possible sampling errors in morphometric measurements. To identify outliers, we employed the criterion of Nunes et al. (2017), which considers data outside ± 2 standard deviations from the mean as outliers. Next, we used Pearson correlation tests with Bonferroni correction for multiple testing ($\alpha = 0.012$, for 4 comparisons). Finally, to evaluate the relationships between morphometric variables and body mass, all morphometric variables were positively correlated among themselves and with body mass ($r = 0.21$–0.55; p-values 0.007 to < 0.001 for all pairwise comparisons; Fig. S3), except the correlation between ulna and tarsus lengths ($r = 0.18$; $p = 0.021$).

Subsequently, we employed a principal component analysis (PCA) to create a composite body size index for each individual. This index was obtained by incorporating all body measurements (culmen, ulna, and tarsus lengths) except body mass, as it could vary depending on the time of capture (i.e., breeding stage or before or after feeding a chick). However, as the first principal component (PC1) only explained 51.8% of the variance in body measurements, we did not consider it useful as an integrative measure of body size. Therefore, we used each body trait and mass independently in subsequent analyses.

We compared body size measurements between sexes and breeding populations using a two-way MANOVA with Wilk's lambda (λ) and a post hoc Fisher least significant difference test. Likewise, univariate normality was evaluated with the Shapiro–Wilk test using the residual values of each body measurement, and multivariate normality was evaluated with the Mardia test of skewness and kurtosis, Henze–Zirkler test, and Royston test with the R package MVN (Korkmaz et al. 2014). Considering the possible variations in the body mass of individuals at the time of capture, we used a general linear model (GLM) where colony, sex, and breeding stage were considered as categorical variables and the date of collection as a covariate. The GLM showed that date ($F_{1,137} = 11.87$, $p < 0.001$) significantly influenced the final model selected. Therefore, we standardized the body mass of all individuals using the adjusted means (covariate = day 52) in the subsequent analyses where this variable was analyzed.

We used regression models to explain the relationships between morphometric structures and body mass, and environmental variables. Prior to conducting regression modelling, correlations between environmental variables (i.e., air temperature, SST, and chlorophyll-a) and latitude (in decimal degrees) were analyzed with Pearson correlation tests with Bonferroni correction for multiple testing ($\alpha = 0.012$). All variables were highly correlated ($r = -0.87$ to 0.95, p-values < 0.001; Fig. S4). Thus, we used a PCA to create an environmental index (PC1: 96.8% of the total variance) to avoid redundancy and multicollinearity between variables. The regression models were run with the environmental index as an explanatory variable and the means of body structures (culmen, ulna, and tarsus lengths), and the adjusted mean of body mass as response variables. We analyzed each explanatory variable in independent models considering the male and female datasets separately. The assumptions of all parametric tests used in this study were assessed and fulfilled by the data. All statistical analyses were carried out in STATISTICA 7.1 (Hill and Lewicki 2007) except for the univariate and multivariate normality tests used for the MANOVA analyses, which we performed in R 3.6.2 (R Core Team 2020). Body traits and mass are reported as the mean ± standard deviation.

Results

After removing outliers, all body traits showed a multivariate normal distribution for all breeding islands. In addition, a global difference was found for body traits (Wilk's $\lambda = 0.33$, $F_{20,20} = 9.14$, $p < 0.001$) and body mass ($F_{5,137} = 17.41$, $p < 0.001$) among the individuals from the different breeding islands. Overall, both size and body mass increased to the north in females and males (between 1 and 9%; Table 2). However, no multivariate global differences between sexes were detected in body traits (Wilk's $\lambda = 0.96$, $F_{4,20} = 1.48$, $p = 0.21$) or body mass ($F_{5,137} = 17.41$, $p < 0.001$). Nevertheless, the post hoc test revealed a significant difference between sexes in the northern colonies of SPM (Fisher LSD test $p = 0.037$) and FSI (Fisher LSD test $p = 0.006$), with males showing larger culmens than those of females. Likewise, males from SPM had longer ulna lengths than females (Fisher LSD test $p = 0.027$; Table 2, Fig. 2).

All body traits and body mass were positively related to the environmental index for both females (culmen: $y = 2.21x + 61.15$, $R^2 = 0.45$, $p < 0.001$; ulna: $y = 1.08x + 103.30$, $R^2 = 0.17$, $p = 0.002$; tarsus: $y = 0.37x + 27.48$, $R^2 = 0.07$, $p = 0.017$; body mass: $y = 46.54x + 580.64$, $R^2 = 0.42$, $p < 0.001$) and males (culmen: $y = 2.22x + 62.03$, $R^2 = 0.46$, $p < 0.001$; ulna: $y = 1.70x + 102.91$, $R^2 = 0.38$, $p < 0.001$; tarsus: $y = 0.56x + 27.73$, $R^2 = 0.19$, $p < 0.001$; body mass: $y = 47.26x + 584.79$, $R^2 = 0.50$, $p < 0.001$; see Fig. 3).

Discussion

Geographical body size variation

Our results describe geographical variation in the body size of red-billed tropicbirds among six breeding colonies distributed throughout the Mexican Pacific. The body traits considered in this study increased from the south to the north and were related to local environmental conditions (i.e., air temperature, SST, and chlorophyll-a). These results agree with those found in other seabird species, such as the European storm petrel (*Hydrobates pelagicus*; Jakubas et al. 2014), streaked shearwater (*Calonectris leucomelas*; Yamamoto et al. 2016), and brown booby (*Sula leucogaster*; Nunes et al. 2017). Studies on seabirds distributed in temperate regions (e.g., European storm petrel and streaked shearwater) have generally found that body size variation conforms to the heat conservation hypothesis (i.e., Bergmann's rule). However, in tropical seabirds, it has been suggested that air temperature is not the only factor involved in shaping phenotypes, and other local oceanographic features may influence this process (Nunes et al. 2017). In our study, the high correlations among environmental variables preclude the identification of the contributions of different factors to variations in body size. However, as other studies have proposed, local oceanographic features, such as chlorophyll-a or ocean temperatures (SST), may be fundamental driving forces behind the geographical body size divergence in seabirds (Moen 1991; Barrett et al. 1997; Nunes et al. 2017). Thus, individuals belonging to northern colonies in the Gulf of California, where primary productivity (annual mean of 2.14 g C m^{-2} d^{-1}) values are high and SST (annual mean of 23.24 °C) is low, were the ones that showed larger body sizes compared to those in the southern Gulf of California (from the mouth to the central region; primary productivity of 0.92–1.52 g C m^{-2} d^{-1} and SST of 24.6–25.6 °C) and Mexican Tropical Pacific (0.82 g C m^{-2} d^{-1} and mean SST of 28 °C) where individuals were smaller (Wilkinson et al. 2009; Escalante et al. 2013).

Intraspecific body size variation among populations may reflect phenotypic plasticity or genetic differences as responses to local environmental conditions (local adaptation). It has been challenging to differentiate between genetic and environmental contributions to phenotypic variations in size. Some studies have established adaptive conclusions

Table 2 Mean ± standard deviation of ulna length, culmen length, tarsus length, and body mass measurements of the red-billed tropicbird (*Phaethon aethereus*) sampled from six colonies on Mexican islands

Breeding island[a] and sample size by sex (n)[b]	Ulna length (mm)		Culmen length (mm)		Tarsus length (mm)		Body mass (g)	
	Male	Female	Male	Female	Male	Female	Male	Female
SNJ ($M = 12$; $F = 11$)	104.37 ± 1.78	105.02 ± 1.16	63.20 ± 3.24	64.41 ± 2.41	28.05 ± 0.68	27.84 ± 0.88	646.67 ± 22.50	636.82 ± 47.61
SPM ($M = 28$; $F = 12$)	104.97 ± 2.56	103.54 ± 2.80	64.92 ± 1.82	63.01 ± 2.65	28.57 ± 0.85	28.07 ± 1.28	621.39 ± 57.76	624.90 ± 46.47
FSI ($M = 10$; $F = 9$)	104.23 ± 1.62	103.91 ± 1.56	64.77 ± 2.57	61.84 ± 2.82	27.74 ± 0.85	27.37 ± 0.70	611.20 ± 38.00	571.78 ± 46.81
ISA ($M = 4$; $F = 7$)	102.48 ± 1.25	103.79 ± 1.80	61.53 ± 2.09	60.77 ± 2.69	27.70 ± 0.80	27.03 ± 0.44	556.25 ± 13.77	579.29 ± 46.85
PBL ($M = 29$; $F = 27$)	101.81 ± 2.10	102.36 ± 3.24	59.92 ± 2.26	59.01 ± 1.75	27.11 ± 1.57	27.42 ± 1.72	548.28 ± 48.85	546.35 ± 46.47
MEP ($M = 15$; $F = 6$)	99.91 ± 1.78	100.88 ± 2.37	59.12 ± 1.71	58.70 ± 0.97	27.33 ± 1.17	27.17 ± 1.09	538.33 ± 51.05	540.0 ± 51.67

[a]Breeding islands: *SNJ* San Jorge; *SPM* San Pedro Mártir; *FSI* Farallón de San Ignacio; *ISA* Isabel; *PBL* Peña Blanca; *MEP* Morros El Potosí

[b] *M* Male; *F* Female

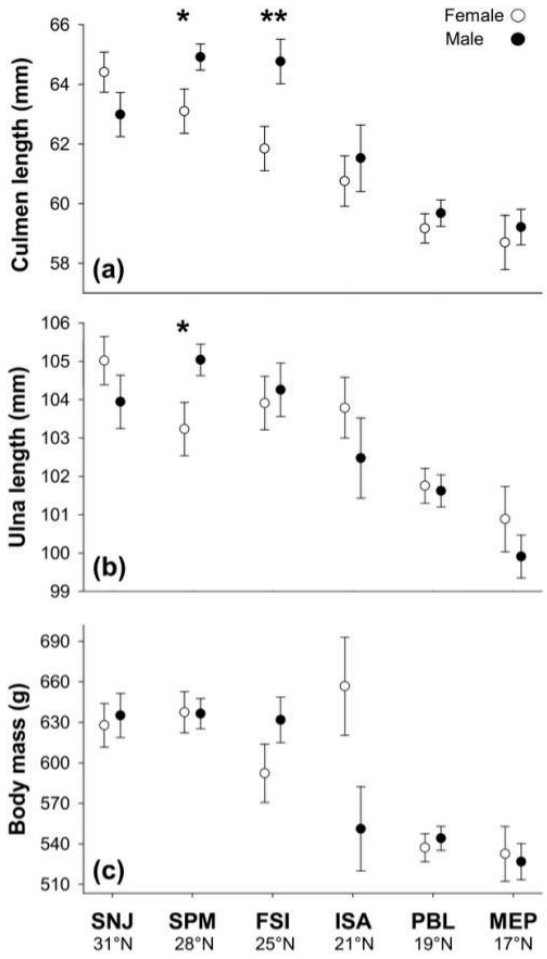


Fig. 2 Least square means ± standard error of **a** culmen length **b**, ulna length (both in mm), and **c** adjusted means ± standard error of body mass (*g*) of females and males of the red-billed tropicbird (*Phaethon aethereus*) distributed along a latitudinal gradient (north to south) in the Gulf of California and Mexican Tropical Pacific. Light and dark symbols, respectively, represent females and males. Adjusted means of body mass at day 52 are shown. *SNJ* = San Jorge; *SPM* = San Pedro Mártir; *FSI* = Farallón de San Ignacio; *ISA* = Isabel; *PBL* = Peña Blanca; *MEP* = Morros El Potosí. *Differences for $p < 0.05$; **differences for $p < 0.01$

based only on phenotypic measures of size (Stillwell 2010) without discerning whether the differences are related to local adaptation or phenotypic plasticity (Jakubas et al. 2014; Yamamoto et al. 2016). Other studies have determined long-term phenotypic changes in body size without evidence of genetic changes or differences in genetic structure (Moen 1991; Teplitsky et al. 2008; Husby et al. 2011). Few studies have attempted to assess the influence of both components in seabirds, for example, in the snow petrel (*Pagodroma nivea*) although body size seems to be partly genetically determined, the relationship between adult size and food supply suggests that chick size may be influenced by food availability (Barbraud et al. 1999). In the study area, red-billed tropicbirds show low levels of genetic structure among colonies based on neutral markers (Castillo-Guerrero et al. 2020). Therefore, subject to certain caveats, we consider that body size variation could be primarily driven by phenotypic plasticity shaped by local oceanographic variables, with population genetic differentiation playing a secondary role.

Within this conceptual framework, local environmental conditions can promote different food availability scenarios among colonies and contribute to the inter-colony variation in body size. For example, the foraging strategies used by seabirds in a specific colony are subject to the resources available in the area (Botha and Pistorius 2018; Clay et al. 2019; Geary et al. 2019). Preliminary studies on the foraging ecology of the Red-billed Tropicbird indicate that the duration and distance of foraging trips vary between individuals from breeding colonies located in the Gulf of California and the Mexican Tropical Pacific, with breeding individuals from the southern colonies (e.g., PBL) making longer and more distant trips compared to those in northern colonies (e.g., SPM; Piña-Ortiz et al. unpubl. data). This result is consistent with the higher productivity registered for the Gulf of California compared to that of the tropical Pacific. In addition, the resources provided to offspring during the chick growth stage are crucial for determining the body size of adult seabirds (Barbraud et al. 1999; Quillfeldt and Peter 2000). Thus, it would be expected that food availability would be low in areas or times of low productivity compared to that in areas or times of high primary productivity, which in turn would be reflected in chick growth rates that depend on the number of feeding events and the nutritional quality of the prey (Barbraud et al. 1999; Quillfeldt et al. 2007; Grissot et al. 2019; Ausems et al. 2020). Under this scenario, food availability controlled by oceanic environmental factors would differ among colonies, resulting in differences in food provisioning and chick growth rates among the study regions. Thus, the phenotypic plasticity driven by differences in prey availability and, consequently, in foraging behavior may affect the provisioning of food resources during chick development and could contribute to the variation in body size observed among colonies.

It has been suggested that populations of seabirds are mainly regulated by food availability (Ashmole 1963; Weimerskirch 2002). In this sense, areas with high productivity could support larger colony sizes. However, on the other hand, in large colonies, density-dependent processes are exacerbated, and breeding success and recruitment decrease (Lewis et al. 2001; Pozzi et al. 2015). This pattern is because greater competition is present in larger

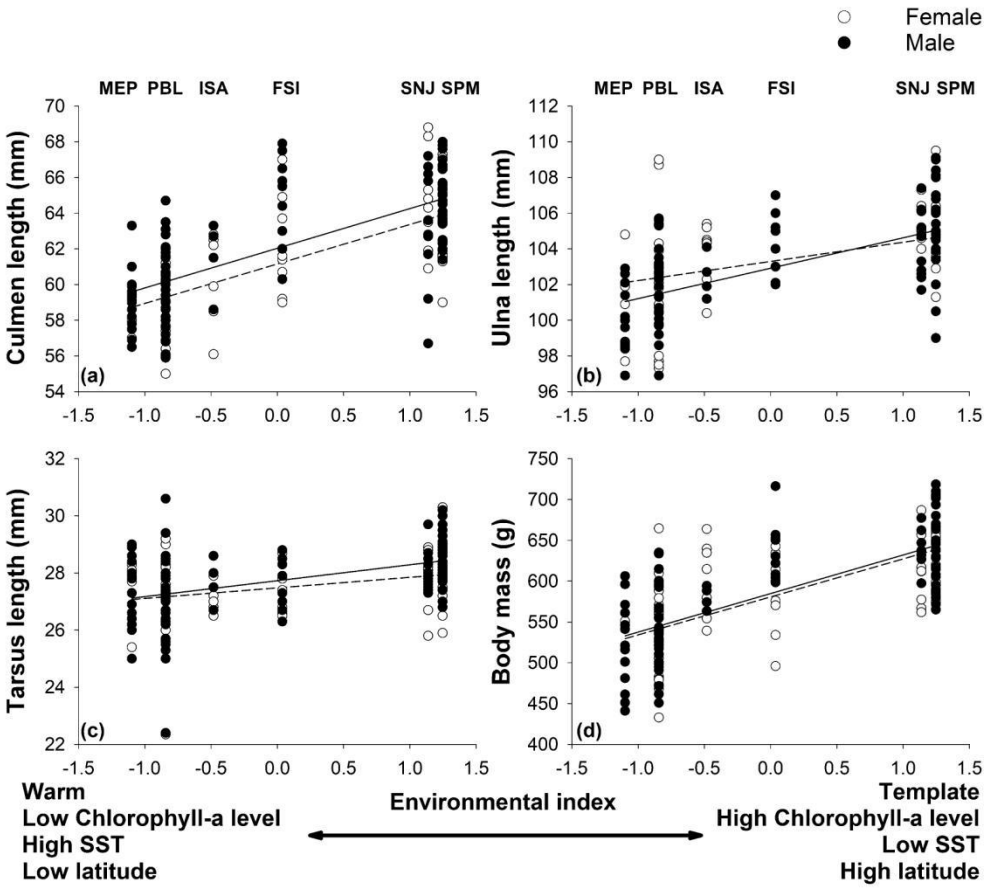


Fig. 3 Relationship between the environmental index and (**a**) culmen length (mm), (**b**) ulna length (mm), (**c**) tarsus length (mm), and (**d**) adjusted means of body mass (*g*) of females ($n = 72$; light dots) and males ($n = 98$; dark dots) of the red-billed tropicbirds in the study colonies. Regression lines estimated by the models used for female and male data sets are shown using dashed and solid lines, respectively. Study locations in the order shown in the figure: *MEP* = Morros El Potosí; *PBL* = Peña Blanca; *ISA* = Isabel; *FSI* = Farallón de San Ignacio; *SNJ* = San Jorge; *SPM* = San Pedro Mártir

colonies, and generally, less food or lower quality is given to the chicks compared to what is observed in smaller colonies. Thus, there could be a relationship between colony size and body size, although this pattern was not evident in preliminary analyses (see results in Table S2). The absence of a pattern may be due to multiple interacting factors that operate at different scales to regulate colony size, including biotic (e.g., predation, competition, and prey availability) and abiotic factors (e.g., availability of nesting sites, availability and extension of foraging grounds, wind speed, sea surface temperature, local climate conditions, and seasonality; e.g., Crespin et al. 2006; Amorim et al. 2009; Keslinka et al. 2019; Pagenaud et al. 2022).

Sexual size dimorphism (SSD)

Overall, the body trait data of red-billed tropicbirds did not support our prediction or the previously reported SSD pattern for the species. Instead, our results showed that individuals belonging to the SPM and FSI colonies were the only ones with significant SSD values (males > females; SSD index of 1.01–1.05 for significant body traits). This result partially agrees with those of Nunes et al. (2013), who determined that breeding males from the Abrolhos Archipelago were larger than females, specifically with regard to the wing chord (SSD index = 1.01) and bill measurements (bill length, nostril-to-bill-tip, and head-plus-bill; SSD index of 1.03, 1.01, and 1.01, respectively).

Many animal taxa exhibit SSD, including seabirds (Fairbairn and Shine 1993; Croxall 1995; Serrano-Meneses and Székely 2006). Latitudinal variations in SSD among populations within a given species have been found in vertebrate and invertebrate groups (Blanckenhorn et al. 2006; Roitberg 2007). Sex-specific selection for body size based on environmental factors (e.g., season length, food availability, or temperature) has been suggested to mediate the relationship between SSD divergence and latitude (Blanckenhorn et al. 2006; Roitberg 2007). However, the underlying mechanisms are often unclear, such that any latitudinal change in an environmental factor that affects one sex more than the other may generate sex-specific variations in body size and consequently affect SSD (Dobson and Wigginton 1996; Blanckenhorn et al. 2006; Tamate and Maekawa 2006; Roitberg 2007). Red-billed tropicbirds appear to partially conform to this pattern, as our study found male-biased SSD in northern colonies with enhanced levels of primary productivity.

The causes behind SSD have long been discussed, yet no definitive consensus has been reached. Currently, three principal hypotheses have been proposed to produce and maintain SSD: (1) sexual selection, (2) fecundity selection, and (3) differential niche-utilization, with the former being the most strongly supported mechanism to explain sexual dimorphism (Andersson 1994; Fairbairn 1997; Figuerola 1999; Serrano-Meneses and Székely 2006). It has been proposed that sexual selection favors smaller male body sizes in species with aerial displays, while larger body sizes are advantageous in species whose males either display or fight on the ground (Figuerola 1999; Székely et al. 2000; Serrano-Meneses and Székely 2006). In this context, sexual selection might be expected to favor larger males in red-billed tropicbirds because aerial displays are conducted in groups that likely involve both sexes (Nelson 2006), although individuals on the ground engage in intense fights and threats when competing for nest hole sites (e.g., Ascension Island; Stonehouse 1962).

In our study, it was expected that not only the SPM and FSI colonies would exhibit SSD but that other colonies with similar or larger population sizes would also exhibit SSD (e.g., PBL is a fivefold larger colony with an island surface area that is smaller than those of the others; Table 1). When competition for nesting crevices is frequent, sexual selection should favor larger males. Hence, sexual selection is not likely the primary mechanism driving the observed SSD in this study. On the other hand, the fecundity selection hypothesis may be ruled out for this species because our results show male-biased SSD, which is the opposite of what is expected with this hypothesis proposed to explain female-biased SSD.

It is essential to mention that the pattern of SSD found in red-billed tropicbirds seems to follow the same pattern as those observed in other seabirds distributed in the Southern Hemisphere (both at intra-specific and family levels; Fairbairn and Shine 1993). For example, Fairbairn and Shine (1993) found that males tended to be larger than females in populations with large average body mass values whose individuals fed in highly productive oceanic areas, especially at high latitudes. However, further studies have shown that body size, SSD, and primary productivity are highly correlated, and applying multivariate analysis to such variables is not appropriate due to co-linearity (Croxall 1995; Serrano-Meneses and Székely 2006). From the results obtained in this study, SSD may be affected by a body size cline because SSD has also been shown to vary geographically in many species (Blanckenhorn et al. 2006; Bidau et al. 2016). Under this scenario, it may be established that environmental variability influences body size and SSD. However, identifying the underlying mechanisms will require further studies that evaluate latitudinal variation due to sex-specific, natural, and sexual selection on body size.

Overall, our results indicate that the body size of red-billed tropicbirds breeding in the Mexican Pacific shows a latitudinal cline, which could be influenced by a set of both abiotic (i.e., air temperature, SST and chlorophyll-a) and biotic (i.e., food availability and foraging behavior) factors that are not mutually exclusive. Furthermore, the SSD detected in two colonies with relatively large mean body sizes, high chlorophyll-a values, and low SST values suggest that variation in productivity among populations could be an essential correlate or mediator of SSD, and future studies should assess whether or how latitudinal variation in body size is related to sex-based size differences and their putative selective causes. In other species, it has been established that there is a relationship between body size, foraging behavior, and fitness (Barbraud et al. 1999). Then, to understand the ecological and evolutionary significance of the body size variation in red-billed tropicbirds is necessary to know the foraging behavior and breeding success under different environmental regimes at different colonies.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10336-022-02037-5.

Acknowledgements We thank Comisión Nacional de Áreas Naturales Protegidas – Islas del Golfo de California (Sinaloa and Sonora), Parque Nacional Isla Isabel, and Departamento de Estudios para el Desarrollo Sustentable de Zona Costera CUCSur – Universidad de Guadalajara for logistical support in the field. We also thank M. Guevara, J. Ventura, C. Robles, C. Franco, L. Lee, A. Fleishman, G. Aréchiga, M. Lerma, A. Rocha, D. González, H. Almanza, M. Silva, V. Huerta, A. Aguilar, S. Castañeda, G. López, and J.I. Piña-Marquina for their assistance in the field and N. Victoria for their assistance with sample processing for DNA sex determination.

Author contributions AP-O and JAC-G originally formulated the idea and analyzed data; AP-O, JAC-G, and SH-V conducted fieldwork; AP-O, JAC-G, GF, and PQ developed methodology; LME-P generated molecular analyses; AP-O, JAC-G, and PQ wrote the original draft, and all coauthors edited and approved the manuscript for publication.

Funding This work was supported by Consejo Nacional de Ciencia y Tecnología (CONACYT; grant numbers CB2010-155353 and I010/176/2012) and the Universidad de Guadalajara. APO was financed with a CONACYT scholarship for doctoral studies abroad (No. 795355).

Availability of data and material The data are available from the Dryad Digital Repository.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The fieldwork, including wildlife management and sampling collection, was conducted with permission from the Dirección General de Vida Silvestre (DGVS, Mexico) under the permits SGPA/DGVS/62712/12, SGPA/DGVS/02923/13, SGPA/DGVS/00404/15, and SGPA/DGVS/02779/21. We complied with all applicable institutional and/or national guidelines for the welfare and conservation of wildlife. All individuals included in this study were not handled for more than 10 min, and the smallest amount of blood was drawn from each individual. While the adults were sampled, we cared for their eggs and/or chicks until their parents returned to the nest. No individuals abandoned their nests after capture.

References

Aljanabi SM, Martinez I (1997) Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. Nucleic Acids Res 25:4692–4693. https://doi.org/10.1093/nar/25.22.4692

Álvarez-Borrego S (2002) Physical oceanography. In: Case TJ, Cody ML, Ezcurra E (eds) A new island biogeography of the Sea of Cortés. Oxford Univ Press, New York, NY, USA, pp 41–59

Álvarez-Borrego S (2012) Phytoplankton biomass and production in the gulf of California: a review. Bot Mar 55:119–128. https://doi.org/10.1515/bot.2011.105

Amorim P, Figueiredo M, Machete M, Morato M, Martins A, Santos RS (2009) Spatial variability of seabird distribution associated with environmental factors: a case study of marine important bird areas in the Azores. ICES J Mar Sci 66:29–40. https://doi.org/10.1093/icesjms/fsn175

Andersson M (1994) Sexual selection. Princeton Univ. Press, New York, NY, USA

Ashmole NP (1963) The regulation of numbers of tropical oceanic seabirds. Ibis 103:458–473. https://doi.org/10.1111/j.1474-919X.1963.tb06766.x

Ashton KG (2002) Patterns of within-species body size variation of birds: strong evidence for Bergmann's rule. Global Ecol Biogeogr 11:505–523. https://doi.org/10.1046/j.1466-822X.2002.00313.x

Ausems ANMA, Skrzypek G, Wojczulanis-Jakubas K, Jakubas D (2020) Sharing menus and kids' specials: Inter- and intraspecific differences in stable isotope niches between sympatrically breeding storm-petrels. Sci Total Environ 728:138768. https://doi.org/10.1016/j.scitotenv.2020.138768

Bandeira V, Virgós E, Barros T, Cunha MV, Fonseca C (2016) Geographic variation and sexual dimorphism in body size of the Egyptian mongoose, *Herpestes ichneumon* in the western limit of its European distribution. Zool Anz 264:1–10. https://doi.org/10.1016/j.jcz.2016.06.001

Barbraud C, Jouventin P (1998) What causes body size variation in the snow petrel pagodroma nivea? J Avian Biol 29:161–171. https://doi.org/10.2307/3677194

Barbraud C, Weimerskirch H, Robertson GG, Jouventin P (1999) Size-related life history traits: insights from a study of snow petrels (*Pagodroma nivea*). J Anim Ecol 68:1179–1192. https://doi.org/10.1046/j.1365-2656.1999.00361.x

Barrett RT, Anker-Nilssen T, Krasnov YV (1997) Can Norwegian and Russian razorbills *Alca torda* be identified by their measurements? Mar Ornithol 25:5–8

Bergmann K (1847) Ueber die Verhältnisse der Wärmeökonomie der Thiere zu ihrer Grösse. Gött Stud 3:595–708

Bidau CJ, Taffarel A, Castillo ER (2016) Breaking the rule: multiple patterns of scaling of sexual size dimorphism with body size in orthopteroid insects. Rev Soc Entomol Argent 75:111–116

Blackburn TM, Gaston KJ, Loder N (1999) Geographic gradients in body size: a clarification of Bergmann's rule. Divers Distrib 5:165–174. https://doi.org/10.1046/j.1472-4642.1999.00046.x

Blanckenhorn WU, Stillwell RC, Young KA, Fox CW, Ashton KG (2006) When Rensch meets Bergmann: does sexual size dimorphism change systematically with latitude? Evolution 60:2004–2011. https://doi.org/10.1554/06-110.1

Botha JA, Pistorius PA (2018) Variability in the foraging distribution and diet of Cape gannets between the guard and post-guard phases of the breeding cycle. Front Mar Sci 5:15. https://doi.org/10.3389/fmars.2018.00015

Castillo-Guerrero JA, Guevara-Medina MA, Mellink E (2011) Breeding ecology of the Red-billed tropicbird *Phaethon aethereus* under contrasting environmental conditions in the gulf of California. Ardea 99:61–71. https://doi.org/10.5253/078.099.0108

Castillo-Guerrero JA, Piña-Ortiz A, Enríquez-Paredes L, van der Heiden A, Hernández-Vázquez S, Saavedra-Sotelo NC, Fernández G (2020) Low genetic structure and diversity of red-billed tropicbirds in the Mexican Pacific. J Field Ornithol 91:142–155. https://doi.org/10.1111/jofo.12334

Clay TA, Oppel S, Lavers JL, Phillips RA, MdeL B (2019) Divergent foraging strategies during incubation of an unusually wide-ranging seabird, the Murphy's petrel. Mar Biol 166:8. https://doi.org/10.1007/s00227-018-3451-7

Crespin L, Harris MP, Lebreton J-D, Frederiksen M, Wanless S (2006) Recruitment to a seabird population depends on environmental factors and on population size. J Anim Ecol 75:228–238. https://doi.org/10.1111/j.1365-2656.2006.01035.x

Croxall JP (1995) Sexual size dimorphism in seabirds. Oikos 73:399–403. https://doi.org/10.2307/3545964

Dale J, Dunn PO, Figuerola J, Lislevand T, Székely T, Whittingham LA (2007) Sexual selection explains Rensch's rule of allometry for sexual size dimorphism. Proc R Soc B 274:2971–2979. https://doi.org/10.1098/rspb.2007.1043

Diop N, Zango L, Beard A, Ba CT, Ndiaye PI, Henry L, Clingham E, Oppel S, González-Solís J (2018) Foraging ecology of tropicbirds breeding in two contrasting marine environments in the tropical Atlantic. Mar Ecol Prog Ser 607:221–236. https://doi.org/10.3354/meps12774

Dobson FS, Wigginton JD (1996) Environmental influences on the sexual dimorphism in body size of western bobcats. Oecologia 108:610–616. https://doi.org/10.1007/BF00329033

Emmrich M, Pédron S, Brucet S, Winfield IJ, Jeppesen E, Volta P, Argillier C, Lauridsen TL, Holmgren K, Hesthagen T, Mehner T (2014) Geographical patterns in the body-size structure of European lake fish assemblages along abiotic and biotic gradients. J Biogeogr 41:2221–2233. https://doi.org/10.1111/jbi.12366

Escalante F, Valdez-Holguín JE, Álvarez-Borrego S, Lara-Lara JR (2013) Temporal and spatial variation of sea surface temperature, chlorophyll a, and primary productivity in the gulf of California. Cienc Mar. 39:203–215. https://doi.org/10.7773/cm.v39i2.2233

Fairbairn DJ (1997) Allometry for sexual size dimorphism: pattern and process in the coevolution of body size in males and females.

Annu Rev Ecol Syst 28:659–687. https://doi.org/10.1146/annurev.ecolsys.28.1.659
Fairbairn J, Shine R (1993) Patterns of sexual size dimorphism in seabirds of the Southern Hemisphere. Oikos 68:139–145. https://doi.org/10.2307/3545319
Figuerola J (1999) A comparative study on the evolution of reversed size dimorphism in monogamous waders. Biol J Linn Soc 67:1–18. https://doi.org/10.1111/j.1095-8312.1999.tb01926.x
Fridolfsson A-K, Ellegren H (1999) A simple and universal method for molecular sexing of non-ratite birds. J Avian Biol 30:116–121. https://doi.org/10.2307/3677252
Friesen VL (2015) Speciation in seabirds: why are there so many species…and why aren't there more? J Ornithol 156:27–39. https://doi.org/10.1007/s10336-015-1235-0
Geary B, Walter ST, Leberg PL, Karubian J (2019) Condition-dependent foraging strategies in a coastal seabird: evidence for the rich get richer hypothesis. Behav Ecol 30:356–363. https://doi.org/10.1093/beheco/ary173
Geist V (1987) Bergmann's rule is invalid. Can J Zool 65:1035–1038. https://doi.org/10.1139/z87-164
González-Zamora DA (2019) Identificación de las áreas de forrajeo prioritarias para las aves marinas anidantes en la isla Peña Blanca, México. Masters thesis, Centro de Investigación Científica y de Educación Superior de Ensenada, Baja California, MX.
Grissot A, Araya-Salas M, Jakubas D, Kidawa D, Boehnke R, Błachowiak-Samołyk K, Wojczulanis-Jakubas K (2019) Parental coordination of chick provisioning in a planktivorous Arctic seabird under divergent conditions on foraging grounds. Front Ecol Evol 7:349. https://doi.org/10.3389/fevo.2019.00349
Hill T, Lewicki P (2007) STATISTICS: methods and applications. StatSoft, Tulsa, Oklahoma, USA
Husby A, Hille SM, Visser ME (2011) Testing mechanisms of Bergmann's rule: phenotypic decline but no genetic change in body size in three passerine bird populations. Am Nat 178:202–213. https://doi.org/10.1086/660834
Jakubas D, Wojczulanis-Jakubas K, Jensen J-K (2014) Body size variation of European storm petrels *Hydrobates pelagicus* in relation to environmental variables. Acta Ornithol 49:71–82. https://doi.org/10.3161/000164514X682904
James FC (1970) Geographic size variation in birds and its relationship to climate. Ecology 51:365–390. https://doi.org/10.2307/1935374
Keslinka LK, Wojczulanis-Jakubas K, Jakubas D, Neubauer G (2019) Determinants of the little auk (*Alle alle*) breeding colony location and size in W and NW coast of Spitsbergen. PLoS ONE 14:e0212668. https://doi.org/10.1371/journal.pone.0212668
Korkmaz S, Goksuluk D, Zararsiz G (2014) MVN: an R package for assessing multivariate normality. The R J 6:151–162
Lavín MF, Castro R, Beier E, Godínez VM (2013) Mesoscale eddies in the gulf of California during summer: characteristics and interaction with the wind stress. J Geophys Res 118:1–15. https://doi.org/10.1002/jgrc.20132
Le Corre M, Jouventin P (1999) Geographical variation in the White-tailed tropicbird *Phaethon lepturus*, with the description of a new subspecies endemic to Europa Island, southern Mozambique Channel. Ibis 141:233–239. https://doi.org/10.1111/j.1474-919X.1999.tb07546.x
Lengkeek W, Didderen K, Côté IM, van der Zee EM, Snoek RC, Reynolds JD (2008) Plasticity in sexual size dimorphism and Rensch's rule in Mediterranean blennies (*Blenniidae*). Can J Zool 86:1173–1178. https://doi.org/10.1139/Z08-103
Lewis S, Sherratt TN, Hamer KC, Wanless S (2001) Evidence of intra-specific competition for food in a pelagic seabird. Nature 412:816–819. https://doi.org/10.1038/35090566
Lluch-Cota SE, Aragón-Noriega EA, Arreguín-Sánchez F, Aurioles-Gamboa D, Bautitsta-Romero JJ, Brusca RC, Cervantes-Duarte R, Cortés-Altamirano R, Del-Monte-Luna P, Esquivel-Herrera A, Fernández G, Hendrickx ME, Hernández-Vázquez S, Herrera-Cervantes H, Kahru M, Lavín M, Luch-Belda D, Lluch-Cota DB, López-Martínez J, Marinone SG, Nevárez-Martínez MO, Ortega-García S, Palacios-Castro E, Parés-Sierra A, Ponce-Díaz G, Ramírez-Rodríguez M, Salinas-Zavala CA, Schwartzlose RA, Sierra-Beltrán AP (2007) The gulf of California: review of ecosystem status and sustainability challenges. Prog Oceanogr 73:1–26. https://doi.org/10.1016/j.pocean.2007.01.013
Longhurst A, Sathyendranath S, Platt T, Caverhill C (1995) An estimate of global primary production in the ocean from satellite radiometer data. J Plankton Res 17:1245–1271. https://doi.org/10.1093/plankt/17.6.1245
Ma S, Tao Z, Yang X, Yu Y, Zhou X, Ma W, Li Z (2014) Estimation of marine primary productivity from satellite-derived phytoplankton absorption data. IEEE J Sel Top Appl Earth Obs Remote Sens 7:3084–3092. https://doi.org/10.1109/JSTARS.2014.2298863
Mancini PL, Bond AL, Hobson KA, Duarte LS, Bugoni L (2013) Foraging segregation in tropical and polar seabirds: testing the intersexual competition hypothesis. J Exp Mar Biol Ecol 449:186–193. https://doi.org/10.1016/j.jembe.2013.09.011
Mercado-Santana JA, Santamaría-del-Ángel E, González-Silvera A, Sánchez-Velasco L, Gracia-Escobar MF, Millán-Núñez R, Torres-Navarrete C (2017) Productivity in the gulf of California large marine ecosystem. Environ Dev 22:18–29. https://doi.org/10.1016/j.envdev.2017.01.003
Moen SM (1991) Morphologic and genetic variation among breeding colonies of the Atlantic puffin (*Fratercula arctica*). Auk 108:755–763. https://doi.org/10.1093/auk/108.4.755
Nelson JB (2006) Pelicans, cormorants, and their relatives, the Pelecaniformes. Oxford Univ Press, New York, NY, USA
Nunes GT, Leal GDR, Campolina C, de Freitas TRO, Efe MA, Bugoni L (2013) Sex determination and sexual size dimorphism in the Red-billed tropicbird (*Phaethon aethereus*) and white-tailed tropicbird (*P. lepturus*). Waterbirds 36:348–352. https://doi.org/10.1675/063.036.0312
Nunes GT, Mancini PL, Bugoni L (2017) When Bergmann's rule fails: evidences of environmental selection pressures shaping phenotypic diversification in a widespread seabird. Ecography 40:365–375. https://doi.org/10.1111/ecog.02209
Orta J, Jutglar F, Garcia EFJ, Kirwan GM (2020) Red-billed Tropicbird (*Phaethon aethereus*). In: del Hoyo J, Elliott A, Sargatal J, Christie DA, de Juana E (eds) Birds of the World. ver. 1.0. Cornell Lab of Ornithology, Ithaca, NY, USA
Pagenaud A, Ravache A, Bourgeois K, Mathivet M, Bourguet É, Vidal E, Thibault M (2022) Nest-site selection and its influence on breeding success in a poorly-known and declining seabird: The tahiti petrel *Pseudobulweria rostrata*. PLoS ONE 17:e0267408. https://doi.org/10.1371/journal.pone.0267408
Pennington JT, Mahoney KL, Kuwahara VS, Kolber DD, Calienes R, Chavez FP (2006) Primary production in the eastern tropical Pacific: a review. Progr Oceanogr 69:285–317. https://doi.org/10.1016/j.pocean.2006.03.012
Piña-Ortiz A, Hernández-Vázquez S, Fernández G, Castillo-Guerrero JA (2018) Distribution and population size of the red-billed tropicbird (*Phaethon aethereus*) in Mexico. Waterbirds 41:135–144. https://doi.org/10.1675/063.041.0204
Pozzi LM, Borboroglu PG, Boersma PD, Pascual MA (2015) Population regulation in magellanic penguins: what determines changes in colony size? PLoS ONE 10:e0119002. https://doi.org/10.1371/journal.pone.0119002
Quillfeldt P, Peter H-U (2000) Provisioning and growth in chicks of Wilson's storm-petrels (*Oceanites oceanicus*) on King George Island, South Shetland islands. Polar Biol 23:817–824. https://doi.org/10.1007/s003000000158
Quillfeldt P, Strange IJ, Masello JF (2007) Sea surface temperatures and behavioural buffering capacity in thin-billed prions *Pachyptila*

belcheri: breeding success, provisioning and chick begging. J Avian Biol 38:298–308. https://doi.org/10.1111/j.2007.0908-8857.03846.x

R Core Team (2020) *R: A language and environment for statistical computing*. R Foundation for statistical computing, Vienna, Austria. < https://www.R-project.org/>. Accessed 25 September 2021

Rensch B (1938) Some problems of geographical variation and species-formation. Proc Linn Soc Lond 150:275–285. https://doi.org/10.1111/j.1095-8312.1938.tb00182k.x

Rodríguez MÁ, López-Sañudo IL, Hawkins BA (2006) The geographic distribution of mammal body size in Europe. Glob Ecol Biogeogr 15:173–181. https://doi.org/10.1111/j.1466-822X.2006.00206.x

Roitberg ES (2007) Variation in sexual size dimorphism within a widespread lizard species. In: Fairbairn DJ, Blanckenhorn WU, Székely T (eds) Sex, size and gender roles: evolutionary studies of sexual size dimorphism. Oxford Univ Press, New York, NY, USA, pp 143–153

Seeholzer GF, Brumfield RT (2017) Isolation by distance, not incipient ecological speciation, explains genetic differentiation in an andean songbird (Aves: *Furnariidae*: *Cranioleuca antisiensis*, line-cheeked spinetail) despite near threefold body size change across an environmental gradient. Mol Ecol 27:279–296. https://doi.org/10.1111/mec.14429

Serrano-Meneses MA, Székely T (2006) Sexual size dimorphism in seabirds: sexual selection, fecundity selection and differential niche-utilisation. Oikos 113:385–394. https://doi.org/10.1111/j.0030-1299.2006.14246.x

Sexton JP, Hangartner SB, Hoffmann AA (2014) Genetic isolation by environment or distance: which pattern of gene flow is most common? Evolution 68:1–15. https://doi.org/10.1111/evo.12258

Simpson JH, Souza AJ, Lavín MF (1994) Tidal mixing in the gulf of California. In: Beven KJ, Chatwin PC, Millbank JH (eds) Mixing and transport in the environment. Wiley, New York, USA, pp 160–182

Stillwell RC (2010) Are latitudinal clines in body size adaptive? Oikos 119:1387–1390. https://doi.org/10.1111/j.1600-0706.2010.18670.x

Stillwell RC, Fox CW (2007) Environmental effects on sexual size dimorphism of a seed-feeding beetle. Oecologia 153:273–280. https://doi.org/10.1007/s00442-007-0724-0

Stonehouse B (1962) The tropic birds (genus *Phaethon*) of ascension island. Ibis 103B:124–161. https://doi.org/10.1111/j.1474-919X.1962.tb07242.x

Székely T, Reynolds JD, Figuerola J (2000) Sexual size dimorphism in shorebirds, gulls, and alcids: the influence of sexual and natural selection. Evolution 54:1404–1413. https://doi.org/10.1111/j.0014-3820.2000.tb00572.x

Tamate T, Maekawa K (2006) Latitudinal variation in sexual size dimorphism of sea-run masu salmon, *Oncorhynchus masou*. Evolution 60:196–201. https://doi.org/10.1111/j.0014-3820.2006.tb01094.x

Teplitsky C, Mills JA, Alho JS, Yarrall JW, Merilä J (2008) Bergmann's rule and climate change revisited: disentangling environmental and genetic responses in a wild bird population. Proc Nat Acad Sci USA 105:13492–13496. https://doi.org/10.1073/pnas.0800999105

Ulian CMV, Rossi MN (2016) Intraspecific variation in body size and sexual size dimorphism, and a test of Rensch's rule in bats. Acta Zool 98:377–386. https://doi.org/10.1111/azo.12183

Weimerskirch H (2002) Seabird Demography and Its Relationship with the Marine Environment. In: Schreiber EA, Burger J (eds) Biology of Marine Birds. CRC Press LLC, Boca Raton, FL USA, pp 115–135

Wilkinson TAC, Wiken E, Bezaury-Creel J, Hourigan TF, Agardy T, Herrmann H, Janishevski L, Madden C, Morgan L, Padilla M (2009) Marine ecoregions of North America. Commission for Environmental Cooperation, Montreal, CAN.

Wojczulanis-Jakubas K, Jakubas D, Welcker J, Harding AMA, Karnovsky NJ, Kidawa D, Steen H, Stempniewicz L, Camphuysen CJ (2011) Body size variation of a high-arctic seabird: the dovekie (*Alle alle*). Polar Biol 34:847–854. https://doi.org/10.1007/s00300-010-0941-6

Yamamoto T, Kohno H, Mizutani A, Yoda K, Matsumoto S, Kawabe R, Watanabe S, Oka N, Sato K, Yamamoto M, Sugawa H, Karino K, Shiomi K, Yonehara Y, Takahashi A (2016) Geographical variation in body size of a pelagic seabird, the streaked shearwater *Calonectris leucomelas*. J Biogeogr 43:801–808. https://doi.org/10.1111/jbi.12654

Chapter II

PARENTAL DUTIES AND FORAGING STRATEGIES OF A TROPICAL PELAGIC SEABIRD (PHAETHON AETHEREUS, AVES: PHAETHONTIDAE) DURING THE BREEDING SEASON

Piña-Ortiz Alberto, González-Zamora Diego Adolfo, Paz Jesica Andrea, Hernández-Vázquez Salvador, Mellink Eric, Bustamante Paco, Quillfeldt Petra, Castillo-Guerrero José Alfredo

Marine Biology Volume 171, article number 64, (2024)
https://doi.org/10.1007/s00227-023-04375-1

Marine Biology (2024) 171:64
https://doi.org/10.1007/s00227-023-04375-1

ORIGINAL PAPER

Parental duties and foraging strategies of a tropical pelagic seabird (*Phaethon aethereus*, Aves: Phaethontidae) during the breeding season

Alberto Piña-Ortiz[1] · Diego Adolfo González-Zamora[2] · Jesica Andrea Paz[3] · Salvador Hernández-Vázquez[4] · Eric Mellink[2] · Paco Bustamante[5] · Petra Quillfeldt[1] · José Alfredo Castillo-Guerrero[4]

Received: 8 September 2023 / Accepted: 6 December 2023

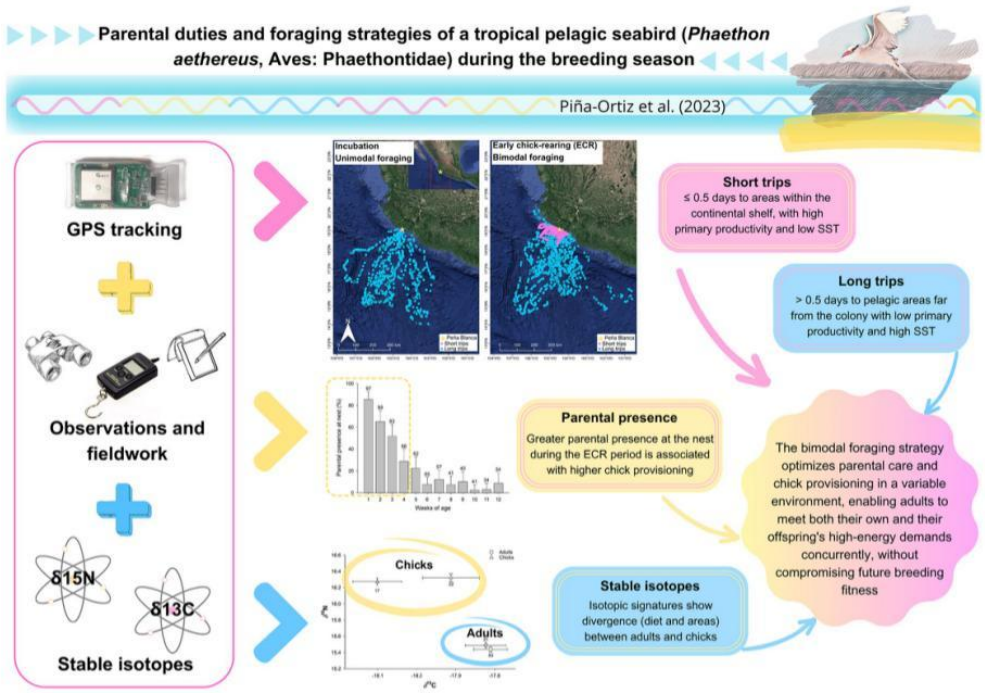
Abstract
Breeding seabirds must balance the energetic demands of feeding themselves and their offspring while coping with the constraints imposed by central-place foraging. As such, foraging strategies and parental care patterns are usually linked. Here, the foraging behavior of the Red-billed Tropicbird (*Phaethon aethereus*; n = 161) of Peña Blanca Islet, Mexico (19° 06′ 11″ N, 104° 29′ 12″ W) during the incubation and chick-rearing (≤ 4 weeks of age) stages was characterized with the aid of GPS loggers. Blood samples from adults and chicks were collected to determine δ^{15}N and δ^{13}C, and parental presence at the nest, meal size, and parent-chick feeding events were recorded. During incubation, parents made long trips offshore to areas far from the colony; however, immediately after their chicks hatched, the parents switched to a bimodal foraging strategy by undertaking both short and long foraging trips. The δ^{15}N values indicated that trophic differences were present between parents and their offspring, with chicks being fed prey enriched in ^{15}N. Parental presence at the nest was greater during early chick-rearing, which was associated with a higher provisioning rate. Parents adopted a strategy in which the parent on nest duty only made short foraging trips to provide for its offspring without leaving it unattended for long periods, while its mate undertook long trips to feed itself. After the early chick-rearing period, the parents gradually reduced the time spent at the nest and increased the time spent foraging, compensating with larger meal sizes for their offspring.

Responsible Editor: V. Paiva .

Extended author information available on the last page of the article

Published online: 30 January 2024

Graphical abstract



Keywords Bimodal foraging · Foraging behavior · GPS-tracking · Parental duties · Red-billed Tropicbird · Stable isotopes

Introduction

The breeding season is an essential and energetically demanding period in the annual cycles of seabirds. During this period, seabird parents must strike a balance between feeding themselves and their offspring given the constraints of central-place foraging (Orians and Pearson 1979; Harding et al. 2013; Cleeland et al. 2014). In the marine environment, resources are generally sparse and patchy, making it difficult for pelagic seabirds to gather enough food for themselves and their chicks (Weimerskirch 2007; Shoji et al. 2015). If food resources in the vicinity of a seabird breeding site are limited, parents may be unable to simultaneously meet the needs of their offspring while maintaining their body condition (Welcker et al. 2009). In response to this limitation, seabird species can employ a bimodal foraging strategy, which has been reported in the families Laridae, Sulidae, Spheniscidae, Diomedeidae, Procellariidae, Alcidae, Fregatidae, and Phaethontidae (Baduini and Hyrenbach 2003; Steen et al. 2007; Welcker et al. 2009; Sommerfeld and Hennicke 2010; Campos et al. 2018; Austin et al. 2019; Phillips et al. 2023).

The bimodal foraging strategy is characterized by either alternating short and long trips or long trips interspersed with multiple short trips (Weimerskirch 1998; Congdon et al. 2005). Short trips enable parents to feed their chicks frequently, albeit at the expense of adult body condition, while long trips allow the adults to restore their reserves (Weimerskirch 1998; Weimerskirch et al. 2003). However, this bimodal foraging strategy may be influenced by the distance of profitable prey patches from the colony (Suryan et al. 2000). The bimodal foraging strategy has also been interpreted as a means of regulating parental investment in offspring (Granadeiro et al. 1998). Originally, the decision to undertake a short or long trip was thought to be under the exclusive control of parental body condition (Weimerskirch 1998). However, chick begging was found to strongly influence seabird parents in later studies (e.g., Quillfeldt 2002; Hamer et al. 2006), although its influence on bimodal foraging has not been investigated. The bimodal foraging strategy is relatively common among seabirds distributed in temperate and tropical-subtropical areas, and the factors affecting

the decisions of the parents to undertake either long or short foraging trips may be species-specific (Baduini and Hyrenbach 2003).

The Red-billed Tropicbird (*Phaethon aethereus*) is a pelagic seabird distributed in the tropical areas of the Atlantic, Indian, and Pacific Oceans (Nelson 2006). This species exhibits foraging plasticity in response to the varying oceanographic conditions in its breeding sites (Castillo-Guerrero et al. 2011; Diop et al. 2018). In the eastern Pacific, red-billed tropicbirds live in the open ocean most of the year, where they forage in patchy oligotrophic waters characterized by shallow thermoclines and low salinity (Spear and Ainley 2005). During the breeding period, which lasts approximately six months, they become central-place foragers, alternating between tending to their chicks in nesting colonies and undertaking foraging trips that take them up to 600 km from their breeding sites (Nelson 2006; Diop et al. 2018).

During the rearing period, parents must feed their chicks frequently. In turn, parents exploit the trophic resources surrounding the colony by undertaking short foraging trips lasting 3–4 h (Sommerfeld and Hennicke 2010; Campos et al. 2018). Nevertheless, the amount of available food near these colonies may be insufficient to maintain parental body condition and meet the needs of growing chicks. In response, red-billed tropicbirds appear to switch from a unimodal to bimodal foraging strategy (see Sommerfeld and Hennicke 2010). Nevertheless, it is unknown whether the use of a bimodal foraging strategy is widespread among tropicbirds or if it is employed only by individuals in breeding colonies located in highly oligotrophic environments or during years of low prey availability (Campos et al. 2018). Furthermore, little information is available regarding the habitat use, behavior, or foraging ecology of the Red-billed Tropicbird (e.g., Diop et al. 2018; Madden et al. 2022, 2023). Indeed, no study has linked foraging variables to Red-billed Tropicbird breeding parental presence at the nest, meal mass, chick-feeding rates, or foraging behavior at sea.

In this study, we assessed the foraging ecology and parental care patterns of breeding red-billed tropicbirds on Peña Blanca Islet, Mexico. We characterized the foraging areas surrounding the islet and their oceanographic characteristics, including sea surface temperature (SST), chlorophyll-a (Chl-a), and bathymetry, and monitored the at-sea behavior of red-billed tropicbirds during the egg incubation and chick-rearing stages with the aid of GPS data loggers over six breeding seasons (2017–2022). In addition, we measured the isotopic values of carbon (δ^{13}C) and nitrogen (δ^{15}N) in whole blood samples from adults and chicks to evaluate if differences were present in the assimilated foods. The presence of parents at nests, feeding frequency, and amount of food given to chicks were also evaluated, considering the age of the chicks. We hypothesized that (1) breeding adults would switch from a unimodal to bimodal foraging strategy between the incubation and chick-rearing stages, undertaking more short than long trips during the latter stage; (2) the bimodal strategy would allow for a high parental presence at the nest and frequent chick feeding during the first weeks after hatching, which would be reflected in the presence of at least one parent at the nest, who would be in charge of undertaking short trips to provide food for the chick, while its mate would undertake long trips for self-provisioning; (3) variations in isotopic composition would be present between the blood of parents and their offspring, with chicks showing enriched δ^{15}N values and impoverished δ^{13}C values compared to those of their parents because chicks would be fed prey from high trophic levels found in areas near the colony; (4) a gradual decrease in adult presence at the nest would be apparent along with an increase in foraging activity, which would be reflected in an increase in chick meal size as the chicks grew.

Materials and methods

Study area

The study was carried out on Peña Blanca (19° 06′ 11″ N, 104° 29′ 12″ W), an islet located 1.9 km from the coast of Colima, Mexico (Supplementary Information, Fig. S1). Peña Blanca supports a colony of 1200–1600 pairs of red-billed tropicbirds (Hernández-Vázquez et al. 2018). According to the climatic conditions and primary productivity of the sea in the region, there are two marked climatic periods during the year: winter-spring (Dec–May) and summer-autumn (Jun–Nov). During winter-spring, SST values range between 23.5 and 30 °C, while salinity (34.3) and Chl-a (up to 10 mg m^{-3}) are both high at the beginning of the period, with Chl-a later decreasing (1 mg m^{-3}). Summer-autumn is characterized by high SST values that increase to 31 °C, while salinity and Chl-a values decrease to 33.5 and ~ 0.1 mg m^{-3}, respectively (Kono-Martínez et al. 2017). The topographic and physiographic features of the coast promote the formation of dynamic mesoscale structures (i.e., marine current systems spanning 1–100 km), such as anticyclonic and cyclonic eddies, over the continental shelf (Salas et al. 2006).

GPS deployment and sampling

Fieldwork was conducted from January to May during six consecutive breeding seasons (2017–2022; Table S1), covering the egg-laying peak (Jan–Feb) and hatching and fledgling periods (Mar–Apr) of this colony (Hernández-Vázquez et al. 2018). GPS data loggers (i-gotU GT-120, Mobile Action, Taiwan; CatLog-S, Catnip Technologies, Hong

Kong, China; and CatLog-S2, Perthold Engineering LLC, Dallas, USA) were attached to 161 breeding adults, which were captured by hand directly from nest burrows during incubation and the first 4 weeks of the chick-rearing period (early chick-rearing; ECR). Each nest was located within a pre-established study plot used for long-term monitoring. The loggers were programmed to record time, latitude, and longitude every 5 min and water-proofed with heat-shrink casing.

Data loggers were attached with TESA® tape (Norderstedt, Germany) to the tops of four to five central rectrices directly below the uropygial gland. The loggers and tape weighed between 8–16 g, which was ~ 2.4% (1.5–2.9%) of adult body mass (536.85 ± 50.56 g; range: 432.9–664.6 g, n = 54; Piña-Ortiz et al. 2023) and below the recommended 3% weight threshold for devices attached to birds (Wilson and McMahon 2006; Vandenabeele et al. 2012). Adults from targeted nests were captured during the first hours of daylight (0600–0900 h) and the last hours before dusk (1800–2000 h) to protect the birds from sunstroke. The handling time never exceeded 10 min. Data loggers were recovered 1–15 days after being attached by carefully removing the tape from the tail feathers. In addition, blood samples (~ 0.5 mL per bird) were obtained by brachial vein puncture with a syringe (3 mL, 23G, 0.5 mm × 16 mm) from adults (n = 84) and chicks (n = 39), including those tagged with GPS devices during the 2020–2022 breeding seasons. The blood samples were transferred to 1.5-mL plastic tubes and kept on ice in the field. Once in the laboratory, they were frozen at − 20 °C for stable isotope analysis. While the adults were handled, assistants cared for the eggs or chicks until the adults were returned to the nests. Continual monitoring of the nests and parental breeding success confirmed that no adults abandoned the nests after being handled.

Analyses of foraging trajectories

We visually reviewed every GPS trajectory obtained from all individuals in Google Earth or CatLog_Data-viewer and removed all anomalous trajectories and those over land. Next, following the approach applied by Diop et al. (2018), we eliminated those fixes that would have resulted in an average velocity of > 80 km h^{-1} (i.e., the species flight speed threshold). Then, foraging parameters from the tracking data were determined in R v. 4.3.1 (R Core Team 2023) with RStudio v. 2023.06.1 + 524 "Mountain Hydrangea" (RStudio Team 2023) using the function 'tripSplit' provided in the 'track2KBA' package (Beal et al. 2021). This function allowed us to split individual GPS trajectories from multiple foraging trips from individual birds, which were separated by the return of the individual to the colony. For each foraging trip, we calculated the maximum linear distance from the colony, the total duration of the trip, and the total distance traveled. Incomplete foraging trips (i.e., foraging trips that could not be fully tracked prior to the return of an individual to the colony) were removed from all subsequent analyses. To guarantee the accurate splitting of individual foraging trips, given that subsequent trips with burrow-nesting species may be lumped into a single trip, a 1.5-km radius filter was applied to the colony to discard these locations (Beal et al. 2021).

A total of 178 complete foraging trips were recorded from 140 Red-billed Tropicbirds during the incubation and ECR stages from 2017 to 2022 (Table S1). Individual foraging trips were classified as either short (≤ 0.5 days) or long (> 0.5 days) using the method proposed by Welcker et al. (2009), who used two log-normal distributions to determine the best fit and set the cut-off value as that which minimized the sum of the variances of both trip types given their log-normal distribution. For subsequent analyses, data from all years were pooled according to the lowest number of complete foraging trips for 2017, 2018, and 2019 (3, 3, and 12 trips, respectively; Table S1, Fig. S1).

Once all individual foraging trips were classified, they were grouped based on behavior with Hidden Markov Models (HMM) with the 'moveHMM' package (Michelot et al. 2016). Four behavioral states were defined based on the speeds and turning angles of the trajectories: resting (low speed and low turning angles), intensive search (low speed and high turning angles), extensive search (high speed and high turning angles), and relocation (high speed and low turning angles). Similar to what has been obtained in other studies that have classified the foraging behavior of tropicbirds (see Diop et al. 2018), the HMM algorithm does not effectively categorize intensive search and resting behaviors (Fig. S2–S5). Therefore, fixes classified as intensive search and resting behaviors were grouped into the single category of "resting-intensive search". Foraging behavior was then inferred from locations classified as "extensive search" and "resting-intensive search". Extensive searches involved individuals foraging over large areas to locate prey patches, whereas intensive searches occurred when individuals conducted area-restricted searches after locating prey (Weimerskirch 2007; Bennison et al. 2017; Clay et al. 2019; dos Santos et al. 2022). Subsequently, "extensive search" and "resting-intensive search" locations were selected to classify the habitat use of red-billed tropicbirds using kernel density estimations (KDEs) through the transformation of all positions into utilization distributions (Wood et al. 2000).

To estimate the kernel utilization distributions (KUD), we made use of the functions 'hr_kde_ref' and 'hr_kde_pi' in the 'amt' package (Signer et al. 2019) to calculate the reference bandwidth and the bandwidth with the plug-in equation method in two dimensions, respectively (Wand and Jones 1994; Gitzen et al. 2006). Due to its simplicity and wide use in other studies, we selected the reference bandwidth as

the ideal smoothing parameter for estimating KUDs (Beal et al. 2021). A smoothing parameter of 6.75 km was used for short trips, and a smoothing parameter of 27.89 km was used for long trips. We calculated the 50% and 95% KUDs using the function 'hr_kde' in the package 'amt' to represent the core and general foraging areas used by adults, respectively (Fig. S6). The representativeness (the degree to which the space use of a tracked sample of animals represents that of the larger population) of short and long foraging trips was tested separately for the incubation and ECR stages, resulting in a low representativeness for the population (Fig. S7–S8). However, the representativeness for short and long foraging trips pooling both breeding stages was suitable (Fig. S9–S10; Beal et al. 2021). The overlap of the KUD contours for core areas between short and long trips was calculated with the function 'hr_overlap' using the utilization distribution overlap index (UDOI). The UDOI ranges from 0 (when two home ranges do not overlap) to 1 (when two utilization distributions are uniformly distributed and show 100% overlap). However, the UDOI can be > 1 if the utilization distributions are non-uniformly distributed and exhibit a high degree of overlap (Fieberg and Kochanny 2005).

Oceanographic variables

The oceanographic characteristics of the core areas (KUD 50%) of short and long foraging trips were assessed. Raster data for SST (°C), Chl-a (mg m^{-3}), and bathymetry (m) were downloaded from the ERDDAP database (http://coastwatch.pfeg.noaa.gov/erddap). The SST and Chl-a values were collected from the Aqua MODIS satellite model "Net Primary Production (NPP), 0.025 degrees, Pacific Ocean, Daytime, 2006–present (8 Day Composite), Lon ± 180." Bathymetry values corresponded to the model "Topography, ETOPO1, 0.0166667 degrees, Global (longitude -180 to 180), (Ice Sheet Surface)." The raster images used for SST and Chl-a had a monthly period from December to May between 2017 and 2022, which were the months during which the foraging tracks were recorded (Hernández-Vázquez et al. 2018). Finally, the values of the oceanographic variables within each core area (i.e., short and long trips) were obtained.

A Chi-square homogeneity test was used to compare the proportion of short and long foraging trips between the incubation and ECR stages. Two-way analyses of variance (ANOVA) were used to compare SST values and Chl-a concentrations between the core areas, including the type of foraging trip (i.e., long or short) and month as categorical factors. Bathymetry differences between the core areas were tested with a one-way ANOVA with the type of foraging trip as a factor.

Parental presence

The presence of parents caring for chicks at the nests was based on the results of monitoring 68, 108, and 100 nests during December–April of the 2020, 2021, and 2022 breeding seasons, respectively. The number of active nests, chick age, and the presence or absence of the parents were recorded within pre-established study plots by punctual observations of all nests in these plots. The time required to survey all pre-established plots was approximately 4 h (0800–1100 h), and the plots were surveyed in the same order each time. We knew the hatching dates for most chicks; however, when hatching occurred between visits, chick age was estimated based on plumage and body measurements. In the 2020 season, we conducted 5 surveys (3 December, 28 January, 10 February, 9 and 21 March). In the 2021 and 2022 seasons, we conducted 9 and 8 surveys during each season (2021: 18 December; 15, 22 and 31 January; 9 and 16 February; 19 and 27 March; and 4 April; 2022: 12 and 14 December; 18 and 24 January; 1 February; and 11, 22, and 31 March), respectively. On Peña Blanca, red-billed tropicbirds are not sexually dimorphic; thus, it was not possible to sex the adult individuals. However, to identify each member of a breeding pair, individuals were tagged with alphanumeric bands affixed to the tarsus.

Stable isotope analyses

To assess differences in the assimilated foods between parents and offspring, carbon and nitrogen isotope analyses were conducted on whole blood samples that reflected the dietary integration period of 2–4 weeks prior to sampling (Bearhop et al. 2002). Therefore, the blood samples taken from the individuals in this study during the ECR (adults = 53 [2020 = 15, 2021 = 16, and 2022 = 22], chicks = 17 [2020 = 5, 2021 = 1, and 2022 = 11]) and late chick-rearing (LCR; adults = 31 [2020 = 8, 2021 = 11, and 2022 = 12], chicks = 22 [2020 = 9, 2021 = 3, and 2022 = 10]) periods should reflect the dietary items consumed during those stages. Blood samples were oven-dried at 50 °C for 24–48 h and then finely ground and homogenized. Subsamples (0.3–0.5 mg) were packed in tin capsules and analyzed for %N, %C, δ^{15}N, and δ^{13}C using a Flash 2000 elemental analyzer (Thermo Scientific, Milan, Italy) coupled with a Delta V Plus isotope ratio mass spectrometer with a Conflo IV interface (Thermo Scientific, Bremen, Germany). Analyses were conducted at the Littoral, Environnement et Societés (LIENSs) Joint Research Unit stable isotope facility (CNRS – La Rochelle Université, France). Results are expressed as δ (‰) for δ^{15}N and δ^{13}C and were calibrated against the international isotopic references of atmospheric nitrogen for δ^{15}N and Vienna-Pee Dee Belemnite for δ^{13}C. The analytical precision was ± 0.15 ‰ δ^{15}N and ± 0.10

‰ for $\delta^{13}C$ based on the internal standards USGS-61 and USGS-62, which were inserted every ten measurements. All samples had low C:N mass ratios (< 4.0), indicating low lipid content, and no lipid extraction was required (Cherel et al. 2005).

Physiological and metabolic differences between seabird adults and chicks may influence their isotopic ratios (Harding et al. 2008; Sears et al. 2009; Micklem et al. 2021). Therefore, the isotopic composition between parents and offspring may reflect some differences not linked to dietary intake. As chicks grow, their $\delta^{15}N$ values gradually change due to ontogenetic changes in tissue turnover rates (Harding et al. 2008; Sears et al. 2009; Micklem et al. 2021). Blood analyses of captive African penguins (*Spheniscus demersus*) showed that chick growth resulted in a depletion of ^{15}N in whole blood of 0.30 ‰ with respect to that of the adults (neither breeding, fasting, nor molting) under the dietary regime established by the researchers (see Micklem et al. 2021 for details). To compare adult and chick $\delta^{15}N$ values, we adjusted our $\delta^{15}N$ data by subtracting 0.30 ‰ from the adult values.

General linear models (GLM) were used to assess the differences in $\delta^{15}N$ and $\delta^{13}C$ isotope ratios between chicks and adults (i.e., age class), including the effects of the breeding season (2020–2022) and breeding stage (ECR and LCR) as factors and sample collection date (days since 1 January) as a continuous predictor. All GLMs were applied based on complete initial models that considered all variables and interactions. Subsequently, all non-significant interactions and variables ($P < 0.05$) were eliminated to simplify the models. The initial models, which included all variables and interactions, are presented in Table 3. However, the accompanying statistics correspond to the level at which non-significant interactions and variables were removed from the model.

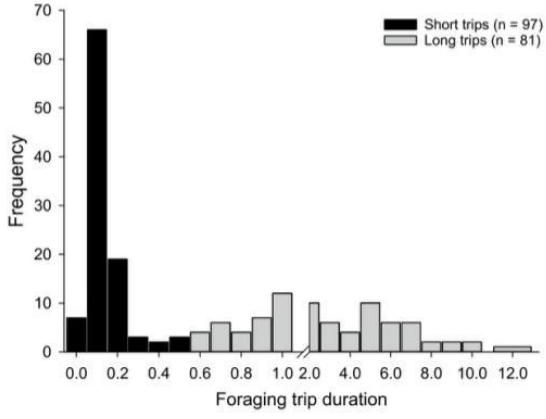


Fig. 1 Frequency distribution of foraging trip duration (days) of the Red-billed Tropicbird of Peña Blanca Islet, Mexico. Short trips are indicated by dark bars, and long trips are indicated by gray bars

Table 1 Number and proportion of long and short foraging trips in red-billed tropicbirds in the incubation and early chick-rearing (ECR) stages from 2017 to 2022 at Peña Blanca Islet, Mexico

Foraging trip length	Incubation $n = 11$		Early chick-rearing $n = 167$	
	Number of trips	Proportion (%)	Number of trips	Proportion (%)
Long	10	90.9	71	42.5
Short	1	9.1	96	57.5

A Chi-square test revealed significant differences in the distribution of the number of long and short foraging trips between the incubation and ECR stages (Chi-square test, $X1 = 7.90$, $df = 1$, $P = 0.005$)

Furthermore, t-tests that included a Bonferroni correction ($\alpha = 0.025$, 2 comparisons) were used to compare differences in isotopic signatures between adults and chicks during the ECR and LCR stages.

Chick feeding events

Parental food supply and chick meal sizes were determined in the 2020, 2021, and 2022 breeding seasons by weighing 82 chicks (1–12 weeks old) three times (events) each day at dawn (0700 h), noon (1300 h), and dusk (1800 h). Chick body mass was measured with a portable electronic scale to the nearest 5 g. For each breeding season, chicks were selected from nests located in five different islet sections exhibiting different nest densities. Body mass was measured in 34, 20, and 28 chicks in either March or April of 2020 (13–20 March), 2021 (27 March–2 April), and 2022 (23–31 March), respectively. The maximum number of nests with chicks and the highest annual values of Chl-a surrounding the study area are present during these months (Hernández-Vázquez et al. 2018). For each time event, the weighing time was ~ 40 min, and each chick was always weighed in the same order. Increases in chick mass between weighing events were attributed to parental feeding and were used to estimate daily feeding events and meal sizes.

Due to the sensitivity of the scale and the unlikeliness of adults feeding chicks with only small amounts of food, all increases in weight < 10 g were omitted from the analyses. To obtain the number of feeding events per day, we divided the number of events in which a chick gained weight by the number of days it was weighed. Mass increments between weighing events can be considered a proxy of the meal sizes parents deliver. However, with this approach, the amount of food is inevitably underestimated because chicks also lose mass through excretion. Therefore, we evaluated mass loss after feeding events using linear mixed models (LMMs), to estimate the relationship between chick mass loss rates at

Table 2 Foraging and spatial ecology parameters (mean ± SD) of red-billed tropicbirds on short and long trips during incubation and ECR stages at Peña Blanca Islet during six consecutive breeding seasons (2017–2022)

Foraging parameters	Short trips	Long trips
n =	41	49
Number of foraging trips	97	82
Trip duration (days)	0.1 ± 0.1	3.7 ± 2.9
Maximum distance to colony (km)	31.1 ± 21.3	245.8 ± 173.1
Total distance travelled (km)	75.3 ± 52.7	771.8 ± 554.6
Direction (°)	228.2 ± 55.6	205.8 ± 51.6
Spatial ecology parameters		
Number of foraging trips	39	35
Core areas (50% KUD; km^2)	545.2 ± 635.4	10,761.9 ± 8072.4
General areas (95% KUD; km^2)	2169.1 ± 2745.6	46,701.8 ± 35,222.5
Core areas overlap between foraging trip category	$2.24 \times 10^{-2} \pm 0.0$	
General areas overlap between foraging trip category	0.10 ± 0.00	

Breeding season and reproductive stage data are pooled. Direction (°) was measured from the origin (islet) to the furthest point of the foraging trip. The overlap of the core areas (50% KUD) was estimated between trip categories using the utilization distribution overlap index (UDOI). *n* = number of individuals from which the foraging and spatial ecology parameters were obtained. The UDOI ranges from 0 (when two home ranges do not overlap) to 1 (when two utilization distributions are uniformly distributed and exhibit 100% overlap; Fieberg and Kochanny 2005)

post-feeding intervals and initial mass, chick age, and meal size, with individuals considered a random factor.

The rate of mass loss following a feeding event was related to the initial mass of the chick and meal size but not to chick age [ANOVA Satterthwaite's method, Meal size: $F_{(1,273)} = 11.90$, $P < 0.001$; Initial mass: $F_{(1,273)} = 7.45$, $P = 0.006$; Age: $F_{(1,273)} = 1.96$, $P = 0.16$]. The equation obtained, $Masslossrate\left(gh^{-1}\right) = -0.235 - 0.030(mealsize) - 0.009(initialmass)$, was used to estimate mass loss between weighing events and correct the calculated meal size and the total amount of food provided to the chicks. Meal size corrections were made under the following extreme possibilities: (1) the maximum possible meal size, assuming that chicks were fed immediately before weighing, and (2) the minimum possible meal size (no adjustment required), assuming that the chicks were fed just after they were weighed. Considering such possibilities, a consumption threshold (i.e., the range between these two possibilities) was obtained for each age group. In addition, we recorded culmen, ulna, and tarsus lengths for all chicks on both the first and last weighing days (Table S2). All statistical analyses were conducted in STATISTICA 7.1 (Hill and Lewicki 2007), except for the LMMs, which were implemented with the 'lme4' package in R (Bates et al. 2015). All values are expressed as mean ± SD throughout the results section. The significance level was set to $P \leq 0.05$.

Results

Foraging strategy of breeding adults

Overall, the foraging trips exhibited a bimodal distribution, with more short trips (97 trips; up to 0.5 d) than long trips (81 trips; up to 12 d; Fig. 1). Short and long foraging trips were recorded for all breeding seasons (see Table S3). However, during the incubation period, tropicbirds conducted long trips > 90% of the time. After the eggs hatched, tropicbirds undertook long and short trips 42.5 and 57.5% of the time, respectively (Table 1). This pattern was consistent for both reproductive stages throughout the breeding season of the species (Fig. S1). Short trips took the tropicbirds 31 km from the islet, lasted 0.14 ± 0.10 d (mean ~ 3 h, range 1.03–12.85 h, n = 97), and covered a mean total distance of ~ 75 km. Individuals on long trips traveled approximately 770 km (mean maximum distance from the colony of 245 ± 173 km, n = 81), with these trips lasting from 0.60 to 12.3 d (Table 2). Most trips were conducted southwest of the islet (Table 2; Fig. S1). The core (50% KUD) and general (95% KUD) areas used by red-billed tropicbirds on long trips were an order of magnitude larger in size than those of the short trips (Table 2, Fig. 2 and S2).

The core area of the short trips was located over the continental shelf and exhibited mean SST values ranging from 25.5 to 28.5 °C and mean Chl-a concentrations as high as 14 mg m^{-3} (Fig. 3). In contrast, the long-trip core area was located in deeper waters with higher SST (27.5–29.0 °C) and lower Chl-a concentrations (< 1 mg m^{-3}; Fig. 3) than those of the short-trip core area. The bathymetry and oceanographic variables were significantly different between the two core areas throughout the breeding seasons of the Red-billed Tropicbird in the study area (ANOVA, Depth: $F_{(1,5058)} = 534.13$, $P < 0.001$; SST: $F_{(5,181667)} = 238.46$, $P < 0.001$; Chl-a: $F_{(5,181775)} = 950.33$, $P < 0.001$; Fig. 3a–c). In addition, the overlap between the short- and long-trip core areas was minimal ($2.24 \times 10^{-2} \pm 0.00$), underlining different spatial utilization related to trip type (Fig. 2, Table 2).

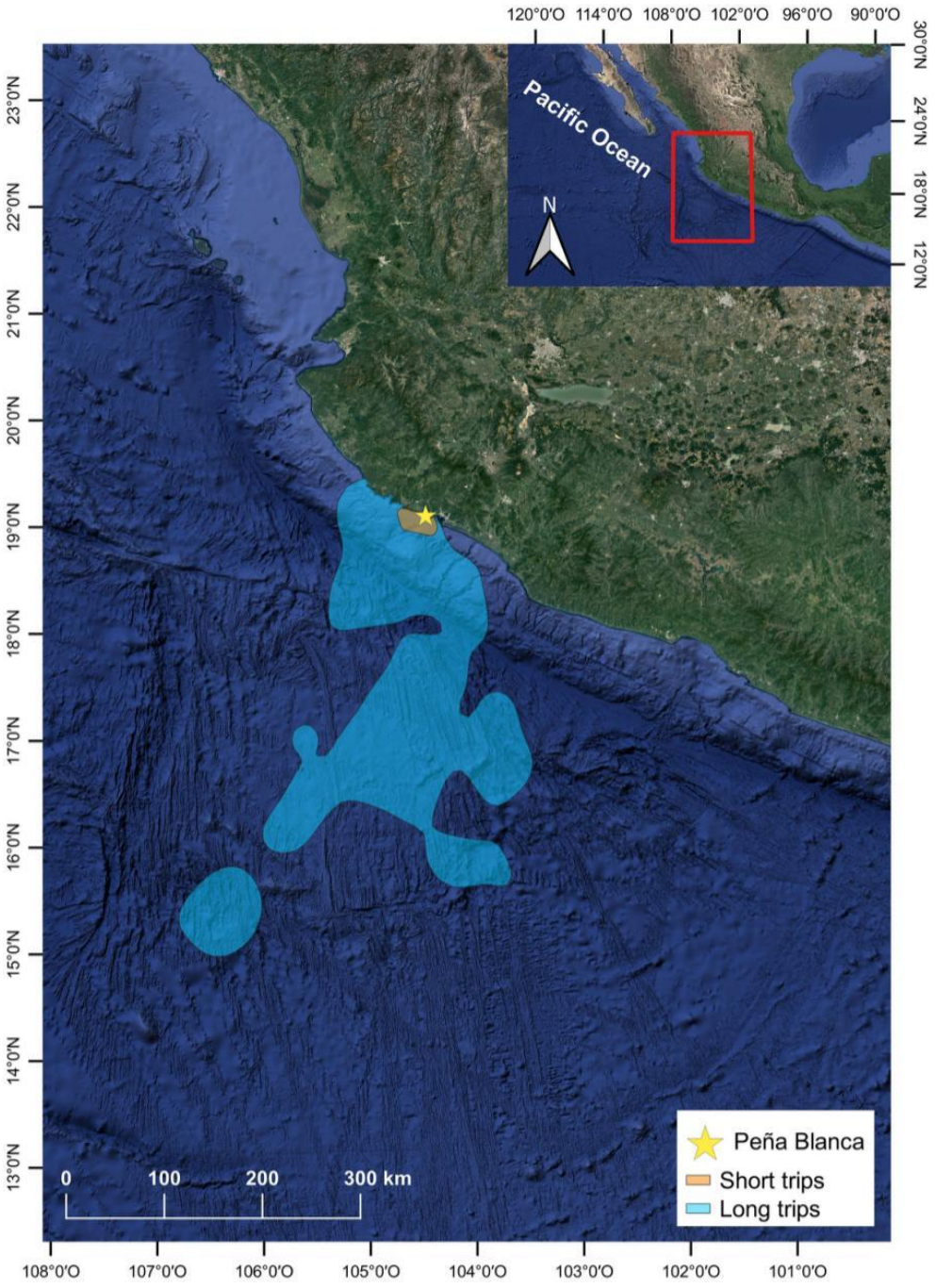


Fig. 2 Utilization distributions of core areas (50% KUD) used by red-billed tropicbirds on short and long foraging trips during the incubation and early chick-rearing (ECR) stages between 2017–2022 on Peña Blanca Islet, Mexico

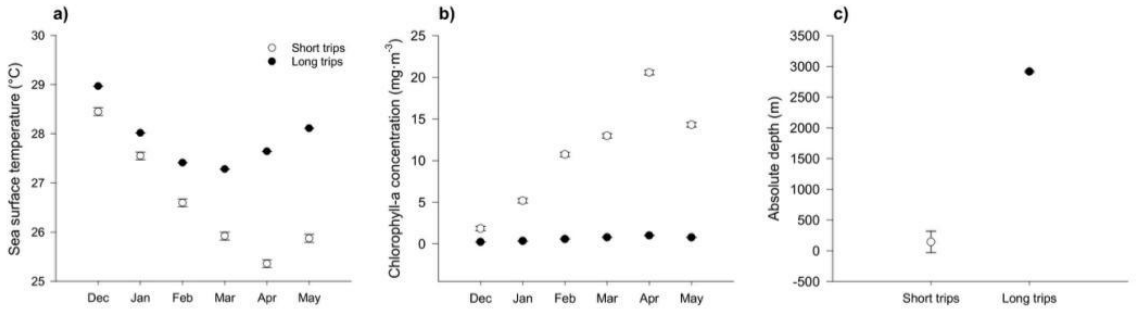


Fig. 3 Unweighted monthly mean ± S.E. of **a** sea surface temperature, **b** chlorophyll-a concentrations, and **c** bathymetry (absolute value) in the core areas (50% KUD) of red-billed tropicbirds during short and long foraging trips during incubation and early chick-rearing (ECR) stages over six breeding seasons (2017–2022) at Peña Blanca Islet, Mexico

Presence of adults at the nest

During nests checks, a total of 709 records (241 presence and 468 absence) allowed us to determine that parental presence gradually decreased during the first 4 weeks after chick hatching (86% during week 1, 29% during week 4). After the chicks reached 4 weeks of age, parental presence remained low until fledgling, fluctuating between 2–23% (Fig. 4a).

Variation in isotopic values between adults and chicks

The δ^{15}N and δ^{13}C values differed significantly between adults and chicks (GLM, δ^{15}N: $F_{(1,116)} = 299.23$, $P < 0.001$; δ^{13}C: $F_{(1,119)} = 16.10$, $P < 0.001$), with a significant year-stage interaction for δ^{15}N ($F_{(2,116)} = 5.10$, $P = 0.008$). The δ^{15}N values from ECR individuals in 2021 were higher than those from 2020 ($t = -0.35$, $P < 0.001$), and the values from LCR individuals during 2021 were higher than those from 2020 and 2022 (t-tests with Bonferroni correction, $t = -0.54$, $P < 0.001$ and $t = 0.50$, $P < 0.001$, respectively). Furthermore, the δ^{15}N values differed between these stages during 2021 ($t = -0.36$, $P < 0.001$). All other factors and interactions were not significant (Table 3). Chicks (in both the ECR and LCR stages) exhibited significantly higher δ^{15}N and lower δ^{13}C values than those of the adults (Fig. 5). The δ^{15}N and δ^{13}C values of chicks were also consistent between years. Late-reared chicks had similar δ^{15}N values and slightly lower but not significantly different δ^{13}C values than those of early-reared chicks (Table 3; Fig. 5).

Meal sizes provided to the chicks

The chicks were fed more than once per day from 2–4 weeks of age, with the average amount of food they received not exceeding 50 g per meal (Fig. 4b,c). From the fifth week of age onward, the chicks were fed approximately once per day, although the feeding frequency increased in weeks 7 and 9 and then continued to increase as the chicks approached fledgling age. The amount of food delivered to the chicks increased during the first 5 weeks and peaked in weeks 6–7, after which it gradually decreased (Fig. 4b,c). Chicks received between 8.5 to 127 g of food, with the greatest amount being given when the chicks were 5–7 weeks in age (Fig. 4c).

Discussion

Foraging strategy of breeding adults

This research demonstrates 1) Red-billed Tropicbird adults switch from unimodal to bimodal foraging strategies immediately after their chicks hatch, 2) the existence of different core utilization areas between adults on short and long foraging trips, 3) a pattern of parental presence in the nest during chick development, and 4) the presence of differences in the isotopic signatures of blood samples from adults and chicks during ECR and LCR. Adults appear to use short trips to feed chicks and long trips to feed themselves, indicating that bimodal foraging is bound to patterns of parental care. During the first few weeks of life, chicks require high parental presence at the nest and must be fed frequently, and consequently, parents take turns caring for their chicks. While one parent remains at the nest to care for its chick, making short trips to feed its offspring, its mate conducts long trips to feed itself. Adults gradually decrease the time they spend at the nest and increase the time they spend foraging. During this time, parents carry more food to their chicks as they grow, with the maximum chick meal sizes being delivered when the chicks are ~ 6 weeks old (Fig. 4c).

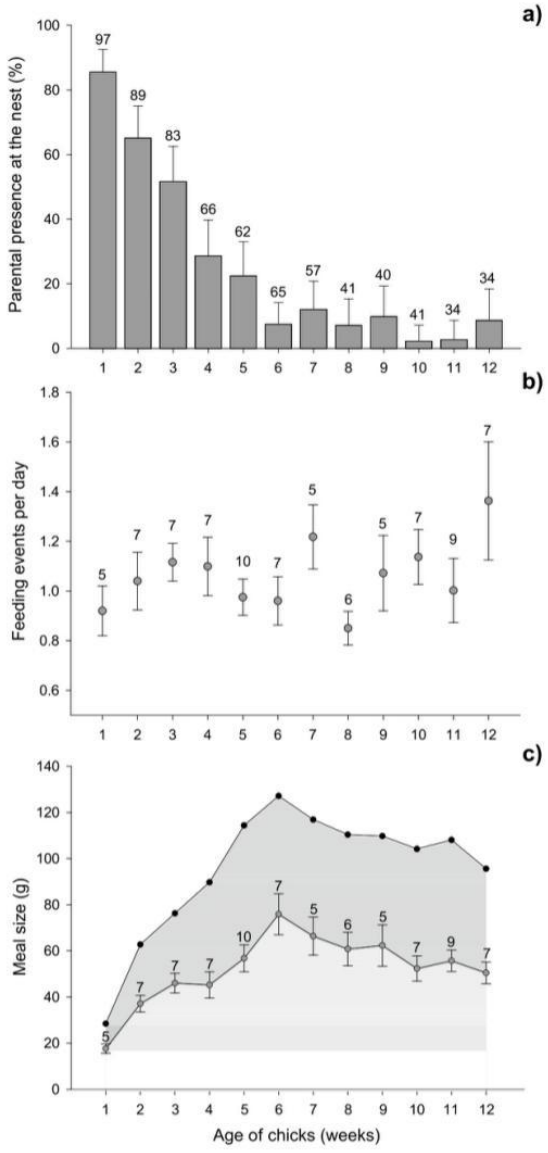


Fig. 4 Parental care and chick feeding of red-billed tropicbirds breeding on Peña Blanca Islet, Mexico. (a) Presence of the parents in active nests during the chick-rearing stage in the 2020–2022 breeding seasons; (b) mean ± S.E. of chick-feeding rates per day; and (c) mean ± S.E. of chick meal size, and fitted lines (black dots) of the total amount of food delivered to 1–12 week old Red-billed Tropicbird chicks (N = 82) during the breeding seasons of 2020 to 2022. The gray shading represents the threshold between the minimum and maximum consumption estimates. The numbers above each error bar indicate the sample sizes from which the mean and S.E. were calculated [number of records for (a) and number of nests for (b) and (c)]

Bimodal foraging

Recent studies on the foraging ecology of red-billed tropicbirds have provided insights into their foraging grounds at-sea and colony-specific foraging movements (Diop et al. 2018; Madden et al. 2022, 2023). However, this study is the first to identify a bimodal foraging strategy during the chick-rearing period in this species (Fig. 1, Table S3). In other colonies, the frequency distribution of the duration of Red-billed Tropicbird foraging trips also shows a bimodal pattern during chick-rearing (see Fig. S12; data from Madden et al. 2022, 2023). Together these findings may indicate that bimodal foraging is an intrinsic mechanism of the species and perhaps even for Phaethontidae (Le Corre et al. 2003; Sommerfeld and Hennicke 2010; Campos et al. 2018; Phillips et al. 2023), instead of being adopted occasionally to cope with low resource availability or only by specific populations, as has been reported for other seabirds such as the Cory's Shearwater (*Calonectris borealis*; Granadeiro et al. 1998).

Bimodal foraging in which parents alternate or mix short and long trips is a behavioral strategy that is mainly implemented by pelagic seabirds (e.g., Procellariiformes, Alcids, Sphenisciformes, Suliformes, and Phaethontiformes) while caring for their chicks to meet the conflicting energy demands of self-maintenance and chick feeding (Weimerskirch et al. 1993, 1994; Weimerskirch 1998; Ropert-Coudert et al. 2004; Congdon et al. 2005; Steen et al. 2007; Welcker et al. 2009; Sommerfeld and Hennicke 2010; Saraux et al. 2011; Shoji et al. 2015; Campos et al. 2018, Austin et al. 2019; Phillips et al. 2023). Adult seabirds that engage in bimodal foraging are generally thought to make long trips to productive areas to feed themselves and avoid the high travel costs of commuting, whereas short trips to resource-poor areas near their nesting colonies are conducted to obtain resources for their offspring (Weimerskirch et al. 1994; Weimerskirch 1998; Jakubas et al. 2012; Welcker et al. 2012). Our findings indicate that red-billed tropicbirds follow this general pattern, making short trips (average of ~ 30 km) to areas around their colony and long trips (> 240 km) to pelagic areas far from the colony. However, during long trips, red-billed tropicbirds forage in areas with lower Chl-a concentrations and higher SST than those used during short trips (Fig. 3a,b).

Variation in isotopic values between adults and chicks

The different isotopic signatures in the blood samples from adults and chicks strongly suggest that parents and offspring consume different prey from different areas. In particular, parents fed their offspring with prey enriched in ^{15}N, which may be associated with elevated energetic content that could

Table 3 Summary statistics of the general linear models (GLMs) evaluating the effects of the breeding season, age class (adults and chicks), and reproductive stage (early chick-rearing, ECR; late chick-rearing, LCR) on the nitrogen (δ^{15}N) and carbon (δ^{13}C) isotope ratios measured in whole blood samples of red-billed tropicbirds from 2020 to 2022 at Peña Blanca Islet, Mexico

Comparison	Factors and interactions	δ^{15}N			δ^{13}C		
		F	df	*p*	F	df	*p*
Adults and chicks at ECR and LCR	Year·age class·stage	0.73	2, 110	0.49	0.66	2, 110	0.52
	Year·age class	0.16	2, 112	0.85	1.59	2, 114	0.21
	Year·stage	5.10	2, 116	**0.008**	1.34	2, 112	0.27
	Age class·stage	1.12	1, 115	0.29	2.34	1, 116	0.13
	Year	83.89	2, 116	**< 0.001**	7.44	2, 119	**< 0.001**
	Age class	299.23	1, 116	**< 0.001**	16.10	1, 119	**< 0.001**
	Stage	0.36	1, 114	0.55	1.83	1, 117	0.18
	Date	18.90	1, 116	**< 0.001**	3.02	1, 118	0.09
Final selected model		Date + age class + year + year·stage			Age class + year		

Sample sizes are as follows: Adults: ECR = 53, LCR = 31; Chicks: ECR = 17, and LCR = 22. Initial models included all terms listed in the table. Significant terms were retained in the final models and are shown in bold

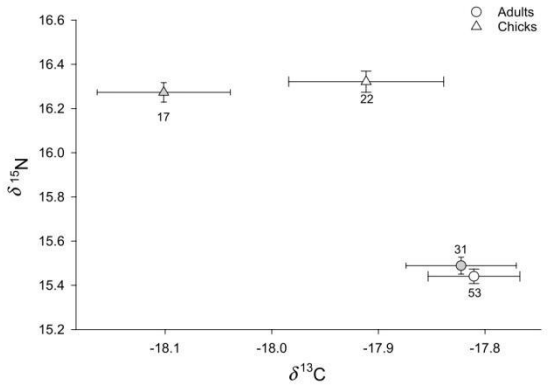


Fig. 5 Stable isotope ratios (δ^{15}N and δ^{13}C) of whole blood in Red-billed Tropicbird adults and chicks during early chick-rearing (ECR; white symbols) and late chick-rearing (LCR; gray symbols) at Peña Blanca Islet, Mexico, during the breeding seasons of 2020 to 2022. Symbols differentiating adults (circles) and chicks (triangles) are shown. The δ^{15}N and δ^{13}C values represent means ± SE. The number of samples analyzed is indicated next to each symbol. Adult δ^{15}N values were adjusted by subtracting 0.30 ‰ to account for metabolic differences between adult and chick seabirds

improve growth and body condition (Albano et al. 2011; González-Medina et al. 2017). The isotopic δ^{15}N composition differed by ~ 0.90 ‰ and 0.78 ‰ between adults and chicks during the ECR and LCR periods, respectively. Hence, the isotopic signatures are consistent with the change in feeding grounds exhibited by adult red-billed tropicbirds during the chick-rearing period being associated with the specific function of providing food and care to their offspring.

However, the question arises of why parents make long trips to less productive pelagic areas when short trips seem more profitable. Tropicbirds are distributed in tropical oceanic areas characterized by low resource productivity and predictability (Weimerskirch 2007). The use of both coastal and pelagic areas during the chick-rearing period could be linked to the energetic and nutritional demands of offspring, resource availability, and inter- and intraspecific competition for shared resources. According to the Chl-a values, a proxy of primary productivity, obtained during the study period and the relationship between areas with high primary productivity and fish and crustacean aggregation zones for spawning, rearing, and feeding (Franco-Gordo et al. 2008; Ambriz-Arreola et al. 2012; Capuzzo et al. 2017; Kozak et al. 2020), greater resource availability in foraging areas close to the colony was expected, as Chl-a values near the colony were higher than those offshore (Fig. 3b). Hence, utilizing areas near the colony requires less travel time, which would be reflected in more time spent caring for chicks and a regular food supply. However, this would involve a

trade-off because competition near the colony may also be high; therefore, the resource availability in these areas would likely be depleted at some point during the season (Birt et al. 1987; Weber et al. 2021). Thus, exclusively using nearby areas to obtain prey for chick provisioning would result in resources becoming scarce prior the chick is old enough for the parents to begin making longer trips.

On the other hand, foraging far from the colony may prove profitable for parents trying to maintain their own body condition, either because they are more successful in capturing prey far from the colony or because they encounter low competition, as has been observed in other tropical seabirds (Weimerskirch 1998; Austin et al. 2019). Peña Blanca supports approximately 2,500–3,300 breeding adults, without considering that several thousand other seabirds also breed on the islet and use nearby foraging areas (Hernández-Vázquez et al. 2017). These breeding adults exploit an area of ~ 550 km^2 surrounding the colony during chick-rearing, resulting in a high density of users per km^2. Thus, despite the expense of traveling further, individuals forage in oceanic areas with low primary productivity but with few users per km^2, which may increase capture success and reduce competition for prey (Weimerskirch 2007). Furthermore, red-billed tropicbirds have been reported to be mostly solitary at sea, exhibiting an opportunistic foraging pattern that often depends on predatory fish for prey availability and foraging over wide areas with very low densities to avoid intra- or interspecific feeding flocks (Spear and Ainley 2005). Extending travel time and distance to these areas may also increase the probability of finding high-quality prey, as these prey are usually scarce and less reliably caught (Shoji et al. 2015). In addition, the observed pattern of directionality of most Red-billed Tropicbird foraging trips towards the southwest of the islet could be influenced by wind patterns (southwest during the breeding season), resource distribution, or even by inter-colony segregation of foraging areas (Tarroux et al. 2016; Goto et al. 2017). However, future research is required to elucidate which factors and the extent to which they influence the directionality of foraging trips of breeding red-billed tropicbirds.

Relationship between bimodal foraging and parental care

The bimodal foraging strategy in seabirds is assumed to have evolved in response to the scarcity of resources near their colonies, with parents meeting their energetic requirements and those of their chicks, by using different foraging areas and increasing the size of those areas to reduce competition for prey (Welcker et al. 2012). This strategy evolved in pelagic foragers in response to prolonged parental care and the constraints of central-place foraging (Ropert-Coudert et al. 2004). For red-billed tropicbirds, which have a breeding period of about 112–125 days until the fledgling leaves the nest (Castillo-Guerrero et al. 2011; Boeken 2016), this strategy seems to be an appropriate mechanism to deal with these constraints, especially at the beginning of the chick-rearing phase when the demands of altricial chicks are high (e.g., brooding, feeding, and protection) and must be met by their parents (Wittenberger and Tilson 1980; Dial 2003). Parental presence of red-billed tropicbirds from other colonies has been reported in ~ 82% and 10% of nests with small and large chicks, respectively (Nelson 2006). These findings are similar to our results, as the parents in our study exhibited a higher presence at the nest and a stable upward chick-feeding frequency during the ECR period than during the later rearing stages (Fig. 4a,b).

Based on what has been reported of the foraging behavior of Adélie penguins (*Pygoscelis adeliae*), there are two stages that parents must navigate during chick-rearing to deliver food to their chicks efficiently (Ropert-Coudert et al. 2004). The first stage comprises the beginning of brooding when the chicks are small, and parents frequently deliver food to maximize their food intake. If parents can maximize the rate at which energy is supplied to their chicks, the likelihood of reproductive success will increase. Conversely, when the sizes of the chicks and parents are the same, the travel time of foraging trips increases. Optimally, parents should alternate short trips and long trips, as this maximizes the rate at which they obtain food for themselves and, consequently, improves their future breeding fitness (Ropert-Coudert et al. 2004).

Meal sizes provided to the chicks

The results of this study suggest that the foraging ecology of red-billed tropicbirds is linked to their parental duties. The Red-billed Tropicbird adults of Peña Blanca seem to maximize food delivery to their chicks during the ECR stage by making both short and long trips. Young chicks, which lack the reserves to withstand prolonged periods of fasting, require regular parental provisioning during the first weeks after hatching, as this is a particularly critical stage for chick survival (Phillips and Hamer 1999). Once the Red-billed Tropicbird chicks nearly reach adult size at 5–6 weeks of age (Table S2), parents can spend more time foraging and less time at the nest (see Fig. 4a), as the lipid reserves of chicks allow them to tolerate longer periods of fasting, which the parents compensate for by increasing meal size (see Fig. 4c; Chaurand and Weimerskirch 1994), although larger meal sizes and food provisioning gradually decrease prior to fledgling when parents are unable or unwilling to maintain earlier levels of food provisioning (Riou and Hamer 2010; Riou et al. 2012; Arnold et al. 2016). This is

consistent with what has been reported for other seabirds in which high levels of coordination between mates regarding their foraging trips ensure a consistent supply of food for their chicks, although this coordination begins to decline as the chick-rearing period progresses (Tyson et al. 2017; Wojczulanis-Jakubas et al. 2018). However, these patterns can vary between species and even between conspecific individuals (Clutton-Brock 1991; McGraw et al. 2010; Royle et al. 2012).

On the other hand, studies of sooty shearwaters (*Ardenna grisea*) have shown that the parental decision to make either short or long trips after feeding chicks depends exclusively on the mass of the adult and not on other factors (e.g., chick nutritional status, duration of the previous trip, or endogenous rhythm), as birds always undertake long trips when their body mass falls below the threshold of 750 g (Weimerskirch 1998). Although we weighed the adults in this study, we did so only once when we retrieved the GPS devices. This prevented us from assessing whether adults undertook long trips when they reached a mass threshold, and we cannot rule out the involvement of parental body condition in foraging decisions or that factors influencing parental decisions to undertake long or short foraging trips may be species-specific (Baduini and Hyrenbach 2003). Further research employing a body condition index in red-billed tropicbirds could demonstrate the existence of a threshold value for this behavioral decision.

Bimodal foraging strategy could be controlled by different, but not mutually exclusive, factors to regulating parental investment in offspring (Granadeiro et al. 1998) or exclusively by adult body condition (Weimerskirch 1998). The extent to which each factor influences the foraging decisions of Red-billed Tropicbird parents is a key question that should be answered in future studies.

Conclusion

Based on the GPS data obtained from the foraging trips conducted by red-billed tropicbirds of Peña Blanca Islet, we can conclude that parents switched from a unimodal foraging strategy during the incubation stage to a bimodal foraging strategy once their chicks hatched. Parents undertook short and long foraging trips during the ECR period (chicks < 4 weeks old). Short trips were made to shallower areas (depths < 200 m) surrounding the breeding site with high Chl-a concentrations and low SST, whereas long foraging trips were made to deeper, less productive waters. The most plausible explanation for this bimodal strategy is that red-billed tropicbirds undertook long foraging trips to arrive in areas with low oceanic productivity that were undisputed by other birds and thus had low user density, thereby increasing the probability of finding high-quality prey by extending their travel time and distance. Concurrently, parental presence at the nest was greater during the ECR period, which was associated with a higher rate of chick-feeding. The foraging strategy used by red-billed tropicbirds in this study is therefore clearly linked to parental duties. As young chicks require a high level of parental care at the nest and frequent feedings, parents alternated caring for their chicks. The parent on duty made short trips to provide for their chick without leaving it unattended for long periods, while its mate made long trips to feed itself. Adults then gradually reduced the time spent at the nest and increased the time spent foraging, compensating with larger meal sizes for their chicks as they grew. Our results seem to indicate that parental obligations trigger foraging decisions in red-billed tropicbirds during the chick-rearing period. The bimodal foraging strategy used by adults is a means to simultaneously meet their own high energetic demands and those of their young during the breeding season without sacrificing their own future breeding fitness.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00227-023-04375-1.

Acknowledgements We thank Departamento de Estudios para el Desarrollo Sustentable de Zona Costera CUCSur – Universidad de Guadalajara for logistical support in the field. We also thank Captain A. Alvízar-Martínez for helping us access the islet and the fieldwork assistants for their hard work. Furthermore, we thank A. Espino-Herrera, S. Gómez-Hernández, L. Aguilar-Nuño, and L. Estrada-Osuna for their help with sample processing in the laboratory; M. Austad, M. Brault-Favrou, and C. Churlaud for training and sample preparation; J. Cullen for his valuable support in using the 'amt' package; and G. Guillou from the 'Plateforme analyses isotopiques' of LIENSs for running stable isotope analyses. We also thank the two anonymous reviewers for their comments and suggestions, which greatly improved the manuscript. PB is an honorary member of the IUF (Institut Universitaire de France).

Author contributions Conceptualization: AP-O and JAC-G; Methodology: AP-O, JAC-G, PQ and PB; Fieldwork: AP-O, DAG-Z, SH-V and JAC-G; Formal analysis and investigation: AP-O, JAC-G, DAG-Z, JAP and EM; Writing—original draft preparation: AP-O and JAC-G; Writing—review and editing: AP-O, DAG-Z, JAP, SH-V, EM, PB, PQ and JAC-G; Funding acquisition: AP-O, SH-V and JAC-G; Resources: AP-O, PQ and JAC-G; Supervision: PQ and JAC-G.

Funding This work was supported by Programa para el Desarrollo Profesional Docente para el tipo Superior (UDG-PTC-1516) and Universidad de Guadalajara. GPS devices and Stable Isotope analysis were partly covered by Justus Liebig University. AP-O was financed with a CONAHCYT scholarship for doctoral studies abroad (No. 795355) and received a Student Research Grant from The Pacific Seabird Group (PSG SRGA 2021) to partly finance the stable isotope analysis. The CPER (Contrat de Projet Etat-Région) and the FEDER (Fonds Européen de Développement Régional) for funding the IRMS of LIENSs laboratory.

Data availability The data sets generated and analyzed during the current study are available in the OSF repository: https://osf.io/xw4ab/?view_only=deea243f23514f50bea27506c3407eab

Declarations

Conflict of interest The authors have no competing interests to declare that are relevant to the content of this work.

Ethics approval Fieldwork, including wildlife management and sampling, was conducted with the permission of the Dirección General de Vida Silvestre (DGVS, Mexico) under permits SGPA/DGVS/00404/15, SGPA/DGVS/01919/17, SGPA/DGVS/02779/21, and SPARN/DGVS/01482/22. All applicable institutional and/or national guidelines for the welfare and conservation of wildlife were followed. Individuals in this study were not handled for more than 10 min. The smallest amount of blood was collected from each animal. While the adults were sampled, we cared for their eggs and chicks until their parents returned to the nest. No adults abandoned their nests after capture. Further monitoring after this research allowed us to verify that the chicks were not abandoned by their parents after being handled for the purposes of this work.

Consent to participate N/A.

Consent to publish N/A.

References

Albano N, Masero JA, Sánchez-Guzmán JM, Villegas A, Santiago-Quesada F (2011) Effects of diet on growth-related patterns of energy and macronutrient assimilation efficiency in a semi-precocial bird, the Gull-billed Tern *Gelochelidon nilotica*. Ardea 99:93–101. https://doi.org/10.5253/078.099.0111

Ambriz-Arreola I, Gómez-Gutiérrez J, Franco-Gordo MC, Lavaniegos BE, Godínez-Domínguez E (2012) Influence of coastal upwelling–downwelling variability on tropical euphausiid abundance and community structure in the inshore Mexican central Pacific. Mar Ecol Prog Ser 451:119–136. https://doi.org/10.3354/meps09607

Arnold JM, Nisbet ICT, Oswald SA (2016) Energetic constraint of non-monotonic mass change during offspring growth: a general hypothesis and application of a new tool. J Anim Ecol 85:476–486. https://doi.org/10.1111/1365-2656.12467

Austin RE, De Pascalis F, Arnould JPY, Haakonsson J, Votier SC, Ebanks-Petrie G, Austin T, Morgan G, Bennett G, Green JA (2019) A sex-influenced flexible foraging strategy in a tropical seabird, the magnificent frigatebird. Mar Ecol Prog Ser 611:203–214. https://doi.org/10.3354/meps12859

Baduini CL, Hyrenbach KD (2003) Biogeography of Procellariiform foraging strategies: does ocean productivity influence provisioning? Mar Ornithol 31:101–112

Bates D, Maechler M, Bolker B, Walker S, Christensen RHB, Singmann H, Dai B, Grothendieck G (2015) lme4: linear mixed-effects models using "Eigen" and S4. R Package Version 1(1–10):1–98

Beal M, Oppel S, Handley J, Pearmain EJ, Morera-Pujol V, Carneiro APB, Davies TE, Phillips RA, Taylor PR, Miller MGR, Franco AMA, Catry I, Patrício AR, Regalla A, Staniland I, Boyd C, Catry P, Dias MP (2021) track2KBA: An R package for identifying important sites for biodiversity from tracking data. Methods Ecol Evol 12:2372–2378. https://doi.org/10.1111/2041-210X.13713

Bearhop S, Waldron S, Votier SC, Furness RW (2002) Factors that influence assimilation rates and fractionation of nitrogen and carbon stable isotopes in avian blood and feathers. Physiol Biochem Zool 75:451–458. https://doi.org/10.1086/342800

Bennison A, Bearhop S, Bodey TW, Votier SC, Grecian WJ, Wakefield ED, Hamer KC, Jessopp M (2017) Search and foraging behaviors from movement data: A comparison of methods. Ecol Evol 8:13–24. https://doi.org/10.1002/ece3.3593

Birt VL, Birt TP, Goulet D, Cairns DK, Montevecchi WA (1987) Ashmole's halo: direct evidence for prey depletion by a seabird. Mar Ecol Prog Ser 40:205–208

Boeken M (2016) Breeding Success of Red-Billed Tropicbirds *Phaethon aethereus* on the Caribbean Island of Saba. Ardea 104:263–271. https://doi.org/10.5253/arde.v104i3.a8

Campos LFAS, Andrade AB, Bertrand S, Efe MA (2018) Foraging behavior and at-sea distribution of White-Tailed Tropicbirds in tropical ocean. Braz J Biol 78:556–563. https://doi.org/10.1590/1519-6984.173578

Capuzzo E, Lynam CP, Barry J, Stephens D, Forster RM, Greenwood N, McQuatters-Gollop A, Silva t, van Leeuwen SM, engelhard GH, (2017) A decline in primary production in the North Sea over 25 years, associated with reductions in zooplankton abundance and fish stock recruitment. Glob Chang Biol 24:e352–e364. https://doi.org/10.1111/gcb.13916

Castillo-Guerrero JA, Guevara-Medina MA, Mellink E (2011) Breeding ecology of the Red-billed tropicbird *Phaethon aethereus* under contrasting environmental conditions in the Gulf of California. Ardea 99:61–71. https://doi.org/10.5253/078.099.0108

Chaurand T, Weimerskirch H (1994) The regular alternation of short and long foraging trips in the blue petrel Halobaena caerulea: a previously undescribed strategy of food provisioning in a pelagic seabird. J Anim Ecol 63:275–282. https://doi.org/10.2307/5546

Cherel Y, Hobson KA, Hassani S (2005) Isotopic discrimination factors between food and blood and feathers of captive penguins: implications for dietary studies in the wild. Physiol Biochem Zool 78:106–115. https://doi.org/10.1086/425202

Clarke JR (2001) Partitioning of foraging effort in Adélie penguins provisioning chicks at Béchervaise Island, Antarctica. Polar Biol 24:16–20. https://doi.org/10.1007/s003000000168

Clay TA, Oppel S, Lavers JL, Phillips RA, Brooke M-de L (2019) Divergent foraging strategies during incubation of an unusually wide-ranging seabird, the Murphy's petrel. Mar Biol 166:8. https://doi.org/10.1007/s00227-018-3451-7

Cleeland JB, Lea M-A, Hindell MA (2014) Use of the Southern Ocean by breeding Short-tailed shearwaters (*Puffinus tenuirostris*). J Exp Mar Biol Ecol 450:109–117. https://doi.org/10.1016/j.jembe.2013.10.012

Clutton-Brock TH (1991) The evolution of parental care. Princeton University Press, Princeton, New Jersey

Congdon BC, Krockenberger AK, Smithers BV (2005) Dual-foraging and co-ordinated provisioning in a tropical Procellariiform, the Wedge-tailed Shearwater. Mar Ecol Prog Ser 301:293–301. https://doi.org/10.3354/meps301293

Crawley MJ (2007) The R book. Wiley, Chichester

Dial KP (2003) Evolution of Avian Locomotion: Correlates of Flight Style, Locomotor Modules, Nesting Biology, Body Size,

Development, and the Origin of Flapping Flight. Auk 120:941–952. https://doi.org/10.1642/0004-8038(2003)120[0941:EOALCO]2.0.CO;2

Diop N, Zango L, Beard A, Ba CT, Ndiaye PI, Henry L, Clingham E, Oppel S, González-Solís J (2018) Foraging ecology of tropicbirds breeding in two contrasting marine environments in the tropical Atlantic. Mar Ecol Prog Ser 607:221–236. https://doi.org/10.3354/meps12774

dos Santos I, Ramos JA, Ceia FR, Rodrigues I, Almeida N, Antunes S, Carreiro AR, Matos DM, Lopes RJ, Geraldes P, Paiva VH (2022) Sexual segregation in the foraging distribution, behaviour, and trophic niche of the endemic Boyd's shearwater (*Puffinus lherminieri boydi*). Mar Biol 169:144. https://doi.org/10.1007/s00227-022-04127-7

Fieberg J, Kochanny CO (2005) Quantifying Home-Range Overlap: The Importance of the Utilization Distribution. J Wildl Manage 69:1346–1359. https://doi.org/10.2193/0022-541X(2005)69[1346:QHOTIO]2.0.CO;2

Franco-Gordo C, Godínez-Domínguez E, Suárez-Morales E, Freire J (2008) Interannual and seasonal variability of the diversity and structure of ichthyoplankton assemblages in the central Mexican Pacific. Fish Oceanogr 17:178–190. https://doi.org/10.1111/j.1365-2419.2008.00463.x

Gitzen RA, Millspaugh JJ, Kernohan BJ (2006) Bandwidth Selection for Fixed-Kernel Analysis of Animal Utilization Distributions. J Wildl Manage 70:1334–1344. https://doi.org/10.2193/0022-541X(2006)70[1334:BSFFAO]2.0.CO;2

González-Medina E, Castillo-Guerrero JA, Herzka SZ, Fernández G (2017) Flexibility in food resource allocation in parents and selectivity for offspring: variations in $\delta^{15}N$ and $\delta^{13}C$ values during breeding of the blue-footed booby. Mar Biol 164:38. https://doi.org/10.1007/s00227-017-3070-8

Goto Y, Yoda K, Sato K (2017) Asymmetry hidden in birds' tracks reveals wind, heading, and orientation ability over the ocean. Sci Adv 3:e1700097. https://doi.org/10.1126/sciadv.1700097

Granadeiro JP, Nunes M, Silva MC, Furness RW (1998) Flexible foraging strategy of Cory's shearwater, *Calonectris diomedea*, during the chick-rearing period. Anim Behav 56:1169–1176. https://doi.org/10.1006/anbe.1998.0827

Hamer KC, Quillfeldt P, Masello JF, Fletcher K (2006) Sex differences in provisioning rules: responses of Manx shearwaters to supplementary chick feeding. Behav Ecol 17:132–137. https://doi.org/10.1093/beheco/arj008

Harding AMA, Hobson KA, Wojciech W, Dmoch K, Karnovsky NJ, Van Pelt TI, Lifjeld JT (2008) Can stable isotope ($\delta^{13}C$ and $\delta^{15}N$) measurements of little auk (*Alle alle*) adults and chicks be used to track changes in high-Arctic marine foodwebs? Polar Biol 31:725–733. https://doi.org/10.1007/s00300-008-0413-4

Harding A, Paredes R, Suryan R, Roby D, Irons D, Orben R, Renner H, Young R, Barger C, Dorresteijn I, Kitaysky A (2013) Does location really matter? An inter-colony comparison of seabirds breeding at varying distances from productive oceanographic features in the Bering Sea. Deep-Sea Res II Top Stud Oceanogr 94:178–191. https://doi.org/10.1016/j.dsr2.2013.03.013

Hernández-Vázquez S, Castillo-Guerrero JA, Mellink E, Almaguer-Hernández AM (2018) Colony Size and Breeding Success of Red-billed Tropicbird (*Phaethon aethereus*) on Peña Blanca Island, Colima, México. Waterbirds 41:128–134. https://doi.org/10.1675/063.041.0203

Hernández-Vázquez S, Mellink E, Castillo-Guerrero JA, Rodríguez-Estrella R, Hinojosa-Larios JA, Galván-Piña VH (2017) Ecología reproductiva del bobo café (*Sula leucogaster*) en tres islas del Pacífico tropical Mexicano. Ornitol Neotrop 28:57–66https://doi.org/10.58843/ornneo.v28i0.146

Hill T, Lewicki P (2007) STATISTICS: methods and applications. StatSoft, Tulsa, Oklahoma, USA.

Jakubas D, Iliszko L, Wojczulanis-Jakubas K, Stempniewicz L (2012) Foraging by little auks in the distant marginal sea ice zone during the chick-rearing period. Polar Biol 35:73–81. https://doi.org/10.1007/s00300-011-1034-x

Kono-Martínez T, Ortega-Ortiz CD, Olivos-Ortiz A, Torres-Orozco E, González-Rodríguez E (2017) Oceanographic conditions and marine mammals: identifying a potential relationship in the coastal region of the Mexican Central Pacific. Rev Biol Mar Oceanogr 52:479–494. https://doi.org/10.4067/S0718-19572017000300006

Kozak ER, Franco-Gordo C, Godínez-Domínguez E, Suárez-Morales E, Ambriz-Arreola I (2020) Seasonal variability of stable isotope values and niche size in tropical calanoid copepods and zooplankton size fractions. Mar Biol 167:37. https://doi.org/10.1007/s00227-020-3653-7

Le Corre M, Cherel Y, Lagarde F, Lormée H, Jouventin P (2003) Seasonal and inter-annual variation in the feeding ecology of a tropical oceanic seabird, the red-tailed tropicbird *Phaethon rubricauda*. Mar Ecol Prog Ser 255:289–301. https://doi.org/10.3354/meps255289

Madden H, Satgé Y, Wilkinson B, Jodice PGR (2022) Foraging ecology of Red-billed Tropicbird *Phaethon aethereus* in the Caribbean during early chick rearing revealed by GPS tracking. Mar Ornithol 50:165–175

Madden H, Boehm H, Mielke L (2023) Foraging Ecology of Red-Billed Tropicbirds on Saba, Caribbean Netherlands, During Early Chick-Rearing. Ardea 111:1–18. https://doi.org/10.5253/arde.2022.a14

McGraw L, Székely T, Young LJ (2010) Pair bonds and parental behaviour. In: Székely T, Moore AJ, Komdeur J (eds) Social behaviour: Genes, ecology and evolution. Cambridge University Press, Cambridge, UK, pp 271–301

Michelot T, Langrock R, Patterson TA (2016) moveHMM: an R package for the statistical modelling of animal movement data using hidden Markov models. Methods Ecol Evol 7:1308–1315. https://doi.org/10.1111/2041-210X.12578

Micklem IA, Connan M, Stander N, McQuaid CD (2021) Influence of ontogeny on stable isotope ratios and trophic discrimination factors of African penguin (*Spheniscus demersus*) tissues. Mar Biol 168:96. https://doi.org/10.1007/s00227-021-03877-0

Nelson JB (2006) Pelicans, cormorants, and their relatives, the Pelecaniformes. Oxford University Press, New York, USA

Orians GH, Pearson NE (1979) On the theory of central place foraging. In: Horn JD, Mitchell RD, Stairs GR (eds) Analysis of Ecological Systems. Ohio State University Press, Columbus, Ohio, USA, pp 154–177

Phillips RA, Hamer KC (1999) Lipid reserves, fasting capability and the evolution of nestling obesity in procellariiform seabirds. Proc R Soc B Biol Sci 266:1329–1334. https://doi.org/10.1098/rspb.1999.0783

Phillips JA, Guilford T, Fayet AL (2023) How do resource distribution and taxonomy affect the use of dual foraging in seabirds? A review. Behav Ecol 34:769–779. https://doi.org/10.1093/beheco/arad052

Piña-Ortiz A, Castillo-Guerrero JA, Enríquez-Paredes LM, Fernández G, Hernández-Vázquez S, Quillfeldt P (2023) Body size variation in a tropical seabird along a latitude-productivity gradient. J Ornithol 164:327–339. https://doi.org/10.1007/s10336-022-02037-5

QGIS.org, 2020. QGIS Geographic Information System. QGIS Association. http://www.qgis.org

Quillfeldt P (2002) Begging in the absence of sibling competition in Wilson´s storm-petrels, *Oceanites oceanicus*. Anim Behav 64:579–587. https://doi.org/10.1006/anbe.2002.3090

R Core Team (2023) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria

Riou S, Hamer KC (2010) Lipid metabolism, begging behaviour and nestling obesity in a pelagic seabird. Funct Ecol 24:340–346. https://doi.org/10.1111/j.1365-2435.2009.01651.x

Riou S, Chastel O, Hamer KC (2012) Parent–offspring conflict during the transition to independence in a pelagic seabird. Behav Ecol 23:1102–1107. https://doi.org/10.1093/beheco/ars079

Ropert-Coudert Y, Wilson RP, Daunt F, Kato A (2004) Patterns of energy acquisition by a central place forager: benefits of alternating short and long foraging trips. Behav Ecol 15:824–830. https://doi.org/10.1093/beheco/arh086

Royle N, Smiseth P, Kölliker M (2012) The evolution of parental care. Oxford University Press, Oxford, UK

RStudio Team (2023) RStudio: integrated development for R. RStudio, PBC, Boston, MA

Salas J, Gomis D, Olivos-Ortiz A, García-Uribe G (2006) Seasonal hydrodynamical features on the shelf of Colima (west coast of Mexico). Sci Mar 70:719–726. https://doi.org/10.3989/scimar.2006.70n4

Saraux C, Robinson-Laverick SM, Le Maho Y, Ropert-Coudert Y, Chiaradia A (2011) Plasticity in foraging strategies of inshore birds: how Little Penguins maintain body reserves while feeding offspring. Ecology 92:1909–1916. https://doi.org/10.1890/11-0407.1

Sears J, Hatch SA, O'Brien DM (2009) Disentangling effects of growth and nutritional status on seabirds stable isotope ratios. Oecologia 159:41–48. https://doi.org/10.1007/s00442-008-1199-3

Shoji A, Aris-Brosou S, Fayet A, Padget O, Perrins C, Guilford T (2015) Dual foraging and pair coordination during chick provisioning by Manx shearwaters: empirical evidence supported by a simple model. J Exp Biol 218:2116–2123. https://doi.org/10.1242/jeb.120626

Signer J, Fieberg J, Avgar T (2019) Animal movement tools (amt): R package for managing tracking data conducting habitat selection analyses. Ecol Evol 9:880–890. https://doi.org/10.1002/ece3.4823

Sommerfeld J, Hennicke JC (2010) Comparison of trip duration, activity pattern and diving behaviour by Red-tailed Tropicbirds (*Phaethon rubricauda*) during incubation and chick-rearing. Emu 110:78–86. https://doi.org/10.1071/MU09053

Spear LB, Ainley DG (2005) At-sea distributions and abundance of tropicbirds in the eastern Pacific. Ibis 147:353–366. https://doi.org/10.1111/j.1474-919x.2005.00411.x

Steen H, Vogedes D, Broms F, Falk-Petersen S, Berge J (2007) Little auks (*Alle alle*) breeding in a High Arctic fjord system: bimodal foraging strategies as a response to poor food quality? Polar Res 26:118–125. https://doi.org/10.1111/j.1751-8369.2007.00022.x

Suryan RM, Irons DB, Benson J (2000) Prey switching and variable foraging strategies of Black-Legged Kittiwakes and the effect on reproductive success. The Condor 102:374–384. https://doi.org/10.1650/0010-5422(2000)102[0374:PSAVFS]2.0.CO;2

Tarroux A, Weimerskirch H, Wang S-H, Bromwich DH, Cherel Y, Kato A, Ropert-Coudert Y, Varpe Ø, Yoccoz NG, Descamps S (2016) Flexible flight response to challenging wind conditions in a commuting Antarctic seabird: do you catch the drift? Anim Behav 113:99–112. https://doi.org/10.1016/j.anbehav.2015.12.021

Tyson C, Kirk H, Fayet A, Van Loon EE, Shoji A, Dean B, Perrins C, Freeman R, Guilford T (2017) Coordinated provisioning in a dual-foraging pelagic seabird. Anim Behav 132:73–79. https://doi.org/10.1016/j.anbehav.2017.07.022

Vandenabeele SP, Shepard EL, Grogan A, Wilson RP (2012) When three per cent may not be three per cent; device-equipped seabirds experience variable fight constraints. Mar Biol 159:1–14. https://doi.org/10.1007/s00227-011-1784-6

Wand MP, Jones MC (1994) Kernel Smoothing. Chapman and Hall/CRC, New York, USA

Weber SM, Richardson AJ, Brown J, Bolton M, Clark BL, Godley BJ, Leat E, Oppel S, Shearer L, Soetaert KER, Weber N, Broderick AC (2021) Direct evidence of a prey depletion "halo" surrounding a pelagic predator colony. PNAS 118:e2101325118. https://doi.org/10.1073/pnas.2101325118

Weimerskirch H (1998) How can a pelagic seabird provision its chick when relying on a distant resource? Cyclic attendance, foraging decision and body condition in sooty shearwaters. J Anim Ecol 67:99–109. https://doi.org/10.1046/j.1365-2656.1998.00180.x

Weimerskirch H (2007) Are seabirds foraging for unpredictable resources? Deep Sea Res Part II Top Stud Oceanogr 54:211–223. https://doi.org/10.1016/j.dsr2.2006.11.013

Weimerskirch H, Salamolard M, Sarrazin F, Jouventin P (1993) Foraging strategy of wandering albatrosses through the breeding season: a study using satellite telemetry. Auk 110:325–342. https://doi.org/10.1093/auk/110.2.325

Weimerskirch H, Chastel O, Ackermann L, Chaurand T, Cuenot-Chaillet F, Hindermeyer X, Judas J (1994) Alternate long and short foraging trips in pelagic seabird parents. Anim Behav 47:472–476. https://doi.org/10.1006/anbe.1994.1065

Weimerskirch H, Ancel A, Caloin M, Zahariev A, Spagiari J, Kersten M, Chastel O (2003) Foraging efficiency and adjustment of energy expenditure in a pelagic seabird provisioning its chick. J Anim Ecol 72:500–508. https://doi.org/10.1046/j.1365-2656.2002.00720.x

Welcker J, Harding AMA, Karnovsky NJ, Steen H, Strøm H, Gabrielsen GW (2009) Flexibility in the bimodal foraging strategy of a high Arctic alcid, the little auk *Alle alle*. J Avian Biol 40:388–399. https://doi.org/10.1111/j.1600-048X.2008.04620.x

Welcker J, Beiersdorf A, Varpe Ø, Steen H (2012) Mass fluctuations suggest different functions of bimodal foraging trips in a central-place forager. Behav Ecol 23:1372–1378. https://doi.org/10.1093/beheco/ars131

Wilson RP, McMahon CR (2006) Measuring devices on wild animals: what constitutes acceptable practice? Front Ecol Environ 4:147–154. https://doi.org/10.1890/1540-9295(2006)004[0147:MDOWAW]2.0.CO;2

Wittenberger JF, Tilson RL (1980) The evolution of monogamy: hypotheses and evidences. Annu Rev Ecol Evol Syst 11:197–232. https://doi.org/10.1146/annurev.es.11.110180.001213

Wojczulanis-Jakubas K, Araya-Salas M, Jakubas D (2018) Seabird parents provision their chick in a coordinated manner. PLoS ONE 13:e0189969. https://doi.org/10.1371/journal.pone.0189969

Wood AG, Naef-Daenzer B, Prince PA, Croxall JP (2000) Quantifying habitat use in satellite-tracked pelagic seabirds: application of kernel estimation to albatross locations. J Avian Biol 31:278–286. https://doi.org/10.1034/j.1600-048X.2000.310302.x

Authors and Affiliations

Alberto Piña-Ortiz[1] · Diego Adolfo González-Zamora[2] · Jesica Andrea Paz[3] · Salvador Hernández-Vázquez[4] · Eric Mellink[2] · Paco Bustamante[5] · Petra Quillfeldt[1] · José Alfredo Castillo-Guerrero[4]

✉ Alberto Piña-Ortiz
Alberto.Pina-Ortiz@bio.uni-giessen.de

[1] Department of Animal Ecology and Systematics, Justus Liebig University (JLU) Giessen, Heinrich-Buff-Ring 26, 35392 Giessen, Hessen, Germany

[2] Departamento de Biología de la Conservación, Centro de Investigación Científica y de Educación Superior de Ensenada, Carretera Ensenada-Tijuana 3918, C.P. 22860 Baja California, México

[3] Instituto de Investigaciones Marinas y Costeras (IIMyC), Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Mar del Plata, Argentina

[4] Departamento de Estudios para el Desarrollo Sustentable de Zonas Costeras, Centro Universitario de la Costa Sur, Universidad de Guadalajara, Gómez Farías 82, San Patricio-Melaque, C.P. 48980 Municipio de Cihuatlán, Jalisco, México

[5] Littoral, Environnement et Sociétés (LIENSs), UMR 7266 CNRS – La Rochelle Université, 2 Rue Olympe de Gouges, 17000 La Rochelle, France

Chapter III

TROPHIC PLASTICITY OF A TROPICAL SEABIRD REVEALED THROUGH DNA METABARCODING AND STABLE ISOTOPE ANALYSES

Marcuk Vladislav[†], Piña-Ortiz Alberto[†], Castillo-Guerrero José Alfredo, Masello Juan F., Bustamante Paco, Griep Sven, Quillfeldt Petra

Marine Environmental Research Volume 199, 106627, (2024)
https://doi.org/10.1016/j.marenvres.2024.106627

[†]These authors contributed equally to this work

Marine Environmental Research 199 (2024) 106627

Contents lists available at ScienceDirect

Marine Environmental Research

journal homepage: www.elsevier.com/locate/marenvrev

Trophic plasticity of a tropical seabird revealed through DNA metabarcoding and stable isotope analyses

Vladislav Marcuk [a,1], Alberto Piña-Ortiz [a,*,1], José Alfredo Castillo-Guerrero [c], Juan F. Masello [d,e], Paco Bustamante [f], Sven Griep [g], Petra Quillfeldt [a]

[a] Department of Animal Ecology and Systematics, Justus Liebig University Giessen, Heinrich-Buff-Ring 26, 35392, Giessen, Germany
[c] Departamento de Estudios para el Desarrollo Sustentable de la Zona Costera, Centro Universitario de la Costa Sur, Universidad de Guadalajara, Gómez Farías 82, San Patricio-Melaque, Municipio de Cihuatlán, Jalisco, C.P. 48980, Mexico
[d] Department of Animal Behaviour, Bielefeld University, Germany
[e] Department of Biological Sciences, University of Venda, Private Bag X5050, Thohoyandou, 0950, South Africa
[f] LIttoral, Environnement et Societés (LIENSs), UMR 7266 CNRS – La Rochelle Université, 17000, La Rochelle, France
[g] Institute for Bioinformatics and Systems Biology, Justus Liebig University Giessen, Heinrich-Buff-Ring 58, D-35392, Giessen, Germany

ARTICLE INFO

Keywords:
Birds
$\delta^{15}N$
$\delta^{13}C$
Dietary plasticity
Feeding strategy
Gulf of California
Marine ecology
Mexican tropical Pacific
Red-billed tropicbird
Spatio-temporal dietary divergence

ABSTRACT

DNA metabarcoding and stable isotope analysis have significantly advanced our understanding of marine trophic ecology, aiding systematic research on foraging habits and species conservation. In this study, we employed these methods to analyse faecal and blood samples, respectively, to compare the trophic ecology of two Red-billed Tropicbird (*Phaethon aethereus*; Linnaeus, 1758) colonies on Mexican islands in the Pacific. Trophic patterns among different breeding stages were also examined at both colonies. Dietary analysis reveals a preference for epipelagic fish, cephalopods, and small crustaceans, with variations between colonies and breeding stages. Isotopic values ($\delta^{15}N$ and $\delta^{13}C$) align with DNA metabarcoding results, with wider niches during incubation stages. Differences in diet are linked to environmental conditions and trophic plasticity among breeding stages, influenced by changing physiological requirements and prey availability. Variations in dietary profiles reflect contrasting environmental conditions affecting local prey availability.

1. Introduction

The understanding of food webs and species interactions of marine biota can provide insights into feeding strategies, population dynamics and their functional role in species-prey interactions (Hedd et al., 2001; Fauchald et al., 2011; Ceia et al., 2012; Lynam et al., 2017). Seabirds are found at most trophic levels of the marine food web, some of them being among top predators, playing a determining role in the flow of energy in marine environments (Shealer, 2001; Grémillet and Boulinier, 2009; Astarloa et al., 2021). Seabirds have a breeding cycle that lasts several months (Schreiber and Burger 2001; Nelson 2006) during which they need to find food for themselves and their offspring (Ricklefs, 1983; Roby, 1991), causing prey depletion and modifying the trophic structure of the ecosystem (Weber et al., 2021).

The diet composition of seabirds can be influenced by both intrinsic (e.g., energy demands, competition, foraging behaviour) and extrinsic factors (e.g., environmental conditions, prey availability, anthropogenic activities; Masello et al., 2010; Quillfeldt et al., 2013; Dehnhard et al., 2016; Gaglio et al., 2018; Soanes et al., 2021). In particular, extrinsic factors appear to strongly influence foraging decisions of tropical seabirds, as they rely mostly on unpredictable food resources in a highly heterogeneous environment characterized by oligotrophic oceanic waters (Weimerskirch, 2007; Soanes et al., 2021). Variations in local environmental conditions can drive to divergent foraging patterns between conspecific seabird colonies (Dunphy et al., 2020; Soanes et al., 2021; Jacoby et al., 2023). Likewise, seabird prey composition is likely to vary within colonies due to temporal differences in prey abundance, changing nutritional requirements, foraging behaviour, interaction with

* Corresponding author.
E-mail addresses: vladislav.marcuk@gmail.com (V. Marcuk), Alberto.Pina-Ortiz@bio.uni-giessen.de (A. Piña-Ortiz), alfredo.castillo@academicos.udg.mx (J.A. Castillo-Guerrero), juan.masello@uni-bielefeld.de (J.F. Masello), paco.bustamante@univ-lr.fr (P. Bustamante), sven.griep@gmx.de (S. Griep), Petra.Quillfeldt@bio.uni-giessen.de (P. Quillfeldt).
[1] These authors contributed equally to this work and share first authorship.

https://doi.org/10.1016/j.marenvres.2024.106627
Received 4 May 2024; Received in revised form 19 June 2024; Accepted 28 June 2024
Available online 29 June 2024

other birds (i.e., competition) and energetic demands (Navarro et al., 2014; González-Medina et al., 2017; et al., 2018; Lerma et al., 2022).

The foraging plasticity in seabirds refers to their ability to adjust feeding strategies in response to changing environmental conditions and resources (Paiva et al., 2010; Dehnhard et al., 2016; Gaglio et al., 2018). These adjustments may include changes in diet, selection of foraging habitats, and diving depth during foraging (Masello et al., 2010; Dehnhard et al., 2016). Understanding foraging plasticity is crucial to assess the adaptability of seabirds in response to environmental changes, such as climate change and fluctuations in food availability (Barrett et al., 2007). Conventional approaches, such as the analysis of the crop, stomach contents and regurgitates, provided valuable insight into the prey spectrum and preferences of focal seabird species (Chiaradia et al., 2003; Barrett et al., 2007). However, some of them (e.g., stomach contents) require an invasive approach for the extraction of sample material (Chiaradia et al., 2003). Therefore, less invasive methods such as stable isotope analysis and DNA metabarcoding, are now frequently used to replace the conventional approaches (Deagle et al., 2007; Inger and Bearhop, 2008).

The ratio of nitrogen isotopes (e.g., $\delta^{14}N/\delta^{15}N$) has been measured in feathers, blood or other tissue samples to infer the trophic level of the assimilated prey at different time scales (Inger and Bearhop, 2008). On the other hand, DNA metabarcoding is nowadays frequently used in terrestrial and marine ecosystems and has already been successfully implemented in studies involving different animals, including seabirds (Valentini et al., 2009; Crisol-Martínez et al., 2016; McInnes et al., 2017; Kleinschmidt et al., 2019; Masello et al., 2021; Alemany et al., 2023). DNA metabarcoding also allows the detection of soft prey often overlooked in conventional analyses. It supports a higher taxonomic resolution compared to conventional methods (e.g., regurgitates or stomach contents), depending on the availability of suitable primers and complementary gene sequences in data banks (e.g., GenBank) for prey taxa (McInnes et al., 2017). Thus, combining both approaches can improve inferences about seabird trophic ecology (Carreiro et al., 2020; Ceia et al., 2022).

The Red-billed Tropicbird (*Phaethon aethereus*; Linnaeus, 1758) is a pelagic-pantropical seabird that inhabits a range of coastal and oceanic habitats for breeding, with a global population of ~16,000–30,000 mature individuals (Birdlife International, 2024). Despite some spatial variability in prey composition, Exocoetidae (flying fish) and Carangidae (jacks, jack mackerels, etc.) were commonly reported as preferred prey items (Stonehouse, 1962; Castillo-Guerrero et al., 2011; Diop et al., 2018; Madden et al., 2022, 2023). In the Pacific Ocean, prey mainly on flying fish and cephalopods (Nelson, 2006; Almaguer-Hernández, 2016). In contrast to the two congeneric species, little is known about the trophic ecology of the species (see Table S1), including the Eastern Pacific population with globally/regionally important colonies occurring along varied oceanographic conditions along the Mexican Pacific coast (e.g., Peña Blanca and San Pedro Mártir islands; Piña-Ortiz et al., 2018). The study of the trophic ecology of tropical seabirds provides a better understanding of marine ecosystems and the challenges they face (e.g., changes in food availability, pollution, anthropogenic activities), which can improve conservation and management strategies (Gagné et al., 2018a, 2018b; Gatt et al., 2020; Lois et al., 2022). This knowledge is essential for marine conservation, detection of marine environmental changes and sustainable management of fishery resources (Parsons et al., 2008; Lyday et al., 2015).

We used DNA metabarcoding and stable isotope ($\delta^{15}N$ and $\delta^{13}C$) analyses to investigate the prey composition of red-billed tropicbirds in the Mexican Pacific, focusing on 1) comparing the diet between San Pedro Mártir and Peña Blanca, two of the most important colonies of the species in the region, located in contrasting oceanic systems in the region (upwelling vs. oligotrophic, respectively); and 2) comparing the diet between the breeding stages for each colony (courtship vs. incubation vs. early chick-rearing vs. late chick-rearing). We hypothesize that dissimilar marine environmental conditions will result in differences in the diet. We expected that the diet of the San Pedro Mártir colony, being reliant on predictable food resources, would be strongly influenced by temporal changes, especially in response to environmental variations (e.g., El Niño-Southern Oscillation), resulting in significant shifts in productivity and prey availability (Velarde et al., 2004; et al., 2013). In contrast, the Peña Blanca colony in oligotrophic waters will be less affected by the changes in prey abundances, thus expected to show less variability across the breeding season (e.g., Lerma et al., 2020).

2. Materials and methods

2.1. Study site and sample collection

We conducted the study at two sites (San Pedro Mártir Island [28°22′ N 112°19′ W] and Peña Blanca Islet [19°06′ N, 104°29′ W]; Fig. 1) from January to May 2021. San Pedro Mártir Island, located in the Gulf of California, is surrounded by an upwelling system fuelled by nutrient-rich waters that exchange with the Pacific Ocean. This exchange involves deep water inflow (200–600 m) and surface water outflow (0–200 m; Escalante et al., 2013). In contrast, Peña Blanca is a tropical islet located close to the coast of Colima (Mexican Tropical Pacific), which is primarily affected by the open, oligotrophic oceanic waters (Hernández-Vázquez et al., 2018). These two sites are the most important colonies of the species in the region in terms of colony size. San Pedro Mártir hosts 150–190 breeding pairs, while the Peña Blanca colony has 1200–1650 breeding pairs (Tershy and Breese, 1997; Piña-Ortiz et al., 2018). On both islands, we collected faecal samples from adults at different breeding stages: courtship (adults inside crevice but without clutch), incubation, early chick-rearing (chicks ≤5 weeks old), and late chick-rearing (≥6 weeks old) by inspecting active nests in different areas. Chicks of six weeks of age were assigned to the late chick-rearing stage, based on parental nest attendance rates (Stonehouse, 1962) and the body mass of the chicks, which reached adult weight during that post-natal period (adult mass: 536.85 ± 50.56 g; range: 432.9–664.6 g, $n = 54$; Piña-Ortiz et al., 2023). Nest cavities were labelled, and birds sampled were marked with alphanumeric rings on the tarsus to avoid resampling. In total, we collected 71 samples at San Pedro Mártir (courtship = 25, incubation = 19, early chick-rearing = 10, and late chick-rearing = 17), and 61 samples in Peña Blanca (courtship = 19, incubation = 24, early chick-rearing = 12, and late chick-rearing = 6). All individuals were captured by hand in the nest cavities. Once the birds were captured, they were placed on the legs of a staff member, who had covered his lap with a piece of stretch film or tinfoil to allow the bird to defecate naturally on it. The person in charge of this procedure used a new piece of foil for each individual and took all necessary hygienic measures, such as washing hands including alcohol and wearing latex gloves, to minimise possible contamination of the sample. The handling period for courtship and late chick-rearing individuals was set to a maximum of 60 min, while for incubating and early chick-rearing adults, it was kept to 30 min. All individuals were released immediately post-defecation back to the nest cavity. Subsequent monitoring of breeding success during the season allowed us to determine that no individuals abandoned or failed breeding following the handling of individuals. Faecal samples were collected in 1.5 mL plastic tubes and suspended in absolute ethanol. Additionally, blood samples (about 0.5 mL per bird) were collected from breeding adults during courtship (San Pedro Mártir = 16, Peña Blanca = 11), incubation (San Pedro Mártir = 16, Peña Blanca = 15), early chick-rearing (San Pedro Mártir = 12, Peña Blanca = 18), and late chick-rearing (San Pedro Mártir = 18, Peña Blanca = 9) by brachial vein puncture (3 mL syringe, 23G, 0.5 mm × 16 mm). Both blood and faecal samples were stored in a portable freezer (−2 °C; GoSun®) in the field and then frozen in the laboratory at −20 °C pending preparation for further analysis.

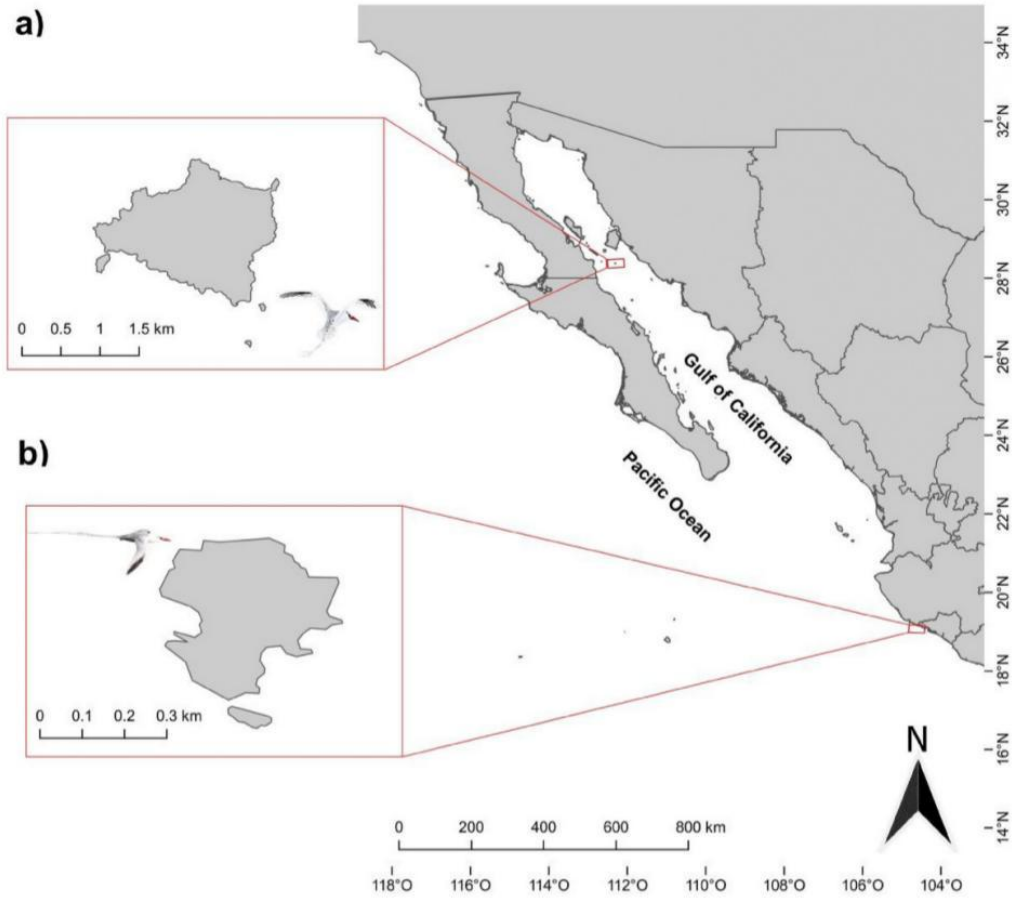


Fig. 1. Geographic locations of Red-billed Tropicbird (*Phaethon aethereus*) colonies sampled in this study. **a)** San Pedro Mártir Island and, **b)** Peña Blanca Islet.

2.2. DNA isolation and library preparation

We performed DNA extractions following the manufacturer's instructions for the Qiagen Fast DNA Stool Mini Kit (QIAGEN GmbH, Germany). For PCR amplifications, we used a Metazoa COI primer set to identify prey at family level. Based on our prior knowledge of the diet of the focal study species (Table S1), two more specific 16S rDNA primer pairs were used to identify the two main prey categories (fish and cephalopods; Table S2). For PCR amplifications, a 20 μL reaction volume was prepared, containing 10 μL Qiagen Multiplex PCR Buffer, 5.1 μL double-sterilized water, 0.1 μL BSA, 0.4 μL forward primer (10 μM), and 0.4 μL reverse primer (10 μM), along with 4 μL or 6 μL of the DNA template. PCRs were run following the protocol for the Qiagen Multiplex PCR Buffer (for annealing temperatures, see Table S2). For the fish and cephalopod primers, a touchdown PCR reaction was used, where the annealing temperature was decreased after each cycling step by $\Delta t = -1$ °C to optimize amplification. The adapter PCR products were inspected using the QIAxcel Advanced-System sequencer (QIAGEN), with products showing DNA concentrations below 0.5 ng/μL being repeated with more DNA template.

Amplicons resulting from our adapter PCR reactions underwent purification using the illustra™ ExoProStar™ 1-STEP kit (Cytiva, Amersham, UK), and we combined the amplicons of each samples following Swift et al. (2018). Subsequently, we prepared the Illumina library using the Nextera XT DNA Library Preparation Kit (Illumina, San Diego, CA). Index PCR amplifications were carried with a 30 μL PCR reaction volume, including 7.5 μL Qiagen Multiplex PCR buffer, 13.3 μL double-sterilized water, 10 μM primer (2.1 μL forward and 2.1 μL reverse primer for all three specific primers), and 5 μL of the DNA template. PCRs were run following the protocol for the Qiagen Multiplex PCR Buffer (with annealing temperature 56 °C). Subsequently, amplicons were purified using a SequalPrep™ Normalization kit (InvitrogenTM, Massachusetts, USA). The library was sequenced using 250-bp paired-end reads on a MiSeq desktop sequencer (Illumina) at SEQ-IT (SEQ-IT GmbH and Co.KG, Kaiserslautern, Germany).

2.3. Bioinformatic analysis

To obtain a list of molecular operational taxonomic units (MOTUs), we ran a custom workflow (Masello et al., 2021) in GALAXY (The Galaxy Community, 2022). The workflow included the following steps: 1) assessing sequence quality with FASTQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc) (accessed on Sep 5, 2023), 2) adapter and quality trimming of the paired-end reads with TRIMMOMATIC (minimum quality score of 20 over a sliding window of 4 bp; Bolger et al., 2014), 3) merging of the overlapping paired-end read pairs using FLASH (Magoč and Salzberg, 2011), 4) transforming sequence files to FASTA with the FASTX-Toolkit (http://hannonlab.cshl.edu/fastx_toolkit/) (accessed on Sep 5, 2023), 5) extracting amplicons from the FASTA files in MOTHUR (Schloss et al., 2009), 6) removing identical

replicates (dereplicate, plus strand), 7) detecting and removing chimeric sequences (de novo, minimal abundance ratio of parent vs. chimera 2, 'no' vote pseudo count 1.4, 'no' vote weight 8, minimum number of differences in segment 3, minimum divergence from closest parent 0.8, minimum score 0.28), and 8) clustering sequences into MOTUs, rejecting if identity was lower than 0.97, with VSEARCH (Rognes et al., 2016). Finally, using the BLASTn algorithm (Altschul et al., 1990), we matched MOTU sequences to reference sequences in the National Center for Biotechnology Information (NCBI) GenBank nucleotide database, employing a cut-off of 90% minimum sequence identity and a maximum e-value of 0.00001. For the taxonomic assignments, we used the percentage similarity of the query and the reference sequences, retaining a BLASTn assignment match greater than 98%, and a minimum sequence length of 190 bp since short fragments are less expected to contain trustworthy taxonomic information (Deagle et al., 2009; Vesterinen et al., 2013). We allocated MOTUs to the species level only in cases when all retained hits of a MOTU, with the same quality criteria (sequence identity, sequence length, e-value), corresponded to the same species. Otherwise, we assigned the MOTU to the lowest shared taxonomic level, (e.g., genus or family; Kleinschmidt et al., 2019). The raw data set included a wide range of unspecific, contaminant DNA (e.g., human, bacteria) that could be excluded as potential prey taxa based on previous literature (Stonehouse, 1962; Nelson, 2006; Castillo-Guerrero et al., 2011; Almaguer-Hernández, 2016; Diop et al., 2018; Madden et al., 2022, 2023). Non-prey MOTUs included various taxa of the orders Insecta, Reptilia and Aves, which were omitted during the validation steps, as they were ecologically irrelevant or very distant distribution ranges. As in Masello et al. (2021), records with <10 reads or in singular MOTUs, where the read number accounted <1% of the maximum count were excluded from the analysis.

In order to restrict secondary prey identifications, we applied two approaches previously employed in other studies using DNA metabarcoding (see Hardy et al., 2017; Ando et al., 2020; Nimz et al., 2022). Firstly, we assumed that fish and squid were the primary prey based on previously published studies on the diet of the species, while other matches such as Gastropoda, Copepoda, Branchiopoda, Bivalvia, and Isopoda were probably due to secondary predation, accidental ingestion during foraging, prey parasites or even contamination of samples. Secondly, using prey diet information, we examined potential cases of secondary predation by considering both the co-occurrence of prey items and their distributions.

For the three main prey groups—fish, cephalopods, and crustaceans—we calculated the frequency of occurrence (FO; Formula 1) and the relative read abundance (RRA; Formula 2). We utilized the RRA to enhance our interpretation of FO, as recommended by previous studies (Barrett et al., 2007; McInnes et al., 2017; Young et al., 2020). In this context, FO is defined as:

$$FO = (n / t) * 100 \quad (1)$$

where n represents the number of samples in which we detected prey DNA, and t is the total number of samples in which DNA from the considered prey group was present. We defined the RRA as:

$$RRA = (reads \ / \ total\ number\ of\ reads) * 100 \quad (2)$$

representing the percentage ratio of reads in relation to the total number of reads recorded for the respective MOTU.

Moreover, while DNA metabarcoding is a powerful tool for obtaining comprehensive insights into a species' diet using a non-invasive approach and small sample sizes, several disadvantages are associated with the method (Ando et al., 2020). Sample contamination, whether from the laboratory, field environment, or secondary prey, poses a significant issue. Additionally, the preselection and use of DNA barcoding markers can affect taxonomic resolution and detectability (Ando et al., 2020). Technical biases such as inappropriate PCR settings, DNA host amplification, and PCR inhibition, as well as the selection of bioinformatic scripts and the steps to compile the reference database, are also crucial considerations (Ando et al., 2020).

2.4. Stable isotope analyses

Blood reflects a dietary integration period of 2–4 weeks prior to sampling for carbon and nitrogen isotopic analyses (Bearhop et al., 2002). Therefore, blood samples obtained from adults in our study are expected to represent the diet consumed during each respective stage. We oven-dried (50 °C) the blood samples and then we finely ground them. We packed subsamples (0.3–0.5 mg) in tin capsules to be analysed for %N, %C, δ^{15}N and δ^{13}C using a Flash 2000 elemental analyser (Thermo Scientific, Milan, Italy) coupled with a Delta V Plus isotope ratio mass spectrometer with a Conflo IV interface (Thermo Scientific, Bremen, Germany). We carried out the analyses at the 'Littoral, Environnement and Societés (LIENSs)' Joint Research Unit stable isotope facility (CNRS – La Rochelle Université, France). Results are expressed as δ (‰) for δ^{15}N and δ^{13}C, calibrated against the international isotopic references (atmospheric nitrogen for δ^{15}N and Vienna-Pee Dee Belemnite for δ^{13}C). The analytical precision was ±0.15 ‰ for δ^{15}N and ±0.10 ‰ for δ^{13}C based on internal standards USGS-61 and USGS-62 inserted every ten measurements. All samples had a low C:N mass ratio (<4.0), indicating low lipid content, so we did not perform any lipid extraction (Cherel et al., 2005).

2.5. Statistical analyses

We assessed species diversity using rarefaction curves and determined the percentage of samples covering the four breeding stages at each study site, confirming the adequacy of our sample sizes. This analysis was performed using the 'iNEXT' package (Hsieh et al., 2016) within R v4.1.8 (R Core Team). We compared prey composition between sites and breeding stages employing a Permutational Analysis of Variance (PERMANOVA) test with the 'VEGAN' package (Oksanen et al., 2018). We integrated various effects into the Adonis base model, including sites, collection date (Julian calendar), and chick age, ensuring a clear distinction between early and late chick-rearing stages. Interaction effects in the model (e.g., stage*collection date, stage*age) were also explored. We selected the optimal model based on our significance level and further evaluated it using the Akaike Information Criterion (AIC). The model with the lowest score was deemed optimal for our specific data set. We employed a non-metric multidimensional scaling (NMDS) with Bray-Curtis dissimilarity for binary data to illustrate the dissimilarity in prey composition between the stages and islands. We estimated the stress level in both study sites using the 'VEGAN' package (Oksanen et al., 2018). A stress level <0.05 is considered as an excellent agreement, a stress level below 0.1 very good, and below 0.2 as good for representing the data set (Masello et al., 2023). In our models, the stress was 0.07–0.08. A hierarchical cluster analysis was employed to assess dissimilarity among prey MOTUs, utilizing Ward's cluster method with a Manhattan distance measure. The frequencies of the respective taxonomic class (i.e., species, genus or family) to which the readings were assigned were visualised in a heat map using the corresponding add-on in OriginPro Lab (Version, 2023; OriginLab Corporation, Northampton, MA, USA).

To examine differences in adult δ^{15}N and δ^{13}C isotope ratios we conducted general linear models (GLM) considering sites (San Pedro Mártir and Peña Blanca) and breeding stages (courtship, incubation, early chick-rearing, and late chick-rearing) as categorical factors, and the sample collection date as a continuous predictor. GLMs were based on complete initial models that included all variables and interactions. To compare differences between adults in isotopic values during the breeding stages, we employed t-tests with Bonferroni correction (α = 0.05, 1 comparison, and α = 0.017, 3 comparisons for site and breeding stage, respectively). Moreover, we assessed niche breadth among breeding stages within and across sites by calculating two-dimensional

isotopic niches using standard ellipses areas, as implemented in the 'SIBER' package (Jackson et al., 2011). Specifically, for the quantification of niche breadth and comparisons across stages and sites, we employed the standard ellipse area corrected for small sample sizes (SEA_C). Simultaneously, Bayesian standard ellipses (SEA_B) were employed to evaluate the proportion of overlapping area among stages and sites (Jackson et al., 2011). For the rest of analyses, we conducted all statistical tests at a significance level of $\alpha = 0.05$, and the results are presented as the mean ± standard deviation.

3. Results

3.1. Diversity and total identified taxa

From a total of 131 samples, 97 (74%) successfully amplified with the Metazoa primers, 47 (36%) with the Fish primers and 11 (8%) with the Cephalopoda primers (Fig. S1). We identified 20 different MOTUs (6 species, 9 genus, and 13 families) for the San Pedro Mártir data set and 22 different MOTUs (4 species, 9 genus, 13 families) for the Peña Blanca data set, with the highest MOTUs numbers recorded for courtship individuals in San Pedro Mártir and incubating birds in Peña Blanca (Table S3). In San Pedro Mártir, the average number of taxa detected for

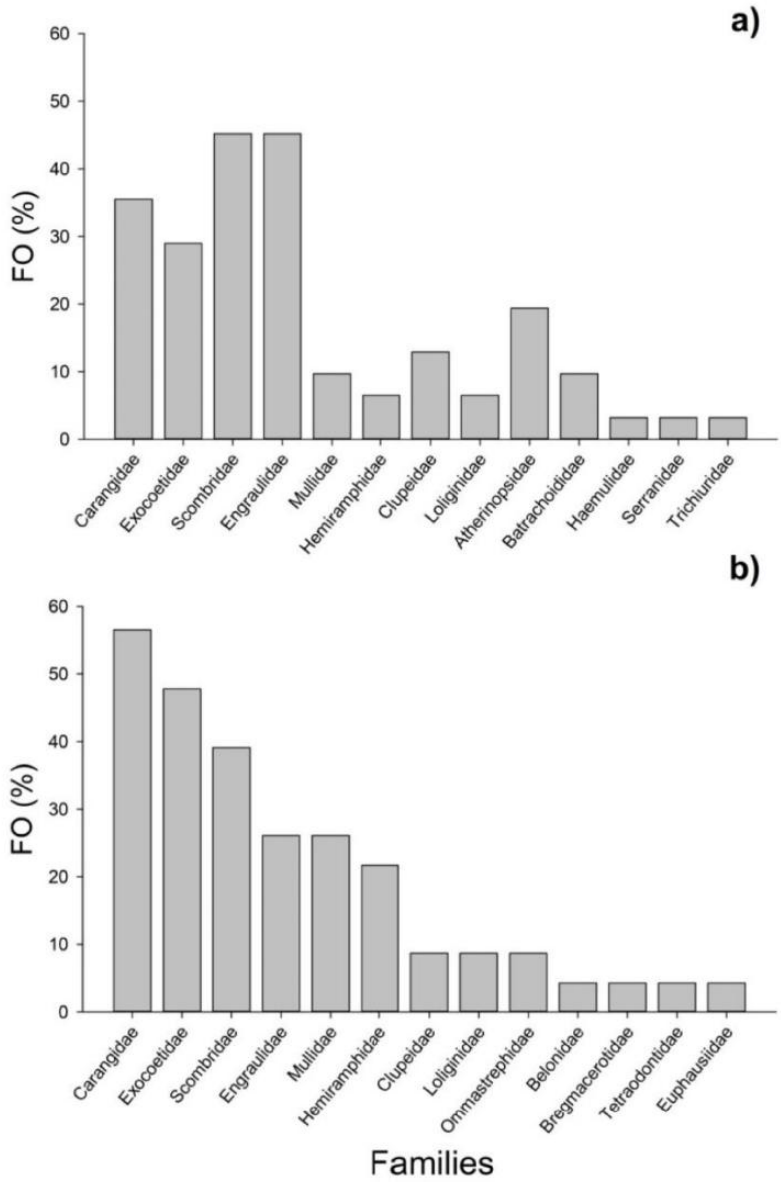


Fig. 2. Bar charts showing the frequency of occurrence (% of samples; FO) of the predominant prey families in the diet of the Red-billed Tropicbird (*Phaethon aethereus*) in **a)** San Pedro Mártir Island and **b)** Peña Blanca Islet estimated by metabarcoding.

each breeding stage was 2.8 ± 1.2 (n = 13) for individuals at courtship, 2.8 ± 1.8 ($n = 5$) at incubation, 2.5 ± 1.0 ($n = 6$) at early chick-rearing, and 2.9 ± 1.2 ($n = 7$) at late chick-rearing stage. For Peña Blanca, the numbers of taxa were 2.3 ± 1.4 ($n = 8$) at courtship, 3.2 ± 1.5 ($n = 6$) at incubation, 3.5 ± 1.3 ($n = 6$) at early chick-rearing, and 4.7 ± 2.3 ($n = 3$) at late chick-rearing (Fig. S1). Rarefaction curves indicated that sufficient species coverage was obtained with the sample sizes for most breeding stages at both sites (species coverage between 73 and 85%), except for the incubation stage, which explained only around 55% and 45% of the variation for San Pedro Mártir and Peña Blanca, respectively (Fig. S2). Hence, we encourage readers to take and use the results of this stage with due discretion.

The most common prey items at family level for both study sites were Carangidae (San Pedro Mártir: 35.5%, Peña Blanca: 56.5% of samples), Scombridae (San Pedro Mártir: 45.2%, Peña Blanca: 39.1%), Exocoetidae (San Pedro Mártir: 29%, Peña Blanca: 47.8%), and Engraulidae (San Pedro Mártir: 45.2%, Peña Blanca: 26.1%; Fig. 2). For San Pedro Mártir, the Californian anchovy (*Engraulis mordax*) was the most frequent prey for courtship individuals (Table 1). The topsmelt silverside (*Atherinops affinis*) was the most frequently consumed prey at incubation and early chick-rearing stages, while *Scomber* sp. and the Californian anchovy formed the most frequently observed prey of late chick-rearing adults (Table 1, Fig. 3; for RRA values see Table S4). On the other hand, Carangidae and Engraulidae represented concurrently the most frequent prey of Peña Blanca courtship individuals. The Bullet tuna (*Auxis rochei*), and *Scomber* sp. were the predominant prey for incubation individuals, while *Cypselurus* sp. was the main prey for early chick-rearing individuals (Table 1). For late chick-rearing adults *Caranx* sp., an unidentified Carangidae, and an unidentified Engraulidae were the most frequent prey (Table 1; Fig. 3). Crustaceans were only recorded as prey from Peña Blanca courtship individuals, and cephalopods were omnipresent in courtship and early chick-rearing adults in San Pedro Mártir and in all breeding stages, except for the courtship at Peña Blanca, reaching a low FO (8–33%) throughout the data set (Table 1).

3.2. Multivariate analysis of spatio-temporal divergence in prey selection

The multivariate analysis of prey composition revealed spatial divergence between sites ($AIC_{Perm} = -60.93$, Permanova test, $pseudoF_1 = 4.16$, $p = 0.005$; Table 2). Likewise, differences in the diet between the breeding stages were present in the Peña Blanca population (Permanova test, $pseudoF_{1,3} = 3.06$, $p = 0.003$), and from San Pedro Mártir population, however for the latter the prey composition between breeding stages varied in interaction with the collection date (Permanova test, $pseudoF_{1,26} = 3{,}88$, $p = 0.002$; Table 2). The dietary composition for the early and late chick-rearing stages showed significant differences between breeding adults in Peña Blanca (Permanova test, $pseudoF_{1,7} = 3.06$, $p = 0.05$). The cluster analysis comparing the FO highlighted the formation of two clusters between the breeding stages for the San Pedro Mártir data set, with the highest dissimilarity being estimated between courtship and late chick-rearing individuals and incubation and early chick-rearing birds (Fig. 3a). In contrast, for Peña Blanca, only the individuals in incubation and late-chick rearing had a notable overlap between the prey incidences (Fig. 3b). The NMDS analysis highlighted similar divergence between the stages in San Pedro Mártir (stress level = 0.076), indicating a certain degree of overlap between courtship and late chick-rearing individuals, as well as between incubation and early chick-rearing individuals, while in Peña Blanca (stress level = 0.076) there was an overlap in prey composition between most breeding stages, with the exception of the incubation and early chick-rearing stages (Fig. 4a and b).

3.3. Stable isotopes

The $\delta^{15}N$ and $\delta^{13}C$ values differed significantly between sites (GLM, $\delta^{15}N$: $F_{1,106} = 525.76$, $p < 0.001$; $\delta^{13}C$: $F_{1,106} = 24.0$, $p < 0.001$), and breeding stages ($\delta^{15}N$: $F_{3,106} = 6.50$, $p < 0.001$; $\delta^{13}C$: $F_{3,106} = 8.61$, $p < 0.001$), and the interaction between these factors was non-significant (Table 3). In general, individuals from San Pedro Mártir had significantly higher $\delta^{15}N$ and $\delta^{13}C$ than adults from Peña Blanca (Fig. 5). San Pedro Mártir individuals at the incubation stage had significantly lower $\delta^{13}C$ values compared to the rest of the stages (Paired *t*-test, $t_{5,6} = 0.79$, $p < 0.001$, $t_{6,7} = 0.57$, $p = 0.017$ and $t_{6,8} = 0.60$, $p < 0.002$ for courtship, early and late chick-rearing stages, respectively), while late chick-rearing individuals at Peña Blanca had significantly higher $\delta^{15}N$ values than those from incubation stage ($t_{2,4} = 0.63$, $p = 0.005$; Fig. 5). The standard ellipse areas estimated for each breeding stage at the study sites exhibited dissimilarities, with the widest isotopic niches during incubation at both colonies (SEA_C: San Pedro Mártir = 0.55 and Peña Blanca = 0.31), whereas the narrowest isotopic niches were estimated during early chick-rearing from San Pedro Mártir and late chick-rearing from Peña Blanca (SEA_C: 0.29 and 0.15, respectively; Fig. 5, Table 4). Likewise, all pairwise comparisons of niche breadth between breeding stages showed significant differences in their ellipse areas ($p < 0.001$; Table S5). The isotopic niches of the breeding stages in San Pedro Mártir had an overlap ranging from 28% to 39%, while in Peña Blanca, they ranged from 22% to 34% (Table S6). Particularly, the highest overlap in San Pedro Mártir occurred between the courtship and late chick-rearing, while in Peña Blanca it was observed between the early and late chick-rearing stages (Fig. 5, Table S6).

4. Discussion

4.1. Literature review on the diet

The results of this study agreed with previously reported data on the prey composition of the species, that consumed fish, cephalopods, and crustaceans (Stonehouse, 1962; Nelson 2006; Castillo-Guerrero et al., 2011; Almaguer-Hernández, 2016; Diop et al., 2018; Madden et al., 2022, 2023; Table S1). Unlike previous studies that relied on regurgitates (Stonehouse, 1962; Castillo-Guerrero et al., 2011; Almaguer-Hernández, 2016; Diop et al., 2018; Madden et al., 2022, 2023; Table S1) or stomach contents (North, 1946), our study highlights the advantages of DNA metabarcoding on faeces over conventional methods (e.g., Guillerault et al., 2017; Snider et al., 2022; Allen et al., 2023). Specifically, we achieved higher taxonomic resolution, identifying to species level more accurately than most previous studies, and obtained high amplification success even with minimal sample amounts. In contrast, when collecting regurgitates from colonies, prey may be at a significant stage of digestion that renders them unidentifiable (Scribner and Bowman, 1998; Barrett et al., 2007). This constrain for identifying digested prey at the species level may result in the omission of key dietary information required to understand marine food webs (Barrett et al., 2007; Allen et al., 2023; Querejeta et al., 2023). In addition, faeces collection does not affect the breeding success of the species studied by not interfering with prey captured by individuals (e.g., Almaguer-Hernández, 2016), and the number of samples necessary for a complete dietary mapping is only a fraction compared to studies using regurgitates or stomach contents.

In this study, the availability of trophic resources for both colonies was inferred from bibliographic information, considering the location, environmental characteristics, and phenology of the prey. However, since the presence, distribution, and abundance of prey can change annually, direct sampling of the fish community or other potential prey would have provided a more accurate assessment of prey availability. Nonetheless, this approach presents significant logistical and financial challenges. In that sense, environmental DNA (eDNA) analysis presents an alternative approach, to assessing biodiversity and identifying organisms in different habitats and oceans (e.g., Lima-Mendez et al., 2015; Andruszkiewicz et al., 2017; Deiner et al., 2017; Djurhuus et al., 2018). It can provide a detailed view of species composition, facilitating the inference of co-occurrence patterns and the detection of organisms

Table 1
Frequency of occurrence (% of samples; FO) for main prey categories consumed by Red-billed Tropicbird (*Phaethon aethereus*) at the study sites (San Pedro Mártir and Peña Blanca islands), and during four defined breeding stages (COU – courtship, INC – incubation, ECR – early chick-rearing, LCR – late chick-rearing). Prey depth ranges (m) are shown in the table. Sample sizes correspond to the number of DNA extractions from faecal samples, and in brackets to the number of successfully sequenced samples.

							San Pedro Mártir				Peña Blanca			
Phylum	Class	Order	Family	Species	Common Name	Depth[a]	COU	INC	ECR	LCR	COU	INC	ECR	LCR
							$n = 25$ (13)	$n = 19$ (5)	$n = 10$ (6)	$n = 17$ (7)	$n = 19$ (8)	$n = 24$ (6)	$n = 12$ (6)	$n = 6$ (3)
Arthropoda	Malacostraca	Euphausiacea	Euphausiidae		Krill	0–600	–	–	–	–	12	–	–	–
Mollusca	Cephalopoda	Myopsida	Loliginidae		Pencil squids	0–1000	7.7	–	16.7	–	–	16.7	16.7	–
		Oegopsida	Ommastrephidae		Flying squid	–	–	–	–	–	–	–	16.7	33.3
Chordata	Actinopterygii	Atheriniformes	Atherinopsidae	*Atherinops affinis*	Topsnelt silverside	0–26	–	60	50	–	–	–	–	–
		Beloniformes	Exocoetidae		Flying fish	0–20[b]	7.7	40	50	14.3	12.5	33.3	100	66.7
				Cheilopogon sp.		0–5[b]	–	–	–	–	–	–	50	–
				Cypselurus sp.		0–20[b]	–	20	33.3	–	12.5	16.7	83.3	33.3
			Hemiramphidae		Halfbeak	–	–	–	–	28.6	25	–	50	–
				Oxyporhamphus sp.	Bigwing halfbeak	0–?	–	–	–	–	12.5	–	50	–
			Belonidae		Needlefish	–	–	–	–	–	–	16.7	–	–
		Perciformes	Carangidae		Jacks and pompanos	0–380	23	–	16.7	28.6	62.5	33.3	50	100
				Caranx sp.	Jacks	0–380	–	–	–	–	25	16.7	–	66.7
				Decapterus macrosoma	Shortfin scad	20–214	–	–	16.7	28.6	25	16.7	50	33.3
				Selar crumenophthalmus	Bigeye scad	0–170	–	–	–	–	25	–	–	–
			Haemulidae		Grunts	–	7.7	–	–	–	–	–	–	–
			Mullidae	*Mulloidichthys* sp.	Goatfishes	2–113	–	20	33.3	–	25	16.7	33.3	33.3
			Serranidae	*Diplectrum* sp.	Sandperches	1–160	–	–	–	14.3	–	–	–	–
		Scombriformes	Trichiuridae	unid.	Cutlassfishes	2–500	7.7	–	–	–	–	–	–	–
			Scombridae			0–300	53.8	20	33.3	57.1	–	83.3	33.3	66.7
				Auxis rochei	Bullet tuna	0–200	–	–	–	–	–	33.3	16.7	33.3
				Scomber japonicus	Pacific chub mackerel	0–300	7.7	20	–	28.6	–	16.7	–	–
				Scomber sp.	Club Mackerels	–	30.8	20	33.3	57.1	–	33.3	16.7	33.3
				Scomberomorus concolor	Monterey Spanish mackerel	15–?	15.4	–	–	–	–	–	–	–
		Tetraodontiformes	Tetraodontidae		Puffer-Fishes	0–483	–	–	–	–	12.5	–	–	–
		Clupeiformes	Clupeidae		Herrings	–	38.5	20	–	42.8	–	16.7	–	–
				Sardinops sagax	Californian pilchard	0–200	7.7	20	–		–	–	–	–
				Sardinops sp.	Sardines	0–200	23.1	20	–	42.8		–		
			Engraulidae		Anchovies	–	46.1	40	33.3	57.1	37.5	16.7	–	66.7
				Engraulis mordax	California anchovy	0–310	46.1	40	33.3	57.1	–	–	–	–
		Batrachoidiformes	Batrachoididae		Toadfishes	1–225	7.7	20	16.7	–	–	–	–	–
		Gadiformes	Bregmacerotidae	*Bregmaceros bathymaster*	East Pacific codlet	0–1246	–	–	–	–	–	16.7	–	–

? – Depth range unknown.
[a] Depth range derived from Froese and Pauly, 2024; Robertson and Allen (2024).
[b] Flying fish is encountered gliding over the sea level, indicated by the value 0 m here.

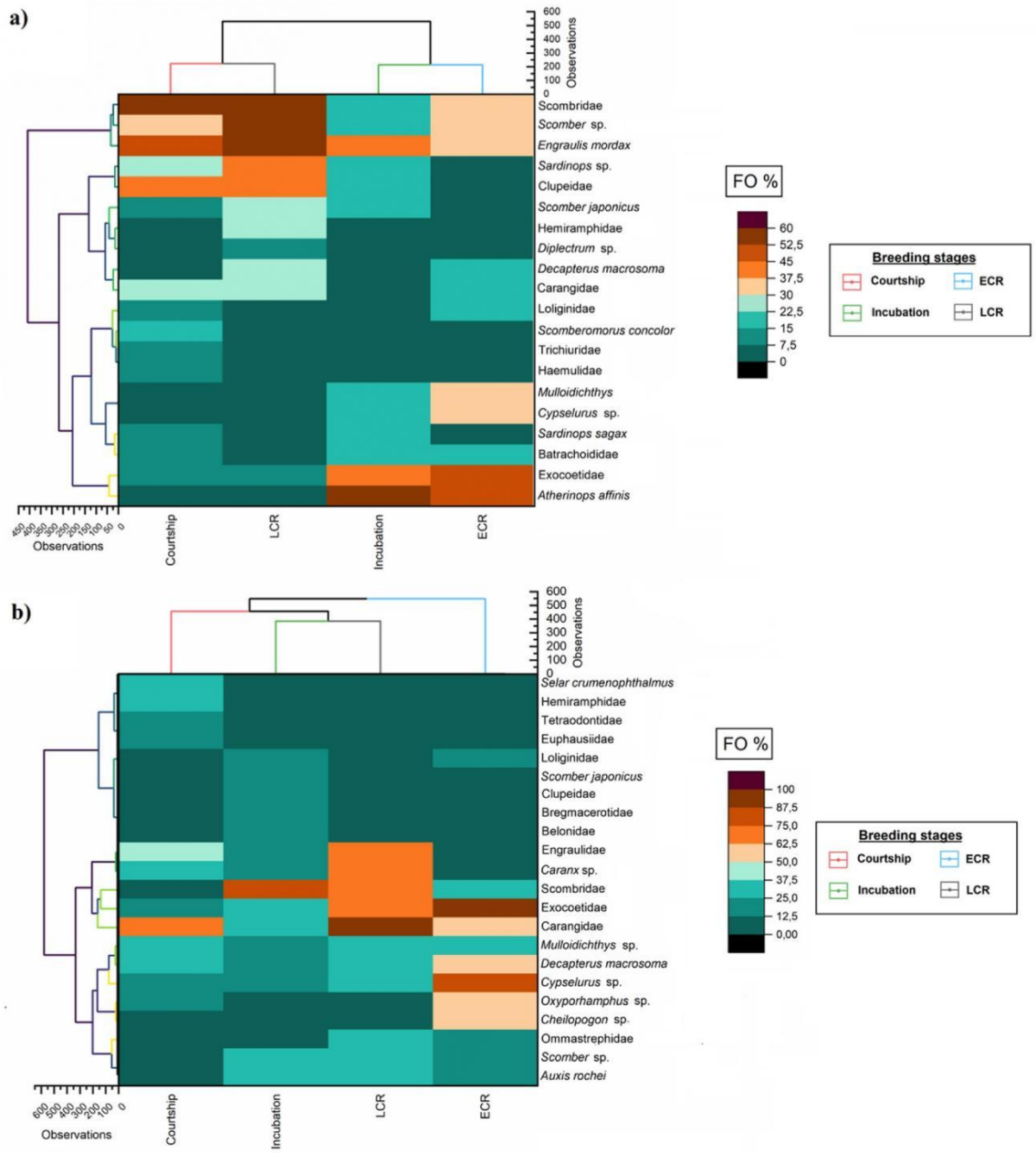


Fig. 3. Cluster maps highlighting the hierarchical cluster analysis performed for the MOTUs using the frequency of occurrence (FO) of the respective taxonomic class with the associated reads and the breeding stages (ECR = early chick-rearing, LCR = late chick-rearing, Ward's cluster method CityBlock or Manhattan type) with the dendograms of each study site [**a)** San Pedro Mártir Island, **b)** Peña Blanca Islet] are outlined below showing associated observation clusters. MOTUs were clustered based on the number of generated observations.

across wide marine areas (Djurhuus et al., 2020).

The integration of metabarcoding and stable isotope data revealed that red-billed tropicbirds hold a top-predator role in marine pelagic systems, primarily preying on mesopelagic and epipelagic open-ocean fish species and to a lesser extent, benthic and reef-associated fish, cephalopods and crustaceans. The Californian anchovy, Pacific chub mackerel and South American pilchard (*Sardinops sagax*) were identified as main prey for San Pedro Mártir, whereas flying fish were prominent in the diet from Peña Blanca individuals, consistent with prior studies (Stonehouse, 1962; Castillo-Guerrero et al., 2011; Almaguer-Hernández, 2016; Diop et al., 2018; Madden et al., 2022, 2023). Specifically, Spotfin flying fish (*Cheilopogon furcatus)*, Stained flying fish (*C. spilonotopterus*), Whitetip flying fish (*C. xenopterus)*, and *Cypselurus* sp. (likely Ornamented flying fish [*C. callopterus*]) were noted prey for Peña Blanca

Table 2
Results of Permutational analysis of variance (Permanova) test using the Adonis base model with added effects for each study location and different taxonomic resolution. ECR – early chick rearing, LCR – late chick rearing. Significant *p*-values: * = 0.05, ** = 0.01, *** = 0.001.

Model	AIC	R	F	*p*	Significance level
General data set (combined)					
Sites	−60.93	0.07	4.16	**0.005**	**
San Pedro Mártir					
stages	−33.05	0.16	1.62	0.095	
collection date	−44.74	0.77	2.35	**0.002**	**
age	−29.33	0.26	1.17	0.239	
*stages*age*	−30.44	0.33	1.37	0.094	
*stages*collection date*	−83.21	0.96	3.88	**0.002**	**
*chickstages ~ ECR*LCR*	−13.76	0.17	2.39	0.067	
Peña Blanca					
stages	−30.43	0.33	3.06	**0.003**	**
collection_date	−19.80	0.65	0.71	0.873	
age(metric)	−20.56	0.20	0.68	0.897	
*stages*collection date*	−29.60	0.81	0.95	0.553	
*stages*age(metric)*	−24.05	0.37	1.27	0.182	
*chickstages ~ ECR*LCR*	−16.77	0.30	3.06	**0.050**	*

(Almaguer-Hernández, 2016). In addition, Carangidae dominated in Peña Blanca diet, with MOTUs like *Caranx* sp. and Shortfin scad (*D. macrosoma*), aligning with previous records (Almaguer-Hernández, 2016; Diop et al., 2018). Among the most frequent prey of the San Pedro Mártir individuals, the topsmelt silverside (FO = 19.3%) was identified, which simultaneously represent the first record for the diet of the species.

Clupeidae and Hemiramphidae were previously recorded in the prey of the focal species like the Pacific thread herring (*Opisthonema libertate*) and *Oxyporhamphus* sp. (i.e., Bigwing halfbeak [*O. micropterus*]; Castillo-Guerrero et al., 2011; Almaguer Hernández, 2016). Belonidae was found in the diet of Peña Blanca individuals, recently recorded in the Caribbean and the Eastern Atlantic populations (Diop et al., 2018; Madden et al., 2022). Other fish taxa identified had not been previously recorded and represent probably opportunistic prey, evidenced by low read counts or single records (e.g., Trichiuridae, Batrachoididae, Tetraodontidae, *Diplectrum* sp. and *Bregmaceros bathymaster*). Although the possibility that these observations are the result of secondary predation (i.e., DNA carry-over from ingested prey) cannot be excluded either.

Further comparison of the prevalence of cephalopods and crustaceans in this study with previous research on the diet of tropicbirds reveals a minor role of both taxa in the colonies studied (Table S1). Therefore, the observed diet profiles align more closely with Caribbean and Atlantic Red-billed Tropicbird populations, where fish predominantly or entirely comprise the diet (Diop et al., 2018; Madden et al., 2022 et al., 2023). Despite previous studies indicating low crustacean frequencies, squid consistently appeared in regurgitates, ranging between 0 and 30% prevalence (Stonehouse 1962; Nelson 2006; Castillo-Guerrero et al., 2011; Almaguer-Hernández, 2016; Madden et al., 2023). Our findings suggest a lower importance of cephalopods as prey, nevertheless it is crucial to address the potential biases that influence low detection rates. While the cephalopod primers used here were confirmed to detect unspecific, non-target taxa with a generally low match rate possibly introducing a bias (non-target prey recorded in 96.2% of all reads; Young et al., 2020), we find this scenario highly unlikely for the following reasons. The COI and 16S regions have been widely employed for the amplification and identification of DNA from vertebrates and cephalopods in dietary studies (e.g., Carreiro et al., 2020; Young et al., 2020; Nimz et al., 2022); besides, the primers set was used for cephalopods already and was validated (see Peters et al., 2015; Berry et al., 2017; de Jonge et al., 2021). Also, we used two different primers to target cephalopods, leaving a low likelihood that cephalopods were possibly overlooked. Conversely, recent studies have pointed out the relatively lower success rates of 16S assays in dietary DNA studies of pelagic seabirds (Doyle and Adams, 2018; Nimz et al., 2022). Therefore, it is advisable to perform an initial experiment with a subset of samples to identify the most effective primer sets for full analyses. This recommendation is based on the fact that new primer sets emerge rapidly, and the success of primer assays depends on factors such as primer binding efficiency and the availability of prey sequence data (Nimz et al., 2022). Additionally, it is worth mentioning that detection of uncommon or poorly studied taxa as cephalopods could be limited by the lack of complementary DNA sequences in genetic reference databases (i.e., Genbank), although a matter of time for sequence databases to improve the widely used metabarcoding targets to achieve more accurate taxonomic identifications (e.g., de Jonge et al., 2021).

4.2. Divergence in prey selection between breeding colonies

In accordance with our expectations, the diet of red-billed tropicbirds varied between breeding sites, reflecting differences in prey composition and abundance between regional marine systems (upwelling vs. oligotrophic). These differences could be linked to factors such as food availability, reflected in differences in foraging behaviour and competition for resources. The individuals from San Pedro Mártir exhibited lower prey diversity than those from Peña Blanca, with individuals from both sites primarily relying on fishes, comprising their diets mainly by 4–5 prey species (FO range: 19.5–45%, total RRA >80%; Tables 2 and S2, Fig. S3). Notably, individuals at each site prey on abundant species in their respective regions (e.g., *E. mordax*, *Sardinops* sp. [*sagax*] at SPM), showcasing trophic plasticity throughout their distribution range (Castillo-Guerrero et al., 2011; Diop et al., 2018; Madden et al., 2022, 2023). Trophic plasticity, a common strategy among widely distributed species, allows the use of fluctuating food resources driven by environmental variability in their home ranges (Carlig et al., 2019; Jafari et al., 2021; Song et al., 2021). Breeding seabirds can adjust their foraging behaviour based on prey accessibility near their colonies (McInnes et al., 2017; Jacoby et al., 2023; Querejeta et al., 2023). Indeed, variations in the availability and abundance of the main prey items could lead to differences in foraging behaviour and diet between breeding sites (Ainley et al., 1996; Mellink et al., 2001; Castillo-Guerrero et al., 2016). Foraging behaviour in red-billed tropicbirds varies according to the local marine environmental features (e.g., coastal upwelling vs. oceanic; Diop et al., 2018). The areas surrounding our study sites, used as tropicbird foraging grounds, exhibit contrasting local features (e.g., chlorophyll-a, air and sea surface temperature [SST]; Piña-Ortiz et al., 2023), as they are located in distinct marine ecoregions (see details Spalding et al., 2007). San Pedro Mártir, located in the Gulf of California, is an upwelling system, while Peña Blanca (Mexican Tropical Pacific) is primarily influenced by open, oligotrophic oceanic waters (Hernández-Vázquez et al., 2018). Preliminary data on foraging ecology indicate divergent foraging parameters between these colonies, with individuals from Peña Blanca undertaking longer and more distant trips compared to those from San Pedro Mártir (Figs. S4 and S5; Piña-Ortiz et al., unpubl. data). This suggests that differences in foraging behaviour are likely driven by variations in prey availability and resource distribution between the two locations.

Predators sharing common prey are thought to occupy similar ecological niches, potentially leading to competition for finite resources (Holt, 2009). During the breeding season, seabirds become central-place foragers, and those with overlapping breeding periods or confined foraging habitats often face heightened competition due to limited resource availability. Documented evidence indicates that intraspecific competition is more intense than interspecific competition among seabirds (Grémillet et al., 2004; Masello et al., 2010; Paredes et al., 2012; Rosciano et al., 2016; Lee et al., 2021). While San Pedro Mártir supports a greater diversity of breeding seabirds (8 species vs. 2 species), Peña Blanca has larger population sizes (~7500 vs. ~16,500 breeding seabird pairs; Hernández-Vázquez et al., 2017; Piña-Ortiz et al., 2018; Castillo-Guerrero et al., 2022). Therefore, we expect a scenario of increased

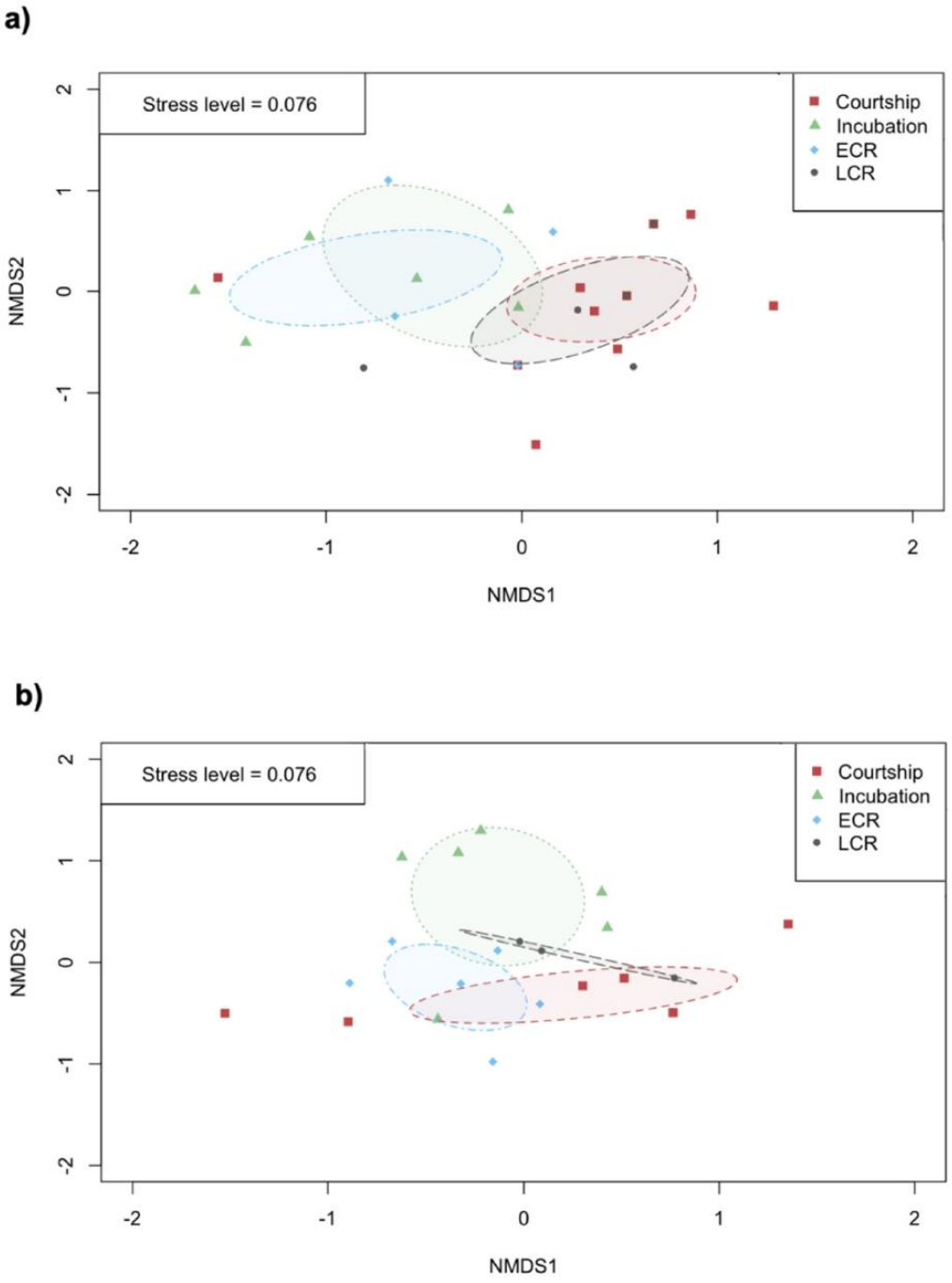


Fig. 4. Non-metric multidimensional scaling plots (Bray-Curtis k = 2) showing the dissimilarity patterns in prey compensation using the MOTUs between the four breeding stages (ECR = early chick-rearing, LCR = late chick-rearing) at **a)** San Pedro Mártir Island (stress level = 0.076) and, **b)** Peña Blanca Islet (stress level = 0.076).

competition for resources at the Peña Blanca colony, supported by differential foraging effort between study sites, which could result in a decrease in the abundance of preferred prey, and consequently, may result in a more diverse diet (Optimal foraging theory; MacArthur and Pianka, 1966; Stephens and Krebs, 1986; Ratcliffe et al., 2018). Actually, studies have demonstrated that high intraspecific competition increases population niche width and individual specialization (Svanbäck and Bolnick, 2005, 2007; Ratcliffe et al., 2018). However, our results show a

Table 3
Summary statistics of the final selected general linear model (GLM) evaluating the effects of the site and breeding stage (courtship, incubation, early chick-rearing and late chick-rearing) on the nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) isotope ratios measured in whole blood of red-billed tropicbirds from the 2021 breeding season at San Pedro Mártir Island (temperate) and Peña Blanca Islet (tropical), Mexico. Sample sizes are as follows, courtship (San Pedro Mártir = 16, Peña Blanca = 11), incubation (San Pedro Mártir = 16, Peña Blanca = 15), early chick-rearing (San Pedro Mártir = 12, Peña Blanca = 18) and late chick-rearing (San Pedro Mártir = 18, Peña Blanca = 9). Significant terms are shown in bold.

Factors and interactions	$\delta^{15}N$			$\delta^{13}C$		
	F	*df*	*p*	F	*df*	*p*
site*stage	1.94	3, 106	0.13	2.70	3, 106	0.05
site	525.76	1, 106	< **0.001**	24.0	1, 106	< **0.001**
stage	6.50	3, 106	< **0.001**	8.61	3, 106	< **0.001**
date	1.83	1, 106	0.18	1.92	1, 106	0.17

surprisingly similar prey range and comparable niche breadth between sites (Layman's metric of convex hull area [TA]: San Pedro Mártir = 0.090 and Peña Blanca = 0.086; Fig. 2 and S6). Although resource availability and colony size generate a competition gradient (Ashmole, 1963; Gaston et al., 2007), this competition will not necessarily translate into an expansion of niche breadth (Correa and Winemiller, 2014). In fact, variability in niche breadth and individual specialization could arise from an "ecological opportunity" (e.g., spatio-temporal availability of resources) to exploit different prey rather than direct competition (see Araújo et al., 2012; Correa and Winemiller, 2014). Despite our findings agreeing on a relationship between resource availability and competition, disentangling the role of each in the diet of the Red-billed Tropicbird remains challenging to obtain. Future research could explore the diet of breeding seabirds from both sites and assess the interplay with the intra- and interspecific competition.

Moreover, the isotopic differences between sites closely mirrored those identified in the dietary analysis. Despite variations in consumed species, attributing all nitrogen value differences solely to the feeding ecology of predominant prey proved challenging. Both sites featured predominantly planktivorous (e.g., Atherinopsidae, Exocoetidae, Engraulidae) and secondarily carnivorous fish species (e.g., Scombridae, Carangidae). However, observed isotopic variations may be significantly influenced by spatio-temporal fluctuations in isotopic baselines between sites, driven by environmental differences in the foraging grounds (Quillfeldt et al., 2005; Cherel and Hobson, 2007; Bond and Jones, 2009). The isotopic levels of red-billed tropicbirds from both study sites align with those of other seabirds in their respective regions. By comparing $\delta^{15}N$ values in their blood with those of other seabirds known to consume fish and cephalopods, consistent trophic positions were observed (Peña Blanca: ~17.7‰; San Pedro Mártir: 18.6–20.4‰; Aurioles-Gamboa et al., 2013; Castillo-Guerrero et al., unpubl. data). This consistency underscores the ecological connections within the seabird community in each marine region. Additionally, the observed $\delta^{15}N$ values in zooplankton, cephalopods, and flying fish across the study sites further support these ecological connections (Peña Blanca: ~10.7‰, 16.2‰, and 16.5‰; San Pedro Mártir: ~12.3‰, 16.3‰, and 16.1‰, respectively; Aurioles-Gamboa et al., 2013; López-Ibarra et al., 2018; Castillo-Guerrero et al., unpubl. data). Considering trophic

Table 4
Summary of the SIBER computational analysis evaluating the two-dimensional isotopic niche breadth based on Bayesian ellipses for red-billed tropicbirds from different breeding stages at San Pedro Mártir Island and Peña Blanca Islet during the 2021 breeding season. The Layman's metric of convex hull area (TA) and the area of the corrected standard ellipse (SEA_C). Sample sizes are as follows, courtship (San Pedro Mártir = 16, Peña Blanca = 11), incubation (San Pedro Mártir = 16, Peña Blanca = 15), ECR (San Pedro Mártir = 12, Peña Blanca = 18) and LCR (San Pedro Mártir = 18, Peña Blanca = 9).

Breeding stage	TA		SEA_C	
	San Pedro Mártir	Peña Blanca	San Pedro Mártir	Peña Blanca
Courtship	1.19	0.34	0.42	0.18
Incubation	1.36	0.71	0.55	0.31
Early chick-rearing	0.55	0.70	0.29	0.26
Late chick-rearing	0.88	0.24	0.32	0.15

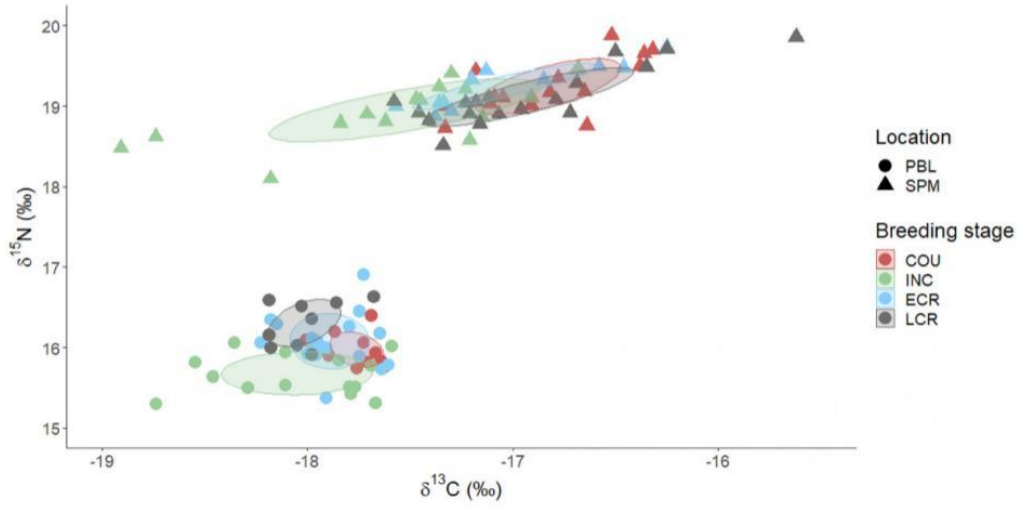


Fig. 5. Bayesian standard ellipse areas (SEA_B) estimated from stable isotope ratios ($\delta^{15}N$ and $\delta^{13}C$) of whole blood in red-billed tropicbirds during courtship [COU], incubation [INC], early chick-rearing [ECR] and late chick-rearing [LCR] at San Pedro Mártir [circle] and Peña Blanca [triangle] during the 2021 breeding season. Sample sizes are as follows, courtship (San Pedro Mártir = 16, Peña Blanca = 11), incubation (San Pedro Mártir = 16, Peña Blanca = 15), ECR (San Pedro Mártir = 12, Peña Blanca = 18) and LCR (San Pedro Mártir = 18, Peña Blanca = 9).

enrichment factors (2–3.5‰ blood tissue; Bond and Jones, 2009), the δ^{15}N values observed in red-billed tropicbirds are within the expected range for piscivorous seabirds in both study locations.

4.3. Temporal variation in prey composition

Our findings highlight the trophic plasticity of red-billed tropicbirds across the breeding season. Resource partitioning and adaptive foraging behaviour in response to changing requirements during different breeding stages has been documented in several seabird species (Navarro et al., 2014; Dehnhard et al., 2016; Booth et al., 2018; Gaglio et al., 2018; Soanes et al., 2021). In the case of San Pedro Mártir red-billed tropicbirds, diet has been found to be influenced by breeding stage and collection date, suggesting that variations may be attributed, at least in part, to differential prey selection between stages (see below), and changes in prey availability related to local oceanographic conditions, such as SST, primary productivity and seasonal hydrographic circulation (Sánchez-Velasco et al., 2009; Garcés-Rodríguez et al., 2021). For Peña Blanca, variations in diet were only influenced by breeding stage. Simultaneous sampling of all breeding stages at San Pedro Mártir allowed us to identify changes in diet associated with both stage and date. However, at Peña Blanca, sampling at different breeding stages followed a more chronological pattern, which limited our ability to discern the role of temporal food availability in dietary variation related to breeding stage. In addition, important inter-annual climatic variations are observed in the region, mainly linked to the ENSO cycle. Our findings highlight the relevance of local conditions on food availability, highlighting the importance of conducting research under variable conditions to understand the constraints and trophic dynamics of each colony.

In the Gulf of California, small pelagic fish availability is impacted by thermal fronts and mesoscale eddies, influencing the abundance of key prey species like the Californian anchovy, Pacific chub mackerel and South American pilchard (Garcés-Rodríguez et al., 2021). The absence of tropical fish, as flyingfish, during low SST in winter-spring contrasts with their increased presence during spring-summer, affecting prey availability for red-billed tropicbirds (Froese and Pauly, 2024). During courtship, individuals make various adjustments, including changes in behaviour, nest selection, defence, mating and egg production, aiming for higher energetic and physiological compensation compared to non-breeders. Previous studies suggest that a higher food quality and lipid metabolic profile during the pre-laying period are linked to increased breeding success and earlier reproduction in the season (González-Medina et al., 2018). In the case of red-billed tropicbirds, timely breeding is crucial, as both colonies are synchronised with peaks in food availability and breeding of other seabirds (Tershy and Breese, 1997; Hernández-Vázquez et al., 2017). A delay in the onset of breeding could increase competition for limited resources and reduce breeding success. As courting individuals exhibited higher δ^{15}N values compared to incubating individuals, indicating distinct foraging patterns. Unlike individuals in advanced breeding stages (e.g., incubation or chick-rearing), courting birds are not bound by temporal constraints or the physiological demand to provision for a chick. Their foraging trips, although the range is largely unknown, is comparable to adults during the late chick-rearing stage, where adults can spend more time foraging for oceanic prey offshore that is contemporary available. In Peña Blanca, Carangidae, particularly bigeye scads with high lipid content (3.31 ± 0.25%; Chedoloh et al., 2011), dominated the diet during courtship (FO ~40%). Conversely, courting individuals in San Pedro Mártir showed a diet reflecting the occurrence of profitable prey, including the Californian anchovy and *Scomber* sp.

Red-billed tropicbirds exhibit biparental care, with parental shifts during incubation lasting up to 12 days, significantly more to the <1–3 days recorded for the early chick-rearing stage (Fig. S5; Piña-Ortiz et al., 2024). Prolonged shifts during incubation require parents to endure prolonged fasting periods, emphasising the importance of diet adjustments to maintain their metabolic needs. This probably involves the choice of larger prey or prey with higher lipid content, providing sustained energy release (Hilton et al., 2000; Jacobs et al., 2011). At Peña Blanca, the predilection of *Scomber* sp. (likely *japonicus*) for incubating individuals aligns with this strategy, given their higher muscle lipid content in larger individuals (size >20 cm: 23.2 ± 1.2%, vs. <20 cm: 8.5 ± 0.5%; Shulgina et al., 2019). A similar pattern was observed at San Pedro Mártir, where topsmelt silversides and Californian anchovies contributed significantly to the diet.

The dietary challenges faced by red-billed tropicbirds during chick-rearing are notable. For instance, adults exhibit a bimodal foraging strategy during this stage to meet the needs for chicks and themselves. Parents adjust their foraging behaviour, opting for shorter trips to coastal areas to enhance chick feeding frequency. Short foraging trips limit the effective time available for foraging, ensuring that adults can return faster to the nest and feed the chick more frequently (Piña-Ortiz et al., 2024). Although coastal areas offer cost-effectiveness, competition (particularly intraspecific, see above) there is markedly higher due to the predictability of food resources (see Weimerskirch, 2007), which poses a trade-off for red-billed tropicbirds, as foraging entirely near coastal areas could inflict finite resource depletion and increase competition (Weber et al., 2021). During chick-rearing, red-billed tropicbirds seem to adjust their prey choice, feeding selectively on higher caloric prey to meet the nutritional needs of the chick's growth. In fact, the stable isotope values varied between adults and chicks for both rearing stages in Peña Blanca, indicating that the diet selection for the adults is likely more selective, choosing prey that is abundant around pelagic waters, whereas prey provided to chicks are obtained closer to the coast and with higher δ^{15}N enrichment, as they require to fulfil their energetic demands (Piña-Ortiz et al., 2024). Previous research in seabirds linked higher-quality food, characterized by elevated caloric, protein, and lipid levels, with better weight gain, body condition, and higher breeding success (Albano et al., 2011; van Donk et al., 2017; González-Medina et al., 2017). Our study was limited to the analysis of adult faecal samples, but further studies should consider incorporating faecal samples of chicks, to confirm dietary divergence between age groups. Due to the differences in isotopic values between adults and chicks, we can assume that our data set reflects the diet of the adults rather than the prey selected for the chicks. For instance, early chick-rearing adults in Peña Blanca primarily consumed flying fish, representing a consistent and abundant prey resource in oligotrophic waters. In San Pedro Mártir, cold SST during winter-spring may limit flying fish availability, leading adults to shift preference towards other abundant epipelagic fish like topsmelt silversides and Californian anchovies.

After about the fifth week of age, chicks experience longer periods of parental absence in the nest, corresponding to a reduction in the food supply. This prompts adults to modify their foraging behaviour, spending less time to the nest but compensating with more time foraging (Stonehouse, 1962; Piña-Ortiz et al., 2024). Likely, adults select prey with elevated lipid content or greater body mass to try the enhanced fasting endurance of older chicks, potentially favouring larger or slower-digesting prey with a higher lipid profile to provide offspring. During this stage, San Pedro Mártir adults consumed *Scomber* sp., *Sardinops* sp., and *E. mordax*, representing prey with high internal fat content (e.g., *S. sagax* 14.5% lipid content; Clark et al., 2010) or abundant during that period. In Peña Blanca, late chick-rearing adults mainly consumed Carangidae and Engraulidae, likely the most profitable prey in the area considering spawning events. *Caranx* sp. (likely *C. caballus*) could be encountered more often once the adults start foraging offshore on more pelagic sites as indicated by the lower δ^{13}C levels, despite that the species in question could represent in general a profitable prey due to the moderate lipid levels (3.74 ± 0.41 g/100 g body mass, Murillo et al., 2014).

In addition, fisheries in the Midriff Islands region, where San Pedro Mártir is located, have a significant impact on the small pelagic stocks,

which could affect the availability of prey for red-billed tropicbirds (Cisneros et al., 1990; Cisneros-Mata et al., 1995; Morales-Bojórquez et al., 2021). A decline in South American pilchards has shown a direct correlation between catch-per-unit rate and the proportion in the diet of pelagic seabirds in the Midriff region, suggesting targeted prey capture by industrial fisheries could induce temporal shifts and depletion in prey availability, prompting birds to adjust their diet or explore less affected foraging grounds (Velarde et al., 2013). For red-billed tropicbirds, most of the main prey items identified in this study coincide with the target species of small pelagic fishing fleets in the Gulf of California (Martínez-Zavala et al., 2010), which does not rule out the possibility that dietary variation could be influenced by the regional fisheries. However, future research must be conducted in the long term to elucidate the precise impact of fisheries on the diet and foraging patterns of the species.

5. Conclusion

Our study provides a comprehensive account on the diet of the Red-billed Tropicbird (*P. aethereus)* for two ecologically contrasting study sites (upwelling vs. oligotrophic) along the Mexican Pacific coast using DNA metabarcoding and stable isotope values. Our data highlights that breeding adults exhibit a divergent dietary profile between sites, regardless of epipelagic fish being the predominant prey for both locations. Spatial divergences in the diet were linked predominantly to prey availability and abundance determined by environmental parameters. Both colonies showed further trophic plasticity between the breeding stages, which seems to be related to changing physiological requirements (e.g., metabolic changes during different breeding stages), and environmental (SST and chlorophyll-a fluctuation) and biotic patterns (prey availability and competition). Due to the prolonged breeding cycle, red-billed tropicbirds adjust their diet continuously in response to the nutritional requirements associated with the respective breeding stage and the environmental changes taking place, efficiently utilizing profitable prey that is available through the breeding season. Although our study only covered one season of the breeding ecology of this species, our data provide an insight into the dietary plasticity of this species. Further research incorporating more breeding colonies, additional samples and multiple study years would be highly desirable to facilitate our understanding of the foraging ecology of red-billed tropicbirds.

Funding

This work was supported by the Sonoran Joint Venture [MBJV/F20AP11531-00]; Deutsche Ornithologische Gesellschaft [DO-G, Forschungsföderung to VM]; Universidad de Guadalajara; Justus Liebig University [Erasmus+ Traineeship Programme to APO]; CONAHCYT [scholarship for doctoral studies abroad No. 795355 to APO]; and the Pacific Seabird Group [student research grant - PSG SRGA 2021 to APO]. We further acknowledge CPER (Contrat de Projet Etat-Région) and the FEDER (Fonds Européen de Développement Régional) for funding the IRMS of LIENSs laboratory.

CRediT authorship contribution statement

Vladislav Marcuk: Writing – review & editing, Writing – original draft, Visualization, Validation, Project administration, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Alberto Piña-Ortiz:** Writing – review & editing, Writing – original draft, Visualization, Validation, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **José Alfredo Castillo-Guerrero:** Writing – review & editing, Visualization, Validation, Resources, Methodology, Investigation, Funding acquisition, Formal analysis. **Juan F. Masello:** Writing – review & editing, Validation, Supervision, Software, Resources, Methodology, Investigation, Formal analysis. **Paco Bustamante:** Writing – review & editing, Validation, Resources, Methodology. **Sven Griep:** Writing – review & editing, Validation, Software. **Petra Quillfeldt:** Writing – review & editing, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

We thank Departamento de Estudios para el Desarrollo Sustentable de Zonas Costeras CUCSur – Universidad de Guadalajara and Comisión Nacional de Areas 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 A. Alvízar-Martínez and Captain E. Ramírez-León for helping us access the islands and the fieldwork assistants for their hard work. Furthermore, we thank A. Espino-Herrera, S. Gómez-Hernández, L. Aguilar-Nuño, and L. Estrada-Osuna for their help with sample processing in the laboratory; S. Wagner, M. Austad, M. Brault-Favrou, and C. Churlaud for training and sample preparation; and G. Guillou from the 'Plateforme analyses isotopiques' of LIENSs for running stable isotope analyses. PB is an honorary member of the IUF (Institut Universitaire de France).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marenvres.2024.106627.

References

Ainley, D.G., Spear, L.B., Allen, S.G., Ribic, C.A., 1996. Temporal and spatial patterns in the diet of the common murre in California waters. Condor 98, 691–705. https://doi.org/10.2307/1369852.

Albano, N., Masero, J.A., Sánchez-Guzmán, J.M., Villegas, A., Santiago-Quesada, F., 2011. Effects of diet on growth-related patterns of energy and macronutrient assimilation efficiency in a semi-precocial bird, the gull-billed tern *Gelochelidon nilotica*. Ardea 99, 93–101. https://doi.org/10.5253/078.099.0111.

Alemany, I., Pérez-Cembranos, A., Pérez-Mellado, V., Castro, J.A., Picornell, A., Ramon, C., Jurado-Rivera, J.A., 2023. DNA metabarcoding the diet of *Podarcis* lizards endemic to the Balearic Islands. Current Zoology 69, 514–526. https://doi.org/10.1093/cz/zoac073.

Allen, W.J., Waller, L.P., Barratt, B.I.P., Dickie, I.A., 2023. Puke or poop? Comparison of regurgitate and faecal samples to infer alpine grasshopper (*Paprides nitidus* Hutton) diet in experimental plant communities. Ecol. Evol. 13, e10444 https://doi.org/10.1002/ece3.10444.

Almaguer-Hernández, A.M., 2016. Ecología reproductiva y trófica del rabijunco pico rojo (*Phaethon aethereus*) (Linnaeus 1758) en la isla Peña Blanca, Colima, México [Reproductive and trophic ecology of the red-billed tropicbird (*Phaethon aethereus*) (Linnaeus 1758) on Peña Blanca Island, Colima, Mexico]. *Bachelor thesis.* Universidad de Guadalajara.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., 1990. Basic local alignment search tool. J. Mol. Biol. 215, 403–410. https://doi.org/10.1016/S0022-283.

Ando, H., Mukai, H., Komura, T., Dewi, T., Ando, M., Isagi, Y., 2020. Methodological trends and perspectives of animal dietary studies by noninvasive fecal DNA metabarcoding. Environmental DNA 2, 391–406. https://doi.org/10.1002/edn3.117.

Andruszkiewicz, E.A., Starks, H.A., Chavez, F.P., Sassoubre, L.M., Block, B.A., Boehm, A. B., 2017. Biomonitoring of marine vertebrates in Monterey Bay using eDNA metabarcoding. PLoS One 12, e0176343. https://doi.org/10.1371/journal.pone.0176343.

Araújo, M.S., Bolnick, D.I., Layman, C.A., 2012. The ecological causes of individual specialisation. Ecol. Lett. 14, 948–958. https://doi.org/10.1111/j.1461-0248.2011.01662.x.

Ashmole, N.P., 1963. The regulation of numbers of tropical oceanic birds. Ibis 103b, 458–473. https://doi.org/10.1111/j.1474-919X.1963.tb06766.x.
Astarloa, A., Glennie, R., Chust, G., García-Baron, I., Boyra, G., Martínez, U., Rubio, A., Louzao, M., 2021. Niche segregation mechanisms in marine apex predators inhabiting dynamic environments. Divers. Distrib. 27, 799–815. https://doi.org/10.1111/ddi.13229.
Aurioles-Gamboa, D., Rodríguez-Pérez, M.Y., Sánchez-Velasco, L., Lavín, M.F., 2013. Habitat, trophic level, and residence of marine mammals in the Gulf of California assessed by stable isotope analysis. Mar. Ecol. Prog. Ser. 488, 275–290. https://doi.org/10.3354/meps10369.
Barrett, R.T., Camphuysen, K.C.J., Anker-Nilssen, T., Chardine, J.W., Furness, R.W., Garthe, S., Hüppop, O., Leopold, M.F., Montevecchi, W.A., Veit, R.R., 2007. Diet studies of seabirds: a review and recommendations. ICES (Int. Counc. Explor. Sea) J. Mar. Sci. 64, 1675–1691. https://doi.org/10.1093/icesjms/fsm152.
Bearhop, S., Waldron, S., Votier, S.C., Furness, R.W., 2002. Factors that influence assimilation rates and fractionation of nitrogen and carbon stable isotopes in avian blood and feathers. Physiol. Biochem. Zool. 75, 451–458. https://doi.org/10.1086/342800.
Berry, T.E., Osterrieder, S.K., Murray, D.C., Coghlan, M.L., Richardson, A.J., Grealy, A.K., Stat, M., Bejder, L., Bunce, M., 2017. DNA metabarcoding for diet analysis and biodiversity: a case study using the endangered Australian sea lion (*Neophoca cinerea*). Ecol. Evol. 7, 5435–5453. https://doi.org/10.1002/ece3.3123.
BirdLife International, 2024. Species factsheet: *Phaethon aethereus*. Downloaded from. https://datazone.birdlife.org/species/factsheet/red-billed-tropicbird-phaethon-aethereus. (Accessed 1 February 2024).
Bolger, A.M., Lohse, M., Usadel, B., 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30, 2114–2120. https://doi.org/10.1093/bioinformatics/btu170.
Bond, A.L., Jones, I.L., 2009. A practical introduction to stable-isotope analysis for seabird biologists: approaches, cautions and caveats. Mar. Ornithol. 37, 183–188.
Booth, J.M., Steinfurth, A., Fusi, M., Cuthbert, R.J., McQuaid, C.D., 2018. Foraging plasticity of breeding Northern Rockhopper Penguins, *Eudyptes moseleyi*, in response to changing energy requirements. Polar Biol. 41, 1815–1826. https://doi.org/10.1007/s00300-018-2321-6.
Carlig, E., Blasi, D.D., Ghigliotti, L., Pisano, E., Koubbi, P., Vacchi, M., 2019. Diversified feeding strategies of *Pleuragramma antarctica* (notothenidae) in the southern ocean. Polar Biol. 42, 2045–2054. https://doi.org/10.1007/s00300-019-02579-0.
Carreiro, A.R., Paiva, V.H., Medeiros, R., Franklin, K.A., Oliveira, N., Fagundes, A.I., Ramos, J.A., 2020. Metabarcoding, stables isotopes, and tracking: unraveling the trophic ecology of a winter-breeding storm petrel (*Hydrobates castro*) with a multimethod approach. Mar. Biol. 167, 14. https://doi.org/10.1007/s00227-019-3626-x.
Castillo-Guerrero, J.A., Guevara-Medina, M.A., Mellink, E., 2011. Breeding ecology of the Red-billed Tropicbird *Phaethon aethereus* under contrasting environmental conditions in the Gulf of California. Ardea 99, 61–71. https://doi.org/10.5253/078.099.0108.
Castillo-Guerrero, J.A., Lerma, M., Mellink, E., Suazo-Guillén, E., Peñaloza-Padilla, E.A., 2016. Environmentally-mediated flexible foraging strategies in brown boobies in the Gulf of California. Ardea 104, 33–47. https://doi.org/10.5253/arde.v104i1.a3.
Castillo-Guerrero, J.A., Piña-Ortiz, A., Ventura-Trejo, J., Zatarain-González, D.J., 2022. Population status and priority marine areas for seabirds on san Pedro Mártir island. Sonoran Joint Venture – Recent News. https://sonoranjv.org/seabirds-san-pedro-martir-island/. (Accessed 29 January 2024).
Ceia, F.R., Phillips, R.A., Ramos, J.A., Cherel, Y., Vieira, R.P., Richard, P., Xavier, J.C., 2012. Short- and long-term consistency in the foraging niche of wandering albatrosses. Mar. Biol. 159, 1581–1591. https://doi.org/10.1007/s00227-012-1946-1.
Ceia, F.R., Xavier, J.C., Carreiro, A.R., Dos Santos, I., Cherel, Y., 2022. Conventional and modern approaches to study seabird trophic ecology and diet. In: Ramos, J.A., Pereira, L. (Eds.), Seabird Biodiversity and Human Activities, 1. CRC Press, pp. 19–35.
Chiaradia, A., Costalunga, A., Kerry, K., 2003. The diet of little penguins (*Eudyptula minor*) at phillip island, victoria, in the absence of a major prey—pilchard (*Sardinops sagax*). Emu 103, 43–48. https://doi.org/10.1071/MU02020.
Chedoloh, R., Karrila, T.T., Pakdeechanuan, P., 2011. Fatty acid composition of important aquatic animals in Southern Thailand. Int. Food Res. J. 18, 783–790.
Cherel, Y., Hobson, K.A., Weimerskirch, H., 2005. Using stable isotopes to study resource acquisition and allocation in procellariiform seabirds. Oecologia 145, 533–540. https://doi.org/10.1007/s00442-005-0156-7.
Cherel, Y., Hobson, K.A., 2007. Geographical variation in carbon stable isotope signatures of marine predators: a tool to investigate their foraging areas in the Southern Ocean. Mar. Ecol. Prog. Ser. 329, 281–287. https://doi.org/10.3354/meps329281.
Cisneros-Mata, M.A., Nevárez-Martínez, M.O., Hammann, M.G., 1995. The rise and fall of the Pacific sardine *Sardinops sagax caeruleus* Girard, in the Gulf of California Mexico. CalCOFI Report 36, 136–143.
Cisneros, M.A., Estrada, J., Montemayor, G., 1990. Growth, mortality and recruitment of exploited small pelagic fishes in the Gulf of California, Mexico. Fishbyte 8, 15–17. https://hdl.handle.net/20.500.12348/3174.
Clark, T.D., Brandt, W.T., Nogueira, J., Rodriguez, L.E., Price, M., Farwell, C.J., Block, B. A., 2010. Postprandial metabolism of Pacific bluefin tuna (*Thunnus orientalis*). J. Exp. Biol. 213, 2379–2385. https://doi.org/10.1242/jeb.043455.
Correa, S.B., Winemiller, K.O., 2014. Niche partitioning among frugivorous fishes in response to fluctuating resources in the Amazonian floodplain forest. Ecology 95, 210–224. https://doi.org/10.1890/13-0393.1.
Crisol-Martínez, E., Moreno-Moyano, L.T., Wormington, K.R., Brown, P.H., Stanley, D., 2016. Using next-generation sequencing to contrast the diet and explore pest-reduction services of sympatric bird species in macadamia orchards in Australia. PLoS One 11, e0150159. https://doi.org/10.1371/journal.pone.0150159.
Deagle, B.E., Gales, N.J., Evans, K., Jarman, S.N., Robinson, S., Trebilco, R., Hindell, M. A., 2007. Studying seabird diet through genetic analysis of faeces: a case study on macaroni penguins (*Eudyptes chrysolophus*). PLoS One 2, e831. https://doi.org/10.1371/journal.pone.0000831.
Deagle, B.E., Kirkwood, R., Jarman, S.N., 2009. Analysis of Australian fur seal diet by pyrosequencing prey DNA in faeces. Mol. Ecol. 18, 2022–2038. https://doi.org/10.1111/j.1365-294X.2009.04172.x.
Dehnhard, N., Ludynia, K., Masello, J.F., Voigt, C.C., McGill, R.A.R., Quillfeldt, P., 2016. Plasticity in foraging behaviour and diet buffers effects of inter-annual environmental differences on chick growth and survival in southern rockhopper penguins *Eudyptes chrysocome chrysocome*. Polar Biol. 39, 1627–1641. https://doi.org/10.1007/s00300-015-1887-5.
Deiner, K., Bik, H.M., Mächler, E., Seymour, M., Lacoursière-Roussel, A., Altermatt, F., Creer, S., Bista, I., Lodge, D.M., de Vere, N., Pfrender, M.E., Bernatchez, L., 2017. Environmental DNA metabarcoding: transforming how we survey animal and plant communities. Mol. Ecol. 26, 5872–5895. https://doi.org/10.1111/mec.14350.
de Jonge, D.S.W., Merten, V., Bayer, T., Puebla, O., Reusch, T.B.H., Hoving, H.-J.T., 2021. A novel metabarcoding primer pair for environmental DNA analysis of Cephalopoda (Mollusca) targeting the nuclear 18S rRNA region. R. Soc. Open Sci. 8, 201388 https://doi.org/10.1098/rsos.201388.
Diop, N., Zango, L., Beard, A., Ba, C.T., Ndiaye, P.I., Henry, L., Clingham, E., Oppel, S., González-Solís, J., 2018. Foraging ecology of tropicbirds breeding in two contrasting marine environments in the tropical Atlantic. Mar. Ecol. Prog. Ser. 607, 221–236. https://doi.org/10.3354/meps12774.
Djurhuus, A., Pitz, K., Sawaya, N.A., Rojas-Márquez, J., Michaud, B., Montes, E., Muller-Karger, F., Breitbart, M., 2018. Evaluation of marine zooplankton community structure through environmental DNA metabarcoding. Limnol Oceanogr. Methods 16, 209–221. https://doi.org/10.1002/lom3.10237.
Djurhuus, A., Closek, C.J., Kelly, R.P., Pitz, K.J., Michisaki, R.P., Starks, H.A., Walz, K.R., Andruszkiewicz, E.A., Olesin, E., Hubbard, K., Montes, E., Otis, D., Muller-Karger, F. E., Chavez, F.P., Boehm, A.B., Breitbart, M., 2020. Environmental DNA reveals seasonal shifts and potential interactions in a marine community. Nat. Commun. 11, 254. https://doi.org/10.1038/s41467-019-14105-1.
Doyle, E., Adams, N., 2018. DNA extraction and amplification of regurgitate and faecal samples from Buller's Shearwater (*Puffinis bulleri*). *Report prepared for Northern New Zealand Seabird Trust*. 8p. Auckland. https://hdl.handle.net/10652/4609.
Dunphy, B.J., Vickers, S.I., Zhang, J., Sagar, R.L., Landers, T.J., Bury, S.J., Hickey, A.J.R., Rayner, M.J., 2020. Seabirds as environmental indicators: foraging behaviour and ecophysiology of common diving petrels (*Pelecanoides urinatrix*) reflect local-scale differences in prey availability. Mar. Biol. 167, 53. https://doi.org/10.1007/s00227-020-3672-4.
Escalante, F., Valdez-Holguín, J.E., Álvarez-Borrego, S., Lara-Lara, J.R., 2013. Temporal and spatial variation of sea surface temperature, chlorophyll a, and primary productivity in the Gulf of California. Cienc. Mar. 39, 203–215. https://doi.org/10.7773/cm.v39i2.2233.
Fauchald, P., Skov, H., Skern-Mauritzen, M., Johns, D., Tveraa, T., 2011. Wasp-waist interactions in the north sea ecosystem. PLoS One 6, e22729. https://doi.org/10.1371/journal.pone.0022729.
Froese, R., Pauly, D. (Eds.), 2024. FishBase: World Wide Web Electronic Publication. www.fishbase.org. (Accessed 12 February 2024).
Gaglio, D., Cook, T.R., McInnes, A., Sherley, R.B., Ryan, P.G., 2018. Foraging plasticity in seabirds: a non-invasive study of the diet of greater crested terns breeding in the Benguela region. PLoS One 13, e0190444. https://doi.org/10.1371/journal.pone.0190444.
Gagné, T.O., Hyrenbach, K.D., Hagemann, M.E., Bass, O.L., Pimm, S.L., MacDonald, M., Peck, B., Van Houtan, K.S., 2018a. Seabird trophic position across three ocean regions tracks ecosystem differences. Front. Mar. Sci. 5, 317. https://doi.org/10.3389/fmars.2018.00317.
Gagné, T.O., Hyrenbach, K.D., Hagemann, Van Houtan, K.S., 2018b. Trophic signatures of seabirds suggest shifts in oceanic ecosystems. Sci. Adv. 4, eaao3946 https://doi.org/10.1126/sciadv.aao39.
Garcés-Rodríguez, Y., Sánchez-Velasco, L., Parés-Sierra, A., Jiménez-Rosenberg, S.P.A., Márquez-Artavia, A., Flores-Morales, A.L., 2021. FISH larvae distribution and transport on the thermal fronts in the Midriff Archipelago region, Gulf of California. Continent. Shelf Res. 218, 104384 https://doi.org/10.1016/j.csr.2021.104384.
Gaston, A.J., Ydenberg, R.C., Smith, G.E.J., 2007. Ashmole's halo and population regulation in seabirds. Mar. Ornithol. 35, 119–126.
Gatt, M.C., Reis, B., Granadeiro, J.P., Pereira, E., Catry, P., 2020. Generalist seabirds as biomonitors of ocean mercury: the importance of accurate trophic position assignment. Sci. Total Environ. 740, 140159 https://doi.org/10.1016/j.scitotenv.2020.140159.
González-Medina, E., Castillo-Guerrero, J.A., Herzka, S.Z., Fernández, G., 2017. Flexibility in food resource allocation in parents and selectivity for offspring: variations in $\delta^{15}N$ and $\delta^{13}C$ values during breeding of the blue-footed booby. Mar. Biol. 164, 38. https://doi.org/10.1007/s00227-017-3070-8.
González-Medina, E., Castillo-Guerrero, J.A., Herzka, S.Z., Fernández, G., 2018. High quality diet improves lipid metabolic profile and breeding performance in the blue-footed booby, a long-lived seabird. PLoS One 13, e0193136. https://doi.org/10.1371/journal.pone.0193136.
Grémillet, D., Boulinier, T., 2009. Spatial ecology and conservation of seabirds facing global climate change: a review. Mar. Ecol. Prog. Ser. 391, 121–137. https://doi.org/10.3354/meps08212.

Grémillet, D., Dell'Omo, G., Ryan, P.G., Peters, G., Ropert-Coudert, Y., Weeks, S.J., 2004. Offshore diplomacy, or how seabirds mitigate intra-specific competition: a case study based on GPS tracking of Cape gannets from neighbouring colonies. Mar. Ecol. Prog. Ser. 268, 265–279. https://doi.org/10.3354/meps268265.

Guillerault, N., Bouletreau, S., Iribar, A., Valentini, A., Santoul, F., 2017. Application of DNA metabarcoding on faeces to identify European catfish Silurus glanis diet. J. Fish. Biol. 90, 2214–2219. https://doi.org/10.1111/jfb.13294.

Hardy, N., Berry, T., Kelaher, B.P., Goldsworthy, S.D., Bunce, M., Coleman, M.A., Gillanders, B.M., Connell, S.D., Blewitt, M., Figueira, W., 2017. Assessing the trophic ecology of top predators across a recolonisation frontier using DNA metabarcoding of diets. Mar. Ecol. Prog. Ser. 573, 237–254. https://doi.org/10.3354/meps12165.

Hedd, A., Gales, R., Brothers, N., 2001. Foraging strategies of shy albatross *Thalassarche cauta* breeding at Albatross Island, Tasmania, Australia. Mar. Ecol. Prog. Ser. 224, 267–282. https://doi.org/10.3354/meps224267.

Hernández-Vázquez, S., Castillo-Guerrero, J.A., Mellink, E., Almaguer-Hernández, A.M., 2018. Colony size and breeding success of red-billed tropicbird (*Phaethon aethereus*) on Peña Blanca island, Colima, méxico. Waterbirds 41, 128–134. https://doi.org/10.1675/063.041.0203.

Hernández-Vázquez, S., Mellink, E., Castillo-Guerrero, J.A., Rodríguez-Estrella, R., Hinojosa-Larios, J.A., Galván-Piña, V.H., 2017. Ecología reproductiva del bobo café (*Sula leucogaster*) en tres islas del Pacífico tropical Mexicano. Ornitol. Neotrop. 28, 57–66. https://doi.org/10.58843/ornneo.v28i0.146.

Hilton, G.M., Furness, R.W., Houston, D.C., 2000. A comparative study of digestion in North Atlantic seabirds. J. Avian Biol. 31, 36–46. https://doi.org/10.1034/j.1600-048X.2000.310106.x.

Holt, R.D., 2009. Bringing the Hutchinsonian niche into the 21st century: ecological and evolutionary perspectives. Proc. Natl. Acad. Sci. USA 106, 19659–19665. https://doi.org/10.1073/pnas.0905137106.

Hsieh, T.C., Ma, K.H., Chao, A., 2016. iNEXT: an R package for rarefaction and extrapolation of species diversity (Hill numbers). Methods Ecol. Evol. 7, 1451–1456. https://doi.org/10.1111/2041-210X.12613.

Inger, R., Bearhop, S., 2008. Applications of stable isotope analyses to avian ecology. Ibis 150, 447–461. https://doi.org/10.1111/j.1474-919X.2008.00839.x.

Jacobs, S.R., Edwards, D.B., Ringrose, J., Elliott, K.H., Weber, J.-M., Gaston, A.J., 2011. Changes in body composition during breeding: reproductive strategies of three species of seabirds under poor environmental conditions. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 158, 77–82. https://doi.org/10.1016/j.cbpb.2010.09.011.

Jacoby, J., Mancini, P.L., Bertrand, S.L., Efe, M.A., Bugoni, L., Nunes, G.T., 2023. Biogeographic variation on dietary aspects of a widely distributed seabird. Mar. Biol. 170, 21. https://doi.org/10.1007/s00227-022-04171-3.

Jackson, A.L., Inger, R., Parnell, A.C., Bearhop, S., 2011. Comparing isotopic niche widths among and within communities: SIBER – stable Isotope Bayesian Ellipses in R. J. Anim. Ecol. 80, 595–602. https://doi.org/10.1111/j.1365-2656.2011.01806.x.

Jafari, V., Maccapan, D., Careddu, G., Caputi, S.S., Calizza, E., Rossi, L., Costantini, M.L., 2021. Spatial and temporal diet variability of Adélie (*Pygoscelis adeliae*) and Emperor (*Aptenodytes forsteri*) Penguin: a multi tissue stable isotope analysis. Polar Biol. 44, 1869–1881. https://doi.org/10.1007/s00300-021-02925-1.

Kleinschmidt, B., Burger, C., Dorsch, M., Nehls, G., Heinänen, S., Morkūnas, J., Žydelis, R., Moorhouse-Gann, R.J., Hipperson, H., Symondson, W.O.C., Quillfeldt, P., 2019. The diet of red-throated divers (*Gavia stellata*) overwintering in the German Bight (North Sea) analysed using molecular diagnostics. Mar. Biol. 166, 77. https://doi.org/10.1007/s00227-019-3523-3.

Lee, W.Y., Park, S., Kim, K.W., Kim, J.-H., Gal, J.-K., Chung, H., 2021. Inter-specific and intra-specific competition of two sympatrically breeding seabirds, chinstrap and gentoo penguins, at two neighboring colonies. Animals 11, 482. https://doi.org/10.3390/ani11020482.

Lerma, M., Castillo-Guerrero, J.A., Hernández-Vázquez, S., Garthe, S., 2020. Foraging ecology of a marine top predator in the Eastern Tropical Pacific over 3 years with different ENSO phases. Mar. Biol. 167, 88. https://doi.org/10.1007/s00227-020-03699-6.

Lerma, M., Dehnhard, N., Castillo-Guerrero, J.A., Fernández, G., 2022. Nutritional state variations in a tropical seabird throughout its breeding season. J. Comp. Physiol. B 192, 775–787. https://doi.org/10.1007/s00360-022-01456-3.

Lima-Mendez, G., Faust, K., Henry, N., Decelle, J., Colin, S., Carcillo, F., Chaffron, S., Ignacio-Espinosa, J.C., Roux, S., Vincent, F., Bittner, L., Darzi, Y., Wang, J., Audic, S., Berline, L., Bontempi, G., Cabello, A.M., Coppola, L., Cornejo-Castillo, F.M., D'Ovidio, F., De Meester, L., Ferrera, I., Garet-Delmas, M.-J., Guidi, L., Lara, E., Pesant, S., Royo-Llonch, M., Salazar, G., Sánchez, P., Sebastian, M., Souffreau, C., Dimier, C., Picheral, M., Searson, S., Kandels-Lewis, S., Tara, Oceans Coordinators, Gorsky, G., Not, F., Ogata, H., Speich, S., Stemmann, L., Weissenbach, J., Wincker, P., Acinas, S.G., Sunagawa, S., Bork, P., Sullivan, M.B., Karsenti, E., Bowler, C., de Vargas, C., Raes, J., 2015. Determinants of community structure in the global plankton interactome. Science 348, 1262073. https://doi.org/10.1126/science.1262073, 1262073.

López-Ibarra, G.A., Bode, A., Hernández-Trujillo, S., Zetina-Rejón, M.J., Arreguín-Sánchez, F., 2018. Trophic position of twelve dominant pelagic copepods in the eastern tropical Pacific Ocean. J. Mar. Syst. 187, 13–22. https://doi.org/10.1016/j.jmarsys.2018.06.009.

Lois, N.A., Balza, U., Brasso, R., Dodino, S., Pütz, K., Polito, M.J., Riccialdelli, L., Ciancio, J., Quillfeldt, P., Mahler, B., Rey, A.R., 2022. Mercury and stable isotopes portray colony-specific foraging grounds in southern rockhopper penguins over the Patagonian Shelf. Mar. Pollut. Bull. 184, 114137 https://doi.org/10.1016/j.marpolbul.2022.114137.

Lyday, S.E., Ballance, L.T., Field, D.B., Hyrenbach, K.D., 2015. Shearwaters as ecosystem indicators: towards fishery-independent metrics of fish abundance in the California Current. J. Mar. Syst. 146, 109–120. https://doi.org/10.1016/j.jmarsys.2014.08.010.

Lynam, C.P., Llope, M., Möllmann, C., Helaouët, P., Bayliss-Brown, G.A., Stenseth, N.C., 2017. Interaction between top-down and bottom-up control in marine food webs. Proc. Natl. Acad. Sci. USA 114, 1952–1957. https://doi.org/10.1073/pnas.1621037114.

Madden, H., Satgé, Y., Wilkinson, B., Jodice, P.G., 2022. Foraging ecology of Red-billed tropicbrid *Phaethon aethereus* in the Caribbean during early chick rearing revealed by GPS tracking. Mar. Ornithol. 50, 165–175.

Madden, H., Boehm, H., Mielke, L., 2023. Foraging ecology of red-billed tropicbirds on saba, caribbean Netherlands, during early chick-rearing. Ardea 111, 1–18. https://doi.org/10.5253/arde.2022.a14.

Magoč, T., Salzberg, S.L., 2011. FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics 27, 2957–2963. https://doi.org/10.1093/bioinformatics/btr507.

Martínez-Zavala, M. de los A., Nevárez-Martínez, M.O., Anguiano-Carrazco, M.L., Santos-Molina, J.P., Godínez-Cota, A.R., 2010. Captura de peces pelágicos menores en el golfode California, temporada de pesca 2007-2008. Ciencia Pesquera 18, 5–18.

Masello, J.F., Mundry, R., Poisbleau, M., Demongin, L., Voigt, C.C., Wikelski, M., Quillfeldt, P., 2010. Diving seabirds share foraging space and time within and among species. Ecosphere 1, 1–28. https://doi.org/10.1890/ES10-00103.1.

Masello, J.F., Barbosa, A., Kato, A., Mattern, T., Medeiros, R., Stockdale, J.E., Kümmel, M.N., Bustamante, P., Belliure, J., Benzal, J., Colominas-Ciuró, R., Menéndez-Blázquez, J., Griep, S., Goesmann, A., Symondson, W.O.C., Quillfeldt, P., 2021. How animals distribute themselves in space: energy landscapes of Antarctic avian predators. Movement Ecology 9, 1–25. https://doi.org/10.1186/s40462-021-00255-9.

Masello, J.F., Schumm, Y.R., Griep, S., Quillfeldt, P., 2023. Using next-generation sequencing to disentangle the diet and incidence of intestinal parasites of falkland flightless steamer duck *Tachyeres brachypterus* and patagonian crested duck *Lophonetta specularioides* sharing a South atlantic island. Genes 14, 731. https://doi.org/10.3390/genes14030731.

MacArthur, R.H., Pianka, E.R., 1966. On optimal use of a patchy environment. Am. Nat. 100, 603–609. https://doi.org/10.1086/282454.

McInnes, J.C., Alderman, R., Lea, M.-A., Raymond, B., Deagle, B.E., Phillips, R.A., Stanworth, A., Thompson, D.R., Catry, P., Weimerskirch, H., Suazo, C.G., Gras, M., Jarman, S.N., 2017. High occurrence of jellyfish predation by black-browed and Campbell albatross identified by DNA metabarcoding. Mol. Ecol. 26, 4831–4845. https://doi.org/10.1111/mec.14245.

Mellink, E., Domínguez, J., Luévano, J., 2001. Diet of eastern pacific Brown boobies *Sula leucogaster brewsteri* on Isla san jorge, north-eastern Gulf of California, and an april comparison with diets in the middle Gulf of California. Mar. Ornithol. 29, 23–28.

Morales-Bojórquez, E., Nevárez-Martínez, M.O., García-Alberto, G., Villalobos, H., Aguirre-Villaseñor, H., Larios-Castro, E., González-Peláez, S.S., Arizmendi-Rodríguez, D.I., Martínez-Zavala, M.D., 2021. Interaction between marine fauna and the small pelagic fishery in the coastal environment of the Gulf of California, Mexico. Front. Mar. Sci. 8, 669176 https://doi.org/10.3389/fmars.2021.669176.

Murillo, E., Rao, K.S., Durant, A.A., 2014. The lipid content and fatty acid composition of four eastern central Pacific native fish species. J. Food Compos. Anal. 33, 1–5. https://doi.org/10.1016/j.jfca.2013.08.007.

Navarro, J., Moreno, R., Braun, L., Sanpera, C., Hennicke, J.C., 2014. Resource partitioning between incubating and chick-rearing brown boobies and red-tailed tropicbirds on Christmas Island. Zool. Stud. 53, 27. https://doi.org/10.1186/s40555-014-0027-1.

Nelson, J.B., 2006. Pelicans, Cormorants, and Their Relatives, the Pelecaniformes. Oxford University Press.

Nimz, I., Renshaw, M.A., Baczenas, J., Vanderlip, C., Hyrenbach, K.D., Iacchei, M., 2022. MetaBARFcoding: DNA-barcoding of regurgitated prey yields insights into Christmas shearwater (*Puffinus nativitatis*) foraging ecology at Hōlanikū (Kure Atoll) Hawai'i. Environmental DNA 4, 254–268. https://doi.org/10.1002/edn3.263.

North, M.E.W., 1946. Malt island-A bird-rock in the Gulf of aden. Ibis 88, 478–501. https://doi.org/10.1111/j.1474-919X.1946.tb03501.x.

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Henry, M., Stevens, H., Szoecs, E., Wagner, H., 2018. Package 'vegan' Community Ecology Package.

Paiva, V.H., Geraldes, P., Ramírez, I., Meirinho, A., Garthe, S., Ramos, J.A., 2010. Foraging plasticity in a pelagic seabird species along a marine productivity gradient. Mar. Ecol. Prog. Ser. 398, 259–274. https://doi.org/10.3354/meps08319.

Paredes, R., Harding, A.M.A., Irons, D.B., Roby, D.D., Suryan, R.M., Orben, R.A., Renner, H., Young, R., Kitaysky, A., 2012. Proximity to multiple foraging habitats enhances seabirds' resilience to local food shortages. Mar. Ecol. Prog. Ser. 471, 253–269. https://doi.org/10.3354/meps10034.

Parsons, M., Mitchel, l I., Butler, A., Ratcliffe, N., Frederiksen, M., Foster, S., Reid, J.B., 2008. Seabirds as indicators of the marine environment. ICES (Int. Counc. Explor. Sea) J. Mar. Sci. 65, 1520–1526. https://doi.org/10.1093/icesjms/fsn155.

Peters, K.J., Ophelkeller, K., Bott, N.J., Deagle, B.E., Jarman, S.N., Goldsworthy, S.D., 2015. Fine-scale diet of the Australian sea lion (*Neophoca cinerea*) using DNA-based analysis of faeces. Mar. Ecol. 36, 347–367. https://doi.org/10.1111/maec.12145.

Piña-Ortiz, A., Castillo-Guerrero, J.A., Enríquez-Paredes, L.M., Fernández, G., Hernández-Vázquez, S., Quillfeldt, P., 2023. Body size variation in a tropical seabird along a latitude-productivity gradient. J. Ornithol. 164, 327–339. https://doi.org/10.1007/s10336-022-02037-5.

Piña-Ortiz, A., González-Zamora, D.A., Paz, J.A., Hernández-Vázquez, S., Mellink, E., Bustamante, P., Quillfeldt, P., Castillo-Guerrero, J.A., 2024. Parental duties and foraging strategies of a tropical pelagic seabird (*Phaethon aethereus*, Aves:

phaethontidae) during the breeding season. Mar. Biol. 171, 64. https://doi.org/10.1007/s00227-023-04375-1.
Piña-Ortiz, A., Hernández-Vázquez, S., Fernández, G., Castillo-Guerrero, J.A., 2018. Distribution and population size of the red-billed tropicbird (*Phaethon aethereus*) in Mexico. Waterbirds 41, 135–144. https://doi.org/10.1675/063.041.0204.
Querejeta, M., Lefort, M.-C., Bretagnolle, V., Boyer, S., 2023. Metabarcoding fecal samples to investigate spatiotemporal variation in the diet of the endangered Westland Petrel (*Procellaria westlandica*). Avian Conserv. Ecol. 18, 17. https://doi.org/10.5751/ACE-02410-180117.
Quillfeldt, P., McGill, R.A.R., Furness, R.W., 2005. Diet and foraging areas of Southern Ocean seabirds and their prey inferred from stable isotopes: review and case study of Wilson's storm-petrel. Mar. Ecol. Prog. Ser. 295, 295–304. https://doi.org/10.3354/meps295295.
Quillfeldt, P., Masello, J.F., Navarro, J., Phillips, R.A., 2013. Year-round distribution suggests spatial segregation of two small petrel species in the South Atlantic. J. Biogeogr. 40, 430–441. https://doi.org/10.1111/jbi.12008.
Ratcliffe, N., Adlard, S., Stowasser, G., McGill, R., 2018. Dietary divergence is associated with increased intra-specific competition in a marine predator. Sci. Rep. 8, 6827. https://doi.org/10.1038/s41598-018-25318-7.
Ricklefs, R.E., 1983. Some considerations on the reproductive energy's of pelagic seabirds. Stud. Avian Biol. 8, 84–94.
Robertson, D.R., Allen, G.R., 2024. Shorefishes of the Tropical Eastern Pacific: Online Information System. Version 3.0 Smithsonian Tropical Research Institute, Panamá. https://biogeodb.stri.si.edu/sftep/en/pages. (Accessed 12 February 2024).
Roby, D.D., 1991. Diet and postnatal energetics in convergent taxa of plankton-feeding seabirds. The Auk 108, 131–146. https://doi.org/10.1093/auk/108.1.131.
Rognes, T., Flouri, T., Nichols, B., Quince, C., Mahé, F., 2016. VSEARCH: a versatile open source tool for metagenomics. PeerJ 4, e2584. https://doi:10.7717/peerj.2584.
Rosciano, N.G., Polito, M., Raya Rey, A., 2016. Do penguins share? Evidence of foraging niche segregation between but not within two sympatric, central-place foragers. Mar. Ecol. Prog. Ser. 548, 249–262. https://doi.org/10.3354/meps11689.
Sánchez-Velasco, L., Lavín, M.F., Peguero-Icaza, M., León-Chávez, C.A., Contreras-Catala, F., Marinone, S.G., Gutiérrez-Palacios, I.V., Godínez, V.M., 2009. Seasonal changes in larval fish assemblages in a semi-enclosed sea (Gulf of California). Continent. Shelf Res. 29, 1697–1710. https://doi.org/10.1016/j.csr.2009.06.001.
Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B., Thallinger, G.G., Van Horn, D.J., Weber, C.F., 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl. Environ. Microbiol. 75, 7537–7541. https://doi.org/10.1128/AEM.01541-09.
Schreiber, E.A., Burger, J., 2001. Biology of Marine Birds. CRC Press, Florida.
Scribner, K.T., Bowman, T.D., 1998. Microsatellites identify depredated waterfowl remains from glaucous gull stomachs. Mol. Ecol. 7, 1401–1405. https://doi.org/10.1046/j.1365-294x.1998.00434.x.
Shealer, D.A., 2001. Foraging behavior and food of seabirds. In: Schreiber, E.A., Burger, J. (Eds.), Biology of Marine Birds. CRC Press, Florida, pp. 137–178.
Shulgina, L.V., Davletshina, T.A., Pavlovsky, A.M., Solodova, E.A., Pavel, K.G., 2019. Composition of lipids and fatty acids in muscle tissue of chub mackerel *Scomber japonicus*. Izvestiya TINRO 196, 193–203. https://doi.org/10.26428/1606-9919-2019-196-193-203 (In Russian).
Snider, A.M., Bonisoli-Alquati, A., Pérez-Umphrey, A.A., Stouffer, P.C., Taylor, S.S., 2022. Metabarcoding of stomach contents and fecal samples provide similar insights about Seaside Sparrow diet. Ornithological Applications 124, duab060. https://doi.org/10.1093/ornithapp/duab060.
Soanes, L.M., Green, J.A., Bolton, M., Milligan, G., Mukhida, F., Halsey, L.G., 2021. Linking foraging and breeding strategies in tropical seabirds. J. Avian Biol. 52, 1–12. https://doi.org/10.1111/jav.02670.
Song, P.-Q., Zhang, H.-S., Zheng, X.-Q., Li, Y.-Y., Lin, L.-S., Li, Y., 2021. Trophic plasticity of Bombay duck (*Harpadon nehereus*) in the south-central east China sea based on stable isotope evidence. Front. Mar. Sci. 8, 728773 https://doi.org/10.3389/fmars.2021.728773.
Spalding, M.D., Fox, H.E., Allen, G.R., Davidson, N., Ferdaña, Z.A., Finlayson, M., Halpern, S., Jorge, M.A., Lombana, A., Lourie, S.A., Martin, K.D., McManus, E., Molnar, J., Recchia, C.A., Robertson, J., 2007. Marine ecoregions of the world: a bioregionalization of coastal and shelf areas. Bioscience 57, 573–583. https://doi.org/10.1641/B570707.
Stephens, D.W., Krebs, J.R., 1986. Foraging Theory. Princeton University Press.
Stonehouse, B., 1962. The tropic birds (genus *Phaethon*) of Ascension Island. Ibis 103, 124–161. https://doi.org/10.1111/j.1474-919X.1962.tb07242.x.
Svanbäck, R., Bolnick, D.I., 2005. Intraspecific competition affects the strength of individual specialization: an optimal diet theory model. Evol. Ecol. Res. 7, 993–1012.
Svanbäck, R., Bolnick, D.I., 2007. Intraspecific competition drives increased resource use diversity within a natural population. Proceedings of the Royal Society B 274, 839–844. https://doi.org/10.1098/rspb.2006.0198.
Swift, J.F., Lance, R.F., Guan, X., Britzke, E.R., Lindsay, D.L., Edwards, C.E., 2018. Multifaceted DNA metabarcoding: validation of a noninvasive, next-generation approach to studying bat populations. Evolutionary Applications 11, 1120–1138. https://doi.org/10.1111/eva.12644.
Tershy, B.R., Breese, D., 1997. The birds of San Pedro Mártir Island, Gulf of California, Mexico. Western Birds 28, 96–107.
The Galaxy Community, 2022. The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2022 update. Nucleic Acids Res. 50, W345–W351. https://doi.org/10.1093/nar/gkac247.
Valentini, A., Pompanon, F., Taberlet, P., 2009. DNA barcoding for ecologists. Trends Ecol. Evol. 24, 110–117. https://doi.org/10.1016/j.tree.2008.09.011.
van Donk, S., Camphuysen, K.C.J., Shamoun-Baranes, J., van der Meer, J., 2017. The most common diet results in low reproduction in a generalist seabird. Ecol. Evol. 7, 4620–4629. https://doi.org/10.1002/ece3.3018.
Velarde, E., Ezcurra, E., Anderson, D.W., 2013. Seabird diets provide early warning of sardine fishery declines in the Gulf of California. Sci. Rep. 3, 1332. https://doi.org/10.1038/srep01332.
Velarde, E., Ezcurra, Cisneros-Mata, M.A., Lavín, M.F., 2004. Seabird ecology, el Niño anomalies, and prediction of sardine fisheries in the Gulf of California. Ecol. Appl. 14, 607–615. https://doi.org/10.1890/02-5320.
Vesterinen, E.J., Lilley, T., Laine, V.N., Wahlberg, N., 2013. Next generation sequencing of fecal DNA reveals the dietary diversity of the widespread insectivorous predator daubenton's bat (*Myotis daubentonii*) in southwestern Finland. PLoS One 8, e82168. https://doi.org/10.1371/journal.pone.0082168.
Weber, S.B., Richardson, A.J., Brown, J., Bolton, M., Clark, B.L., Godley, B.J., Leat, E., Opper, S., Shearer, L., Soetaert, K.E.R., Weber, N., Broderick, A.C., 2021. Direct evidence of a prey depletion "halo" surrounding a pelagic predator colony. Proc. Natl. Acad. Sci. USA 118, e2101325118. https://doi.org/10.1073/pnas.2101325118.
Weimerskirch, H., 2007. Are seabirds foraging for unpredictable resources? Deep Sea Res. Part II Top. Stud. Oceanogr. 54, 211–223. https://doi.org/10.1016/j.dsr2.2006.11.013.
Young, M.J., Dutoit, L., Robertson, F., van Heezik, Y., Seddon, P.J., Robertson, B.C., 2020. Species in the faeces: DNA metabarcoding as a method to determine the diet of the endangered yellow-eyed penguin. Wildl. Res. 47, 509–522. https://doi.org/10.1071/WR19246.

Appendix I

SUPPLEMENTARY MATERIAL

CURRICULUM VITAE

ACKNOWLEDGEMENTS

Supplementary Material

The supplementary material was stored on a CD, which is attached to this thesis. The files are organised in folders and are arranged according to the chapters that form this dissertation.

Chapter I | Geographical body size variation in a tropical seabird along a latitude-productivity gradient — Electronic supplementary material

Table S1. Summary of external measurement of red-billed tropicbirds (*Phaethon aethereus*) during the breeding season (October-May) in 2012-2020 at six breeding colonies in the Mexican Pacific. Values are presented as means ± standard deviation and ranges in parenthesis. Upper and lower values for each morphological trait represent males and females, respectively. SNJ = San Jorge; SPM = San Pedro Mártir; FSI = Farallón de San Ignacio; IS = Isabel; PBL = Peña Blanca; MEP = Morros El Potosí; M = Male; F = Female; N/A = Not available

Table S2. Summary statistics of general linear models (GLMs) evaluating the effects of sex, colony size, and environmental index (sea surface temperature, chlorophyll-a, air temperature, and latitude) on the culmen length (mm), ulna length (mm), tarsus length (mm), and body mass (g) in red-billed tropicbirds from six breeding colonies in the Gulf of California and Mexican Tropical Pacific.

Figure S1. Body size traits measurements taken in red-billed tropicbird's individuals. HPB = Head-plus-bill; NTBT = Nostril-to-bill-tip; CL = Culmen length; BD = Bill depth; BW = Bill width.

Figure S2. Agarose gel electrophoresis (2.0% stained with ethidium bromide [0.5 μg/ml]) of genomic DNA fragments amplified from blood samples of red-billed tropicbirds (Phaethon aethereus) with 2550/2718 primers. Esc = 280-bp and 400-bp homemade ladder, H = females, M = males, 5H7 = female blue-footed booby (Sula nebouxii) with sexual size dimorphism used as a control, H42 = male blue-footed booby used as a control, C- = negative control.

Figure S3. Scatterplot matrices of paired Pearson's correlations among culmen, ulna, tarsus, and body mass of red-billed tropicbirds samples on this study.

Figure S4. Scatterplot matrices of paired Pearson's correlations among sea surface temperature (SST), chlorophyll-a (Chl-a), air temperature (AT), and latitude (Lat/dec).

Chapter II | Parental duties and foraging strategies of a tropical pelagic seabird (*Phaethon aethereus*, Aves: Phaethontidae) during the breeding season — Electronic supplementary material

Table S1. Periods of GPS attachment, the number of individuals captured, devices recovered, and the number of complete foraging trips in Red-billed Tropicbird individuals at Peña Blanca Islet during the 2017–2022 breeding seasons.

Table S2. Mean ± standard deviation of the measurements of ulna, culmen and tarsus lengths of Red-billed Tropicbird chicks sampled from 2020 to 2022 at Peña Blanca Islet, Mexico.

Fig. S1 Foraging trips of red-billed tropicbirds during the incubation and early chick-rearing (ECR) stages between 2017–2022 on Peña Blanca Islet, Mexico. The coloured lines indicates the foraging trips carried out during each breeding season, and the yellow star is the study colony.

Fig. S2 Estimates of speeds and turning angles for intensive search and resting behaviours of GPS fixes from red-billed tropicbirds on Peña Blanca islet using the HMM algorithm. a) Box plot of speeds for intensive foraging and resting behaviours; b) turning angle frequency histogram for intensive searches; c) turning angle frequency histogram for intensive searches.

Fig. S3 a) GPS track data with foraging trips made by individual B55*_048V showing local time (UTC-06:00) and distance from the colony (km) for each GPS fix. b) Individual foraging trip as highlighted in a) showing how intensive foraging locations (red dots) almost always occur during resting periods or directly preceding resting locations (yellow dots). Each fix is coloured according to the classification assigned from the HMM algorithm (yellow = resting, red = intensive search, cyan = relocation, and dark blue = extensive search).

Fig. S4 a) GPS track data with foraging trips made by individual B33_B06B showing local time (UTC-06:00) and distance from the colony (km) for each GPS fix. b) Individual foraging trip as highlighted in a) showing how intensive foraging locations (red dots) almost always occur during resting periods or directly preceding resting locations (yellow dots). Each fix is coloured according to the classification assigned from the HMM algorithm (yellow = resting, red = intensive search, cyan = relocation, and dark blue = extensive search).

Fig. S5 a) GPS track data with foraging trips made by individual D03_B66B showing local time (UTC-06:00) and distance from the colony (km) for each GPS fix. b) Individual foraging trip as highlighted in a) showing how intensive foraging locations (red dots) almost always occur during resting periods or directly preceding resting locations (yellow dots). Each GPS fix is coloured according to the classification assigned from the HMM algorithm (yellow = resting, red = intensive search, cyan = relocation, and dark blue = extensive search).

Fig. S6 Kernel Utilization Distributions (KUD) of core (50%, filled polygons) and general (95%, solid lines) areas used by red-billed tropicbirds on short (left panel) and long (right panel) foraging trips during the incubation and early chick-rearing (ECR) stages between 2017–2022 on Peña Blanca Islet, Mexico.

Chapter III | Trophic plasticity of a tropical seabird revealed through DNA metabarcoding and stable isotopes — Electronic supplementary material

Table S1 Diet with the main prey groups of the family Phaethontidae, published studies from 1947–2023. The values shown are percentage composition of food samples (percentage of total food volume). n = Total number of food items.

Table S2 Annealing temperatures and primer sequences for the Adapter PCR reactions.

Table S3 Summary of number of MOTUs with associated number of identified families, genus and species of each study colony and site.

Table S4 Read abundances (% total number of reads; RRA) for main prey categories consumed by Red-billed Tropicbird (*Phaethon aethereus*) at the study sites (San Pedro Mártir and Peña Blanca islands), and during four defined breeding stages (COU – courtship, INC – incubation, ECR – early chick-rearing, LCR – late chick-rearing). Prey depth ranges (m) are shown in the table.

Table S5 Multiple pairwise comparison procedures (Tukey test) of Bayesian standard ellipses areas (SEA_B) to assess niche width differences between breeding stages for each study site, and *p*-values in brackets. PBL = Peña Blanca, SPM = San Pedro Mártir, COU = Courtship, INC = Incubation, ECR = Early chick-rearing, LCR = Late chick-rearing. Significant *p*-values (< 0.05).

Table S6 Overlap (%) of the standard Bayesian ellipse areas (SEA_B) of each breeding stage pairwise for San Pedro Mártir Island and Peña Blanca islet. PBL = Peña Blanca, SPM = San Pedro Mártir, COU = Courtship, INC = Incubation, ECR = Early chick-rearing, LCR = Late chick-rearing.

Fig. S1 Infographic with Adapter PCR amplification results with the associated study sites and breeding stages (ECR – early chick-rearing, LCR – late chick-rearing) along violin plots representing the average number of taxa. and a) San Pedro Mártir Island b) Peña Blanca Islet. Violin plots associated with the islands representing descriptive statistics (mean prey taxa, min. and max. values) of MOTUs recorded for each site, including the raw data distribution for each breeding stage and associated study population. Illustrations by VM.

Fig. S2 iNEXT rarefaction curves showing the interpolated observed and extrapolated numbers of MOTUs (left) and the estimated species coverage (right) of each breeding stage for a) San Pedro Mártir Island and b) Peña Blanca Islet.

Fig. S3 Senker plots illustrate the relative read abundance of ingested fish prey taxa at the four breeding stages on *left* San Pedro Mártir = SPM and *right* Peña Blanca = PBL. Fish families are represented by a colour code and their respective symbols. Indian red nodes representing individuals in courtship stage, green in incubation stage, sky blue early chick-rearing (ECR) stage, and light grey late chick-rearing (LCR) stage.

Fig. S4 Foraging trips parameters of red-billed tropicbirds (*Phaethon aethereus*) at San Pedro Mártir Island (individuals = 16, short trips = 10 and long trips = 28) and Peña Blanca Islet (individuals = 19 short trips = 37 and long trips = 26) during the 2021 breeding season.

Fig. S5 Foraging trips of red-billed tropicbirds during the incubation (red lines), early chick-rearing (ECR; yellow lines), and late chick-rearing (LCR; black lines) stages on San Pedro Mártir Island (upper panels) and Peña Blanca Islet (lower panels) during the 2021 breeding season. The star indicate the location of the study colonies. Individuals sampled are as follows, incubation (San Pedro Mártir = 7 and foraging trips = 10; Peña Blanca = 5 and foraging trips = 5), ECR (San Pedro Mártir = 5 and foraging trips = 14; Peña Blanca = 14; and foraging trips = 58) and LCR (San Pedro Mártir = 4 and foraging trips = 14).

Fig. S6 Convex hull area (TA) density plot based on δ^{15}N and δ^{13}C (‰) values in red-billed tropicbirds from Peña Blanca (PBL) and San Pedro Mártir (SPM) during the 2021 breeding season. Black dots represent their means, and shaded boxes represent the 50, 75 and 95% confidence intervals going from dark gray to light gray, respectively.

Acknowledgements

To the institutions that were involved in my research either academically, logistically or financially: Justus-Liebig-Universität Gießen - AG Verhaltensökologie und Ökophysiologie, Universdad de Guadalajara - Departamento de Estudios para el Desarrollo Sustentable de Zonas Costeras, LIttoral ENvironnement et Sociétés (LIENSs) - La Rochelle Université, Universidad Autónoma de Baja California - Facultad de Ciencias Marinas, Comisión Nacional de Areas Naturales Protegidas (CONANP – Islas del Golfo de California – Reserva de la Biósfera Isla San Pedro Mártir), The Pacific Seabird Group (PSG), the American Ornithological Society (AOS).

To the following projects that financed the research that made up this thesis: CONACYT grant numbers CB2010-155353 and I010/176/2012; Universidad de Guadalajara UDG-PTC-1516; The Sonoran Joint Venture MBJV / F20AP11531-00.

To Consejo Nacional de Humanidades, Ciencia y Tecnología (CONAHCYT-Mexico) for awarding me a scholarship (No. 795355) to be able to carry out my doctoral studies abroad.

I would like to thank Prof. Dr. Petra Quillfeldt for giving me the opportunity to carry out my doctoral studies under her supervision. From the very beginning, her confidence in my project and the academic freedom she gave me to make the necessary adjustments were essential for its successful completion.

I would like to express my gratitude to Dr. José Alfredo Castillo Guerrero for his continuous support at all stages of my PhD. I greatly appreciate his active participation in this research work, as well as the trust he placed in me to collaborate and lead in the field campaigns carried out with him and his team (Equipo Aves - UDG) in Mexico.

To Prof. Dr. Paco Bustamante for giving me the opportunity to carry out my Stable Isotope and Mercury analyses in the "Plateforme analyses isotopiques" and "Analyses Elémentaires" laboratories under his supervision at the CNRS - La Rochelle University.

To Dr. Luis Manuel Enriquez Paredes for his collaboration in this project through the molecular analyses carried out in the Molecular Ecology Laboratory "Dr. Jorge De la Rosa Velez" of the Marine Sciences Faculty at the Universidad Autónoma de Baja California.

To Dr. Juan F. Masello for his participation in this project, specifically in the chapter on feeding ecology, as he was a key player in the bio-informatics analysis.

To Dr. Salvador Hernández Vázquez for his collaboration in the fieldwork on Peña Blanca islet, and for the exchange of ideas during the field trips.

To Vladislav Marcuk for his participation in our seabird projects in Mexico, including his master's degree, which is linked to this doctoral work.

A mi familia por su apoyo y confianza, las cuales nunca me han faltado. Ustedes saben cuánto los amo y que cada una de mis metas cumplidas, es también de ustedes. LO HEMOS LOGRADO!!!

To my friends who were always there to give me words of support. Whether inside or outside of academia, every conversation was useful for me to move forward and achieve my goal. It has been done also because of you.

To all colleagues who helped in any way to bring this research to a conclusion. Whether it was their support in the field, in the lab, or even giving me their time to talk and listen to their views on any doubts or questions I had at the time. It would be unfair of me to list only a few names, when I had the opportunity to receive help from countless others.

To my dear Rabis, I feel that I have not yet been able to give back to you to the extent that I would like to, but I am full of appreciation and determination to continue learning about you and the wildlife of the oceans and continents. I recognise that this work is only a small step in comparison to the immensity of my goal, but I maintain a positive attitude and a firm belief that together with like-minded people we will succeed in protecting you and your habitats.

Last but not least to Mother Nature. Having the opportunity to get to know the biodiversity in each of the places I have visited during my life has allowed me to understand that without Pachamama we are nothing, and yet it seems that every day we try harder to disconnect from her.

Appendix II

LOW GENETIC STRUCTURE AND DIVERSITY OF RED-BILLED TROPICBIRDS IN THE MEXICAN PACIFIC

José Alfredo Castillo-Guerrero, Alberto Piña-Ortiz, Luis Enríquez-Paredes, Albert M. van der Heiden, Salvador Hernández-Vázquez, Nancy C. Saavedra-Sotelo, Guillermo Fernández

Journal of Field Ornithology 91(2):142–155, (2020)
https://doi.org/10.1111/jofo.12334

Contributions: sampling collection and bird handling in the field, lab work, data analyses and partial manuscript writing and editing.

Appendix III

TEMPORAL AND SEX-BASED VARIATION IN ORGANOCHLORINE PESTICIDE LEVELS IN THE BLUE-FOOTED BOOBY IN TWO COASTAL COLONIES OF SINALOA, MEXICO

Alberto Piña-Ortiz, Juan Pablo Ceyca-Contreras, Carlos Eduardo Covantes-Rosales, Miguel Betancourt-Lozano, José Alfredo Castillo-Guerrero

Marine Pollution Bulletin 164:112050, (2021)
https://doi.org/10.1016/j.marpolbul.2021.112050

Contributions: Lead author, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing.

Marine Pollution Bulletin 164 (2021) 112050

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Temporal and sex-based variation in organochlorine pesticide levels in the blue-footed booby in two coastal colonies of Sinaloa, Mexico

Alberto Piña-Ortiz [a,1], Juan Pablo Ceyca-Contreras [a,b], Carlos Eduardo Covantes-Rosales [a], Miguel Betancourt-Lozano [a], José Alfredo Castillo-Guerrero [c,*]

[a] Unidad Mazatlán en Acuicultura y Manejo Ambiental, Centro de Investigación en Alimentación y Desarrollo A.C., Av. Sábalo-Cerritos s/n, Estero del Yugo, Mazatlán, Sinaloa CP82100, Mexico
[b] Laboratorio de Ornitología, Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León, Ciudad Universitaria, San Nicolás de los Garza, Nuevo León CP66450, Mexico
[c] Departamento de Estudios para el Desarrollo Sustentable de la Zona Costera, Centro Universitario de la Costa Sur, Universidad de Guadalajara, Gómez Farías 82, San Patricio-Melaque, Municipio de Cihuatlán, Jalisco CP48980, Mexico

ARTICLE INFO

Keywords:
Biomarker
Coastal ecosystem
Organochlorine pesticide
Sula nebouxii
Sex-related difference
Sentinel species

ABSTRACT

The temporal, inter-site, and sex-based variation of 19 organochlorine pesticides (OCPs) in blood plasma samples collected from blue-footed boobies of two islands in Sinaloa, Mexico, was evaluated. The effect of OCPs was evaluated with the heterophil/lymphocyte ratio, micronucleated erythrocyte frequency, and scaled mass index. The OCP-group levels decreased as the breeding season progressed, and interannual (but not inter-colony) differences were detected. Intra-annual variation in OCP levels seemed to reflect run-off inputs, although other environmental processes may better explain the variation between years. Sex-based differences in OCP levels were likely related to ecological and physiological processes linked to breeding (e.g., egg-laying and use of lipid reserves). No correlations between OCP-group levels and biomarkers were detected. Small pelagic fishes are the main prey sources of blue-footed boobies and the targets of regional industrial fisheries, and thus blue-footed booby OCP levels could reflect ecosystem health and indicate potential risks for human consumers.

1. Introduction

Mexico has more than 20 million ha of agricultural land, and Sinaloa is one of the most important agricultural states in the country, devoting approximately 1.2 million ha to grow 53 different crops (SIAP, 2010). The wide-ranging and intensive agricultural development of the coastal plain of this state poses a high risk to the environment, primarily due to the presence of organochlorine pesticides (OCPs; Osuna-López et al., 2009). Over the last three decades, OCP values in the water, sediments, and several aquatic wildlife species (mainly fish, mollusks, and crustaceans) in the coastal ecosystems adjacent to these agricultural areas have exceeded the permissible limits (Carvalho et al., 2002; Osuna-Flores and Riva, 2002; Osuna-López et al., 2009; García-de la Parra et al., 2012; Montes et al., 2012). Most OCPs are banned and actual levels are associated with historical contamination; however, some compounds (e.g., lindane and DDTs) maintain high presence and concentrations in water, sediments, and wildlife, and it has been suggested that they are still being used despite the restrictions (García-de la Parra et al., 2012; Arellano-Aguilar et al., 2017). Likewise, studies of seasonal fluctuations in the levels of these chemical compounds in coastal lagoon sediments have been carried out, and higher concentrations have been detected during the rainy season and the beginning of the dry season, mainly due to runoff from the agricultural drains that surround these sites (Montes et al., 2012; García-de la Parra et al., 2013). However, information on the bioaccumulation and fluctuation patterns of OCPs in top marine predators is scarce, and improved knowledge of the risks that pollution poses to fishery resources and how these may impact human and ecosystem health is needed.

Seabirds are recognized as sentinels of marine and coastal environments and provide early warnings of pollution problems caused by OCPs at local and regional scales (Muñoz-Cifuentes et al., 2003; Vander-Pol and Becker, 2007; Mellink et al., 2009). Organic pollutants are usually

* Corresponding author.
E-mail addresses: albertopinaortiz@gmail.com (A. Piña-Ortiz), juan.ceycacn@uanl.edu.mx (J.P. Ceyca-Contreras), carlos.covantes@uan.edu.mx (C.E. Covantes-Rosales), mbl@ciad.mx (M. Betancourt-Lozano), alfredocas@gmail.com (J.A. Castillo-Guerrero).
[1] Department of Animal Ecology and Systematics, Justus Liebig University (JLU) Giessen, Heinrich-BuffRing 26, 35,392 Giessen, Germany.

https://doi.org/10.1016/j.marpolbul.2021.112050
Received 11 July 2020; Received in revised form 31 December 2020; Accepted 2 January 2021
Available online 27 January 2021

analyzed in seabird eggs or tissues (e.g., liver, muscle, and subcutaneous fat). However, pollutant concentrations in eggs only reflect the loads acquired by females (Bourgeon et al., 2013; Trefry et al., 2013) and should not be used with species that show low fecundity and/or low reproductive success (e.g., Fregatidae, Phaethontidae, Diomedeidae). Fat tissue sampling is a destructive procedure, which can be an obstacle for long-term monitoring programs and can be biased given that sampling is often limited to carcasses or injured birds that have been euthanized for welfare reasons (Henriksen et al., 1998; Knudsen et al., 2007; Espín et al., 2016). In contrast, whole blood and/or plasma sampling is non-destructive and useful for assessing and monitoring recent pollutant exposure (Bustnes et al., 2004; Pérez et al., 2008; Mallory et al., 2010; Espín et al., 2016). Likewise, blood samples can be used to measure the effects of pollutants on hematological parameters (e.g., white blood cell counts), oxidative stress, genetic damage, plasma biochemistry, and enzymatic activity (Bustnes et al., 2004; Bourgeon et al., 2012; Sonne et al., 2013; De Mas et al., 2015). Based on these biomarkers, blood and/or plasma samples have been used in wildlife monitoring programs to determine OCP levels in seabird populations at both intra- and interannual scales (Bustnes et al., 2005, 2006; Colabuono et al., 2016).

Food ingestion is the main mechanism of exposure to OCPs and other organic pollutants in seabirds (Borgå et al., 2004; Burger and Gochfeld, 2004). Once assimilated, these highly lipophilic compounds are retained in fat tissues and are slowly excreted over months to decades (Peakall, 1986). Exposure and vulnerability to OCPs can differ significantly among seabird species due to differences in feeding strategies, life histories, breeding cycles, behavior, physiology, and habitat use (Borgå et al., 2004). In fact, OCP load levels are usually different among species with sex-based trophic segregation (Norstrom et al., 1986; Carravieri et al., 2014). Also, among years or within the breeding season, OCP loads can vary in individuals of the same population (van den Brink et al., 1998; Bustnes et al., 2004). For instance, interannual differences in OCP levels in great black-backed gulls (*Larus marinus*) have been attributed to variations in blood lipid levels and body condition due to changes in the availability of different prey types between years (Bustnes et al., 2005). Furthermore, OCP concentrations have been found to be lower in seabirds that breed on oceanic islands compared to those of birds that breed and feed near the coast and/or in industrialized or agricultural areas (Furness and Camphuysen, 1997; Burger and Gochfeld, 2001; Roscales et al., 2011). Regardless of the availability of pollutants in either habitats or prey, like in other animals, pollutant susceptibility in seabird species often varies with age, reproductive stage, and gender (Burger, 1993).

As stressors, OCPs can affect the health of seabirds in different ways (Greichus and Hannon, 1973; Walker and Knight, 1981; Yamashita et al., 1993), which may be assessed using various biomarkers, such as the heterophil/lymphocyte (H/L) ratio that has been used to monitor immune function in wild birds and may be correlated with antibody production (Davis, 2005; Bustnes et al., 2004). Similarly, a micronucleus (MN) test applied in seabird erythrocytes may be used as a biomarker analysis to assess genetic damage caused by chemical substances in contaminated areas (Ceyca et al., 2014; De Mas et al., 2015; Oudi et al., 2019).

The blue-footed booby (*Sula nebouxii*) is a long-lived seabird that displays flexible foraging behavior and breeding investments in response to varying environmental conditions and prey availability (Castillo-Guerrero and Mellink, 2011; Ancona et al., 2012; Gilmour et al., 2018). This species feeds primarily on small pelagic fishes, such as the Pacific thread herring (*Opisthonema libertate*), Pacific anchoveta (*Cetengraulis mysticetus*), and Common halfbeak (*Hyporhamphus unifasciatus;* Castillo-Guerrero and Mellink, 2011, González-Medina et al., 2017), by plunge-diving to depths between 3 and 5 m in foraging trips that range up to 30–50 km from the breeding colonies (Zavalaga et al., 2007, 2008; Weimerskirch et al., 2009). The blue-footed booby is sexually dimorphic, with females being heavier (30–32%) and larger (5–10%) than males (Nelson, 1978; Torres and Drummond, 1999). As a long-lived apex predator and central place forager, the blue-footed booby can be used as a biomonitor to assess OCP residue levels in coastal ecosystems.

In this study, concentrations of OCP residues were measured in blue-footed booby plasma during two breeding seasons (2011 and 2013) on two islands near the coast of Sinaloa, Mexico. Temporal fluctuations of OCP plasma levels were assessed during both reproductive stages (i.e., incubation and chick-rearing) and years in an inshore island surrounded by agricultural fields employing sophisticated technologies. In addition, the inter-colony and sex-based variation of these pollutants was measured throughout both seasons in two breeding colonies (i.e., offshore island and inshore island). The relationships between pesticide concentrations and the H/L ratio, frequency of micronucleated erythrocytes (MNE), and body condition based on the scaled mass index (SMI) in blue-footed boobies was evaluated. Considering the above, we predicted the following: 1) OCP concentrations in the plasma of blue-footed boobies would decrease as their breeding season progressed due to the lower availability of pesticides in the region, which is related to the cessation of continental runoff at the end of the rainy season (Montes et al., 2012). 2) OCP concentrations in the plasma will differ between sexes due to physiological aspects related to sexually dimorphic differences in fat storage (males are smaller and lighter than females; Nelson, 1978, Torres and Drummond, 1999) and the transfer of highly lipophilic pollutants to eggs by females (Verreault et al., 2006). 3) When compared to offshore colony individuals, inshore colony individuals will show higher OCP concentrations due to the proximity of agricultural areas. 4) Individuals with higher plasma OCP levels will show a higher H/L ratio and MNE frequency and lower SMI index values compared to those of individuals with low OCP levels.

2. Methods

2.1. Study area and data collection

Samples were collected in two islands located in the southeastern portion of the Gulf of California near the coast of Sinaloa (Fig. 1). Farallón de San Ignacio (FSI; 25° 26′ 12" N, 109° 22′ 38" W; 16 ha) is a rocky island devoid of vegetation located approximately 22 km from the northern coast of Sinaloa. It is surrounded by waters with depths between 200 and 500 m and supports a colony of 2500 blue-footed booby pairs (González-Bernal et al., 2002; Guevara-Medina et al., 2008). El Rancho (RI; 25° 09′ 14" N, 108° 22′ 19" W; 380 ha) is a sandy island located in the northern portion of the mouth of Santa María Bay (SMB). This island is home to a breeding population of approximately 3000 blue-footed booby pairs (Castillo-Guerrero et al., 2014). SMB is the largest coastal lagoon in Sinaloa and is separated from the Gulf of California by Altamura island, a 45-km-long sandy bar that protects this lagoon from wave action (Alvarez-Arellano and Gaitán, 1994). The lagoon is surrounded by technified (131,243 ha) and rainfed (10,071 ha) agriculture as well as shrimp farms (7700 ha) and several human settlements (180,596 inhabitants; Páez-Osuna et al., 2007; INEGI, 2009, 2010). The continental waters that reach SMB mainly come from the Mocorito river basin and a wide network of agricultural drains, which transport waste from aquacultural and agricultural practices and the cities of Culiacán and Guamúchil (Páez-Osuna et al., 2007; Acosta-Velázquez and Vázquez-Lule, 2009; Montaño-Ley and Páez-Osuna, 2014).

At FSI, 66 blue-footed booby samples (35 females and 31 males) were collected during three reproductive periods (courtship = 47, incubation = 13, and chick-rearing = 6) during the 2013 breeding season (November 2012 to May 2013). At RI, 83 samples (39 females and 44 males) were collected during two reproductive periods (incubation = 28 and chick-rearing = 55) during the 2011 season (December 2010 to May 2011). In addition, in this same colony, 52 samples (34 females and 18 males) were collected during three reproductive periods (courtship =

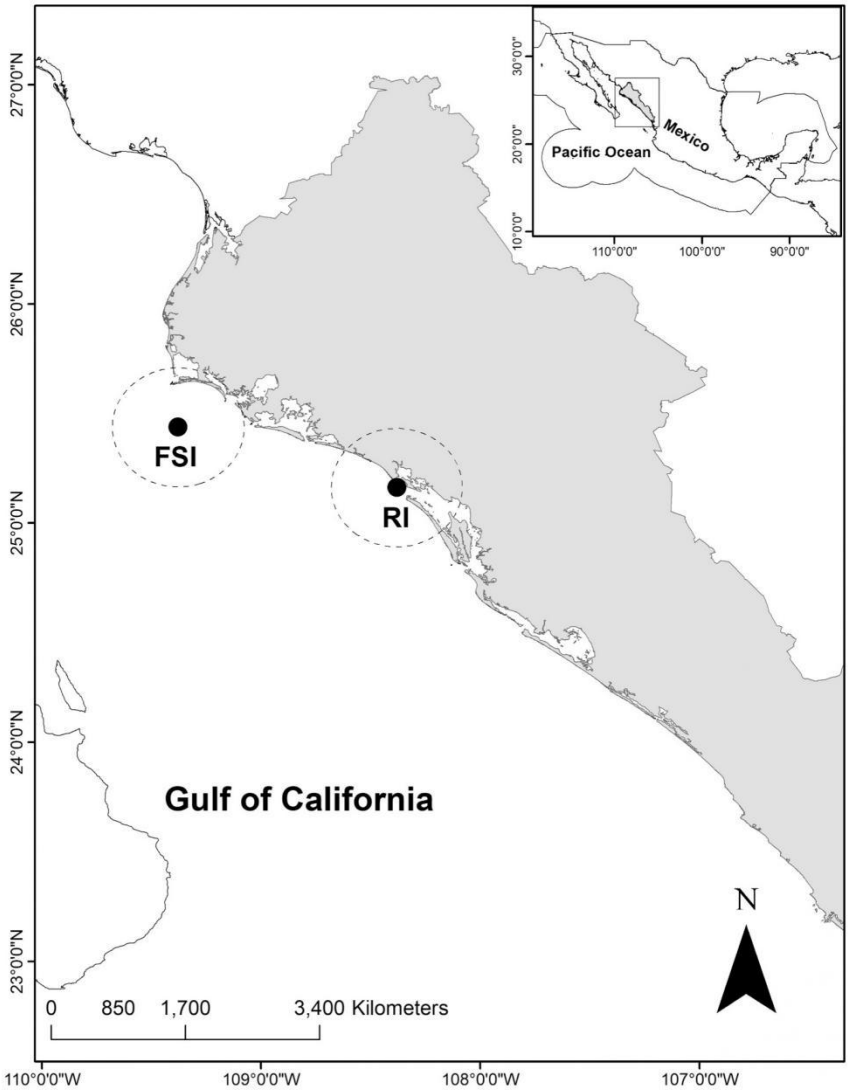


Fig. 1. Study area showing the location of the blue-footed booby breeding colonies. Dashed lines show the mean foraging range (km) used by the blue-footed booby during the breeding season in the Gulf of California, according to Weimerskirch et al. (2009). FSI = Farallón de San Ignacio; RI = El Rancho. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

22, incubation = 21, and chick-rearing = 9) during the 2013 season (February 2013 to May 2013). New individuals for each reproductive stage were selected; however, for a small group of individuals, we were able to collect blood samples throughout the breeding season (see Table S3 and Fig. S2). Individuals subjected to one-time or repeated sampling were not pooled for any statistical analysis. The birds were captured by hand directly at the nest, and blood samples were obtained by brachial vein puncture with a syringe (3 mL, 25G, 0.5 mm × 16 mm). The collected blood was used to prepare three blood smears on glass slides (see Section 2.3), and the remaining blood sample was deposited in 1.5-mL plastic tubes with EDTA K_2 (0.25 M, 100 μL) and centrifuged at 2700 x*g* for 15 min at the study site. The plasma obtained was stored at −18 °C until further analysis in the laboratory. Before the birds were released, they were weighed, and morphometric measures of the tarsus, ulna, and culmen were taken.

2.2. Organochlorine pesticide extraction and analyses

Liquid-liquid extraction of the pesticides in plasma was carried out following the methodology of Matos-Lino et al. (1998) with modifications. Briefly, 1-mL plasma samples were placed in 10-mL glass tubes with 0.2 g of Na_2Cl and 2.5 mL of a 9:1 hexane:acetone solution. The tubes were vortex stirred for 1 min and allowed to stand for 5 min. Each sample was separated into two phases. The upper phase was extracted with a pipette and placed in a 5-mL glass tube. Then, 2.5 mL of a hexane: acetone solution was added to the remaining lower phase, and the aforementioned steps were repeated. The obtained upper phases were combined and concentrated by evaporation and rotation in a miniVAP® (Grabner Instruments, Vienna, Austria) with nitrogen flow. The resulting extract was resuspended in 1 mL of hexane. Subsequently, samples were cleaned with silica gel (EPA 3630c; EPA, 1996). The extract was

then transferred to a polypropylene column with a fiberglass lower-plug that had been previously prepared with 1 g of commercial sand (Sigma Aldrich®) and 1 g of activated silica gel mixed with hexane. After adding the extract, an additional 1 g of commercial sand was added. The extracts were eluted using 10 mL of hexane and then 10 mL of a 1:1 hexane:dichloromethane solution. The final extracts were concentrated by evaporation and rotation in the miniVAP with nitrogen flow and finally resuspended in 200 μL of hexane.

The samples were analyzed by gas chromatography with a Hewlett-Packard 5890 Series II gas chromatograph (Palo Alto, USA) equipped with two electron capture detectors coupled to different columns [Restek Corporation, Bellefonte, USA; Rtx-CLPesticides (cat. #11141) and Rtx-CLPesticides 2 (cat. #11324)] measuring 30 m with a 0.25-cm diameter and 0.2-μm film thickness that were joined with a Press-Tight® Y-universal deactivated connector (Restek Corporation; cat. # 20405261) to a Rxi® guard pre-column (Restek Corporation; cat. # 10039) measuring 5 m with a 0.32-mm diameter. Nitrogen was used as the carrier and auxiliary gas. A total of 2 μL of the extract was injected in splitless mode. The temperature conditions of the injector and detectors were 290 and 300 °C, respectively. The initial oven temperature was 150 °C. Two temperature gradients were programmed: the first from 5 to 245 °C/min and the second from 10 to 310 °C/min for 5 min. A mixture of standard solutions [Restek Corporation; Organochlorine Pesticide Resolution Check Mix [(cat. # 32454)] was used to identify the following organochlorine pesticides: aldrin; dieldrin; endrin; endrin aldehyde; endrin ketone; α- and β-endosulfan; endosulfan sulfate; heptachlor; heptachlor epoxide; α-, β-, γ- and δ-hexachlorocyclohexane (HCH); trans- and cis-chlordane; 4,4′-dichlorodiphenyldichloroethylene (4,4′-DDE); 4,4′-dichlorodiphenyldichloroethane (4,4′-DDD); and 4,4′-dichlorodiphenyltrichloroethane (4,4′-DDT). The detection limits (LOD) of the OCPs analyzed in plasma were between 7.5 and 7.8 ng/mL, and the quantification limits (LOQ) were between 22.5 and 23.3 ng/mL. The plasma concentrations of the pesticides are expressed in ng/mL.

2.3. Biomarkers and body condition analyses

We estimated the H/L ratio by a differential leucocyte count using the blood smears and light microscopy. The blood smears were air-dried in the field. In the laboratory, they were subsequently fixed with methanol (80%) and prepared by Wright-Giemsa staining (Woronzoff-Dashkoff, 2002). Lymphocytes and heterophils were identified using an optical microscope with an oil immersion objective (100 x magnification) and the Clinical Hematology Atlas of Birds (Clark et al., 2009). The differential leucocyte count consisted of counting 100 white blood cells (granular and nongranular) and recording the frequency of each cell type (Houwen, 2001).

The MN test consisted of determining the frequency of MNE in the blood smears via microscopy (Schmid, 1975). Micronuclei are small bodies similar to the nucleus that are formed due to chromosome loss (clastogenic events) or failures in the mitotic apparatus (aneuploidogenic events) during cell division (Schmid, 1975; Fenech, 2000). The blood smears that were fixed with 80% methanol were prepared by acridine orange fluorescent staining (Hayashi et al., 1983). We counted the frequency of MNE per 10,000 erythrocytes using a fluorescence microscope (100 x magnification). The MNE had to be at least one-third smaller and have the same colour and intensity as the nucleus as well as being completely separated from the nucleus and not refractory to be counted (Grisolia, 2002). A zig-zag model was implemented to read all blood smears (both for the H/L ratio and MN test) to avoid crossing the same field more than once. To assess the body condition of individuals, we used the method proposed by Peig and Green (2009) and estimated the SMI using the ulna and body mass of the blue-footed boobies.

2.4. Statistical analyses

For the statistical analyses, we used the total concentrations by affinity group: ΣDDT (4,4′-DDE, 4,4′-DDD, and 4,4′-DDT), ΣHCH (HCH-α, −β, −γ, and -δ), ΣDrins (aldrin, dieldrin, endrin, endrin aldehyde, and endrin ketone), ΣHeptachlor (heptachlor and heptachlor epoxide), ΣChlordane (trans- and cis-chlordane), and ΣEndosulfan (α-and β-endosulfan and endosulfan sulfate). Due to the differences in the magnitude of the OCP concentrations in the plasma (ng/mL; wet weight), a logarithmic transformation (base 10) was carried out for all data groups (Zar, 2010). Further Pearson correlations were performed between all OCP groups (ΣDDT, ΣHCH, ΣDrins, ΣHeptachlor, ΣChlordane, and ΣEndosulfan).

For the inter-site and sex-based comparisons of the OCP concentrations in blue-footed booby blood plasma, we used the samples collected in FSI and RI during the 2013 season. General linear models (GLM) were applied to analyze the concentrations of each group of OCPs, including the site (RI and FSI), sex (male and female), and reproductive period (courtship, incubation, and chick-rearing) as factors and the sample collection date (days from December 1) as a continuous predictor. To evaluate interannual differences in the OCP concentrations in blue-footed booby blood plasma, GLMs were applied using the data collected in RI during the 2011 and 2013 breeding seasons. We applied the GLMs separately for each group of OCPs, considering year (2011 and 2013) and reproductive period (incubation and chick-rearing) as factors and the sample collection date as a continuous predictor. Likewise, to evaluate the effects of the OCP residues on the H/L ratio, MNE frequency, and SMI values, we also used GLMs. The H/L ratio, MNE frequencies, and SMI values were considered as dependent variables in separate analyses, using breeding stage and sex as fixed factors and the concentrations of each organochlorine affinity group (i.e., ΣDDT, ΣHCH, ΣDrins, ΣHeptachlor, ΣChlordane, and ΣEndosulfan). All GLMs were applied based on complete initial models that considered all variables and interactions. Subsequently, the non-significant interactions and variables were eliminated to simplify the models. Statistical analyses were performed with a significance level of 5% ($p < 0.05$), and the OCP concentrations are reported as mean ± standard error.

3. Results

All of the OCPs that were analytically considered in this study were detected in the blue-footed booby plasma samples. In total, 16 compounds were detected at FSI in 2013, while 15 and 14 compounds were detected at RI in 2011 and 2013, respectively (Table 1). The most frequent OCP residues (by affinity group) in the blue-footed boobies were ΣDrins (147 out of 201 individuals), ΣHCH (129), ΣChlordane (87), and ΣDDT (72). Likewise, ΣDrins (64.63–765.24 ng/mL) and ΣHCH (73.59–76.29 ng/mL) were the predominant compounds detected in blue-footed booby individuals throughout the 2011 breeding season at RI, followed by ΣChlordane (24.31–63.50 ng/mL) and ΣHeptachlor (3.56–7.85 ng/mL), while in 2013, the blue-footed boobies exhibited a different pattern [ΣHCH (0.72–30.47 ng/mL) > ΣDrins (1.54–15.76 ng/mL) > ΣEndosulfan (BDL - 9.39 ng/mL) > ΣChlordane (0.90–3.84 ng/mL)]. At FSI, the observed pattern was quite similar to what was observed at RI in 2011 (ΣDrins > ΣHCH > ΣChlordane > ΣDDT; Table 1). Overall, the results of the correlation analysis between the OCP groups showed positive pairwise correlations between all groups, and high pairwise correlations between groups with major frequencies and concentrations in blue-footed booby plasma was detected (r values from 0.53 to 0.68; DF = 2, and $p < 0.00001$, in all pairwise tests; Supplemental material, Appendix 1; Fig. S1).

The OCP concentrations of each affinity group in blue-footed booby plasma were not significantly different between sites (p-values varied between 0.15 and 0.70; see Table S1), except for the ΣHeptachlor group, which showed higher levels in RI than those of FSI, but these differences were related to the breeding stage ($F_{2,100} = 3.35$, $p = 0.04$; Table S1). For RI, an increase of the ΣHeptachlor levels was observed through the different stages, while in FSI, the concentrations were low during courtship and subsequently increased during incubation and decreased

Table 1
Mean concentrations of OCPs (ng/mL, ww) analyzed in blue-footed booby (*Sula nebouxii*) plasma samples obtained from two coastal breeding colonies in Sinaloa, México. S.E. = standard error; FRC = frequency; BDL = below detectable level; FSI = Farallón de San Ignacio; RI = El Rancho. The total by each affinity group is resalted in bold.

Compounds	RI (Dec 2010 - May 2011)						RI (Feb-May 2013)									FSI (Nov 2012 - May 2013)								
	Incubation (n = 28)			Chick-rearing (n = 55)			Courtship (n = 22)			Incubation (n = 21)			Chick-rearing (n = 9)			Courtship (n = 47)			Incubation (n = 13)			Chick-rearing (n = 6)		
	Mean	S.E.	FRC	Mean	S.E.	FRC	Mean	S.E.	FRC	Mean	S.E.	FRC	Mean	S.E.	FRC	Mean	S.E.	FRC	Mean	S.E.	FRC	Mean	S.E.	FRC
4,4'-DDT	0.46	0.38	2	0.45	0.32	3	BDL	–	–	BDL	–	–	BDL	–	–	BDL	–	–	BDL	–	–	BDL	–	–
4,4'-DDE	6.01	2.14	10	9.61	4.83	27	2.59	0.98	10	0.09	0.06	2	0.16	0.16	1	2.61	0.81	18	0.50	0.28	3	BDL	–	–
4,4'-DDD	1.11	0.81	2	2.05	2.05	1	BDL	–	–	BDL	–	–	BDL	–	–	BDL	–	–	BDL	–	–	BDL	–	–
ΣDDT	**7.58**	**3.33**	**11**	**12.11**	**7.19**	**27**	**2.59**	**0.98**	**10**	**0.09**	**0.06**	**2**	**0.16**	**0.16**	**1**	**2.61**	**0.81**	**18**	**0.50**	**0.28**	**3**	**BDL**	**–**	**–**
HCH-α	45.91	24.27	13	7.38	3.91	17	0.02	0.02	1	3.70	2.97	4	0.10	0.10	1	2.37	1.12	17	0.81	0.56	2	BDL	–	–
HCH-β	30.38	9.74	12	66.06	15.84	43	30.40	28.17	16	7.03	4.25	8	0.62	0.36	4	6.61	2.18	29	8.02	6.36	6	0.20	0.20	1
HCH-γ	BDL	–	–	BDL	–	–	0.03	0.03	1	0.23	0.23	1	BDL	–	–	0.04	0.03	3	BDL	–	–	BDL	–	–
HCH-δ	BDL	–	–	0.16	0.16	1	0.01	0.01	1	1.61	1.53	2	BDL	–	–	0.73	0.47	7	BDL	–	–	BDL	–	–
ΣHCH	**76.29**	**34.01**	**21**	**73.59**	**19.91**	**43**	**30.47**	**28.23**	**17**	**12.56**	**8.98**	**8**	**0.72**	**0.46**	**4**	**9.76**	**3.80**	**29**	**8.83**	**6.92**	**6**	**0.20**	**0.20**	**1**
Aldrin	1.38	0.96	2	13.35	10.47	10	2.57	2.57	1	3.80	2.16	5	BDL	–	–	2.03	0.85	6	0.78	0.78	1	BDL	–	–
Dieldrin	1.51	0.88	3	BDL	–	–	0.16	0.16	1	0.07	0.07	1	BDL	–	–	2.10	1.49	5	BDL	–	–	BDL	–	–
Endrin	3.33	1.44	7	1.17	1.17	1	BDL	–	–	BDL	–	–	BDL	–	–	0.01	0.01	2	BDL	–	–	BDL	–	–
Endrin ketone	750.05	374.42	23	50.10	10.29	31	6.71	1.60	16	11.90	5.83	14	1.54	0.41	7	18.18	7.77	38	15.93	9.55	9	2.45	1.02	4
Endrin aldelhyde	8.98	8.07	5	BDL	–	–	BDL	–	–	BDL	–	–	BDL	–	–	1.94	1.94	2	BDL	–	–	BDL	–	–
ΣDrins	**765.24**	**385.76**	**25**	**64.63**	**21.93**	**31**	**9.44**	**4.33**	**17**	**15.76**	**8.06**	**14**	**1.54**	**0.41**	**7**	**24.26**	**12.06**	**40**	**16.71**	**10.33**	**9**	**2.45**	**1.02**	**4**
Heptachlor	7.85	4.91	4	3.56	1.10	11	BDL	–	–	1.83	1.39	2	0.02	0.02	1	0.26	0.13	5	0.19	0.19	1	BDL	–	–
Heptachlor epoxide	BDL	–	–	BDL	–	–	BDL	–	–	0.69	0.56	3	BDL	–	–	0.09	0.09	1	BDL	–	–	BDL	–	–
ΣHeptachlor	**7.85**	**4.91**	**4**	**3.56**	**1.10**	**11**	**BDL**	**–**	**–**	**2.53**	**1.95**	**4**	**0.02**	**0.02**	**1**	**BDL**	**–**	**5**	**0.19**	**0.19**	**1**	**BDL**	**–**	**–**
Trans-chlordane	24.31	8.56	8	62.76	17.16	29	2.89	2.51	8	1.51	1.02	6	0.33	0.11	5	1.83	0.62	24	0.29	0.18	3	BDL	–	–
Cis-chlordane	BDL	–	–	0.73	0.54	2	0.95	0.54	3	0.73	0.73	1	0.57	0.57	1	1.11	0.63	3	1.92	1.33	3	0.72	0.72	1
ΣChlordane	**24.31**	**8.56**	**8**	**63.50**	**17.71**	**30**	**3.84**	**3.05**	**8**	**2.24**	**1.74**	**7**	**0.90**	**0.68**	**5**	**2.94**	**1.25**	**24**	**2.21**	**1.51**	**4**	**0.72**	**0.72**	**1**
α-endosulfan	1.13	0.79	2	3.15	2.88	3	0.38	0.36	2	0.19	0.16	2	BDL	–	–	0.34	0.24	5	BDL	–	–	BDL	–	–
β-endosulfan	3.29	1.15	12	0.19	0.15	2	0.04	0.04	1	BDL	–	–	BDL	–	–	BDL	–	–	BDL	–	–	BDL	–	–
Endosulfan sulfate	0.19	0.13	2	BDL	–	–	8.97	8.47	2	0.18	0.12	2	BDL	–	–	0.18	0.10	3	0.17	0.17	1	BDL	–	–
ΣEndosulfan	**4.61**	**2.07**	**13**	**3.35**	**3.03**	**5**	**9.39**	**8.87**	**3**	**0.37**	**0.29**	**4**	**BDL**	**–**	**–**	**0.52**	**0.34**	**8**	**0.17**	**0.17**	**1**	**BDL**	**–**	**–**

during the chick-rearing stage.

Sex-based variation was detected on five out of six OCP groups, although these differences were related to the breeding stage (All p-values <0.05; Table S1). Overall, the ΣHCH, ΣDrins, and ΣChlordane groups showed similar patterns between stages. During courtship, females had higher concentrations of these OCP groups than those of males, although only ΣDrins showed significant differences [post-hoc test (LSD) = 0.308, p = 0.033]. During the incubation stage, an inverse pattern was observed, with ΣHCH being the only group showing significant differences [post-hoc test (LSD) = −0.587, p = 0.006]. No sex-based differences were detected during the chick-rearing period, although these OCP concentrations decreased in both sexes during this stage (Fig. 2). Particularly for females, a negative trend was noted between the concentrations of most OCP groups and the progression of the breeding season. In fact, during the last reproductive stage, only four groups of OCPs were detected (Fig. 2).

The concentrations of OCP groups in the blue-footed booby samples decreased as the two breeding seasons progressed, with significant decreases detected for ΣDrins ($F_{1,105} = 7.74$, $p = 0.006$, SE = 0.005, $r = 0.46$ and $r^2 = 0.21$), Heptachlor ($F_{1,110} = 4.21$, $p = 0.04$, SE = 0.001, $r = 0.17$ and $r^2 = 0.02$), and Chlordane ($F_{1,108} = 4.74$, $p = 0.03$, SE = 0.002, $r = 0.18$ and $r^2 = 0.03$; Fig. 3; Table S1), indicating a higher uptake of pollutants during the beginning of the breeding season (autumn-winter) compared to those of the final phases (spring-summer). Regarding interannual variation, significant differences between years were detected in ΣDDT, ΣHCH, ΣEndosulfan, ΣDrins, and ΣChlordane (p-values <0.05; Table S1), with higher levels in 2011 compared to those of 2013 for the first three groups, while ΣDrins and ΣChlordane showed the opposite pattern (2011 < 2013; Fig. 4).

No significant OCP-group effects were observed on the indicators of the health status of the blue-footed booby (H/L ratio, SMI values, or MNE frequency, p-values = 0.25–0.94, 0.31–0.91, and 0.21–0.68 for each affinity group, respectively; see Table S2 for detailed statistical results).

4. Discussion

Our results showed that all OCP residues analyzed were present in the plasma samples from blue-footed boobies that breed on the Sinaloa coast. An important feature of OCPs is their high biological persistence (see Willett et al., 1998; Borgå et al., 2004). In recent years, due to restrictions and bans imposed since the 1970s in many countries worldwide, gradual reductions in environmental OCP concentrations have been observed (Wang et al., 2016). However, in historically agricultural regions, the concentrations of some OCPs still exceed safe levels, which poses a risk to adjacent ecosystems (Ene et al., 2012; García-de la Parra et al., 2012, 2013; Sun et al., 2018), and most OCPs are still present in measurable concentrations. The OCP concentrations by affinity group in blue-footed booby plasma were generally higher than those that have been reported for seabirds with similar diets and foraging areas in other tropical regions of the world (e.g., Grand Connétable, French Guiana; Sebastiano et al., 2016, 2017). However, these concentrations were also lower than the reported negative effect thresholds (i.e., behavioral, developmental, neurological, and reproductive) or lethal levels (Keith, 1966; Greichus and Hannon, 1973; Gress et al., 1973; Jehl, 1973; Blus, 1982; Peakall and Fox, 1987; Elliott et al., 1988) for seabirds. Nevertheless, our results indicate that blue-footed boobies in this area of the Gulf of California are exposed to a vast diversity of OCPs, and this does not rule out the possibility that cumulative effects may be caused by interactive toxicity.

The frequency and concentrations of OCPs measured in blue-footed booby plasma suggest two different general patterns. The concentrations of 4,4′-DDE and HCH-β were higher than those of their respective parental compounds (Table 1), which reflects historical use and

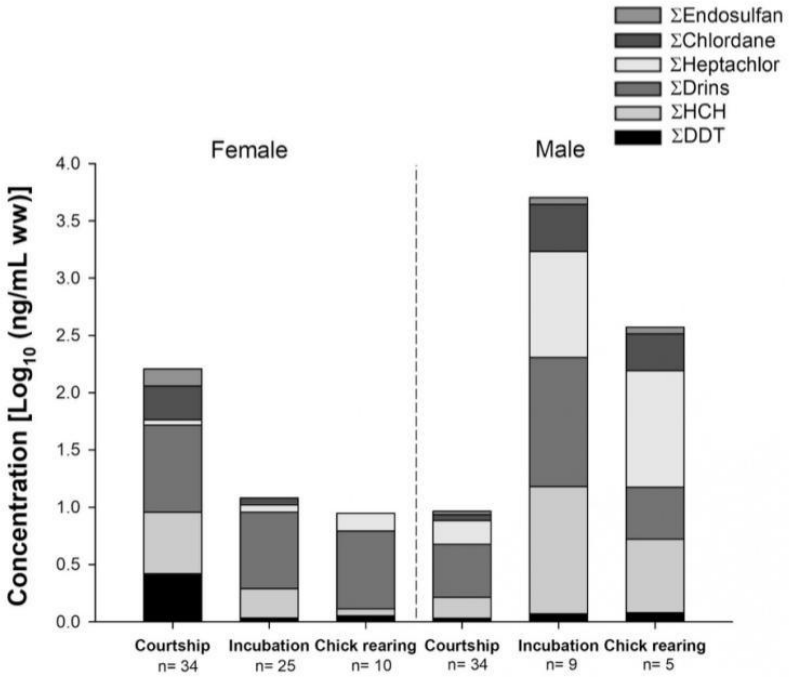


Fig. 2. Adjusted means of organochlorine pesticide (OCP) concentrations by affinity group (Log_{10}-transformed; ng/mL ww) in the plasma samples of blue-footed booby females and males from Farallón de San Ignacio (FSI) and El Rancho (RI) during the 2013 breeding season. Different reproductive stages and adjusted covariate means at day 69 are shown. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

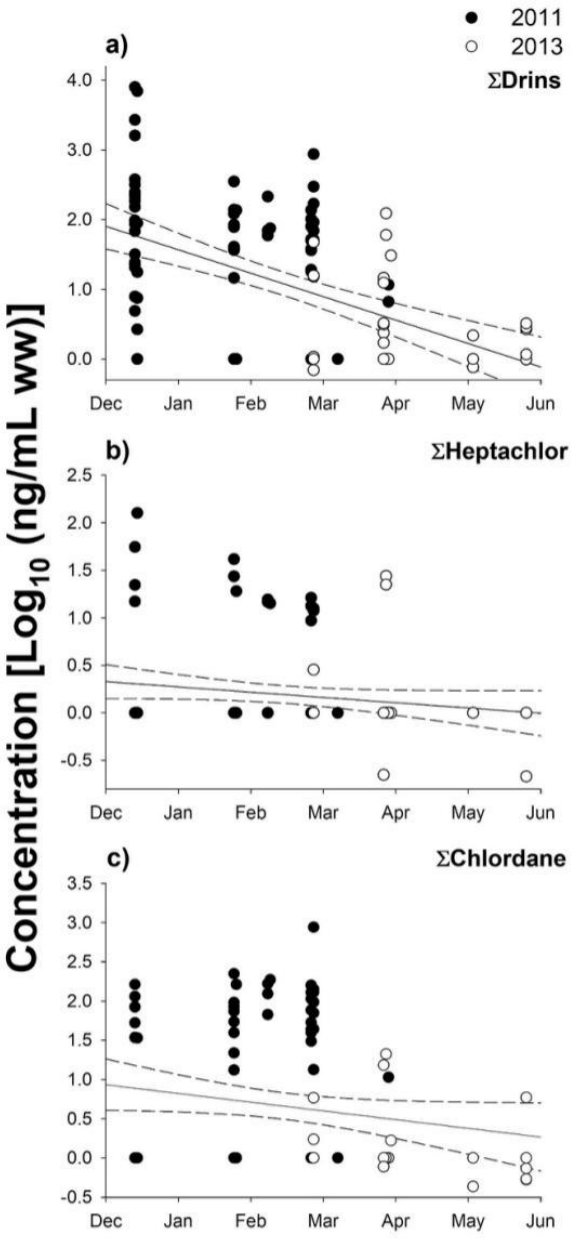


Fig. 3. Concentrations of ΣDrins (a), ΣHeptachlor (b), and ΣChlordane (c) (Log_{10}-transformed; ng/mL ww) in blue-footed booby plasma samples collected at El Rancho (RI) island during the 2011 ($n = 83$) and 2013 ($n = 30$) breeding seasons. The trendline and confidence intervals for each OCP group is shown. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

exposure and a reduction or disuse of these pesticides in agricultural activities within the study area. In contrast, OCPs, such as endrin ketone, heptachlor, endosulfan sulfate, and α- and β-endosulfan, were present in higher concentrations in the plasma samples, which could be linked to relatively recent exposure events given that the half-life of these OCPs in both the environment and biota is relatively short (days to weeks; ATSDR, 2007, ATSDR, 2019, Weber et al., 2010). In general, the OCP

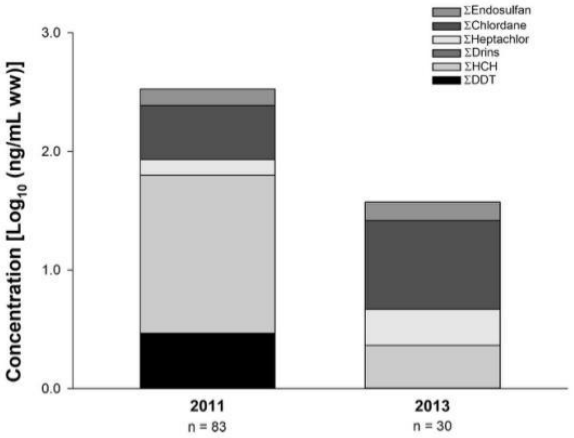


Fig. 4. Adjusted means of organochlorine pesticide (OCP) concentrations by affinity group (Log_{10}-transformed; ng/mL ww) in the blue-footed booby plasma samples collected in El Rancho (RI) during the 2011 and 2013 breeding seasons. Adjusted covariate means at day 74 are shown. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

levels in the seabirds indicated that the presence of most compounds was related to historical exposure; however, some compounds may be still in use in the coastal ecosystems of the region despite legal restrictions in Mexico.

During the breeding season, the OCP plasma concentrations found in this study seem to be related to physiological (e.g., fat storage, egg-laying), ecological (e.g., prey availability), and environmental factors (e.g., run-off to coastal systems). The differences in the OCP concentrations between males and females during the early breeding stages may be due to both physiological aspects and diet differences. During the courtship stage, blue-footed booby females have high levels of triglycerides that are related to the consumption of prey with high lipid content [e.g., the Pacific anchovy (*C. mysticetus*) and Pacific thread herring (*O. libertate*)] as well as with high $\delta^{15}N$ values (González-Medina et al., 2018). Therefore, the high concentrations of OCPs observed during courtship in the blue-footed booby females may be related to the increased consumption of high-quality prey, which consequently boosts blood lipid levels and is reflected in higher plasma OCP residues. Lower OCP levels in males during courtship are consistent with this explanation because they do not need to mobilize lipids to form and lay eggs.

During the incubation stage, the OCP levels decreased in the plasma of females, probably because of the allocation of nutrients (mainly protein and lipids) for egg formation (Bond and Diamond, 2010). It is expected that a proportion of highly lipophilic organochlorine compounds that are bonded to blood lipids and destined for embryo development are transferred to the eggs (Bogan and Newton, 1977; Elliott et al., 2007; Bourgeon et al., 2013). On the other hand, blue-footed boobies show sex-specific body mass regulation (Velando and Alonso-Alvarez, 2003). Males are smaller and lighter than females (Nelson, 1978) and during high-demand experimental conditions (i.e., primary feathers trimmed and enlarged brood sizes), males have been found to maintain a fixed body mass at the expense of the conditions of chicks and mates, which suggests that males are unable to adjust their body mass because they were functioning under their maximum physiological capacity (Velando and Alonso-Alvarez, 2003). The incubation stage of the blue-footed booby spans approximately 40 days, with equitable incubation shifts between sexes that last an average of 4–7 h, depending on brood size and food availability (for Gulf of California colonies, see Castillo-Guerrero and Mellink, 2011). Each incubation shift leads to a period of fasting and possibly the use of fat reserves, during which fat-

soluble organochlorines that are stored in these reserves will be mobilized to other tissues through the bloodstream, increasing the OCP concentrations in the blood and other tissues (Södergren and Ulfstrand, 1972; Bogan and Newton, 1977; Henriksen et al., 1996). Then, under the assumption that males function under their physiological maximum, males would be more prone to use (and mobilize) stored lipids during incubation periods and thus would show elevated OCP levels in the blood compared to those of females. At the end of the breeding season, during the chick-rearing stage, the OCP levels decreased in both sexes. This seems to be related to environmental availability (Montes et al., 2012) and the consumption of similar prey in equal proportions by both sexes during this stage (González-Medina et al., 2017).

In addition to differences related to sex and breeding stage, a consistent temporal pattern was present in both years. The gradual reduction of most OCP groups in plasma levels in the blue-footed booby could be mainly related to environmental processes. In Sinaloa, the input of OCPs from agricultural areas to coastal ecosystems increases during the rainy season (late July to October; see Osuna-Flores and Riva, 2002, Montes et al., 2012) due to runoff that carries sediments (CONAGUA, 2011). The inputs of these sediments to coastal systems decline and reach their lowest levels during the spring and beginning of summer (Montes et al., 2012). On the other hand, in the same colony, changes in $\delta^{15}N$ and $\delta^{13}C$ values were found to be indicative of differences in foraging regions or prey as the breeding season progressed (González-Medina et al., 2017). Thus, this pattern of a gradual decrease in OCP residues from winter to spring in the plasma samples of blue-footed boobies can be related to both changes in diet and environmental OCP availability in coastal Sinaloa.

Another possibility that we considered was that breeding performance was linked to the OCP load. At the beginning of the breeding season, many individuals with highly variable attributes (age, condition, and OCP load) are available and willing to reproduce; however, as the season progresses, some individuals fail to breed or desert. This may be linked to the OCP load, with successful breeders having lower OCP loads than those of unsuccessful breeders who either failed to breed or deserted. Thus, the temporal pattern of OCP concentrations could be due to such "selection" instead of reflecting an actual decrease within individuals. We were able to test if a pattern was present within individuals that reflected the general pattern (i.e., 8 individuals with three repeated measures during the breeding season). In these individuals, the OCP loads (Drins, HCHs and DDTs) decreased gradually and in a similar magnitudes to that of the general pattern. As such, possible low-OCP individual "selection" seems unlikely (see results in Table S3). In general, the intra-annual differences in the OCP plasma concentrations found in the blue-footed boobies in this study seem to be related to both physiological (e.g., fat storage, egg-laying, and sexual body size dimorphism) and ecological factors (e.g., food resources and environmental OCP availability), although they are influenced to a greater extent by the latter.

Concerning the inter-site comparison, the similarity of concentrations for most of the OCP groups between breeding locations could be related to blue-footed booby feeding behavior. During foraging, blue-footed boobies remain close to their breeding islands (foraging range: 30–40 km) in coastal areas associated with upwelling in which elevated concentrations of their main prey are available (e.g., sardines and anchovies; Zavalaga et al., 2007, Weimerskirch et al., 2009). In the case of FSI, the island is located approximately 24 km from the mainland (Fig. 1), and so it is highly probable that individuals from this colony made foraging trips to coastal areas and used similar foraging habitats to those of the blue-footed boobies of the RI colony.

Considering that runoff is one of the main ways to incorporate OCP residues into coastal environments (Sarkar et al., 2008; Lin et al., 2012), years with higher rainfall levels are expected to result in higher OCP inputs at these sites. In the study area, the amount of accumulated rainfall throughout the year before the 2013 breeding season (431.5 mm) was almost 2-fold greater than the total amount of accumulated rainfall before the 2011 season (262.8 mm; CONAGUA, 2020). This pattern coincided with the variations between years for ΣDrins, ΣHeptachlor, and ΣChlordane; however, for the rest of the groups, the opposite was observed. Therefore, it is highly probable that other factors, such as upwelling, atmospheric transport, and even changes in soil properties in the study zone, may have influenced the dynamics of OCP residues that are input to SMB, which is similar to what has been reported for other coastal regions (Li et al., 2007; Sarkar et al., 2008; Li et al., 2016; Ya et al., 2017). Furthermore, some studies have argued that interannual variations in OCP blood levels are due to physiological processes, such as changes in blood lipid levels and body condition, which may be linked to different feeding conditions (i.e., changes in prey availability; Bustnes et al., 2005, 2017). The blue-footed booby preys mainly on small pelagic fish, and the composition of its diet varies according to oceanographic conditions (e.g., ENSO cycle; Castillo-Guerrero and Mellink, 2011, Ancona et al., 2012). As such, diet differences between years should influence circulating OCP levels in the blood. However, during the study years, there were slight variations in regional oceanographic anomalies. For example, the mean sea surface temperature anomaly was −0.7 °C in 2011 and -0.5 °C in 2013, indicating that the cold-rich phase of the ENSO cycle was present during both years (Fiedler and Mantua, 2017). Therefore, we consider that this study was conducted under conditions of adequate food supply, and presumably, the availability of prey resources between years should have been similar.

The lack of correlations between OCP affinity groups and health biomarkers did not concur with our objective of linking the effects of OCPs to the physiological state of the seabirds (see Bustnes et al., 2004; Barbosa et al., 2013; Oudi et al., 2019). This could be related to the lack of specificity or sensitivity of the selected biomarkers. It is worth noting that ecological context could affect the biomarker response; therefore, ecological factors, such as food availability, predation pressure, or climatic conditions, may induce different responses (Bourgeon et al., 2012). For example, the H/L ratio is a biomarker that is used by researchers to study responses to stress and has been associated with a wide variety of stressors, such as infections, long-distance migrations, and contamination, as well as other measures of individual health and quality (Bustnes et al., 2004; Davis et al., 2004; Lobato et al., 2005; Davis et al., 2008). Therefore, on one hand, we believe that the lack of consistent relationships between OCP affinity group levels and the health biomarkers analyzed in the current study was a result of these biomarkers not responding specifically to the effects of the OCPs. On the other hand, it is quite feasible that the health biomarkers measured in blue-footed booby individuals show some OCP-associated influence after a certain threshold is reached, and OCP levels below that threshold would not have notable effects on the state of the individuals.

Finally, the Sinaloa coast is one of the most important areas of the Mexican Pacific in terms of fishing (CONAPESCA, 2017). The sardine and anchovy fishery shares its target species (i.e., the Pacific thread herring and Pacific anchovy) with the blue-footed booby. This fishery is the second-most important in the state, taking in catches of approximately 81,000 tons (out of a total of 309,000 tons) following the shrimp fishery (> 84,000 tons), and both fisheries constitute almost 10% of the fish production in Mexico (CONAPESCA, 2017; Hernandez-Padilla et al., 2017). The volume obtained by the anchovy and sardine fishery on the Sinaloa coast is mainly used for the production of fishmeal, which is used as a food base in aquaculture and livestock in Mexico, while a percentage is exported to other countries for the same purpose (CONAPESCA, 2017). Hence, direct consumption and the use of these products as a base for livestock production implies that humans are exposed to OCP residues. In particular, the levels of four OCP groups (ΣDrins, ΣHeptachlorine, ΣChlordane, and ΣEndosulfan) detected in blue-footed booby plasma samples were above the Minimum Risk Levels (MRLs) proposed by the Agency for Toxic Substances and Disease Registry (ATSDR). Therefore, monitoring programs using seabirds as sentinels could lead to early warnings regarding the health of human populations

in the event of possible environmental pollution scenarios given that both blue-footed boobies and humans exploit the same resources.

5. Conclusions

The presence of all OCPs measured in the blue-footed booby plasma samples confirms the high biological persistence of these compounds in the environment, even with the legal restrictions that Mexico has imposed for their application. The sex-based variation detected in this study suggests that both intrinsic and extrinsic factors could be causing these variations. The high concentrations of all OCP affinity groups in the blue-footed boobies at the beginning of their breeding season suggests an elevated environmental availability of these pollutants that gradually decreases as the breeding season progresses and the rainy season ends and the dry season begins. The OCP residues were not related to the health biomarkers evaluated in blue-footed booby breeding individuals, which may be due to either the wide-ranging responses of these biomarkers or that the detected pollutant levels were below their negative-effect thresholds. Finally, this study shows the potential of seabirds as sentinels of pollution levels (specifically OCPs) in marine and coastal environments as well as the potential risks associated with the consumption of aquacultural and fishery products from the coastal ecosystems of Northwestern Mexico.

CRediT authorship contribution statement

Alberto Piña-Ortiz: Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Juan Pablo Ceyca-Contreras:** Investigation, Supervision, Visualization, Writing – review & editing. **Carlos Eduardo Covantes-Rosales:** Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – review & editing. **Miguel Betancourt-Lozano:** Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing. **José Alfredo Castillo-Guerrero:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Visualization, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We thank Comisión Nacional de Áreas Naturales Protegidas – Islas del Golfo de California – Sinaloa for logistic support, and to M. Guevara, E. González-Medina, A. Mendoza, M. Leal, M. Arvizú, S. Rendón, M. Lerma, F. Quesada, A. Leal, D. Brito, C. Franco, N. Albano, and J. Velázquez for fieldwork help. Laboratory work was completed in the Laboratorio de Cromatografía de gases del Centro de Investigación en Alimentación y Desarrollo A.C., Unidad Mazatlán with assistance of G. Aguilar-Zárate and C. Vargas. We thank Andrea Lievana MacTavish for English editing. We thank our two anonymous reviewers for their helpful comments that improved the manuscript.

Funding

This work was supported by the Fondo Mexicano para la Conservación de la Naturaleza A.C. [grant number PIE-2012-A-P-C-IGSI-12-12], Consejo Nacional de Ciencia y Tecnología [CONACYT, grant number I010/176/2012], and the Universidad de Guadalajara. APO was also supported with a scholarship provided by Centro de Investigación en Alimentación y Desarrollo A.C., and CECR and JPCC were supported with scholarships provided by CONACYT [grant numbers 326429 and 317081, respectively].

Ethical approval

The fieldwork -including wildlife management and sampling collection- was carried out with permission from the Dirección General de Vida Silvestre (DGVS, Mexico) under the permit numbers SGPA/DGVS/62712/12 and SGPA/DGVS/02923/13. We complied with all applicable institutional and/or national guidelines for the welfare and conservation of wildlife. The blue-footed boobies used for this study were not handled for more than 10 min, and as the smallest blood amount as possible was drawn for each individual. While the adults were sampled, we cared for their eggs and/or chicks until their parents returned to nest. No individual abandoned the nest after capture.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2021.112050.

References

Acosta-Velázquez J. and Vázquez-Lule A.D. 2009. Caracterización del sitio de manglar Santa María – La Reforma. Pp:1–18 In: Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (CONABIO) (ed.). Sitios de manglar con relevancia biológica y con necesidades de rehabilitación ecológica. CONABIO, México, D.F.

Agency for Toxic Substances and Disease Registry (ATSDR), 2007. Toxicological Profile for Heptachlor and Heptachlor Epoxide. Department of Health and Human Service. Atlanta, Georgia.

Agency for Toxic Substances and Disease Registry (ATSDR), 2019. Toxicological Profile for Endrin (Draft for Public Comment). Department of Health and Human Service. Atlanta, Georgia.

Alvarez-Arellano, A.D., Gaitán, M.J., 1994. Lagunas Costeras y el Litoral Mexicano: Geología. In: de la Lanza-Espino, G., Cáceres-Martínez, C. (Eds.), Lagunas Costeras y el Litoral Mexicano. UNAM-UABCS, México, D.F, pp. 13–74.

Ancona, S., Calixto-Albarrán, I., Drummond, H., 2012. Effect of El Niño on the diet of a specialist seabird, *Sula nebouxii*, in the warm eastern tropical Pacific. Mar. Ecol. Prog. Ser. 462, 273–286. https://doi.org/10.3354/meps09851.

Arellano-Aguilar, O., Betancourt-Lozano, M., Aguilar-Zárate, G., Ponce de Leon-Hill, C., 2017. Agrochemical loading in drains and rivers and its connection with pollution in coastal lagoons of the Mexican Pacific. Environ. Monit. Assess. 189, 270.

Barbosa, A., De Mas, E., Benzal, J., Diaz, J.I., Motas, M., Jerez, S., Pertierra, L., Benayas, J., Justel, A., Lauzurica, P., García-Peña, F.J., Serrano, T., 2013. Pollution and physiological variability in gentoo penguins at two rookeries with different levels of human visitation. Antarct. Sci. 25, 329–338. https://doi.org/10.1017/S0954102012000739/.

Blus, L.J., 1982. Further interpretation of the relation of organochlorine residues in brown pelican eggs to reproductive success. Environmental Pollution Series A, Ecological and Biological 28, 15–33. https://doi.org/10.1016/0143-1471(82)90042-3.

Bogan, J.A., Newton, I., 1977. Redistribution of DDE in Sparrowhawks during starvation. Bull. Environ. Contam. Toxicol. 18, 317–321. https://doi.org/10.1007/BF01683425.

Bond, A.L., Diamond, A.W., 2010. Nutrient allocation for egg production in six Atlantic seabirds. Can. J. Zool. 88, 1095–1102. https://doi.org/10.1139/Z10-082.

Borgå, K., Fisk, A.T., Hoekstra, P.F., Muir, D.C.G., 2004. Biological and chemical factors of importance in the bioaccumulation and trophic transfer of persistent organochlorine contaminants in Arctic marine food webs. Environ. Toxicol. Chem. 23, 2367–2385. https://doi.org/10.1897/03-518.

Bourgeon, S., Leat, E.K.H., Magnusdóttir, E., Fisk, A.T., Furness, R.W., Strøm, H., Hanssen, S.A., Petersen, Æ., Olafsdóttir, K., Borgå, K., Gabrielsen, G.W., Bustnes, J. O., 2012. Individual variation in biomarkers of health: influence of persistent organic pollutants in Great skuas (*Stercorarius skua*) breeding at different geographical locations. Environ. Res. 118, 31–39. https://doi.org/10.1016/j.envres.2012.08.004.

Bourgeon, S., Leat, E.K.H., Furness, R.W., Borgå, K., Hanssen, S.A., Bustnes, J.O., 2013. Dietary versus maternal sources of organochlorines in top predator seabird chicks: an experimental approach. Environ. Sci. Technol. 47, 5963–5970. https://doi.org/10.1021/es400442q.

Burger, J., 1993. Metals in avian feathers: bioindicators of environmental pollution. Rev. Environ. Toxicol. 5, 203–311.

Burger, J., Gochfeld, M., 2001. Effects of chemicals and pollution on seabirds. In: Schreiber, E.A., Burger, J. (Eds.), Biology of Marine Birds. CRC Press, Boca Raton, FL, pp. 485–525.

Burger, J., Gochfeld, M., 2004. Marine birds as sentinels of environmental pollution. Ecohealth 1, 263–274. https://doi.org/10.1007/s10393-004-0096-4.

Bustnes, J.O., Hanssen, S.A., Folstad, I., Erikstad, K.E., Hasselquist, D., Skaare, J.U., 2004. Immune function and organochlorine pollutants in Arctic breeding glaucous

gulls. Arch. Environ. Contam. Toxicol. 47, 530–541. https://doi.org/10.1007/s00244-003-3203-6.
Bustnes, J.O., Skaare, J.U., Berg, V., Tveraa, T., 2005. Interseasonal variation in blood concentrations of organochlorines in great black-backed gulls (*Larus marinus*). Environ. Toxicol. Chem. 24, 1801–1806. https://doi.org/10.1897/04-203R.1.
Bustnes, J.O., Tveraa, T., Henden, J.A., Varpe, O., Janssen, K., Skaare, J.U., 2006. Organochlorines in Antarctic and Arctic avian top predators: A comparison between the south polar skua and two species of northern hemisphere gulls. Environ. Sci. Technol. 40, 2826–2831. https://doi.org/10.1021/es051920q.
Bustnes, J.O., Bårdsen, B.-J., Moe, B., Herzke, D., Hanssen, S.A., Sagerup, K., Bech, C., Nordstad, T., Chastel, O., Tartu, S., Gabrielsen, G.W., 2017. Temporal variation in circulating concentrations of organochlorine pollutants in a pelagic seabird breeding in the high Arctic. Environ. Toxicol. Chem. 36, 442–448. https://doi.org/10.1002/etc.3560.
Carravieri, A., Bustamante, P., Tartu, S., Meillère, A., Labadie, P., Budzinski, H., Peluhet, L., Barbraud, C., Weimerskirch, H., Chastel, O., Cherel, Y., 2014. Wandering albatrosses document latitudinal variations in the transfer of persistent organic pollutants and mercury to Southern Ocean predators. Environ. Sci. Technol. 48, 14746–14755. https://doi.org/10.1021/es504601m.
Carvalho, F.P., González-Farias, F., Villeneuve, J.P., Cattini, C., Hernandez-Garza, M., Mee, L.D., Fowler, S.W., 2002. Distribution, fate and effects of pesticide residues in tropical coastal lagoons of northwestern Mexico. Environ. Technol. 23, 1257–1270. https://doi.org/10.1080/09593332308618321.
Castillo-Guerrero, J.A., Mellink, E., 2011. Occasional inter-sex differences in diet and foraging behavior of the blue-footed booby: maximizing chick rearing in a variable environment? J. Ornithol. 152, 269–277. https://doi.org/10.1007/s10336-010-0575-z.
Castillo-Guerrero, J.A., González-Medina, E., Fernández, G., 2014. Seabird colonies of the small islands of Bahía Santa María-La Reforma, Sinaloa, México. Waterbirds 37, 439–445. https://doi.org/10.1675/063.037.0412.
Ceyca, J.P., Torres-Bugarín, O., Castillo-Guerrero, J.A., Betancourt-Lozano, M., 2014. Seabird embryos as biomonitors of micronuclei genotoxic agents: potential application for the coast of Mexico. Avian Biology Research 7, 223–234. https://doi.org/10.3184/175815514X14162211300859.
Clark, P., Boardman, W., Raidal, S., 2009. Atlas of Clinical Avian Hematology. Wiley-Blackwell, West Sussex, U.K.
Colabuono, F.I., Vander-Pol, S.S., Huncik, K.M., Taniguchi, S., Petry, M.V., Kucklick, J.R., Montone, R.C., 2016. Persistent organic pollutants in blood samples of southern giant petrels (*Macronectes giganteus*) from the South Shetland Islands, Antarctica. Environ. Pollut. 216, 38–45. https://doi.org/10.1016/j.envpol.2016.05.041.
Comisión Nacional de Acuacultura y Pesca (CONAPESCA), 2017. Anuario Estadístico de Acuacultura y Pesca 2017. Comisión Nacional de Acuacultura y Pesca. Mazatlán, Sinaloa.
Comisión Nacional del Agua (CONAGUA). 2011. Estadísticas agrícolas de los distritos de riego. Año Agrícola 2009-2010. SEMARNAT. México, D.F.
Comisión Nacional del Agua (CONAGUA). 2020. Información Estadística Climatológica: Información de Estaciones Climatológicas. SEMARNAT. México, D.F. https://smn.conagua.gob.mx/es/climatologia/informacion-climatologica/informacion-estadistica-climatologica. (Consulted on: 15/05/20).
Davis, A.K., 2005. Effect of handling time and repeated sampling on avian white blood cell counts. Journal of Field Ornithology 76, 334–338. https://doi.org/10.1648/0273-8570-76.4.334.
Davis, A.K., Cook, K.C., Altizer, S., 2004. Leukocyte profiles in wild house finches with and without mycoplasmal conjunctivitis, a recently emerged bacterial disease. Ecohealth 1, 362–373. https://doi.org/10.1007/s10393-004-0134-2.
Davis, A.K., Maney, D.L., Maerz, J.C., 2008. The use of leukocyte profiles to measure stress in vertebrates: a review for ecologists. Funct. Ecol. 22, 760–772. https://doi.org/10.1111/j.1365-2435.2008.01467.x/.
De Mas, E., Benzal, J., Merino, S., Valera, F., Palacios, M.J., Cuervo, J.J., Barbosa, A., 2015. Erythrocytic abnormalities in three Antarctic penguin species along the Antarctic peninsula: biomonitoring of genomic damage. Polar Biol. 38, 1067–1074. https://doi.org/10.1007/s00300-015-1667-2.
Elliott, J.E., Norstrom, R.J., Keith, J.A., 1988. Organochlorines and eggshell thinning in northern gannets (Sula bassanus) from eastern Canada, 1968-1984. Environ. Pollut. 52, 81–102. https://doi.org/10.1016/0269-7491(88)90083-8.
Elliott, J.E., Morrissey, C.A., Henny, C.J., Inzunza, E.R., Shaw, P., 2007. Satellite telemetry and prey sampling reveal contaminant sources to Pacific northwest ospreys. Ecol. Appl. 17, 1223–1233. https://doi.org/10.1890/06-1213. 17555230.
Ene A., Bogdevich O. y Sion A. 2012. Levels and distribution of organochlorine pesticides (OCPs) and polycyclic aromatic hydrocarbons (PAHs) in topsoils from SE Romania. Sci. Total Environ. 439: 76–86. doi:https://doi.org/10.1016/j.scitotenv.2012.09.004.
EPA, 1996. Method 3630C silica gel cleanup. In: U.S. Enviromental Agency's Office (Ed.), CD-ROM Disclaimer-1 Revision 1 (EPA. U.S.A).
Espín S., García-Fernández A.J., Herzke D., Shore R.F, van Hattum, Martínez-López E., Coeurdassier M., Eulaers I., Fritsch C., Gómez-Ramírez P., Jaspers V.L.B., Krone O., Duke G., Helander B., Mateo R., Movalli P., Sonne C. and van den Brink N.W. 2016. Tracking pan-continental trends in environmental contamination using sentinel raptors-what types of samples should we use? Ecotoxicology 25:777–801. doi: https://doi.org/10.1007/s10646-016-1636-8.
Fenech, M., 2000. The in vitro micronucleus technique. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis 455, 81–95. https://doi.org/10.1016/S0027-5107(00)00065-8.
Fiedler, P.C., Mantua, N.J., 2017. How are warm and cool years in the California current related to ENSO? J. Geophys. Res. Oceans 122, 5936–5951. https://doi.org/10.1002/2017JC013094.
Furness, R.W., Camphuysen, C.J., 1997. Seabirds as monitors of the marine environment. ICES Journal of Marine Sciences 54, 726–737. https://doi.org/10.1006/jmsc.1997.0243.
García-de la Parra, L.M., Cervantes-Mojica, L.J., González-Valdivia, C., Martínez-Cordero, F.J., Aguilar-Zárate, G., Bastidas-Bastidas, P., Betancourt-Lozano, M., 2012. Distribution of pesticides and PCBs in sediments of agricultural drains in the Culiacan Valley, Sinaloa, Mexico. Arch. Environ. Contam. Toxicol. 63, 323–336. https://doi.org/10.1007/s00244-012-9780-5.
García-de la Parra L.M., González-Valdivia C., Cervantes-Mojica L. J., Aguilar-Zárate G., Bastidas P., Betancourt-Lozano M. 2013. Plaguicidas y PCB en sedimentos de granjas camaronícolas en un sistema costero de Sinaloa, México. Pp. 1–18 In: Botello A.V., Páez-Osuna F., Mendez L., Betancourt M., Álvarez Borrego S. and Lara R. (eds.). Pacífico Mexicano. Contaminación e impacto ambiental: diagnóstico y tendencias. UAC, UNAM-ICMYL, CIAD-Mazatlán, CIBNOR, CICESE.
Gilmour, M.E., Castillo-Guerrero, J.A., Fleishman, A.B., Hernández-Vázquez, S., Young, H.S., Shaffer, S.A., 2018. Plasticity of foraging behaviors in response to diverse environmental conditions. Ecosphere 9 (7), e02301. https://doi.org/10.1002/ecs2.2301.
González-Bernal, M.A., Mellink, E., Fong-Mendoza, J.R., 2002. Nesting birds of Farallón de San Ignacio, Sinaloa, México. Western Birds 33, 254–257.
González-Medina, E., Castillo-Guerrero, J.A., Herzka, S.Z., Fernández, G., 2017. Flexibility in food resource allocation in parents and selectivity for offpring: variations in δ15N and δ13C values during breeding of the blue-footed booby. Mar. Biol. 164, 38. https://doi.org/10.1007/s00227-017-3070-8.
González-Medina, E., Castillo-Guerrero, J.A., Herzka, S.Z., Fernández, G., 2018. High quality diet improves lipid metabolic profile and breeding performance in the blue-footed booby, a long-lived seabird. PLoS One 13 (2), e0193136. https://doi.org/10.1371/journal.pone.0193136.
Greichus, Y.A., Hannon, M.R., 1973. Distribution and biochemical effects of DDT, DDD and DDE in penned double-crested cormorants. Toxicol. Appl. Pharmacol. 26, 483–494. https://doi.org/10.1016/0041-008x(73)90286-x.
Gress, F., Risebrough, W., Anderson, D.W., Kiff, L.F., Jehl, J.R.J., 1973. Reproductive failures of double-crested cormorants in Southern California and Baja California. The Wilson Bulletin 85, 197–208.
Grisolia, C.K., 2002. A comparison between mouse and fish micronucleus test using cyclophosphamide, mytomycin C and various pesticides. Mutation Research/Genetic Toxicology and Environmental Mutagenesis 518, 145–150. https://doi.org/10.1016/S1383-5718(02)00086-4.
Guevara-Medina M.A., Castillo-Guerrero J.A. and González-Bernal M.A. 2008. Presencia y abundancia de aves de la isla Farallón de San Ignacio, Sinaloa. Huitzil 9:20–28. doi:10.28947/hrmo.2008.9.2.75.
Hayashi, M., Sofuni, T., Ishidate, M.J., 1983. An application of Acridine Orange fluorescent staining to the micronucleus test. Mutat. Res. Lett. 120, 241–247. https://doi.org/10.1016/0165-7992(83)90096-9.
Henriksen, E.O., Gabrielsen, G.W., Skaare, J.U., 1996. Levels and congener pattern of polychlorinated biphenyls in kittiwakes (*Rissa tridactyla*) in relation to mobilization of body-lipids associated with reproduction. Environ. Pollut. 92, 27–37. https://doi.org/10.1016/0269-7491(95)00087-9.
Henriksen, E.O., Gabrielsen, G.W., Skaare, J.U., 1998. Validation of the use of blood samples to assess tissue concentrations of organochlorines in glaucous gull (*Larus hyperboreus*). Chemosphere 37, 2627–2643. https://doi.org/10.1016/S0045-6535(98)00162-3.
Hernandez-Padilla, J.C., Ruiz-Barreiro, T.M., Salcedo-Bojorquez, S., Espinosa-Romero, M.J., Zetina-Rejon, M.J., Arreguin-Sanchez, F., 2017. The ecological role of *Opisthonema libertate* and *Cetengraulis mysticetus* on ecosystem order in the southeastern gulf of California, Mexico. Turk. J. Fish. Aquat. Sci. 17, 713–724. https://doi.org/10.4194/1303-2712-v17_4_07.
Houwen, B., 2001. The differential cell count. Lab. Hematol. 7, 89–100.
Instituto Nacional de Estadística y Geografía (INEGI). 2009. Censo Agropecuario 2007: VIII Censo Agrícola, Ganadero y Forestal. Instituto Nacional de Estadística y Geografía. México. https://www.inegi.org.mx/programas/cagf/2007/. (Consulted on: 12/04/20).
Instituto Nacional de Estadística y Geografía (INEGI). 2010. Anuario estadístico y geográfico de Sinaloa 2017. México. http://internet.contenidos.inegi.org.mx/contenidos/Productos/prod_serv/contenidos/espanol/bvinegi/productos/nueva_estruc/anuarios_2017/702825094898.pdf.
Jehl, J.R.J., 1973. Studies of a declining population of Brown pelicans in northwestern Baja California. Condor 75, 69–79. https://doi.org/10.2307/1366536.
Keith, J.O., 1966. Insecticide contamination in wetland habitats and their effects on fish-eating birds. Journal of Applied Ecology (Suppl.) 3, 71–85. https://doi.org/10.2307/2401446.
Knudsen, L.B., Borgå, K., Jørgensen, E.H., van Bavel, B., Schlabach, M., Verreault, J., Gabrielsen, G.W., 2007. Halogenated organic contaminants and mercury in northern fulmars (*Fulmarus glacialis*): levels, relationships to dietary descriptors and blood to liver comparison. Environ. Pollut. 146, 25–33. https://doi.org/10.1016/j.envpol.2006.06.017.
Li, J., Zhang, G., Guo, L., Xu, W., Li, X., Lee, C.S.L., Ding, A., Wang, T., 2007. Organochlorine pesticides in the atmosphere of Guangzhou and Hong Kong: regional sources and long-range atmospheric transport. Atmos. Environ. 41, 3889–3903. https://doi.org/10.1016/j.atmosenv.2006.12.052.
Li, Y., Zhang, H., Li, Q., Zhou, Q., Chen, X., Tu, C., Luo, Y., Christie, P., Hu, X., Li, L., 2016. Characteristics of residual organochlorine pesticides in soils under different land-use types on a coastal plain of the Yellow River Delta. Environ. Geochem. Health 38, 535–547. https://doi.org/10.1007/s10653-015-9738-4.
Lin, T., Li, J., Xu, Y., Liu, X., Luo, C., Cheng, H., Chen, Y., Zhang, G., 2012. Organochlorine pesticides in seawater and the surrounding atmosphere of

themarginal seas of China: spatial distribution, sources and air–water exchange. Sci. Total Environ. 435-436, 244–252. https://doi.org/10.1016/j.scitotenv.2012.07.028.
Lobato, E., Moreno, J., Merino, S., Sanz, J.J., Arriero, E., 2005. Haematological variables are good predictors of recruitment in nestling pied flycatchers (*Ficedula hypoleuca*). Ecoscience 12, 27–34. https://doi.org/10.2980/i1195-6860-12-1-27.1.
Mallory, M.L., Robinson, S.A., Hebert, C.E., Forbes, M.R., 2010. Seabirds as indicators of aquatic ecosystem conditions: a case for gathering multiple proxies of seabird health. Mar. Pollut. Bull. 60, 7–12. https://doi.org/10.1016/j.marpolbul.2009.08.024.
Matos-Lino, C., Ferreira-Azzolini, C.B., Valente-Nunes, D.S., Rocha-Silva, J.M., Noronha-da Silveira, M.I., 1998. Methods for the determination of organochlorine pesticide residues in human serum. J. Chromatogr. B 716, 147–152. https://doi.org/10.1016/S0378-4347(98)00284-9.
Mellink, E., Riojas-López, M.E., Luévano-Esparza, J., 2009. Organchlorine content and shell thickness in brown booby (Sula leucogaster) eggs in the Gulf of California and the southern Pacific coast of Mexico. Environ. Pollut. 157, 2184–2188. https://doi.org/10.1016/j.envpol.2009.02.001.
Montaño-Ley, Y., Páez-Osuna, F., 2014. Assessment of the tidal currents and pollutants dynamics associated with shrimp aquaculture effluents in SAMARE coastal lagoon (NW Mexico). Aquac. Res. 45, 1269–1282. https://doi.org/10.1111/are.12071.
Montes, A.M., González-Farias, F.A., Botello, A.V., 2012. Pollution by organochlorine pesticides in Navachiste-Macapule, Sinaloa, Mexico. Environ. Monit. Assess. 184, 1359–1369. https://doi.org/10.1007/s10661-011-2046-2.
Muñoz-Cifuentes, J., Becker, P.H., Sommer, U., Pacheco, P., Schlatter, R., 2003. Seabird eggs as bioindicators of chemical contamination in Chile. Environ. Pollut. 126, 123–137. https://doi.org/10.1016/S0269-7491(02)00400-1.
Nelson, J.B., 1978. The Sulidae: Gannets and Boobies. Oxford University Press, Oxford.
Norstrom, R.J., Clark, T.P., Jeffrey, D.A., Won, H.T., Gilman, A.P., 1986. Dynamics of organochlorine compounds in herring gulls (*Larus argentatus*): I. distribution and clearance of [^{14}C] DDE in freeliving herring gulls (*Larus argentatus*). Environ. Toxicol. Chem. 5, 41–48. https://doi.org/10.1002/etc.5620050106.
Osuna-Flores, I., Riva, M.C., 2002. Organochlorine pesticide residue concentrations in shrimps, sediments, and surface water from bay of Ohuira, Topolobampo, Sinaloa. Mexico. Bulletin of Environmental Contamination and Toxicology. 68, 532–539. https://doi.org/10.1007/s001280287.
Osuna-López, J.I., Frías-Espericueta, M.G., López-López, G., Zazueta-Padilla, H.M., Izaguirre-Fierro, G., Páez-Osuna, F., Ruiz-Fernández, A.C., Voltolina, D., 2009. Cd, Pb and organochlorine pesticides of *Mytella strigata* (Pelecypoda: Mytilidae) of six coastal lagoons of NW Mexico. Boletín de Investigaciones Marinas y Costeras. 38, 233–239. http://hdl.handle.net/1834/3618.
Oudi, A., Chokri, M.A., Hammouda a., Chaabane R., Badraoui R., Besnard A. and Santos R., 2019. Physiological impacts of pollution exposure in seabird's progeny nesting in a Mediterranean contaminated area. Mar. Pollut. Bull. 142, 196–205. https://doi.org/10.1016/j.marpolbul.2019.02.056.
Páez-Osuna, F., Ramírez-Reséndiz, G., Ruiz-Fernández, A.C., Soto Martínez, M.F., 2007. La Contaminación por Nitrógeno y Fósforo en Sinaloa: Flujos, Fuentes, Efectos y Opciones de manejo. In: Páez-Osuna, F. (Ed.), La Serie las Lagunas Costeras de Sinaloa (UNAM-El Colegio de Sinaloa).
Peakall, D.B., 1986. Accumulation and effects on birds. In: Aaid, J.S. (Ed.), PCBs in the Environment, II. CRC Press, Boca Raton, FL, pp. 31–47.
Peakall, D.B., Fox, G.A., 1987. Toxicological investigations of pollutant related effects in Great Lakes gulls. Environ. Health Perspect. 71, 187–193. https://doi.org/10.1289/ehp.8771187.
Peig, J., Green, A.J., 2009. New perspectives for estimating body condition from mass/length data: the scaled mass index as an alternative method. OIKOS 118, 1883–1891. https://doi.org/10.1111/j.1600-0706.2009.17643.x.
Pérez, C., Velando, A., Munilla, I., López-Alonso, M., Oro, D., 2008. Monitoring polycyclic aromatic hydrocarbon pollution in the marine environment after the prestige oil spill by means of seabird blood analysis. Environ. Sci. Technol. 42, 707–713. https://doi.org/10.1021/es071835d.
Roscales, J.L., González-Solís, J., Jiménez, B., 2011. Geographic and trophic patterns of PCBs and DDTs in Mediterranean and NE Atlantic seabirds: can POPs adversely affect Mediterranean seabirds? Organohalogen Compd. 73, 882–885.
Sarkar, S.K., Bhattacharya, B.D., Bhattacharya, A., Chatterjee, M., Alam, A., Satpathy, K. K., Jonathan, M.P., 2008. Occurrence, distribution and possible sources of organochlorine pesticide residues in tropical coastal environment of India: an overview. Environ. Int. 34, 1062–1071. https://doi.org/10.1016/j.envint.2008.02.010.
Schmid, W., 1975. The micronucleus test. Mutation Research/Environmental Mutagenesis and Related Subjects 31, 9–15. https://doi.org/10.1016/0165-1161(75)90058-8.
Sebastiano, M., Bustamante, P., Costantini, D., Eulaers, I., Malarvannan, G., Mendez-Fernandez, P., Churlaud, C., Blévin, P., Hauselmann, A., Dell'Omo, G., Covaci, A., Eens, M., Chastel, O., 2016. High levels of mercury and low levels of persistent organic pollutants in a tropical seabird in French Guiana, the magnificent frigatebird, *Fregata magnificens*. Environ. Pollut. 214, 384–393. https://doi.org/10.1016/j.envpol.2016.03.070.
Sebastiano, M., Bustamante, P., Eulaers, I., Malarvannan, G., Mendez-Fernandez, P., Churlaud, C., Blévin, P., Hauselmann, A., Covaci, A., Eens, M., Costantini, D., Chastel, O., 2017. Trophic ecology drives contaminant concentrations within a tropical seabird community. Environ. Pollut. 227, 183–193. https://doi.org/10.1016/j.envpol.2017.04.040.
Servicio de Información Agroalimentaria y Pesquera (SIAP). 2010. Anuario Agropecuario. Secretaría de Agricultura y Desarrollo Rural. http://www.siap.gob.mx 09/09/2012. (Consulted on: 24/04/20).
Södergren, A., Ulfstrand, S., 1972. DDT and PCB relocate when caged robins use fat reserves. Ambio 1, 36–40. https://www.jstor.org/stable/4311942.
Sonne, C., Rigét, F.F., Leat, E.H.K., Bourgeon, S., Borgå, K., Strøm, H., Hanssen, S.A., Gabrielsen, G.W., Petersen, A., Olafsdottir, K., Magnusdottir, E., Bustnes, J.O., Furness, R.W., Kjelgaard-Hansen, M., 2013. Organohalogen contaminants and blood plasma clinical–chemical parameters in three colonies of North Atlantic great skua (*Stercorarius skua*). Ecotoxicol. Environ. Saf. 92, 245–251. https://doi.org/10.1016/j.ecoenv.2013.02.012.
Sun, J., Pan, Tsang, D.C., Zhan, Y., Zhu, L., Li, X., 2018. Organic contamination and remediation in the agricultural soils of China: a critical review. Sci. Total Environ. 615, 724–740. https://doi.org/10.1016/j.scitotenv.2017.09.271.
Torres, R., Drummond, H., 1999. Does large size make daughters of the blue-footed booby more expensive than sons? J. Anim. Ecol. 68, 1133–1141. https://doi.org/10.1046/j.1365-2656.1999.00357.x.
Trefry, S.A., Diamond, A.W., Spencer, N.C., Mallory, M.L., 2013. Contaminants in magnificent frigatebird eggs from Barbuda, West Indies. Mar. Pollut. Bull. 75, 317–321. https://doi.org/10.1016/j.marpolbul.2013.07.046.
van den Brink, N.W., Van Franeker, J.A., De Ruiter-Dijkman, E.M., 1998. Fluctuating concentrations of organochlorine pollutants during a breeding season in two Antarctic seabirds: Adélie penguin and southern fulmar. Environ. Toxicol. Chem. 17, 702–709. https://doi.org/10.1002/etc.5620170426.
Vander-Pol, S.S., Becker, P.R., 2007. Monitoring contaminants in seabirds: the importance of specimen banking. Mar. Ornithol. 35, 113–118.
Velando, A., Alonso-Alvarez, C., 2003. Differential body condition regulation by males and females in response to experimental manipulations of brood size and parental effort in the blue-footed booby. J. Anim. Ecol. 72, 846–856. https://doi.org/10.1046/j.1365-2656.2003.00756.x.
Verreault, J., Villa, R.A., Gabrielsen, G.W., Skaare, J.U., Letcher, R.J., 2006. Maternal transfer of organohalogen contaminants and metabolites to eggs of Arctic-breeding glaucous gulls. Environ. Pollut. 144, 1053–1060. https://doi.org/10.1016/j.envpol.2005.10.055.
Walker, C.H., Knight, G.C., 1981. The hepatic microsomal enzymes of sea birds and their interaction with liposoluble pollutants. Aquat. Toxicol. 1, 343–354. https://doi.org/10.1016/0166-445X(81)90027-8.
Wang, B., Wu, C., Liu, W., Teng, Y., Luo, Y., Christie, P., Guo, D., 2016. Levels and patterns of organochlorine pesticides in agricultural soils in an area of extensive historical cotton cultivation in Henan province, China. Environ. Sci. Pollut. Res. 23, 6680–6689. https://doi.org/10.1007/s11356-015-5864-x.
Weber, J., Halsall, C.J., Muir, D., Teixeira, C., Small, J., Solomon, K., Hermanson, M., Hung, H., Bidleman, T., 2010. Endosulfan, a global pesticide: A review of its fate in the environment and occurrence in the Arctic. Sci. Total Environ. 408, 2966–2984. https://doi.org/10.1016/j.scitotenv.2009.10.077.
Weimerskirch, H., Shaffer, S.A., Tremblay, Y., Costa, D.P., Gadenne, H., Kato, A., Ropert-Coudert, Y., Sato, K., Aurioles, D., 2009. Speciesand sex-specific differences in foraging behaviour and foraging zones in blue-footed and brown boobies in the Gulf of California. Mar. Ecol. Prog. Ser. 391, 267–278. https://doi.org/10.3354/meps07981.
Willett, K.L., Ulrich, E.M., Hites, R.A., 1998. Differential toxicity and environmental fates of Hexachlorocyclohexane isomers. Environ. Sci. Technol. 32, 2197–2207. https://doi.org/10.1021/es9708530.
Woronzoff-Dashkoff, K.K., 2002. The Wright-Giemsa stain Secretsrevealed. Clin. Lab. Med. 22, 15–23. https://doi.org/10.1016/S0272-2712(03)00065-9.
Ya, M., Wu, Y., Li, Y., Wang, X., 2017. Anthropogenic organochlorine compounds as potential tracers for regional water masses: A case study of estuarine plume, coastal eddy, wind-driven upwelling and long-range warm current. Chemosphere 170, 75–82. https://doi.org/10.1016/j.chemosphere.2016.12.010.
Yamashita, N., Tanabe, S., Ludwig, J.P., Kurita, H., Ludwig, M.E., Tatsukawa, R., 1993. Embryonic abnormalities and organochlorine contamination in double-crested cormorants (*Phalacrocorax auritus*) and Caspian terns (*Hydroprogne caspia*) from the upper Great Lakes in 1998. Environ. Pollut. 79, 163–173. https://doi.org/10.1016/0269-7491(93)90066-W.
Zar J.H. 2010. Biostatistical Analysis.5th ed. Pearson Prentice Hall, New Jersey, U.S.A.
Zavalaga, C.B., Benvenutti, S., Dall'Antonia, L., Emslie, S.D., 2007. Diving behavior of the blue-footed boobies *Sula nebouxii* in northern Peru in relation to sex, body size and prey type. Mar. Ecol. Prog. Ser. 336, 291–303. https://doi.org/10.3354/meps336291.
Zavalaga, C.B., Benvenutti, S., Dall'Antonia, L., Emslie, S.D., 2008. Foraging areas of breeding blue-footed boobies *Sula nebouxii* in northern Peru, as determined by direction recorders. J. Avian Biol. 39, 405–412. https://doi.org/10.1111/j.2008.0908-8857.04275.x.

Appendix IV

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

ALBERTO PIÑA-ORTIZ, VLADISLAV MARCUK, SALVADOR GÓMEZ-HERNÁNDEZ, JOSÉ ALFREDO CASTILLO-GUERRERO, PETRA QUILLFELDT

Marine Ornithology Volume 53 Issue 1, 75－82, (2025)

Article accepted and *In press*

Contributions: Lead author, Conceptualization, Fieldwork, Formal analysis and investigation, Methodology, Writing—original draft preparation, Writing—review and editing, Visualization, Funding acquisition, Resources

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

ALBERTO PIÑA-ORTIZ[1]*, VLADISLAV MARCUK[1], SALVADOR GÓMEZ-HERNÁNDEZ[2], JOSÉ ALFREDO CASTILLO-GUERRERO[2], & PETRA QUILLFELDT[1]

[1]*Department of Animal Ecology and Systematics, Justus Liebig University Giessen, Heinrich-Buff-Ring 26, 35392, Giessen, Germany*
**(Alberto.Pina-Ortiz@bio.uni-giessen.de)*
[2]*Departamento de Estudios para el Desarrollo Sustentable de la Zona Costera, Centro Universitario de la Costa Sur, Universidad de Guadalajara, Gómez Farías 82, San Patricio-Melaque, Municipio de Cihuatlán, Jalisco, 48980, Mexico*

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 five-week-old chick. The adult was ringed, and a GPS tag (CatLog-S2; Perthold Engineering LLC, 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 were used to identify the main prey categories—fish and cephalopods—based on prior knowledge of the species' diet (Berry et al., 2017; Waap, 2015). 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).

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.

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; Tershy & Breese, 1997). 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).

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

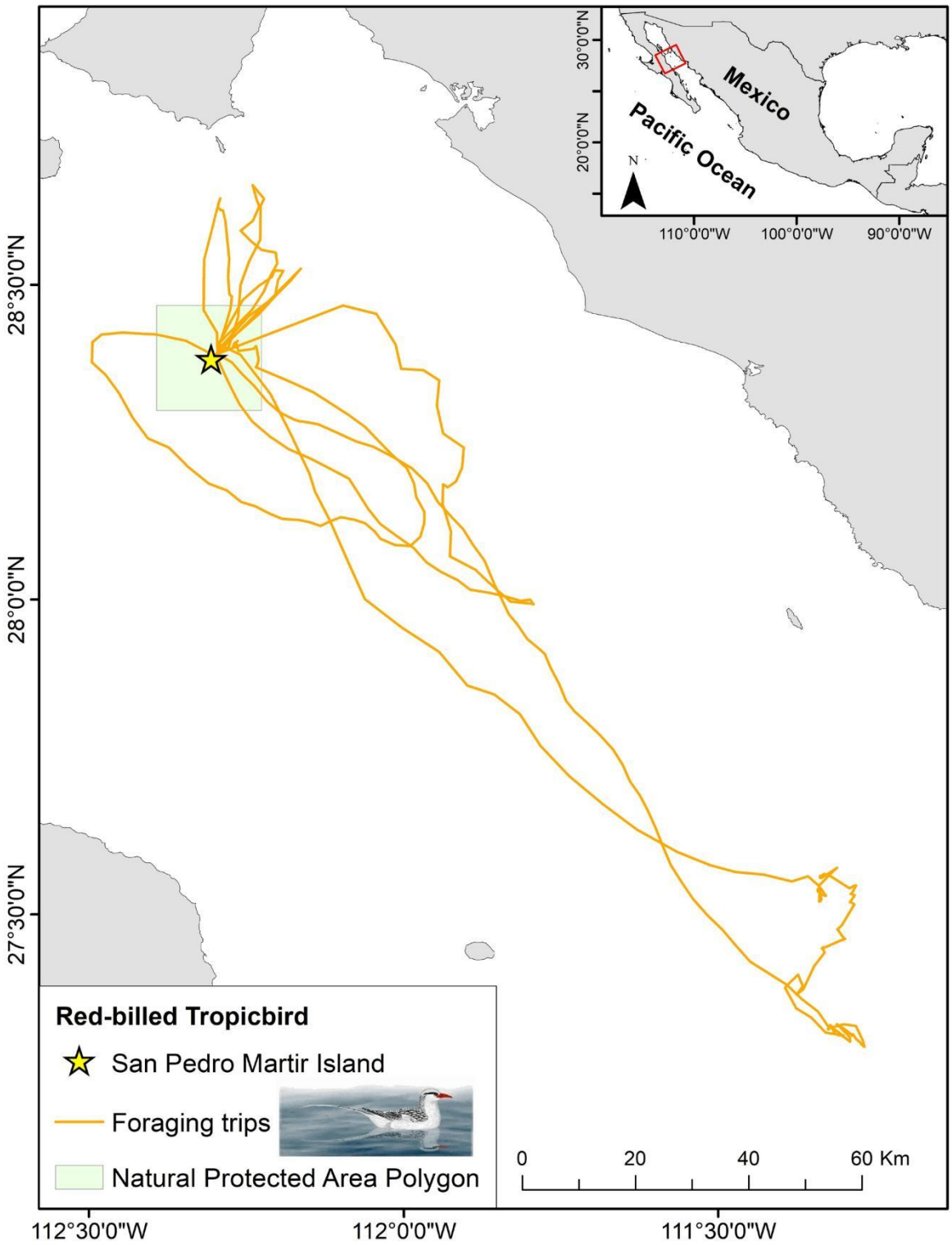


Fig. 2. Foraging trips made by the Red-billed Tropicbird *Phaethon aethereus* female parent prior to its chick excreting a wire fragment on 17 March 2021. The polygon of the Natural Protected Area is indicated in light green (Diario Oficial de la Federación, 2002).

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 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.

REFERENCES

Almaguer-Hernández, A. M. (2016). *Ecología Reproductiva y Trófica del Rabijunco Pico Rojo* Phaethon aethereus *(Linnaeus, 1758) en la Isla Peña Blanca, Colima, México* [Bachelor's thesis, Universidad de Guadalajara].

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology, 215*(3), 403–410. https://doi.org/10.1016/S0022-2836(05)80360-2

Barrett, R. T., Camphuysen, C. J., Anker-Nilssen, T., Chardine, J. W., Furness, R. W., Garthe, S., Hüppop, O., Leopold, M. F., Montevecchi, W. A., & Veit, R. R. (2007). Diet studies of seabirds: A review and recommendations. *ICES Journal of Marine Science, 64*(9), 1675–1691. https://doi.org/10.1093/icesjms/fsm152

Beal, M., Oppel, S., Handley, J., Pearmain, E. J., Morera-Pujol, V., Carneiro, A. P. B., Davies, T. E., Phillips, R. A., Taylor, P. R., Miller, M. G. R., Franco, A. M. A., Catry, I., Patrício, A. R., Regalla, A., Staniland, I., Boyd, C., Catry, P., & Dias, M. P. (2021). track2KBA: An R package for identifying important sites for biodiversity from tracking data. *Methods in Ecology and Evolution, 12*(12), 2372–2378. https://doi.org/10.1111/2041-210X.13713

Berry, T. E., Osterrieder, S. K., Murray, D. C., Coghlan, M. L., Richardson, A. J., Grealy, A. K., Stat, M., Bejder, L., & Bunce, M. (2017). DNA metabarcoding for diet analysis and biodiversity: A case study using the endangered Australian Sea Lion (*Neophoca cinerea*). *Ecology and Evolution, 7*(14), 5435–5453. https://doi.org/10.1002/ece3.3123

Cartraud, A. E., Le Corre, M., Turquet, J., & Tourmetz, J. (2019). Plastic ingestion in seabirds of the western Indian Ocean. *Marine Pollution Bulletin, 140*(March), 308–314. https://doi.org/10.1016/j.marpolbul.2019.01.065

Castillo-Guerrero, J. A., Guevara-Medina, M. A., & Mellink, E. (2011). Breeding ecology of the Red-billed Tropicbird *Phaethon aethereus* under contrasting environmental conditions in the Gulf of California. *Ardea, 99*(1): 61–71. https://doi.org/10.5253/078.099.0108

Castillo-Guerrero, J. A., Piña-Ortiz, A., Ventura-Trejo, J., & Zatarain-González, D. J. (2022, May 24). *Population status and priority marine areas for seabirds on San Pedro Martir Island*. Sonoran Joint Venture. https://sonoranjv.org/seabirds-san-pedro-martir-island/

Castro-Hernández, J. J., & Santana-Ortega, A. T. (2000). *Synopsis of biological data on the Chub Mackerel (*Scomber japonicus *Houttuyn, 1782)*. FAO Fisheries Synopsis No. 157. Food and Agriculture Organization of the United Nations. https://www.fao.org/4/x4287e/x4287e.pdf

Cisneros, M. A., Estrada, J., & Montemayor, G. (1990). Growth, mortality and recruitment of exploited small pelagic fishes in the Gulf of California, Mexico. *Fishbyte, 8*(1), 15–17. https://hdl.handle.net/20.500.12348/3174

Cisneros-Mata, M. A., Nevárez-Martínez, M. O., & Hammann, M. G. (1995). *The rise and fall of the Pacific sardine,* Sardinops sagax caeruleus *Girard, in the Gulf of California, Mexico. CalCOFI Reports, 36*, 136–143. https://calcofi.org/downloads/publications/calcofireports/v36/CalCOFI_Rpt_Vol_36_1995.pdf

Cisneros-Montemayor, A. M., Cheung, W. W. L., & Ota, Y. (Eds.). (2019). *Predicting future oceans: Sustainability of ocean and human systems amidst global environmental change*. Elsevier Science. https://doi.org/10.1016/C2018-0-02416-0

Deagle, B. E., Kirkwood, R., & Jarman, S. N. (2009). Analysis of Australian Fur Seal diet by pyrosequencing prey DNA in faeces. *Molecular Ecology, 18*(9), 2022–2038. https://doi.org/10.1111/j.1365-294X.2009.04158.x

Diario Oficial de la Federación. (2002, June 13). *Decreto por el que se declara área natural protegida con la categoría de reserva de la biosfera, la región denominada Isla San Pedro Mártir, ubicada en el Golfo de California, frente a las costas del Municipio de Hermosillo, Estado de Sonora, con una superficie total de 30,165-23-76.165 hectáreas*. https://simec.conanp.gob.mx/pdf_decretos/25_decreto.pdf

Diop, N., Zango, L., Beard, A., Ba, C. T., Ndiaye, P. I., Henry, L., Clingham, E., Oppel, S., & González-Solís, J. (2018). Foraging ecology of tropicbirds breeding in two contrasting marine environments in the tropical Atlantic. *Marine Ecology Progress Series, 607*, 221–236. https://doi.org/10.3354/meps12774

Galaxy Community. (2022). The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2022 update. *Nucleic Acids Research, 50*(W1), W345–W351. https://doi.org/10.1093/nar/gkac247

Hammer, S., Nager, R.G., Johnson P.C.D., Furness, R.W., & Provencher, J.F. (2016). Plastic Debris in Great Skua (Stercorarius skua) Pellets Corresponds to Seabird Prey Species. Marine Pollution Bulletin, 103(1–2), 206–210. https://doi.org/10.1016/j.marpolbul.2015.12.018

Holland, E.R., Mallory, M.L., & Shutler, D. (2016). Plastics and Other Anthropogenic Debris in Freshwater Birds from Canada. Science of the Total Environment, 571, 251–258. https://doi.org/10.1016/j.scitotenv.2016.07.158

Hunter, J. R., & Dorr, H. (1982). Thresholds for filter feeding in Northern Anchovy, *Engraulis mordax*. *CalCOFI Reports, 23*, 198–204. https://calcofi.org/downloads/publications/calcofireports/v23/CalCOFI_Rpt_Vol_23_1982.pdf

Hyrenbach, K. D., Hester, M. M., Johnson, J. A., Lyday, S., Bingham, S., & Pawloski, J. (2013). First evidence of plastic ingestion by White-tailed Tropicbirds from O'ahu, Hawai'i. *Marine Ornithology, 41*(2), 167–169. Retrieved from http://www.marineornithology.org/article?rn=1035

Kibria, M. G., Masuk, N. I., Safayet, R., Nguyen, H. Q. & Mourshed, M. (2023). Plastic waste: Challenges and opportunities to mitigate pollution and effective management. *International Journal of Environmental Research, 17*, Article 20. https://doi.org/10.1007/s41742-023-00507-z

Laist, D. W. (1997). Impacts of marine debris: Entanglement of marine life in marine debris including a comprehensive list of species with entanglement and ingestion records. In J. M. Coe & D. B. Rogers (Eds.), *Marine debris: Sources, impacts, and solutions* (pp. 99–139). Springer. https://doi.org/10.1007/978-1-4613-8486-1_10

Leray, M., Yang, J. Y., Meyer, C. P., Mills, S. C., Agudelo, N., Ranwez, V., Boehm, J. T., & Machida, R. J. (2013). A new versatile primer set targeting a short fragment of the mitochondrial COI region for metabarcoding metazoan diversity: Application for characterizing coral reef fish gut contents. *Frontiers in Zoology, 10*, Article 34. https://doi.org/10.1186/1742-9994-10-34

Lo, N. C. H., Dorval, E., Funes-Rodríguez, R., Hernández-Rivas, M. E., Huang, Y., & Fan, Z. (2010). Utilities of larval densities of Pacific Mackerel (Scomber japonicus) off California, USA, and west coast of Mexico from 1951 to 2008, as spawning biomass indices. *Ciencia Pesquera, 18*(2), 59–75. https://www.gob.mx/imipas/documentos/revista-ciencia-pesquera-volumen-18-numero-2

Louw, G. G., van der Lingen, C. D., & Gibbons, M. J. (1998). Differential feeding by Sardine *Sardinops sagax* and Anchovy *Engraulis capensis* recruits in mixed shoals. *South African Journal of Marine Science, 19*(1), 227–232. https://doi.org/10.2989/025776198784126647

Madden, H., Boehm, H., & Mielke, L. (2023). Foraging ecology of Red-Billed Tropicbirds on Saba, Caribbean Netherlands, during early chick-rearing. *Ardea, 111*(2), 463–475. https://doi.org/10.5253/arde.2022.a14

Madden, H., & Eggermont, E. (2020). First evidence of plastic ingestion by Red-billed Tropicbirds *Phaethon aethereus* from St. Eustatius, Caribbean Netherlands. *Marine Ornithology, 48*(2), 157–160. https://doi.org/10.5038/2074-1235.48.2.1366

Madden, H., Satgé, Y., Wilkinson, B., & Jodice, P. G. R. (2022). Foraging ecology of Red-billed Tropicbird *Phaethon aethereus* in the Caribbean during early chick rearing revealed by GPS rracking. *Marine Ornithology, 50*(2): 165–175. https://doi.org/10.5038/2074-1235.50.2.1486

Marcuk, V., Piña-Ortiz, A., Castillo-Guerrero, J. A., Masello, J. F., Bustamante, P., Griep, S., & Quillfeldt, P. (2024). Trophic plasticity of a tropical seabird revealed through DNA metabarcoding and stable isotope analyses. *Marine Environmental Research, 199*(July), Article 106627. https://doi.org/10.1016/j.marenvres.2024.106627

Masello, J. F., Barbosa, A., Kato, A., Mattern, T., Medeiros, R., Stockdale, J. E., Kümmel, M. N., Bustamante, P., Belliure, J., Benzal, J., Colominas-Ciuró, R., Menéndez-Blázquez, J., Griep, S., Goesmann, A., Symondson, W. O. C., & Quillfeldt, P. (2021). How animals distribute themselves in space: Energy landscapes of Antarctic avian predators. *Movement Ecology 9*, Article 24. https://doi.org/10.1186/s40462-021-00255-9

McInnes, J. C., Alderman, R., Lea, M.-A., Raymond, B., Deagle, B. E., Phillips, R. A., Stanworth, A., Thompson, D. R., Catry, P., Weimerskirch, H., Suazo, C. G., Gras, M., & Jarman, S. N. (2017). High occurrence of jellyfish predation by Black-browed and Campbell albatross identified by DNA metabarcoding. *Molecular Ecology, 26*(18), 4831–4845. https://doi.org/10.1111/mec.14245

National Oceanic and Atmospheric Administration. (2024, March 22). *What is marine debris?* Marine Debris Program. https://marinedebris.noaa.gov/discover-marine-debris/what-marine-debris

Navarro, A., Pérez Luzardo, O., Gómez, M., Acosta-Dacal, A., Martínez, I., de la Rosa, J. F., Macías-Montes, A., Suárez-Pérez, A., & Herrera, A. (2023). Microplastics ingestion and chemical pollutants in seabirds of Gran Canaria (Canary Islands, Spain). *Marine Pollution Bulletin, 186*(January), Article 114434. https://doi.org/10.1016/j.marpolbul.2022.114434

Nelson, J. B. (2006). *Pelicans, cormorants, and their relatives: The Pelecaniformes*. Oxford University Press. https://global.oup.com/academic/product/pelicans-cormorants-and-their-relatives-9780198577270

Nevárez-Martínez, M. O., Lluch-Belda, D., Cisneros-Mata, M. A., Santos-Molina, J. P., Martínez-Zavala, M. A., & Lluch-Cota, S. E. (2001). Distribution and abundance of the Pacific Sardine (*Sardinops sagax*) in the Gulf of California and their relation with the environment. *Progress in Oceanography, 49*(1–4), 565–580. https://doi.org/10.1016/S0079-6611(01)00041-6

Nunes, L. S., Silva, A. G., Espínola, L. A., Blettler, M. C. M., & Simões, N. R. (2021). Intake of microplastics by commercial fish: A Bayesian approach. *Environmental Monitoring and Assessment 193*, Article 402. https://doi.org/10.1007/s10661-021-09156-1

O'Connell, C. P., & Zweifel, J. R. (1972). A laboratory study of particulate and filter feeding of the Pacific Mackerel, *Scomber japonicus*. *Fishery Bulletin, 70*(4), 973–981. https://swfsc-publications.fisheries.noaa.gov/publications/CR/1972/7230.pdf

Ostle, C., Thompson, R. C., Broughton, D., Gregory, L., Wootton, M., & Johns, D. G. (2019). The rise in ocean plastics evidenced from a 60-year time series. *Nature Communications, 10*, Article 1622. https://doi.org/10.1038/s41467-019-09506-1

Petry, M. V., da Silva Fonseca, V. S., & Scherer, A. L. (2007). Analysis of stomach contents from the Black-Browed Albatross, *Thalassarche melanophris*, on the coast of Rio Grande do Sul, Southern Brazil. *Polar Biology, 30*, 321–325. https://doi.org/10.1007/s00300-006-0186-6

Piña-Ortiz, A., González-Zamora, D. A., Paz, J. A., Hernández-Vázquez, S., Mellink, E., Bustamante, P., Quillfeldt, P., & Castillo-Guerrero, J. A. (2024). Parental duties and foraging strategies of a tropical pelagic seabird (*Phaethon aethereus*, Aves: Phaethontidae) during the breeding season. *Marine Biology, 171*, Article 64. https://doi.org/10.1007/s00227-023-04375-1

Piña-Ortiz, A., Hernández-Vázquez, S., Fernández, G., & Castillo-Guerrero, J. A. (2018). Distribution and population size of the Red-billed Tropicbird (*Phaethon aethereus*) in Mexico. *Waterbirds, 41*(2), 135–144. https://doi.org/10.1675/063.041.0204

Provencher, J. F., Bond, A. L., Avery-Gomm, S., Borrelle, S. B., Bravo Rebolledo, E. L., Hammer, S., Kühn, S., Lavers, J. L., Mallory, M. L., Trevail, A., & van Franeker, J. A. (2017). Quantifying ingested debris in marine megafauna: A review and recommendations for standardization. *Analytical Methods, 2017*(9), 1454–1469. https://doi.org/10.1039/C6AY02419J

R Core Team. (2023). *R* (version 4.3.1) [Computer software]. The R Foundation for Statistical Computing. https://www.r-project.org/

Rapp, D. C., Youngren, S. M., Hartzell, P., & Hyrenbach, K. D. (2017). Community-wide patterns of plastic ingestion in seabirds breeding at French Frigate Shoals, Northwestern Hawaiian Islands. *Marine Pollution Bulletin, 123*(1–2), 269–278. https://doi.org/10.1016/j.marpolbul.2017.08.047

Robards, M. D. (1993). *Plastic ingestion by North Pacific seabirds*. Report NOAA-43ABNF203014. US Department of Commerce.

Roman, L., Schuyler, Q. A., Hardesty, B. D., & Townsend, K. A. (2016). Anthropogenic debris ingestion by avifauna in eastern Australia. *PLoS ONE, 11*(8), Article e0158343. https://doi.org/10.1371/journal.pone.0158343

RStudio Team. (2023). *RStudio Desktop* (version 2023.06.1 + 524 'Mountain Hydrangea') [Computer software]. RStudio. https://posit.co/download/rstudio-desktop/

Ryan, P. G. (2016). Ingestion of plastics by marine organisms. In H. Takada & H. K. Karapanagioti (Eds.), *Hazardous chemicals associated with plastics in the marine environment* (pp. 235–266). Springer Cham. https://doi.org/10.1007/698_2016_21

Schmaltz, E., Melvin, E. C., Diana, Z., Gunady, E. F., Rittschof, D., Somarelli, J. A., Virdin, J., Dunphy-Daly, M. M. (2020). Plastic pollution solutions: Emerging technologies to prevent and collect marine plastic pollution. *Environment International, 144*(November), Article 106067. https://doi.org/10.1016/j.envint.2020.106067

Schuyler, Q., Hardesty, B. D., Wilcox, C., & Townsend, K. (2014). Global analysis of anthropogenic debris ingestion by sea turtles. *Conservation Biology, 28*(1), 129–139. https://doi.org/10.1111/cobi.12126

Schwartzkopf, B. D., Dorval, E., James, K. C., Walker, J. M., Snodgrass, O. E., Porzio, D. L., & Erisman, B. E. (2022). *A summary report of life history information on the central subpopulation of Northern Anchovy (*Engraulis mordax*) for the 2021 stock assessment*. NOAA Technical Memorandum NMFS-SWFSC-659. Southwest Fisheries Science Center, National Oceanic and Atmospheric Administration. https://doi.org/10.25923/ckvg-va49

Sileo, L., Sievert, P. R., Samuel, M. D., & Fefer, S. I. (1990). Prevalence and characteristics of plastic ingested by Hawaiian seabirds. In R. S. Shomura & M. L. Godfrey (Eds.), *Proceedings of the 2nd International Conference on Marine Debris, 02–07 April 1989, Honolulu, Hawai'i* (pp. 665–681). NOAA Technical Memorandum NOAA-TM-NMFS-SWFSC-154. US Department of Commerce, National Oceanic and Atmospheric Administration.

Sindermann, C. J. (Ed.). (1995). *Ocean pollution: Effects on living resources and humans*. CRC Press.

Spear, L. B., Ainley, D. G., & Ribic, C. A. (1995). Incidence of plastic in seabirds from the tropical Pacific, 1984–91: Relation with distribution of species, sex, age, season, year and body weight. *Marine Environmental Research, 40*(2), 123–146. https://doi.org/10.1016/0141-1136(94)00140-K

Stonehouse, B. (1962). The tropic birds (genus *Phaethon*) of Ascension Island. *Ibis, 103B*(2), 124–161. https://doi.org/10.1111/j.1474-919X.1962.tb07242.x

Su, L., Xiong, X., Zhang, Y., Wu, C., Xu, X., Sun, C., & Shi, H. (2022). Global transportation of plastics and microplastics: A critical review of pathways and influences. *Science of the Total Environment, 831*(July), Article 154884. https://doi.org/10.1016/j.scitotenv.2022.154884

Tershy, B. R., & Breese, D. (1997). The birds of San Pedro Mártir Island, Gulf of California, Mexico. *Western Birds, 28*(2), 96–107. https://westernfieldornithologists.org/publications/journal/journal-volume-28-2/

Tokunaga, Y., Okochi, H., Tani, Y., Niida, Y., Tachibana, T., Saigawa, K., Katayama, K., Moriguchi, S., Kato, T., & Hayama, S. (2023). Airborne microplastics detected in the lungs of wild birds in Japan. *Chemosphere, 321*(April), Article 138032. https://doi.org/10.1016/j.chemosphere.2023.138032

Vandenabeele, S. P., Shepard, E. L., Grogan, A. & Wilson, R. P. (2012). When three per cent may not be three per cent: Device-equipped seabirds experience variable fight constraints. *Marine Biology, 159*, 1–14. https://doi.org/10.1007/s00227-011-1784-6

van der Lingen, C. D. (1994). Effect of particle size and concentration on the feeding behaviour of adult pilchard *Sardinops sagax*. *Marine Ecology Progress Series, 109*(1), 1–13. https://doi.org/10.3354/meps109001

van Franeker, J. A., & Meijboom, A. (2002). *LITTER NSV: Marine litter monitoring by Northern Fulmars (A pilot study)*. Alterra-rapport No. 401. Alterra. https://edepot.wur.nl/45695

Velarde, E., Ezcurra, E., & Anderson, D. W. (2013). Seabird diets provide early warning of sardine fishery declines in the Gulf of California. *Scientific Reports 3*, Article 1332. https://doi.org/10.1038/srep01332

Vesterinen, E. J., Lilley, T., Laine, V. N., & Wahlberg, N. (2013). Next generation sequencing of fecal DNA reveals the dietary diversity of the widespread insectivorous predator Daubenton's Bat (*Myotis daubentonii*) in southwestern Finland. *PLoS ONE, 8*(11), Article e82168. https://doi.org/10.1371/journal.pone.0082168

Waap, S. (2015). *Trophic relationships among pelagic predators of the deep seas of the Madeira islands* [Doctoral dissertation, Cardiff University]. Online Research @ Cardiff. https://orca.cardiff.ac.uk/id/eprint/75359/

Wang, G.-X., Huang, D., Ji, J.-H., Völker, C., & Wurm, F. R. (2021). Seawater-degradable polymers—fighting the marine plastic pollution. *Advanced Science, 8*(1), Article 2001121. https://doi.org/10.1002/advs.202001121

Wayman, C., Fernández-Piñas, F., Fernández-Valeriano, R., García-Baquero, G. A., López-Márquez, I., González-González, F., Rosal, R., & González-Pleiter, M. (2024). The potential use of birds as bioindicators of suspended atmospheric microplastics and artificial fibers. *Ecotoxicology and Environmental Safety, 282*(September), Article 116744. https://doi.org/10.1016/j.ecoenv.2024.116744

Willis, K. A., Serra-Gonçalves, C., Richardson, K., Schuyler, Q. A., Pedersen, H., Anderson, K., Stark, J. S., Vince, J., Hardesty, B. D., Wilcox, C., Nowak, B. F., Lavers, J. L., Semmens, J. M., Greeno, D., MacLeod, C., Frederiksen, N. P. O., & Puskic, P. S. (2022). Cleaner seas: Reducing marine pollution. *Reviews in Fish Biology and Fisheries, 32*, 145–160. https://doi.org/10.1007/s11160-021-09674-8

Wilson, R. P. & McMahon, C. R. (2006). Measuring devices on wild animals: What constitutes acceptable practice? *Frontiers in Ecology and the Environment, 4*(3), 147–154. https://doi.org/10.1890/1540-9295(2006)004[0147:MDOWAW]2.0.CO;2

Yorio, P., Marinao, C., Kasinsky, T., Ibarra, C., & Suárez, N. (2020). Patterns of plastic ingestion in Kelp Gull (*Larus dominicanus*) populations breeding in northern Patagonia, Argentina. *Marine Pollution Bulletin, 156*(July), 111240. https://doi.org/10.1016/j.marpolbul.2020.111240

Young, M. J., Dutoit, L., Robertson, F., van Heezik, Y., Seddon, P. J., & Robertson, B. C. (2020). Species in the faeces: DNA metabarcoding as a method to determine the diet of the endangered Yellow-eyed Penguin. *Wildlife Research, 47*(6), 509–522. https://doi.org/10.1071/WR19246