

# TROPHIC WEB OF THE MALPELO FAUNA AND FLORA SANCTUARY, COLOMBIA

THESIS

TO OBTAIN THE DEGREE OF DOCTOR IN MARINE SCIENCES

BY

M.C. COLOMBO ESTUPIÑÁN-MONTAÑO

LA PAZ, B.C.S., AUGUST 12, 2022

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#### COLOMBO ESTUPIÑAN MONTAÑO P R E S E N T E

Por este conducto me permito comunicarle que el H. Colegio de Profesores de este Centro, en su reunión O-350-21, celebrada el día viernes 12 de noviembre de 2021, ACORDÓ otorgar su solicitud para que con base en el artículo 33 fracción III y 34 del REP-IPN, redactar el documento de tesis en idioma inglés y con una estructura organizada por "capítulos" que es diferente a la establecida en el artículo 34.

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## DEDICATORY

A mis padres José Felix Estupiñán Montaño y Rosario Tulia Montaño,

A mis hermanos Joe Estupiñán Montaño y Josephoenix Estupiñán Montaño,

A mi hija Yda Sabina Estupiñán Mora,

A mi compañera Anika Mora Coral,

A mis compañeros de lucha Daniel Javier Villalobos Ramírez, Harrinzon Lozano Campaz,

Jaiver Rojas Cundumí y Nancy Murillo Bohórquez,

A mi tia Adriana Estupiñán Castro,

A toda mi familia de Ecuador y de Colombia.

Y finalmente, al Santuario de Fauna y Flora Malpelo.

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## ABSTRACT

Trophic networks represent the interactions between species in ecosystems, allowing the identification of key species and processes in the ecosystem. This study structurally analyzes the terrestrial (RT) and marine (RM) food webs of Malpelo Island, Colombia, based on isotopic ( $\delta^{13}$ C,  $\delta^{15}$ N) and topological analyses. Of the isotopically analyzed samples, 67 terrestrial and 140 marine, RT and RM reflected values between -30.3% and -14.8% for  $\delta^{13}$ C and from 3.7\% to 19.4% for  $\delta^{15}$ N, indicating 3 and 5 trophic levels for RT and RM, respectively. Using mixing models, high connectivity between terrestrial and marine ecosystems was evidenced due to the higher contribution of marine nutrients to the terrestrial environment contributed by the Nazca booby seabird Sula granti and the low contribution of plants (mosses). Topologically, the network models were constructed with 170 nodes, 27 for the RT and 140 for the RM. Connectivity patterns suggest the existence of 3 and 4 terrestrial and marine trophic sub-networks, respectively. Additionally, there is a high recurrence of competition processes and trophic chain of three steps in length. Eight trophogroups (RT: detritus, crabs, lizards and lizards; RM: zooplankton, crabs, Carangidae and Scombridae) were identified as nodes centralizing trophic flows. The RT presented shorter trophic chains in contrast to the RM, characterized by long chains. Both networks were formed by groups of organisms with high trophic interaction and different connectivity patterns constituted by few key trophogroups. This indicates that the structural characteristics and ecological dynamics of these ecosystems can be modified if their main components are disturbed. It also confirms the role of S. granti as a keystone species functioning as a bridge between the two ecosystems. It is hoped that this study will contribute to the understanding of the dynamics and design of management measures for Malpelo Island.

*Key words:* Isotopic analysis, Topological analysis, Trophic chain, Trophic connectivity, Trophic levels, Trophic web.

## RESUMEN

Las redes tróficas representan las interacciones entre especies en los ecosistemas, permitiendo identificar aquellas especies y procesos claves del ecosistema. Este estudio analiza estructuralmente las redes tróficas terrestre (RT) y marina (RM) de la Isla Malpelo, Colombia, a partir de análisis isotópicos ( $\delta^{13}C$ ,  $\delta^{15}N$ ) y topológicos. De las muestras analizadas isotópicamente, 67 terrestres y 140 marinas, las RT y RM reflejaron valores entre -30.3% y -14.8% para  $\delta^{13}$ C y desde 3.7‰ a 19.4‰ para  $\delta^{15}$ N, indicando 3 y 5 niveles tróficos para las RT y RM, respectivamente. Usando modelos de mezcla, se evidenció una alta conectividad entre los ecosistemas terrestre y marino debido a la mayor contribución de nutrientes marinos al ambiente terrestre aportados por el ave marina piquero de Nazca Sula granti y al bajo aporte de plantas (musgos). Topológicamente, los modelos de redes fueron construidas con 170 nodos, 27 para la RT y 140 para la RM. Los patrones de conectividad sugieren la existencia de 3 y 4 subredes tróficas terrestres y marinas, respectivamente. Adicionalmente, existe una alta recurrencia de procesos de competencia y cadena tróficas de tres pasos de longitud. Se identificaron ocho trofogrupos (RT: detrito, cangrejos, lagartos y lagartijas; RM: zooplancton, cangrejos, Carangidae y Scombridae) como nodos que centralizan los flujos tróficos. La RT presentó cadenas tróficas más cortas en contraste con la RM, caracterizada por cadenas largas. Ambas redes estuvieron conformadas por grupos de organismos con alta interacción trófica y diferentes patrones de conectividad constituidos por pocos trofogrupos claves. Lo anterior indica que las características estructurales y dinámica ecológica de estos ecosistemas, pueden verse modificadas si sus componentes principales son perturbados. Asimismo, se confirma el rol de S. granti como especie clave funcionando como puente entre ambos ecosistemas. Se espera que este estudio contribuya al entendimiento de la dinámica y diseño de medidas de manejo de la Isla Malpelo.

*Palabras clave:* Análisis isotópicos, Análisis topológicos, Cadena trófica, Conectividad trófica, Niveles tróficos, Red trófica.

## **Chapter 1: GENERAL CONSIDERATIONS**

## **INTRODUCTION**

A food web is the model of the food interactions of species within an ecological community (Smith and Smith, 2018), indicating the transport of nutrients and energy through the different trophic levels (primary producers - primary consumers - secondary consumers - tertiary consumers - quaternary consumers) (Krebs, 2009).

Trophic networks describe feeding relationships (links or connections) between species. However, these relationships may vary depending on energy flow, population dynamics and their functions in the ecosystem. Studies of food webs generate information on community structure at different levels (e.g., individual, intermediate and group) (Pimm, 1980), as well as competition, nutrient dynamics and predation cascade effects (Winemiller and Polis, 1996). This information makes it possible to identify and understand the complex relationships between the individual components of an ecosystem and their characteristics (Balasudaram et al., 2005) or to identify the structural patterns of a network (Milo et al., 2002).

Accordingly, three types of networks can be distinguished (Fig. 1): A) Connectivity networks or topological trophic networks that emphasize trophic relationships between species, represented by links in the network; B) Energy flow networks that quantify the flow of energy from one species to another; and C) Functional webs or trophic interaction trophic webs that represent the importance of each species in maintaining community integrity and reflect the influence on the population growth rate of other species (Paine, 1980).



Figure 1. Classification of food webs according to energy flow and dynamics in species populations. The circles correspond to species. The lines and arrows represent the trophic interaction, and the thickness of the line indicates the intensity of the relationship. Modified from: Paine (1980).

Studying the trophic ecology of species provides information on trophic composition, trophic level, species distribution, energy flow, predation impacts on other species, predator-prey relationship, prey abundance, distribution, food preferences, and dietary changes (Cortés, 1999). Therefore, studying the trophic ecology of species is important for: 1) the construction of food

webs (Navia et al., 2010; Bornatowski et al., 2014a); 2) ecosystem models for the evaluation of the function of each species within the ecosystem; and 3) prediction of changes due to disturbances (e.g., fishing). Likewise, trophic studies enable the identification of the relative frequency of prey in the diet of consumers and indicate the importance of species as links between different trophic levels along the food chain (Bornatowski et al., 2014b).

Currently, different techniques have been developed to improve knowledge and understanding of the trophic dynamics of ecosystems. In this sense, traditional techniques in feeding studies and food web modeling are stomach contents studies analysis (SCA) (Galván-Magaña et al. 2013; Polo-Silva et al. 2013), biochemical tracer analysis (stable isotopes, fatty acids and essential amino acids) (Stowasser et al., 2009; Kim et al., 2012; Li et al. 2016) and mathematical modeling (topology, Ecopath with Ecosim) (Colléter et al., 2015; Zetina-Rejón et al. 2015).

On the one hand, studies with biochemical tracers such as stable isotope analysis (SIA) of carbon ( $\delta^{13}$ C) and nitrogen ( $\delta^{15}$ N) provide insight into food sources, trophic position, ontogenetic changes, ecological niche, food preferences and migration of species (DeNiro and Epstein, 1978; Post, 2002; Graham et al., 2010; Jackson et al., 2011), based on the use of different tissues (e.g., muscle, teeth, liver, vertebrae, among others) that reflect the food synthesized by the consumer over different time and space intervals ranging from periods of several days to several years (Kim et al., 2012; Polo-Silva et al., 2012; Estupiñán-Montaño et al., 2019; Tamburin et al., 2020).

The quantification and analysis of  $\delta^{13}$ C in a specimen allows estimating the nature and origin of the food sources, as the integration of this element into the food webs happens mainly through the different photosynthetic pathways. Therefore, 13C originating from C<sub>3</sub> ( $\delta^{13}$ C: -35‰ to -22‰), C<sub>4</sub> (-17‰ to -9‰) and/or CAM (-34‰ to -10‰) plants can be identified (Ehleringer and Rundel, 1988; Handley and Raven, 1992). In contrast, 15N is considered an indicator of the trophic position of consumers, since the value of this ratio is a product of the nitrogen present in prey, as a result of <sup>15</sup>N-enrichment, through food webs (~3‰, DeNiro and Epstein, 1981). However,  $\delta^{15}$ N varies by consumer type (e.g., carnivores, herbivores) (Fujiwara and Highsmith, 1997), feeding areas (e.g., oceanic zones, mangrove areas, etc.) (Polo-Silva et al., 2013; Estupiñán-Montaño et al., 2019), among other factors, that may affect their composition (e.g., upwelling, currents or oxygen minimum zones) (Sigman et al., 1997; Bauersachs et al., 2009).

Mathematical modeling methods such as network analysis allow the construction of simplified models of an ecosystem in order to evaluate and simulate trophic dynamics. This technique is

based on the topology of the network, represented through *nodes* and *links*. *Nodes* correspond to species (i.e., predator or prey) and *links* represent their interactions (e.g., trophic relationships). These models allow the identification of influential nodes (i.e., species) in a network and their dynamics (Borgatti, 2006). Therefore, these nodes are referred to by ecological theory as keystone species (Mills et al., 1993; Power et al., 1996; Jordán et al., 2006).

In ecological terms, there are two types of networks: unipartite and bipartite. Unipartite networks represent relationships between species that can (potentially) interact with another species, across and between trophic levels (e.g., feeding networks, Fig. 2). Whereas bipartite networks represent the relationship between species from two different groups or two different trophic levels, but not between species of the same group (e.g., mutualistic interactions between animals and plants; Fig. 2B).

Additionally, networks can be divided into directed and undirected, depending on the effect that the species has on other species through interactions. Unipartite networks are generally directed as they illustrate the energy flow of an ecosystem through various trophic levels, and there is a clear differentiation of negative effect from one species to another (e.g., A eats B, B eats C) or positive effect in the opposite direction (e.g., C preys on B, B preys on A). Whereas bipartite networks are non-directed, as the effects of interactions operate in both directions (e.g., a plant has a positive effect for an animal by providing resources, and at the same time the animal has a positive effect for the plant by serving as a pollinator) (Dehling, 2018).

Finally, the joint application of different methodologies (i.e., SIA and topology) constitutes a viable strategy for study, the conservation and management of resources and ecosystems, as it provides a variety of perspectives on the panorama of the interactions between species and the system, based on the increase in the spectrum of description and identification of processes that influence the ecological dynamics of trophic networks. They therefore provide additional tools for the development of appropriate resource management strategies and measures based on the ecosystem and not on individual species (Whipple et al., 2000; Borgatti, 2002).



Figure 2. Ecological networks can be represented as graphs (left side) and matrices (right side). Nodes are colored and represent species or individuals. Links are represented as lines between nodes in graphs, and as squares in matrices. A) Unipartite networks (simple food web). Species from three trophic levels are indicated by different colors, the links are shown as arrows going from the lower trophic level (prey) to the upper trophic level (predator). In the matrix, each column shows the prey per predator. B) Simple bipartite network. Blue circles and yellow squares show species from two trophic levels. In the matrix, black squares indicate the interaction, while in the graph, the width of the lines indicates the frequencies of interactions. Taken from: Dehling (2018).

#### BACKGROUND

Knowledge about trophic dynamics and the role of individual species in the food webs of terrestrial and marine ecosystems of the Malpelo Fauna and Flora Sanctuary (hereafter Malpelo FFS) is scarce. To date, some trophic studies have been conducted on terrestrial (López-Victoria, 2006; López-Victoria and Werding, 2008; López-Victoria and García, 2010; López-Victoria et al., 2009, 2011, 2013) and marine (Estupiñán-Montaño et al., 2017) species, including some studies on migratory movements (Bessudo et al., 2011a, b). This information helps to improve the understanding of the biology and role of species within the ecosystem and, in addition, aids in the modeling and interpretation of the trophic dynamics of the ecosystems of the Malpelo FFS.

Despite this, there are few studies that describe the food webs of the Malpelo FFS, and to date only two studies have been reported that propose energy flow models for the terrestrial food web, and none for the marine food web. The first food web model for the Malpelo FFS was proposed by Wolda (1975). This author studied the flow of energy in the terrestrial ecosystem focusing mainly on the larger species. However, due to the limited information available at the time, some trophic pathways remained unknown. Notwithstanding, the limitations of the study, Wolda (1975) highlighted in his model the role of the marine ecosystem in supplementing and maintaining the terrestrial ecosystem through seabirds (Fig. 3A). Calero et al. (2011) developed the second proposal of trophic structure in the terrestrial ecosystem focusing on invertebrates. These authors described the main energy flows and generated information on the biomass produced by the most representative and abundant groups on the island (e.g., spiders, crabs, earthworms, mealybugs, snails, centipedes, and mites), as well as their relationship with other components of the network, but without presenting detailed information on these other trophic interactions, leaving our understanding of the trophic dynamics of the terrestrial ecosystem of the Malpelo FFS somewhat inconclusive. Nevertheless, they generated a trophic map complementary to the one proposed by Wolda (1975) (Fig. 3B).

As in the earlier study by Wolda (1975), Calero et al. (2011) as well as Wolda (1975), also concluded that seabirds are important for the maintenance of the terrestrial ecosystem due to the contribution of marine nutrients, which are made available to terrestrial communities in different forms (e.g., feathers, carcasses, eggs, chicks, guano, fish and squid; Fig. 3B).

Despite the fact that the Malpelo FFS is a protected area and an important zone for the conservation and aggregation of species, there is still a chronic scarcity of data and a corresponding need for further studies to improve knowledge about the functioning of this important Marine Protected Area (MPA) and its relationship with other ecosystems along the Eastern Pacific Ocean, in order to have a better understanding of the ecological integrity of the ecosystems present in the MPA and as a tool for management and conservation.



Figure 3. A) Tentative structure of the food web on the Malpelo Fauna and Flora Sanctuary Malpelo. The four question marks refer to uncertainties in utilization of grass by the herbivores, crabs eating living insects, *Diploglossus* consuming living crabs, and the importance of bird droppings in providing nutrients for the plants. For simplicity, some relationship are omitted. These include lizards and crabs providing food for the scavengers and ticks being eaten by *Anolis*. Modified from: Wolda (1975). B) Food web map of the Malpelo FFS representing the main energy flows, with emphasis on terrestrial invertebrates, highlighting the most representative and abundant groups. The size of the gray boxes indicates the proportion of biomass generated (except for the white boxes, which only indicate the relationship with other invertebrates). Modified from: Calero et al. (2011).

#### **PROBLEM STATEMENT**

Malpelo is the only oceanic island and the largest MPA in the Colombian Pacific. It is a site of great biological and ecological importance for several endemic, resident and migratory species (Plan de Manejo, 2015). Among the most emblematic species are hammerhead sharks (*Sphyrna lewini*) and silky sharks (*Carcharhinus falciformis*), which use the MPA and its surroundings as feeding (Estupiñán-Montaño et al., 2017, 2021), resting, cleaning, and perhaps breeding grounds (Bessudo et al., 2011a, b). These shark species form large concentrations around Malpelo Island and its MPA. Because of this, the area is frequented by 1) national and international tourists who come to see the large shoals of *S. lewini* and *C. falciformis*, and 2) international fishermen, who aim to capture sharks because they represent a high economic value (mainly their fins). The latter activity currently constitutes the main threat to shark populations in the area and, therefore, to the marine ecosystem of the Malpelo FFS.

Hammerhead and silky sharks are considered top predators of the marine ecosystem of the Malpelo FFS (Estupiñán-Montaño et al., 2017), fulfilling important roles in the regulation of the populations of different prey species present in lower trophic levels, a function that can be affected by the fishing pressure to which they are subjected due to their fins. This, together with their biological characteristics (i.e., slow growth, low fecundity, late maturity; Chapman et al., 2005; Simpfendorfer and Heupel, 2012), prevent shark populations from recovering rapidly, which can generate different consequences in the functioning of ecosystems (e.g., trophic cascades; Grubbs et al., 2016; Desbiens et al., 2021). This is one of the bases for management and conservation measures in the MPA, since these species are considered "umbrella" species by the MPA and conservation efforts are focused on them. Partly for this reason, knowledge of the role of other species that are part of the ecosystems present in the Malpelo FFS has been neglected.

Although the Malpelo FFS is part of the Colombian MPA system, the management and conservation measures of this MPA are, to a certain extent, affected by the lack of knowledge of the biology and ecology of the different species. Therefore, this work aims to understand the importance and role of the species (including sharks) in the terrestrial and marine ecosystems of the Malpelo FFS, and to make inferences about the direct or indirect effects that changes in the shark populations could caused along their food webs, in order to generate information to support decision-making for the management and conservation of the MPA.

Based on the above, the following research questions arose:

What are the key components of the terrestrial and marine ecosystems of the Malpelo FFS and how do they help maintain the stability of the trophic dynamics and connectivity between both environments of this important marine protected area?

What is the role of the Malpelo FFS in the feeding ontogeny of hammerhead sharks *Sphyrna lewini* and silky sharks *Carcharhinus falciformis*?

#### JUSTIFICATION

Malpelo Island is the most distant point in the Colombian Pacific. This characteristic and the influence of different factors (e.g., biological, ecological, geological and oceanographic) make this site a place with special dynamics, which are generated by different processes that facilitate the aggregation of species (resident and migratory). There is also a high presence of endemism (terrestrial and marine), which has led this site to currently be the largest MPA in the Colombian Pacific Ocean, being named Malpelo Fauna and Flora Sanctuary (SFF) (Fig. 4; Plan de Manejo, 2015).

The geoform of the Malpelo FFS (i.e., seamount) means that this MPA has large schools of resident fish (e.g., snappers, groupers, carangids, etc.) and the MPA is also frequented by a great variety of migratory marine species (e.g., sharks, tunas, billfishes, dolphins, etc.). These form large concentrations around the Malpelo FFS and use the site and its surroundings as a feeding, resting, cleaning and perhaps breeding area (Bessudo et al., 2011a, b; Estupiñán-Montaño et al., 2017). Additionally, in the terrestrial portion of the MPA there is an ecological community formed mostly by endemic species (e.g., reptiles, micro and macroinvertebrates, etc.) (Plan de Manejo, 2015). It also has the largest nesting population of Nazca booby (*Sula granti*) in the world (López-Victoria and Rozo, 2007; García, 2013). This species is a key element in the relationship between marine and terrestrial environments (Wolda, 1975; López-Victoria et al., 2009).

This great diversity of terrestrial and marine species means that the site is frequently visited by 1) national and international tourists who want to appreciate the underwater landscapes, the large schools of sharks and teleost fish, as well as the large community of seabirds, and 2) artisanal and industrial fishermen, both national and international, who aim to capture different species of commercial interest (e.g., tuna, billfish, sharks, etc.). In this sense, the dynamics of the Malpelo FFS are strongly threatened by fishing due to the decrease in populations of prey species and

predators, habitat degradation due to pollution (e.g., waste dumping, transport of invasive species, habitat degradation, etc.) and tourism (Herrón et al., 2007) (Plan de Manejo, 2015).

Taking into account the above facts, since the creation of Malpelo Island as an MPA, efforts have been carried out for the management and conservation of the area with the objective of maintaining its ecological integrity (Plan de Manejo, 2015). Despite this, the effectiveness of these conservation efforts may be reduced to a considerable extent, by the lack of knowledge of the biology, ecology and trophic dynamics of the terrestrial and marine ecological communities, both resident and migratory that frequent the MPA. This lack of information constitutes a major obstacle in terms of building an adequate understanding of the ecological dynamics of the ecosystems and the role of individual species in maintaining the stability and dynamics of the Malpelo FFS. Therefore, there is an urgent need for additional studies that, based on the application of different methodologies, help to describe and understand the dynamics and trophic characteristics of this important MPA, as well as the identification of key species and their role in the ecosystems they inhabit. Organized en such a way that this information can become a powerful tool when evaluating the direct or indirect effects generated by any given disturbance in the MPA (e.g., fishing, nonnative species, climate change, pollution, etc.) that could be reflected in the functioning of ecosystems, abundance of species, among other factors. Likewise, the collection and analysis of additional biological information will make it possible to achieve conservation objectives and improve the design of appropriate management strategies and measures that would allow decisionmaking to be made from an ecosystem approach, rather than from the perspective of a particular species, based on the premise that species are not alone, but that they interact with others and their environments (Balasudaram et al., 2005).

## HYPOTHESIS

The terrestrial and marine ecosystems of the Malpelo FFS have a high degree of mutual dependence and trophic interaction influenced by the Naza booby (*Sula granti*) due to its role in the transport of nutrients of marine origin to the terrestrial environment (Wolda, 1975; López-Victoria et al., 2009). For this reason, the following hypotheses were put forward:

- Detritus will reflect similar  $\delta^{13}$ C signals to that of basal marine organisms and *S. granti*'s eggs, as the latter reflect the isotopic signal of the food consumed by their mothers during egg formation.
- The terrestrial and marine ecosystems reflect high isotopic overlap values, which are indicative of the close relationship between them and the integration of marine nutrients into terrestrial species.
- Due to the role of *Sula granti* in both ecosystems (predator and prey), this component would function as an intermediary in the topology of the food webs, acting as a bridge node between both environments.

On the other hand, considering the role of sharks as regulators of marine ecosystems, their high frequency and abundance around the Malpelo FFS, the presence of ontogenetic changes in their feeding, and that these organisms do not use the vicinity of Malpelo Island as a principal feeding area, which gives rise to the following hypotheses:

- Hammerhead (*Sphyrna lewini*) and silky (*Carcharhinus falciformis*) sharks would reflect different δ<sup>13</sup>C values with respect to that of the other components of the Malpelo FFS, due to ontogenetic changes in feeding.
- Hammerheads (*S. lewini*) and silky sharks (*C. falciformis*) would reflect low centrality (topology) values due to their low participation in the food web of the Mapelo FFS.

## **OBJECTIVES**

## **General objective**

To describe the trophic structure of the terrestrial and marine ecosystems of the Malpelo Fauna and Flora Sanctuary (FFS), using biochemical techniques and mathematical modeling.

## **Specific objectives**

- 1. Construct the terrestrial and marine food webs of the Malpelo FFS.
- 2. To identify and describe the role of the species in the terrestrial and marine food webs of the Malpelo FFS.

- 3. To describe the trophic connectivity between the terrestrial and marine ecosystems of the Malpelo SFF.
- 4. To explain the trophic importance of the Malpelo SFF in the feeding ontogeny of the most frequent and abundant sharks around the island.

### **STUDY AREA**

Malpelo Island (Fig. 4A) is the summit of a submarine mountain range called "Dorsal Malpelo", which was formed approximately 20 million years ago, but stabilized during and after the Miocene period (von Prahl, 1990). This submarine mountain extends in a NE-SW direction, with a length of approximately 150 miles (241 km) and a width of 50 miles (80.5 km) (Fig. 4B, red polygon), ascending from about 4000 m depth (Lonsdale and Klitgord, 1978; Fig. 3B, red polygon) and the section that comprises Malpelo Island reaches a maximum height of 300 m.a.s.l. (Fig. 4C).

Malpelo Island constitutes the westernmost insular point of the Colombian territory in the Pacific Ocean (Plan de Manejo, 2015) and is the largest marine reserve in the Colombian Pacific, denominated the Malpelo FFS. It is located ~390 km from the coast of Buenaventura in the Colombian Pacific (4°00'05.63" N; 81°36'36.41" W; Fig. 4B [Plan de Manejo, 2015]). The SFF Malpelo is composed of 11 islets (Fig. 4A) and a protected area of ~2.7 million hectares (Fig. 3B; yellow polygon; [Ministerio de Ambiente y Desarrollo Sostenible, 2017]). Due to its geographic location, it is influenced by several marine currents (Fig. 4D), which allows the aggregation of species due to its high productivity as a result of an annual upwelling that supplies nutrients from deeper waters (Plan de Manejo, 2015).



Figure 4. Study area in the Eastern Pacific Ocean. A) Geographic location of the Malpelo Fauna and Flora Sanctuary. B)
 Polygon of the marine protected area (yellow) of the Malpelo Fauna and Flora Sanctuary and polygon of the Malpelo Dorsal (Red). C) Lateral view of the bathymetric profile of Malpelo Island (Plan de Manejo 2015). D) Marine currents that influence the dynamics of Malpelo Island. Currents near the surface (Yellow arrows discontinued lines), geostrophic currents in the upper layer (Blue arrows continued lines) CC: California Current, NEC: North Equatorial Current, NECC: North Equatorial Countercurrent, SEC: South Equatorial Current, CRCC: Costa Rica Coastal Current, HC: Humboldt Current, COLC: Colombian Current (and subsurface currents) ESC: Equatorial subcurrent, N/SSSCC: North/South subsurface countercurrent, EMC: Eastern Mexican Current, PCS: Peru-Chile subcurrent. Taken and modified from: Kessler (2006).

This orographic system is the result of the interaction that occurred more than 20 million years ago between the Galapagos "*hot spot*" and the Coco-Nazca accretion center. This interaction that generated high volcanic activity that gave rise to Malpelo Island (De Mets et al., 1990; Detrick et al., 2002; Fig. 5).



Figure 5. Relationship of Malpelo Island with respect to the Cocos and Nazca tectonic plates. Star: represents the geographic location of Malpelo Island.

Despite its physical characteristics, different plant and animal species have established on the island (Plan de Manejo, 2015). Twenty-eight species of terrestrial plants have been identified, mainly represented by algae, lichens (i.e., *Caloplaca* sp., *Candelabria* sp., *Lecidea* sp., and *Pyxine* sp.), a moss (*Octoblepharum albidum*), a C<sub>4</sub> grass (*Paspalum* sp.), a legume, a fern (*Pityrogramma calomelanos*), and some shrubs that have not yet been classified (von Prahl, 1990; González-Román et al., 2014).

Conversely, the fauna on the island is represented by ~40 species of invertebrates (Wolda, 1975; Plan de Manejo, 2015), including ants (*Odontomachus bauri*), beetles (*Platynini* sp.), an endemic decapod crustacean (*Johngarthia malpilensis*), three species of endemic reptiles (*Anolis agassizi*, *Diploglossus millepunctatus*, and *Phyllodactus transversalis*), and a high diversity of birds (>60 species, Plan de Manejo, 2015), mainly represented by *S. granti*, which has established the largest nesting colony worldwide on Malpelo Island (López-Victoria and Rozo, 2007; García, 2013).

## **METHODOLOGY FOR STABLE ISOTOPES**

Stable isotope analysis was carryied out in the Stable Isotope Laboratory of the Instituto Andaluz de Ciencias de la Tierra in Granada (CSIC.UGR), Spain. The isotopic composition (i.e., carbon and nitrogen) of all of the samples of the terrestrial and marine specimens was determined using an online Carlo Erba NA 1500 NC elemental analyzer coupled online via ConFlo III interface to a Delta Plus XP mass spectrometer (EA-IRMS; ThermoQuest). The stable isotopes were reported as  $\delta$  values per thousand (‰) based on the following equation:

$$\delta^{13}$$
C or  $\delta^{15}$ N =  $\left(\frac{R_{sample}}{R_{standard}} - 1\right) \times 1000$ 

where *R* is the isotope ratio ( ${}^{13}C/{}^{12}C$  or  ${}^{15}N/{}^{14}N$ ) of the sample or the standard (V-PDB and AIR for carbon and nitrogen, respectively). Commercial CO<sub>2</sub> and N<sub>2</sub> were used as the internal standard for the isotopic analyses. Internal standards of -30.63% and -11.65% (V-PDB) were used for  $\delta^{13}C$  analysis and internal standards of -1.0% and +16.0% (AIR) for  $\delta^{15}N$ . A precision factor was calculated after a correction for the mass spectrometer daily drift from standards systematically interspersed in analytical batches; variability was  $\pm 0.1\%$  for  $\delta^{13}C$  and  $\delta^{15}N$ . The standards used for the measurement of carbon and nitrogen were V-PDB (Vienna-PDB) and atmospheric nitrogen (AIR), respectively. Reference gases and in-house standards (with different C:N ratios and isotopic composition) were calibrated against International Reference Materials for carbon (USGS-24 and IAEA-C6) and nitrogen (IAEA-N1, IAEA-N2, and IAEA-N3).

# Chapter 2: TERRESTRIAL FOOD WEB OF THE MALPELO FAUNA AND FLORA SANCTUARY, COLOMBIA

# 2.1. Terrestrial trophic structure of the Malpelo Fauna and Flora Sanctuary, Colombia, based on analyses of $\delta^{13}$ C y $\delta^{15}$ N

#### INTRODUCTION

Knowledge of the current structure of food webs, and the factors that preceded this structure, is one of the major objectives of ecology in areas of highly diversified and abundant ecosystems and communities. Added to this is the objective of environmental conservation, which focuses on preserving biodiversity ("flagship", charismatic, endemic, rare, or otherwise threatened species (Tylianakis et al., 2010).

The study of trophic interactions is an important step to explain community dynamics and the impacts that individual species have on them (Navia et al., 2010; Bornatowski et al., 2014). This in turn enables the modeling of food webs, generating additional information on ecological processes (e.g., competition, omnivory), nutrient flow, cascade effects (Winemiller and Polis, 1996) and community structure (Pimm, 1980), thereby contributing to a deeper understanding of the complex relationships between components and their properties (Balasundaram et al., 2005). The collection and analysis of such information is essential for the effective design of management and conservation strategies based on ecosystem functioning (Whipple et al., 2000; Borgatti, 2002).

Malpelo is a small oceanic island that harbors high terrestrial and marine biodiversity (Plan de Manejo, 2015). Due to this fact, and also to its geographic location, the island constitutes the largest marine protected area (MPA) in the Colombian Pacific, declared as the Malpelo Fauna and Flora Sanctuary in 1995 (Fig. 4B [Ministry of Environment and Sustainable Development, 2017]). It has been listed as part of the World Heritage for Humanity (UNESCO) and has received other important nominations in recognition of its high conservation value (Management Plan, 2015). These characteristics make the Malpelo FFS a site of great economic (e.g., ecotourism), scientific (e.g., "living laboratory"), and political importance for the Colombian Pacific (Plan de Manejo, 2015).Despite its great importance, little is known about the community dynamics of the ecosystems present in the MPA. This is apparent from the scarcity of studies focused on network analysis, of which only two studies that address this topic are reported. The first one modeled the terrestrial food web of the Malpelo FFS from direct observation and trophic studies of several terrestrial species, representing energy flows (Fig. 4A; Wolda 1975).The second study focused on the terrestrial invertebrate community, representing energy flows and biomass production (Fig 4B;

Calero et al., 2011). Both reveal the important role of the Nazca booby *Sula granti* in the maintenance of the terrestrial ecosystem. However, these studies do not describe the levels of organization (i.e., trophic levels) of the terrestrial food web of the Malpelo FFS, and the flow of matter and energy along them (Vásquez, 1998). This continued lack of information constitutes a major obstacle to understanding the terrestrial trophic dynamics of the Malpelo FFS.

Trophic studies on different species (e.g., terrestrial and marine) focus on the use of biochemical tracers, i.e., stable isotope analysis (SIA) of C and N, asthe composition of these elements in different species can provide important insights into the diet integrated by an organism during a certain period (from days to years) (MacNeil et al., 2005; Logan and Lutcavage, 2010; Kim et al., 2012). SIA generates information on the sources of primary productivity of a food web from the  $\delta^{13}$ C signal (France, 1995), while  $\delta^{15}$ N reflects the position (Hussey et al., 2015) and trophic level of individual species (Vander Zanden et al., 1997) within a food web. Additionally, SIA helps to describe and understand other ecological aspects, including isotopic niche, habitat use, and trophic structure (Layman et al., 2017; Newsome et al., 2007), as well as ecological characteristics such as trophic diversity, i.e., functional richness (Layman et al., 2007; Jackson et al., 2011), isotopic diversity (Layman et al., 2007), and isotopic evenness (Layman et al., 2007; Rigolet et al., 2015).

Therefore, the objectives of this chapter were: 1) to describe the terrestrial trophic structure of the Malpelo FFS from the isotopic signals of some terrestrial components, 2) to determine the number of levels that structure the terrestrial trophic pyramid of the Malpelo FFS, 3) to estimate the degree of trophic interaction (isotopic overlap) between components, and 4) to draw some inferences about key ecological characteristics of the food web (e.g., resource use and degree of omnivory).

#### **MATERIALS AND METHODS**

### Study area

Malpelo Island (Fig. 4A) is the summit of a submarine mountain range called the Malpelo Ridge, which extends in a NE-SW direction; it is approximately 241.4 km long by 80.5 km wide (Fig. 4B, red polygon). The island has a maximum height of 300 m above sea level and the ridge rises from a maximum depth of approximately 4,000 m (Fig. 4C) (more details in Chapter 1).
# **Collection of samples**

Samples of 16 terrestrial species/groups (Table 1) were collected in 2018–2019 in Malpelo FFS, Colombia (Fig. 4A). Samples of terrestrial vertebrates consisted of 1–2 cm of tissue collected from the posterior portion of the tail of *A. agassizi* and *D. millepunctatus*, and body feathers of *S. granti*. For invertebrates, such as the land crab *J. malpilensis*, one of the hind limbs was collected, whereas invertebrates (i.e., millipedes, isopods, spiders, worms, crickets, and ants; Table 1) were collected whole.

All collected samples were placed in pre-labeled zip-lock plastic bags and were kept frozen on board the Pacific Diving Company's M/N Seawolf for subsequent transfer to the laboratory. The sampling procedures were endorsed by Parques Nacionales de Colombia, through Memorandum 20177730007973 of 30 May 2017, issued by the Planning and Management Group.

# Sample preparation and analysis

Samples were washed with distilled water, freeze-dried in an oven at 60 °C for 24 h, and ground to a fine powder with an agate mortar. Approximately 0.23 to 4.40 mg of powder were obtained for each terrestrial sample and packed in  $3.2 \times 4$ -mm tin capsules.

The C:N ratio was estimated and compared to reference values; a C:N value  $\leq 3.5$  indicates no effect of lipid contents (Post et al., 2007), whereas values > 3.5 suggest high lipid content.  $\delta^{13}$ C values of some terrestrial organisms (Table 1) with C:N values >3.5 were mathematically normalized according to Kiljunen et al. (2006):

$$\delta^{13}C_{adjusted} = \delta^{13}C_{measured} + \boldsymbol{D} \times \left(\boldsymbol{I} + \frac{\boldsymbol{3.90}}{\boldsymbol{1} + \boldsymbol{287}/\boldsymbol{L}}\right)$$

Where  $\delta^{13}C_{adjusted}$  is the  $\delta^{13}C$  after normalization and  $\delta^{13}C_{measured}$  is the  $\delta^{13}C$  obtained from the sample without lipid removal. D = 7.018, I = 0.048, and L is the proportional lipid content of the sample, estimated as  $L = -20.54 + (7.24 \times C:N)$  (Post et al., 2007).

Arthropods (i.e., ants, isopods, and millipedes; Table 1) were analyzed without extracting lipids because these organisms have an exoskeleton characterized by high chitin contents (e.g., Liu et al., 2019), which are reflected in high C:N values (>3.5). Therefore,  $\delta^{13}$ C values of arthropods with C:N values <7.0 were not normalized mathematically (Schimmelmann and DeNiro, 1986; Webb

et al., 1997; Pringle and Fox-Dobbs, 2008). Otherwise,  $\delta^{13}$ C values were normalized according to Post et al. (2007).

Feathers of the main seabird present on the island, Nazca Booby *Sula granti*, were cleaned of surface lipids and contaminants using a 2:1 chloroform:methanol solution, followed by two successive methanol rinses (Jaeger et al., 2009). The  $\delta^{13}$ C values of *S. granti* eggs were mathematically normalized because lipid extraction can alter  $\delta^{15}$ N by washing out nitrogenous compounds. In this case, the formula proposed by Elliot et al. (2014) was used:

$$\delta^{13}C_{\text{lipid-extracted}} = \delta^{13}C_{\text{non-extracted}} + 1.47 - 2.72 \times Log_{10} \text{ (C:N)}$$

Where  $\delta^{13}C_{\text{lipid-extracted}}$  is the  $\delta^{13}C$  after normalization and  $\delta^{13}C_{\text{non-extracted}}$  is the  $\delta^{13}C$  obtained from the sample without lipid removal.

Stable isotope analyses were carried out in the Stable Isotope Laboratory of the Instituto Andaluz de Ciencias de la Tierra in Granada (CSIC-UGR), Spain (more details in Chapter I).

#### **Trophic position**

The trophic position (TP) of all organisms was estimated using a Bayesian method within the R statistical environment (R Core Team 2018), employing the *tRophicPosition* package version 0.7.5 (Quezada-Romegialli et al., 2018). Analyses of TP were calculated based on the  $\delta^{13}$ C and  $\delta^{15}$ N values of all organisms and using the isotopic values of C<sub>3</sub> plants (mosses) and detritus of Malpelo Island (Table 1) as isotopic baselines. A Bayesian model of two baselines and two trophic discrimination factors (TDF) was run with 2 Markov Chain Monte Carlo (MCMC) and 20000 adaptive interactions, assuming a baseline taxa ( $\lambda$ ) = 1. Accordingly, each organisms' TP value was determined using the average TDF for terrestrial ecosystem ( $\Delta^{13}$ C = 0.5 ± 0.19‰ SD and  $\Delta^{15}$ N = 2.3 ± 0.24‰ SD [McCutchan et al., 2013]).

#### **Community trophic structure**

To investigate important aspects of the trophic structure of the terrestrial components, the same metrics derived from isotopic values of  $\delta^{13}$ C and  $\delta^{15}$ N of consumer's tissues were applied as in the so-called Layman's metrics (Layman et al., 2007), adopting a Bayesian approach adapted by Jackson et al. (2011).

Cada	Таха			C:N	8	$\delta^{13}C$	(‰)		δ <sup>15</sup> N (‰)			
Code	Scientific name	Common name		Mean $\pm$ SD	Min	Max	Mean	SD	Min	Max	Mean	SD
1	Anolis agassizi	Lizard	8	$3.5\pm0.10$	-18.2	-16.0	-16.8	0.67	13.5	15.5	14.4	0.58
2	Araneae	Spiders	7	$5.3\pm1.31$	-21.0	-17.5	-19.6	1.27	16.8	21.3	19.5	1.59
3	Diploglossus millepunctactus	Dotted galliwasp	9	$3.6\pm0.64$	-18.3	-15.0	-15.8	1.03	13.6	15.3	14.5	0.48
4	_	Detritus	5	$5.4\pm0.22$	-20.1	-18.4	-18.9	0.66	9.5	13.0	10.8	1.3
5	Gryllidae	Crickets	5	$4.5\pm0.42$	-20.6	-18.4	-19.3	0.53	9.7	13.4	11.9	0.92
6	_	Guano	1	1.2	_	_	-19.3	_	_	_	14.2	_
7	Hymenoptera	Wasps, bees	4	$4.5\pm0.36$	-20.3	-19.4	-20.0	0.39	11.0	14.3	13.3	1.53
8	Sula granti	S. granti's eggs	8	$4.3\pm0.41$	-19.5	-18.2	-18.6	0.44	13.1	14.1	13.6	0.36
9	Isopoda	Mealybugs	9	$7.0\pm0.29$	-17.5	-15.0	-16.4	0.82	15.1	17.9	16.8	1.04
10	Johngarthia malpilensis	Terrestrial crabs	12	$3.2\pm 0.07$	-17.1	-15.5	-16.5	0.45	14.9	17.0	15.8	0.6
11	Diplopoda	Millepedes	15	$6.3\pm0.91$	-22.9	-18.6	-20.7	1.17	6.4	15.7	11.7	2.38
12	Lumbriculidae	Earthwors	6	$4.8\pm0.34$	-18.7	-17.0	-18.1	0.6	17.2	20.4	19.1	1.12
13	Microcoryphia	Rock hoppers	7	$4.1\pm0.30$	-21.4	-19.6	-20.5	0.6	10.3	19.4	14.1	3.41
14	Odontomachus sp.	Ants	12	$4.1\pm0.41$	-18.4	-16.3	-17.2	0.82	15.5	19.2	16.7	1.02
15	_	Mosses	8	$15.9\pm1.71$	-30.3	-28.7	-29.4	0.62	3.8	10.1	7.4	2.25
16	Sula granti	S. granti's feathers	9	$3.3\pm 0.05$	-16.7	-16.1	-16.3	0.20	13.5	15.2	14.3	0.49

**Table 1.** Some species of the terrestrial ecosystem of the Malpelo Fauna and Flora Sanctuary, Colombia, showing scientific and common names, as well as their minimum (Min), maximum (Max), mean, and standard deviation (SD) values of  $\delta^{13}$ C and  $\delta^{15}$ N, together with the estimated C:N ratio for each one of them.

The metrics includes the ranges between the lowest and highest  $\delta^{13}$ C and  $\delta^{15}$ N values in each group's isotopic space. The carbon range (CR) gives information about the range of resources used (higher diversity in the exploitation of basal carbon that can sustain a food chain) and the nitrogen range (NR) indicates the organisms' degree of omnivory (consumption of organisms from different trophic levels). In terms of the standard deviation of the nearest neighbor distance (SDNND) as a measure of distribution/dispersal of individuals within an isotopic space: lower values of SDNND mean a smaller distance between individuals and represents the trophic redundancy of species in the community, measuring the degree of species packing (Layman et al., 2007). The isotopic diversity of the population, being calculated as the average Euclidean distance of each individual of a population to the centroid  $\delta^{13}$ C– $\delta^{15}$ N of this population. The total area (TA) encompasses all the isotopic values within each group (i.e., all the specimens of a given family) and provides information on the total isotopic niche space occupied by the relevant population.

## Niche width and isotopic overlap

Isotopic niche was quantified for individuals and age groups, using the Stable Isotopic Bayesian Ellipses method in R (SIBER; Jackson et al., 2011). This analysis is based on calculated ellipses from a covariance matrix, which defines its forms and areas (Jackson et al., 2011) to estimate the width of the isotopic niches (Total area [TA] and Standard Ellipse Corrected Area [SEA<sub>C</sub>]).

Isotopic overlap was estimated using the nicheROVER package in R (Lysy et al., 2014), which is a Bayesian method that calculates the probability of overlap between niche pairs using multidimensional information as niche indicators (e.g., stable isotopes). The probabilistic density of niche overlap was calculated by running  $10^4$  iterations and 95% confidence interval (IC) of the data from each species or group occurring within their respective isospace, providing directional niche overlap estimates (e.g., x vs y and y vs x), according to the distributions of a specific species in the multivariate niche space (Lysy et al., 2014).

# RESULTS

From 16 terrestrial components of the Malpelo FFS, 125 samples (i.e., muscle, whole animals, leaves, eggs, and bird feathers) were analyzed for  $\delta^{13}$ C and  $\delta^{15}$ N (Table 1). The  $\delta^{13}$ C values ranged from -30.3% to -15.0% ( $-18.8 \pm 3.32\%$ ), with mosses (C<sub>3</sub> plants) and mealybugs (Isopoda)

showing the lowest and highest  $\delta^{13}$ C values respectively. For its part,  $\delta^{15}$ N showed values between 3.8‰ and 19.5‰, with C<sub>3</sub> plants being the most impoverished terrestrial elements, while the spider moss (Araneae) group had the highest  $\delta^{15}$ N values (Table 1).

According to  $\delta^{13}$ C and  $\delta^{15}$ N values, the estimated TPs values with 95% CI ranged between 1.0– 5.6 (mean: 2.7). The lowest estimated TPs (without considering basal sources) were given by the cricket (Gryllidae) and millipede (Diplopoda) groups, while the highest TPs were given by the spider (Araneae) and ant (*Odontomachus* sp.) groups (Table 2). These results suggest that the terrestrial trophic chain of the Malpelo FFS is composed of five trophic levels (TL): 1) TL-I; primary producers, 2) TL-II; herbivores (TP: 1.0–1.9), 3) TL-III; omnivores I (2.0–2.9), 4) TL-IV; omnivores II (3.0–3.5) and 5) decomposers and scavengers (Fig. 6A and B).



**Figure 6. A.** Average isotopic values (± standard deviation) of the different components of the terrestrial ecosystem of the Malpelo Fauna and Flora Sanctuary, Colombia, representing the trophic level to which they belong. **B.** Trophic pyramid of the terrestrial ecosystem of the Malpelo Fauna and Flora Sanctuary, Colombia.

On the other hand, measures of trophic structure (Layman metrics) suggest that the Malpelo FFS has an ecological community with high omnivory (NR; 17.6‰), use of different basal sources (CR; 15.4‰) and high isotopic diversity (TA: 134.71‰<sup>2</sup>), suggesting an average species distance (SD) of 3.68 and a trophic redundancy (nearest neighbor distance; NND) of 0.39. These results are

reflective of a trophic community with highly omnivorous individuals making use of several (or all) terrestrial basal sources, as well as highly redundant species/groups (Table 2).

Isotopic niche analysis (SEA<sub>C</sub>) showed that the terrestrial ecological community of the Malpelo FFS presented values ranging from  $0.82\%^2$  (*Johngarthia malpilenesis*) to  $8.46\%^2$  (Diplopoda) (Table 2; Fig. 3). This variability in isotopic niche breadth shows diversity of trophic interaction probabilities among species/groups in the terrestrial ecosystem of the Malpelo FFS. Of the 210 (105 in both directions: *x* vs *y*; *y* vs *x*) possible trophic interactions, 80.9% (*x* vs *y*) and 87.6% (*y* vs *x*) of them represented low overlap probabilities (0–30%), followed by 8.6% (*x* vs *y*) and 6.7% (*y* vs *x*) with intermediate probabilities (31–60%), while 10.5% (*x* vs *y*) and 5.7% (*y* vs *x*) had high probabilities (61–100%) of isotopic overlap (Table 3; Fig. 7).

 Table 2. Layman's metrics estimated for each of the components of the terrestrial ecosystem of the Malpelo Fauna and Flora Sanctuary, Colombia, together with the estimate of the trophic position, represented by values with 95% confidence interval (CI) and their modal value. NR: Nitrogen range, NC: Carbon range, TA: Total area, CD: centroid of their distribution, NND: nearest neighbor distance, SDNND: standard deviation of the nearest neighbor distance, SEAc: Standard corrected area.

Crown	ND	NC	Τ 4	CD	NND	CDNND	SEA	Trophic p	osition
Group	INK	NC	IA	CD	ININD	SDININD	SEAC	95% IC	Mode
Hymenoptera	3.29	0.82	1.27	1.19	1.09	1.08	2.60	1.6 - 2.7	2.2
Isopoda	2.85	2.46	3.42	1.15	0.58	0.30	2.86	3.1 - 4.2	3.7
Odontomachus sp.	3.71	2.03	5.02	1.13	0.54	0.57	2.88	3.2 - 4.0	3.6
Gryllidae	3.72	2.28	3.50	1.34	1.20	0.69	4.12	1.0 - 2.0	1.5
Araneae	4.47	3.48	6.46	1.67	0.99	1.00	6.36	4.4 - 5.6	4.9
Mycrocorifia	9.14	1.75	10.99	2.65	1.39	0.76	7.59	1.7 - 3.3	2.5
Lumbriculidae	3.25	1.69	1.23	0.93	0.74	0.64	1.40	4.1 - 5.2	4.7
Diplopoda	9.27	4.33	20.05	2.22	1.05	0.75	8.46	1.3 - 2.0	1.7
Anolis agassizi	1.94	2.17	1.73	0.72	0.48	0.42	1.40	2.2 - 3.0	2.6
Diploglossus millepunctactus	1.74	3.27	2.86	0.90	0.57	0.52	1.78	2.2 - 3.1	2.6
Johngarthia malpilensis	2.09	1.60	1.54	0.61	0.34	0.14	0.82	2.8 - 3.6	3.2
C <sub>3</sub> Plants	6.38	1.62	4.42	2.03	0.73	0.41	3.33	_	1.0
Detritus	_	_	_	_	_	_	_	_	1.0
Guano	_	_	_	_	_	_	_	2.1 - 3.0	2.6
Sula granti's eggs	_	_	_	_	_	_	_	1.8 - 2.7	2.3
Sula granti's feathers	_	_	_	_	_	_	_	2.1 - 3.0	2.6



Figure 7. Niche and isotopic overlap of the different components of the terrestrial ecosystem of the Malpelo Flora and Fauna Sanctuary, Colombia.



Figure 8. Average isotopic values (± standard deviation) of the different components of the terrestrial ecosystem of the Malpelo Fauna and Flora Sanctuary, Colombia, representing three potential trophic sub-networks. Each color represents the members of each community.

## DISCUSSION

This study is the first work that describes the terrestrial trophic structure of the Malpelo FFS, based on the use of chemical tracers as elements that reflect the food synthesized by organisms.

The  $\delta^{13}$ C and  $\delta^{15}$ N analyses suggest that the terrestrial trophic chain of the Malpelo FFS is short and organized into five trophic levels (Fig. 6) with an absence of top predators: 1) primary producers and basal sources (NT-I; plants and detritus), 2) herbivores (NT-II; Diplopoda and Gryllidae), 3) omnivores I (NT-III; Hymenoptera, Microcoryphia, *Sula granti*, *A. agassizi*, and *D. millepuntactus*), 4) omnivores II (NT-IV; *J. malpilensis*), and 5) decomposers and scavengers (Isopoda, *Odontomachus* sp, Araneae, and Lumbriculidae) (Table 2; Fig. 6A and B).

The above may be the result of four characteristics: 1) an ecosystem with low primary productivity (Jaarsma et al., 1998; Townsend et al., 1998) that depends mainly on nutrients of marine origin and not on the productivity of terrestrial plants (Wolda et al., 1975; von Parhl, 1990; López-Vicoria et al., 2009), 2) the limited size of the ecosystem (Schoener, 1989; Vander Zanden et al., 1999; Post, 2002), since Malpelo Island has an extension of 1.2 km2 (Graham, 1975), 3) history of community organization (e.g., colonization and evolution in situ), since this may induce restrictions in the development of the food web and thus limit the type of species that can be linked to the community and the food web (Post, 2002) and 4) omnivory processes could influence the size of the food web (Schoener, 1989; Post et al., 2000).

Ecosystems with these attributes tend to have few trophic levels (Pimm, 2002) due to the absence of top predators (Post 2002), as opposed to those ecosystems (e.g., the marine environment) with long and complex food webs (Briand and Cohen, 1987; Bengtsson, 1994) due to the presence of top predators that have the ability to feed over large areas (Pimm, 2002). However, the existence of other mechanisms may also influence the size of food webs, but more studies are needed to validate each of the hypotheses related to this issue (Post, 2002).

	Heminop	1	Odontomac		1	Mycrocor	Lumbriculi	2	Anolis	Diploglossus	Jhongarthia	1	S. granti	Detritu	S. granti
Groups	tera	Isopoda	hus sp.	Gryllidae	Araneae	yphia	dae	Diplopoda	agassizi	millepunctatus	malpilensis	C <sub>3</sub> Plants	feathers	S	eggs
Hymenoptera	_	0.4	1.6	37.6	4.3	91.3	0.1	96.6	0.4	0.7	0.0	0.0	0.0	6.0	3.4
Isopoda	0.2	—	78.7	6.1	16.8	0.4	11.1	4.8	10.7	9.9	37.4	0.0	2.4	1.8	0.5
Odontomachus sp.	0.8	73.7	-	7.7	33.4	1.5	19.9	12.0	10.7	9.4	34.9	0.0	2.0	1.7	0.6
Gryllidae	20.7	3.4	4.4	_	1.2	45.2	0.2	83.0	6.9	3.6	0.9	0.0	0.2	45.1	13.2
Araneae	2.0	4.7	11.7	0.7	_	34.3	12.8	7.9	0.6	0.6	1.3	0.0	0.1	0.1	0.1
Microcoryphia	30.4	0.2	0.8	20.0	17.0	_	0.2	72.1	0.1	0.2	0.0	0.0	0.0	6.3	0.8
Lumbriculidae	0.3	15.7	36.1	0.6	74.1	1.8	_	4.2	0.5	0.5	4.6	0.0	0.2	0.1	0.0
Diplopoda	27.1	1.1	2.4	31.1	3.0	61.8	0.3	_	1.1	0.8	0.2	0.0	0.0	10.2	3.2
Anolis agassizi	0.3	35.1	38.5	21.1	5.0	0.6	1.3	10.2	_	76.3	21.1	0.0	24.4	9.6	8.9
Diploglossus	0.2	22.1	22.0	7.6	2.0	0.4	1.0	2.0	56.1		22.4	0.0	21.1	2.1	0.1
millepunctatus Johngarthia	0.3	23.1	23.0	/.6	3.8	0.4	1.2	3.8	56.1	_	23.4	0.0	21.1	3.1	2.1
malpilensis	0.0	88.8	87.4	7.5	15.3	0.1	12.3	2.9	32.8	32.4	_	0.0	12.2	2.5	0.0
C <sub>3</sub> Plants	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	_	0.0	0.0	0.0
S. granti feathers	0.0	31.1	30.5	5.8	4.2	0.0	2.3	1.3	90.3	91.6	35.4	0.0	_	3.4	0.2
Detritus	5.0	1.3	1.3	62.7	0.4	25.9	0.1	64.0	3.2	1.5	0.4	0.0	0.2	_	2.8
S. granti eggs	8.3	6.0	6.4	84.2	2.2	12.9	0.0	76.3	27.7	11.2	0.1	0.0	0.1	21.9	_

Table 3. Isotopic overlap between the different components of the terrestrial ecosystem of the Malpelo FFS, Colombia, represented in percent probability.

Based on the trophic structure outlined above, NT-II (herbivores) was represented by mainly herbivorous groups, such as crickets (Gryllidae; Aguirre et al., 1987) and millipedes (Diplopoda; Bueno-Villegas, 2012). However, it is known that some species of crickets can demonstrate omnivorous and carnivorous feeding behavior (Aguirre et al., 1987) and may even consume carrion (Martin-Vega et al., 2013). Meanwhile, some species of millipedes have been observed to form important associations with guano (Deharveng and Bedos, 2012), which allows them to have great colonization success.

If the above is true: 1) the crickets of the Malpelo FFS could indicate that this group of organisms potentially takes advantage of different NTs of the terrestrial food web of the Malpelo FFS, consuming other NT-III invertebrates and consuming decomposing organic matter (DOM) and 2) the millipedes could be using seabird guano as an additional food source, which is plausible due to the large amount of guano present in the Malpelo FFS (López-Victoria et al., 2009). Despite the potential resource use (between trophic levels and different basal sources) by crickets (CR; Table 2), the millipede group showed greater omnivory than crickets (NR; Table 2).

This difference in NR and CR between millipedes and crickets may be due to two reasons: 1) millipedes, in addition to consuming decomposing plant matter, ingest significant proportions of seabirds' guano (see Chapter 4), thus increasing their  $\delta^{15}$ N and  $\delta^{13}$ C values due to isotopic fractionation during the process of matter degradation (Wynn, 2007; Lerch et al., 2010), and 2) crickets in the Malpelo FFS could be both herbivores and scavengers (see Chapter 4). This would be reflected in the increased NR and CR of both invertebrate groups, with NR and CR being influenced by the consumption of different types of food (e.g., plants and detritus [see Chapter 4]), thus modifying the isotopic signals integrated in their tissues. These hypotheses could be partially validated by the high values of isotopic overlap (88.2%) between crickets and millipedes and the low interaction (37.8%) between millipedes and crickets, the latter influenced by the degree of specialization of millipedes which mostly consume decomposing plant matter (Bueno-Villegas, 2012).

NT-III (omnivores I) was represented by large terrestrial vertebrates (i.e., *A. agassizi* and *D. millepunctatus*) and some small invertebrates (i.e., Hymenoptera and Microcoryphia). In this sense, *A. agassizi* and *D. millepunctatus* are two lizard species that share TPs (both 2.6), suggesting similar trophic resource use (similar NR and CR; Table 2) and, thus, a high trophic interaction between them (Table 3). These results may be due to the fact that *D. millepuntactus* is an

opportunistic and scavenging species, which are behaviors that make this species able to consume items from different TLs (high degree of omnivory; López-Victoria et al., 2011), such as: *J. malpilensis* (TL-IV), *D. millepunctatus* (TL-III), amphibious crabs (*Grapsus grapsus*; TL-IV; [see Chapter 2.1]), *S. granti* and its derivatives (eggs and chicks; TL-III), as well as *A. agassizi* (TL-III) and human food remains (López-Victoria et al., 2011). The opposite case is that of *A. agassizi*, which is a species with a more restricted niche, specializing in the consumption of small invertebrate species (i.e., small *J. malpilensis* (TL-IV), insects (TL-II), and occasionally earthworms), as well as seabird excrement, *S. granti* eggs (TL-III) and, occasionally, human food remains (López-Victoria et al., 2011). The above facts suggestthat *D. millepuntatus* occupies a wider trophic spectrum than *A. agassizi* (they share only three prey items); however, both species present similar isotopic niches (SEA<sub>C\_D. millpuntatus</sub> =  $1.78\%c^2$  SEA<sub>C\_A. agassizi</sub> =  $1.40\%c^2$ ). These facts demonstrate the high degree of trophic interaction between the two species (*D. millepuntatus* vs *A. agassizi* = 82.1% and *A. agassizi* vs *D. millepunctatus* = 64.4%), as well as the similar use of TLs (NR) and food sources (CR), which is also reflected in the similarity of their functions (isotopic redundancy) in the terrestrial ecosystem of the Malpelo FFS (Table 2).

On the other hand, the small invertebrates belonging to this TL are omnivorous species. For example, Hymenoptera consume a high variety of animal species (e.g., Diptera, Isopoda, Microcoryphia; Calero et al., 2011) and some plants (Fernández-Gayubo and Pujade-Villar, 2015). This is similar to the Microcoryphia group who feed on guano, detritus, carcasses (Calero et al., 2011), algae, lichens and remains of other arthropods (Bech de Roca et al., 2015). This similarity between the feeding habits of both groups of invertebrates reveals the high degree of interaction (Table 3) and their functional similarity (redundancy; Table 2). Therefore, a TL formed by two types of organisms (vertebrates and invertebrates) that show a high degree of interaction is evident, a finding that was also suggested by Calero et al. (2011).

Only one species was reported in NT-IV, the crab *J. malpilensis*, which is considered the largest terrestrial consumer in the Malpelo FFS (López-Victoria and Werding, 2008). This confirms the high trophic positions estimated in this study (TP: 2.8–3.6), as a result of its high degree of omnivory and opportunism (López-Victoria and Werding 2008). This reflects direct consumption of juvenile conspecifics (TL-IV), *D. millepunctatus* (TL-III), *A. agassizi* (TL-III), *S. granti* and their derivatives (TL-III), basal components (i.e., detritus and occasionally algae, microalgae and plants), human and seabird food waste, carcasses, and occasionally insects (TL-II) and intertidal

organisms (López-Victoria and Werding, 2008). This would explain the higher NR values (Table 2) and frequent trophic interactions (isotopic overlap) with the other consumer levels (i.e., *A. agassizi* and *D. millepunctatus*; Table 3; Fig. 7). Additionally, the isotopic overlap indicates high interactions with the group of scavengers and decomposers, such as ants (*Odontomachus* sp.) (Ehmer and Hölldobler, 1996; Camargo and Oliveira, 2012) and mealybugs (Isopoda) (Melic, 2015) (Table 3, Fig. 7). The high degree of trophic interactions between these organisms indicates a "shared use of DOM" (i.e., detritus and carcasses) between *J. malpilensis* and ants and mealybugs, suggesting the participation of *J. malpilensis* in DOM reuse processes, with respect to other potentially scavenging species such as *D. millepuntactus* (López-Victoria et al., 2011).

All of the above is evidence of high rates of trophic interaction and intragremial predation, largely due to the omnivorous habits of various components of the ecological community of the Malpelo FFS. This leads to the conclusion that the terrestrial ecosystem of the Malpelo FFS is composed of a large number of redundant species (NND <0.70 and SDNND <0.60; Table 2) and that the recurrence of omnivorous processes in the terrestrial food web of the Malpelo FFS is important for the maintenance of the ecological dynamics of the ecosystem (see Chapter 2.2). This feature may also be indicative of the terrestrial ecosystem's resilience to disturbances (see Chapter 2.2).

In conclusion, the food web of the Malpelo FFS is composed of five trophic steps: 1) primary producers (NT-I), 2) herbivores (NT-II), 3) omnivores I (NT-III), 4) omnivores II (NT-IV), and 5) decomposers and scavengers (Fig. 6). In addition, the terrestrial ecosystem of the Malpelo FFS reflects high degrees of intragremial interaction, omnivory and redundancy, which together with the absence of top predators (Tonn and Magnuson, 1982; Spiller et al., 1998) and the high presence of redundant species (Elmqvist et al., 2003), would indicate the capacity of this ecosystem to mitigate the effects of different disturbances (Mantel, 2003).

In addition to the above, the relative contribution of potential basal sources to each organism (see Chapter 4) and the formation of three sub-networks (see Chapter 2.2) in the terrestrial ecosystem of the Malpelo FFS suggest that the ecological community of this environment is isotopically comprised of three trophic sub-networks that have high (60–100%) and intermediate (30–59%) interaction among the organisms of each sub-network (Table 3). The first one is formed by *J. malpilensis*, ants (*Odontomachus* sp.) and mealybugs. The second, composed of *D. millepunctatus*, *A. agassizi*, guano, and *S. granti* and their derivatives, is closely related to the first

sub-web, while the third sub-network is composed of small invertebrates (i.e., Diplopoda, Gryllidae, Heminoptera, Araneae, Lombricullidae) whose trophic source is plants and detritus (Fig. 8). The above facts strongly support the hypothesis with respect to the importance of marine nutrients for the terrestrial ecological community of the Malpelo FFS, as has been suggested by direct observation studies (Wolda, 1975; López-Victoria et al., 2009; Calero et al., 2011) as well as studies with biochemical tracers (see Chapter 2.1) and is probably responsible for the formation of three sub-networks in the terrestrial ecosystem of the Malpelo FFS (see Chapter 2.2).

In conclusion, this study contributes to a better understanding of the functioning of an important area for the conservation and protection of species and helps to generate additional tools for the development of management and conservation measures focused on the integral whole (ecosystem) and not on the particular individual (species). Nevertheless, it is recommended that further studies be conducted to confirm, corroborate and strengthen the findings and conclusions generated in this study (length of the trophic chain, formation of sub-networks, ecological processes, isotopic signals of more species, etc.), in order to better understand the dynamics of the ecosystem, to identify major processes of change that are occurring, and to develop additional knowledge as to its ability to adapt to different processes of change (e.g., environmental, pollution and other impacts of human activities).

# 2.2. Terrestrial food web of the Malpelo Fauna and Flora Sanctuary, Colombia: a structural analysis from a topological approach

# **INTRODUCTION**

Knowledge of the structure of food webs and the factors underlying such structures is one of the major objectives of ecology, particularly in areas where tyhere is a great range of species-rich communities. In addition, there is the objective of environmental conservation, which aims to preserve biodiversity ("flagship", charismatic, endemic, rare, or otherwise threatened species [Tylianakis et al., 2010]). However, food webs are not only a set of species that interact with each other (Montoya et al., 2006; Bascompte and Jordano, 2007), since these interactions originate characteristic attributes and patterns of ecosystems that can in turn generate different mechanisms that model and modify the structure and functioning of the web, playing an important role in maintaining ecosystem stability (de Ruiter et al., 2005; Tylianakis et al., 2010).

Therefore, focusing on a single component of the web (e.g., a specific species orgroup) neglects the fact that its existence and survival depends on interactions with other components (Bascompte et al., 2006; Fontaine et al., 2006). Thus, focusing on species conservation does not necessarily maintain the structural integrity of the web, whereas preserving the structure of the web helps maintain biodiversity (Bascompte et al., 2006; Bastolla et al., 2009). However, another important factorffor achieving these objectives is the preservation of emergent web characteristics (e.g., stability), which require monitoring of attributes such as: connectivity (the degree of connection, linkage diversity, average path length), compartmentalization, and nesting (particularly when species or habitats are threatened by pollutants or other disturbances [Tylianakis et al., 2010]). The approach to resource and habitat management and conservation must consider the ecosystem holistically (Raffaelli, 2006; Pranovi and Link, 2009), taking into account its structure and functioning through energy flows, trophic relationships (Feng et al., 2017; Gamito et al, 2020) and species interaction strength (Werner and Peacor, 2003; Preisser et al., 2005).

Understanding the trophic interactions between the components of a web is an important step in explaining community dynamics and the impacts that species have on web compartments (Navia et al., 2010; Bornatowski et al., 2014). This in turn helps to understand how effects (direct and indirect) on web components can propagate throughout the system, affecting abundance and connectivity with other components (Werner and Peacor, 2003), possibly resulting in local extinctions leading to secondary extinctions and/or influencing populations of coexisting species (Pimm and Lawton, 1980). This implies that conservation priorities must be in accordance with the maintenance of web stability. Therefore, drawing inferences on the propagation of direct and indirect effects within webs facilitates a better understanding of how energy flow (Stevens et al., 2000; Navia et al., 2010), the position (e.g., centrality) and role of species are affected. Moreover, the ongoing monitoring of key web attributes allows inferences about the propagation of each species and the consequences for them of interactions with other species and other secondary effects (Dambacher et al., 2010; Navia et al., 2010), generating additional valuable information for achieving conservation objectives.

Notwithstanding the importance of describing food webs (Bascompte, 2009) and understanding the effects of different factors (e.g., environmental and anthropogenic) for the dynamics, productivity and stability of ecosystems (Rezende et al., 2009; Zetina-Rejón et al., 2015) as supports for conservation, there have been few efforts to improve the understanding of the community dynamics of a complex oceanic system where ecological dynamics are highly influenced by sea-land interaction (Polis et al., 1997; Caut et al., 2012), as is Malpelo Island.

Studies of food webs generate information on competition, nutrient dynamics, cascade effects (Winemiller and Polis, 1996) and community structure at different levels (individual, intermediate and group [Pimm, 1980]), making it possible to understand complex relationships between components and their properties (Balasudaram et al., 2005) and to identify structural patterns (Milo et al., 2002). This in turn enables the design of ecosystem-based management and conservation strategies (Whipple et al., 2000; Borgatti, 2002), utilizing methods that generate simplified models of food webs, i.e., topology, which facilitate the evaluation and prediction of the qualitative dynamics of the model based on the concept of community structure, which within the context of a web is visualized with nodes and links, where nodes correspond to species (i.e., predator or prey) and links represent their trophic interactions. Moreover, the topological properties of webs provide essential measures for common problems in graph theory, which, in turn, can be applied in ecology. For example, the identification of highly influential nodes in webs (Borgatti, 2005) can be represented in ecology as keystone species (Mills et al., 1993; Jordán et al., 2006).

Considering the above, the geographic isolation of Malpelo Island places the island in a location where several marine currents converge (Fig. 4d; see Chapter I), which makes it an ideal site for the aggregation of endemic and migratory species, resulting in an ideal study area for the analysis of food webs. The high ecological values of the area has led to Malpelo Island currently being the largest marine protected area (MPA) in the Colombian Pacific, known as the Malpelo Fauna and Flora Sanctuary (SFF) (Fig. 4B; see Chapter I [Ministerio del Ambiente y Desarrollo Sostenible, 2017]). The area has also been included as part of the World Heritage for Humanity by UNESCO, among other nomuinations in recognition of its high conservation value (Plan de Manejo, 2015); however, the lack of information on its ecological dynamics has meant that the MPA's management and conservation strategies are carried out from an individual approach (i.e., species or groups) and not from an ecosystem approach.

Currently, some studies on food habits for terrestrial and marine species of the Malpelo FFS have been reported (Table 4); however, studies related to their food webs are more scarce. To date, there are only two studies focused on the analysis of food webs. The first one modeled the terrestrial food web of the Malpelo FFS from direct observation and trophic studies of a few terrestrial species, thus representing some energy flows within the system; but, due to the paucity of information, this study left many more questions regarding energy flow unanswered (Fig. 3A; see Chapter 1 [Wolda, 1975]). The second study focused on the terrestrial invertebrate community, representing energy fluxes and biomass production (Fig 3B; see Chapter I [Calero et al., 2011]). Both studies highlight the importance of the Nazca booby *Sula granti* in maintaining the terrestrial ecosystem. However, these studies do not describe the attributes and structural patterns of the web, nor do they identify those key species that keep the system stable. This leaves the trophic dynamics of the terrestrial ecosystem still poorly understood and, furthermore, tends to promote management and conservation measures that focus on particular species rather than adopting an 'all-of-ecosystem' perspective. Therefore, the identification of structural patterns and the role of species cancontribute to an integrated approach to conservation efforts.

Based on these premises, the objectives of this chapter were: 1) to describe the structure of the terrestrial food web of Malpelo FFS from a topological approach, 2) to identify key species, 3) to identify the formation of terrestrial trophic sub-webs as indicators of stability and resilience to disturbances, and 4) to evaluate the resilience of the food web.

#### **MATERIALS AND METHODS**

#### Study area

Malpelo Island (Fig. 4A) is the summit of a submarine mountain range called the Malpelo Ridge, which extends in a NE-SW direction; it is approximately 241.4 km long by 80.5 km wide (Fig. 4B, red polygon). The island has a maximum height of 300 m above sea level and the ridge of which it forms a part rises from approximately 4,000 m depth (Fig. 4C) (more details in Chapter 1).

#### **Dietary analysis**

For the construction of the trophic relationships between terrestrial species of the Malpelo FFS, an adjacency matrix comprised of 27 trophogroups was elaborated. The matrix was fed with binary data (0, 1) representing the trophic relationships between trophogroups *i* and *j*. Therefore, if trophogroups *i* and *j* had any trophic relationship (i.e., eats or is eaten) they were represented by the value of 1; on the contrary, if trophogroups *i* and *j* did not have any trophic relationship, they were represented by the value of 0.

The information defining the trophic relationships between trophogroups in the terrestrial ecosystem of the Malpelo FFS was obtained from trophic studies in the study area (Table 4). For those trophogroups for which trophic information was not available in the study area, this information was obtained from trophic studies of similar species (Table 4).

# **Topological analysis**

# Identification of key trophogroups

The identification of key trophogroups was based on the estimation of local indicators (i.e., topological centrality indices) that provided an idea of their positional importance. Accordingly, seven primary indicators were estimated: 1) *node degree index* (*DC*), 2) *betweenness index* (*BC*), 3) *closeness index* ( $C_i$ ), 4) *Eigen centrality* (*EC*), 5) *clustering coefficient* (*CC*<sub>individual</sub>) of a web, 6) *subgraph centrality* (*SC*) of the node and 7) *centrality of an odd subgraph* (*SC*<sub>odd</sub>) (Table 5).

Once the centrality indices were calculated, a Pearson correlation analysis (r) was performed to identify those indices that had a high significant correlation (r > 70%, p = 0.05) and could indicate similarities in the identification of key trophogroups of the Malpelo FFS.

Species/groups	Commun name	Author(s) and years
Arthropods		
Johngarthia malpilensis	Terrestrial crab	López-Victoria y Werding (2008)
Diplopoda	Millipedes	Villegas (2012), Melic (2015)
Odontomachus sp.	Ants	Ehmer y Hölldobler (1996); Ávila et al., (2010); Camargo y Oliveira (2012)
Gryllidae	Crickets	Aguirre-Segura y Barranco-Vega (2015)
Birds		
Sula sula*	Red footed boby	López-Victoria y Gacría (2010)
Sula granti*	Nazca booby	García y López-Victoria (2007); López-Victoria et al. (2009)
-		Lánaz Viataria y Warding (2008): Lánaz Viataria et al. (2011)
Migratory birds	-	Lopez- victoria y werding (2008), Lopez- victoria et al, (2011)
Reptiles		
Anolis agassizi	Lizard	
Diploglossus millepunctatus	Dotted lizard	Lopez-Victoria (2006); Lopez-Victoria et al., (2011)

 Table 4. Bibliographic review of studies of food habits of some species present in the terrestrial ecosystem of the Malpelo Fauna and Flora Sanctuary, Colombia, for the construction of trophic relationships (adjacency matrix).

 Phyllodactylus transversalis
 Geko
 López-Victoria (2006); López-Victoria et al., (2013)

 \*Nazca booby Sula granti r represented by juveniles and adults combined, due to the similarity in feeding habits (López-Victoria et al. 2009). Bold: Components with higher centrality values.

To facilitate comparison and identification of key trophogroups, centrality indices were scaled between 0 and 1. All analyses were performed in R statistical software (R Team Core, 2018).

#### Community substructures in the trophic webs

Substructures in food webs are defined as sets of species that are more closely interconnected with each other than with other species in the rest of the web (Rezende et al., 2009). To identify these substructures in the terrestrial ecosystem of the Malpelo FFS, the *fast greedy* subweb identification algorithm (Newman and Girvan, 2004) of the R software package *igraph* (version 1.2.6) was applied (Csardi and Nepusz, 2006).

## Motifs

One method to explore patterns of trophic assemblages is the identification of *motifs*, which are recurrent patterns of connectivity that constitute subgraphs that can include any number of nodes and links. Studies of trophic webs have focused primarily on 3-node *motifs* as ecological theory has relied on several of these patterns (Baiser et al., 2016). Furthermore, the identification of *motifs* in webs requires algorithms that are computationally demanding.

There are 13 possible configurations of 3-node *motifs* (Fig. 9), four of which have been related to ecological processes: 1) apparent competition occurs when two species are preyed upon by a common predator (Fig. 9a), 2) a tri-trophic chain is formed by three nodes where energy flows from base sources to higher consumers (Fig. 9b), 3) exploitative competition is an indirect competition that occurs when a resource is shared by two consumers (Fig. 9d), and 4) omnivory occurs when a species feeds on more than one trophic level (Fig. 9e). In accordance with the above, the recurrence of 3-node *motifs* present in the terrestrial food web of the Malpelo FFS was evaluated. The presence or absence of some type of motifs is related to the persistence and resilience of the food web (Stouffer and Bascompte, 2011).

For the identification of *motifs* in the terrestrial food web of the Malpelo FFS, the R software package *igraph* (Csardi and Nepusz, 2006) was used.



Figure 9. Subgraphs representing the 13 types of 3-node *motifs* present in directed webs. Five of these *motifs* are based on ecological theory. **a.** Apparent competition, **b.** Tritrophic chain, **d.** Exploitative competition, **e.** Omnivory. Taken from: Elhesha et al. (2017).

Table 5. Topological indices used in the terrestrial food web analysis of the Malpelo Fauna and Flora Sanctuary, Colombia, represented by index name, formula, description of
variables and method, and the reference from which each index was obtained.

Indiaca	Formula	Descr	iption	Reference	
muices	Formula	Variable	Methods		
Degree $(DC_i)$	$DC_i = D_{\mathrm{in},i} + D_{\mathrm{out},i}$	$DC_{in}$ is the number of predators and $DC_{out}$ is the number of prey.	The normalized node degree ( <i>DC</i> ) represents the number (in percent) of connections between pairs of nodes and is the sum of the input (predators) and output (prey) connections.		
Betweenness centrality $(BC_i)$	$BC_{i} = \frac{2 \times \sum_{j < k} \frac{\mathbf{g}_{jk}(\mathbf{i})}{\mathbf{g}_{jk}}}{(N-1) \times (N-2)}$	BC is the frequency of species i on the shortest path between species j and k. N is the number of species, $g_{jk}$ is the number of minimum isometric paths between species j and k, and $g_{jk}(i)$ is the number of species i in the shortest path between j and k ( $i \neq j$ , $i \neq k$ ). The higher value of BC <sub>i</sub> indicates the species(s) that has greater control of the information in the web.	The intermediation index ( <i>BC</i> ) represents the capacity to control the exchange of information in the food web.	Wasserman y Faust (1994)	

Closseness (C <sub>i</sub> )	$C_i = \frac{N-1}{\sum_{j=1}^N d_{ij}}$	<i>C</i> indicates the sum of the minimum distances from a species to all other species in the web, N is the number of species, and $d_{ij}$ is the length of the shortest path between species <i>i</i> and <i>j</i> .	The closeness index indicates the speed of information transmission through the food web. That is, species with lower Ci values transmit information more quickly through the network than those with higher values.	Wasserman y Faust (1994)
Eigen Centrality (EC)	$\lambda C_{eiv} = A \times C_{eiv}$	$\lambda$ is a constant, $C_{eiv}$ is an eigenvector of the adjacency matrix A with eigenvalue $\lambda$ .	The closeness index ( <i>C</i> ) indicates the speed of information transmission through the food web. That is, species with lower $C_i$ values transmit information more rapidly through the web than those with higher $C_i$ values.	Bonacich (1987)
Clustering coefficient (CC <sub>i</sub> )	$CC_i = rac{1}{N} \sum_{i=1}^{N} rac{2E_i}{k_i(k_i-1)}$	$N_i$ with $k_i$ neighbors, $E_i$ is defined as the number of links between the $k_i$ neighbors. The clustering coefficient is the ratio of the number of ties between neighbors to the number of ties between neighbors ( $E_i$ ) and the potential number of links ( $k_i$ ( $k_i - 1$ ) / 2 among neighbors.	The clustering coefficient ( $CC_i$ ) measures how densely connected a node is to its immediate neighbors. If $CC_i = 1$ , all neighbors are connected to each of the nodes; but, if $CC_i = 0$ , none of its direct neighbors are connected to the other nodes.	Watts y Strogatz (1998)
Subgraph centrality (SC)	$SC(i) = \sum_{j=1}^{N} [\gamma_j(i)]^2 e^{\lambda j}$ $Eq. 2$ $SC(i) = \sum_{j=1}^{N} [\gamma_j(i)]^2 \cosh \lambda_j + \sum_{j=1}^{N} [\gamma_j(i)]^2 \sin h\lambda_j$ $= SC_{ext}(i) + SC_{odd}(i)$ $Eq. 3$ $SC_{odd} = \sum_{r=1}^{\infty} \frac{\mu_{2r}(i)}{(2r)!}$	Where (i) is the <i>i</i> <sup>th</sup> component of the <i>j</i> <sup>th</sup> eigenvector of the adyacence matrix A and $\lambda_j$ is the corresponding <i>j</i> <sup>th</sup> eigenvalor (Eq. 1). SC(i) counts all closed paths (CWs) in the web, which can be of even (SCeven) and odd (SC <sub>odd</sub> ) length. CWs of even length can move back and forth in subgraphs that have no cycles (i.e., acyclic), while odd CWs do not contain contributions from acyclic subgraphs. Consequently, SC(i) can be divided into two terms by considering the even and odd CWs in the food web (Eq. 2).	The subgraph centrality index (SC) of the node characterizes the importance of a node in all existing subgraphs in the web. The SC of a vertex $i$ is defined as the "sum" of closed paths (CWs) of different lengths in the web, starting and ending at vertex $i$ .	Estrada y Rodríguez- Velázquez, 2005a Estrada y Rodríguez- Velázquez, 2005b

$$C_i = \frac{N-1}{\sum_{j=1}^{N} d_{ij}}$$

		Therefore, $SC_{odd}(i)$ can be expressed in terms of the number of odd-length CWs using the expression in Equation 3.		
Anidación (NODF)	$NODF = \frac{\sum N_{paired}}{\left[\frac{n(n-1)}{2}\right] + \left[\frac{m(m-1)}{2}\right]}$	<i>NODF</i> is the nesting measurement, $N_{\text{paired}}$ is the degree of nesting matching, n(i-1)/2 y $m(m-1)/2$ are the nesting pairing degrees for columns <i>n</i> and rows <i>m</i> , respectively.	<i>NODF</i> is the nesting measure of the web. This method returns values from 0 to 100, indicating that values equal to 0 indicate no nesting, while values equal to 100 suggest perfect nesting.	Almeida-Neto et al., 2008
Modularity	$M_{\scriptscriptstyle W}(P) = \sum_{\scriptscriptstyle s=1}^{\scriptscriptstyle N_{\scriptscriptstyle M}} \left[ \left( rac{w_{\scriptscriptstyle s}^{\scriptscriptstyle in}}{W}  ight) - \left( rac{w_{\scriptscriptstyle s}^{\scriptscriptstyle a \scriptscriptstyle l \scriptstyle l$	$W = \sum_{i \ge j} w_{ij}$ is the sum of the weights of all predator-prey interactions throughout the web. $w^{in}s$ is the sum of the weights of the linkages $w_{ij}$ within each compartment <i>s</i> , and $w^{all}s = \sum_{ils} \sum_{j} w_{ij}$ is the sum of the weights of the interactions involving species <i>i</i> within module <i>s</i> with all other species.	Modularity is the number that illustrates how much a given web can be organized into communities or subwebs. Modularity captures how good a partition is compared to a randomly intertwined web.	Newman and Girvan (2004)
Connectance	$C = L/S^2$	<i>L</i> is the link and <i>S</i> are the species.	Connectance is the ratio of observed linkages to all possible linkages, including cannibalism and mutual predation (Polis, 1991), within a food web.	
Average path length (APL)	$APL = \frac{1}{N(N-1)} \sum_{ij=1, i \neq j}^{N} d_{ij}$	The distance $d_{ij}$ between nodes <i>i</i> and <i>j</i> is defined by the number of vertices that make up the shortest path connecting two nodes. If nodes <i>i</i> and <i>j</i> are not connected, then $d_{ij} = N$ .	It is the average distance between any pair of nodes.	Travers and Milgram (1969)

#### **Topological properties and resilience of the trophic network**

The structure of the terrestrial food web of the Malpelo FFS was analyzed based on several global web indicators, such as: number of trophogroups (N), connectance, clustering coefficient ( $CC_{global}$ ), modularity, diameter, average path length (APL), web centralization and global nesting (NODF) (description in Table 5). This last web attribute was estimated with the RInSp package (Almeida-Neto et al., 2008).

The resilience of the terrestrial food web of the Malpelo FFS was evaluated based on the consecutive removal of nodes (i.e., trophogroups). This removal was done to analyze the resilience of the web through the alterations of its attributes. For this purpose, two types of scenarios representing "attacks" or "failures" were simulated following Albert et al. The "attacks" are impacts directed at particular nodes; in this case the nodes are removed in descending order according to the value of their centrality in the web (Zetina-Rejón et al., 2022). For analytical purposes, three criteria were used to determine the order of node removal: 1)  $DC_{All}$ , 2) BC and 3) SC. These indices were selected considering their high correlation with respect to other centrality indices (described above).

The "failures" are random errors that could occur in the web, pursuant to which nodes were randomly removed three times. Once the nodes were removed, changes in four web properties were evaluated, such as: size (e.g., APL), degree of clustering, connectivity and centralization of the web.

This analysis was designed in the R statistical platform (R Core Team, 2018), based on the simulation approach proposed by Albert et al. (2000).

## RESULTS

The representation of the terrestrial food web of the Malpelo FFS consisted of a total of 27 trophogroups, eight of which were identified at the species level and 19 at the group level (e.g., class, order, family or type), which presented 98 trophic links (Fig. 10A).

According to Pearson's correlation analysis (r),  $DC_{all}$  showed highly significant correlations with C, BC, EC, and  $SC_{odd}$ . On the other hand, SC reflected a highly significant correlation with  $DC_{out}$ while CC presented low correlations with respect to the other centrality indices. These results suggest functional similarity for the trophogroups; therefore, different indicators were chosen to give an idea of consumer/prey ( $DC_{all}$ ), "bridging" (BC), high interaction in trophic subgroups (SC) and clustering (CC) roles.



Figure 10. A. Terrestrial food web of the Malpelo Fauna and Flora Sanctuary, Colombia, showing three trophic communities. B. Simplification of the three communities present in the terrestrial food web and their interactions. Community 1: yellow nodes, Community 2: purple nodes, Community 3: green nodes. Red arrows: diameter of the web.

#### Identification of key species in the terrestrial food web

According to local centrality measures, the DC<sub>All</sub> suggests that the trophogroups that exerted the greatest consumer ( $DC_{in}$ ) and prey ( $DC_{out}$ ) roles were decomposing organic matter (DOM), followed by *J. malpilensis*, *A. agassizi*, *D. millepunctatus*, Staphylinidae, ants, plants, corpses, insects, and flies (Table 6; Fig. 10A and Fig. 11).

On the other hand, *C*, *BC*, and *EC* suggest that the trophogroups of greatest proximity (*C*), intermediacy (*BC*), and influence (*EC*) were DOM, *J. malpilensis*, *A. agassizi*, and *D. milepunctatus* (Fig. 11; Table 5). In addition, it is worth mentioning the group of plants, ants, insects and flies, which showed significant EC values (>40% and <50%; Table 6; Fig. 11).

With respect to individual *CC*, microalgae, chicks and eggs of *S. granti*, gastropods, spiders, and food remains (from seabirds and humans) showed the highest values (between 70–100%), followed by myriapods, isopods, insects, Staphylinidae, *S. granti*, migratory birds, invertebrates, plants, crickets, excrement, feathers, flies, carcasses, earthworms and ants with intermediate values

(between 30–69%). While *A. agassizi*, *D. millepunctatus*, MOD, *J. malpilensis*, Phylodactylus transversalis, and *S. sula* were the trophogroups with the lowest individual CC (0–29%) (Table 6).



Figure 11. Representation of the trophogroups of the terrestrial food web of the Malpelo Fauna and Flora Sanctuary, indicating the values of some centrality indexes. MOD: Decomposing organic matter.

Finally, the trophogroups that had the highest participation in trophic subgroups were plants and *S. granti*, reflecting the highest *SC* values (Table 6; Fig. 11). This confirms that the indicators used may be capturing different dimensions of the role of species within the Malpelo FFS food web.

and meso-	and meso-scale indices, represented in normalized values. DC: Degree, C: Closeness, BC: Intermediation, EC: Eigen centrality,									
	<b>SC</b> <sub>odd</sub> : Odd subgraph centrality, <b>SC</b> : Subgraph centrality, and <b>CC</b> : Clustering coefficient.									
Code	Species	DCIn	DCout	DCAllo	С	BC	EC	SC	SCodd	CC
1	Microalgaes	0.00	0.22	0.00	0.27	0.00	0.15	0.15	0.06	1.00
2	Johngarthia malpilensis	0.74	0.67	0.78	0.67	0.38	0.94	0.26	0.55	0.15
3	Excrement	0.16	0.22	0.13	0.29	0.08	0.19	0.08	0.17	0.40
4	Sula granti feathers	0.16	0.22	0.13	0.36	0.12	0.21	0.06	0.06	0.40
5	Sula granti eggs	0.05	0.44	0.13	0.34	0.00	0.33	0.21	0.46	0.80
6	Sula granti chicks	0.00	0.33	0.04	0.29	0.00	0.22	0.21	0.23	1.00
7	Sula granti	0.00	0.56	0.13	0.36	0.02	0.24	0.96	0.50	0.60

0.22

0.78

0.00

0.38

0.04

0.36

0.56

0.85

0.38

8

Carcasses

Table 6. Centrality indices of the terrestrial food web of the Malpelo Fauna and Flora Sanctuary, Colombia, represented in local and meso-scale indices, represented in normalized values. *DC*: Degree, *C*: Closeness, *BC*: Intermediation, *EC*: Eigen centrality, *SC*: Subgraph centrality, and *CC*: Clustering coefficient

9	Diploglossus millepunctatus	0.47	0.44	0.48	0.47	0.07	0.65	0.15	0.29	0.26
10	Anolis agassizi	0.68	0.44	0.65	0.71	0.35	0.80	0.15	0.29	0.28
11	Insects	0.00	0.78	0.22	0.38	0.01	0.43	0.50	0.89	0.62
12	Plants	0.00	1.00	0.30	0.44	0.06	0.43	1.00	1.00	0.47
13	Food remains	0.00	0.56	0.13	0.34	0.00	0.35	0.33	0.50	0.80
14	DOM	1.00	0.67	1.00	1.00	1.00	1.00	0.18	0.27	0.18
15	Invertebrates	0.00	0.44	0.09	0.31	0.01	0.23	0.20	0.18	0.50
16	Earthworms	0.05	0.22	0.04	0.25	0.00	0.24	0.05	0.05	0.33
17	Flies	0.11	0.67	0.26	0.38	0.03	0.42	0.25	0.52	0.39
18	Ants	0.32	0.56	0.39	0.44	0.05	0.45	0.13	0.25	0.31
19	Crickets	0.21	0.33	0.22	0.38	0.05	0.30	0.05	0.05	0.43
20	Gasteropods	0.05	0.33	0.09	0.29	0.00	0.23	0.06	0.06	1.00
21	Staphylinidae	0.37	0.11	0.26	0.41	0.02	0.39	0.02	0.03	0.61
22	Phollydactylus transversalis	0.26	0.00	0.13	0.29	0.02	0.20	0.00	0.00	0.10
23	Millipedes	0.16	0.11	0.09	0.27	0.00	0.17	0.02	0.03	0.67
24	Spiders	0.05	0.22	0.04	0.25	0.00	0.15	0.08	0.12	1.00
25	Sula sula	0.00	0.22	0.00	0.00	0.00	0.00	0.13	0.08	0.00
26	Migratory birds	0.16	0.22	0.13	0.29	0.01	0.25	0.04	0.08	0.60
27	Isopoda	0.16	0.11	0.09	0.27	0.00	0.17	0.02	0.03	0.67

## Communitary sub-webs in terrestrial food webs

The terrestrial food web of the Malpelo FFS showed a modularity value of 24.2% and a nesting of 41.5%, resulting in a compartmentalized web with three community sub-webs (Fig. 10A, B). Community 1 consisted of 10 trophogroups, community 2 consisted of 6 trophogroups and community 3 presented 11 species.

## Motifs

Of the 13 possible *motifs* of 3-nodes present in directed webs, the terrestrial food web of the Malpelo FFS (Fig. 9) was represented by 12 of them, forming a total of 592 global *motifs* (Table 7). According to the above and to ecological theory, the most recurrent *motifs* were those related to apparent competition (42.1%; Fig. 9a), followed by tri-trophic chains (18.8%; Fig. 9b), exploitative competition processes (11.3%; Fig. 9d) and, to a lesser extent, omnivory processes (10.5%; Fig. 9e) (Table 7).

Likewise, the motifs present in each of the terrestrial trophic communities of the Malpelo FFS were identified, identifying 47 *motifs* of 3-nodes in community 1, 11 *motifs* in community 2, and 90 *motifs* in community 3 (Table 6). Communities 1, 2, and 3 showed the highest frequency of

apparent competition processes (40.4%, 27.3%, and 30.0%; respectively) with respect to the other ecological processes (Table 7).

matifa		Recurrence (n)									
monjs	Global	Community 1	Community 2	Community 3							
а	249	19	3	30							
b	111	11	4	11							
с	51	4	0	5							
d	67	5	1	13							
f	62	3	3	24							
g	20	2	0	0							
h	13	0	0	3							
i	3	0	0	0							
j	6	2	0	3							
k	4	0	0	1							
1	3	1	0	0							
m	3	0	0	0							
n	0	0	0	0							
Total	592	47	11	90							

 Table 7. Recurrence of 3-node *motifs* in each of the communities observed in the terrestrial food web of the Malpelo Fauna and Flora Sanctuary, Colombia. Motifs related to ecological theory: a. Apparent competition, b. Tri-trophic chain, d. Exploitative competition, e. Omnivory.

The terrestrial food web of the Malpelo FFS reflected a diameter equal to 4 (Fig. 10A) and an APL of 2.17. Likewise, the web was represented by a connectance of 13.4%, a global  $CC_{global}$  of 37.3%, a degree of compartmentalization of 24.2%, a nesting (NODF) of 41.5% and a centralization of trophic relationships of 35.4%. According to resilience analyses, these structural attributes can be drastically affected if some of their components suffer any direct perturbation (i.e., attack) that modifies their local attributes (i.e., *DC*, *BC*, and *SC*).

On the other hand, the terrestrial ecosystem of the Malpelo FFS shows a high percentage (>50%) of tolerance to the effects of "failure or error" in the web nodes in terms of DC (Fig. 12), BC (Fig. 13) and SC (Fig. 14). However, direct removal or "attack" and "failure or error" of nodes based on the importance of the three selected centrality indices (i.e., DC, BC, and SC), reflect a drastic loss in web connectivity (Figs. 12–14).

According to the above, the elimination of 11.1-48.1% of the nodes with higher values of *DC* and *BC* could drastically modify the global attributes of the web (Figs. 12 and 13). While,

according to the *SC*, between 40.7-55.5% of the trophogroups could affect the web structure in the face of a perturbation (Fig. 14).



**Figure 12.** Resilience of the terrestrial food web of the Malpelo Fauna and Flora Sanctuary, Colombia, based on the "attack" of the nodes according to their nodal degree values (*BC*), to simulate the modification of some global attributes of the web. **Attack:** Red dots. **Error:** Green, yellow and blue dots.



**Figure 13.** Resilience of the terrestrial food web of the Malpelo Fauna and Flora Sanctuary, Colombia, based on the "attack" of the nodes according to their values of intermediation (*BC*), to simulate the modification of some global attributes of the web. **Attack:** Red dots. **Error:** Green, yellow and blue dots.



**Figure 14.** Resilience of the terrestrial food web of the Malpelo Fauna and Flora Sanctuary, Colombia, based on the "attack" of the nodes according to their subgraph centrality (*SC*) values, to simulate the modification of some global attributes of the web. **Attack:** Red dots. **Error:** Green, yellow and blue dots.

#### DISCUSSION

The isolation of Malpelo Island means that its ecological dynamics are closely related to the surrounding marine ecosystem. In addition, this isolation makes this site an ideal place for the aggregation of species (endemic and migratory). This allows the existence of ecological processes that contribute to maintain its functioning in a stable way, but at the same time, it is highly vulnerable to disturbances due to the complexity of the mechanisms that provide its stability. Despite its importance as an MPA, few studies have been developed in the Malpelo FFS to understand its ecological processes. Topological studies contribute to reveal aspects of the Malpelo FFS, such as: key species, compartmentalization, trophic interaction patterns (*motifs*) and other attributes that model the web that are still unknown for this MPA.

## Key trophogroups in the terrestrial food web

The trophic dynamics of ecosystems can be dominated by some highly important components, so that their identification based on their positional role in the food web (i.e., connectivity,

proximity or intermediation [Gómez et al., 2003]) as a relevant feature for ecosystem functioning would increase the understanding and efficiency of management and conservation efforts (Capocefalo et al., 2018).

The terrestrial ecosystem of the Malpelo FFS is a system with special dynamics due to its isolation and climatic conditions influenced by the surrounding water, all the water masses that in this area converge (Whittaker and Fernández, 2007), its interaction with the marine environment (Wolda, 1975; von Prahl, 1999; López-Victoria et al., 2009) and external forces (e.g., solar indicative radiation [Gómez-Navarro et al., 2012; García-Velero et al., 2012]), which is reflected in low terrestrial biodiversity of both fauna and flora, and numerous endemisms (Management Plan, 2015).

The trophic dynamics of the terrestrial ecosystem of the Malpelo FFS is governed by five trophogroups: MOD, *A. agassizi*, *D. millepuntactus*, *J. malpilensis*, and *S. granti*. These five components manifest higher centrality values (i.e.,  $DC_{ALL}$ , *BC*, *EC*, and *SC*). This suggests that these trophogroups are key to this ecosystem, being highly relevant for web cohesion and connectance (Palacio-Arce, 2014). Thus, these elements have a high interaction in the different trophic pathways of the system, contributing to the integration and availability of nutrients from the sea.

This can be explained by two reasons: 1) generalist feeding habits of the components (López-Victoria et al., 2009). For example, *D. millepuntactus* and *J. malpilensis* have similar feeding habits. These species consume almost all components of the terrestrial ecosystem and even their own species (cannibalism) and each other (López-Victoria et al., 2008, 2011, 2013) and 2) the close trophic relationship of *D. millepuntactus*, *J. malpilensis*, and *A. agassizi* with *S. granti* and

The trophic dynamics of ecosystems can be dominated by some highly important components. Hence the identification of dominant species based on their positional role in the food web (i.e., connectivity, proximity or intermediation [Gómez et al., 2003]) as a relevant feature for ecosystem functioning could increase the efficiency of management and conservation efforts (Capocefalo et al., 2018).

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This can be explained by two reasons: first, generalist feeding habits of the components (López-Victoria et al., 2009). For example, *D. millepuntactus* and *J. malpilensis* have similar feeding habits. These species consume almost all components of the terrestrial ecosystem and even their own species (cannibalism) and each other (López-Victoria et al., 2008, 2011, 2013). Second, the close trophic relationship of *D. millepuntactus*, *J. malpilensis*, and *A. agassizi* with *S. granti* and its derivatives (chicks and eggs), and food remains. For example, the low primary productivity influence of the terrestrial ecosystem is supplanted by marine nutrients transported by *S. granti* (Wolda, 1975; López-Victoria et al., 2009) and deposited in the form of guano and other derivatives (i.e., carcasses, feathers, eggs, and chicks [López-Victoria et al., 2008, 2011, 2013]). Guano is integrated through small consumers, while the other derivatives are mainly consumed by larger organisms (e.g., *D. millepuntactus*, *J. malpilensis*, and *A. agassizi* [López-Victoria et al., 2008, 2011, 2013]). The above is a reflection of the high interaction of *S. granti* in the formation of subgroups (high *SC* and  $CC_{individual}$  values, Table 1) due to its role as an integrator of marine nutrients to the terrestrial ecosystem.

On the other hand, DOM was the component with the highest centrality in the terrestrial web. Its high centrality is related to its origin, since this component comes from dead animals, guano, food remains, etc. (López-Victoria et al., 2009) and from microbial degradation (Ramírez et al., 2010), leaving a high amount of nutrients available for detritivorous organisms (Andramunio-Acero and Caraballo, 2012). Thus, DOM is a key component for connectivity between base and intermediate levels (Abarca-Arenas et al., 2007; Xochihua-Simón, 2009; Molina-Hernández, 2001). Although DOM is not a living component, it should be considered in web analyses (Miranda

et al., 2013) as it regulates "bottom-up" processes and can shape the trophic structure of ecosystems (Pimm et al., 1991; Menge, 2000; Molina-Hernández, 2011). Therefore, their exclusion from analyses would affect the interpretation and modeling of trophic dynamics (Pimm et al., 1991; Menge, 2000; Molina-Hernández, 2011).

The presence of substantial "bottom-up" processes in the terrestrial ecosystem of the Malpelo FFS (Menge, 2000) controls and regulates the abundance of organisms (Cury et al., 2003; Scheffer et al., 2005; Hernández et al., 2008) their role it particularly significant given that primary productivity is limited due to the low diversity and abundance of plants (Pimm et al., 1991). Therefore, the trophic dynamics of this ecosystem are governed by the accumulation of detritus (Wolda, 1975; López-Vicoria et al., 2009), mainly through two processes: 1) transformation of carcasses (e.g., chicks and juveniles) and other derivatives (e.g., feathers and eggs) of *S. granti* due to the action of large consumers, who make them available to the other components of the web and, 2) the deposition of seabird guano deposited in the terrestrial ecosystem.

In addition to the "bottom-up" control mechanisms present in terrestrial ecosystems benefiting from marine nutrients ("donor-controller system" [Pimm et al., 1991]), its trophic structure (shape and energy flow) can also be shaped by the super-generalist species present in it. However, the role of a particular species may vary within an ecosystem, both in space and time; thus, a keystone species in one scenario may have relatively little influence on community dynamics in another scenario (de Ruiter et al., 2005). Therefore, it is necessary to understand the trophic ecology of the super-generalist species of the Malpelo FFS (e.g., *D. millepunctatus* and *J. malpilensis*) at different times of the year, so that we can understand how the dynamics and structure of the web can be modeled over time.

# Community sub-web in terrestrial food web

The presence of three trophic sub-webs in the terrestrial ecosystem of the Malpelo FFS reflects the degree of complexity of the ecosystem and its ability to remain stable in the face of disturbances by reducing the propagation of direct and indirect effects throughout the entire web (Albert et al., 2000). The presence of these three trophic sub-webs can be associated with four factors: First, the body size of the species (Cohen et al., 2003; Stouffer et al., 2005). Sub-web 1 is composed of large species, *J. malpilensis* (6.56 cm carapace width; Lopez-Victoria and Werding, 2008), *D. millepunctatus* (23.5 cm total length; Lopez-Victoria et al., 2011) and some migratory birds. This

allows them to interact with large (e.g., *S. granti* and its derivatives, *A. agassizi* [26-33 cm total length; Lopez-Victoria et al., 2011]) and small components of the web (sub-webs 2 and 3; e.g., insects, ants, Diplopoda, *P. transversalis*, etc.) as food sources.

The above has important implications for the conservation and management of the Malpelo FFS, since the stability of the terrestrial ecosystem may, to a large extent, be subject to secondary extinctions and loss of species as a result of environmental changes, habitat loss, modification of interactions with other species (Solé and Montoya, 2001; Dunne et al, 2002; Srinivasan et al., 2007), changes in species abundance and diversity (Tylianakis et al., 2010), as well as the fact that the persistence of a particular species can be influenced by another group of species with which it interacts (Tylianakis et al., 2010).

Therefore, management and conservation strategies should be based on the ecosystem and not only on individual species, due to the complexity of the system and the function of each component within the web. The second factor that influences the presence of sub-webs is the preferences and feeding behavior (Allesina and Pascual, 2009; Guimerá et al., 2010) of the components of the subwebs, which is closely related to the first factor. Trophic sub-webs are formed mainly by interaction between generalist/opportunistic organisms and specialist organisms. For example, *J. malpilensis* and *D. millepunctatus* consume many components of the web, including microorganisms, marine algae, seabirds and their derivatives; they also prey on each other and are cannibalistic (Lopez-Victoria and Werding, 2008; Lopez-Victoria et al., 2011). On the other hand, *A. agassizi* has a more restricted niche (specialists) feeding on small prey and being an important food source for large consumers (Lopez-Victoria et al., 2011), which helps to strengthen interactions with components of other trophic communities. For its part, *P. transversalis* specializes in preying on insects and small prey (Lopez-Victoria et al., 2013); while insects and other invertebrates present in the food web of the Malpelo FFS maintain a close relationship with DOM degradation and soil transformation (Calero et al., 2011; Bueno-Villegas, 2012).

The third factor influencing the presence of subgroups is the evolutionary histories of the species (Cattin et al., 2004), the limited habitat size and range and abundance of ecological niches (Guimerá et al., 2010) and their distribution, and the habitat boundaries (Allesina and Pascual, 2009) within which they interact. This is confirmed by the isolation of Malpelo Island (~390 km distance from the coast), the great depths (>4000 m) that separate it from the mainland (Fig. 4B), its type of formation (volcanic origin; von Prahl, 1999) and its high degree of endemism (Plan de

Manejo, 2015), all of which indicates that Malpelo Island has never been connected to other islands or continents constituting a barrier for colonization by both terrestrial and marine organisms (Graham, 1975). This would explain how evolutionary histories together with habitat characteristics favor the formation of trophic communities in the Malpelo FFS.

Finally, the length of the trophic chain as an influential factor in the formation of sub-webs (Mantel et al., 2004; Newth, 2005) as a consequence of the close relationships between the components of the web and the low productivity, as a reflection of the size of the web (diameter = 4). This factor applies to the Malpelo FFS, as its terrestrial ecosystem has a trophic chain structured in five trophic levels: 1) primary producers and base sources (TL-I; plants and detritus), 2) primary consumers (NT-II; Diplopoda and Gryllidae), 3) secondary consumers (NT-III; Hymenoptera, Microcoryphia, *Sula granti, A. agassizi*, and *D. millepuntactus*), 4) tertiary consumers (NT-VI; *J. malpilensis*), and 5) decomposers and scavengers (Isopoda, *Odontomachus* sp, Araneae, and Lumbriculidae) (see Chapter 2.1). Thus, the above results reveal the complexity of the Malpelo FFS system and the existence of mechanisms that may facilitate stability and adaptation in the face of some type of disturbance.

# Motifs

Interspecific trophic interactions (i.e., competition, predation, herbivory, etc.) present in an ecological community play an important role in the abundance and dynamics of its populations. The analysis of motifs can help to identify patterns of connections present in food webs that are relevant to ecosystem functioning (Almaas et al., 2007). The topology of such motifs indicates the most frequent processes by which energy is transferred within the system (Milo et al., 2002).

The interspecific interactions of the terrestrial ecological community of the Malpelo FFS are dominated by four ecological processes: apparent competition, exploitative competition, tritrophic chain and omnivory. Of these, competition (apparent and exploitative) and omnivory are important processes involved in regulating the dynamics of the terrestrial ecosystem of the Malpelo FFS. In the competition processes, apparent competition (one predator consumes two prey) was the most recurrent with exploitative competition (two predators consume one prey) apparent to a lesser degree. The higher recurrence of apparent competition suggests a high indirect trophic interaction between predators (mainly between *J. malpilensis* and *D. millepunctatus*) which may be due to: 1) low prey species diversity ( $n_{terrestrial} = ~45$  species [Plan de Manejo, 2015]) and 2) similarity in feeding habits of the large predators on the island (i.e., *J. malpilensis* and *D. millepunctatus* [López-Victoria and Werding, 2008; López-Victoria et al., 2011]). In contrast, the lower frequency of exploitative competition patterns can be explained by possible competitive exclusion processes of large predators associated with prey abundance at different times of the year (Wolda, 1975). For example, 1) during the nesting season of *S. granti* the large predators could make use of the same abundant resource (i.e., eggs and chicks of *S. granti*), 2) an increase in the availability of potential prey for other consumers during the rainy season when many organisms leave their burrows (M. López-Victoria, *personal observation*), reducing competition among them, and 3) at some times of the year *D. millepunctatus* could be consuming *J. malpilensis* causing a reduction of competition among predators with similar niches, thus allowing their coexistence (Wolda, 1975).

On the other hand, omnivory is another recurrent process in the terrestrial ecosystem of the Malpelo FFS, which suggests that this process contributes to the proper functioning of the system, participating in the maintenance of connectivity (Pimm and Lawton, 1978; Pimm, 1982), stability (Emerson and Yearsley, 2004; Namba et al., 2008) and the persistence of the web as a mechanism to regulate disturbances (Vandermeer, 2006). However, the lower recurrence rate of omnivory in the Malpelo FFS compared to the motifs of competition (apparent and exploitative) and tri-trophic chain may be related to three factors: 1) the size of the trophic chain, the Malpelo FFS has a short trophic web made up of five levels (see Chapter 2.1), 2) the high degree of generalist/opportunist organisms (McCann and Hastings, 1997) and 3) variations in ecological processes influenced by the availability of food at different times of the year (e.g., rainy vs dry seasons). Despite the above, the presence of omnivory in the Malpelo FFS suggests that this process could be a regulatory mechanism in the maintenance of the island's trophic dynamics (Vandermeer, 2006). Thus, an increase in the rate of omnivory in the Malpelo FFS could affect the dynamics of the ecosystem (Pimm and Lawton, 1978; Pimm, 1982).

## **Resilience of the terrestrial food web**

The stability of complex systems is generally attributed to the functional entanglement of the webs, which is defined by the relationships between their components (Albert et al., 2000). Therefore, each type of web presents different degrees of robustness to direct disturbances ("attack") or random failures. Thus, the malfunction of any element of the system can increase the

distances between the remaining components due to the elimination of some pathways that contribute to the connectivity of the web (Albert et al., 2000).

The stability of the terrestrial food web of the Malpelo FFS depends mainly on the role of five trophogroups: DOM, *J. malpilensis*, *A. agassizi*, *D. millepunctatus*, and *S. granti* (Fig. 5). Therefore, the disturbance of one or more of these trophogroups would reduce the stability and persistence (Solé and Montoya, 2001) of the terrestrial food web of the Malpelo FFS. If the trophogroups with higher  $DC_{All}$  and BC, i.e., DOM, *J. malpilensis*, *A. agassizi*, and *D. millepunctatus*, were to suffer a disturbance, some properties of the web could be drastically modified, as in the case of central energy pathways, global  $CC_{global}$  and connectivity among species (Fig. 12). This can be explained in two ways: 1) modifications in the patterns of trophic sub-web formation that would change the speed of propagation of the direct and indirect effects of a disturbance throughout the web (Albert et al., 2000) and 2) modification in the patterns of web functioning (i.e., *motifs*). Modifications in the frequency of omnivory processes could modify the effects of bottom-up control of trophic flows throughout the web (Thompson et al., 2007). Thus, the presence of all of these mechanisms contributes to reducing the severity or duration of disturbance (Vandermeer, 2006).

On the other hand, some species with intermediate values of  $DC_{All}$ , BC, and SC may have different effects (positive and/or negative) on other properties of the web, i.e., the APL. If we consider the definition of APL –shortest average distance between the most distant nodes–, its variation in the terrestrial web of the Malpelo FFS could have different implications on the speed of propagation of disturbances along the web. If this is true, a direct attack or disturbance on trophogroups such as *D. millepunctatus*, Staphylidae, plants, ants, crickets, and caddis flies could increase the APL values considering the scenarios of importance in *DC*, *BC*, and *SC* (Figs. 12–14) of the trophogroups. Whereas direct affectation of the other trophogroups could reflect a reduction in the APL value (Figs. 12–14). This could lead to the interruption of energy flows between trophic levels since, as APL increases, the number of short chains is reduced and longer chains are formed, which usually leads to lower efficiency in energy transfer. The opposite would happen if the APL decreases (a greater number of short chains and greater efficiency in energy transfer). This is because trophogroups of great importance in *DC*, *BC*, and *SC* scenarios participate mostly in trophic pathways, concentrating and distributing energy along the web.
It is worth noting that although *D. millepunctatus* is a key trophogroup of the terrestrial ecosystem of the Malpelo FFS (Fig. 10, Table 7), in the context of "hit-and-miss" scenarios, if this species is directly affected, it would "not generate" substantial modifications in the web attributes. This could be explained by the high similarity in trophic habits with *J. malpilensis*, since the affectation on redundant species, i.e., *D. millepunctatus* and *J. malpilensis*, may generate a greater diversity of behavioral responses to perturbations (Elmqvist et al., 2003), contributing to the reduction of web-wide effects (Mantel, 2003); whereas the systematic involvement of redundant species could lead to secondary extinctions (Dunne et al., 2002).

On the other hand, SC showed a different pattern in the order of importance of trophogroups compared to DC<sub>All</sub> and BC (Fig. 10, Table 7). In this sense, the SC suggests that trophogroups such as plants, S. granti, carcasses, insects, food debris, J. malpilensis, flies, and S. granti eggs may have greater effects on the centralization of energy and CC<sub>individual</sub> among species (Fig. 14). This is consistent with the idea suggesting that the terrestrial ecosystem depends on the marine ecosystem, as S. granti contributes marine nutrients to the terrestrial ecosystem in different ways (Wolda, 1975; López-Victoria et al., 2009). In addition, J. malpilensis is one of the components that has a high interaction with S. granti and its derivatives (e.g., feathers, eggs, chicks [López-Victoria et al., 2009]) and a high interaction within and between trophic subgroups, which allows the concentration of a large part of the energy in these components. Thus, the stability and robustness of the terrestrial web may also be affected if species with high SC values suffer a disturbance. If the prey of S. granti (marine origin) suffer any alteration due to any disturbance (e.g., environmental changes or anthropogenic activities), these effects could be reflected in the terrestrial ecosystem, which would lead to a modification of the interaction patterns due to the participation of J. malpilenesis as an energy integrator and could, therefore, generate an alteration in the stability of the web.

These results demonstrate that despite the fact that the trophic dynamics of the terrestrial ecosystem of the Malpelo FFS are centered on a few trophogroups, each component fulfills different functions in the system, functions that are not only linked to the degree of connectivity (high DC), but also to their degree of participation in energy flow (high BC) and high interaction with other trophogroups to form sub-webs (high SC). This enhances the integration and availability of allochthonous inputs (marine nutrients [Wolda, 1975]) for the whole ecological community,

thus generating different processes that maintain the stability of the ecosystem (e. g., motifs, communities, etc.).

On the other hand, the connectivity of the terrestrial food web of the Malpelo FFS was negatively affected by the removal or "attack" of the four most connected trophogroups (*DC*) and intermediates (*BC*), i.e., DOM, *J. malpilensis*, *A. agassizi*, and *D. millepunctatus* (Figs. 6 and 7). This suggests a high fragility of the web in the face of disturbances that adversely affect these components, as the resilience of the web is linked to the homogeneity of the connectivity distribution, which is maintained by a few highly connected species that when removed drastically alter the web topology and decrease the ability of the components to interact with the other components (Albert et al., 2000; Tylianakis et al., 2010).

Contrary to *DC* and *BC*, *SC* values suggest that *S. granti* is the component that can generate the greatest impact on the connectivity of the terrestrial web of the Malpelo FFS. This is consistent with previous hypotheses mentioning the importance of *S. granti* in the maintenance of the terrestrial food web due to the contribution of marine nutrients (Wolda, 1975; von Prahl, 1990; López-Victoria et al., 2009). Therefore, a disturbance affecting its food sources and altering its trophic habits could generate modifications in the subgroups of the terrestrial web and, therefore, affect its stability.

#### CONCLUSIONS

This study showed that the functioning of the terrestrial ecosystem of the Malpelo FFS is governed by a few trophogroups (DOM, *J. malpilensis*, *D. millepunctatus*, and *A. agassizi*). These findings reflect an ecological community with a food web compartmentalized into three communities formed by groups of organisms with high trophic interaction, which is related to their body sizes (Cohen et al., 2003; Stouffer et al., 2005), food preferences (Allesina and Pascual, 2009; Guimerá et al., 2010), evolutionary histories (Cattin et al., 2004), habitat and reduced niche (Guimerá et al., 2010), and the length of the trophic chain (Mantel et al., 2004; Newth, 2005 [five trophic levels; see Chapter 2.1]). In addition, the high degree of interaction between key trophogroups (e.g., *D. millepuntactus*, *J. malpilensis*, and *A. agassizi*) with *S. granti* and their derivatives (e.g., chicks and eggs), as well as with food scraps, indicates that these species maintain a close relationship with the other components within the system's trophic pathways, being in charge of integrating, making available and distributing marine nutrients throughout the food web.

Despite the fact that the web is centered on a few trophogroups, when they are attacked by some disturbance they can affect (positively or negatively) the dynamics of the entire ecosystem, altering its stability and robustness. Likewise, it is evident that other components of the ecosystem, as they fulfill different functions in the web, can also generate changes in their structural attributes (e.g., APL, compartmentalization, etc.) if they are affected by a perturbation.

The high recurrence of 3-node *motifs* reveals that energy is transferred throughout the web through processes of competition (apparent and exploitative) and omnivory. Moreover, it explains how two of the major predators of the system (i.e., *D. millepuntactus* and *J. malpilensis*) with similar trophic habits can coexist in a small environment where resources are limited due to low primary production (low plant cover). Therefore, these patterns also constitute an important characteristic that allows the terrestrial ecosystem of the Malpelo FFS to remain stable.

In addition, the presence of "top-down" processes as a regulatory mechanism for the stability of the terrestrial system of the Malpelo FFS is evident due to the high centrality of DOM and the recurrence of ominivory processes. Similarly, it is clear that DOM is an important element to include in the modeling of food webs, since its exclusion from the analyses would prevent the elucidation of important regulatory processes (e.g., "bottom-up") of the system (Pimm et al., 1991; Menge, 2000; Molina-Hernández, 2011); particularly in those ecosystems where there is low primary productivity and few plant inputs (Pimm et al., 1991), as in the Malpelo FFS.

The findings of this study can contribute to the management and conservation policies of the MPA, since the identification of key species, trophic communities and ecological patterns suggest that the design and implementation of management and conservation measures should be based on 1) the ecosystem and not on individuals or flagship species and 2) the role of each component in the integration and distribution of energy throughout the web should be taken into account.

This is the first study in the Malpelo FFS that reveals different structural characteristics of the terrestrial ecosystem and shows how its attributes can be altered if its components are directly or indirectly affected. However, more studies are needed to: 1) complement the web modeling and elucidate different patterns related to seasonal variations, 2) strengthen the description of the terrestrial food web of the Malpelo FFS as the results obtained here may be affected by the taxonomic resolution used (Mantel et al., 2004) and, 3) understand the formation of and relationships between omnivory and other recurrent ecological processes (e.g., competition) that allow the system to remain stable and connected throughout the year, and in turn, help to model

the trophic dynamics at different times of the year, relevant to questions such as: how do the magnitudes of the different ecological processes vary, and how do competitive exclusion processes contribute to the maintenance of system stability and species coexistence?

## Chapter 3. MARINE FOOD WEB OF THE MALPELO FAUNA AND FLORA SANCTUARY, COLOMBIA

# 3.1. Marine community trophic structure of Malpelo Island, Colombia, based on the use of bio-markers

#### **INTRODUCTION**

Seamounts are one of the most predominant geographic features on earth, and due to their volcanic nature, they are mostly located on oceanic ridges, generating near mid-ocean ridges in the interior of plates over upwelling plumes (hotspots) and in convergent island arc environments (Wessel, 2007).

Seamounts induce local currents that enhance the upwellings around them, thereby allowing the transport of nutrients from the seafloor to the ocean surface, enhancing primary production and supporting a wide variety of life (Rogers, 1994), sustaining important ecological communities and providing habitats for commercially important species, such as snappers, groupers, etc. (Wessel, 2007).

Malpelo is a small oceanic island of volcanic origin which is part of a subset of seamounts that exceed sea level (Price and Cluge, 1992) and, in turn, support a high diversity of fauna and flora (Plan de Manejo, 2015). These characteristics make Malpelo Island the largest marine protected area (MPA) in the Colombian Pacific, named as a Fauna and Flora Sanctuary (SFF) Malpelo (Fig. 4, see Chapter 1 [Ministerio de Ambiente y Desarrollo Sostenible, 2017]) and World Heritage for Humanity (UNESCO), among other important nominations for conservation (Management Plan, 2015).

All this makes the location an important place for the development of marine ecology and evolution studies (e.g., Price and Cluge, 2002). However, as in most tropical ecosystems, trophic information (e.g., diet and food webs) is limited, and this leaves its trophic architecture poorly understood (Link, 2002) or largely unknown. This limits our ability to understand the ecological dynamics (e.g., trophic relationships, energy flow, etc.) of this important MPA and its adaptive capacity in the face of different disturbances such as climate change, as well as hindering the development of adequate management plans and measures for the MPA.

In this context, to broaden our knowledge of the ecological dynamics of ecosystems ir is imperative to improve the understanding of energy and nutrient flows through trophic networks (Sardenne et al., 2017). As they are organized in trophic chains based on energy transfer (trophic levels) (Lindeman, 1942), they can broaden understanding of the nature and magnitude of

interactions in the formation of complex trophic networks (Post, 2002; Bascompte et al., 2005). In this way, the effects of various pressures throughout the webs (i.e., fisheries, climate change, etc.) can be assessed (Litzow et al. [Litzow et al., 2006; Hebert et al., 2008]), and the information thereby produced also provides tools to assess the persistence and resilience of ecosystems in the face of disturbances (Wilson et al., 2010).

Generally, trophic studies on marine species use biomarkers, i.e., stable isotopes of C ( $\delta^{13}$ C) and N ( $\delta^{15}$ N), which have the advantage of reflecting the diet integrated by a consumer during a certain period of time (from days to years, depending on the tissue analyzed [MacNeil et al., 2005; Logan and Lutcavage, 2010; Kim et al., 2012]). In this sense,  $\delta^{13}$ C is considered an indicator of the sources of primary production in a food web (i.e., benthic vs pelagic [France, 1995]), whereas  $\delta^{15}$ N allows estimation of the position (Hussey et al., 2015) and trophic level of species (Vander Zanden and Cabana Rasmussen, 1997) within a food web. Added to this, stable isotope analysis (SIA) is a powerful tool that can facilitate the description of ecological aspects, i.e., isotopic niche, habitat use, and trophic structure (Layman et al., 2017; Newsome et al., 2007) and components of trophic diversity, i.e., functional richness (Layman et al., 2007; Jackson et al., 2011), isotopic diversity (Layman et al., 2007), and isotopic evenness (Layman et al., 2007; Rigolet et al., 2015). Therefore, the objectives of this chapter were: 1) describe the trophic structure of the Malpelo FFS from the marine isotopic space estimated with the isotopic signals of the food web components, 2) determine the number of trophic levels that make up the marine food web of the Malpelo FFS, 3) estimate the isotopic niche breadth of each trophic level and their degree of trophic interaction (isotopic overlap), 4) make some inferences about key ecological characteristics of the web (e. g., richness, diversity and isotopic regularity) and 4) estimate the degree of trophic fractionation between each trophic level, as a potential "correction factor" for future trophic studies in the Malpelo FFS.

#### **MATERIALS AND METHODS**

#### Study area

Malpelo Island (Fig. 4A) is the summit of a submarine mountain range called the Malpelo Ridge, which extends in a NE-SW direction; it is approximately 241.4 km long by 80.5 km wide (Fig. 4B, red polygon). The island has a maximum height of 300 m above sea level and rises from approximately 4,000 m depth (Fig. 4C) (more details in Chapter 1).

#### **Collection of samples**

Sample of 39 marine species/families of marine organisms (Table 8) were collected between 2017–2021 in Malpelo FFS, Colombia (Fig. 4A). A total of 297 samples were obtained at different depths (between 10–30 m) by scuba diving at sites around Malpelo Island. Muscle tissue of teleost fishes and rays was obtained with a harpoon and/or Hawaiian hook, and from fish that had been illegally caught and seized by the authorities. Carcharhinidae and Sphyrnidae muscle tissue was obtained from Estupiñán-Montaño et al. (2017).

Plankton samples were collected around Malpelo FFS with a "bongo" type net of 68, 90, and 294  $\mu$ m mesh size; surface tows were conducted from the M/N Seawolf inflatable boats for 10 min at each sampling site around the island. Samples of the other marine species/groups (e.g., algae, crustaceans, gastropods, and oysters; Table 8) were collected by hand.

All collected samples were placed in pre-labeled zip-lock plastic bags, except for the plankton samples, which were stored in 250 ml plastic bottles. Samples were kept frozen on board the Pacific Diving Company's M/N Seawolf for subsequent transfer to the laboratory. The sampling procedures were endorsed by Parques Nacionales de Colombia, through Memorandum 20177730007973 of 30 May 2017, issued by the Planning and Management Group.

#### Sample preparation and analysis

Samples were washed with distilled water, freeze-dried in an oven at 60 °C for 24 h, and ground to a fine powder with an agate mortar. Approximately 0.26 to 3.70 mg of powder were obtained for each terrestrial sample and packed in  $3.2 \times 4$ -mm tin capsules.

The C:N ratio was estimated and compared to reference values; a C:N value  $\leq 3.5$  indicates no effect of lipid contents (Post et al., 2007), whereas values > 3.5 suggest high lipid content.  $\delta^{13}$ C values of terrestrial and marine samples (Table 1) with C:N values > 3.5 were mathematically normalized according to Kiljunen et al. (2006):

$$\delta^{13}C_{\text{adjusted}} = \delta^{13}C_{\text{measured}} + D \times \left(I + \frac{3.90}{1 + \frac{287}{L}}\right)$$

Where  $\delta^{13}C_{adjusted}$  is the  $\delta^{13}C$  after normalization and  $\delta^{13}C_{measured}$  is the  $\delta^{13}C$  obtained from the sample without lipid removal. D = 7.018, I = 0.048, and L is the proportional lipid content of the sample, estimated as  $L = -20.54 + (7.24 \times C:N)$  (Post et al., 2007).

Feather of the seabird (Nazca Booby *Sula granti*) were cleaned of surface lipids and contaminants using a 2:1 chloroform:methanol solution, followed by two successive methanol rinses (Jaeger et al., 2009). The  $\delta^{13}$ C values of *S. granti* eggs were mathematically normalized because lipid extraction can alter  $\delta^{15}$ N by washing out nitrogenous compounds. In this case, the formula proposed by Elliot et al. (2014) was used:

$$\delta^{13}C_{\text{lipid-extracted}} = \delta^{13}C_{\text{non-extracted}} + 1.47 - 2.72 \times Log_{10} \text{ (C:N)}$$

Where  $\delta^{13}C_{\text{lipid-extracted}}$  is the  $\delta^{13}C$  after normalization and  $\delta^{13}C_{\text{non-extracted}}$  is the  $\delta^{13}C$  obtained from the sample without lipid removal.

Extraction of lipids and urea from elasmobranch muscle samples (i.e., sharks and rays; Table 1) was performed following the procedure described by Kim and Koch (2012). Stable isotope analyses were carried out in the Stable Isotope Laboratory of the Instituto Andaluz de Ciencias de la Tierra in Granada (CSIC-UGR), Spain (more details in Chapter 1).

#### Niche width and isotopic overlap

Isotopic niche was quantified for individuals and age groups, using the Stable Isotopic Bayesian Ellipses method in R (SIBER; Jackson et al., 2011). This analysis is based on calculated ellipses from a covariance matrix, which defines its forms and areas (Jackson et al., 2011) to estimate the width of the isotopic niches (Total area [TA] and Standard Ellipse Corrected Area [SEA<sub>C</sub>]).

Isotopic overlap was estimated using the nicheROVER package in R (Lysy et al., 2014), which is a Bayesian method that calculates the probability of overlap between niche pairs using multidimensional information as niche indicators (e.g., stable isotopes). The probabilistic density of niche overlap was calculated by running  $10^4$  iterations and 95% of confidence interval (IC) for the data from each species or group occurring within their respective isospace, providing directional niche overlap estimates (e.g., x vs y and y vs x), according to the distributions of a particular species in the multivariate niche space (Lysy et al., 2014).

#### Trophic position and trophic factor discrimination

The trophic position (TP) of all organisms was estimated implementing a Bayesian method within the R statistical environment (R Core Team 2018), employing the *tRophicPosition* package version 0.7.5 (Quezada-Romegialli et al., 2018). Analyses of TP were calculated using the  $\delta^{13}$ C and  $\delta^{15}$ N values of all organisms, with the isotopic values of macroalgae and phytoplankton of Malpelo Island (Table 8) serving as the isotopic baseline. A Bayesian model of two baselines and two trophic discrimination factors (TDF) was run with 2 Markov Chain Monte Carlo (MCMC) and 20000 adaptive interactions, assuming a baseline taxa ( $\lambda$ ) = 1. Accordingly, each organism's TP value was determined using the TDF for teleost fish (muscle;  $\Delta^{13}$ C = 1.3 ± 0.30% SD and  $\Delta^{15}$ N = 2.9 ± 0.32% SD [McCutchan et al., 2013]), for sharks ( $\Delta^{13}$ C = 0.99 ± 0.33% SD and  $\Delta^{15}$ N = 2.29 ± 0.22% SD [Hussey et al., 2010]). For other organisms, the TDF was taken from the average for aquatic ecosystems ( $\Delta^{13}$ C = 0.4 ± 0.17% SD and  $\Delta^{15}$ N = 2.3 ± 0.28% SD [McCutchan et al., 2013]).

The estimated TPs were classified into six trophic levels (TL) according to their food preferences: TL-I; Base or primary producers (TP  $\leq$ 1), TL-II; Herbivores (1.1< TP <2.1), TL-III; Omnivores (I) showing a preference for vegetables (2.1< TP <2.9), TL-IV; Omnivores (II) showing a preference for animals (2.9< TP <3.7), TL-V; Carnivores showing a preference for large decapods, cephalopods, and fish (3.7< TP <4.5), and TL-VI; Tope predators (TP >4.5) (Stergiou y Karpouzi, 2002).

Subsequently, the average trophic discrimination fractionation (TDF<sub>Malpelo</sub>) of  $\Delta\delta^{13}$ C and  $\Delta\delta^{15}$ N between levels was estimated. For this, the average value of each isotope was subtracted from the upper TL minus the TL immediately below. For example, the average  $\delta^{13}$ C and/or  $\delta^{15}$ N value of TL-V minus the average  $\delta^{13}$ C and/or  $\delta^{15}$ N value of TL-IV, and so on down to the primary producers. Finally, a global average TDF<sub>Malpelo</sub> value was obtained for  $\Delta\delta^{13}$ C and  $\Delta\delta^{15}$ N present within the marine ecosystem.

#### **Isotopic diversity**

Previous studies have proposed the convex hull area (TA) as a measure of the functional space occupied by a species or community (Cornwell et al., 2006). Thus, the TA would in effect represent functional richness estimated as the amount of functional space occupied by the species or community (Villéger et al., 2008). From an isotopic point of view, the TA represents the isotopic

diversity or richness (IRic [Cucherousset and Villéger, 2015]). Therefore, several indices were produced in order to reveal various facets of isotopic diversity, such as: isotopic divergence index (*IDiv*), isotopic dispersion index (*IDis*), equality index (*IEve*) and uniqueness index (*IUni*).

*IDiv* measures the distribution of points within the TA (Villéger et al., 2008, Cucherousset and Villéger, 2015). *IDiv* is minimal (i.e., tends to 0) when most points are close to the center of gravity of the TA, i.e., when organisms with the most extreme isotopic values (e.g., primary producers and/or top predators) in a community are rare. *IDiv* has a value of 1 when all points are located at the edges of the AT, i.e., when organisms with the most extreme isotopic values dominate the food web (Cucherousset and Villéger, 2015).

IEve measures the regularity in the distribution of organisms (Cucherousset and Villéger 2015). *IEve* has a value of 0 when most organisms are clustered within a small region of isotope space and only a few of them are located further away from the main group (e.g., most species are strictly herbivorous and there are only few predators in the community). *IEve* has a value of 1 when organisms are distributed throughout the isotopic space (Cucherousset and Villéger, 2015).

*IUni* is considered the inverse of the average isotopic redundancy, with isotopic redundancy reflecting the average proximity of organisms in isotopic space (Cucherousset and Villéger, 2015). *IUni* equals 0 when each organism has at least the same position as another organism within the isotopic space (e.g., communities made up of paired species with similar diets). The *IUni* has a value close to 1 when most organisms are isolated in isotopic space, i.e., their isotopic values are different from all other species (e.g., invertebrate communities with the highest abundance of species being the only detritivorous species consuming terrestrial debris [Cucherousset and Villéger, 2015]).

Additionally, the isotopic similarity index (*ISim*) was estimated as a measure of the ratio between the insertion volume and the union volume of two groups of species (in this study trophic level) in isotopic space (Villéger et al., 2011). ISim values range from 0 (when the two groups of organisms occupy totally different parts of the isotope space, e.g., primary consumers and top predators) to 1 (when they occupy the same portion of the isotope space) (Cucherousset and Villéger, 2015). Due to the influence exerted by the size of the TA of each group on the calculation of *ISim*, the isotopic nesting index (*INes*) was calculated as a complementary indicator. This indicator is the ratio between the intersection volume and the minimum volume occupied by a group (Cucherousset and Villéger, 2015). The *ISim* and *INes* indices were used to compare the

basal TL with the other TLs, with the objective of assessing the similarity of the basal  $\delta^{13}$ C and the  $\delta^{13}$ C reflected in each TL of the network, to be used as an indicator of basal source use.

The aforementioned indices were calculated for the groups of species with similar TLs, with the objective of having a preliminary estimation of the functional diversity by TLs for the Malpelo FFS web. The analysis was designed in this way due to the low number of samples for some species/groups (Table 9).

All isotopic diversity indices were estimated using the method generated by Cucherousset and Villéger (2015), available in the on-line version of the paper. (https://dx.doi.org/10.1016/j.ecolind.2015.03.032).

#### RESULTS

The relevant isotopes of a total of 296 samples were analyzed, grouped into 12 taxa (Table 9) comprising 39 genus/families/groups (Table 8). Of the groups analyzed, 41.5% corresponded to teleosts, 20.3% crustaceans, 9.1% elasmobranchs, 7.8% macroplankton, 5.7% seabirds, and the remaining 15.5% was composed of algae, bivalves, cephalopods, corals, sponges, gastropods, and microplankton (Table 9).

The taxonomic groups analyzed occupied a marine isotope space between -23.2% and -10.0% for  $\delta^{13}$ C and from 4.6% to 16.9% for  $\delta^{15}$ N, with macroplankton showing the lowest values of  $\delta^{13}$ C and  $\delta^{15}$ N, while crabs of the family Grapsidae presented the highest values of both isotopes (Table 8).

The calculation of the TPs for the ecological community of the Malpelo FFS produced values between 1.0 and 5.5 (95% CI). These results suggest that the trophic pyramid of the Malpelo FFS marine ecosystem consists of seven levels: 1) TL-I = primary producers, 2) TL-II = herbivores, 3) TL-III = omnivores I, 4) TL-IV = omnivores II, 5) TL-V = carnivores, 6) TL-VI = top predators (Table 1; Fig. 15 and 16), and 7) decomposers (not included in this study).

The broad marine isotopic space reflected different isotopic niche amplitudes (Niche<sub>SIA</sub>) among taxa, so that crustaceans was the group that presented the highest niche<sub>SIA</sub> (TA:  $68.03\%^2$ ; SEA<sub>C</sub>: 22.54‰<sup>2</sup>). In contrast, squids (TA:  $0.22\%^2$ ; SEA<sub>C</sub>:  $0.31\%^2$ ) and corals (TA:  $0.03\%^2$ ; SEA<sub>C</sub>:  $0.13\%^2$ ) presented the lowest niche<sub>SIA</sub> (Table 10; Fig. 17).



Figure 15. Marine isotopic space of the Malpelo Fauna and Flora Sanctuary, Colombia, represented by mean values  $\pm$  standard deviation of  $\delta^{13}$ C and  $\delta^{15}$ N and estimation of the range of trophic position of each marine ecosystem component. Identification code: see Table 8.



Figure 16. Marine trophic pyramid of the Malpelo Fauna and Flora Sanctuary, indicating the mean values  $\pm$  standard deviation (SD) of  $\delta^{13}C(A)$ ,  $\delta^{15}N(B)$ , and the average trophic discrimination factor per trophic level.

Code	Familias/groups	10	C:N	C:N $\delta^{13}C$ (‰)					δ <sup>15</sup> ]	N (‰)		Trophic position		
Code	rainines/groups	п	$Mean \pm SD$	Min	Max	Mean	SD	Min	Max	Mean	SD	IC 95%	Mode	
1	Padina sp.	6	$14.6\pm2.71$	-21.0	-17.1	-18.7	1.58	4.6	5.6	5.2	0.46	_	1.0	
2	Green algaes	4	$13.3\pm1.42$	-18.1	-16.0	-17.2	0.93	6.5	7.8	7.3	0.63	_	1.0	
3	Arcidae	1	3.6	_	_	-17.8	_	_	_	9.0	_	_	2.2	
4	Balanidae	2	$4.3\pm0.35$	-18.1	-15.7	-16.9	1.70	9.5	10.0	9.8	0.35	2.4 - 2.8	2.6	
5	Balistidae	3	$3.3\pm 0.00$	-18.1	-17.8	-18.0	0.19	12.1	13.1	12.7	0.53	3.1 - 3-4	3.2	
6	Unidentified shrimp	8	$4.8\pm 0.45$	-18.7	-16.5	-17.3	0.69	8.2	11.6	9.3	1.03	1.9 - 2.7	2.3	
7	Carangidae	12	$3.4\pm 0.20$	-18.3	-17.6	-18.0	0.19	11.9	13.7	12.8	0.53	3.1 - 3.5	3.3	
8	Carcharhinidae	12	$3.0\pm 0.07$	-16.7	-16.1	-16.3	0.21	14.8	15.9	15.3	0.33	4.8 - 5.3	5.0	
9	Chaetodontidae	2	$3.6\pm0.21$	-17.5	-17.3	-17.4	0.12	12.7	14.3	13.5	1.12	3.2 - 3.8	3.5	
10	Unidentified crustaceans	3	$6.2\pm1.55$	-19.0	-11.7	-16.4	4.03	7.1	9.1	8.0	1.03	1.5 - 2.2	1.8	
11	Dendrophylliidae	3	$3.6\pm 0.70$	-23.0	-21.8	-22.5	0.60	4.7	6.6	5.5	0.96	1.0 - 1.2	1.0	
12	Epialtidae	1	7.5	_	-	-13.2	_	_	_	7.0	_	_	1.4	
13	Porifera	5	$4.0\pm0.20$	-16.3	-14.9	-15.7	0.58	4.9	9.4	7.2	1.96	1.0 - 2.0	1.4	
14	Exocoetidae	4	$3.6 \pm 0.10$	-17.8	-16.8	-17.5	0.47	9.6	10.9	10.3	0.55	2.2 - 2.6	2.4	
15	Unidentified gasteropds	4	$4.1\pm0.42$	-19.1	-15.4	-16.9	1.58	7.1	11.2	9.5	1.85	1.8 - 3.0	2.4	
16	Gecarcinidae	2	$6.2\pm0.85$	-15.7	-14.9	-15.3	0.60	8.2	8.2	8.2	0.01	1.7 - 2.0	2.0	
17	Grapsidae	21	$5.3\pm1.58$	-19.6	-10.0	-14.6	3.45	6.9	16.9	10.5	3.48	2.0 - 4.5	3.3	
18	Inachidae	3	$6.2\pm0.55$	-15.0	-12.6	-14.2	1.41	8.7	9.4	8.9	0.41	2.0 - 2.4	2.2	
19	Lophiidae (Juv.)	5	$4.2\pm0.33$	-18.9	-18.1	-18.6	0.36	8.6	13.3	11.1	1.97	2.2 - 3.2	2.7	
20	Lutjanidae	36	$3.5\pm 0.28$	-19.3	-16.4	-17.4	0.62	9.8	15.0	13.7	1.10	3.3 - 3.8	3.5	
21	Macroplankton	23	$6.6\pm1.28$	-23.2	-17.8	-21.4	1.02	4.6	10.8	7.2	1.53	1.0 - 2.0	1.5	
22	Malacanthidae	8	$3.3\pm 0.04$	-18.8	-18.0	-18.5	0.27	11.7	14.0	12.9	0.79	3.1 - 3.6	3.3	
23	Microplankton	9	$7.9\pm 0.72$	-20.7	-15.5	-18.6	1.79	4.8	8.3	6.0	1.06	_	1.0	
24	Myliobatidae	1	3.5	_	_	-16.0	_	_	_	13.1	_	_	3.8	
25	Ommastrephidae (Juv.)	5	$4.1\pm0.13$	-18.1	-17.4	-17.7	0.31	10.1	10.8	10.5	0.26	2.7 - 3.1	2.9	
26	Ostreoida	9	$4.0\pm0.53$	-20.1	-18.3	-19.4	0.46	4.8	7.8	6.1	0.98	1.0 - 1.4	1.1	

 Table 8. Values of  $\delta^{13}$ C and  $\delta^{15}$ N of key some families/groups of organisms present in the marine ecosystem of the Malpelo Fauna and Flora Sanctuary, Colombia, with calculations of in minimum (Min), maximum (Max), mean, standard deviation (SD), C:N ratio and trophic position (95% confidence interval and mode). Code = the number representing each species in the figures. n = number of samples for each group.

27	Palinuridae	4	$4.0\pm0.06$	-16.2	-15.8	-15.9	0.16	12.2	12.6	12.4	0.17	3.5 - 3.9	3.7
28	Parthenopidae	2	$8.2\pm0.35$	-17.0	-11.5	-14.2	3.90	5.9	6.5	6.2	0.46	1.0 - 1.2	1.0
29	Penaeidae	12	$4.5\pm 0.48$	-20.1	-17.7	-19.7	0.65	7.5	9.5	8.4	0.60	1.8 - 2.3	2.0
30	Pomacanthidae	3	$3.3\pm 0.06$	-18.1	-18.0	-18.0	0.03	12.4	13.7	12.9	0.71	3.1 - 3.5	3.3
31	Scombridae	12	$3.6 \pm 0.43$	-17.9	-15.9	-17.1	0.56	12.0	14.6	13.2	0.80	3.1 - 3.7	3.4
32	Scorpaenidae	2	$3.4\pm 0.07$	-17.8	-17.8	-17.8	0.04	14.8	15.0	14.9	0.13	3.8 - 4.2	4.0
33	Serranidae	34	$3.7\pm 0.59$	-21.5	-16.2	-18.1	1.20	8.4	15.0	12.7	1.48	2.9 - 3.6	3.3
34	Sphyrnidae	14	$3.1\pm 0.07$	-16.6	-14.8	-16.0	0.50	15.0	16.4	15.9	0.42	4.9 - 5.5	5.2
35	Squillidae	1	4.7	_	_	-16.4	_	_	_	11.5	_	_	3.3
36	Stromatidae	1	3.3	_	_	-17.3	_	_	_	12.3	_	_	3.7
37	Sulidae	17	$3.8\pm 0.60$	-20.0	-16.1	-17.5	1.35	13.1	15.2	14	0.56	4.1 - 4.6	4.3
38	Synodontidae	1	4.3	_	_	-18.3	_	_	_	8.6	_	_	2.1
39	Xanthidae	1	7.7	—	_	-11.8	—	_	_	6.9	_	_	1.3

 Table 9.  $\delta^{13}$ C and  $\delta^{15}$ N values of key some components of the marine ecosystem of the Malpelo Fauna and Flora Sanctuary, Colombia, with calculations of minimum (Min), maximum (Max), mean, standard deviation (SD) values. n = number of samples for each group.

Group	10		δ <sup>13</sup> C (	%0)			δ <sup>15</sup> N	[ (‰)	
Oroup	n	Min	Max	Mean	SD	Min	Max	Mean	SD
Algaes	10	-21.0	-16.0	-18.1	1.50	4.6	7.8	6.1	1.19
Seabirds	17	-20.0	-16.1	-17.5	1.35	13.1	15.2	14.0	0.56
Bivalves	10	-20.1	-17.8	-19.2	0.68	4.8	9.0	6.4	1.30
Cephalopods	5	-18.1	-17.4	-17.7	0.31	10.1	10.8	10.5	0.26
Corals	3	-23.0	-21.8	-22.5	0.60	4.7	6.6	5.5	0.96
Crustaceans	60	-20.1	-10.0	-16.2	3.08	5.9	16.9	9.5	2.53
Elasmobranch	27	-16.7	-14.8	-16.1	0.40	13.1	16.4	15.5	0.68
Sponges	5	-16.3	-14.9	-15.7	0.58	4.9	9.4	7.2	1.96
Gasteropods	4	-19.1	-15.4	-16.9	1.58	7.1	11.2	9.5	1.85
Macroplankton	23	-23.2	-17.8	-21.4	1.02	4.6	10.8	7.2	1.53
Microplankton	9	-20.7	-15.5	-18.6	1.79	4.8	8.3	6.0	1.06
Teleosts	123	-21.5	-15.9	-17.8	0.86	8.4	15.0	13.0	1.42



Figure 17. Total isotopic niche of the groups presents in the marine ecosystem of the Malpelo Fauna and Flora Sanctuary, Colombia, represented by total area (TA).

 Table 10. Isotopic niche by groups of organisms present in the Malpelo Fauna and Flora Sanctuary, Colombia, calculated by total area (TA), standard ellipse area (SEA) and standard ellipse area corrected (SEA<sub>C</sub>).

Groups	TA	SEA	SEAc
Oroups		$(\%^2)$	
Macroalgaes	7.47	4.53	5.09
Seabirds	3.99	1.79	1.91
Bivalves	2.94	1.41	1.59
Squids	0.22	0.23	0.31
Corals	0.03	0.06	0.13
Crustaceans	68.03	22.14	22.52
Elasmobranchs	3.56	0.82	0.85
Sponges	3.08	2.93	3.91
Gastropods	5.68	7.22	10.83
Macroplankton	17.18	4.87	5.10
Microplankton	9.98	5.95	6.79
Teleosts	20.72	3.32	3.35

The wide isotopic spacing and length of the food web (seven levels) suggest the generation of different levels of trophic interaction between the components of the different TLs. According to the isotopic overlap analysis (30 possible interactions, 15 in each direction), the degree of interspecific interaction was 66.7% (x v's y direction), with the lowest overlap probabilities (overlap probability: 0–29%), while the highest probabilities (60–100%) had a value of 20%, with intermediate probabilities (30–59%) showing the lowest frequency of 13.3%. In the opposite

direction (y v's x), the frequency of the lowest interactions was 80%, followed by high and intermediate trophic interactions with values of 13.3% and 5.7% respectively (Table 11; Fig. 18).

	marme 100	sa web oi u	е марею г	auna and fic	ora Sanctual	y, Colombi	a.	
Tranhia lavala	тіі	тіп	ті пі	TI VI	TIV	TI VI	TA	SEAc
I ropine levels	1 L-1	1 L-11	1 L-111	1 L- V I	1 L- V	1 L- V I	$(\%^2)$	$(\%^2)$
TL-I	-	95.3	21.9	12.6	0.0	0.0	12.59	5.56
TL-II	57.8	_	29.0	32.3	0.0	0.0	48.26	10.56
TL-III	19.2	72	_	92.7	2.1	0.0	19.79	5.81
	2.5	10.7	50.0		20.0	<b>-</b> 4	76.10	11.00
TL-VI	2.5	19.7	50.9	-	28.0	5.4	76.19	11.99
TL-V	0.0	0.1	21.7	99.8	_	10.8	3.98	1.91
TL-VI	0.0	0.0	0.2	99.9	28.8	—	1.63	0.55

Table 11. Overlap values (%) and isotopic niche (TA = total area,  $SEA_C$  = corrected ellipse area) between trophic levels of themarine food web of the Malpelo Fauna and Flora Sanctuary, Colombia.



Figure 18. Overlap and isotopic niche (represented by total area and area of the corrected ellipse) between trophic levels of the marine ecosystem of the Malpelo Fauna and Flora Sanctuary, Colombia.

Additionally, the overall TDF<sub>Malpelo</sub> of the different TLs of the marine ecosystem of the Malpelo FFS was between -2.98% and +7.26% for  $\Delta\delta^{13}$ C and  $\Delta\delta^{15}$ N. The highest TDF<sub>Malpelo</sub> of  $\Delta\delta^{13}$ C was between TLs-II and -III (Table 12, Fig. 3A), while the highest TDF<sub>Malpelo</sub> of  $\Delta\delta^{15}$ N was between TLs-III and -VI (Table 12, Fig. 3B). These results indicate that the overall mean TDF<sub>Malpelo</sub> (±SD) for the Malpelo FFS is  $0.43 \pm 1.27\%$  and  $1.88 \pm 0.80\%$  for  $\delta^{13}$ C and  $\delta^{15}$ N respectively.

Trophic level	δ <sup>13</sup> C (	%0)	δ <sup>15</sup> N (%	00)
	Range	$Mean \pm SD$	Range	Mean $\pm$ SD
TL-II – TL-I	-4.89 to +6.84	$-1.07\pm2.83$	-1.65 to +4.64	$+1.06\pm1.44$
TL-III – TL-II	+0.29 to +6.81	$+2.15\pm1.37$	-0.15 to +5.99	$+2.66\pm1.28$
TL-IV – TL-III	-4.32 to +7.26	$+0.03\pm1.92$	-2.98 to +6.95	$+2.78\pm1.95$
TL-V - TL-IV	-2.81 to +1.29	$-0.24\pm1.29$	+0.35 to +2.47	$+1.32\pm0.63$
TL-VI – TL-V	+0.42 to +2.60	$+1.31\pm0.41$	+0.47 to +2.38	$+1.60\pm0.41$

 Table 12. Trophic discrimination factor between the different trophic levels of the food web of the Malpelo Fauna and Flora

 Sanctuary, Colombia, represented by range and mean values ± standard deviation (SD).

Isotopic diversity indices suggest that the global isotopic space of the Malpelo FFS has an *IRic* of 0.512; 0.711 for *IDiv*, 0.745 for *IEve* and 0.381 for *IUni*. For their part, the isotopic diversity values by TLs presented values of IRic between 0.005 to 0.193; *IDiv* from 0.331 to 0.935, *IEve* from 0.416 to 0.708 and *IUni*presented values from 0.257 to 0.740 (Table 13).

The similarity indices showed basal  $\delta^{13}$ C values and the other TLs between 0.139 to 0.311 for *ISim* and from 0.403 to 1 for *INes*.

 Table 13. Isotopic diversity for each trophic level of the marine ecosystem of the Malpelo Fauna and Flora Sanctuary, Colombia.

 IRic = Isotopic Richness Index, IDiv = Isotopic Diversity Index, IEve = Evenness Index, IUni = Redundancy Index.

Parameters	TL-I	TL-II	TL-III	TL-VI	TL-V	TL-VI
IRic	0.005	0.193	0.032	0.031	0.005	_
IDiv	0.634	0.712	0.664	0.935	0.331	_
IDis	0.451	0.329	0.349	0.383	0.138	_
IEve	0.527	0.628	0.607	0.416	0.708	_
IUni	0.537	0.740	0.283	0.257	0.600	_

#### DISCUSSION

The objective of this chapter is to describe the marine trophic structure of the Malpelo FFS, which contributes to reduce the information gaps that exist for the MPA and, in turn, generates new tools that help to improve the understanding of the trophodynamics of the Malpelo FFS. Based on the results of the study, it is suggested that the marine ecosystem of the Malpelo FFS has a trophic web composed of six TLs: 1) primary producers (TL-I), 2) herbivores (TL-II), 3) omnivores I (TL-III), 4) omnivores II (TL-IV), 5) carnivores (TL-V), and 6) top predators (TL-VI) (Figs. 15 and 16) and groups of organisms with a high degree of multifunctionality that allow energy exchange between sea and land.

The marine food web of the Malpelo FFS showed global intermediate values (0.350–0.640) in terms of richness (*IRic*<sub>Global</sub>), diversity (*IDiv*<sub>Global</sub>), and isotopic redundancy (*IUni*<sub>Global</sub>), as well as

high values of evenness ( $IEve_{Global} = 0.650-1$ ). These isotopic diversity values reflect a long marine food chain (six links), in which 12 feeding strategies are present (phytoplanktivores, zooplanktivores, zoobenthivores, planktivores, herbivores, omnivores, carnivores, filter feeders, sediment feeders, suspenchivores, detritivores, and scavengers) corresponding to the potential use of different basal sources.

Of the wide range of  $\delta^{13}$ C in the marine isotopic space of the Malpelo FFS, most organisms presented values between -20% and -15% (n = 226, excluding phytoplankton and macroalgae). This could suggest that the marine food web is mostly supported by marine phytoplankton (-20.7% to -15.5% [this study]) and macroalgae (-21.0% to -16% [this study]); but with a high diversity of carbon utilization patterns ( $\delta^{13}C_{Mapelo} = -23.2\%$  to -10.0%). Thus, other basal sources that were not analyzed in this study, such as seagrasses (-15% to -3% [Fry and Sherr, 1984]), particulate organic matter (POM; -23.2% to -20.9% [Riera et al., 2002]), sedimentary organic matter (SOM; -21.8% to -19.2% [Riera et al., 2002]), terrestrial inputs (-30% to -10% [Fry and Sherr, 1984]), among others, could be elements of great importance in providing additional carbon for the marine food web.

An example of the above is the potential terrestrial inputs to the sea, which can contribute nutrients in two ways: 1) the Malpelo FFS hosts the largest colony of Nazca boobies (*Sula granti*) in the world (~80,000 individuals) (López-Victoria and Rozo, 2007; Garcia 2013), which deposit large amounts of guano; this, together with other terrestrial detritus, is transported towards the marine environment due to the washing of the island by the action of precipitation. In this way, these compounds (detritus+guano) would feed the benthic energy pathways and would favor filterfeeding organisms (benthic and pelagic), 2) the consumption of terrestrial organisms, either in the form of carcasses and/or animal remains, by marine species of the intertidal zone that have both herbivorous and scavenging habits, which would function as vectors in the transport of terrestrial nutrients to the marine environment.

This would explain the high values of  $\delta^{13}$ C (-10‰; Grapsidae; Table 8), which would be related to the potential contributions of seagrasses to the marine food web. However, further studies analyzing all potential basal sources present in the Malpelo FFS are needed to validate these hypotheses.

From a functional point of view within each TL, the isotopic diversity analysis suggests that TL-II presents the highest IRic with respect to the other TLs (Table 13) as a consequence of the

niche size (TA<sub>TL-II</sub>: 48.26‰<sup>2</sup>, SEA<sub>C</sub>: 10.56‰<sup>2</sup>), which would lead to a higher functional diversity of species (Dézerald et al., 2018). This high isotopic richness is associated with: 1) the isotopic variability of basal sources (Brind'Amour and Dubois, 2013; Belle and Cabana, 2020) related to the different forms of carbon fixation by primary producers during photosynthesis (Belle and Cabana, 2020), as some biological and biochemical processes (e.g., respiration) can influence dissolved inorganic  $\delta^{13}$ C values, leaving spatial and temporal variability in basal  $\delta^{13}$ C values (France and Cattaneo, 1998; Finlay, 2003) and 2) the diversity of feeding strategies displayed by the groups comprising this TL (11 families/groups). Eight feeding mechanisms can be identified in this TL: phytoplanktivorous (zooplankton), zooplanktivorous (Penaeidae), herbivorous (Gecarcinidae, Xanthidae), omnivorous (zooplankton, Epialtidae, Gecarcinidae, Penaeidae), carnivores (Epialtidae, Synodontidae, Xanthidae), filter feeders (sponges, Ostraoidea), sediment feeders (Epialtidae) and suspensivores (Ostraoidea). This high diversity of feeding strategies is the manifestation of a high degree of isotopic diversity (*IDis*), sameness (*IEve*) and redundancy (*IUni*) present at this level (Table 13), which could be related to ontogenetic changes in habitat use and the food preferences (Varisco et al., 2015) of different species.

TL-III and -IV presented similar *IRic* values (Table 13). However, both TLs varied widely in their isotopic niche sizes (TA; Table 11). These differences in the TA of each TL were mainly due to the results for the crab *Grapsus grapsus* (Grapsidae), which presented the highest  $\delta^{13}$ C and  $\delta^{15}$ N values in the marine isotopic space ( $\delta^{13}$ C: –11.3% to –10.0% and  $\delta^{15}$ N: 14.8% to 16.9%; Fig. 15 and 17). Despite the similarity in *IRic* values between these two TLs, TL-III was characterized by being comprised of organisms that account for six functional roles, such as: phytoplanktivores (Arcidae), zooplanktivores (Balanidae, Exocoetidae), suspensivores (Balanidae), detritivores (Balanidae), omnivores (unidentified shrimp,), carnivores (Gastropods, Ommastrephidae) and zoobenthivores (Lophiidae).

Despite the number of functional roles (n = 6) present in this TL, a low value of *IUni* was estimated (0.283). This result suggests that most of the community elements in this TL (8 in this study) were formed by pairs of isotopically similar components and/or probably with similar diets. This suggests interspecific trophic interactions between the components of this TL. However, such interactions could occur at low intensity (*IEve* = 0.607) due to the different feeding mechanisms of each group, as well as the variability of carbon sources (see above) of the basal sources that support the food web. For example, the families Serranidae (code 33; Fig. 15) and Balistidae (code

5; Fig. 15) show similar isotopic values (Table 8). However, the family Serranidae includes species with planktivorous (i.e., *Paranthias colonus* [Froese and Pauly 2022]) and carnivorous habits (i.e., *Dermatolepis dermatolepis, Mycteroperca olfax* [Froese and Pauly, 2022]), whereas the species of the family Balistidae are zooplanktivorous and zoobenthivorous (Froese and Pauly, 2022). These differences in feeding strategies would explain the high *IEve* values (0.607) obtained in this study and would indicate use of food chains supported by several basal sources.

On the other hand, TL-IV was represented by 12 families/groups, reflecting a total of eight functional roles grouped into planktivores (Carangidae, Serranidae, Stromatidae), zooplanktivores and zoobenthivores (Pomacanthidae), herbivores (Grapsidae, Pomacanthidae), omnivores, detritivores and scavengers (Grapsidae), and carnivores (Grapsidae, Lutjanidae, Stromatidae, Serranidae, Carangidae).

The above would explain the high isotopic diversity of TL-VI (*IDiv*; Table 13), reflecting the multifunctionality of several species. For example, the Serranidae and Carangidae families include organisms with both planktivorous and carnivorous habits (Froese and Pauly, 2022) and other families, such as Grapsidae (crabs), have omnivorous, detritivorous, carnivorous, and even scavenging species (Grapsidae). For example, *G. grapsus* (Grapsidae) is a species that inhabits the intertidal zone (Gianuca and Vooren, 2007) and has the ability to interact between marine and terrestrial ecosystems; the species has been observed consuming a great variety of elements, including detritus, feathers, regurgitated fish, *S. leucogaster* and other terrestrial crabs (Gianuca and Vooren, 2007).

If the behavior of this species in the Malpelo FFS is similar to that of other localities (e.g., Brazil [Gianuca and Vooren, 2007]), in the intertidal zone of the Malpelo FFS the diet of *G. grapsus* would likely include: 1) large quantities of seabird guano (dry and wet) since, due to the steep slopes on the island and the population size of *S. granti* in the Malpelo FFS, seabird's guano is present throughout the island and is transported to the marine environment (intertidal and pelagic zone) due to rainfall and 2) the potential consumption of land crabs (*Jhongarthia malpilensis*), *S. granti* (feathers and carcasses), and carcasses of other organisms.

The above would explain the high isotopic values of *G. grapsus* (Fig. 17) in the marine isotopic space of the Malpelo FFS, the large TA of TL-IV (without *G. grapsus* = 46.44‰<sup>2</sup>; with = 76.19‰<sup>2</sup>) and the similarity of the  $\delta^{15}$ N values of *G. grapsus* (mean ± SD; -15.8 ± 0.75‰) and *J. malpilensis* ( $\delta^{15}$ N = -15.8 ± 0.60‰ [see Chapter 4]) as potential consumers of *S. granti* and its

derivatives (guano, chicks, carcasses, feathers, etc. [López-Victoria and Werding, 2008]). On the other hand, the high  $\delta^{13}$ C values ( $-10.4 \pm 0.49\%$ ) of *G. grapsus*, could indicate that this species is also integrating the isotopic signals of seagrasses (-15% to -3% [Fry and Sherr, 1984]) and consuming some products of terrestrial origin.

In contrast to the other TLs, TLs-V and -VI presented the lowest isotopic richness, with TL-V presenting a low *IRic* value of 0.005 (TA =  $3.98\%^2$ ) and TL-VI a small total isotopic area ( $1.63\%^2$ ). These isotopic richness values suggest a high degree of trophic specialization of the components that made up these levels. TL-V consisted of three species; an eagle ray (*Aetobatus laticeps*), a scorpion fish (*Pontinus clemensi*) and a seabird (*S. granti*); while TL-VI was comprised of silky sharks, *Carcharhinus falciformis* (Carcharhinidae) and hammerhead sharks, *Sphyrna lewini* (Sphyrnidae).

The above indicates that the main components of both trophic levels (TL-V and -VI) exhibit carnivorous feeding strategies. For example, *P. clemensi* consumes small crustaceans (shrimps and crabs), cephalopods (octopuses, squids and cuttlefishes) and teleost fishes [Ortega-García, 2018]), *S. granti* consumes mostly flying fish and squids [López-Victoria and Estela, 2007; López-Victoria et al., 2009]), while *A. laticeps* mainly consumes gastropods, and to a lesser extent some crustaceans (Serrano-Flores, 2017). For its part, *C. falciformis* is a mostly piscivorous species (Estupiñán-Montaño et al., 2017), while *S. lewini* is a mostly teutophagous species (Estupiñán-Montaño et al., 2009).

So, the degree of specialization in feeding mechanisms shown in TLs-V and -VI would explain the high values of *IEve* and *IUni* (Table 13). This suggests that the components, despite reflecting the same feeding mechanism (carnivores), are not concentrated in a certain area of the isotopic space of their TLs (Fig. 15 and 17) due to their food preferences, habitat use and ontogenetic changes (Estupiñán-Montaño et al., 2017, 2021a). A clear example of this is *S. lewini*, which shows ontogenetic changes in feeding habits over the course of its life cycle (Estupiñán-Montaño et al., 2021a). These changes in feeding and habitat uses by *S. lewini* suggest that the species is an important element linking coastal and oceanic energy flows (Estupiñán-Montalo et al., 2021a, b).

The different TLs proposed for the marine ecosystem of the Malpelo FFS reflect different degrees of interaction (isotopic overlap; Table 11, Fig. 18). These levels of interaction between TLs can be explained by: 1) a high diversity of feeding mechanisms, which allows some components of each TL to cross the "boundaries" between them, 2) organisms that expand the

isotopic space of their TL by the use of different food sources (e.g., *G. grapsus* interacting between the marine and terrestrial environments), 3) seasonal and ontogenetic changes in habitat use (Varisco et al., 2015; Estupiñán-Montaño et al., 2021a), 4) high degrees of omnivory, as several organisms feed in different TLs, and 5) the presence of diverse basal sources that support different TL food chains, which would be supported by the high nesting values (*INes*) between basal  $\delta^{13}$ C and  $\delta^{13}$ C of the other TLs (*INes*; TL-II = 1, TL-III = 0.926, TL-IV = 0.856, TL-V = 0.403). Thus, trophic interactions would be an important mechanism for energy exchange from the pelagic to the benthic environment and from land to sea, also influencing marine secondary production (Ying et al., 2020). For example, some mobile invertebrates (TL-III and -IV) and suspenchivorous organisms (TL-II) that feed on plankton are consumed by demersal predators, and thus act in the transfer of energy from pelagic to benthic environments (Funes et al., 2018; Woodland and Secor, 2013; Zhou, 2006).

Additionally, the estimated TDFs for the Malpelo FFS showed a global average (TDF<sub>Malpelo</sub>) of  $0.43 \pm 1.27\%$  and  $1.88 \pm 0.80\%$  for  $\Delta\delta^{13}$ C and  $\Delta\delta^{15}$ N respectively. These values were in the range of TDFs estimated in previous studies ( $\Delta\delta^{13}$ C = -1.9 and +5.6% [Busst and Britton, 2017; Sacramento et al., 2016; Britton and Busst, 2017] and  $\Delta\delta^{15}$ N = +3.3 to +6.9% [Mill et al., 2007; Busst and Britton, 2017: Sacramento et al., 2016; Britton and Busst, 2017]. The similarity of the calculated values for  $\Delta\delta^{13}$ C<sub>Malpelo</sub> to those proposed by Post et al. (2007) and McCutchan et al. (2013) ( $0.4 \pm 0.17\%$  and  $0.4 \pm 1.3\%$  respectively) was observed; while the values for  $\Delta\delta^{15}$ N<sub>Malpelo</sub> were different from those proposed by the same authors ( $2.3 \pm 0.28\%$  SD [McCutchan et al., 2003] and  $3.4 \pm 1.0\%$  [Post et al., 2007]).

It is likely that the differences in the TDF values between this and other studies could be due to several reasons, including but not limited to the following: 1) this study only used three basal sources (Table 8) to estimate TDFs between the base and TL-II. Therefore, the exclusion of other basal sources (e.g., detritus, microphytobenthos, etc.), as suggested by the wide range of  $\delta^{13}$ C and different  $\delta^{15}$ N signals from primary consumers, could influence the average TDF value between TLs, 2) methodological differences between studies (e.g., feeding under controlled conditions, sample treatment [McCutchan et al., 2003]). In this regard, in some cases samples in this study were treated to remove lipids and urea (e.g., elasmobranchs) while in other cases they were untreated (e.g., teleosts, gastropods, crustaceans), with mathematically normalization being

applied when necessary (C:N ratio >3.5), 3) the portion of the organisms (part of muscle tissue or the whole organism) analyzed isotopically (McCutchan et al., 2003), 4) this study did not consider some conditions or characteristics of the organisms studied (e.g., health, physical condition, sex, age, etc. [Vander-Zande and Rasmussen, 2001; Vanderklift and Ponsard, 2003; McCutchan et al., 2003; Busst et al., 2015; Britton and Busst, 2017]), 5) the turnover rate of the analyzed tissue, as this may change due to differential digestion during assimilation and metabolic processes (McCutchan et al., 2003; Matsubayashi et al., 2018), as TDFs tend to decrease with increasing dietary isotopic values (Caut et al., 2009; Mohan et al., 2016), 6) the proportion of animals in the population consuming the same type of diet (McCutchan et al., 2003), which could indicate individual species variation (Gutmann-Roberts et al., 2017; Estupiñán-Montaño et al., 2021a) originating substantial change in individual isotopic ratios (Hobson and Clark, 1992) and 7) environmental conditions (e.g., salinity and temperature [Vanderklift and Ponsard, 2003; Barnes et al., 2007; Medina-Contreras et al., 2020]). For example, salinity is a variable that can influence  $\delta^{13}$ C values, since it is generally controlled by the amount of freshwater entering the system (Medina-Contreras et al., 2020).

The above factors, combined with the large freshwater inputs from the terrestrial ecosystem of the Malpelo FFS containing high amounts of detritus and guano (different  $\delta^{13}$ C and  $\delta^{15}$ N signals) due to rainfall, could modify the oceanographic conditions of the island, and would produce significant sources of variation during the calculation of TDFs (McCutchan et al., 2003). Therefore, the TDF estimates in this study should be interpreted with caution and considered as a first approximation of the fractionation that could be occurring in the different trophic pathways of the Malpelo FFS food web.

In conclusion, this study of the marine trophic structure of the Malpelo FFS was based on the analysis of 39 families/groups, mostly represented by teleosts, followed by crustaceans, elasmobranchs, macroplankton and seabirds, with other groups present to a lesser extent (Table 8). The low representativeness of some groups was related to the difficulty of conducting field trips. Malpelo Island is located at a great distance from the Colombian mainland (~390 km away [Plan de Manejo, 2015]), and has a small terrestrial area (1. 2 km<sup>2</sup>; Graham, 1975). Hence, it does not have enough space for the creation of adequate infrastructure that would allow marine sampling for long periods of time. For this reason, the field trips conducted in this study were subject to the availability of support provided by a tourism company, Pacific Dive and its M/N Sea Wolff during

several tourism trips to the island. In addition to this, many of the samples analyzed came from seizures of illegal fishing, to which we obtained access thanks to the support of Parques Nacionales de Colombia and the SFF Malpelo team.

Despite the above, the number of samples and the groups they represented was sufficient to enable the elucidation of a marine food web composed of six trophic levels: 1) primary producers (TL-I), 2) herbivores (TL-II), 3) omnivores I (TL-III), 4) omnivores II (TL-IV), 5) carnivores (TL-V), and 6) top predators (T-VI). Each TL showed a different degree of complexity due to the presence of 12 feeding mechanisms, i.e., phytoplanktivores, zooplanktivores, zoobenthivores, planktivores, herbivores, omnivores, carnivores, filter feeders, sediment feeders, suspenchivores, detritivores, and scavengers. This in turn generated a high trophic interaction between TLs due to the multifunctionality of some species within and between different TLs, as well as the trophic interaction between marine and terrestrial environments, with some species mediating the flow of energy between these ecosystems, as is the case of *G. grapsus*.

Although only a relatively limited number of basal sources were analyzed in this study, the wide range of  $\delta^{13}$ C values encountered suggests that other basal sources also contribute to the marine food web of the Malpelo FFS. Therefore, it is necessary to analyze the full diversity of basal sources of the Malpelo FFS in order to improve the understanding of energy flow through the different trophic pathways and likewise, to verify the estimates of TDF for this ecosystem  $(\Delta\delta^{13}C_{Malpelo} = 0.42 \pm 1.27\%; \Delta\delta^{15}N_{Malpelo} = 1.88 \pm 0.80\%)$ , taking into account the potential pathways of variation that exist within this complex process.

Describing trophic pathways and energy flow on small oceanic islands with large seabird communities is complicated by the different sources of energy that can enter the system due to the land-sea interface, where marine organisms can interact between both ecosystems and terrestrial organic material is deposited in the sea due to precipitation.

Finally, this is the first study that describes the marine trophic structure of the largest MPA in the Colombian Pacific, the Malpelo FFS (Ministerio de Ambiente y Desarrollo Sostenible, 2017), from the use of trophic bio-markers, and makes evident how the lack of inter-institutional cooperation can limit the scope of research, especially in remote areas. Therefore, interinstitutional cooperation plays an important role in improving our understanding of the dynamics of these isolated ecosystems and providing more tools for the development of appropriate management and conservation measures for these ecosystems.

### 3.2. Structural analysis of the marine food web of the Malpelo Fauna and Flora Sanctuary, Colombia, from a topological approach

#### **INTRODUCTION**

An important topic in terms of enhancing our knowledge of ecological dynamics of ecosystems and improve species conservation strategies is to broaden the understanding of energy and nutrient fluxes through the study of food webs (Sardenne et al., 2017), because their analysis can make important contributions to understanding the diversity and magnitude of interactions that take place in the formation and functioning of complex food webs (Post, 2002; Bascompte et al., 2005). In this way, the effects of various pressures along the webs (i.e., fisheries, climate change, etc.) can be assessed (Litzow et al., 2005). [Litzow et al., 2006; Hebert et al., 2008]) and it can also help to generate tools to assess the persistence and resilience of ecosystems in the face of disturbances (Wilson et al., 2010).

For all of the reasons outlined above, the description of food webs (Bascompte, 2009) and the understanding of the effects of different forcing factors (e.g., environmental and anthropogenic) on ecosystem dynamics, productivity and stability (Rezende et al., 2009; Zetina-Rejón et al., 2015) have important implications for conservation. Despite this, apart from a couple of now quite dated studies on the ecology of Malpelo Island described above, little effort has been made to gather additional ecological information from the area in order to improve our understanding of the community dynamics of the Malpelo FFS, a complex oceanic system whose ecological dynamics are highly influenced by the oceanic processes that converge around it (Rodríguez-Rubio and Stuardo, 2002; Rodríguez-Rubio et al., 2003).

Studies of food webs generate information on different ecological aspects (e.g., competition, nutrient dynamics, cascade effects [Winemiller and Polis, 1996], community structure at different levels [Pimm, 1980], etc.), making it possible to understand the complex relationships between components and their properties (Balasundaram et al., 2005) and to identify structural patterns (Milo et al., 2002). This information constitutes a vital input for improving the design of ecosystem-based management and conservation strategies (Whipple et al., 2000; Borgatti, 2002), based on the elaboration of simplified models of trophic networks, i.e., topology, which can be used to evaluate and predict the qualitative dynamics of the ecosystem based on the community structure. In this sense, a network is visualized with nodes and links, where nodes correspond to

species (i.e., predator or prey) and links represent their trophic interactions. Added to this, the quantification of topological properties of networks provides information that can be used to address different ecological aspects (e.g., identification of keystone species [Mills et al., 1993; Jordán et al., 2006] or competition processes [Baiser et al., 2016]).

To date there are no studies related to the description of the marine food web of the Malpelo FFS, and studies focused on trophic aspects of marine species are still scarce. Among the trophic studies reported for the species of this ecosystem are those related to the feeding habits of seabirds, the Nazca booby Sula granti and the red-footed booby S. sula, being two species that have a high preference for consuming fish of the families Exocoetidae, Hemiramphidae, as well as juveniles of the families Carangidae, Scombridae and small (Garcia and Lopez-Victoria, 2007; Lopez-Victoria and Garcia, 2010). Studies on the trophic preferences of hammerhead sharks Sphyrna *lewini* and silky sharks *Carcharhinus falciformis* are also reported based on analysis of  $\delta^{13}$ C and  $\delta^{15}$ N. One of these studies suggests that both species occupy high trophic levels (trophic position >5.0) in the food chain of the Malpelo FFS, with high preference for the use of oceanic zones as feeding areas (Estupiñán-Montaño et al., 2017a). The above facts show that there is still a deficiency of in situ studies analyzing trophic aspects that are useful for the characterization and modeling of the marine food web of the Malpelo FFS, which places severe limits on our ability to understand the trophic dynamics of this ecosystem. Partly as a consequence of this deficiency, existing management and conservation measures in this MPA tend to focus on particular species and not on a global perspective. That is to say, a perspective that takes into account the characteristics of the ecosystem as a whole. For this reason, the identification of structural patterns and the role of species can make a significant contribution to the planning and implementation of conservation efforts in an integrated manner.

Based on the above, the objectives of this chapter were: 1) to describe the marine food web structure of the Malpelo FFS from a topological approach, 2) to identify key species, 3) to identify the formation of marine trophic communities as attributes of stability and resilience to disturbances, and 4) to evaluate the resilience of the web.

#### **MATERIALS AND METHODS**

#### Study area

Malpelo Island (Fig. 4A) is the summit of a submarine mountain range called the Malpelo Ridge, which extends in a NE-SW direction; it is approximately 241.4 km long by 80.5 km wide (Fig. 4B, red polygon). The island has a maximum height of 300 m above sea level and emerges from approximately 4,000 m depth (Fig. 4C) (more details in Chapter 1).

#### **Dietary analysis**

In order to construct a simplified model of the trophic relations between the marine species of Malpelo FFS, an adjacency matrix was elaborated comprising 143 trophogroups (defined as an aggregation of biological groups based on trophic similarity). Binary data (0,1) representing the trophic relations between trophogroups *i* and *j* was then fed into the matrix. In this sense, if the trophogroups *i* and *j* presented some type of trophic relation (i.e., eats or is eaten by the other) it was represented with a value of 1, whereas if there was no trophic relation between the respective trophic groups it was represented with the value of 0.

Due to the paucity of information on the diet and feeding habits of the marine species that inhabit Malpelo FFS, the information used to define the trophic relations between the trophogroups of the ecosystem was obtained from a revision of the web page www.fishbase.org (for all of the fish species studied [Froese y Pauly, 2022]), such information being complemented by bibliographies of trophic studies of the main marine birds present in the area (García and López-Victoria, 2007; López-Victoria and García, 2010), squid and cuttlefish (Jereb and Roper, 2005a,b), crustaceans (e.g., Kneib and Weeks, 1990; Mc Tigue and Zimmerman, 1991; Díaz-Arredondo and Guzmán-de-Próo, 1995; Kyomo, 1999; Gianuca and Vooren, 2007), sharks (Estupiñán-Montaño et al., 2009, 2017b; Cárdenas-Palomo et al., 2018; Morales-Serran 2020), bivalve molluscs and gasteropods (e.g., Galtsoff, 1964; Kohn, 1983; Morton, 1983) and invertebrates (e.g., Goreau et al., 1971; Gili et al., 1984; Yeates et al., 1993; Okada et al., 2005; Smith et al., 2006; von Ruckert and Giani, 2008; Leys and Hill, 2012; Li et al., 2020). In all instances these corresponded to species reported as inhabiting the Malpelo FFS, being grouped according to Families (Table 14).

Tabla 14. Indices of centrality for teh marine trophic web of the Malpelo FFS, Colombia, including local and meso-scale indices represented by normalized values. DC: Degree, C: Closeness, BC: Intermediation, EC: Eigen centrality, SC: Centrality of subgraphs, CC: Coefficient of grouping. ID: Identification code of each trophogroup.

ID	Trophogroups	DCin	DCout	DC <sub>All</sub>	С	BC	EC	SC
1	Phytoplankton	0.000	0.387	0.365	0.651	0.073	0.398	0.642
2	Acanthuridae	0.191	0.048	0.175	0.515	0.069	0.200	0.000
3	Detritus	0.000	0.452	0.429	0.640	0.121	0.436	1.000
4	Algaes	0.000	0.532	0.508	0.668	0.306	0.438	0.121
5	Hidrozoa	0.043	0.274	0.286	0.593	0.052	0.304	0.025
6	Corals	0.064	0.097	0.127	0.465	0.009	0.132	0.000
7	Zooplankton	0.043	1.000	1.000	0.968	1.000	0.817	0.558
8	Crustaceans	0.106	0.694	0.746	0.914	0.556	0.717	0.044
9	Molluscs	0.085	0.452	0.492	0.802	0.197	0.495	0.014
10	Nematodes	0.000	0.032	0.016	0.072	0.000	0.009	0.013
11	Apogonidae	0.149	0.016	0.111	0.515	0.005	0.170	0.000
12	Gonostomatidae	0.085	0.016	0.063	0.428	0.001	0.115	0.000
13	Poliquetes	0.064	0.452	0.476	0.714	0.087	0.548	0.053
14	Crabs	0.000	0.484	0.460	0.691	0.166	0.452	0.033
15	Myctophidae	0.106	0.113	0.175	0.598	0.030	0.217	0.001
16	Isopoda	0.106	0.435	0.492	0.738	0.105	0.555	0.029
17	Balistidae	0.191	0.129	0.254	0.708	0.053	0.335	0.000
18	Echinoderms	0.128	0.274	0.349	0.588	0.069	0.373	0.009
19	Annelids	0.064	0.113	0.143	0.461	0.010	0.159	0.000
20	Clupeidae	0.128	0.242	0.317	0.745	0.044	0.450	0.006
21	Belonidae	0.149	0.032	0.127	0.553	0.007	0.193	0.000
22	Mugilidae	0.234	0.081	0.238	0.646	0.025	0.335	0.001
23	Shrimps	0.000	0.548	0.524	0.745	0.277	0.533	0.029
24	Engraulidae	0.170	0.258	0.365	0.770	0.081	0.483	0.003
25	Carangidae	0.809	0.274	0.857	1.000	0.505	1.000	0.004
26	Lolliginidae	0.128	0.161	0.238	0.598	0.019	0.353	0.003
27	Mastigoteuthidae	0.000	0.048	0.032	0.395	0.000	0.099	0.014
28	Sciaenidae	0.298	0.129	0.333	0.732	0.038	0.498	0.001
29	Haemulidae	0.255	0.097	0.270	0.685	0.027	0.421	0.001
30	Sparidae	0.383	0.065	0.333	0.668	0.050	0.435	0.000
31	Labridae	0.170	0.048	0.159	0.563	0.008	0.248	0.001
32	Centriscidae	0.106	0.016	0.079	0.479	0.001	0.163	0.000
33	Mullidae	0.234	0.032	0.190	0.543	0.093	0.268	0.000
34	Scombridae	1.000	0.194	0.921	0.937	0.863	0.874	0.002
35	Trichiuridae	0.489	0.032	0.381	0.702	0.071	0.526	0.000
36	Ammodytidae	0.128	0.032	0.111	0.548	0.003	0.219	0.001

37	Lutjanidae	0.489	0.048	0.397	0.662	0.180	0.451	0.000
38	Scaridae	0.085	0.048	0.095	0.529	0.005	0.147	0.000
39	Syngnathidae	0.043	0.048	0.063	0.519	0.001	0.149	0.001
40	Lobsters	0.085	0.113	0.159	0.533	0.007	0.214	0.003
41	Gasteropods	0.149	0.339	0.429	0.679	0.087	0.461	0.011
42	Euphausiids	0.064	0.258	0.286	0.640	0.085	0.320	0.007
43	Mysids	0.128	0.242	0.317	0.691	0.030	0.453	0.022
44	Ostracods	0.000	0.129	0.111	0.346	0.010	0.120	0.018
45	Bivalves	0.021	0.355	0.349	0.598	0.075	0.401	0.016
46	Gerreidae	0.000	0.032	0.016	0.321	0.000	0.056	0.013
47	Serranidae	0.340	0.097	0.333	0.720	0.070	0.391	0.001
48	Argonautidae	0.064	0.097	0.127	0.501	0.004	0.151	0.000
49	Carcharhinus falciformis	0.426	0.000	0.302	0.567	0.160	0.308	0.000
50	Ancistrocheuridae	0.000	0.065	0.048	0.336	0.002	0.079	0.013
51	Ommastrephidae	0.383	0.194	0.460	0.776	0.208	0.442	0.001
52	Pholidoteuthidae	0.000	0.048	0.032	0.206	0.002	0.038	0.013
53	Thysanoteuthidae	0.043	0.081	0.095	0.538	0.008	0.156	0.000
54	Vitreledonellidae	0.000	0.016	0.000	0.086	0.000	0.010	0.013
55	Hemiramphidae	0.021	0.161	0.159	0.598	0.038	0.214	0.001
56	Coryphaenidae	0.340	0.097	0.333	0.651	0.098	0.392	0.000
57	Diodontidae	0.191	0.048	0.175	0.519	0.021	0.221	0.000
58	Tetraodontidae	0.128	0.097	0.175	0.553	0.118	0.180	0.000
59	Chelonidae	0.191	0.016	0.143	0.492	0.092	0.158	0.000
60	Triaenodon obesus	0.277	0.016	0.206	0.465	0.034	0.185	0.000
61	Carcharhinus galapagensis	0.362	0.000	0.254	0.543	0.048	0.254	0.000
62	Muraenidae	0.128	0.048	0.127	0.510	0.009	0.162	0.000
63	Synodontidae	0.000	0.081	0.063	0.343	0.006	0.065	0.013
64	Holocentridae	0.298	0.065	0.270	0.629	0.034	0.399	0.000
65	Priacanthidae	0.213	0.016	0.159	0.524	0.089	0.217	0.000
66	Pomacentridae	0.128	0.048	0.127	0.496	0.011	0.180	0.000
67	Monacanthidae	0.149	0.048	0.143	0.524	0.013	0.173	0.000
68	Octopodidae	0.170	0.177	0.286	0.646	0.033	0.338	0.001
69	Xanthidae	0.128	0.016	0.095	0.392	0.109	0.098	0.000
70	Carpiliidae	0.043	0.016	0.032	0.301	0.001	0.060	0.007
71	Chaenopsidae	0.021	0.000	0.000	0.261	0.000	0.033	0.000
72	Chaetodontidae	0.064	0.032	0.063	0.436	0.003	0.106	0.000
73	Chanidae	0.191	0.000	0.127	0.501	0.004	0.203	0.000

74	Cirrhitidae	0.191	0.000	0.127	0.483	0.007	0.184	0.000
75	Gobiidae	0.340	0.097	0.333	0.629	0.039	0.467	0.000
76	Foraminifera	0.106	0.048	0.111	0.372	0.006	0.110	0.000
77	Stomatopods	0.085	0.242	0.286	0.651	0.061	0.328	0.003
78	Congridae	0.021	0.032	0.032	0.328	0.001	0.068	0.000
79	Polynemidae	0.128	0.016	0.095	0.423	0.003	0.128	0.000
80	Exocoetidae	0.021	0.129	0.127	0.572	0.026	0.183	0.001
81	Merluccidae	0.191	0.081	0.206	0.608	0.093	0.265	0.000
82	Paralichthydae	0.277	0.048	0.238	0.629	0.031	0.308	0.000
83	Platycephalidae	0.064	0.016	0.048	0.281	0.000	0.067	0.000
84	Atherinidae	0.021	0.032	0.032	0.365	0.000	0.070	0.000
85	Fistulariidae	0.383	0.016	0.286	0.646	0.244	0.274	0.000
86	Bothidae	0.064	0.048	0.079	0.380	0.004	0.115	0.000
87	Blenniidae	0.106	0.016	0.079	0.354	0.005	0.095	0.000
88	Siganidae	0.000	0.016	0.000	0.123	0.000	0.008	0.013
89	Centracanthidae	0.000	0.016	0.000	0.123	0.000	0.008	0.013
90	Gobiesocidae	0.043	0.000	0.016	0.325	0.000	0.054	0.000
91	Istiophoridae	0.660	0.000	0.476	0.776	0.420	0.448	0.000
92	Lethrinidae	0.000	0.016	0.000	0.181	0.000	0.016	0.013
93	Ostraciidae	0.043	0.016	0.032	0.321	0.002	0.047	0.000
94	Lophiidae	0.043	0.016	0.032	0.407	0.000	0.074	0.000
95	Ocythoidae	0.000	0.016	0.000	0.181	0.000	0.016	0.013
96	Scorpaenidae	0.085	0.048	0.095	0.384	0.001	0.103	0.000
97	Xiphiidae	0.404	0.016	0.302	0.598	0.325	0.252	0.000
98	Scomberesocidae	0.000	0.048	0.032	0.357	0.000	0.077	0.013
99	Dussumieriidae	0.000	0.016	0.000	0.181	0.000	0.016	0.013
100	Gempylidae	0.128	0.065	0.143	0.598	0.007	0.267	0.000
101	Kyphosidae	0.234	0.032	0.190	0.651	0.017	0.283	0.000
102	Sponges	0.064	0.177	0.206	0.613	0.077	0.215	0.002
103	Labrisomidae	0.021	0.000	0.000	0.239	0.000	0.029	0.000
104	Lobotidae	0.021	0.000	0.000	0.239	0.000	0.029	0.000
105	Batrachoididae	0.000	0.016	0.000	0.130	0.000	0.016	0.013
106	Malacanthidae	0.021	0.000	0.000	0.261	0.000	0.033	0.000
107	Uranoscopidae	0.000	0.016	0.000	0.075	0.000	0.008	0.013
108	Opistognathidae	0.021	0.000	0.000	0.261	0.000	0.033	0.000
109	Pomacanthidae	0.213	0.000	0.143	0.483	0.008	0.172	0.000
110	Rhincodon typus	0.043	0.000	0.016	0.304	0.000	0.048	0.000

111	Sphyraenidae	0.234	0.032	0.190	0.543	0.005	0.303	0.000
112	Bolitaenidae	0.000	0.016	0.000	0.249	0.000	0.036	0.013
113	Enoploteuthidae	0.000	0.048	0.032	0.304	0.001	0.060	0.014
114	Histioteuthidae	0.000	0.048	0.032	0.332	0.000	0.073	0.013
115	Echeneidae	0.000	0.016	0.000	0.249	0.000	0.036	0.013
116	Teuthidae	0.000	0.016	0.000	0.249	0.000	0.036	0.013
117	Bramidae	0.000	0.016	0.000	0.249	0.000	0.036	0.013
118	Onychoteuthidae	0.000	0.065	0.048	0.361	0.001	0.085	0.013
119	Pristigasteridae	0.149	0.016	0.111	0.588	0.005	0.206	0.000
120	Noemidae	0.064	0.032	0.063	0.582	0.008	0.133	0.000
121	Gonatidae	0.149	0.065	0.159	0.646	0.016	0.233	0.000
122	Octopoteuthidae	0.000	0.032	0.016	0.284	0.000	0.052	0.013
123	Sphyrna lewini	0.574	0.000	0.413	0.613	0.229	0.356	0.000
124	Ophichthidae	0.000	0.016	0.000	0.108	0.000	0.012	0.013
125	Balans	0.000	0.016	0.000	0.079	0.000	0.004	0.013
126	Tripterygiidae	0.021	0.000	0.000	0.133	0.000	0.016	0.000
127	Stromateidae	0.191	0.016	0.143	0.533	0.020	0.189	0.000
128	Luvaridae	0.043	0.016	0.032	0.388	0.005	0.059	0.007
129	Trachipteridae	0.000	0.016	0.000	0.100	0.000	0.007	0.013
130	Amphitretidae	0.000	0.016	0.000	0.100	0.000	0.007	0.013
131	Vampyroteuthidae	0.000	0.016	0.000	0.100	0.000	0.007	0.013
132	Zanclidae	0.043	0.000	0.016	0.203	0.000	0.026	0.000
133	Sternoptychidae	0.064	0.016	0.048	0.432	0.005	0.065	0.000
134	Paralepididae	0.085	0.016	0.063	0.328	0.003	0.069	0.000
135	Briozoa	0.021	0.016	0.016	0.307	0.000	0.051	0.000
136	Ariidae	0.000	0.016	0.000	0.050	0.000	0.003	0.013
137	Leiognatidae	0.000	0.016	0.000	0.065	0.000	0.006	0.013
138	Cnidarians	0.000	0.016	0.000	0.000	0.000	0.000	0.013
139	Triglidae	0.000	0.016	0.000	0.105	0.000	0.008	0.013
140	Ophidiidae	0.191	0.048	0.175	0.388	0.010	0.151	0.000
141	Sula granti	0.191	0.000	0.127	0.440	0.004	0.177	0.000
142	Sula sula	0.064	0.000	0.032	0.192	0.000	0.032	0.000
143	Tursiops truncatus	0.234	0.000	0.159	0.487	0.016	0.214	0.000

#### Topological analysis

#### Identification of key trophogroups

The identification of key trophogroups was carried out by estimating local indicators (i.e., topological centrality indices) that provided information on their positional importance. In this way, five indicators were estimated: 1) degree index (*DC*), 2) betweenness index (*BC*), 3) closeness index (*C<sub>i</sub>*), 4) Eigen centrality (*EC*) and 5) subgraph centrality (*SC*) of the (Table 15).

To facilitate comparison and identification of key trophogroups, centrality indices were scaled between 0 and 1. All analyses were performed in R statistical software (R Team Core, 2018).

#### Community substructures in the trophic web

Substructures in food webs are defined as sets of species that are more closely interconnected with each other than with other species in the rest of the web (Rezende et al., 2009). To identify these substructures in the marine ecosystem of the Malpelo FFS, the *fast greedy* subnetwork identification algorithm (Newman and Girvan, 2004) of the R software package *igraph* (version 1.2.6) was applied (Csardi and Nepusz, 2006).

#### Motifs

One method than can be used to explore trophic interconnectivity patterns is the identification of *motifs*, which are recurrent patterns of connectivity that constitute subgraphs that can include any number of nodes and linkages. Studies of food webs have focused primarily on 3-node *motifs* as ecological theory has relied on several of these patterns (Baiser et al., 2016).

There are 13 possible configurations of 3-node *motifs* (Fig. 19). Four of them are related to ecological processes: 1) apparent competition: this occurs when two species are preyed upon by a common predator (Fig. 19a), 2) tri-trophic chain: formed by three nodes where energy flows from basal sources to higher consumers (Fig. 19b), 3) exploitative competition: this is an indirect competition and occurs when a resource is shared by two consumers (Fig. 19d) and 4) omnivory: this occurs when a species feeds on more than one trophic level (Fig. 19e). In accordance with the above, the recurrence of 3-node *motifs* present in the marine food web of the Malpelo FFS was

evaluated as an indicator of ecological regulation mechanisms, and of their relationship with the persistence and resilience of the web (Stouffer and Bascompte, 2011).

The R software package *igraph* (Csardi and Nepusz, 2006) was used to identify these *motifs* in the marine web of the Malpelo FFS.



**Figure 19.** Subgraphs representing the 13 types of 3-node *motifs* present in directed networks. Four of these *motifs* are based on ecological theory. **a.** Apparent competition, **b.** Tri-trophic chain, **d.** Exploitative competition, **e.** Omnivory. **Taken from:** Elhesha et al., (2017).

~		Descr	Description					
Indices	Formula	Variable	Methods					
Degree ( <i>DC<sub>i</sub></i> )	$DC_i = D_{\mathrm{in},i} + D_{\mathrm{out},i}$	$DC_{in}$ is the number of predators and $DC_{out}$ is the number of prey.	The normalized node degree ( <i>DC</i> ) represents the number (in percent) of connections between pairs of nodes and is the sum of the input (predators) and output (prey) connections.					
Betweenness centrality ( <i>BC</i> <sub>i</sub> )	$BC_{i} = \frac{2 \times \sum_{j < k} \frac{\mathbf{g}_{jk}(\mathbf{i})}{\mathbf{g}_{jk}}}{(N-1) \times (N-2)}$	BC is the frequency of species i on the shortest path between species j and k. N is the number of species, $g_{jk}$ is the number of minimum isometric paths between species j and k, and $g_{jk}(i)$ is the number of species i in the shortest path between j and k ( $i \neq j$ , $i \neq k$ ). The higher value of BC <sub>i</sub> indicates the species(s) that has greater control of the information in the web.	The intermediation index $(BC)$ represents the capacity to control the exchange of information in the food web.	Wasserman y Faust (1994)				
Closseness $(C_i)$	$C_i = \frac{N-1}{\sum_{j=1}^{N} d_{ij}}$	<i>C</i> indicates the sum of the minimum distances from a species to all other species in the web, N is the number of species, and $d_{ij}$ is the length of the shortest path between species <i>i</i> and <i>j</i> .	El índice de cercanía indica la velocidad de transmisión de la información a través de la red trófica. Es decir, especies con menores valores de $C_i$ transmiten la información más rápidamente a través de la red, que aquellas con mayores valores.	Wasserman y Faust (1994)				
Eigen Centrality ( <i>EC</i> )	$\lambda C_{eiv} = A \times C_{eiv}$	$\lambda$ is a constant, $C_{eiv}$ is an eigenvector of the adjacency matrix A with eigenvalue $\lambda$ .	The closeness index ( <i>C</i> ) indicates the speed of information transmission through the food web. That is, species with lower $C_i$ values transmit information more rapidly through the web than those with higher $C_i$ values	Bonacich (1987)				

Clustering coeffienciet (CC <sub>i</sub> )	$CC_{i} = \frac{1}{N} \sum_{i=1}^{N} \frac{2E_{i}}{k_{i}(k_{i}-1)}$	$N_i$ with $k_i$ neighbors, $E_i$ is defined as the number of links between the $k_i$ neihborns. The clustering coefficient is the ratio of the number of ties between neighbors to the number of ties between neighbors ( $E_i$ ) and the potential number of links ( $k_i (k_i - 1) / 2$ among neighbors.	The clustering coefficient $(CC_i)$ measures how densely connected a node is to its immediate neighbors. If $CC_i = 1$ , all neighbors are connected to each of the nodes; but, if $CC_i = 0$ , none of its direct neighbors are connected to the other nodes	Watts y Strogatz (1998)	
Subgraph centrality (SC)	$SC(i) = \sum_{j=1}^{N} [\gamma_j(i)]^2 e^{\lambda j}$ $Eq. 2$ $SC(i) = \sum_{j=1}^{N} [\gamma_j(i)]^2 \cosh \lambda_j + \sum_{j=1}^{N} [\gamma_j(i)]^2 \sin h\lambda_j$ $= SC_{exs}(i) + SC_{edd}(i)$ $Eq. 3$ $SC_{odd} = \sum_{r=1}^{\infty} \frac{\mu_{2r}(i)}{(2r)!}$	Where (i) is the <i>i</i> <sup>th</sup> component of the <i>j</i> <sup>th</sup> eigenvector of the adyacence matrix A y $\lambda_j$ is the corresponding <i>j</i> <sup>th</sup> eigenvalor (Eq. 1). <i>SC</i> (i) counts all closed paths (CWs) in the web, which can be of even (SCeven) and odd ( <i>SC</i> <sub>odd</sub> ) length. CWs of even length can move back and forth in subgraphs that have no cycles (i.e., acyclic), while odd CWs do not contain contributions from acyclic subgraphs. Consequently, <i>SC</i> (i) can be divided into two terms by considering the even and odd CWs in the food web (Eq. 2). Therefore, <i>SC</i> <sub>odd</sub> (i) can be expressed in terms of the number of odd-length CWs using the expression in Equation 3.	The subgraph centrality index ( <i>SC</i> ) of the node characterizes the importance of a node in all existing subgraphs in the web. The <i>SC</i> of a vertex $i$ is defined as the "sum" of closed paths ( <i>CWs</i> ) of different lengths in the web, starting and ending at vertex $i$ .	Estrada y Rodríguez- Velázquez, 2005a Estrada y Rodríguez- Velázquez, 2005b	
Anidación (NODF)	$NODF = \frac{\sum N_{paired}}{\left[\frac{n(n-1)}{2}\right] + \left[\frac{m(m-1)}{2}\right]}$	<i>NODF</i> is the nesting measurement, $N_{\text{paired}}$ is the degree of nesting matching, n(i - 1)/2 y $m(m - 1)/2$ are the nesting pairing degrees for columns <i>n</i> and rows <i>m</i> , respectively.	<i>NODF</i> is the nesting measure of the web. This method returns values from 0 to 100, indicating that values equal to 0 indicate no nesting, while values equal to 100 suggest perfect nesting.	Almeida-Neto et al., 2008	
Modularity $M_{W}(P) = \sum_{s=1}^{N_{M}} \left[ \left( \frac{w_{s}^{in}}{W} \right) - \left( \frac{w_{s}^{all}}{2W} \right)^{2} \right]$		$W = \sum_{i \ge j} w_{ij}$ is the sum of the weights of all predator-prey interactions throughout the web. $w^{in}s$ is the sum of the weights of the linkages $w_{ij}$ within each compartment <i>s</i> , y $w^{all}s = \sum_{ijs} \sum_{j} w_{ij}$ is the sum of the weights of the interactions involving species <i>i</i> within module <i>s</i> with all other species.	Modularity is the number that illustrates how much a given web can be organized into communities or subwebs. Modularity captures how good a partition is compared to a randomly intertwined web.	Newman and Girvan (2004)	
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Connectance $C = L/S^2$		L are the link and S are the species.	Connectance is the ratio of observed linkages to all possible linkages, including cannibalism and mutual predation (Polis, 1991), within a food web.		
Average path length (APL)	$APL = \frac{1}{N(N-1)} \sum_{ij=1, i \neq j}^{N} d_{ij}$	The distance $d_{ij}$ between nodes <i>i</i> and <i>j</i> is defined by the number of vertices that make up the shortest path connecting two nodes. If nodes <i>i</i> and <i>j</i> are not connected, then $d_{ij} = N$ .	It is the average distance between any pair of nodes.	Travers and Milgram (1969)	

#### Minimum spanning tree

The *minimum spanning tree* (MST) is a special type of tree that minimizes the lengths of tree edges. In this sense, a food web can be summarized in a MST, in which all groups link to basal groups following the shortest food chain and removing links that do not appear in shorter chains (Garlaschelli et al., 2003).

Considering the above, the energy distribution along the marine food web of the Malpelo FFS was evaluated using the MST approach, which was created using the mst function of the R software package igraph (Csardi and Nepusz, 2006).

#### Topological properties and resilience of the food web

The structure of the marine food web of the Malpelo FFS was analyzed based on several global network indicators, such as: number of trophogroups (N), connectance, clustering coefficient ( $CC_{global}$ ), modularity, diameter, average path length (APL), network centralization and global nesting (NODF) (description in Table 15). This last network attribute was estimated with the RInSp package (Almeida-Neto et al., 2008).

The resilience of the marine food web of the Malpelo FFS was evaluated based on the consecutive removal of nodes (i.e., trophogroups). The removal of nodes was done in order to analyze the resilience of the network through the alteration of its attributes. For this purpose, two types of scenarios were simulated: 1) "attacks" and 2) "failures", following Albert et al. (2000). "Attacks" are impacts directed at particular nodes. In this case, nodes are removed in descending order according to their centrality value in the network (Zetina-Rejon et al., 2022). In this case, three criteria were used to determine the order of node removal: 1)  $DC_{All}$ , 2) BC, and 3) SC, as indicators of different functional characteristics of each node.

The "failures" are random errors that could occur in the network. These nodes were removed randomly for three times. Once the nodes were removed considering both scenarios (attack and error), changes in four network properties were evaluated, such as: size (e.g., APL), degree of clustering, connectivity and centralization of the network.

This analysis was designed in the R statistical platform (R Core Team, 2018), based on the simulation approach proposed by Albert et al. (2000) modified by Zetina-Rejón et al. (2022).

#### RESULTS

The marine food web of the Malpelo FFS was constructed with 143 trophogroups, of which eight were integrated at the species level, while the others were included at the group level (e.g., family, class, order). Accordingly, the network reflected a total of 838 trophic links (Fig. 20A).



Figure 20. A. Marine food web of the Malpelo Fauna and Flora Sanctuary, Colombia, showing four trophic communities. B. Simplification of the four communities present in the marine food web and their interactions. Community 1: yellow nodes, Community 2: green nodes, Community 3: pink nodes, Community 4: orange nodes. Red arrows: diameter of the web.

#### Identification of key trophogroups in marine food web

Based on the local centrality indicators, the *DC* suggests that the trophogroups with the highest consumer role ( $DC_{in}$ ) were tunas (Fam. Scombridae; ID: 34), followed by carangids (Fam. Carangidae; ID: 25), hammerhead sharks (*Sphyrna lewini*; ID: 123), silky sharks (*Carcharhinus falciformis*; ID: 49), snappers (Fam. Lutjanidae; ID: 37) and pomfrets (Fam. Trichiuridae; ID: 35) (Table 14; Fig. 20A). While the trophogroups acting mostly as prey ( $DC_{out}$ ) were: zooplankton (ID: 7), followed by crustaceans (ID: 8), shrimps (ID: 23), crabs (ID: 14), mollusks (ID: 9), polychaetes (ID: 13), and isopods (ID: 16) (Table 14; Fig. 20A).

On the other hand, the C index suggests that 49.7% of trophogroups showed high closeness to the other network components (C  $\geq$ 0.50; Fig. 21), whereas only 2.8% and 11.2% of trophogroups were high intermediates (*BC*) and influence (*EC*) in the network, respectively. The most relevant nodes in energy transfer in terms of *C*, *BC*, and *EC* were: zooplankton, tunas (Fam. Scombridae),

carangids (Fam. Carangidae), and crustaceans (Fig. 21). Unlike the previous centrality indices, the SC indicated that the trophogroups contributing most to the formation of trophic subgroups were: detritus (ID: 3), phytoplankton (ID: 1), and zooplankton (Table 14; Fig. 21).



Figure 21. Representation of the key trophogroups of the marine food web of the Malpelo Fauna and Flora Sanctuary, indicating the values of some centrality indices. Note: The identification codes of the trophogroups can be found in Table 1.

#### Community sub-webs in the marine foodweb

The marine food web of the Malpelo FFS showed a modularity value of 27.5% and nestedness of 27.8%, resulting in a compartmentalized web consisting of four community sub-webs (Figs. 20A, B). Community 1 consisted of 18 trophogroups (Fig. 20A and B [yellow nodes]), community 2 consisted of 47 components (Fig. 20A and B [green nodes]), community 3 presented 51 trophogroups (Fig. 20A and B [pink nodes]), and community 4 was composed of 27 trophogroups (Fig. 20A and B [orange nodes]).

#### Motifs

Of the possible 13 types of 3-node *motifs* present in directed webs, the marine food web of the Malpelo FFS included 12 of them (Table 16), generating a total of 14621 global *motifs*. Of these, 44.1% represented exploitative competition (Fig. 19d), 28.7% of them were apparent competition (Fig. 19a), and 16.8% and 7.7% represented tri-trophic chains (Fig. 19b) and omnivory (Fig. 19e) respectively (Table 16).

**Table 16.** Recurrence of 3-node *motifs* in each of the communities observed in the marine food web of the Malpelo Fauna and Flora Sanctuary, Colombia. **Motifs related to ecological theory: a.** Apparent competition, **b.** Tri-trophic chain, **d.** Exploitative

Motifs	Community 1	Community 2	Community 3	Community 4	Global web
а	13	286	769	148	4192
b	2	169	288	76	2457
c	0	0	72	5	247
d	54	1155	263	217	6441
e	1	181	171	47	1129
f	0	0	16	2	63
g	0	0	29	1	53
h	0	0	2	0	6
i	0	0	1	0	1
j	0	0	3	0	4
k	0	0	19	0	21
1	0	0	7	0	7
m	0	0	0	0	0
Total	70	1791	1640	496	14621

Additionally, 3-node *motifs* were identified within each marine trophic community of the Malpelo FFS, so that 70 *motifs* were identified in community 1, 1791 in community 2, 1640 in community 3, and 496 in community 4 (Table 16). Within each trophic community, the most recurrent motifs were of the exploitative competition type with 77.1%, 64.5%, and 43.8% for communities 1, 2, and 4 respectively, while in community 3, apparent competition was the most frequent with 46.9% (Table 16).

#### Minimum spanning tree

The marine food web of the Malpelo FFS was modeled in the form of a *minimum spanning tree* (representing the most efficient routes in energy transfer) in which the energy generated is transferred more rapidly through the web by means of two short routes: the first, and smallest, route supported by algae (ID: 2; Fig. 22) and the second, the route with the largest number of short chains (larger in size), is supported by phytoplankton (ID: 1; Fig. 22). The trophic chain supported by phytoplankton shows six "branches" as a representation of the shortest or most efficient trophic chains in the transfer of energy from the "environment", thus showing the relative importance of some trophogroups in the flow and distribution of energy to the other members of the network (Fig. 22).

#### Topological properties and resilience of the food web

The Malpelo FFS consisted of a marine food web with a diameter of 7 steps in length (Fig. 20A) and an APL of 2.25 steps. In addition, this web reflected low connectivity (4.1%) and centralization of trophic relationships (4.2%), as well as intermediate coefficients of clustering ( $CC_{global}$ : 21.5%), compartmentalization (27.5%) and nesting (NODF: 27.8%). According to the above, and considering resilience models, these structural attributes could be drastically altered if the local properties of some trophogroups (i.e., *DC*, *BC*, and *SC*) are directly affected (i.e., attack; Figs. 23–25).

On the contrary, the marine food web of the Malpelo FFS showed a high percentage (>50%) of tolerance to the effects of "failures or errors" in the web nodes in terms of *DC* (Fig. 23), *BC* (Fig. 24), and *SC* (Fig. 25). Despite this, direct removal or "attack" and "failures or errors" on those nodes of high centrality (i.e., *DC*, *BC*, and *SC*) generate loss of network connectivity in a cascading fashion, acting more rapidly in the case of direct attacks (Figs. 23–25). This same pattern was observed for the centralization and clustering attributes in the *DC* (Fig. 23) and *BC* (Fig. 24) scenarios.

Consequently, the removal of 21.7-56.6% of the nodes with higher *DC* and *BC* values could have different effects on some global attributes of the network (Fig. 6 and 7). Whereas, according to the *SC*, the affectation between 18.2-79.0% of trophogroups could alter the network structure in the face of some direct perturbation (Fig. 25).



Figure 22. Representation of the *minimun spanning tree* of the marine trophic web of the Malpelo Fauna and Flora Sanctuary, Colombia, indicating the shortest routes of energy glow from the "environment" to the groups making up the web.



Figure 23. Resilience of the marine food web of the Malpelo Fauna and Flora Sanctuary, Colombia, based on the an "attack" on the nodes according to their node degree (DC) values, to simulate the modification of some global attributes of the web. Attack: Red dots. Error: Green, yellow and blue dots.



**Figure 24.** Resilience of the marine food web of the Malpelo Fauna and Flora Sanctuary, Colombia, based on the an "attack" on the nodes according to their values of intermediation (*BC*), to simulate the modification of some global attributes of the web. **Attack:** Red dots. **Error:** Green, yellow and blue dots.



Figure 25. Resilience of the marine food web of the Malpelo Fauna and Flora Sanctuary, Colombia, based on an the "attack" on the nodes according to their subgraph centrality (*SC*) values, to simulate the modification of some global attributes of the web. Attack: Red dots. Error: Green, yellow and blue dots.

#### DISCUSSION

#### Identification of key trophogroups in the marine trophic web

The positional role (i.e., connectivity, proximity or intermediation) of a species within a food web is an attribute of great importance for understanding the functioning of ecosystems, since their dynamics can be governed by a relatively small number of highly significant components (Gómez et al., 2003).

The trophic dynamics of the marine ecosystem of the Malpelo FFS is governed by six trophogroups that reflected high centrality values (i.e., *DC*, *BC*, *EC*, and *SC*): zooplankton (ID: 7), tunas (Fam. Scombridae; ID: 34), carangids (Fam. Carangidae; ID: 25), crustaceans (ID: 8), phytoplankton (ID: 1), and detritus (ID: 3) (Table 14; Fig. 21). This indicates the relative significance of these trophogroups as key elements in maintaining the cohesion and connectedness (Palacio-Arce, 2014) of the marine food web of the Malpelo FFS, conferring stability and robustness (Albert et al., 2000).

The most relevant aspect of the role of these key trophogroups in the marine food web of the Malpelo FFS may lie mainly in their extensive participation in short chains (MST), which allows energy flow to be more efficient along the web (Fig. 22, red nodes).

This may be associated with several distinct factors: first, the variety of feeding strategies by key trophogroups, such as Fam. Scombridae and Fam. Carangidae (Froese and Pauly, 2022), which gives them a high capacity to interact with primary producers (e.g., phytoplankton) and primary consumers (e.g., zooplankton), the main genedarians and species responsible for making energy available, respectively.

For example, the carangids (i.e., *Caranx caninus*, *C. lugubris*, *Decapterus macarellus*, *Seriola rivoliana*, *Trachinotus paitensis*, *T. stilbe*, and *Uraspis helvola*) and scombroids (i.e., *Acanthocybium solandrii*, *Thunnus albacares*, and *T. obesus*) that are most common in the Malpelo FFS manifest different feeding mechanisms, e.g., carnivores (consumption of fish, crustaceans, cephalopod mollusks), zooplanktivores, zoobenthivores and herbivores (Froese and Pauly, 2022).

This would signify a relatively major participation in trophic pathways due to the consumption of prey highly related to phytoplankton (Fig. 22, red nodes, IDs: 25 and 34). 2) The importance of Fam. Carangidae, Fam. Scombridae, crustaceans and zooplankton for trophogroups with high consumer roles (high *DC*<sub>in</sub> >0.45; Table 14) such as other large-sized carangids (e.g., *S. rivoliana*; [Froese and Pauly 2022]), *S. lewini* (Estupiñán-Montaño et al., 2009, 2017a), *C. falciformis* (Estupiñán-Montaño et al., 2017a), and sailfishes (Fam. Istiophoridae [Froese and Pauly, 2022]).

This confers them a high degree of intermediation (*BC* >0.50; Table 14) in the concentration and distribution of energy throughout the entire network. 3) The variety in size shown by carangids (Froese and Pauly, 2022) and tunas (Froese and Pauly, 2022) allows them to consume both small and medium-sized prey. This in turn makes them highly consumable by a number of predators during different life stages. For example, crustaceans, small coastal and oceanic fishes are important prey of juvenile *S. lewini* (Estupiñán-Montaño et al., 2009, 2021; Flores-Martínez et al., 2016) and *C. falciformis* (Cabrera-Chávez-Acosta et al., 2010; Flores-Martínez et al., 2016]). Whereas adult sharks prefer the consumption of larger prey, such as squid (*S. lewini* [Estupiñán-Montaño et al., 2009, 2021]) and tuna (*C. falciformis* [Estupiñán-Montaño et al., 2017b, Duffy et al., 2015). Another case of large consumers in the Malpelo FFS is *S. rivoliana*, which due to its large size and mesopredator status (up to 160 cm TL; trophic position: 4.4 [Froese and Pauly, 2022]), can consume small carangids (e.g., *Elagatis bipinnulata*), medium-sized serranids (e.g., *Mycteroperca olfax*) and perhaps some sea urchins (*personal observation* Daniel J. Villalobos-Ramírez and Jaiver Rojas-Cundumí). The detritus, phytoplankton, and zooplankton were found to be key elements in the formation of trophic sub-networks (high *SC*); consequently, these groups could be of great relevance to the interaction routes for the generation of different ecological processes (i.e., exploitative and apparent competition, see below) that model the trophic dynamics of the marine ecosystem of the Malpelo FFS. The importance of these groups in the formation of trophic sub-networks may be related to two reasons: First, phytoplankton could constitute the basal source that provides most support to the marine trophic chains of the Malpelo FFS (Fig. 22), since it is highly abundant in the area due to the high amount of nutrients present in the ecosystem as a consequence of different oceanographic characteristics including: 1) constant upwelling that brings nutrient-rich deep water upwelling throughout the year (Rodríguez-Rubio and Stuardo, 2002; Rodríguez-Rubio et al., 2003), 2) the confluence of various ocean currents (Kessler, 2006; Fig. 4C) and water masses (Fiedler and Talley, 2006), and 3) cyclonic and acyclonic circulations (Rodríguez-Rubio and Stuardo 2002; Rodríguez-Rubio et al., 2003).

This favors the creation of large phytoplankton patches due to the abundance of nutrients (Rodríguez-Rubio and Stuardo, 2002), giving rise to the high importance of zooplankton in the formation of subgrids (*SC*), since these microorganisms are mainly responsible for making the energy generated by phytoplankton available to the other consumers (important "bridges"; Fig. 19 and 22).

Second, detritus, being a component originating from dead organisms and from the microbial degradation of matter (Ramírez et al., 2010), constitutes an important alternative source of nutrients for detritivorous and decomposer organisms (Andramunio-Acero and Caraballo, 2021), which transform dead organic matter and convert it into reusable energy for other heterotrophic organisms (Vásquez, 1998).

#### Community sub-webs in the marine food web

The formation of trophic communities is a feature of networks, reflecting the evolution of complex ecosystems and conferring a certain degree of tolerance to the effects of disturbances (Verdu and Valiente-Banuet, 2008; Thébault and Fontaine, 2010; Stouffer and Bascompte, 2011). In this sense, the marine ecosystem of the Malpelo FFS could be considered a complex system, due to its degree of compartmentalization (*NODF*: 27.8% [Almeida-Neto et al., 2008]) and the

presence of four trophic communities (Fig. 20A and B), each of which shows different degrees of nesting among their components (*NODF*: 20–50%).

The formation of the trophic communities of the Malpelo FFS marine ecosystem and their different degrees of nesting can be related to four factors. First, the body size of the species (Cohen et al., 2003; Stouffer et al., 2005), which allows them to interact with prey of sizes appropriate to their size and the dimensions of their body structures (i.e., mouth width). A clear example of this is the organisms that make up community 1. These are small species that feed on other small species such as the monkfish *Lophiodes spilurus* (Fam. Lophiidae; up to 30 cm maximum TL; ID: 94), the dwarf pipefish *Doryrhamphus excisus* (Fam. Syngnathidae; up to 7 cm TL; ID: 39) and the cardinal fish *Apogon stradorsalis* (Fam. Apogonidae; 8.9 cm TL; ID: 11) (Froese and Pauly, 2022) (Froese and Pauly, 2022).

Unlike community 3, it is composed of medium and large organisms, i.e., mesopredators (carangids, tunas, etc.) and top predators (sharks). This gives the organisms of each community the ability to interact with components within and between communities, consuming prey of different sizes at different life stages. Whereas community 2 is composed of organisms which interact with basal organisms (e.g., filter feeders, detritivores, planktivores), and community 4 is composed of small (e.g., crabs, bivalves, stomatopods, etc.), medium (e.g., Serranidae [*Mycteroperca olfax*: up to 120 cm TL; Froese and Pauly 2022]) and large species (e.g., *C. galapagensis* [370 cm TL; Froese and Pauly, 2022], *Triaenodon obesus* [213 cm TL; Froese and Pauly, 2022]), which mostly interact with microorganisms and species of low trophic levels, suggesting that this group of organisms may be an important component in connecting the other communities with that community highly related to basal sources.

Second, the preferences and feeding behavior (Allesina and Pascual, 2009; Guimará et al., 2010) of the components of each community, as the sub-networks are formed by generalist and specialist organisms (Almeida-Neto et al., 2008), which is closely linked to the first factor (body size). In this sense, communities 1 and 2 are mostly composed of planktivorous organisms (e.g., Centriscidae [Froese and Pauly, 2022], corals [Goreau et al., 1971]), herbivores (e.g., gastropods [Kohn, 1983], Pomacentridae [Froese and Pauly, 2022]), and filter feeders (e.g., Sponges [Gili et al., 1984; Leys and Hill, 2012], Ostraciidae [Galtsoff, 1964; Morton, 1983]).

Communities 3 and 4 are composed of specialist organisms (hammerhead sharks [Estupiñán-Montaño et al., 2009], silky sharks [Estupiñán-Montaño et al., 2017a,b], Fam. Istiophoridae [LoorAndrade et al., 2017], Xiphiidae [Loor-Andrade et al., 2017]), opportunists (e.g., whale shark [Colman, 1997], whitetip sharks *Triaenodon obesus* [Randall, 1977], Fam. Merluccidae [Orrego and Mendo, 2012; Varela and Pincay-Espinoza, 2019]), and some omnivores (e.g., Lutjanidae [Froese and Pauly, 2022], chernas [Froese and Pauly, 2022]) that occupy high and intermediate trophic positions (TP: 3.0–5.5), meaning that they can interact with prey from different trophic levels.

The third factor, the habitat size and niches (Guimerá et al., 2010) which limit their distribution and the habitat boundaries (Allesina and Pascual, 2009) within which they interact. This is supported by the isolation of Malpelo Island (~390 km distance from the coast), the great depths (>4000 m) that separate it from the mainland (Fig. 4B), its type of formation (volcanic origin [von Prahl, 1999]) and its high degree of endemism (Plan de Manejo, 2015), all of which indicates that Malpelo Island has never been connected to other islands or continents. This has created a barrier for both terrestrial and marine organisms to colonize this ecosystem (Graham, 1975). This would explain how the characteristics of the habitat and its relationships with the surrounding environment favor the formation of trophic communities in the Malpelo FFS.

Finally, the length of the trophic chain as an influential factor in the formation of trophic communities (Mantel et al., 2004; Newth, 2005) as a consequence of the close relationships between the components of the network and the high productivity of the system, resulting in a network containing seven steps (diameter). The marine ecosystem of the Malpelo FFS has a long trophic chain made up of seven trophic levels: 1) primary producers and basal sources (trophic level [TL-I]: algaes, detritus, and phytoplankton), 2) herbivores (NT-II), 3) omnivores I (NT-III), 4) omnivores II (NT-IV), 5) carnivores (NT-V), 6) top predators (NT-VI), and 7) scavengers and decomposers (see Chapter 3. 1). Thus, the above results reveal the presence of two sets of species: a) species with many interactions and b) species with fewer interactions, but with a high relationship with those highly connected species (Palacio-Arce, 2014). Therefore, the formation of different trophic communities in the marine ecosystem of the Malpelo FFS demonstrates the complexity of the ecosystem as an important mechanism that facilitates stability against the effects of some type of disturbance.

#### Motifs

The inter-specific interactions present in the marine food web of the Malpelo FFS are mostly governed by processes of exploitative competition (44.1% [two predators consume one prey]) and to a lesser extent by apparent competition (28.7% [one predator consumes two prey]), tri-trophic chain (16.8% [hierarchical interaction from the base to higher consumers]) and omnivory (7.7% [one consumer feeds on different trophic levels]). These results suggest that the ecological processes that may have the greatest influence on the regulation of the dynamics of the marine ecosystem of the Malpelo FFS are exploitative and apparent competition.

The high frequency of exploitative competition could suggest a significant indirect interaction between consumers of different trophic levels. For example, at lower trophic levels, organisms of the families Clupeidae and Exocoetidae, being important consumers of zooplankton, show some degree of competition for the consumption of this food resource. At intermediate trophic levels, we could refer to the competition existing between the families Carangidae and Scombridae (mesopredators), which consume similar fish species (e.g., other carangids, etc.), squids, plankton, etc. [Froese and Pauly, 2022]), as well as the families Lutjanidae and Serranidae, which reflect high isotopic overlap values in the surroundings of the Malpelo FFS. This may also be related to the high consumption of small fish (Estupiñán-Montaño et al., in review).

At higher trophic levels (top predators), a clear example is silky sharks and hammerhead sharks who share prey such as similar types of cephalopods (e.g., Ommastrephidae, Anchistrocheuridae), but in different degrees of importance (Estupiñán-Montaño et al., 2009, 2017a, b). This type of interaction can be seen between and within each trophic level, which generates significant interactions of exploitative competition (6441 interactions) throughout the network (Table 16), suggesting that this type of process generates substantial indirect competition for the food resource when it is limited in the Malpelo FFS. As a consequence of this, one of the prey species could be benefited if the other species is adversely affected by predation processes.

Considering the above, it is possible that the relative frequency of ecological processes (i.e., exploitative competition, apparent competition and omnivory) in the marine ecosystem of the Malpelo FFS obtained in this study is obscured somewhat due to the level of taxonomic resolution used in this study (Mantel et al., 2004). This could be explained by the high availability of resources in the Malpelo FFS (Plan de Manejo, 2015) as a result of its high primary productivity (Rodríguez-Rubio and Stuardo, 2002; Rodríguez-Rubio et al., 2003). If this is true, the frequency

with which a species is consumed by two predators (exploitative competition), or two prey species are consumed by the same predator (apparent competition), could be over or underestimated.

Therefore, by having a higher taxonomic resolution in the modeling of the Malpelo FFS network, the processes of competitive exclusion and resource partitioning could be better evidenced and modeled. An example that could support this hypothesis is the case of two sympatric shark species, hammerhead sharks (*S. lewini*) and silky sharks (*C. falciformis*). Both species consume the same type of squid (i.e., *Dosidicus gigas*); however, *D. gigas* represents about 20% (%IRI) of the food consumed by *S. lewini* (Estupiñán-Montaño et al., 2009), while for *C. falciformis* this prey only represents 0.2% (Estupiñán-Montaño et al., 2017b), which is a reflection of the low trophic interaction (competitive exclusion) between them (Estupiñán-Montaño et al., 2017a), indicating that the exploitative competition processes between them could be of low frequency and intensity.

This would suggest that other consumer species may also have different degrees of competition and/or resource sharing, which can be deciphered from: i) specific trophic studies and ii) greater taxonomic resolution when modeling the marine food web of the Malpelo FFS.

This highlights the need to carry out more studies to generate more detailed information on the trophic aspects of different groups (species level) of the marine ecosystem of the Malpelo FFS, in order to improve the modeling and understanding of the food web and its ecological dynamics. Despite the low taxonomic resolution used in this study and the scarcity of trophic information of the species present in the marine ecosystem of the Malpelo FFS, the results of this work constitute an important starting point for the reduction of information gaps and the improvement of the understanding of the dynamics of this ecosystem. Therefore, these results should be interpreted with caution, taking into account the limitations underlying this research.

#### Topological properties and resilience of the food web

The marine ecosystem of the Malpelo FFS is isolated from other ecosystems (i.e., the mainland and other oceanic and coastal islands), which means that its ecological dynamics are influenced by different oceanographic conditions, such as convergence of water masses (Whittaker and Fernández-Palacios, 2007), constant upwelling (Rodríguez-Rubio and Stuardo, 2002; Rodríguez-Rubio et al., 2003) and external forcings (Gómez-Navarro et al., 2012; García-Velero et al., 2012), to which is added its interaction with the terrestrial environment (see Chapter 3.1) and its geoform

(seamount; [Plan de Manejo, 2015]). These factors generate favorable conditions for the aggregation of a high variety of species, resulting in the highly diverse marine ecosystem of the Malpelo FFS with the presence of numerous endemic species (Plan de Manejo, 2015).

This in turn leads to the trophic dynamics of the marine ecosystem of the Malpelo FFS being dominated by a few key trophogroups (i.e., zooplankton, Fam. Scombridae and Fam. Carangidae, crustaceans, phytoplankton and detritus). This would indicate that these marine components play important roles in maintaining cohesion, connectivity (Palacio-Arce, 2014), stability and robustness of the food web against the effects of any disturbance (Albert et al., 2000).

Based on the above, if one or several of the key trophogroups were to present an anomaly in their functions, either due to a direct disturbance ("attack") or due to a "failure or error", the global properties of the marine food web of the Malpelo FFS could show changes in their structural attributes, such as: the central energy pathways, global  $CC_{global}$  and connectivity (Fig. 23–25), as well as in the efficiency of energy flow (MST), which in turn could lead to three major consequences: 1) changes in the patterns of sub-network formation (trophic communities), so that the speed of propagation of the direct and indirect effects of disturbances would be modified (Albert et al., 2000), 2) modification in the patterns of web functioning (i.e., *motifs*), and 3) an increase in the number of short or long trophic chains (MST), thus modifying the efficiency of the network in terms of the transfer of energy from the base to the other levels of the network (Fig. 22).

For example, if three key trophogroups with higher  $DC_{ALL}$  and BC values were directly disturbed, the APL would tend to increase (Fig. 23). This means that an increase in the shortest average distance between the farthest nodes would occur, which would in turn have different implications on the speed of energy propagation along the web. Thus, if there is a direct attack on key trophogroups such as the Carangidae and Scombridae families and crustaceans, the APL could increase considering the *DC* and *BC* scenarios (Figs. 23–25). This, in turn, would have different implications on the efficiency of energy flow (Fig. 22).

If this is correct, the speed and routes of energy transfer along the network could be altered, due to the disruption of trophic pathways as a consequence of the elimination of highly connected (DC) and high pathway participation (BC) nodes reflecting: 1) a reduction in the number of short chains (no disturbance = 24 chains, Fig. 5; no Fam. Carangidae = 21 chains; no Fam. Scombridae = 23; without crustaceans = 22 chains), and thus an increase in long chains, so that energy transfer

efficiency would be reduced, 2) modifications in trophic community formation patterns (unaltered network = 4 trophic communities; without Fam. Carangidae = 6; without Fam. Scombridae = 9 and without crustaceans = 6), 3) alterations in the recurrence of network functioning patterns (i.e., motifsglobal), with reductions of 10.3%, 11.4%, and 9.4% of *motifs* without the presence of Fam. Carangidae, Fam. Scombridae and crustaceans respectively, and 4) cascading effects on patterns of connectivity, clustering (nodes: 1–3; Figs. 23 and 24) and energy centralization (nodes: 1–3; Fig. 23).

This could have important implications on the speed of propagation of the effects of disturbances throughout the network (Albert et al., 2000), and this would generate drastic impacts on some species, modifying their ecological attributes within the marine web of the Malpelo FFS.

In another scenario, the *SC* showed different key trophogroups to those suggested by *DC* and *BC* (Table 14), where detritus, phytoplankton, and zooplankton are the trophogroups that participate most actively in the formation of subgroups (high *SC*), suggesting similar effects with respect to high *DC* and *BC* species (see above), affecting APL, energy centralization, connectivity and global  $CC_{global}$  (Fig. 25). These results suggest the presence of high-intensity "bottom-up" control processes, which have been identified for small islands (Wang et al., 2020) and could apply to the Malpelo FFS (120 ha [Graham 1975]). This type of control is mainly generated by phytoplankton (Li et al., 2020) and its interaction with zooplankton, which connect primary producers with other consumers in the food chain (Smith et al., 2006; Li et al., 2020). For this reason this interaction constitutes a link of vital importance in the marine food web (Li et al., 2020), such that a perturbation or modification in the ecological attributes (e.g., abundance, biomass) of phytoplankton can be reflected in zooplankton and thus be transferred to the other trophic levels in an inverted cascade effect.

The presence of "bottom-up" control in the Malpelo FFS may be the consequence of its high primary productivity, which promotes the increase of secondary productivity in this ecosystem (CCO and DIMAR 2019). This is influenced by oceanographic conditions that are the main modulators of the dynamics of the pelagic environment of the Malpelo FFS, due to its close relationship with the intertropical convergence zone (Devis-Morales et al., 2008; Amador et al., 2006; Villegas et al., 2016) which determines the system of currents converging at this location (Rodríguez-Rubio et al., 2002, 2003) and which also, through upwelling processes, promotes the

transport of subsurface water of lower temperature and oxygen concentration, as well as having higher salinity and higher nutrient concentration (Devis-Motarles et al., 2008).

Therefore, "bottom-up" effects on the marine ecosystem of the Malpelo FFS could be modified if: 1) changes in phytoplankton biomass, density and species composition are affected by seasonal changes (Von Ruckert and Giani, 2008, Li et al., 2020) and physical or chemical factors (e.g., nutrient concentration; Carpenter et al., 1985; Doi et al., 2013). For example, Li et al. (2020) found in their study area in Lake Nansi (Northern China) that bottom-up effects were stronger during the wet season. This could also be reflected in the Malpelo FFS, since during the wet season the marine ecosystem of the Malpelo FFS receives large inputs of water with high concentrations of seabird guano from the terrestrial ecosystem. This guano deposited in the sea increases the concentrations of natural nitrogen and phosphorus in the pelagic zone, affecting the biomass, density and species composition of phytoplankton during this time of the year, influencing the community structure of this ecosystem.

Additionally, "bottom-up" and "top-down" processes function in a balanced manner (Smith and Lancelot 2004) to regulate ecosystem structure and functions (Wang et al., 2020) of the Malpelo FFS. Thus, an alteration in phytoplankton could modify the equilibrium relationship between "bottom-up" and "top-down" processes, which could generate modifications in the ecological dynamics of the Malpelo FFS; however, more studies are needed to validate this hypothesis and evaluate how these changes affect the structure and marine trophic dynamics of the Malpelo FFS due to different variables (e.g., seasonal changes).

#### CONCLUSIONS

The trophic dynamics of the marine ecosystem of the Malpelo FFS are centralized (i.e.,  $DC_{ALL}$ , BC, EC, and SC) within six key trophogroups (i.e., phytoplankton, zooplankton, Fam. Scombridae and Carangidae, crustaceans, and detritus), which maintain the cohesion and connectivity of the marine food web of the Malpelo FFS and allow the ecosystem to remain stable and robust. This is achieved by the high participation of all of these components in the short trophic chains (*minimum spanning tree*) of the Malpelo FFS, helping to maintain the highest efficiency of energy flow throughout the network, taking advantage of the high primary productivity generated in the Malpelo FFS in a more efficient way.

This is closely related to the variety of feeding strategies of the different key trophogroups, which allows them to make use of different resources, from basal trophic sources (primary producers) to prey of similar trophic levels (intra-trophic interaction). This is demonstrated by the role of the Fam. Carangidae and Scombridae as important consumers of the network (recipients and distributors of energy; high  $DC_{in}$  and BC) and of detritus, phytoplankton, and zooplankton as important prey ( $DC_{out}$ ) and trophic group formers (high SC). This confers to these key trophogroups an important role in the processes of generation and provision of energy to the different trophic levels of the network, which means that these key trophogroups have a high relevance in the formation of the ecological processes that mainly modulate the marine food web of the Malpelo FFS (i.e., exploitative and apparent competition).

In addition, phytoplankton, being a highly abundant basal source in the Malpelo FFS, is the primary trophic base supporting the marine trophic chains of the ecosystem, due to its high availability as a result of the different oceanographic characteristics that are generated around the Malpelo FFS (e.g., upwelling, convergence of water masses, etc.), which makes this ecosystem a highly productive environment throughout the year.

The marine ecosystem of the Malpelo FFS presents a compartmentalized (four trophic communities) and fragmented (nesting: 27.8%) marine food web, which indicates the degree of complexity of the ecosystem and its capacity to withstand the effects of disturbances. The key characteristics of the network structuring are mainly related to the body size of the species (Cohen et al., 2003; Stouffer et al., 2005), the preferences and feeding behavior of the trophogroups (subnetworks formed by generalist and specialist organisms [Allesina and Pascual, 2009; Guimará et al., 2010]), the habitat (distribution and boundaries [Allesina and Pascual, 2009]) and limited range of niches in which all trophogroups interact (Guimerá et al., 2010), and finally, the size of the trophic chain (Mantel et al., 2004; Newth, 2005) with a length of seven trophic levels: 1) primary producers and basal sources (TL-I: algae, detritus, and phytoplankton), 2) herbivores (TL-II), 3) omnivores I (TL-III), 4) omnivores II (TL-IV), 5) carnivores (TL-V), 6) top predators (TL-VI), and 7) scavengers and decomposers (see Chapter 3.1).

All this as a consequence of the close relationships between network components and the high productivity of the ecosystem. Nonetheless, the complexity of the ecosystem and its ability to withstand the effects of disturbances may be affected if key trophogroups suffer direct disturbance, leading to modification of the structural attributes of the web (e.g., compartmentalization, nesting,

APL, energy centralization, and clustering), generating different consequences on the efficiency of energy transfer (MST), as well as on the degree of interspecies interaction.

The inter-specific interactions present in the marine food web of the Malpelo FFS are mostly governed by two processes of competition (exploitative and apparent). This indicates that these ecological processes have the greatest influence in regulating the dynamics of the marine ecosystem of the Malpelo FFS. However, their intensity and frequency may be affected by the level of taxonomic resolution used in this study (Mantel et al., 2004). Therefore, further research is needed to model the food web with a higher degree of taxonomic resolution, allowing greater clarity with respect to the nature and relative frequency of these processes in the marine network of the Malpelo FFS.

This study is the first work that models the marine food web of the Malpelo FFS, generating the first arguments for understanding the ecological dynamics that keep this ecosystem stable and robust. It also shows the complexity of the marine ecosystem, and, shows how fragile it can be if any of its key components are affected. In this way, the results presented here serve as a baseline for the identification of species/groups of great importance and the identification of processes that regulate the maintenance of the ecological dynamics of the marine ecosystem of the Malpelo FFS. The results of this study have great implications for the development of management and conservation strategies for the MPA, as it allows improving the focus and efficiency of conservation and ecosystem management efforts (Capocefalo et al., 2018) and suggests that these efforts should be developed from a global approach, considering the ecosystem as a whole (ecosystem approach) rather than focusing only on the conservation of particular species (i.e., flagship species).

## Chapter 4: THE TROPHIC INTERRELATIONSHIP BETWEEN THE ECOSYSTEM OF THE MALPELO FAUNA AND FLORA SANCTUARY, COLOMBIA

# 4.1. Trophic connectivity between the terrestrial and marine ecosystems of Malpelo Island, Colombia

#### **INTRODUCTION**

Communities categorized as discrete can be open and connected in innumerable ways due to external factors (Holt, 1993; Menge, 1995; Schindler et al., 1996; Polis et al., 1996; Rooney et al., 2003) that allow the basic components of food webs (i.e., nutrients, detritus, and organisms) to cross the spatial limits of ecosystems (Polis et al., 1997a). This is related to the shape (complexity; Kent and Wong, 1982) and size of the ecosystem (Post et al., 2000; Vander Zanden and Vadeboncoeur, 2002; McCann et al., 2005; Dolson et al., 2009). The strength of the interactions of mobile generalist predators can be limited (McCann et al., 2005) by the degree of accessibility to different ecosystems (Dolson et al., 2009). These spatial processes impact the trophic structure and dynamics of ecosystems. This is evidenced by allochthonous inputs from different sources (e.g., transport of detritus and nutrients by mobile consumers) that can in turn influence energy, carbon, and nutrient reservoirs (i.e., nitrogen and phosphorus) (Polis and Hurd, 1996a; Polis et al., 1997b; Schindler and Scheuerell, 2002).

A key element in the trophic dynamics of islands and coastal areas is subsidy from a donor habitat through marine allochthonous inputs (Polis and Hurd, 1996a; Polis et al., 1997b). Although islands can have low terrestrial primary productivity (Caut et al., 2012), they can support high abundance and biomass (i.e., secondary production) of consumers, such as spiders, scorpions, lizards, and rodents that are subsidized by marine contributions (Sánchez-Piñero and Polis, 2000; Moore et al., 2004). On islands, these allochthonous inputs are mainly incorporated from two sources: 1) seabird colonies and 2) marine detritus transported across beaches (Polis and Hurd 1995, 1996b). This also contributes to combating nutrient limitation (i.e., nitrogen and phosphorus) of primary producers.

Malpelo Island is a small oceanic island (1.2 km<sup>2</sup>; Graham, 1975) located in the Colombian Pacific. Its geographical isolation and position at the convergence of several marine currents (Fig. 4, see Chapter 1) mean that this island is an ideal place for the aggregation of species (endemic and migratory). This has led to this island becoming part of the largest marine protected area in the Colombian Pacific, the Malpelo Fauna and Flora Sanctuary (FFS) (Fig. 4, see Chapter 1) (Ministerio de Ambiente y Desarrollo Sostenible, 2017). It is a World Heritage Site (UNESCO)

and is included in other important lists for the conservation of species (Plan de Manejo, 2015). These characteristics and its importance for ecological communities mean that Malpelo FFS is an ideal site for the study of trophic interactions between ecosystems and of the input of marine nutrients to the terrestrial environment, due to its low terrestrial primary productivity. Considering its topography, complicated access, and the presence of the largest nesting colony of the Nazca booby *Sula granti* (>80,000 individuals; López-Victoria and Rozo 2007; García 2013), terrestrial ecosystem structure and trophic dynamics could be directly and/or indirectly affected by the input of marine nutrients in the form of *S. granti* guano, chicks, food remains, and carcasses, denoting high connectivity between ecosystems (Wolda, 1975; von Prahl, 1990; López-Victoria et al., 2009).

Variations in the donor-controlled habitat (i.e., marine ecosystem; Polis et al., 1997a) could cause modifications in the community ecology of Malpelo FFS (Wolda, 1975), with drastic consequences on species composition and trophic dynamics at landscape scales (Polis and Hurd, 1995; Nakano et al., 1999). Changes in the feeding habits of *S. granti* could result in changes to the role this seabird plays in the trophic connectivity between the two ecosystems (Wolda, 1975; von Prahl, 1990; López-Victoria et al., 2009). Several studies have indicated that of the total energy contributed by *S. granti*, 99% corresponds to guano, 0.64% to eggs and chicks, and 0.06% to carcasses (López-Victoria et al., 2009). These are important dietary components of the dotted galliwasp *Diploglossus millepunctatus*, the Malpelo anole *Anolis agassizi*, the terrestrial crab *Johngarthia malpilensis*, and other invertebrates (López-Victoria, 2006, López-Victoria et al., 2011).

Trophic studies based on the observation of terrestrial macro-species from Malpelo FFS (e.g., *A. agassizi, D. millepunctatus, J. malpilensis, Phyllodactylus transversalis* [López-Victoria, 2006; López-Victoria and Werding, 2008; López-Victoria et al., 2011; López-Victoria et al., 2013]), including trophic relationships with *S. granti*, have shown the importance of this avian species in the trophic dynamics (López-Victoria et al., 2009) and stability of the terrestrial ecosystem, due to energy input from the sea (Wolda, 1975; von Parhl, 1990; López-Victoria et al., 2009). However, previous trophic studies based on direct observations, as well as stomach contents analysis carried out on several terrestrial species of Malpelo FFS, should be complemented with other methods to strengthen the hypothesis raised by other studies (i.e., Wolda, 1975; von Parhl, 1990; López-Victoria et al., 2009). Stable isotope analysis (SIA) is a complementary approach that

counters some of the limitations of previous studies, e.g., use of stomach contents analysis and direct observations, which only provide a temporal snapshot of food ingested. SIA allows the identification of sources of carbon and nitrogen that constitute food assimilated over the short- and long-term (e.g., Kim et al., 2012; Estupiñán-Montaño et al., 2019). The isotopic signal depends on the trophic level and origin of the diet, as well as on ingestion rates, accumulation, turnover rates of assimilated tissue, and growth, among other factors (Fry and Arnorld, 1982; Tieszen et al., 1983).

Three main objectives were addressed in this study to determine the degree of coupling between the terrestrial and marine environments of Malpelo Island: 1) the assessment of  $\delta^{13}$ C and  $\delta^{15}$ N values of the biological components of the terrestrial and marine environments of Malpelo FFS; 2) the identification of the primary sources that support the terrestrial food web; and 3) the evaluation of the trophic connectivity between the two ecosystems in Malpelo FFS. The general aim was to provide new evidence that *S. granti* is the main mediator in the transfer of matter and energy between the two ecosystems and generate new ideas to clarify some hypotheses, such as: i) the terrestrial food web has a low dependence on terrestrial C<sub>3</sub> plants due to their low abundance; therefore, terrestrial debris should provide the greatest contribution to the different components of the terrestrial food web; ii) the  $\delta^{13}$ C of terrestrial debris is similar and/or varies slightly in relation to  $\delta^{13}$ C of basal sources and consumers at low marine trophic levels, as well as *S. granti* eggs; iii) terrestrial C<sub>3</sub> plants should reflect high values of  $\delta^{15}$ N, since N is found in high concentrations in *S. granti* guano; and iv) terrestrial and marine ecosystems should evidence high isotopic overlap as a result of the high connectivity between them.

#### **MATERIALS AND METHODS**

#### Study area

Malpelo Island (Fig. 4A) is the summit of a submarine mountain range called the Malpelo Ridge, which extends in a NE-SW direction; it is approximately 241.4 km long by 80.5 km wide (Fig. 4B, red polygon). The island has a maximum height of 300 m above sea level and emerges from approximately 4,000 m depth (Fig. 4C) (more details in Chapter 1).

#### Sample collection

Samples of 16 terrestrial and 38 marine species/functional groups (Table 17) were collected in 2017–2021 in Malpelo FFS, Colombia (Fig. 4A, see Chapter I). All terrestrial samples were collected in October 2018. Samples of terrestrial vertebrates consisted in 1–2 cm of tissue collected from the posterior portion of the tail of *A. agassizi* and *D. millepunctatus*, and body feathers of *S. granti*. For invertebrates such as the land crab *J. malpilensis*, one of the hind limbs was collected, whereas invertebrates (i.e., millipedes, isopods, spiders, worms, crickets, and ants; Table 17) were collected whole.

Marine samples were obtained at different depths (between 10–30 m) by scuba diving at different sites around Malpelo Island. Muscle tissue of teleost fishes and rays was obtained with a harpoon and/or Hawaiian hook, and from fish that had been illegally caught and seized by the authorities. Scalloped hammerhead (*Sphyrna lewini*) and silky shark (*Carcharhinus falciformis*) muscle tissue was obtained from Estupiñán-Montaño et al. (2017).

Plankton samples were collected around Malpelo Island with a "bongo" type net of 68, 90, and 294  $\mu$ m mesh size; surface tows were conducted from the M/N Seawolf inflatable boats for 10 min at each sampling site around the island. Samples of the other marine species/groups (e.g., algae, crustaceans, gastropods, and oysters; Table 17) were collected by hand.

All collected samples (terrestrial and marine) were placed in pre-labeled zip-lock plastic bags, except for the plankton samples, which were stored in 250 ml plastic bottles. Samples were kept frozen on board the Pacific Diving Company's M/N Seawolf for subsequent transfer to the laboratory. Sampling procedures were endorsed by Parques Nacionales de Colombia, through Memorandum 20177730007973 of 30 May 2017, issued by the Planning and Management Group.

#### Sample preparation and analysis

Samples were washed with distilled water, freeze-dried in an oven at 60 °C for 24 h, and ground to a fine powder with an agate mortar. Approximately 0.23 to 0.97 mg of powder were obtained for each terrestrial sample and packed in  $3.2 \times 4$ -mm tin capsules.

The C:N ratio was estimated and compared to reference values; a C:N value  $\leq 3.5$  indicates no effect of lipid contents (Post et al., 2007), whereas values > 3.5 suggest high lipid content.  $\delta^{13}$ C values of terrestrial and marine samples (Table 17) with C:N values > 3.5 were mathematically normalized according to Kiljunen et al. (2006):

$$\delta^{13}C_{adjusted} = \delta^{13}C_{measured} + D \times \left(I + \frac{3.90}{1 + \frac{287}{L}}\right)$$

Where  $\delta^{13}C_{adjusted}$  is the  $\delta^{13}C$  after normalization and  $\delta^{13}C_{measured}$  is the  $\delta^{13}C$  obtained from the sample without lipid removal. D = 7.018, I = 0.048, and L is the proportional lipid content of the sample, estimated as  $L = -20.54 + (7.24 \times C:N)$  (Post et al., 2007).

Arthropods (i.e., ants, isopods, and millipedes; Table 17) were analyzed without extracting lipids because these organisms have an exoskeleton characterized by high chitin contents (e.g., Liu et al., 2019), which are reflected in high C:N values (>3.5). Therefore,  $\delta^{13}$ C values of arthropods with C:N values <7.0 were not normalized mathematically (Schimmelmann and DeNiro, 1986; Webb et al., 1997; Pringle and Fox-Dobbs, 2008). Otherwise,  $\delta^{13}$ C values were normalized according to Post et al. (2007).

S. granti feathers were cleaned of surface lipids and contaminants using a 2:1 chloroform:methanol solution, followed by two successive methanol rinses (Jaeger et al., 2009). The  $\delta^{13}$ C values of S. granti eggs were mathematically normalized because lipid extraction can alter  $\delta^{15}$ N by washing out nitrogenous compounds. In this case, the formula proposed by Elliot et al. (2014) was used:

$$\delta^{13}C_{\text{lipid-extracted}} = \delta^{13}C_{\text{non-extracted}} + 1.47 - 2.72 \times Log_{10} \text{ (C:N)}$$

Where  $\delta^{13}C_{\text{lipid-extracted}}$  is the  $\delta^{13}C$  after normalization and  $\delta^{13}C_{\text{non-extracted}}$  is the  $\delta^{13}C$  obtained from the sample without lipid removal.

Extraction of lipids and urea from elasmobranch muscle samples (i.e., sharks and rays; Table 1) was performed following the procedure described by Kim and Koch (2012). Stable isotope analyses were carried out in the Stable Isotope Laboratory of the Instituto Andaluz de Ciencias de la Tierra in Granada (CSIC-UGR), Spain (more details in Chapter I).

#### Relative contribution of potential basal sources

The relative contribution of potential terrestrial basal sources to the diet of terrestrial consumer groups was estimated with the package *simmr* (version 0.3) in R (R Core Team, 2018). This model

uses a Bayesian isotopic framework based on  $\delta^{13}$ C and  $\delta^{15}$ N values to estimate the proportional contribution of potential prey (in this case, basal sources) to a consumer's diet (Parnell et al., 2013), including variability in model inputs such as trophic discrimination factor (TDF) values of consumers.

The following four steps were implemented: 1) we selected two potential basal sources: terrestrial C<sub>3</sub> plants and terrestrial detritus (sources that consist of decomposing organic matter [DOM] and seabird feces [López-Victoria et al., 2009]); 2) all terrestrial organisms were considered potential prey, due to feeding preferences (López-Victoria, 2006; López-Victoria and Werding, 2008; López-Victoria et al., 2011), and also consumers (i.e., mixing), except C<sub>3</sub> plants and detritus (basal sources); 3) due to the lack of specific TDFs for each terrestrial organism, we used the estimated mean TDF for terrestrial ecosystems ( $\Delta^{13}C = 0.5 \pm 0.19\%$  SD and  $\Delta^{15}N =$ 2.3±0.24‰ SD [McCutchan et al., 2003]), to minimize sources of uncertainty (i.e., environmental and physiological factors, trophic position, metabolic rates, growth rates [Phillips et al., 2014]), to which mixing models are highly sensitive (Bond and Diamnon, 2011, Phillips et al., 2014); and 4) the mixing model was adjusted to verify that the TDFs, potential prey, and consumers were consistent with the assumptions of the model (Smith et al., 2013). The mixing model adjustment was run with 10<sup>3</sup> iterations with a 95% probability for the mixing polygon (Smith et al., 2013). The model was considered adequate if isotopic values were within 1% of the mixing model polygons (Reum et al., 2020). Finally, if the model was correctly adjusted, we ran the mixing model with the isotopic values of terrestrial Malpelo FFS consumer groups (Table 17). The mixing model was run with 10<sup>6</sup> iterations, 10<sup>4</sup> burn-in period, 100 thinning period, and 4 Markov Chain Monte Carlo (MCMC).

The basal  $\delta^{13}C_{detritus}$  of the terrestrial ecosystem was compared to the  $\delta^{13}C$  values of the following five marine groups: macroalgae, marine crabs, zooplankton, *S. granti* eggs, and flying fish. The values of the marine groups were corrected with the mean TDF for marine environments  $(\Delta^{13}C = 0.4 \pm 0.17\% \text{ SD} [\text{McCutchan et al., 2003}])$  and compared statistically with a non-parametric paired test (Wilcoxon rank sum test).

#### <sup>15</sup>N-enrichment

<sup>15</sup>N enrichment of terrestrial components was estimated using  $\delta^{15}$ N values of detritus, eggs, and feathers of *Sula granti* as reference, as this species provides marine nutrients to the terrestrial ecosystem (García and López-Victoria, 2007; López-Victoria et al., 2009). Relative <sup>15</sup>N enrichment was calculated using the algorithm proposed by Estrada et al. (2006):

Enrichment in 
$$Y = \left(\frac{\delta^z Y_x - \delta^z Y_{S. granti \text{ eggs and/or feathers}}}{\delta^z Y_{S. granti \text{ eggs and/or feathers}}}\right)$$

where: *Y* is the element of interest ( $^{15}$ N), *z* is the atomic mass of the element, and *x* are the terrestrial components (i.e., plants, *A. agassizi*, *J. malpilensis*, *D. millepunctatus*, ants, millipedes, and Isopoda; Table 17) relative to the reference component (i.e., *Sula granti* eggs and feathers).

#### Niche amplitude and isotopic overlap

To quantify the isotopic niche and isotopic overlap between ecosystems (terrestrial [with and without  $C_3$  plants] vs. marine), we used the Stable Isotope Bayesian Ellipses (SIBER [Jackson et al., 2011]) method available in the R package (R Development Core Team, 2008). This analysis estimates ellipses that represent the "core isotopic niche" (Standard Ellipse Corrected Area, SEA<sub>C</sub>) using a Bayesian approach and calculating covariance matrices that define the shapes and areas of the ellipses (Jackson et al., 2011).

The ellipses were corrected using a posteriori randomly replicated sequences (SEA<sub>C</sub> = standard ellipse area correction [Jackson et al., 2011]) and they represent the isotopic niche width of consumers. In addition, this method allows the estimation of the isotopic niche overlap of the consumer (in this study, isospace), based on the overlap between ellipses (Newsome, 2007).

SIBER results were supported by the nicheROVER package in R (Lysy et al., 2014), which uses a probabilistic method to calculate niche regions and pairwise niche overlap using multidimensional niche indicator data. The niche regions are defined as the joint probability density function of the multidimensional niche indicators at a user-defined probability *alpha* (95%), while the package provides directional estimations of niche overlap (x vs y and y vs x), according to the species-specific distributions in the multivariate niche space (Lysy et al., 2014).

#### RESULTS

A total of 403 samples were collected in Malpelo FFS, 26.6% of which (n = 107) corresponded to the terrestrial ecosystem and 73.4% (n = 296) to the marine ecosystem (Table 17).

#### Carbon and nitrogen stable isotopes

 $\delta^{13}$ C values of the terrestrial ecosystem ranged from -30.3% to -15.0% and  $\delta^{15}$ N ranged from 3.7% to 21.3% (Table 17). Terrestrial C<sub>3</sub> plants (mosses) had the lowest average  $\delta^{13}$ C value (-30.3%) and the dotted galliwasp *Diploglossus millepunctatus* had the highest average value (-15.0%), with a total range in  $\delta^{13}$ C values of 15.3% (Fig. 26). The lowest  $\delta^{15}$ N value corresponded to the terrestrial C<sub>3</sub> plants (3.7%), whereas the highest value was obtained for arthropods from the family Araneae (21.3%), with a  $\delta^{15}$ N range of 17.6% (Fig. 26).

The carbon isotopic space of the marine ecosystem ranged from -23.2% to -10.0% for  $\delta^{13}$ C and from 4.5% to 16.9% for  $\delta^{15}$ N (Table 1). In this ecosystem, corals Dendrophylliidae had the lowest average  $\delta^{13}$ C value (-22.5%) and the scalloped hammerhead shark *Sphyrna lewini* (Sphyrnidae; -14.8%) had the highest average value, with a range of 7.7% (Fig. 27). Brown algae (Dictyotaceae) showed the lowest  $\delta^{15}$ N values (4.5%), while the scalloped hammerhead shark had the most positive value (16.4%), with a range of 11.8% (Fig. 27).



**Figure 26.** Terrestrial isospace of the Malpelo Fauna and Flora Sanctuary, Colombia, represented by the  $\delta^{13}$ C and  $\delta^{15}$ N values (mean ± standard deviation) of sixteen components of its trophic web. **Note:** the numbers correspond to each species identified.



Figure 27. Marine isospace of the Malpelo Fauna and Flora Sanctuary, Colombia, represented in average values ( $\pm$  standard deviation) of  $\delta^{13}$ C and  $\delta^{15}$ N of 39 different consumer groups (species/families/orders) of the marine trophic web.

Cada	Taxa		sotopic v	C:N		δ <sup>13</sup> C (% <i>o</i> )				δ <sup>15</sup> N (%0)				
Code	Scientific name	Common name	_ n	Mean ± SD	Min	Max	Mean	SD	Min	Max	Mean	SD		
				Torrostrial F	cosystem									
1	Anolis agassizi	Lizards	8	35 + 0.10	_18 2	-16.0	-16.8	0.67	13.5	15 5	144	0.58		
2	Araneae	Spiders	8	$5.3 \pm 0.10$ $5.3 \pm 1.31$	_21.0	_17.5	-19.6	1.27	16.8	28.4	20.6	49		
2	Diploglossus millenunctatus	Dotted galliwasp	9	$3.5 \pm 0.64$	_18.3	_15.0	_15.8	1.03	13.6	15.3	14.5	0.48		
1		Detritus	5	$5.0 \pm 0.04$	20.1	-13.0	18.0	0.66	9.5	13.0	10.8	13		
т 5	- Creullidae	Crickets	5	$3.4 \pm 0.22$	-20.1	-10.4	10.3	0.00	9.5	13.0	11.0	0.02		
5	Orymdae	Guere	1	$4.5 \pm 0.42$	-20.0	-10.4	-19.3	0.55	9.1	13.4	14.2	0.92		
0		Guano	1	1.2	-	-	-19.5	-	-	-	14.2	-		
/	Hymenoptera	$\mathbf{N}$	4	$4.5 \pm 0.36$	-20.3	-19.4	-20.0	0.39	11.0	14.5	13.5	1.53		
8	Sula granti <sup>8</sup>	Nazca booby (eggs)	8	$4.3 \pm 0.41$	-19.5	-18.2	-18.6	0.44	13.1	14.1	13.6	0.36		
9	Isopoda	Mealybugs	9	$7.0 \pm 0.29$	-17.5	-15.0	-16.4	0.82	15.1	17.9	16.8	1.04		
10	Johngarthia malpilensis	Terrestrial crabs	12	$3.2\pm0.07$	-17.1	-15.5	-16.5	0.45	14.9	17.0	15.8	0.6		
11	Diplopoda	Millepede	15	$6.3\pm 0.91$	-22.9	-18.6	-20.7	1.17	6.4	15.7	11.7	2.38		
12	Lumbriculidae	Worm	6	$4.8\pm 0.34$	-18.7	-17.0	-18.1	0.6	17.2	20.4	19.1	1.12		
13	Mycrocorifia	Rock jumpers	7	$4.1\pm0.30$	-21.4	-19.6	-20.5	0.6	10.3	19.4	14.1	3.41		
14	Odontomachus sp.	Ants	12	$4.1\pm0.41$	-18.4	-16.3	-17.2	0.82	15.5	19.2	16.7	1.02		
15	_	Mosses	8	$15.9\pm1.71$	-30.3	-28.7	-29.4	0.62	3.8	10.1	7.4	2.25		
16	Sula granti <sup>§</sup>	Nazca booby (feathers)	9	$3.3\pm0.05$	-16.7	-16.1	-16.3	0.2	13.5	15.2	14.3	0.49		
				Marine Eco	osystem									
1	_	Green algaes	6	14.6±2.71	-21.0	-17.1	-18.7	1.58	4.6	5.6	5.2	0.46		
2	Padina sp.	Brown algaes	4	13.3±1.42	-18.1	-16.0	-17.2	0.93	6.5	7.8	7.3	0.63		
3	Arcidae	_	1	3.6	_	_	-17.8	_	_	_	9.0	_		

### **Table 17.** Components of the terrestrial and marine ecosystems of the Malpelo Flora and Fauna Sanctuary represented by taxa with scientific and common names, number of samples (n), and average isotopic values $\pm$ standard error (SE) of $\delta^{13}$ C and $\delta^{15}$ N.

4	Balanidae <sup>‡</sup>	-	2	$4.2\pm0.35$	-18.1	-15.7	-16.9	1.70	9.5	10.0	9.8	0.35
5	Balistidae	Triggerfishes	3	$3.3\pm 0.00$	-18.1	-17.8	-18.0	0.19	12.1	13.1	12.7	0.53
6	_	Unidentified shrimp	8	$4.8\pm 0.45$	-18.7	-16.5	-17.3	0.69	8.2	11.6	9.3	1.03
7	Carangidae <sup>‡</sup>	Jacks	12	$3.4\pm 0.20$	-18.3	-17.6	-18.0	0.19	11.9	13.7	12.8	0.53
8	Carcharhinidae	Requiem sharks	12	$3.0\pm 0.07$	-16.7	-16.1	-16.3	0.21	14.8	15.9	15.3	0.33
9	Chaetodontidae <sup>‡</sup>	Butterflyfishes	2	$3.5\pm 0.21$	-17.5	-17.3	-17.4	0.12	12.7	14.3	13.5	1.12
10	_	Unidentified crustaceans	3	$6.2\pm1.55$	-19.0	-11.7	-16.4	4.03	7.1	9.1	8.0	1.03
11	Dendrophylliidae <sup>‡</sup>	Anthozoos	3	$3.6\pm0.70$	-23.0	-21.8	-22.5	0.60	4.7	6.6	5.5	0.96
12	Epialtidae <sup>‡</sup>	Crabs	1	7.5	_	_	-13.2	_	_	_	7.0	_
13	_	Sponges <sup>‡</sup>	5	$4.0\pm0.20$	-16.3	-14.9	-15.7	0.58	4.9	9.4	7.2	1.96
14	Exocoetidae <sup>‡</sup>	Flyingfishes	4	$3.6\pm0.10$	-17.8	-16.8	-17.5	0.47	9.6	10.9	10.3	0.55
15	_	Unidentified gastropds <sup>‡</sup>	5	$4.1\pm0.36$	-19.1	-15.4	-16.9	1.58	7.1	13.5	10.3	2.39
16	Gecarcinidae <sup>‡</sup>	Crabs	2	$6.2\pm 0.85$	-15.7	-14.9	-15.3	0.60	8.2	8.2	8.2	0.01
17	Grapsidae <sup>‡</sup>	Amphibian crabs	21	$5.3\pm1.58$	-19.6	-10.0	-14.6	3.45	6.9	16.9	10.5	3.48
18	Inachidae <sup>‡</sup>	Spider crabs	3	$6.2\pm0.55$	-15.0	-12.6	-14.2	1.41	8.7	9.4	8.9	0.41
19	Lophiidae <sup>‡</sup>	Rapes	5	$4.2\pm0.33$	-18.9	-18.1	-18.6	0.36	8.6	13.3	11.1	1.97
20	Lutjanidae <sup>‡</sup>	Snappers	36	$3.5\pm 0.28$	-19.3	-16.4	-17.4	0.62	9.8	15.0	13.7	1.10
21	_	Macroplankton <sup>‡</sup>	23	$6.6\pm1.28$	-23.2	-17.8	-21.4	1.02	4.6	10.8	7.2	1.53
22	Malacanthidae	Tilefishes	8	$3.3\pm 0.04$	-18.8	-18.0	-18.5	0.27	11.7	14.0	12.9	0.79
23	_	Microplankton <sup>‡</sup>	9	$7.9\pm 0.72$	-20.7	-15.5	-18.6	1.79	4.8	8.3	6.0	1.06
24	Myliobatidae	Eagle rays	1	3.5	_	_	-15.2	_	_	_	13.1	_
25	Ommastrephidae <sup>‡</sup>	Squids	5	$4.1\pm0.13$	-18.1	-17.4	-17.7	0.31	10.1	10.8	10.5	0.26
26	Ostreoida <sup>‡</sup>	Oysters	9	$4.0\pm0.53$	-20.1	-18.3	-19.4	0.46	4.8	7.8	6.1	0.98
27	Palinuridae <sup>‡</sup>	Lobsters	4	$4.0\pm0.06$	-16.2	-15.8	-15.9	0.16	12.2	12.6	12.4	0.17
28	Parthenopidae <sup>‡</sup>	Crabs	2	$8.2\pm0.35$	-17.0	-11.5	-14.2	3.90	5.9	6.5	6.2	0.46
29	Penaeidae <sup>‡</sup>	Shrimp	12	$4.5\pm 0.48$	-20.1	-17.7	-19.7	0.65	7.5	9.5	8.4	0.60
30	Pomacanthidae	Angelfish	3	$3.3\pm 0.06$	-18.1	-18.0	-18.0	0.03	12.4	13.7	12.9	0.71

31	Scombridae <sup>‡</sup>	Tunas	12	$3.6\pm0.43$	-17.9	-15.9	-17.1	0.56	12.0	14.6	13.2	0.80
32	Scorpaenidae	Scorpion fish	2	$3.3\pm 0.07$	-17.8	-17.8	-17.8	0.04	14.8	15.0	14.9	0.13
33	Serranidae <sup>‡</sup>	Groupers	34	$3.7\pm 0.59$	-21.5	-16.2	-18.1	1.20	8.4	15.0	12.7	1.48
34	Sphyrnidae	Hammerhead sharks	14	$3.1\pm 0.07$	-16.6	-14.8	-16.0	0.50	15.0	16.4	15.9	0.42
35	Squillidae <sup>‡</sup>	Mantis shrimp	1	4.7	_	—	-16.4	_	_	_	11.5	_
36	Stromatidae	Butterfishes	1	3.3	_	_	-17.3	_	_	_	12.3	_
37	$Sulidae^{\dagger}$	Nazca booby	17	$3.8\pm 0.60$	-20.0	-16.1	-17.5	1.35	13.1	15.2	14.0	0.56
38	Synodontidae <sup>‡</sup>	Lizardfish	1	4.3	_	_	-18.3	_	_	_	8.6	_
39	Xanthidae <sup>‡</sup>	Crabs	1	7.7	_	—	-11.8	_	_	_	6.9	_

§ Species present in both ecosystems.

†  $\delta^{13}$ C values corrected with Elliot et al. (2014).

 $\ddagger\,\delta^{13}C$  values corrected with Kiljunen et al. (2006).

#### Contribution of terrestrial basal sources to the trophic web

The fitted model (i.e., mixing polygons, subsequent predictive validations), suggested that these results explained the uncertainty of the TDFs and of the isotopic values of the 13 consumer groups. Therefore, the implementation of the mixing model was adequate to estimate the relative contribution of the different basal sources, confirmed by the Gelman-Rubin (Rhat) convergence diagnostic statistics, which was 1.00 for all parameters and suggested that there was convergence.

The organic matter present in the soil ( $\delta^{13}C = -20.1\%$  to -17.3%) (Figs. 28 and 29), reflected the isotopic signal of organic matter transferred from marine primary production. These results suggest a high input of  $\delta^{13}C$  from detritus towards the lizard *Anolis agassizi*, the crab *Johngarthia malpilensis*, and the dotted galliwasp *D. millepunctatus*. Terrestrial C<sub>3</sub> plants contributed mainly to the Orders Hymenoptera, Diplopoda, and Microcoryphia, which presented low  $\delta^{13}C$  values, resulting in a greater contribution probability from terrestrial C<sub>3</sub> plants (Fig. 28, Table 18).



**Figure 28. A.** Information of the sources used in the stable isotope mixing models. **B.** Estimation of the relative contribution of the terrestrial basal sources to the diet of the secondary consumers of the terrestrial ecosystem. **C.** Estimation of the contribution probability (in %) if the terrestrial basal sources to the terrestrial ecosystem in Malpelo Fauna and Flora Sanctuary, Colombia.

Consumers	Contribution p	robability (%)
Consumers	C <sub>3</sub> Plants	Detritus
Hymenoptera	78.0	22.0
Isopoda	29.5	70.5
Odontomachus sp.	25.3	74.7
Gryllidae	54.2	45.8
Araneae	67.0	33.0
Microcoryphia	93.6	6.4
Lumbriculidae	41.7	58.3
Diplopoda	78.5	21.5
Anolis agassizi	10.4	89.6
Diploglossus millepunctatus	9.8	90.2
Johngarthia malpilensis	9.2	90.8

 Table 18. Comparison of the relative contribution probability of two terrestrial basal sources of the Malpelo Flora and Fauna Sanctuary, with respect to all terrestrial consumer groups.

The basal  $\delta^{13}C_{detritus}$  of the terrestrial ecosystem was contrasted with the  $\delta^{13}C_{Corrected*TDF}$  values of five marine groups:  $\delta^{13}C_{macroalgae}$  (Wilcoxon rank sum test, W = 14, P = 0.21),  $\delta^{13}C_{phytoplankton}$ (Wilcoxon rank sum test, W = 24, P = 90),  $\delta^{13}C_{marine crabs}$  (Wilcoxon rank sum test, W = 84.5, P = 0.37), and  $\delta^{13}C_{S. granti eggs}$  (Wilcoxon Rank sum test, W = 33, P = 0.07), with statistically significant differences between the basal source of detritus and  $\delta^{13}C_{zooplankton}$  (Wilcoxon rank sum test, W = 110, P = 0.002) (Fig. 29).



Figure 29. Statistical comparison of the  $\delta^{13}$ C values (mean ± SD) of the terrestrial detritus with respect to the basal sources and organisms of low marine trophic levels of the Malpelo Fauna and Flora Sanctuary, Colombia.

#### <sup>15</sup>N-enrichment

The range of  $\delta^{15}$ N values indicated that terrestrial C<sub>3</sub> plants, as well as crickets, millipedes, and Hymenoptera, presented values compatible with terrestrial primary production (Craine et al., 2009; Amundson et al., 2003). However, species such as *A. agassizi*, *D. millepunctatus*, *J. malpilensis*, Isopoda, Araneae, Lumbricullidae, and *Odontomachus* sp. were enriched in <sup>15</sup>N, presenting values incompatible with a diet based on the primary productivity of the island (i.e., C<sub>3</sub> plants) and with some groups associated with organic matter decomposition processes (e.g., consumption of detritus) (Fig. 30). High  $\delta^{15}$ N levels of terrestrial animals could be related to the high trophic level of *S. granti* (trophic level = 4.2 [3.9–4.4, CI 95%]; Estupiñán-Montaño et al., unpublished), due to prey consumed in the marine environment.

#### Isotopic niche and isotopic overlap

The wide isotopic range of carbon, and especially of nitrogen, in the terrestrial ecosystem reflected an isospace (TA<sub>Terrestrial</sub>) of 134.7‰<sup>2</sup> and an isotopic niche (SEA<sub>C\_terrestrial</sub>) of 30.4‰<sup>2</sup> (Fig. 31A). After excluding terrestrial C<sub>3</sub> plants, the isospace and the isotopic niche were 65.1‰<sup>2</sup> and 17.3‰<sup>2</sup>, respectively (Fig. 6B). The isospace and isotopic niche of the marine ecosystem (TA<sub>marine</sub> = 117.2‰<sup>2</sup> and SEA<sub>C\_marine</sub> = 21.0‰<sup>2</sup>) were very similar to those of the terrestrial ecosystem, excluding C<sub>3</sub> plants (Fig. 31A, B).



**Figure 30.** Isotopic enrichment (mean ± SD) of <sup>15</sup>N of the various components of the terrestrial ecosystem of the Malpelo Fauna and Flora Sanctuary, Colombia, related to feathers (green), eggs (orange) of *Sula granti*, and detritus (grey).
Taking into account the low contribution of terrestrial C<sub>3</sub> plants to the terrestrial trophic web, two isotopic overlap scenarios were considered: one including C<sub>3</sub> plants and one excluding them. The terrestrial (C<sub>3</sub> plants; red box, Fig. 31A) and marine isospaces reflected an isotopic overlap of 0.85 (SIAR overlap; Fig. 31A), suggesting an overlap probability of 65% (nicheROVER) between the two ecosystems. In contrast, the marine isospace indicated a higher overlap probability with the terrestrial isospace (76%; Fig. 31A). In the second scenario, the estimated isotopic overlap between the terrestrial and marine isospaces was 0.71 (SIAR overlap; Fig. 31B), corresponding to 82% (terrestrial vs. marine) and 70% (marine vs. terrestrial) overlap between the two isospaces (Fig. 31B).

# DISCUSSION

Some isolated systems, such as oceanic islands, can support relatively complex food webs due to the input of nutrients via seabirds (Polis and Hurd, 1996; Polis et al., 1997a; Ellis, 2005). This allows a connection between low-productivity habitats ("receptor habitats") and environments with higher primary productivity ("donor habitats"); these processes drive the trophic and ecological dynamics of connected ecosystems (Polis and Hurd, 1995; 1996a, Polis and Strang, 1996; Polis et al., 1996, 1997a; Anderson and Polis, 1999; Caut et al., 2012).

The terrestrial ecosystem of Malpelo FFS is a small insular system with a limited capacity for atmospheric nitrogen fixation; it is therefore highly dependent on external nitrogen. *Sula granti* plays an important role in supplying nitrogen from the marine environment (Wolda, 1975; López-Victoria et al., 2009), resulting in an increase in the isotopic nitrogen concentration of the terrestrial environment. This seabird provides high quantities of nutrients in the form of guano, feathers, eggs, carcasses, chick remains, juveniles, and adults, in addition to food waste of marine origin, such as fish and squid (López-Victoria and Werding, 2008; López-Victoria et al., 2009, 2013). This highlights its importance in the transport of nutrients from the marine to the terrestrial ecosystem (Burger et al., 1978; López-Victoria et al., 2009). The same seabird-dependent process of transfer of energy and matter has been observed in the islands of the Gulf of California, Mexico (Anderson and Polis, 1999; Sánchez-Piñero and Polis, 2000), in Baccalieu Island, Canada (Duda et al., 2020), the Pacific and Indian Oceans, as well as in the Mediterranean Sea (Caut et al., 2012).



Figure 31. Isotopic overlap between the terrestrial and marine ecosystems of the Malpelo Fauna and Flora Sanctuary, Colombia, represented by two scenarios A. Terrestrial isospace including terrestrial C<sub>3</sub> plants *vs* the marine isospace. B. Terrestrial isospace excluding terrestrial C<sub>3</sub> plants and the marine isospace.

Terrestrial macro-species (i.e., A. agassizi, D. millepunctatus, and J. malpilensis) had similar  $\delta^{13}$ C values to those of the marine ecosystem; they were supported by the presence of seabirds as nutrient assimilators from the marine to the terrestrial ecosystem (Caut et al., 2012). This could be due to: 1) the similarity in isospace amplitude between the terrestrial (excluding terrestrial C<sub>3</sub> plants) and marine ecosystems; 2) the high isotopic overlap between the two ecosystems; and 3) the similarity between the  $\delta^{13}C$  of terrestrial detritus, S. granti eggs, marine macroalgae, and marine crustaceans. The high contribution of detritus to terrestrial consumers (Fig. 28B) suggests that the carbon in terrestrial organisms comes from the marine environment (Table 17). Their  $\delta^{13}$ C signals are similar to those of marine primary producers in Malpelo FFS (i.e., macroalgae: -21.0% to -16.0%; phytoplankton: -20.7% to -15.5% [this study]), as a result of transport and deposition of nutrients by S. granti and its "byproducts" (López-Victoria and Werding, 2008; López-Victoria et al., 2009, 2013), and not from terrestrial primary producers (i.e., terrestrial C<sub>3</sub> plants). Conversely, grasses (i.e., *Paspalum* sp.) are  $C_4$  plants, and similarly to  $C_3$  plants, they have a high C:N ratio (C<sub>3</sub> Malpelo Island = 13.5-18.7); thus, they would not be the main source of protein of the terrestrial ecosystem. However, it should be noted that no samples of C4 or CAM plants were collected, mainly due to the reduced plant cover in the study area.

On the contrary, the orders Diplopoda (millipedes) and Microcoryphia reflected a higher contribution of terrestrial C<sub>3</sub> plants (Fig. 31B), which is consistent with the food preferences of these taxa (Bueno-Villegas, 2012; Bach de Roca et al., 2015). These results reinforce the hypothesis that suggests a high reliance and trophic interaction between the marine and terrestrial ecosystems of Malpelo FFS (Wolda, 1975; López-Victoria et al., 2009).

The decomposition of naturally <sup>15</sup>N-enriched guano and seabird tissue (Anderson and Polis 1999) could be further <sup>15</sup>N enriched due to the volatilization of <sup>14</sup>N (Lindeboom 1984; Mulder et al., 2011) and to the fast mineralization of uric acid to ammonium ( $NH_4^+$ ) from guano (Wainright et al., 1998). This leads to greater isotopic fractionation, provoking <sup>15</sup>N-enrichment of the residual  $NH_4^+$  reservoir (Mizutani and Wada, 1988; Wainright et al., 1998). Plants fertilized with guano have <sup>15</sup>N-enriched values (Anderson and Polis, 1999), similar to the soil (Croll et al., 2005; Maron et al., 2006). Conversely, organisms that consume guano and those who include other seabird byproducts in their diet (i.e., feathers, eggs, carcasses [Barrett et al., 2005; López-Victoria et al., 2009]) have higher  $\delta^{15}$ N values; consequently, they have a higher trophic position than their prey (e.g., seabirds) or present higher tissue <sup>15</sup>N-enrichment.

In this regard, terrestrial C<sub>3</sub> plants of Malpelo FFS should reflect <sup>15</sup>N-enrichment, as has been documented for islands in the Gulf of California (C<sub>3</sub> plants =  $24.5 \pm 1.1\%$ , C<sub>4</sub> =  $24.3 \pm 1.4\%$ ; Barrett et al., 2005). However, terrestrial C<sub>3</sub> plants of Malpelo FFS evidenced a different pattern (low  $\delta^{15}$ N values; Fig. 26). Values found for these plants are consistent with atmospheric nitrogen fixation and were impoverished in <sup>15</sup>N relative to the eggs and feathers of *S. granti* (Fig. 30). Similar results were reported for Possession Island in the Indian Ocean (plants =  $5.2 \pm 1.05\%$  SD, seabirds =  $9.34 \pm 0.45\%$  SD, enrichment = -0.44 [Caut et al., 2012]). Therefore, it seems that terrestrial C<sub>3</sub> plants of Malpelo FFS do not obtain N indirectly from guano nor from the solids of seabirds (Caut et al., 2012). Primary consumers (i.e., Isopoda and ants *Odontomachus* sp.) and terrestrial secondary consumers (i.e., *A. agassizi*, *D. millepunctatus*, and *J. malpilensis*), incorporate <sup>15</sup>N directly from the consumption of *S. granti* and its byproducts (López-Victoria and Werding, 2008; López-Victoria et al., 2009, 2013). This indicates <sup>15</sup>N-enrichment relative to the eggs and feathers of *S. granti* (Fig. 30).

The *S. granti* colony positively impacts terrestrial communities of Malpelo FFS due to the high contributions of guano and other "byproducts" that terrestrial species consume directly (Polis and Hurd, 1996; Sánchez-Piñero and Polis, 2000). This is reflected in the high abundances of *J. malpilensis* (estimated population: 833,000 individuals [López-Victoria and Werding, 2008]), *D. millepunctatus* (12,000–18,000 individuals [López-Victoria et al., 2011]), and *A. agassizi* (60,000–102,000 individuals [López-Victoria et al., 2011]) present in Malpelo FFS. In contrast, the large *S. granti* colony could negatively affect the population of terrestrial C<sub>3</sub> plants (28 species [González-Román et al., 2014]) by reducing their cover on the island.

This phenomenon has been observed on Malpelo island (S. Bessudo Lion, *personal communication*). It could be related to: 1) the high concentrations of guano during the dry season that could exceed the concentration limits of essential nutrients and eventually toxify the soil and limit the development of plants; this could also prevent the establishment of native plants in places where there is a high density of seabirds (Boutin et al., 2011; Sánchez-Piñero and Polis, 2000) and 2) the reduction of nutrients due to guano washing off during the rainy season, which limits soil formation and affects the adequate development of plants (Caita and Guerrero, 2000).

There is a high input of nutrients (mainly from marine origin) from the terrestrial environment (e.g., organic matter, seabird guano, etc.) into the sea at Malpelo FFS, due to runoff from frequent and abundant rains between May and December (annual precipitation ~2,500 mm [von Prahl,

1990; López-Victoria and Estela, 2007]). Terrestrial nutrients could affect primary producers locally, altering the typical values of marine primary productivity surrounding Malpelo FFS and modifying seasonal marine trophic dynamics (Ishida, 1996; Wait et al., 2005); as a result, this would be reflected in their isotopic values. Despite the contributions of terrestrial nutrients to the sea and the effects that these contributions may have on the dynamics of this ecosystem, more studies are necessary to validate these hypotheses and identify other trophic connectivity routes between the terrestrial and marine ecosystems of Malpelo FFS.

Finally, an important control by the "donor" habitat (marine ecosystem) over the "receptor" habitat (terrestrial habitat) was evidenced by the transport and contribution of matter and energy between ecosystems (Polis et al., 1997a). The transport of nutrients from sea to land in Malpelo FFS is governed mainly by *S. granti*. However, there are other inputs in the sea-land interface, which are generated in the intertidal zone when *J. malpilensis* and *D. millepunctatus* consume marine algae and marine crabs (*Grapsus grapsus*), respectively (López-Victoria et al., 2009, 2013). Nevertheless, this source of input of marine nutrients into the terrestrial ecosystem has not been studied in detail. More studies are necessary to estimate the contribution of the intertidal zone and terrestrial ecosystem in Malpelo FFS. In turn, this would improve ecological knowledge regarding the dynamics of this small oceanic island.

Given the impact exerted by the donor habitat on the receptor habitat, it is possible that an eventual disturbance of marine populations may alter food webs, due to the transitional interphase between the marine and insular environment (Sullivan and Manning, 2019). The present study documented trophic interactions between marine and terrestrial ecosystems, providing support to how diverse species can cross the limits of distinct environments (e.g., terrestrial and aquatic). Furthermore, this study evidenced how stable isotope analysis constitutes a useful tool in the identification of trophic interactions between terrestrial and marine ecosystems.

# Chapter 5: FEEDING ONTOGENY OF THE MOST ABUNDANT AND FREQUENT SHARK SPECIES AROUND THE MALPELO FAUNA AND FLORA SANCTUARY, COLOMBIA

# 5.1. Ontogenetic feeding ecology of the scalloped hammerhead (*Sphyrna lewini*) in the Colombian eastern tropical Pacific

#### **INTRODUCTION**

The ontogenetic changes in diet suggest shifts in foraging behavior during the species' life cycle (Werner et al., 1984; Newman et al., 2012), which could cause modifications in their habitat use (coastal vs oceanic). These trophic patterns could alter the community structure (Estrada et al., 2006) and the local trophic dynamics (Polis et al., 1996; Polis and Strong, 1996). For this reason, quantitative diet changes in the ontogeny of the species are important as they provide new information about the niche and the ecological role of species in an ecosystem (Braga et al., 2012), along with the effects of predator-prey relationships on ecosystem structure (Juanes et al., 2001; Newman et al., 2012). However, the existing research of trophic ontogeny of large predators (i.e., sharks) could be complicated due to the migratory movements of these animals and the inaccessibility of their habitats, which makes it difficult to gather information about their life cycle (Hanze et al., 2012). The current study addresses some of the information gaps regarding early stages of life and habitat changes of chondrichthyan, as such information is crucial to elaborate appropriate management and conservations measures, considering that the survival and recruitment of juveniles is vital to maintain the health of these populations due to their vulnerability to commercial fishing (Baum et al., 2003; Dulvy et al., 2008), as well as their ecological importance as top predators (Cortés, 2002; Baum et al., 2003; Grubbs, 2010).

The trophic studies and ontogenetic diet shift of different species of sharks have based their methodology on stomach content analysis (SCA) and stable isotope ratios (Torres-Rojas et al., 2010, 2013). While the SCA generates information about the prey digested in the recent past (i.e., in the last hours or days), the isotopic analysis of a tissue sample (e.g., muscle, teeth, or liver) reveals the food synthesized by the predator over different time and space scales (Kim et al., 2012), providing the study with additional information about habitat use, migration, feeding preferences, ecological niche, sharing resources, and trophic position of an individual species (e.g., Post, 2002; Newman et al., 2007; Graham et al., 2010; Jackson et al., 2011). The osteological sections formed by accretion (e.g., fish otoliths and shark vertebrae) show the fluctuations in diet and habitat use of sharks et al., species during their lives (Best & Schell, 1996; Kim et al., 2012; Carlisle et al., 2015; Estupiñán-Montaño et al., 2019). Despite the important record that the vertebrae preserve,

these structures have received less attention from researchers, which could be related to the greater demand of time and energy necessary for the extraction and processing of the tissue, since these structures must be systematically sampled with the help of micro-drills (Estupiñán-Montaño et al., 2019) and processed for several days for the adequate extraction of carbonates (Kim et al., 2012), unlike the soft tissues, such as muscle, which have less complex treatment processes (Kim et al., 2012; Tamburin et al., 2019, 2020).

The scalloped hammerhead shark *Sphyrna lewini* is a species with a broad distribution along the eastern tropical Pacific (ETP), including around oceanic islands and seamounts (Compagno, 1984; Klimley, 1981; Klimley & Nelson, 1981), which is partly related to large migratory movements among various oceanic islands of the ETP (Malpelo, Colombia; Cocos Island, Costa Rica; Galapagos Islands, Ecuador [Bessudo et al., 2011a, b]). This characteristic makes *S. lewini* vulnerable to overexploitation, which has led to its classification as a critically endangered species (Rigby et al., 2019) and its inclusion in Appendix II of CITES (CITES, 2013). In spite of this, and its ecological importance as a top predator in the ETP (Torres-Rojas et al., 2010, 2015; Estupiñán-Montaño et al., 2017), detailed studies about the species and its biology are still lacking. Few studies have been carried out to investigate the changes in diet that occur during its life cycle.

The SCA of *S. lewini* suggest that the species consumes a high diversity of prey, such as crustaceans, teleosts, and cephalopods (coastal and oceanic), and even other chondrichthyans (Bethea et al., 2004; Estupiňán-Montaño et al., 2009; Torres-Rojas et al., 2010, 2015; Bornatowski et al., 2014; Galván-Magaña et al., 2013; Flores-Martínez et al., 2016; Rosende-Pereiro et al., 2020). Nonetheless, *S. lewini* has shown a preference for the consumption of oceanic squids (e.g., Ommastrephidae [Estupiñán-Montaño et al., 2009; Galván-Magaña et al., 2013]), which has led some to consider the species to be a predator with a narrow trophic niche at the population level (Levin's index, *Bi* < 0.35 [Estupiñàn-Montaño et al., 2009; Torres-Rojas et al., 2010, 2013; Bornatowski et al., 2014]), but with wide trophic niches at specific levels. For example, the juveniles of the species consume a high variety of pelagic and benthic teleosts (i.e., Albulidae, Arridae, Carangidae, Centropomidae, Haemulidae and Lutjanidae), coastal squids (i.e., Lolliginidae), and some crustaceans (i.e., Penaeidae) (Flores-Martínez et al., 2016; Rosende-Pereiro et al., 2020) in different habitats. Adults of the species tend to occupy narrower trophic niches however,

due to their preferential consumption of oceanic squid (Bi > 0.20 [Estupiñán-Montaño et al., 2009]).

Similarly, the stable isotopes analyses (SIA) of muscle samples of *S. lewini* (Loor-Andrade et al., 2015 [ $-15.9\pm0.4\%$ ], Li et al., 2016 [ $-16.3\pm0.3\%$ ]; Estupiñán-Montaño et al., 2017 [ $-16.0\pm0.48\%$ ]; Rosende-Pereiro et al., 2020 [ $-15.5\pm0.07\%$ ]) have suggested that the species has a wide trophic niche due to the use of coastal (-14.9% to -13.7% [Torres-Rojas et al., 2013; Estupiñán-Montaño et al., 2017]) and oceanic zones (-16.6% to -15.5% Loor-Andrade et al., 2015; Li et al., 2016; Estupiñán-Montaño et al., 2017; Rosende-Pereiro et al., 2020]). In this context, the use of diverse trophic levels (trophic position: 3.8-5.9 [Li et al., 2016; Estupiñán-Montaño et al., 2017]) is a reflection of individual specialization in a diverse range of habitats (Loor-Andrade et al., 2015; Li et al., 2015; Li et al., 2016) and a high degree of trophic plasticity (Torres-Rojas et al., 2013).

The objective of this study was to analyze ontogenetic changes in the diet and habitat use of *S*. *lewini* in the Colombian Eastern Tropical Pacific, using stable isotope ratios of carbon and nitrogen ( $\delta^{13}$ C and  $\delta^{15}$ N) in vertebral collagen. This study generated information about the ecological patterns of *S*. *lewini* throughout its feeding ontogeny, in order to understand the role that the species plays in potential nursery areas (e.g., Colombian mangroves) and the coastal and oceanic food web of the southeastern tropical Pacific.

# **MATERIALS AND METHODS**

# Study area

Malpelo Island (Fig. 4A) is the summit of a submarine mountain range called the Malpelo Ridge, which extends in a NE-SW direction; it is approximately 241.4 km long by 80.5 km wide (Fig. 4B, red polygon). The island has a maximum height of 300 m above sea level and emerges from approximately 4,000 m depth (Fig. 4C) (more details in Chapter 1).

## **Collection of samples**

During 2013, a total of 16 *S. lewini* were confiscated from illegal fishing operations around the "Fauna and Flora Sanctuary Malpelo, Colombia" (Fig. 4B, see Chapter 1). Although sex was not registered due to the lack of pelvic fins and viscera from these illegal captures the total length (TL) was measured (cm) for each specimen. The vertebrae were measured and recorded close to the

head of each organism (Estupiñán-Montaño et al., 2019). Afterwards, they were stored in previously labeled Eppendorf tubes and transported to the Fundación Alium Pacific facilities for further processing.

The neural arc and the connective tissue were removed from all vertebrae, leaving the body of the vertebra completely clean; afterwards, the tissue samples were dehydrated at ambient temperature (Estupiñán-Montaño et al., 2019). The samples were then transferred to the "Laboratorio de Ecología de Peces del Centro Interdisciplinario de Ciencias Marinas del Instituto Politécnico Nacional (La Paz, México)".

# Sample preparation and analysis

Subsamples were obtained from each vertebra using a microdrill and a 0.5-mm diameter bit, to obtain two sets of samples. We drilled twice in each sample location along the entire vertebra from the center to the periphery. In order to remove the inorganic carbon, the first set of samples weighed from 0.48 to 0.98 mg and stored in a desiccant with HCl vapor at 37% for 12–24 hours (Hedges & Stern, 1984). The first set of samples registered %C values between 0.08–14.6% (7.68±3.1%); while the second set of samples weighed between 0.22 and 0.71 mg and was analyzed without any treatment in order to avoid any distortion of the <sup>15</sup>N values (Christiansen et al., 2014), resulting in %C values between 10.6–19.3% (14.1±1.7%). Both sets of vertebral collagen samples were stored in 3.2 × 4 mm tin capsules.

Stable isotope analysis was carried out in the Laboratorio de Biogeoquímica de Isótopos Estables del Instituto Andaluz de Ciencias de la Tierra in Granada (CSIC-UGR), Spain (more details in Chapter I).

### **Trophic position**

The trophic position (TP) of *S. lewini* was assessed by individual and estimated ages associated with vertebral radius (VR), and by the implementation of a Bayesian method within the R statistical environment (R Core Team, 2018), employing the *tRophicPosition* package version 0.7.5 (Quezada-Romegialli et al., 2018). Analyses of TP were then completed using the  $\delta^{13}$ C and  $\delta^{15}$ N values of *S. lewini*; while the isotopic values of the brown algae (*Padina* sp.) of Malpelo Island ( $\delta^{13}$ C = -18.7±1.58‰ SD and  $\delta^{15}$ N = 5.2±0.45‰ SD [ver Capítulo 3.1]) were used as the isotopic baseline. A Bayesian model of one baseline and two trophic discrimination factors (TDF) was run

with 2 Markov Chain Monte Carlo (MCMC) and 20000 adaptive interactions, assuming a  $\lambda$  of the baseline taxa = 1. Accordingly, *S. lewini*'s TP value was determined using a TDF for the tissue-specific (vertebrae) fractionation of phylogenetically similar species to *S. lewini* as well as lemon shark *Negaprion brevirostris* (Vélez-Zuazo & Agnarsson, 2011) with values of  $\Delta^{13}$ C = 3.75±0.44‰ SD and  $\Delta^{15}$ N = 1.45±0.61‰ SD (Hussey et al., 2010) for carbon and nitrogen, respectively.

The estimated TPs were classified into five functional groups, primary carnivores (level 3), intermediate (primary-secondary) carnivores (levels 3.5–4), secondary carnivores (level 4), intermediate (secondary-tertiary) carnivores (levels 4.5–5), tertiary carnivores (level 5 & greater]) (Mearns et al., 1981).

### Bayesian mixing models and prey selection

In order to make inferences about the dietary preferences of S. lewini, we applied Bayesian mixing models, based on the  $\delta^{13}$ C and  $\delta^{15}$ N values of vertebrae, to determine the specie's overall diet during its life cycle (e.g., Kim et al., 2012). The mixing model considered four steps: 1) selection of potential S. lewini SCA prey (e.g., Estupiñán-Montaño et al., 2009; Torres-Rojas et al., 2010, 2015; Bornatowski et al., 2014), and potential prey within Colombian mangrove ecosystems (Medina et al., 2018) as they are important breeding areas for the species (Quintanilla et al., 2015); 2) grouping the potential prey into five categories according to their biological characteristics (Table 19; Phillips et al., 2014); 3) as there are no specific TDFs for S. lewini, we selected the estimated TDFs for other shark species such as the lemon shark N. brevirostris ( $\delta^{13}$ C =  $3.75\pm0.44\%$  and  $\delta^{15}N = 1.45\pm0.61\%$  [Hussey et al., 2010]), a species which is phylogenetically similar to S. lewini (Vélez-Zuazo and Agnarsson 2011) and has approximately 'equivalent' food preferences (e.g., squid, shrimp, fish, elasmobranches [Cortés and Gruber, 1990; Wetherbee et al., 1990; Newman, 2012]), with the objective of minimizing sources of uncertainty (i.e., environmental and physiological factors, trophic position, metabolic rates, growth rates, maturity stages [Phillips et al., 2014]), which make the mixing models highly sensitive (Bond & Diamnon, 2011; Phillips et al., 2014). Finally, the mixing model was adjusted to verify that the TDFs and potential prey groups were consistent with the assumptions of the model (Smith et al., 2013). The mixing model adjustment was run with  $10^3$  iterations with a 95% probability for the mixing

polygon (Smith et al., 2013). The model was considered adequate if the isotopic values were within 1% of the mixing model polygons (Reum et al., 2020).

Later, in order to make inferences about ontogenetic use habitat, we estimated the relative contribution of *S. lewini*, through the package "simmr" (version 0.3) of the isotope mixing model SIAR (stable isotope analysis in R; Parnell et al., 2013) version 3.4.3 in the R statistical platform (R Core Team 2018). This model uses a Bayesian isotopic framework to estimate the proportional contribution of prey to a consumer's diet (Parnell et al., 2013), including variability in model inputs, such as TDF values for sharks. To run the SIAR model, we used the mean  $\delta^{13}$ C and  $\delta^{15}$ N (± SD) values of *S. lewini* (*i.e.*, individuals and age groups), potential prey species (Table 19), and tissue-specific (vertebrae) fractionation ( $\Delta^{13}$ C<sub>N. brevirostris</sub> = 3.75±0.44‰ and  $\Delta^{15}$ N<sub>N. brevirostris</sub> = 1.45±0.61‰; Hussey et al., 2010) as mixture, sources, and correction factors, respectively. The model was run with 10<sup>6</sup> iterations, 10000 burn-in, 100 thin, and 4 MCMC.

# **Feeding ontogeny**

The patterns of isotopic enrichment along the ontogeny of *S. lewini* were inferred from the initial point of sampling located 2 mm from the center of the vertebrae. The relative enrichment of <sup>13</sup>C and <sup>15</sup>N were calculated using the algorithm proposed by Estrada et al. (2006):

Enrichment 
$$Y = \left(\frac{\delta^z Y_{x mm} - \delta^z Y_{2mm}}{\delta^z Y_{2mm}}\right)$$

where *Y* is the element of interest ( $^{13}$ C and  $^{15}$ N), *z* is the atomic mass of the element, and *x* is the location of each vertebral collagen sample, relative to the location of the first sampling point (in mm).

As it was not possible to determine the sex for each analyzed individual, the TL and age were estimated from the average of the regression parameters for both sexes. Thus, the TL for every VR sample was estimated using the following equation (Anislado-Tolentino et al., 2008): TL = 12.65 + 214 × VR. Where *TL* is the estimated total length in centimeters, and *VR* is the vertebral radius distance in centimeters.

Potential prev	δ <sup>13</sup> C	$\delta^{15}N$	Trophic
1 7	(‰)	(‰)	position
Oceanic cephalopods	$-18.0 \pm 0.6$	$10.2 \pm 1.4$	4.14
Dosidicus gigas <sup>a,b</sup>	$-17.8\pm0.4$	$10.1\pm1.3$	4.14 <sup>g</sup>
Sthenoteuthis oualaniensis <sup>a</sup>	$-18.0\pm0.2$	$10.4\pm0.2$	4.09 <sup>g</sup>
Ommastrephes bartramii <sup>c</sup>	$-18.1\pm0.4$	$10.0\pm0.4$	4.20 <sup>g</sup>
Coastal cephalopods	$-16.9 \pm 0.4$	$12.5\pm0.2$	3.90
Lolligunculla (Loliolopsis) diomedeaeª	$-16.9\pm0.4$	$12.5\pm0.2$	3.90 <sup>g</sup>
Oceanic fish	$-16.8 \pm 1.1$	$12.3 \pm 3.7$	4.16
Auxis thazard <sup>d</sup>	$-16.6 \pm 0.1$	$11.0\pm0.1$	4.33 <sup>g</sup>
Auxis spp. <sup>d</sup>	$-15.5\pm0.9$	$12.1 \pm 1.7$	4.33 <sup>g</sup>
Katsuwonus pelamis <sup>d</sup>	$-17.1 \pm 0.5$	$12.3\pm3.0$	4.30 <sup>g</sup>
Thunnus albacares (Ecuador) <sup>d</sup>	$-17.2 \pm 0.2$	$13.3 \pm 1.1$	4.30 <sup>g</sup>
Thunnus albacares (Malpelo) <sup>f</sup>	$-17.5\pm0.4$	$13.7\pm0.7$	4.30 <sup>g</sup>
Scomber japonicus <sup>c</sup>	$-16.8\pm0.1$	$11.6\pm0.5$	3.38 <sup>g</sup>
Mangrove fish <sup>e</sup>	$-18.3 \pm 1.9$	$11.1 \pm 1.1$	3.56 <sup>e</sup>
Spheroides rosenblatti	$-17.3\pm0.1$	$11.8\pm0.1$	3.90
Lutjanus argentiventris	$-17.2\pm0.9$	$13.5\pm0.5$	4.40
Daector dowii	$-18.7\pm0.4$	$11.1\pm0.1$	3.70
Bathigobius andrei	$-18.7\pm0.4$	$9.4\pm0.2$	3.20
Ctenogobius sagittula	$-21.1 \pm 1.3$	$8.1\pm0.6$	2.80
Microgobius tabogensis	$-17.9\pm0.8$	$11.3\pm0.2$	3.80
Larimus argenteus <sup>b</sup>	$-17.4\pm0.5$	$12.6\pm0.7$	3.10 <sup>g</sup>
Coastal crustaceans <sup>e</sup>	$-20.5\pm2.5$	$6.5 \pm 1.3$	<b>2.48</b> <sup>e</sup>
Petrolishes zacae	$-15.7\pm0.7$	$6.6\pm0.8$	2.40
Panopeus chilensis	$-19.3\pm0.8$	$9.4\pm1.1$	3.20
Macrobranchium panamensis	$-20.5 \pm 1.7$	$8.4\pm1.3$	2.90
Alpheus colombiensis	$-22.2\pm0.9$	$6.4\pm0.7$	2.30
Armases occidentale	$-24.8 \pm 0.3$	$3.8 \pm 0.1$	1.60

**Table 19.** Previously published  $\delta^{13}$ C,  $\delta^{15}$ N values (mean ± SD), and trophic position of potential prey of scalloped hammerhead shark (*S. lewini*) in the southeast Pacific Ocean, used in mixing models. Prey selection was based on stomach content studies of *S. lewini* in regions closest to the study area (Estupiñán-Montaño et al., 2009), potential prey of nurseries area at Colombian Pacific coastal zones (Medina et al., 2018) and in the eastern Pacific Ocean (Calle-Morán, 2010, Rosas-Luis et al., 2017).

**References:** A. Bolaños-Martínez (2009); B. Ruíz-Cooley et al. (2010); C. Calle-Morán (2010); D. Rosas-Luis et al. (2017); E. Medina et al. (2018); F. This study; G. Pauly & Zeller (2015).

Additionally, the age (t) from each vertebral collagen sampling point (i.e., every millimeter) was estimated in accordance to the von Bertalanffy growth function:

$$\mathrm{TL} = L_{\infty} \left( 1 - e^{-K(t-t_o)} \right)$$

where  $L_t$  is the predicted length at age t,  $L_{\infty}$  is the asymptotic mean length, K is the growth rate (year <sup>-1</sup>), and  $t_0$  is the theoretical age at which the shark had zero length. Likewise, the year in each millimeter (location of vertebral collagen sample point) was estimated using the following formula:

$$t = -\frac{\ln\left(1-\frac{L_t}{L_{\infty}}\right)}{K} + t_0$$

where the parameters of the von Bertalanffy growth function were:  $L_{\infty} = 370$  cm LT; *K* was 0.11 years<sup>-1</sup>, and  $t_0$  was -1.17 years (Anislado-Tolentino et al., 2008).

# Niche width and isotopic overlap

Isotopic niche was quantified for individuals and age groups, using the Stable Isotopic Bayesian Ellipses (SIBER) method in R (Jackson et al., 2011). This analysis is based on calculated ellipses from a covariance matrix, which defines its forms and areas (Jackson et al., 2011) to estimate the width of the isotopic niches (Standard Ellipse Corrected Area, SEA<sub>C</sub>).

The isotopic overlap was estimated using the nicheROVER package in R (Lysy et al., 2014), which is a Bayesian method that calculates the probability of overlap between niche pairs using multidimensional information as niche indicators (e.g., stable isotopes, environmental variables). The probabilistic density of niche overlap was calculated by running  $10^4$  iterations and 95% of the data from each species or group occurring within their isospaces, providing directional niche overlap estimates (e.g., *x* vs *y* and *y* vs *x*), according to the distributions of a specific species in the multivariate niche space (Lysy et al., 2014).

#### Statistical analysis

The normality and homoscedasticity of the isotopic information was tested by a Shapiro–Wilk test and a Levene's test, respectively. Non-parametric analyses of variance (Kruskall–Wallis test, Wilcoxon signed-rank test) were used to test the isotopic differences among categories (individuals and VR [ages]). A post-hoc test of multiple comparisons (Dunn's test) was then performed to identify the specific differences among categories.

# RESULTS

Cross-sectional sampling and analysis of vertebrae of 16 *Sphyrna lewini* (LEW#) ranged between 145.3 cm TL and 193.2 cm TL (Table 20), with an estimated age of 0.1 to 5.5 years (Table 21). A total of 101 vertebral collagen samples were analyzed.

# **Feeding sources**

The values of  $\delta^{13}$ C ranged from -17.2% to -14.1% (mean±SE,  $-15.2\pm0.06\%$ , V-PDB) (Shapiro, p = 0.001; Levene, p = 0.44). The individual analysis showed that LEW8 ( $-15.6\pm0.23\%$ ), LEW12 ( $-15.7\pm0.36\%$ ), and LEW15 ( $-15.7\pm0.10\%$ ) had the most depleted  $\delta^{13}$ C values, while LEW3 ( $-14.7\pm0.11\%$ ), LEW11 ( $-14.6\pm0.1\%$ ), and LEW13 ( $-14.7\pm0.12\%$ ) showed the highest  $\delta^{13}$ C values (Table 20).

On the other hand, we obtained  $\delta^{13}$ C values of seven age groups (VR, Table 21): the age groups 0.1–0.6 (2 mm in VR) showed the most negative values of  $\delta^{13}$ C compared to other estimated age groups (Fig. 2). Nonetheless, no statistical differences were apparent among age groups (Kruskal-Wallis, p = 0.10, Table 21).



Figure 32. Mean values ( $\pm$ SE) of  $\delta^{13}$ C and  $\delta^{15}$ N for estimated age groups of the scalloped hammerhead shark (*Sphyrna lewini*). Each vertebral radius represents an estimated age group (see legend in figure).

#### **Trophic position**

The values of  $\delta^{15}$ N in the *S. lewini* specimens ranged from 7.6% to 13.0% (11.3±0.09%, AIR) (Shapiro, p < 0.01; Levene, p = 0.31). The individual analyzes showed that LEW1 (9.3±0.40%) and LEW2 (9.4±0.79%) had the most depleted  $\delta^{15}$ N values, while LEW3 (12.2±0.19%) and LEW4 (11.9±0.08%) were the individuals with the highest values of  $\delta^{15}$ N (Table 20). The  $\delta^{15}$ N

values obtained from the seven distinct age groups did not reveal statistical differences among the different age groups (Kruskal-Wallis, p = 0.12; Table 21; Fig. 32).

The TPs estimated for *S. lewini* produced a mode of 4.8 (95% CI = 2.9–6.5); with TPs estimated by individuals producing modes that varied between 3.9 and 5.8 (95% CI = 2.9–6.5; Table 20) and estimated by age groups the mode varied between 4.9 and 5.4 (95% CI = 4.1–6.1; Table 21), respectively.

#### Bayesian mixing models and prey selection

The results obtained by the fitting model (i.e., mixing polygons, subsequent predictive validations), suggest that these results explained the uncertainty of the TDFs and of the isotopic values of the five groups of potential prey. Therefore, the application of the mixing model was adequate to estimate the selection of potential feeding habits of *S. lewini* during its life cycle. Additionally, the mixing model produced a total of 3600 posterior distributions and Gelman-Rubin (Rhat) convergence diagnostic statistics between 1.00 and 1.03 for all parameters, suggesting that there was convergence.

The mixing model suggested a high negative correlation (> 0.50) between the estimated relative contribution of mangrove fish with respect to coastal cephalopods (-0.51) and coastal crustaceans (-0.55); oceanic cephalopods with respect to coastal cephalopods (-0.63) and coastal crustaceans (-0.67). Coastal cephalopods and coastal crustaceans showed a high positive correlation (0.71). The correlation between the other potential groups of prey showed low negative and positive values (< 0.31).

The estimated relative contribution of the potential prey groups of *S. lewini* indicated that the main dietary contribution was provided by coastal crustaceans (median [2.5%-97.5%]; 30.3% [24.5%-37.0%]), followed by oceanic cephalopods (28.0% [12.1%-40.5%]), mangrove teleosts (26.4% [14.6%-36.8%]), coastal cephalopods (9.7% [2.1%-22.9%]), and oceanic teleosts (4.8% [1.1%-12.0%]). The relative contribution of the groups of potential prey for each individual of *S. lewini* indicated different degrees of contribution to the diet at the population-level (Fig. 33).

The mixing model by age group indicated that coastal crustaceans, oceanic cephalopods, and mangrove teleosts, contributed to the diet of *S. lewini* throughout its life cycle (Fig. 34A). Of these three prey groups, coastal crustaceans contributed in a greater proportion to the diet at 0.1-0.6 years (37% [24.3% - 50.1%]), 0.6-1.3 years (36% [20.5% - 45.4%]), 3.7-4.5 years (34% [20.6% - 20.6%)

-43.6%]), and 5.1–5.5 years (35% [7.5% – 52.7%]), while the relative contribution of oceanic cephalopods was highest at 1.3–2.0 years (22% [3.3% – 44.5%]), 2.0–2.7 years (22% [3.9% – 48.2%]), and 2.8–3.6 years (39% [5.2% – 57.9%]) (Fig. 34B).



Figure 33. Individual feeding preferences of the scalloped hammerhead shark (*Sphyrna lewini*), represented by the estimated relative contributions of potential prey groups, reported in contribution percentage values obtained from stable isotope mixing models.



**Figure 34. A.** Stable isotope mixing models. **B.** Estimated prey group contribution to diet of scalloped hammerhead shark (*Sphyrna lewini*) during its life cycle, represented by relative contribution from stable isotope mixing models, and reported in percentage values for estimated age group.

Individuala	5	TL Age	Age	δ <sup>13</sup> C (‰)		δ <sup>15</sup> N (‰)		Trophic position		SEA <sub>C</sub>
Individuals	п	(cm)	(years)	Range	$Mean \pm SE$	Range	$Mean \pm SE$	95% CI	Mode	$(\%^2)$
LEW1	4	145.3	3.4	-15.8 to -14.6	$-15.3\pm0.26$	8.3 to 10.2	$9.3\pm0.40$	3.3–4.5	3.9	2.40
LEW2	5	176.8	4.7	-16.1 to -14.6	$-15.3\pm0.28$	7.6 to 12.2	$9.4\pm0.79$	2.9-4.9	4.9	3.30
LEW3	7	162.1	4.1	-15.1 to -14.4	$-14.7 \pm 0.11$	11.5 to 13.0	$12.2 \pm 0.19$	5.1-6.5	5.8	0.62
LEW4	6	165.8	4.2	-16.2 to -14.7	$-15.5 \pm 0.24$	11.6 to 12.2	$11.9 \pm 0.08$	5.0-6.3	5.6	0.24
LEW5	7	176.8	4.7	-16.7 to -14.3	$-15.2\pm0.64$	11.1 to 12.1	$11.7\pm0.12$	4.9–6.2	5.5	0.28
LEW6	6	162.1	4.1	-15.5 to -14.6	$-15.1 \pm 0.15$	10.4 to 12.0	$11.5\pm0.26$	4.8-6.1	5.4	0.68
LEW7	6	193.2	5.5	-15.8 to -14.4	$-15.2 \pm 0.19$	10.1 to 12.1	$11.0\pm0.29$	4.4–5.6	5.0	1.89
LEW8	4	193.2	5.5	-16.8 to -15.0	$-15.6 \pm 0.23$	11.2 to 12.3	$11.7 \pm 0.18$	4.8-6.1	5.4	0.37
LEW9	6	151.1	3.6	-16.3 to -14.4	$-15.1\pm0.27$	11.1 to 12.1	$11.6 \pm 0.16$	4.8-6.1	5.4	0.32
LEW10	7	165.8	4.2	-17.2 to -14.3	$-15.3\pm0.38$	11.3 to 11.9	$11.7\pm0.08$	4.9–6.1	5.4	0.30
LEW11	6	191.5	5.5	-15.3 to -14.3	$-14.6 \pm 0.15$	10.8 to 11.7	$11.3 \pm 0.15$	4.6-5.8	5.2	0.34
LEW12	6	151.1	3.6	-16.8 to -14.6	$-15.7\pm0.36$	10.7 to 12.2	$11.4\pm0.21$	4.7–5.9	5.2	0.92
LEW13	7	186.0	5.2	-15.2 to -14.3	$-14.7\pm0.12$	10.7 to 12.1	$11.4\pm0.18$	4.7–6.0	5.2	0.50
LEW14	7	171.2	4.5	-15.8 to -14.1	$-15.0 \pm 0.19$	10.9 to 11.9	$11.5 \pm 0.14$	4.7–6.0	5.4	0.20
LEW15	7	191.8	5.5	-16.0 to -15.2	$-15.7 \pm 0.10$	9.1 to 11.5	$10.7\pm0.36$	4.2–5.6	4.8	0.68
LEW16	7	184.1	5.1	-15.2 to -14.6	$-15.0\pm0.08$	10.7 to 12.1	$11.4\pm0.20$	4.7–6.0	5.3	0.22

**Table 20.**  $\delta^{13}$ C and  $\delta^{15}$ N values (range and mean ± SE), total length (TL, cm), estimated age (years), trophic position (95% confidence intervals [CI] and mode), and isotopic niche (area of the corrected standard ellipse [SEA<sub>C</sub>]) for scalloped hammerhead shark (*Sphyrna lewini*) around Malpelo Island, Colombia. **Bold:** Individuals that showed statistically significant differences according to the multiple comparisons test (Dunn's test [Suppl. 1 and 2]).

**Table 21.**  $\delta^{13}$ C and  $\delta^{15}$ N values (range and mean ± SE), trophic position (95% confidence intervals [CI] and mode), isotopic niche (area of the corrected standard ellipse [SEA<sub>C</sub>]), and estimated lengths (TL, cm) and ages (years) of scalloped hammerhead shark *Sphyrna lewini* around Malpelo Island, Colombia.

VD - TI			AgeEstimated		δ <sup>13</sup> C (‰)		δ <sup>15</sup> N (‰)		Trophic position		SEAc
VK II	п	I LEstimated (range) –	Range	$Mean \pm SE$	Range	$Mean \pm SE$	Range	$Mean \pm SE$	95% CI	Mode	$(\%^2)$
2	18	47.3-65.7	0.1–0.6	$0.3\pm0.04$	-16.8 to -14.5	$-15.6\pm0.16$	7.6 to 12.5	$11.3\pm0.26$	4.3–6.0	5.2	2.40
3	15	66.2-87.3	0.6-1.3	$1.0\pm0.06$	-17.2 to -14.4	$-15.2\pm0.22$	8.4 to 12.0	$11.3\pm0.22$	4.5-6.0	5.2	2.53
4	17	88.6-107.9	1.3 - 2.0	$1.7\pm0.05$	-16.4 to -14.1	$-15.0\pm0.15$	9.3 to 13.0	$11.6\pm0.18$	4.8-6.2	5.4	1.55
5	15	110.4-128.0	2.0 - 2.7	$2.4\pm0.05$	-15.8 to -14.4	$-15.1\pm0.12$	9.6 to 12.4	$11.6\pm0.17$	4.7-6.1	5.4	0.86
6	19	131.8-151.1	2.8 - 3.6	$3.1\pm 0.05$	-16.2 to -14.4	$-15.1\pm0.13$	8.3 to 12.2	$11.0\pm0.28$	4.1–5.8	4.9	2.08
7	13	152.6-171.2	3.7-4.5	$4.1\pm0.08$	-15.9 to -14.3	$-15.1\pm0.14$	9.6 to 12.0	$11.1\pm0.18$	4.5-5.8	5.1	1.07
8	4	183.9–191.8	5.1-5.5	$5.2\pm0.09$	-15.6 to -14.4	$-15.0\pm0.26$	10.7 to 11.3	$11.0\pm0.12$	4.5–5.6	5.0	0.59

#### **Feeding ontogeny**

Vertebral collagen  $\delta^{13}$ C of *S. lewini* decreased along the vertebra (from 3 mm until 8 mm VR) (Fig. 35), while  $\delta^{15}$ N increased between 3 mm and 5 mm VR from the center of the vertebra, and then decreased at > 6 mm (Fig. 35). The estimated ages are presented in Table 21 according to each VR.



Figure 35. Isotopic enrichment (mean  $\pm$  SD) of <sup>13</sup>C (black) and <sup>15</sup>N (grey) in the scalloped hammerhead shark (*Sphyrna lewini*) vs vertebral radius, related to the values of the sample points relative to the 2-mm base point (n = 101).

# Niche width and isotopic overlap

The isotopic niche (SEA<sub>C</sub>) estimated for *S. lewini* was  $1.80\%^2$  at population-level, while the SEA<sub>C</sub> at the individual-level was between  $0.20\%^2$  and  $3.30\%^2$ , suggesting that 50% of the individuals had broad isotopic niches (>  $0.50\%^2$ ); while the rest of the individuals had narrow niches (<  $0.50\%^2$ ; Table 20).

Alternatively, the results for isotopic niche by estimated age suggested that the individuals between 5.1–5.5 years had the narrowest niches, followed by intermediate niches for individuals between 2.0–2.7 years and 3.7–4.5 years, and broad isotopic niches between 0.1–0.6 and 0.6–1.3 years (Table 21).

Based on the isotopic overlap, the individual LEW1 evidenced the lowest probabilities of overlap in both directions (< 50%), followed by the individual LEW2 (LEW2 vs all specimens; < 60%); while the other overlap combinations showed the highest probabilities of isotopic overlap (> 60%) (Fig. 36).



Figure 36. Isotopic overlap between individuals of the scalloped hammerhead shark (*Sphyrna lewini*) around Malpelo Island, Colombia.

Meanwhile, the isotopic overlap between ages suggested that the individuals of 5.1-5.5 years showed an overlap probability < 40% with respect to all age groups, while the other ages displayed overlap probabilities > 50% (Fig. 37). The probability of overlap between all ages generated the highest probabilities of overlap (> 70%) (Fig. 37).



Figure 37. Isotopic overlap between estimated ages of the scalloped hammerhead shark (*Sphyrna lewini*) around Malpelo Island, Colombia.

#### DISCUSSION

The investigation of feeding ontogeny using hard anatomic structures has allowed the integration of dietary information throughout the life cycle (Estrada et al., 2006; Kim et al., 2012), a feature that is typically difficult to determine in highly migratory species. The scalloped hammerhead *S. lewini* uses diverse coastal and oceanic feeding areas, which indicate a broad isotopic niche (SEA<sub>C</sub> =  $1.80\%c^2$ ), occupying different trophic levels from primary carnivores (TP 3.0) to tertiary carnivores (> 4.0) (Mearns et al., 1981). These results demonstrate an ontogenetic change in the use of habitat (from coastal to oceanic zones) and consumption of prey during their life cycle.

## **Feeding sources**

Different food webs make up the coastal and oceanic areas used by *S. lewini* as foraging areas along the ETP. The juveniles of the species mainly forage in coastal areas where they consume crustaceans (e.g., Penaeidos), planctivorous fish (i.e., Engraulidae, Carangidae, Haemulidae), benthic fish (e.g., Merluccidae, Paralychthidae, Synodontidae), and coastal cephalopods (e.g., Loliginidae, Octopodidae) (Estupiñán-Montaño et al., 2009; Torres-Rojas et al., 2013; Bornatowski et la. 2014; Flores-Martínez et al., 2016; Rosende-Pereiro et al., 2019); while adults prefer oceanic areas where they have access to larger prey (i.e., Scombridae, Ommastrephidae [Estupiñán-Montaño et al., 2009; Galván-Magaña et al., 2013]).

These food preferences could explain the origin of different sources of basal carbon, reflected in a depletion of  $\delta^{13}$ C associated with migrations form coastal areas to oceanic, pelagic or demersal areas (Cherel et al., 2008) which cause the ontogenetic changes. This is especially true at low latitudes, where C<sub>4</sub> biomass abounds on the continent and seagrasses are present, contributing to higher  $\delta^{13}$ C values at the base of the food chain (Pereira et al., 2007).

Our results suggested that the food sources of *S. lewini* correspond to trophic webs of the ETP and the Malpelo Islands, supported mainly by pelagic phytoplankton ( $\delta^{13}C = -22\%$  a -18%) and benthic macroalgae (-17%) (Peterson & Fry, 1987; France, 1993). The latter is supported by the values of  $\delta^{13}C$  from -17.2% to -14.1%, which after being corrected for the TDF of the vertebrae ( $\Delta^{13}C = 3.75\%$  [Hussey et al., 2010]), showed  $\delta^{13}C_{adjusted}$  values from -20.9% to -17.8%, similar to the basal sources of Malpelo Island (zooplankton: -21.9% to -17.8% and macroalgae *Padina* spp.: -21.0% to -17.1% [ver Capítulo 3.1]).

Isotopic studies in oceanic sharks that are highly migratory and that frequent oceanic islands (i.e., Galapagos Islands, Ecuador) along the ETP, such as the blue shark *Prionace glauca* (Carey et al., 1990; Vandeperre et al., 2014), produced values of  $\delta^{13}$ C from -16.8% to -13.1% (Estupiñán-Montaño et al., 2019). These values suggest that the base of the trophic chain demonstrates low latitudinal variation for the zooplankton of Malpelo Island, Colombia ( $\delta^{13}$ C =  $-20.7\pm1.48\%$  [ver Capítulo 3.1]) and the Galapagos Archipelago ( $-21.6\pm0.52\%$  [Paéz-Rosas et al., 2012], difference = 0.9%), and/or the use of similar feeding zones in the life cycle of both species along the ETP.

The similarities in the values of  $\delta^{13}$ C of *S. lewini* and *P. glauca* could reflect the use of a similar type of feeding area around Malpelo Island and the Galapagos Archipelago. The latitudinal differences in the basal  $\delta^{13}$ C between both top predators could reflect: 1) migratory processes of *S. lewini* (Bessudo et al., 2011a, b) and *P. glauca* (Carey et al., 1990; Vandeperre et al., 2014), and 2) the timeframe represented by vertebral rings (e.g., Kim et al., 2012). On the other hand, the differences in  $\delta^{15}$ N between both species was 4.2‰ (*S. lewini* = 11.3±0.09‰ and *P. glauca* = 15.5±0.20‰ [Estupiñán-Montaño et al., 2019]) which suggested: 1) the use of distinct ecosystems that correspond to different values of basal  $\delta^{15}$ N, with the difference related to oceanographic processes (e.g., upwelling, nitrification/denitrification, assimilation, currents, oxygen minimum layer [Olson et al., 2010; Casciotti et al., 2013; Martin et al., 2019]); 2) greater migratory routes (in distance) by *P. glauca* with respect to *S. lewini*, which permits the use of different geographical areas along the ETP; and 3) the use of different nursing areas (*S. lewini*: coastal zones [Quintanilla et al., 2015; Zanella et al., 2019] and *P. glauca*: oceanic zones [Kubodera et al., 2007]).

On the other hand,  $\delta^{13}$ C values in muscle samples suggested that *S. lewini* frequently uses oceanic zones to feed ( $\delta^{13}$ C<sub>muscle</sub> = -15.9‰ [Loor-Andrade et al., 2015] and -16.3‰ [Li et al., 2016; Estupiñán-Montaño et al., 2017]). These  $\delta^{13}$ C results are similar to those obtained for the interval of 0.1–0.6 years (-15.6‰), which reflect the maternal signature and diet (McMeans et al., 2009; Vaudo et al., 2010; Estupiñán-Montaño et al., 2019). These similarities could be supported by the rate of renewal of muscle tissue (≈11.3 and 18.2 months [MacNeil et al., 2005; Logan and Lutcavage, 2010]) as well as the gestation period of *S. lewini* (10–11 months [Torres-Huertas et al., 2008]). Finally, our results are supported by two factors: 1) migratory patterns of *S. lewini* between oceanic islands and the ETP (Bessudo et al., 2011a, b; Nalesso et al., 2019) as aggregation and feeding zones; and 2) the use of coastal zones as nursery areas (Quintanilla et al., 2015; Zanella & López-Garro, 2015), ecosystems which are characterized by the presence of mangroves and seagrass (Zanella et al., 2019) and continental C<sub>4</sub> biomass contributions. Both factors are confirmed by genetic connectivity between individuals of *S. lewini* samples from Malpelo Island and the provinces of Choco and Nariño, Colombia (Quintanilla et al., 2015), which represent 22.9% and 52.3% of the mangroves in the Colombian Pacific (282835 Ha [Ulloa-Delgado et al., 2004]). These results exemplify the role of Colombian mangroves in the ontogeny of *S. lewini*.

# **Trophic position**

Similar to other sharks, *Sphyrna lewini* is considered to be a species that occupies high positions in the trophic webs of the ETP (3.8–5.9 [Liu et al., 2016; Estupiñán-Montaño et al., 2017]). These observations are consistent with the wide range of TPs (2.9–6.5) estimated in this study.

The different TPs that the juveniles of *S. lewini* occupy throughout their life cycle are reflected in the consumption of prey from low trophic levels (e.g., penaeidos, engraulidos, clupeidos, hemiranfidos [Estupiñán-Montaño et al., 2009; Torres-Rojas et al., 2013; Flores-Martínez et al., 2016]) and some mid-level predators (e.g., Scombridae, Lutjanidae, Ommastrephidae [Estupiñán-Montaño et al., 2009; Galván-Magaña et al., 2013; Torres-Rojas et al., 2013; Flores-Martínez et al., 2016]). While the high TPs (TP > 6.0) are related to the feeding behavior of *S. lewini* adults, which can consume other elasmobranches (Bethea et al., 2004; Bornatowski et al., 2014), along with the consumption of prey from different trophic webs with a  $\delta^{15}$ N-enriched baseline (Vanderklift & Ponsard, 2003; Graham et al., 2010; Tamburin et al., 2019) that could be reflected in the chemical composition of the vertebrae (Estupiñán-Montaño et al., 2019); however, more studies would be necessary to obtain more precise information.

The results of this study suggest that *S. lewini* plays different trophic roles (from primary consumer to tertiary consumer) throughout its life cycle, consuming prey from trophic webs with different  $\delta^{15}N$  baselines; these can change over time due to environmental and/or oceanographic factors (*e.g.*, upwelling, currents), and feeding zones (e.g., oceanic zones, mangroves, reefs), etc. For instance, relatively anoxic zones experience a reduction of NO<sub>3</sub><sup>-</sup> generating residual nitrates enriched in <sup>15</sup>N (Granger et al., 2008) and/or upwelling oceanic areas that could reflect  $\delta^{15}N$  values

of 5–8‰ (Sigman et al., 1997). In the case of Malpelo Islands, upwelling during the whole year (Rodríguez-Rubio & Stuardo, 2002) would favor <sup>15</sup>N-enrichment from deep waters rich in nutrients (Bauersachs et al., 2009). Nonetheless, the absence of fractionation due to assimilation (by the phytoplankton, not depleting nitrates) and the abundance of herbivorous prey would explain the relatively low values of  $\delta^{15}$ N in the vertebrae of *S. lewini* (7.6–13.0‰).

In contrast, the use of habitat occupied by diazotrophic cyanobacteria communities (range: – 5‰ to +2‰ [Bauersachs et al., 2009]), such as the mangrove zones, present depleted  $\delta^{15}$ N values (i.e., detritus: –0.9±0.5‰, leaves: 1.8±0.6‰, sediment: –0.1±0.5‰, and seston: 1.8±1.1‰ [Medina et al., 2018]). The Colombian mangroves are used as nursery areas by *S. lewini* (Quintanilla et al., 2015), where they spend their two first years of life (Zanella et al., 2019). The use of mangroves as nursery areas by *S. lewini* supports the low values of  $\delta^{15}$ N in the vertebrae, which is a reflection of the consumption of coastal prey from low trophic levels, i.e., coastal crustaceans (Estupiñán-Montaño et al., 2009; Flores-Martínez et al., 2016). For example, after being corrected with the TDF, individuals LEW1 and LEW2 showed  $\delta^{15}$ N<sub>corrected</sub> values of 7.8‰ and 7.9‰, respectively, which are relatively similar to the snapping shrimps *Alpheus colombiensis* (6.4±0.7‰ SD [Medina et al., 2018]). These values could be reflecting the use of mangroves as feeding areas for this species. Therefore, the consumption of prey from low trophic levels, the use of mangroves as nursing areas, the consumption of oceanic prey from high trophic levels, and the individual foraging strategies of the species (Fig. 34), allow *S. lewini* to have a diversity of roles in the coastal and oceanic trophic chains of the ETP.

However, more studies concerning the base line along the ETP are necessary given the oceanographic conditions of these zones (e.g., upwelling, currents, etc. [Rodríguez-Rubio & Stuardo, 2002; Rodríguez-Rubio et al., 2007]) which could generate a higher variability in the basal isotopic signals.

# Bayesian mixing models and prey selection

Some crustaceans, teleosts and cephalopods of coastal zones are important prey for juveniles (Torres-Rojas et al., 2008; Flores-Martínez et al., 2016) and adult females (Estupiñán-Montaño et al., 2009). Our results support these conclusions, because this study found that coastal crustaceans made the highest dietary contribution, followed by oceanic cephalopods, mangrove fish, and coastal cephalopods (Fig. 33 and 34). This highlights the importance of coastal crustaceans and

mangrove fish in the diet and habitat selection of *S. lewini*. Accordingly, the input of these two prey groups (crustaceans and mangrove fish) in the diet of *S. lewini* could be related to: 1) feeding behavior of neonates and small juveniles that feed in coastal zones (Flores-Martínez et al., 2016) and nursing areas (mangrove zones), in which they spend their first two years of life (Zanella et al., 2019); 2) availability of accessible prey for juveniles that are adjusting their ability to capture prey; and 3) adult females consuming coastal and benthic prey (Estupiñán-Montaño et al., 2009). These results demonstrate the importance of the Colombian mangrove ecosystems in the ontogeny of *S. lewini*.

The low values of  $\delta^{15}$ N observed in the vertebrae and the isotopic enrichment observed between the first millimeters of the vertebral radius (3-5 mm VR; Fig. 36), could be a reflection of processes associated with maternal transfer processes (inherited proteins [Estupiñán-Montaño et al., 2019]) and the scavenger consumption of high trophic prey hunted by adult females. The explanation to these findings could be due to maternal (Fuller et al., 2004) or embryonic (Vander Zanden et al., 1998) changes during two stages of the gestation period (10-11 months [Torres-Huerta et al., 2008]) of S. lewini. Thus, the first stage would correspond to the initiation of the gestation period, and in this stage, pregnant females spend more time in oceanic areas, consuming prey with a high protein content (e.g., Dosidicus gigas: 78.3-78.5% [Ochoa-Tepelta 2014]). While, in the second stage, pregnant females move towards coastal areas (e.g., nursing areas), where there is a higher availability of prey and easier access for feeding (Estupiñán-Montaño et al., 2009; Torres-Rojas et al., 2008; Flores-Martínez et al., 2016), which would allow them to reduce efforts in the search for food. In this way, the saved energy would be routed to the development and nutrition of the final stage of the embryos, reflected in the depleted  $\delta^{15}N$  from the food webs of the mangrove zones (Medina et al., 2018). Another explanation is their strategy of favoring small, normally herbivorous prey from lower trophic levels.

The mixing model applied to individuals did not evidence an individual feeding pattern in *S. lewini*, unlike in Ecuadorian waters (Loor-Andrade et al., 2015). The observed discrepancies between studies could be related to: 1) the integration of the maternal isotopic signature in the vertebrae of each individual (inherited proteins), reflecting the consumption of coastal prey (i.e., crustaceans and mangrove fishes); 2) migratory movements between coastal and oceanic zones, where they consume prey with a high caloric content (i.e. oceanic cephalopods [Ochoa-Tepelta

2014]); and 3) high isotopic overlap between individuals, suggesting the consumption of similar prey and feeding areas.

#### Feeding ontogeny and habitat use

The studies of the trophic ecology of *S. lewini* suggest changes in the consumption of prey and habitat use changes that are associated with different life cycle stages (Loor-Andrade et al., 2015). Neonates and small juveniles of *S. lewini* prefer to consume small and medium sized prey associated with coastal areas (e.g., Torres-Rojas et la. 2013, Rosende-Pereiro et al., 2020). These observations are confirmed by the  $\delta^{13}$ C values (-17.2% to -14.1%) of the ages 2.0–2.7 and 5.1–5.5, which suggest that *S. lewini* searches for food mainly in highly productive areas, such as mangrove zones (Fig. 34B) along the Colombian Pacific coast. On the contrary, the intermediate ages (2.8–5.0 years) were associated with the use of zones of lesser productivity (i.e., oceanic, pelagic, and benthic; 34B), where they consume larger prey (Estupiñán-Montaño et al., 2009; Galván-Magaña et al., 2013).

Accordingly, the preferences in habitat use and prey consumption of S. lewini highlighted changes in their trophic ecology in relation to age, and supported by SCA (e.g., Estupiñán-Montaño et al., 2009; Rosende-Pereiro et al., 2020) and the findings of the mixing models (Fig. 4), which suggest that such ontogenetic change is carried out at approximately two years of age (Fig. 34B & 35). The explanation for the changes in the use of habitat and consumption of prey of S. lewini could be due to 1) the use of nursery areas for a two-year period (Zanella et al., 2019); 2) abundance and availability of prey; 3) body development and prey-capture skills (Lowe et al., 1996); 4) multiple visits at intermediate ages (1.3–4.5 years) to the nursery areas (Bessudo et al., 2011a,b); and 5) pregnant females spend more time in coastal zones (see section Bayesian mixing models and prey selection) because these areas provide them with sufficient food for the development of their pups, as well as safe zones for giving birth (Estupiñán-Montaño et al., 2019). Therefore, our results suggest that S. lewini has a "cyclical" type of habitat use, with individuals between 0-2 years old using coastal zones as important feeding areas, where they have easy access to small coastal prey (benthic and pelagic). From approximately 2 years of age they migrate to oceanic areas changing their dietary preferences form small prey to large squid (e.g., Ommastrephidae), and finally, return to coastal areas as adults (> 4 years), which is potentially

related to the search for important areas to ensure giving birth to, feeding, and protecting their young (i.e., mangrove areas).

Additionally, we found similar contributions of crustaceans to the diet of individuals of 0.1–1.3 years and 3.7–5.5 years (Fig. 34B) that could be the reflection of maternal transfer processes (McMeans et al., 2009; Vaudo et al., 2010). This hypothesis is partially supported by the isotopic enrichment observed between the 3- and 5-mm VR (Fig. 35). The isotopic enrichment analysis did not evidence a clear pattern for <sup>13</sup>C along the vertebrae (Fig. 35). The reason for these results could be related to: 1) the use of the same feeding areas by juveniles younger than two years of age and pregnant females during the second phase of the gestation period; and 2) sporadic visits of juveniles over two years of age to the nursery areas (Zanella et al., 2019). This would cause the maternal  $\delta^{13}$ C present in *S. lewini* juvenile vertebrae to remain combined (maternal transfer); however, it can be suggested that the metabolic turnover result in the maternal carbon isotopic signal is being "diluted" between 3.1–4.5 years (6–7 mm VR; Fig. 35). In contrast,  $\delta^{15}$ N values presented a higher enrichment between 0.6-2.7 years (3-5 mm VR; Fig. 35). This higher enrichment is the reflection of prey from high trophic levels (e.g., Ommastrephidae) consumed by the mothers during the gestation period (see above). The results for adult individuals indicate a turnover in the vertebrae proteins that results in the primary signals of maternal  $\delta^{15}$ N being "diluted" between 2.0–3.6 years (5–6 mm VR; Fig. 35), which suggest that S. lewini would reflect the isotopic signal of the life cycle of its prey and its age when it was consumed, which is also consistent with the time they spend in the breeding areas (~2 years [Zanella et al., 2019]).

### Niche and isotopic overlap

The SEA<sub>C</sub> estimations indicated that a high number of individuals presented broad isotopic niches (SEA<sub>C</sub> >  $0.70\%c^2$ ; Table 20 and 21). These results, along with the low probability of individual isotopic overlap (Fig. 6) suggest low interspecific competition and a high degree of specialization. This behavior was observed for *S. lewini* in Ecuadorian waters (Loor-Andrade et al., 2015), as well as for the white shark (Kim et al., 2012). These characteristics can be attributed to: 1) changes in prey preference and foraging locations (Kim et al., 2012) in relation to ontogeny (Kim et al., 2012, Loor-Andrade et al., 2015); 2) consumption of the same prey in the same area (Kim et al., 2012); and 3) the consumption of the same prey in the same area or the combination of different types of prey in different localities (Kim et al., 2012). These explanations are

confirmed by the high contribution of coastal crustaceans, mangrove fish, and oceanic cephalopods in individuals (Fig. 3), associated with vertical and horizontal migratory movements (Bessudo et al., 2011a, b).

Moreover, the isotopic niches estimated by age group suggest that juveniles (2.0–2.5 years) and adults (> 5.0 years) occupy narrow niches. These results are a reflection of the migration by pregnant females towards coastal areas to consume prey that are easier to capture and with a high protein content (i.e., shrimp: 82–86% [Rivas-Vega et al., 2001]), as well as the use of nursery zones, as these are places where neonates can have access to abundant and easily captured prey (e.g., crustaceans, mangrove fish, Fig. 35). Conversely, the intermediate ages (2.7–5.0 years) occupied broad isotopic niches (SEA<sub>C</sub> > 0.90; Table 21). These results suggest the exploration and use of new feeding areas and the consumption of a higher diversity of prey. This behavior is associated with morphological changes and the ability to capture potential prey.

#### CONCLUSIONS

This investigation is the first study to describe in detail the ontogenetic trophic ecology of *Sphyrna lewini* of Malpelo Island based on vertebral  $\delta^{13}$ C and  $\delta^{15}$ N values, demonstrating ontogenetic changes in the diet and habitat use at the population level. The results of the study showed that *S. lewini* occupies a wide trophic niche as a result of the use of different habits (coastal and oceanic) and the consumption of prey from different trophic levels throughout its life cycle. These changes reflect a migration from coastal to oceanic zones in juveniles (~2–4 years old), and their return to coastal habitats as adults (> 4 years), potentially related to the use of coastal zones (i.e., mangroves) in the eastern tropical Pacific, both as important feeding areas for neonates and feeding/pupping grounds for adults.

Finally, the enrichment of <sup>15</sup>N in the vertebrae reflects the maternal transfer of nutrients during pregnancy, particularly in the first stages of life. Thus, the isotopic signal could be an indicator of the trophic level of their mothers. In contrast, the <sup>13</sup>C cannot be used in a similar way due to the use of similar feeding zones by neonates and adults. Metabolic turnover processes contribute to a loss of the original isotopic signal. The maternal  $\delta^{13}$ C could be "diluted" between 3.6–4.5 years and the maternal  $\delta^{15}$ N is "diluted" between 2.0–3.6 years of age (Fig. 36). Accordingly, the isotopic signal of neonates and juveniles of *S. lewini* could also be an indicator of prey sources used by their mothers and their trophic level (Estupiñán-Montaño et al., 2019). Therefore, the isotopic

signals of *S. lewini* juveniles should be interpreted with caution when making dietary inferences in this species.

Additionally, the estimates of relative contribution (mixing models) and TPs must be applied with caution due to the uncertainty that can be produced when using diverse TDFs (Hussey et al., 2013; Parnell et al., 2014), particularly when the species demonstrates ontogenetic changes (Hussey et al., 2013).

The results of this study have provided additional information that helps to reduce knowledge gaps pertaining to *S. lewini* along the ETP, and in particular highlights the importance of the mangrove areas of the Colombian Pacific as significant feeding sites for neonates, juveniles, and adults of the species. The use of anatomically hard structures (i.e., vertebrae) is of particular significance, as it integrates information on the dietary ontogeny of shark species throughout its life cycle and provides a better understanding of the trophic characteristics of the species, as well as the variety of roles that they can play in different marine ecosystems

# 5.2. Feeding ontogeny of the silky shark, *Carcharhinus falciformis*, in the eastern tropical Colombian Pacific

#### INTRODUCTION

Temporal, spatial, and behavioral changes in feeding are an inherent characteristic in several shark species (Méndez-Macías et al., 2019; Estupiñán-Montaño et al., 2019, 2021a, b, c). These changes are associated with different aspects of the species life cycle and can generate modifications in community structures (Estrada et al., 2006) and trophic dynamics (Polis et al., 1996; Polis and Strong, 1996). Ontogenetic changes of a species provide information about the niche, ecological role of a species in the ecosystem (Braga et al., 2012), identification of essential areas (Estupiñán-Montaño et al., 2021a, c), as well as specific habitats for each life stage (Loor-Andrade et al., 2015, Estupiñán-Montaño et al., 2021a, b, c). Thus, ontogenetic development should be considered in the management and conservation strategies of organisms (Bethea et al., 2004; Kinney and Simpfendorfer, 2009).

Studies on trophic role and changes in feeding behavior of some shark species have been based on stomach content analysis (SCA; Polo-Silva et al., 2013; Méndez-Macías et al., 2019) and stable isotope analysis (SIA; Li et al., 2014; Páez-Rosas et al., 2018). SCAs generate information on recently consumed prey, in contrast, SIAs reveal the food synthesized in the different tissues of the animal, reflecting different temporal and spatial scales of trophic aspects (Kim et al., 2012). In this way, information is generated on habitat use, migration, food preferences, trophic niche, resource partitioning, trophic position (Post, 2002; Graham et al 2010; Jackson et al., 2021b) and feeding patterns (Estupiñán-Montaño et al., 2021c). Although structures formed by accretion (i.e., vertebrae, otoliths, statoliths), they preserve the trophic chronology (diet and habitat use) of species during their life cycle (Kim et al., 2012; Estupiñán-Montaño et al., 2019; 2021a) they have been little studied.

The silky shark, *Carcharhinus falciformis*, is an oceanic-coastal and circumtropical species (Compagno, 1984) with a geographic distribution in the Eastern Tropical Pacific (ETP), found from the southern portion of the Baja California Peninsula to Peru and in different oceanic islands (Compagno, 1984). *C. falciformis* is characterized by reaching sizes up to 310 cm total length (TL) (Martínez-Ortiz et al., 2007) with females and males maturing at 190 cm TL and 180 cm TL,

respectively (Galván-Tirado et al., 2015). This species is placentate viviparous with low fecundity rate (2–14 embryos), a long gestation period (18–20 months) and a birth size between 60 and 69 cm LT (Galván-Tirado et al., 2015), characteristics that make it vulnerable to overfishing (Schaefer et al., 2021). Its preference for oceanic areas (Li et al., 2014; Estupiñán-Montaño et al., 2017a, b; Páez-Rosas et al., 2018) and affinity to floating objects in the ETP (Filmater et al., 2011, 2016; Duffy et al., 2015), have led to this species being incidentally caught by gillnets, longlines and purse seines (Soriano et al., 2006, Bonfil, 2008, SEMARNAT, 2018). It is listed as a Vulnerable species in the International Union for Conservation of Nature (IUCN) Red List as a Vulnerable species of Wild Fauna and Flora in Appendix II (CITES, 2016). Despite this, and its importance as a top predator in the ETP (Li et al., 2014; Estupiñán-Montaño et al., 2017a, b; Páez-Rosas et al., 2018), studies on its biology and ecology are still scarce, including its feeding ontogeny.

The SCA in *C. falciformis* has evidenced that this species feeds on a wide variety of prey of both coastal and oceanic origin, consuming crustaceans, cephalopods, fish, and occasionally sea turtles (Cabrera-Chávez-Acosta et al., 2010; Duffy et al., 2015; Estupiñán-Montaño et al., 2017a; Flores-Martínez et al., 2017; N'Gouan et al., 2021). In addition, it is considered a consumer that can occupy high levels in ETP food webs in its juvenile and adult stages (Li et al., 2014; Estupiñán-Montaño et al., 2017a; Flores-Martínez et al., 2017; Páez-Rosas et al., 2018), showing specialist habits (Cabrera-Chávez-Acosta et al., 2010; Estupiñán-Montaño et al., 2017b) and generalists (Flores-Martínez et al., 2017); but, with high preference for the consumption of epipelagic and mesopelagic fishes (Ménard et al., 2013; Duffy et al., 2015; Estupiñán-Montaño et al., 2017b).

The SIA ( $\delta^{13}$ C and  $\delta^{15}$ N) in muscle tissue (Rau et al., 1983; Rabehagasoa et al., 2012; Li et al., 2014; Estupiñán-Montaño et al., 2017a, b; Páez-Rosas et al., 2018) have suggested preference for the use of oceanic and coastal zones ( $\delta^{13}$ C: -18.0 a -15.7‰ [Rau et al., 1983; Robehagasoa et al., 2012; Li et al., 2014; Estupiñán-Montaño et al., 2017a, Páez-Rosas et al., 2018]), reflecting narrow (Estupiñán-Montaño et al., 2017a) and broad (Páez-Rosas et al., 2018) isotopic niches and high trophic positions (4.1–5.8 [Li et al., 2014; Estupiñán-Montaño et al., 2014; Estupiñán-Montaño et al., 2014; Estupiñán-Montaño et al., 2014; Estupiñán-Montaño et al., 2015).

Although there is information on the trophic ecology of *C. falciformis* along the ETP, there is limited understanding of the role and use of habitat throughout the life cycle of this species and how these changes are related to the different ETP food webs (e.g., oceanic and coastal webs).

This study aims to analyze changes in feeding and habitat use of C. falciformis in the Colombian ETP, using  $\delta^{13}$ C and  $\delta^{15}$ N in low turnover rate tissues such as vertebrae. This study will generate information on ontogenetic profiles in the feeding of *C. falciformis* in different stages of its life history, based on the analysis of vertebrae, since this tissue, being a structure that forms growth rings, allows us to have a historical record of food preferences and habitat use of the species, thus, expanding the knowledge of the trophic ecology of *C. falciformis* in the ETP and identifying potential feeding areas that may be essential for early life stages (e.g., potential breeding areas).

# **MATERIALES Y MÉTODOS**

#### Study area

Malpelo Island (Fig. 4A) is the summit of a submarine mountain range called the Malpelo Ridge, which extends in a NE-SW direction; it is approximately 241.4 km long by 80.5 km wide (Fig. 4B, red polygon). The island has a maximum height of 300 m above sea level and emerges from approximately 4,000 m depth (Fig. 4C) (more details in Chapter 1).

# Sample collection

In 2013, a total of 12 silky sharks *C. falciformis* were confiscated by illegal fishing operations in the Malpelo Fauna and Flora Sanctuary, Colombia (Fig. 4; see Chapter 1). For each specimen, total length (TL, in cm) and sex were recorded. Vertebrae were collected from the dorsal-anterior part of each shark (between the head and the first dorsal fin) as suggested by Estupiñán-Montaño et al. (2019, 2021a), because vertebrae from this part of the body are recommended for age and growth studies (e.g., Anislado-Tolentino and Robinson-Mendoza, 2001). Once the vertebrae were collected, they were stored in plastic bags previously labeled and kept on ice for further processing.

The vertebrae were completely cleaned, removing the neural arch and connective tissue. Then, the vertebrae were dried at room temperature for further preparation (Estupiñán-Montaño et al., 2019, 2021a).

#### Sample preparation and analysis

With the help of a micro drill and a 0.5-m diameter drill bit, one vertebra of each *C. falciformis* individual was systematically drilled (from the center to the periphery) with a distance of ~1 mm

(Estupiñán-Montaño et al., 2019, 2021a). From each vertebral puncture site, two sets of vertebral collagen subsamples were obtained, thus, one set of samples was analyzed with pretreatment for the removal of inorganic carbon and the other set without any treatment, to avoid any effect on nitrogen values (Christiansen et al., 2014).

To remove residual inorganic carbon the first set of samples were weighed between 0.31–0.80 mg and placed in a desiccator with 37% HCl vapor for a period of 12–24 h (Hedges and Stern, 1984), recording %C<sub>total</sub> between 0.73–14.61% (mean  $\pm$  SD: 7.65  $\pm$  3.68%). The second set of samples analyzed without pretreatment weighed between 0.25–0.64 mg, reflecting %C<sub>total</sub> between 11.99–53.51% (15.43  $\pm$  5.81%). Both sets of vertebral collagen samples were stored in 3.2 × 4-mm tin capsules for isotopic analysis.

The SIA was carried out in theLaboratorio de Biogeoquímica de Isótopos Estables del Instituto Andaluz de Ciencias de la Tierra en Granada (CSIC–UGR), España (more details in Capítulo 1).

#### **Sources food**

To infer the different feeding grounds used by *C. falciformis* in different isotopic spaces of the ETP,  $\delta^{13}$ C and  $\delta^{15}$ N values were obtained from several primary producers and other basal sources (Table 22). The  $\delta^{13}$ C and  $\delta^{15}$ N values of vertebral collagen were corrected following Logan et al. (2020) and Sandoval-Londoño et al. (2022):

$$\Delta^{13}$$
C and  $\Delta^{15}$ N<sub>Corrected</sub> = TP<sub>Silky</sub> × TDF - 1

Where TP<sub>Silky</sub> is the trophic position of each age group estimated in this study, TDF are the trophic discrimination factor values for carbon and nitrogen ( $\Delta^{13}C = 3.75 \pm 0.44\%$  and  $\Delta^{15}N = 1.45 \pm 0.61\%$ ; respectively [Hussey et al., 2010]). The  $\Delta^{13}C$  and  $\Delta^{15}N$  are the corrected carbon and nitrogen values for each sample. Then, the corrected  $\delta^{13}C$  and  $\delta^{15}N$  values were compared with those values of different basal sources obtained in the ETP, and thus, inferring about potential base sources incorporated in the vertebrae of *C. faciformis*, so that these are considered as an indicator of the use of different food webs.

Sources	$\delta^{13}C\pm SD$	$\delta^{15}N\pm SD$	References
Phytoplankton Continental Ecuador	$-22.0\pm0.30$	$8.4\pm0.96$	Calle-Morán (2010)
Phytoplankton Galapagos Island	$-22.1\pm0.35$	$7.4\pm0.23$	Páez-Rosas et al. (2021)
Phytoplankton Malpelo Island	$-18.6\pm1.79$	$6.0\pm1.06$	This study
Phytoplankton Continental Colombia	$-27.1\pm1.49$	$2.2\pm1.50$	Medina-Contreras et al. (2018, 2020)
Detritus Continental Colombia	$-28.5\pm0.60$	$\textbf{-0.9} \pm 0.50$	Medina-Contreras et al. (2018)
Sponges Malpelo Island	$-15.9\pm0.54$	$7.2\pm1.97$	Este estudio
Green algaes Malpelo Island	$-18.7\pm1.59$	$5.2\pm0.47$	Chapter 3
Brown algaes Malpelo Island	$-17.2\pm1.13$	$7.3\pm.78$	This stady
Microphytotobenthos Colombia continental	$-20.6\pm1.7$	$2.7\pm1.1$	Medina-Contreras et al. (2020)

**Table 22.** Some basal sources of pelagic and coastal food webs of the Colombo-Ecuadorian Pacific, represented by mean values  $\pm$  standard deviation (SD) of  $\delta^{13}$ C and  $\delta^{15}$ N isotopes and the bibliographic references from which they were obtained.

# Size and age estimation

To estimate the total length (LT<sub>Estimated</sub>) and age (Age<sub>Estimated</sub>) of each individual, the distance in millimeters of each vertebral sampling perforation was measured, taking the center of the vertebra as the starting point. In this way, different vertebral perforations were obtained, represented as vertebral radius (VR). LT was estimated by separate sexes using the equations:  $LT_{Females} = 17.22 + 24.74 \times VR$  (R<sup>2</sup> = 0.95, *n* = 207 [Gilces-Anchundia, 2013]) and  $LT_{Males} = 16.38 + 31.20 \times VR$  (R<sup>2</sup> = 0.93, *n* = 234 [Gilces-Anchundia, 2013]). In addition, the age (*t*) of each RV (i.e., each millimeter) was estimated with the von Bertalanffy growth function, applying the following formula:

$$t = -\frac{\ln\left(1 - \frac{L_t}{L_{\infty}}\right)}{K} + t_0$$

Where  $L_t$  is the estimated length at age t, L is the asymptotic mean length, K is the growth rate (years<sup>-1</sup>), and  $t_0$  is the theoretical age at which the shark had zero length. Also, age was estimated for each RV by separate sexes. For this purpose, the parameters of the von Bertalanffy growth function were obtained from the Gilces-Anchundia (2013) study (Table 23).

 Tabla 23. Parameters of the von Bertalanffy function to estimate the age of the silky shark Carcharhinus falciformis in the Ecuadorian Pacific, obtained from Gilces-Anchundia (2013).

Parameters	$L\infty$ (in cm)	<i>K</i> (in years)	$t_0$ (in years)
Females	335	0.06	-2.91
Males	326	0.06	-2.85

#### **Feeding ontogeny**

To infer feeding ontogeny processes in *C. falciformis*,  $\delta^{13}$ C and  $\delta^{15}$ N values were analyzed by TL<sub>Estimated</sub> and age<sub>Estimated</sub> for each individual, which were obtained from each VR (see above). Once the TL/Ages<sub>Estimated</sub> were obtained, the sexual maturity stages were determined based on the mean length at maturity (TL<sub>50</sub>) estimated by Galván-Tirado et al. (2015), this being the closest available reference to the study area. Thus, the TL<sub>50</sub> for females and males was 190 cm TL (*n* = 115) and 180 cm TL (*n* = 140), respectively. Therefore, maturity stages were grouped into four categories: juvenile females (34.5–187.7 cm TL), adult females (210.7–316.6 cm TL), juvenile males (57.6–172.4 cm TL) and adult males (202.3–241.3 cm TL).

Once the LT/Ages were estimated, age classes were constructed following the Sturges rule, which allows to improve the representativeness of the data, by applying the following formula:

$$k = 1 + 3.322 \times log_{10}(n)$$

Where k is the number of classes or intervals, n is the total number of samples,  $Log_{10}$  is the common logarithm with base 10. The width of the class interval was estimated as follows: A =  $(\text{Limit}_{\text{Upper}} - \text{Limit}_{\text{Lower}}) / \text{k}$ . Where A is the class or interval width,  $\text{Limit}_{\text{Upper}}$ . and  $\text{Limit}_{\text{Lower}}$ . are the maximum and minimum ages for each RV, respectively, and k is the number of classes or intervals obtained with the Sturges method. Accordingly, eight age groups were obtained: Embryos (-1.1 years), 0.0–2.9 years, 3.0–5.9 years, 6-0–8.9 years, 9.0–11.9 years, 12.0–14.9 years, 15.0–17.9 years, and 18.0–>20.0 years.

#### **Trophic position**

The trophic position (TP) of *C. falciformis* was estimated by categories (individuals, sexes, maturity stages, and estimated length/age associated with vertebral radius [VR]), from the implementation of a Bayesian approach (*tRophicPosition* package, version 0.7.5 [Quezada-Romegialli et al., 2018]) in the R statistical environment (R Development Core Team, 2018). PTs

were estimated with the  $\delta^{13}$ C and  $\delta^{15}$ N values of *C. falciformis* (mixture); while isotopic assignments of pelagic phytoplankton ( $\delta^{13}$ C =  $-18.6 \pm 2.99\%$  SD and 15N =  $6.0 \pm 1.06\%$  SD [this study]) and brown algae Padina sp. ( $\delta^{13}$ C =  $-18.7 \pm 1.58\%$  SD and  $\delta^{15}$ N =  $5.2 \pm 5.17\%$  SD [Estupiñán-Montaño et al., 2021a]) from Malpelo Island, Colombia, were used as a baseline. A Bayesian model of two baselines ("*twoBaselines*") and two trophic discrimination factors (TDFs) were performed with 4 Markov Chain Monte Carlo (MCMC) and 20000 iterations adaptations, assuming a baseline ( $\lambda$ ) = 1. TPs values of *C. falciformis* were determined using TDFs for specific tissues (i.e., vertebrae) of species with "equivalent" feeding habits and high phylogenetic relatedness (Estupiñán-Montaño et al., 2021a) to C. falciformis, such as the lemon shark Negaprion brevirostris (Vélez-Zauzo and Agnarsson, 2011) with values of  $\Delta^{13}$ C =  $3.75 \pm 0.44\%$  and  $\Delta^{15}$ N =  $1.45 \pm 0.61\%$  (Hussey et al., 2010).

The estimated PTs were classified into trophic levels (TL) according to their food preferences: TL-II; herbivores (2.0-2.1), TL-III; omnivores with vegetable preference (2.1< TP <2.9), NT-VI; omnivores with animal preferences (2.9< TP <3.7), NT-V; carnivores with preferences for large decapods, cephalopods and fish (3.7< TP <4.0) and NT-VI; top predators (4.0 < TP) (Stergiou and Karpouzi, 2002).

#### Niche breadth and isotopic overlap

Isotopic niche was quantified for individuals and age groups, using the Stable Isotopic Bayesian Ellipses (SIBER) method in R (Jackson et al., 2011). This analysis is based on calculated ellipses from a covariance matrix, which defines its forms and areas (Jackson et al., 2011) to estimate the width of the isotopic niches (Standard Ellipse Corrected Area, SEA<sub>C</sub>).

The isotopic overlap was estimated using the nicheROVER package in R (Lysy et al., 2014), which is a Bayesian method that calculates the probability of overlap between niche pairs using multidimensional information as niche indicators (e.g., stable isotopes, environmental variables). The probabilistic density of niche overlap was calculated by running  $10^4$  iterations and 95% of the data from each species or group occurring within their isospaces, providing directional niche overlap estimates (e.g., *x* vs *y* and *y* vs *x*), according to the distributions of a specific species in the multivariate niche space (Lysy et al., 2014).
### Statistical analysis

Normality and homoscedasticity of the isotopic data were tested with Shapiro-Wilk and Levene's tests, respectively. Analyses of variance were used to test for isotopic differences between categories. If the samples were normal and homocedastic parametric analyses were applied (i.e., One-way Analysis of Variance - ANOVA and Student's *t*-test); otherwise, non-parametric analyses (i.e., Kruskal-Wallis test, Wilcoxon signed-rank test) were used to test isotopic differences between categories (individuals, sexes, lengths/ages, etc.). A post-hoc test of multiple comparisons (i.e., pairwise *t*-test [parametric] and/or Pairwise Wilcoxon rank sum test [non-parametric]) was designed to identify specific differences between categories.

## RESULTS

From the systematic sampling of vertebrae from 12 *C. falciformis* (FAL#; individual ID) with lengths between 34.5 and 316.6 cm TL (Table 24) and estimated ages between –1.1 and 45 years, a total of 61 vertebral collagen samples were obtained and analyzed (Table 24).

#### **Sources food**

The  $\delta^{13}$ C values of the data set were distributed between -17.8% and -14.0% (mean ± ES;  $-15.5 \pm 0.08\%$ , V-PDB) (Shapiro, p = 0.05; Levene, p = 0.37). Individual analysis indicated that FAL1, FAL2 and FAL7 showed the lowest  $\delta^{13}$ C values, while individuals FAL12 and FAL5 reflected the highest values (Table 24). Despite this, no statistical differences were detected between individuals (Kruskal-Wallis, p = 0.39).

Regarding sex, females (-15.5  $\pm$  0.10% [range: -17.8% to -14.2%]; n = 39) and males (-15.5  $\pm$  0.15% [-16.8% to -14.0%]; n = 22) presented similar  $\delta^{13}$ C values (Wilcoxon rank sum test, W =431.5, p = 0.98), suggesting similar habitat use.

On the other hand, the  $\delta^{13}C_{corrected*TP}$  (-18.5% and -15.5%) and  $\delta^{15}N_{corrected*TP}$  (5.4% and 6.1%) values aligned with some of the basal sources in the ETP (Fig. 38A and B). These results suggest that the basal sources on Malpelo Island (i.e., brown and green algae, sponges, and phytoplankton) are in the  $\delta^{13}C_{corrected*TP}$  and  $\delta^{15}N_{corrected*TP}$  ranges of *C. falciformis* (Fig. 38). These results suggest that *C. falciformis* includes Malpelo Island and its surroundings as feeding grounds at some stage of its life.



Figure 38.  $\delta^{13}$ C (panel A) and  $\delta^{15}$ N (panel B) values corrected with the trophic discrimination factor and trophic position of silky shark *Carcharhinus falciformis* in the Eastern Tropical Pacific (ETP), compared with basal sources from different areas of the ETP and the surroundings of Malpelo Island.

#### Food ontogeny

The analysis by estimated ages suggests that *C. falciformis* from the groups: "Embryos" and 6.0–8.9 years presented the lowest  $\delta^{13}$ C values with respect to the other age groups (Fig. 39; Table 25). However, there was no statistical difference between them (Kruskall-Wallis, p = 0.37). While the 15N showed that sharks from the "Embryos" and age 0.0–2.9 years groups presented the lowest values, but without showing statistical differences with the other groups (Fig. 39; Table 25; Kruskall-Wallis, p = 0.22).

		TI	Aga	$\frac{[SEA_C]}{\delta^{13}C}$		$8^{15}N(\%)$		Trophic position		SEA
Ind.	п	1 L	Age	0 C (760)		0 <sup>-</sup> IN (700)		riopine position		SEAC
		(cm)	(years)	Range	$Mean \pm SE$	Range	$Mean \pm SE$	IC 95%	Mode	$(\%^2)$
FAL1	7	241.3	19.6	-16.6 to -15.0	$-15.7\pm0.22$	10.1 to 11.5	$10.9\pm0.45$	4.0 - 5.3	4.6	0.97
FAL2	5	165.9	8.5	-17.8 to -15.0	$-16.1\pm0.48$	10.6 to 11.7	$11.3\pm0.49$	4.3 - 5.6	4.9	1.83
FAL3	5	177.3	9.6	-16.4 to -14.8	$-15.5\pm0.27$	10.3 to 11.0	$10.7\pm0.29$	3.9 - 5.1	4.5	0.74
FAL4	5	175.3	9.4	-15.6 to -14.8	$-15.2\pm0.17$	10.2 to 12.0	$11.2\pm0.64$	4.0 - 5.2	4.6	1.03
FAL5	4	172.4	9.7	-15.3 to -14.4	$-14.9\pm0.21$	10.6 to 11.0	$10.9\pm0.16$	3.9 - 5.3	4.4	0.31
FAL6	6	210.7	13.6	-16.5 to -14.9	$-15.4\pm0.23$	10.6 to 12.4	$11.3\pm0.72$	4.2 - 5.7	4.9	1.43
FAL7	4	145.2	7.0	-16.8 to -14.8	$-15.7\pm0.43$	9.4 to 11.4	$10.2\pm0.89$	3.4 - 4.9	4.1	3.16
FAL8	3	144.6	6.9	-16.3 to -14.0	$-15.1\pm0.65$	10.1 to 11.2	$10.7\pm0.56$	3.9 - 5.2	4.5	3.05
FAL9	12	316.6	45.4	-16.3 to -15.1	$-15.6\pm0.10$	9.7 to 11.3	$10.5\pm0.41$	3.8 - 5.2	4.5	0.37
FAL10	3	116.2	4.2	-16.1 to -14.8	$-15.4\pm0.37$	10.2 to 10.8	$10.4\pm0.31$	3.7 - 4.9	4.2	1.05
FAL11	4	202.3	13.3	-16.3 to -15.1	$-15.6\pm0.29$	10.7 to 11.6	$11.3\pm0.43$	4.2 - 5.5	4.9	0.79
FAL12	3	125.8	4.9	-15.6 to -14.2	$-14.8\pm0.43$	11.3 to 11.9	$11.6\pm0.29$	4.5 - 5.8	5.1	0.80

**Table 24.** Individual isotopic values of the silky shark *Carcharhinus falciformis* in the tropical Pacific Ocean, represented in values of  $\delta^{13}$ C,  $\delta^{15}$ N, total length (LT), estimated age (years), trophic position and isotopic niche (standard area corrected



Figure 39. Mean ( $\pm$  standard error)  $\delta^{13}$ C and  $\delta^{15}$ N isotopic signals by age groups of the silky shark *Carcharhinus falciformis* in the Eastern Tropical Pacific and around Malpelo Island.

The  $\delta^{13}$ C values by maturity stages suggest that adult females showed higher  $\delta^{13}$ C values compared to the other maturity stages (Fig. 40; Table 26), but without reflecting statistical differences between them (Kruskal-Wallis, p = 0.55). While  $\delta^{15}$ N of juvenile and adult males of *C. falciformis* showed the highest values, being statistically similar to the other maturity stages (Fig. 40; Table 5; Kruskal-Wallis, p = 0.25).



Figure 40. Mean values ( $\pm$  standard error) of  $\delta^{13}$ C and  $\delta^{15}$ N for different stages of sexual maturity of the silky shark *Carcharhinus falciformis* in the Colombian Tropical Eastern Pacific and around Malpelo Island.

## **Trophic position**

Total vertebral collagen samples reflected  $\delta^{15}$ N values between 9.4‰ and 12.4‰ (10.9 ± 0.08‰) (Shapiro, p = 0.97; Levene, p = 0.44). Individually, FAL7, FAL9, and FAL10 presented impoverished values of  $\delta^{15}$ N (Table 24). However, no significant statistical differences were detected (ANOVA  $F_{(1, 59)} = 0.526$ , p = 0.47) (Table 24).

Analysis by sex suggests that females  $(10.9 \pm 0.10\% [9.7\% \text{ to } 12.4\%])$  and males  $(10.8 \pm 0.13\% [9.4\% \text{ to } 11.6\%])$  present similar  $\delta^{15}$ N values (Student's t-test, p = 0.56).

Combining the  $\delta^{15}$ N and  $\delta^{13}$ C values from the total data set suggests that *C. falciformis* presents TPs with a mode of 4.4 (95% CI = 3.9–5.0). Individually, FAL7 and FAL10 showed the lowest and highest TPs, respectively, compared to the other individuals (Table 24). The estimated TP by sex indicates that females (4.7 [3.9–5.4]) and males (4.5 [3.9–5.2]) have similar TPs.

Based on age groups, TP estimation suggests that "Embryos" occupied the lowest TPs and ages 12.0–14.9 years with the highest TPs with respect to the other age groups (Table 4). While by sexual maturity of *C. falciformis* showed that the TPs ranged between 3.8 and 5.6 (95% CI), with adult males reflecting the highest TP (Table 26). These results classify *C. falciformis* between a carnivorous organism with preferences for consuming decapods, cephalopods, and fish (NT-V) and a top predator (NT-VI).

#### Niche breadth and isotopic overlap

The isotopic niche (niche<sub>SIA</sub>) estimated for *C. falciformis* at the population level was SEA<sub>C</sub> of  $1.27\%^2$ . Individually, the nicheSIA was between  $0.31\%^2$  and  $3.16\%^2$ , indicating that 16.7% of the individuals presented reduced niche<sub>SIA</sub> (SEA<sub>C</sub> < $0.50\%^2$ ; Fig. 41, Table 22), whereas the remaining individuals reflected larger niche<sub>SIA</sub> (> $50.0\%^2$ ; Fig. 41, Table 24).

The niche<sub>SIA</sub> obtained by sexes suggests similar niches for females  $(1.27\%^2)$  and males  $(1.37\%^2)$  (Fig. 42). The niche<sub>SIA</sub> by age groups indicates that the age group 9.0–11.9 years presented the lowest niche<sub>SIA</sub>; while ages 0.0–2.9 and 3.0–5.9 years had the highest niche<sub>SIA</sub> relative to the other age groups (Fig. 43; Table 25).



Figure 41. Isotopic overlap between individuals of the silky shark *Carcharhinus falciformis* in the Eastern Tropical Pacific and around Malpelo Island. FAL#: Identification code of each individual.

Age group	п	TL Estimada (cm)	AgeEstimated (years)		δ <sup>13</sup> C (‰)		δ <sup>15</sup> N (‰)		Trophic position	
rige group	11		Range	$Mean \pm SE$	Range	$Mean \pm SE$	Range	$Mean \pm SE$	IC 95%	Mode
"Embrión"	1	34.5	_	-1.1	-	-15.7	_	10.1	_	4.4
0.0 - 2.9	20	56.3 - 94.9	0.3–2.8	$1.3\pm0.22$	-17.8 to -14.2	$-15.5\pm0.19$	9.4 to 12.4	$10.7\pm0.16$	3.8 - 5.2	4.5
3.0 - 5.9	16	97.8 - 136.0	3.1–5.8	$4.5\pm0.24$	-16.8 to -14.0	$-15.4\pm0.17$	9.7 to11.9	$11.0\pm0.15$	4.1 - 5.5	4.7
6.0 - 8.9	9	143.7 - 168.1	6.6-8.7	$7.4\pm0.27$	-16.4 to -15.1	$-15.7\pm0.13$	10.4 to 11.6	$11.1\pm0.15$	4.0 - 5.4	4.5
9.0-11.9	6	167.7 - 187.9	9.2–108	$9.9\pm0.29$	-15.6 to -14.8	$-15.1\pm0.12$	10.4 to 12.0	$10.9\pm0.23$	3.9 - 5.0	4.4
12.0 - 14.9	3	202.3 - 210.7	13.3–13.8	$13.6\pm0.15$	-16.3 to -15.0	$-15.5\pm0.42$	10.8 to 11.6	$11.3\pm0.24$	4.3 - 5.6	4.9
15.0 - 17.9	1	227.5	_	16.0	_	-15.3	_	10.4	_	4.6
$18.0 - \ge 20$	5	241.3 - 316.6	19.6–45.4	$28.5\pm4.80$	-16.3 to -15.1	$-15.4\pm0.22$	10.5 to 11.3	$10.9\pm0.17$	4.0 - 5.3	4.6

 Table 25. Isotopic values by age groups of the silky shark Carcharhinus falciformis in the tropical Pacific Ocean, represented in values of  $\delta^{13}$ C,  $\delta^{15}$ N, estimated total length (LT<sub>Estimated</sub>), estimated age (years), trophic position and isotopic niche (standard area corrected [SEA<sub>C</sub>]).

 Table 26. Isotopic values by sexual maturity stages of the silky shark *Carcharhinus falciformis* in the tropical Pacific Ocean, represented in values of  $\delta^{13}$ C,  $\delta^{15}$ N, sexual maturity, trophic position and isotopic niche (standard area corrected [SEA<sub>C</sub>]).

		1 1	1			~J/		
Sevual maturity	и	δ <sup>13</sup> C (‰)		δ <sup>15</sup> N (‰)		Trophic position		SEAc
Sexual maturity	<i>n</i> _	Range	$Mean \pm SE$	Range	$Mean \pm SE$	IC 95%	Mode	$(\%^2)$
Juv. Females	33	-17.8 to -14.2	$-15.5\pm0.12$	9.7 to 12.4	$10.9\pm0.12$	4.0 - 5.4	4.6	1.46
Adul. Females	6	-15.4 to -15.1	$-15.2\pm0.05$	10.4 to 11.3	$10.8\pm0.14$	3.8 - 4.9	4.3	0.14
Juv. Males	19	-16.8 to $-14.0$	$-15.4\pm0.16$	9.4 to 11.6	$10.7\pm0.13$	3.9 - 5.2	4.5	1.33
Adul. Males	3	-16.3 to -15.0	$-15.9\pm0.44$	11.2 to 11.6	$11.4\pm0.12$	4.4 - 5.6	5.0	1.01



Figure 42. Isotopic overlap between sexes of the silky shark *Carcharhinus falciformis* in the Eastern Tropical Pacific and around Malpelo Island.

Based on niche<sub>SIA</sub> size and individual isotopic overlap, the results suggest the presence of low (0-30%), medium (31-60%), and high (61-100%) overlap probabilities between individuals in both directions. Despite this, FAL12 reflected the highest frequency of low overlap probabilities in both directions (FAL12 vs. all individuals = 50% and vice versa = 100%; Fig. 41) followed by FAL5 (all individuals vs. FAL5 = 83.3%; Fig. 41), all other combinations showed medium and high overlap probabilities in both combinations (>31%; Fig. 41).

On the other hand, isotopic overlap between sexes indicated that females and males have high overlap probabilities (F vs M = 91.7% and M vs F = 90.2%; Fig. 42).

With respect to age group, isotopic overlap estimation suggests the presence of medium to high overlap probabilities among all ages (>35%; Fig. 43).

Isotopic overlap between maturity stages indicated that adult females and males of *C*. *falciformis* had the lowest probabilities (<30%) of overlap with the other maturity stages in one direction (Fig. 44; Table 25); whereas the other combinations had higher probabilities of isotopic overlap in both directions (>80%; Fig. 44; Table 27).



Figure 43. Isotopic overlap between age groups of the silky shark *Carcharhinus falciformis* in the Eastern Tropical Pacific and around Malpelo Island.

 Table 27. Isotopic overlap values (in percentage) between sexual maturity stages of the silky shark Carcharhinus falciformis in the tropical Pacific Ocean, represented in percent probability values in both directions.

Age goups	Adults females	Juveniles females	Adult males	Juvenile males
Adults females	—	99.7	17.7	99.4
Juveniles females	14	_	25.7	86
Adult males	7.4	90.7	_	81
Juvenile males	16	92.1	18.1	_



Figure 44. Isotopic overlap between sexual maturity stages of the silky shark *Carcharhinus falciformis* in the Eastern Tropical Pacific and around Malpelo Island.

### DISCUSSION

Few studies have been conducted on the feeding ontogeny of *C. falciformis* along the ETP. The findings of the present study suggest the existence of changes in habitat use and prey consumption during the life cycle of *C. falciformis*, which are mainly related to sexual maturity, reflecting two movement patterns. This is the first study detailing the feeding ontogeny of *C. falciformis* and shows the first evidence of sex-related preferences in habitat use.

#### **Food sources**

The silky shark *C. falciformis* is a species with a wide distribution throughout the world's oceans (Compagno et al., 1984), characterized by its long migrations (Kohin et al., 2006; Filmater, 2011; Hueter et al., 2018; Schaefer et al., 2019), which makes this species feed in both coastal (Cabrera-Chávez-Acosta et al., 2010; Duffy et al., 2010; Rabehagasoa et al., 2012; Estupiñán-Montaño et al., 2017b) and oceanic (Cabrera-Chávez-Acosta et al., 2010; Flores-Martínez et al., 2016; Estupiñán-Montaño et al., 2017a; Páez-Rosas et al., 2018) areas.

The  $\delta^{13}$ C values (-17.8% to -14.0%) obtained from the vertebral collagen of *C. falciformis* in this study confirm that this species obtains its food in oceanic areas (areas of low primary productivity) and coastal areas (areas of high primary productivity), but with a greater preference for the use of oceanic areas (this study: -15.5 ± 0.08%). These results are similar to SIA in muscle tissue of the species in areas such as the Colombian Pacific and surroundings of Malpelo Island (-16.3 ± 0.06% SE [Estupiñán-Montaño et al., 2017a]), Ecuadorian Pacific (-16.8 ± 0.25% SD [Páez-Rosa et al., 2018]), southwestern Indian Ocean (-16.5 ± 0.4% SD [Rabehagasoa et al., 2021]) and the entire ETP (-17.1 ± 0.35% SD [Li et al., 2014], -16.3 ± 0.40% SD [Galindo-Rosado, 2014]). As well as in vertebral collagen in the ETP (-13.2 ± 0.80% SD [Galindo-Rosado, 2014]) and the Galapagos Islands, Ecuador (-14.3 ± 0.05% SE [Estupiñán-Montaño, 2016]), suggesting a greater use of oceanic areas by *C. falciformis*, along these distribution points.

The  $\delta^{13}$ C isotopic signals from this and other studies throughout the world are supported by SCAs that describe *C. falciformis* with a predator of oceanic trophic habits (Acevedo, 1996; Ménard et al., 2013; Cabrera-Chávez-Acosta et al., 2010; Duffy et al., 2015; Flores-Martínez et al., 2016; Filmater et al., 2016; Estupiñán-Montaño et al., 2017b) and coastal (Cabrera-Chávez-Acosta et al., 2016; Estupiñán-Montaño et al., 2016; Estupiñán-Montaño et al., 2016; Stupiñán-Montaño et al., 2016; Stupiñán-Montaño et al., 2016; Estupiñán-Montaño et al., 2017b).

*C. falciformis* shows a greater preference for the use of oceanic habitats, which is mainly related to two reasons: 1) greater consumption of epipelagic and mesopelagic prey of the Ommastrephidae, Scombridae, Coryphaenidae and Exocoetidae families (Acevedo, 1996; Ménard et al., 2013; Cabrera-Chávez-Acosta et al., 2010; Flores-Martínez et al, 2016; N'Gouan et al., 2012; Estupiñán-Montaño et al., 2017b) and 2) high association to natural (e.g., sticks) and artificial floating objects (FADs), which aggregate a high variety of prey species, such as tuna, mahi-mahi, carangids and pelagic crustaceans (Filmalter et al., 2011, 2016; Duffy et al., 2015; Hueter et al., 2018). Whereas the lower use of coastal areas may be related to the low frequency in the consumption of some small prey such as: fishes of the families Diodontidae and Sciaenidae (Flores-Martínez et al., 2016), cephalopods such as *Loligo* spp. (Acevedo, 1996) and coastal crustaceans such as *Pleurocondes planipes*, Family Portunidae (Cabrera-Chávez-Acosta et al., 2010; Barranco, 2008; Duffy et al., 2015) and sea turtles (e.g., Chelonidae [Acevedo, 1996; Estupiñán-Montaño et al., 2017b]).

The SCA (Cabrera-Chávez-Acosta et al., 2010; N'Gouan et al., 2021) and SIA in muscle ( $\delta^{13}C = -16.8\%$ ; [Páez-Rosas et al., 2018], -16.5% [Estupiñán-Montaño et al., 2017a], -16.3% [Galindo-Rosado, 2014]) and in vertebrae ( $\delta^{13}C = -13.2\%$  [Galindo-Rosado, 2014], -14.3% [Estupiñán-Montaño, 2016]) for both sexes of *C. falciformis*, indicate that females and males present similar food preferences and habitat use. These findings agree with the results obtained in this study (-15.5% in both sexes), as well as their high isotopic overlap, suggesting that both sexes of *C. falciformis* make shared use of resources and habitats along the ETP, associated with an aggregation behavior of organisms composed of both sexes, as has been observed in the surroundings of Malpelo Island, Colombia (personal comment, Daniel Villalobos-Ramírez).

This suggests that *C. falciformis* uses different areas (oceanic and coastal) and different food webs. This is validated by the wide range of  $\delta^{13}$ C (this study: 3.8‰), this is supported by five variables that create a coast-ocean gradient related to the composition of this isotopic ratio (France, 1993; Newsome et al., 2007), which is caused by: i) spatial variation of  $\delta^{13}$ C in primary producers (Goericke and Fry, 1994; Pancost et al., 1997), ii) dissolved CO<sub>2</sub> concentration (Goericke and Fry, 1994), iii) different levels of primary production, iv) micro- and macro-algal composition (Clementz and Koch, 2001) and v) phytoplankton growth rate (France, 1995; Newsome et al., 2007).

According to the above, the food base of C. falciformis comes from food webs of different ecosystems or areas supported mainly by pelagic phytoplankton ( $\delta^{13}C = -22\%$  to -18% [Peterson and Fry, 1987; France 1993]) and benthic macroalgae (-17% [Peterson and Fry, 1987; France 1993]). Thus, C. falciformis could be feeding in some ETP food webs and oceanic islands, such as Malpelo Island, Colombia and Galapagos Islands, Ecuador. This hypothesis can be supported by the  $\delta^{13}C_{\text{corrected}}$  with vertebrate TDF, showing  $\delta^{13}C_{\text{corrected*TP}}$  between -17.4% and -15.5%, similar those of the basal sources from Malpelo Island (phytoplankton = -20.7% to -15.5%; this study, zooplankton: -21.9% to -17.8% and macroalgae = -21.0% to -17.1% [Estupiñán-Montaño et al., 2021a]), Galapagos Islands ( $-21.6 \pm 0.52\%$  [Paéz-Rosas et al., 2012]) and mainland Ecuador  $(-21.6 \pm 0.60\%)$  [Calle-Morán, 2010]). This suggests that C. falciformis could use the surroundings of Malpelo Island as a feeding area at some stage of its life, which contrasts by what was reported by Estupiñán-Montaño et al. (2017a) who concluded that this species does not use Malpelo Island and its surroundings as a feeding area. These conclusions were mainly supported by the preferential consumption of fish of the Scombridae family (Estupiñán-Montaño et al., 2017b), this being a group of fish that visits Malpelo Island occasionally or temporarily. However, lacking isotopic information from the base of the food web around Malpelo Island, the different conclusions of previous studies were limited. Our findings show how having information from basal sources in the study areas improves the understanding of the habitat use of species, especially those that are highly mobile.

Isotopic studies on vertebrae of highly migratory sharks, such as *Prionace glauca* and *Sphyrna lewini* that are distributed in the ETP and frequent oceanic islands (i.e., Malpelo Island, Colombia and Galapagos Island, Ecuador), show  $\delta^{13}$ C values from -17.2% to -13.1% (Estupiñán-Montaño et al., 2019, 2021a), suggesting that these species, along with *C. falciformis*, make use of similar feeding grounds during their life cycle. This similar use of feeding grounds may be associated with the low latitudinal variation of marine zooplankton  $\delta^{13}$ C between Malpelo Island and the Galapagos Islands (0.9% [Estupiñán-Montaño et al., 2021c]). However, the competitive exclusion processes between these species (*C. falciformis*, *P. glauca*, and *S. lewini*) in the ETP, may be due to differences in  $\delta^{15}$ N, which could indicate: 1) use of different ecosystems with different basal  $\delta^{15}$ N values, which may be related to oceanographic processes, such as upwelling, nitrification/denitrification, assimilation, currents, minimum oxygen layer (Olson et al., 2010; Casciotti et al., 2013; Martin et al., 2019) and 2) the higher frequency of vertical and horizontal migratory routes of *P. glauca* and *S. lewini* with respect to *C. falciformis*, exploring different areas of the ETP. Additionally, the low differences in  $\delta^{15}$ N between *S. lewini* and *C. falciformis* (mean difference = 0.4‰) suggest a shared use of habitat with similar oceanographic conditions and uses of breeding areas (e.g., Colombian mangroves [Estupiñán-Montaño et al., 2021a]), at some stage of their ontogeny.

This may be explained by: 1) the slow rate of vertebrae renewal (years), which causes the initial diet isotope (baseline) to be reflected in the tissue over a long period of time (McCutchan et al., 2003), which may result in the  $\delta^{13}$ C similarities of the basal sources on Malpelo Island, suggesting that C. falciformis uses this area and its surroundings as a feeding ground (Fig. 38A), 2) similar  $\delta^{15}$ N isotopic composition of the vertebrae of C. falciformis and some ETP primary producers as suggested by similarities with basal sources from Malpelo Island (Fig. 38B) and 3) use of areas with important N<sub>2</sub> fixation process due to the presence of diazotrophic cyanobacteria ( $\delta^{15}$ N range: -5% to +2% [Bauersachs et al., 2009]), which reflect low values of  $\delta^{15}$ N in particulate organic matter (Carpenter et al., 1997), as could be the Colombian mangroves ( $\delta^{15}$ N; detritus:  $-0.9 \pm 0.5\%$ , leaves:  $1.8 \pm 0.6\%$ , microphytobenthos:  $2.9 \pm 2.94\%$ ; sediment:  $-0.1 \pm 0.5\%$ , seston:  $1.8 \pm 1.1\%$ [Medina-Contreras et al., 2018]). These results suggest that C. falciformis uses the area adjacent to Isla Malpelo and its surroundings as a feeding area and, Colombian mangroves could be potential feeding areas for early life stages of this species; however, the lack of trophic information and the identification of breeding areas for the species, limits the interpretation of some of our results; therefore, more studies are needed that allow us to expand the knowledge about the biology of the species and thus further support our hypotheses.

#### **Trophic position**

Like other sharks, *C. falciformis* is a top predator that occupies different TPs in the ETP food webs (TPs: 3.4–5.4). These results are consistent with previous studies conducted in the ETP (TPs: 2.5–5.8 [Li et al., 2014; Galindo-Rosado, 2014; Flores-Martínez et al., 2016; Estupiñán-Montaño et al., 2017a, b; Páez-Rosas et al., 2018]).

The different trophic roles of *C. falciformis* during its ontogeny in ETP food webs are the result of several ecological and biological aspects. First, wide range of habitat use (coastal and oceanic zones; see above.), due to its high horizontal mobility, which allows it to travel long distances, up to 3000 km [Schaefer et al., 2019]) from aggregation points to different foraging areas (Matich et

al., 2011). This would explain the differences between  $\delta^{13}C_{corrected*TP}$  values of *C. falciformis* and some oceanic and coastal basal sources (Table 22, Fig. 38A) of its range in the ETP.

Second: reduced trophic niche, related to depth, with preferences between 0–80 m (Kohin et al., 2006; Hutchinson et al., 2015) and temperature with preference of 26–30°C Kohin et al., 2006; Musyl et al., 2011), which may result from preferential consumption of coastal and oceanic epipelagic and mesopelagic fishes of the Scombridae families (Cabrera-Chavez-Acosta et al., 2010; Flores-Martínes et al., 2016, Estupiñán-Montaño et al., 2017b), which would reflect basal 15N values of pelagic food webs (Table 22, Fig. 38B).

Third: use of ecosystems or areas with food webs with different basal  $\delta^{15}N$  signals (Rabehagasoa et al., 2012) from areas where NO<sub>3</sub><sup>-</sup> reduction is generated, resulting in <sup>15</sup>N-enriched nitrates (Granger et al., 2008) and upwelling areas that reflect low  $\delta^{15}N$  values (5–8‰ [Sigman et al., 1997]), which is reflected in the vertebrae of *C. falciformis*. In this sense, oceanic islands such as Malpelo Island (Colombia) and Galapagos Islands (Ecuador) could be important feeding sites for this species, since, on these islands, processes favor the transport of nitrogenous material from the bottom to the surface are generated (Palacios et al., 2006), such as year-round upwelling (Malpelo Island; Rodríguez-Rubio and Stuardo, 2002) and large upwelling of phytoplankton due to vertical and horizontal advection processes (Galapagos Islands [Waliser et al., 2005]). This nitrogenous material is used by primary producers who incorporate it into food webs.

Fourth: use of zones with the presence of diazotrophic cyanobacterial communities ( $\delta^{15}N_{range}$ : -5‰ to +2‰ [Bauersachs et al., 2009]), such as mangrove areas which are characterized by reflecting depleted  $\delta^{15}N$  (see above), so, isotopic signals from basal sources of zones could be the reflection of low  $\delta^{15}N$  values in the vertebrae of *C. faclciformis* (see above).

Fifth, the association of *C. falciformis* juveniles to FADs (Filmater et al., 2011, 2016; Duffy et al., 2015), could reflect low trophic levels due to the detrimental quality of food consumed at these sites (Rabehagsoa et al., 2012), which could be explained by the ecological trap hypothesis (Rau et al., 1983; Marsac et al., 2000; Hallier and Gaertner, 2008).

Finally, the different roles of *C. falciformis* in the ETP food webs are a reflection of the consumption of organisms close to the trophic bases, such as crustaceans, small fishes (Engraulidae, Tetraodontidae), and cephalopods (Lolliginidae), and sea turtles (Cabrera-Chávez-Acosta et al., 2010; Flores-Martínez et al., 2016; Estupiñán-Montaño et al., 2017b) to some oceanic mesopredators (Scombridae, Coryphaenidae, Ommastrephidae, Ancistrocheuridae [Cabrera-

Chávez-Acosta et al., 2010; Estupiñán-Montaño et al., 2017b; N'Gouan et al., 2021]). Therefore, *C. falcifomis* is considered a consumer that fulfills different roles in the trophic webs of the ETP (NT-V and -VI [Stergiou and Karpouzi, 2002]) during its life history.

## **Food ontogeny**

Ontogenetic changes in habitat use and prey consumption have been documented for several shark species in the ETP (Polo-Silva et al., 2013; Méndez-Macías et al., 2019; Tamburin et al., 2020), this being a feature not yet widely described for *C. falciformis*. However, it is believed that this species has changes in feeding behavior associated with body growth (Duffy et al., 2015) due to increased swimming speed, visual acuity, mouth size, among others (Scharf et al., 2000; Flores-Martínez et al. 2017), which allow it to capture prey with high locomotion such as scombroids (Duffy et al., 2015; Estupiñán-Montaño et al., 2017b).

Some SCA studies support these observations. For example, juvenile C. falciformis in the Mexican Pacific stay longer in coastal areas consuming abundant and easily accessible prey, i.e., crustaceans, squids such as Dosidicus gigas and Tetraodontidae (Cabrera-Chávez-Acosta et al., 2010; Flores-Martínez et al., 2016). In contrast, adults prefer oceanic areas to consume large prey, i.e., Scombridae (Cabrera-Chávez-Acosta et al., 2010; Flores-Martínez et al., 2016). This is supported by the  $\delta^{13}$ C and  $\delta^{15}$ N values of C. *falciformis* obtained in this study for each sexdifferentiated maturity stage, which show "small" changes in habitat use, as have been suggested for the Mexican Pacific (Cabrera-Chávez-Acosta et al., 2010) and the entire ETP (Duffy et al., 2015). These slight changes in habitat use experienced by C. falciformis are reflective of sexrelated migratory patterns, as has also been suggested for other shark species such as Sphyrna lewini (Estupiñán-Montaño et al., 2021c). Females of C. falciformis moving from oceanic to coastal areas as they grow and mature, periods in which they feed on prey of similar trophic levels (low variation in  $\delta^{15}$ N; Fig. 40) and males tend to move from coastal to oceanic areas to shift consumption from low trophic level prey to larger prey (greater variation in  $\delta^{15}$ N; Fig. 40). The differences in  $\delta^{15}$ N variability between sexes may be explained by the greater nocturnal activity of males, a period in which they make greater vertical migrations to supplement their diet with other types of prey (Compagno et al., 1984).

Additionally, behavioral differences between females and males of *C. falciformis* related to growth/age and sexual maturity may be explained by several reasons: 1) females (juveniles and

adults) and juvenile males tend to consume mainly crustaceans, small sculpins (e.g., *Auxis* spp.) and occasionally sea turtles (Acevedo, 1996; Cabrera-Chávez-Acosta et al., 2010; Filmater et al., 2016; Estupiñán-Montaño et al., 2017b) in coastal areas, as a strategy to reduce energy expenditure, so that they can prepare for reproduction by directing acquired energy towards the growth of reproductive organs (Gerritsen, 1984; Klimley, 1987), 2) juveniles of both sexes feeding around FADs (Filmater et al., 2011, 2016; Duffy et al., 2015), reducing foraging energy expenditure, 3) consumption of low energy quality prey found in FADs reflecting low PT values (ecological trap), 4) adult males preferentially consuming prey of higher trophic levels of oceanic origin (Fig. 40), i.e., squids such as Ancistrocheirus lesueurii, Sthenotuthis oualaniensis and large sculpins such as Thunnus spp. (Filmater et al., 2016; Estupiñán-Montaño et al., 2017b) and 5) opportunistic feeding behavior related to the variety (N'Gouan et al., 2021), abundance and zoogeography of prey (Duffy et al., 2015) in different habitats throughout the year and during their life cycle.

#### Niche breadth and isotopic overlap

Although some shark species fulfill different roles in marine ecosystems (Roff et al., 2016), their feeding preferences have led to categorize them as specialist consumers (Méndez-Macías et al., 2019, Velázquez-Chiquito et al., 2021) and/or generalists (Torres-Rojas et al., 2013, Coiraton and Amezcua 2020). In this sense, *C. falciformis* has been considered a species of both specialist (Cabrera-Chávez-Acosta et al., 2010; Estupiñán-Montaño et al., 2017b; N'Gouan et al., 2021) and generalist/opportunist (Duffy et al., 2015; Filmater et al., 2016; Flores-Martínez et al., 2016) habits.

The different niche<sub>SIA</sub> values obtained in this study (SEA<sub>C</sub> =  $0.14-3.16\%^2$ ) suggest different degrees of specialization. Similar results have been obtained in other ETP areas, such as in the Galapagos Islands, Ecuador (SEA<sub>C</sub> =  $1.9\%^2$  [Estupiñán-Montaño 2016] and  $1.14\%^2$ ; [Páez-Rosas et al., 2018]) and Malpelo Island, Colombia ( $0.20\%^2$  [Estupiñán-Montaño et al., 2017a]). These variations in the amplitude of the niche<sub>SIA</sub> of *C. falciformis* confirm that this species is a consumer with different degrees of trophic specialization (specialist and generalist/opportunist) at different stages of its life history. This type of behavior can be explained by several reasons: 1) availability and abundance of prey that can change in space and time (Stergiou and Fourtouni, 1991; Torres-Rojas et al., 2010; Duffy et al., 2015), 2) regionally specific food habits (Filmater et al., 2016); for

example, SCAs in the Mexican Pacific mention that *C. falciformis* consumes mostly crustaceans and squid (Cabrera-Chávez-Acosta et al., 2010; Flores-Martínes et al., 2017); whereas, in Ecuadorian waters this species has a greater preference for tunas and carangids (Acevedo, 1996; Estupiñán-Montaño et al., 2017b), 3) trophic changes associated with ontogeny (Cabrera-Chávezacosta et al., 2010; Duffy et al., 2015, this study), 4) prey size and energy content (Hart and Ison 1991, Stergiou and Fourtouni, 1991) and 5) effects on feeding due to association to FADs (ecological trapping [Duffy et al., 2015]).

Adding to the above, the different niche<sub>SIA</sub> amplitudes observed in *C. falciformis* (Tables 24–26), together with the intermediate and high degree of isotopic overlap (probability >50%) reflected between individuals (36% [x vs y] and 56% [y vs x]), age group (93. 3% [x vs y] and 60% [y vs x]) and maturity stages (33.3% [x vs y] and 66.7% [y vs x]), suggest different levels of intraspecific competition. For example, females and males of *C. falciformis* from different study areas show high degrees of competition, in regions such as the Mexican Pacific (C $\lambda$  >0.90 [Cabrera-Chávez-Acosta et al., 2010]), the Galapagos Islands, Ecuador, with an isotopic overlap = 0.60 (Estupiñán-Montaño 2016; Páez-Rosas et al., 2018) and West Africa = 0.99 (N'Gouan et al., 2021), while, by maturity stages, low (C $\lambda$  = 0.26–0.30; Flores-Martínez et al., 2016) and high (isotopic overlap >0.44; [Estupiñán-Montaño, 2016; N'Gouan et al., 2021]) degrees of interaction (intraspecific overlap) have been estimated in areas of the Mexican Pacific and Galapagos Islands, Ecuador, respectively.

All of the above would support the different niche<sub>SIA</sub> amplitudes (Tables 24–26) and the different ranges of isotopic overlap (see above) in *C. falciformis*, suggesting a high degree of generalist/opportunistic behavior and low category segregation. Although sexual segregation, by size and sexual maturity, may reduce and/or increase levels of intraspecific competition (Cabrera-Chávez-Acosta et al., 2010), this type of within-species interaction may be compensated by the abundance of prey present in the area (Colwell and Futuyma, 1971) and behavioral differences (Estupiñán-Montaño et al., 2021c; this study). Therefore, the levels of food competition and specialization reflected by *C. falciformis*, are the result of its high adaptability to changes in food webs and prey communities due to different factors, such as seasonality (Duffy et al., 2015). For example, at times when food is abundant, *C. falciformis* may exhibit selective behaviors by restricting resource use and reducing competition; whereas when food is limited or scarce, *C. falciformis* may be a generalist/opportunistic consumer making greater use of available resources

(increasing competition) over time and feeding areas, allowing it to maximize energy use in foraging (Wetherbee et al., 1990).

#### CONCLUSIONS

Few studies have detailed the feeding habits and preferences of *C. falciformis*; this is the first study to present greater details on the feeding ontogeny of *C. falciformis* in the ETP.

*C. falciformis* feeds in both oceanic and coastal areas, with a greater preference for use of oceanic areas due to greater consumption of epipelagic and mesopelagic prey during its life cycle, thus reflecting ontogenetic changes in area use and consumption of prey from different trophic levels. This differential use of these areas (oceanic and coastal) suggests that oceanic islands, such as Malpelo Island, Colombia and Galapagos Islands, Ecuador, could be important feeding sites for this species. Likewise, mangrove areas could constitute potential breeding areas for this species, due to the high similarity in the  $\delta^{13}$ C and  $\delta^{15}$ N values of the vertebrae of *C. falciformis*. The preferences and feeding behavior of *C. falciformis* may be affected by the high degree of association with floating objects (natural and artificial [FADs]), mainly in early life stages.

*C. falciformis* is a consumer located between levels V (tertiary consumer) and VI (top predator), with feeding changes related to two sex-related migratory patterns. Females moving from oceanic to coastal areas and feeding on prey of similar TPs (low  $\delta^{15}$ N variation) and males migrating from coastal to oceanic areas and consuming prey of different TPs during growth (higher  $\delta^{15}$ N variation), which makes *C. falciformis* present both specialist and opportunistic/generalist habits in different phases of its life history. This characteristic results in different degrees of intraspecific competition as a product of its high adaptability to changes in food webs and prey communities due to different factors (e.g., seasonality).

Finally, although the results obtained in this study are supported by several methodologies (e.g., SCA and SIA), further studies are still needed to reinforce our findings and mainly: 1) elucidate and decipher in greater detail the mechanisms and patterns that cause *C. falciformis* to undergo ontogenetic changes in feeding and habitat use, 2) describe in greater detail the trophic patterns of both sexes, considering that each could play different roles and impact food webs in different ways during ontogeny, and 3) clarify the hypothesis of the importance of Colombian mangroves as potential breeding areas for this species.

## **GENERAL RECOMMENDATIONS**

Expand studies on the feeding habits of the different marine and terrestrial species of the Malpelo FFS, from the review of stomach contents (when possible), records of feeding behavior of the species from direct observation during the different activities carried out within the marine protected area (i.e., tourism, research and/or illegal fishing). This would reduce information gaps and improve the modeling of the ecological dynamics of the ecosystems present in the Malpelo FFS.

Continue with the studies of stable isotope analysis, mainly in terrestrial species during different times of the year to try to understand how the large consumers of this ecosystem co-exist in a small environment, such as the Malpelo FFS, and in this way, have a better understanding of the ecological processes that are generated here and that model the terrestrial community dynamics. On the other hand, although it is almost impossible to conduct isotopic analysis of the marine species present in the Malpelo FFS during the same time of the year, it is recommended to continue with the isotopic analysis of the species of this ecosystem, either from sporadic sampling and/or from obtaining samples from seizures of illegal fishing. This information could reveal different ecological aspects of the ecosystem, thus providing important information for decision-making in terms of conservation.

Trophic studies focused on the different species of sharks that reside in the Malpelo FFS are necessary to adequately understand the role of these species within the ecosystem. Some shark species of the Malpelo FFS were not included in this work, such as: whitetip shark (*Triaenodon obesus*), Galapagos shark (*Carcharhinus galapagensis*) and monster shark (*Odontaspis ferox*); therefore, research efforts that focus on these shark species should be conducted to try to understand how these species regulate the ecological dynamics of the Malpelo FFS.

Carry out studies to identify feeding areas that help to understand the relationship of some shark species that frequent oceanic areas (e.g., Malpelo FFS) and coastal areas (e.g., mangroves) during different stages of their life cycle, so that this information can be used to find connectivity between areas and thus generate information for the protection of other essential habitats for the development of sharks during their life cycle.

Involve the different stakeholders (e.g., tourists, researchers, tourism operators, environmentalists, conservation entities, fishermen, etc.) in the research and monitoring activities

(citizen science) of the marine and terrestrial ecological communities of the Malpelo FFS, based on their participation in the collection of information.

Improve inter-institutional cooperation (NGOs, universities and governmental institutions) to enhance research efforts that generate relevant information for the understanding of the ecological dynamics of the Malpelo FFS, and to ensure that this information is actively used in the development of management and conservation measures based on the ecosystem.

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