



OPEN Biogeographic variation in the diet of juvenile blacktip sharks across the Galapagos Archipelago

Savannah J. Ryburn¹✉, Eldridge Wisely², Eduardo Espinoza^{3,4,5}, Daniel Armijos⁶, Haley E. Capone⁷, Diana A. Pazmiño⁶, Alex Hearn^{4,8} & John F. Bruno⁹

Juvenile blacktip sharks (*Carcharhinus limbatus*) are highly abundant in the mangrove-fringed bays throughout the Galapagos Archipelago, utilizing these habitats as nursery grounds. Due to spatial variation in factors including temperature and larval connectivity, fish species richness is higher in the eastern portion of the archipelago. Predator diets often reflect regional prey availability, leading us to hypothesize that juvenile blacktip sharks in the eastern Galapagos would have greater prey taxa richness than those in the west. Here, we used DNA metabarcoding of fecal matter collected via non-lethal cloacal swabs to describe and quantify juvenile blacktip shark diets. We collected samples from 107 sharks across 240 km, spanning 14 bays on four islands, and released all sharks alive. The most common prey taxa identified were Thoburn's mullet (*Mugil thoburni*; 20.0%, based on percent of occurrence), followed by fishes in the genus *Abudefduf* (sergeant-majors; 13.4%), Galapagos ringtail damselfish (*Stegastes beebei*; 12.7%), scalloped hammerhead shark (*Sphyrna lewini*; 11.7%), and yellowtail damselfish (*Stegastes arcifrons*; 10.7%). Prey taxa richness was highest among sharks sampled in the bays of San Cristobal, possibly due to geographic variation in prey availability. Additionally, sharks sampled during a La Niña event in 2021 had higher prey taxa richness than those sampled during an El Niño event in 2023, which could be due to El Niño-induced declines in species richness and abundance resulting from shifts in primary productivity and upwelling. A deeper understanding of shark diets can improve our ability to predict how changes in food web dynamics and prey availability affect shark populations. This knowledge is especially vital in the face of overfishing and climate change, helping to inform more effective conservation strategies and support shark survival.

Keywords Diet reconstruction, fDNA, Cloacal swab, ENSO, Metabarcoding, *Carcharhinus limbatus*

Prey availability and consumption are pivotal in understanding the ecological interactions and behaviors of consumers across different environments. What and how much consumers eat, has a strong effect on their behavior, survival, and fitness, as well as the demography of the population. Moreover, consumer diets vary enormously with seasonality and among locations as a function of caloric demands, prey availability, and the presence or absence of competitors and predators. For example, the diet of pumas (*Felis concolor*) varies by latitude, with temperate populations typically specializing in a few larger prey species, while tropical populations consume a greater diversity of smaller prey¹. Understanding biogeographic variation in consumer diets can provide insight into an animal's ability to adapt to environmental disturbances and fluctuations in prey availability across time and space. For example, wolves (*Canis lupus ligoni*) in Alaska's Alexander Archipelago responded to a decline in ungulate prey abundance and availability by broadening their overall dietary diversity rather than shifting to a single alternative prey source².

¹Environment, Ecology, and Energy Program, The University of North Carolina at Chapel Hill, Chapel Hill, NC 27599–3275, USA. ²Scripps Institution of Oceanography, University of California San Diego, San Diego, CA 92121, USA. ³Galapagos National Park Directorate, Puerto Ayora, Galapagos Islands, Ecuador. ⁴MigraMar, Olema, CA 94950, USA. ⁵The Instituto Nacional de Biodiversidad de Ecuador, Quito, Ecuador. ⁶Galapagos Science Center GSC, Universidad San Francisco de Quito and University of North Carolina at Chapel Hill, Av. Alsacio Northia, Isla San Cristóbal, Galápagos, Ecuador. ⁷Restoration Center, Office of Habitat Conservation, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, Silver Spring, MD 20910, USA. ⁸Colegio de Ciencias Biológicas y Ambientales Universidad San Francisco de Quito USFQ, Diego de Robles y Pampite, Quito 170901, Ecuador. ⁹The Department of Biology, The University of North Carolina at Chapel Hill, Chapel Hill, NC 27599–3280, USA. ✉email: savannahjryburn@gmail.com

The Galapagos Archipelago is one of the most unique biogeographical regions on Earth, shaped by an anomalous ocean climate that fosters distinct marine ecosystems in close proximity³. As a result, its biota includes a unique mix of species typically found in tropical (e.g., manta rays, reef sharks, corals), temperate (e.g., sea lions, kelp), and even subantarctic (e.g., fur seals, penguins, albatross) environments. Species richness of both reef fishes and macro-invertebrates vary throughout the Galapagos³ and the archipelago is divided into bioregions characterized by variable species composition driven by temperature and productivity³. In general, the western portion of the archipelago has cooler water temperatures with more temperate species, while the eastern side of the archipelago is relatively warmer and has more tropical species and greater overall richness. The temperate western regions have the lowest overall fish species richness^{3,4} and exhibit the highest levels of endemism³. These different bioregions and the distinct variation of fish and invertebrate species across the archipelago have led the diet of some predator species, such as the Galapagos sea lion (*Zalophus wollebaeki*), to differ depending on where they are foraging⁵.

While the Galapagos Archipelago's diverse marine ecosystems support a wide array of species, they also provide critical habitats for specific life stages of certain animals, particularly in the case of shark nurseries. Numerous shark species, including the blacktip (*Carcharhinus limbatus*) and scalloped hammerhead (*Sphyrna lewini*), use nursery habitats as juveniles^{6–8}. Shark nurseries are often located in coastal, protected bays and estuaries⁷, which can be susceptible to increased anthropogenic impacts (e.g., pollution, tourism, and fishing)⁹. Shark nurseries are typically in mangrove-fringed bays^{7,9}, which also provide nurseries for other juvenile fishes, potentially increasing the abundance of possible prey for the juvenile sharks¹⁴. It has been hypothesized that sharks use nursery habitats for their greater prey abundance and accessibility to food sources as well as a refuge from predation^{10,11}, although more research is required to fully understand their purpose⁸.

Blacktip sharks have a circumglobal distribution, inhabiting continental and insular shelves in tropical and subtropical regions¹². In the Gulf of Mexico, female blacktip sharks reach maturity at 6–7 years of age (158–162 cm TL), whereas males reach maturity at 4–5 years of age (133–136 cm TL)¹³. The length at birth is estimated to be 50–60 cm TL¹⁴. Juvenile blacktips are also abundant throughout the Galapagos Archipelago^{9,15,16}. Based on previous studies that have used morphological stomach contents analysis, Clupeidae and Sciaenidae fishes had the largest contribution to the blacktip shark's diet worldwide⁸. However, the importance of specific prey species in their diet varies geographically, for example due to the variation in size/age of the sharks analyzed and differentiation in prey availability⁸. While blacktip sharks exhibit ontogenetic dietary shifts¹⁷, research on juvenile blacktip diets has often been limited due to the reliance on morphological stomach content analysis, which can be imprecise^{11,17,18}. Additionally, most studies have focused on juvenile blacktip sharks caught in the Gulf of Mexico^{11,19–23}.

Only one study has investigated the trophic ecology of juvenile blacktips in the Galapagos, in which they used stable isotope analysis (SIA) from sharks caught on two different islands (i.e., Santa Cruz and Santiago)²⁴. While SIA is valuable for determining the relative trophic position of a predator over extended periods, it lacks the taxonomic resolution needed to identify specific prey species. Alternatively, DNA metabarcoding of fecal DNA (fDNA) provides a more precise alternative for diet analysis, enabling researchers to identify prey at higher taxonomic resolutions (e.g., to species). DNA metabarcoding is a technique used to identify many taxa within the same sample and has become a popular method for diet reconstruction studies^{5,25–28}. The use of cloacal swabs to collect fDNA from sharks for DNA metabarcoding is also a less invasive, non-lethal, and more precise method for diet analysis compared to traditional morphological stomach contents analysis^{29–31}. Cloacal swabbing for shark diet reconstruction was first applied by van Zinnicq Bergmann et al. (2021)⁶⁷, who collected samples from captive lemon sharks (*Negaprion brevirostris*) during a controlled feeding trial and from wild bull sharks (*Carcharhinus leucas*). Ryburn et al. (2026)³³ found no statistical difference in overall diet composition when comparing metabarcoded stomach contents and cloacal swabs collected from the same individuals across four shark species: blacktip, bonnethead (*Sphyrna tiburo*), blacknose (*Carcharhinus acronotus*), and Atlantic sharpnose (*Rhizoprionodon terraenovae*).

Here we describe and quantify the diet of the juvenile blacktip shark across 14 bays on four islands in the Galapagos using DNA metabarcoding of fDNA collected with non-lethal cloacal swabs. The purpose of our study was to conduct a biogeographical analysis of juvenile blacktip shark foraging patterns. Given that fish species richness is higher on the eastern side of the archipelago, we hypothesized that the dietary richness of juvenile blacktip sharks would reflect this pattern.

Methods

Shark capture

We sampled 107 juvenile blacktip sharks (694 ± 7 mm stretched total length [STL], 495–940 mm range) across four islands in the Galapagos (i.e., San Cristobal, Isabela, Fernandina, and Santiago; Fig. 1). Sampling took place in 14 bays between June 2021 and July 2023 (Table 1). These bays were chosen based on knowledge from local fishermen and from our team's experience in observing blacktips. San Cristobal had a larger sample size due to ease of daily access because the Galapagos Science Center (GSC) is located on San Cristobal, whereas samples from the other islands were collected during two research cruises in June of 2022 and February of 2023. To capture the sharks, we used gillnets (75 of the 107 sharks), similar to those used by local artisanal fishermen, seine nets (28 of the 107 sharks), and handlines (4 of the 107 sharks). When possible, we used a SonTek CastAway-CTD to measure the water temperature for each sampling before the hooks or net were placed into the water.

A large beach seine, measuring 100 m in length and 3 m in depth with 1.27 cm eye-mesh, was deployed in Puerto Grande to encircle the shallow area along the beach. This net was used exclusively at this site, as Puerto Grande was the only bay with a sandy bottom and a sufficiently large beach to accommodate its use. In contrast, the other 13 bays had rocky bottoms or lacked suitable beaches, making the seine net impractical. As a result, gillnets and handlines were the primary sampling methods used in the other locations. Sampling with the seine

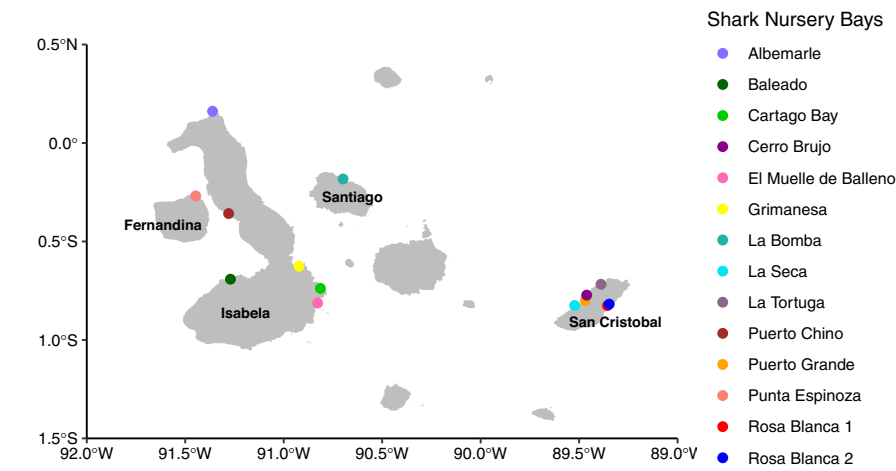


Fig. 1. Map of the Galapagos Archipelago showing the 14 locations where juvenile blacktip sharks were sampled. Each point represents a specific sampling site. Puerto Grande and Cartago Bay are the only two nursery habitats where juvenile blacktip sharks and juvenile scalloped hammerhead sharks co-occur.

Shark nursery bay	Island	Location	Scalloped hammerhead nursery	Year(s) Sampled
Albemarle	Isabela	0° 9'40.14"N, 91°21'40.74"W	No	2022
Baleado	Isabela	0°41'30.12"S, 91°16'13.80"W	No	2022
Cartago Bay	Isabela	0°44'17.66"S, 90°48'48.96"W	Yes	2022, 2023
Cerro Brujo	San Cristobal	0°46'21.67"S, 89°27'42.18"W	No	2021
El Muelle de Balleno	Isabela	0°48'45.36"S, 90°49'39.24"W	No	2022
Grimanesa	Isabela	0°37'33.16"S, 90°55'20.58"W	No	2022
La Bomba	Santiago	0°10'57.78"S, 90°41'55.44"W	No	2022
La Seca	San Cristobal	0°49'31.14"S, 89°31'20.02"W	No	2021
La Tortuga	San Cristobal	0°43'3.30"S, 89°23'20.39"W	No	2021
Puerto Chino	Isabela	0°21'29.76"S, 91°16'47.22"W	No	2022
Puerto Grande	San Cristobal	0°48'3.86"S, 89°28'9.56"W	Yes	2021
Punta Espinoza	Fernandina	0°16'6.84"S, 91°26'47.16"W	No	2022
Rosa Blanca 1	San Cristobal	0°49'32.28"S, 89°21'21.64"W	No	2021
Rosa Blanca 2	San Cristobal	0°49'5.68"S, 89°20'49.56"W	No	2021, 2023

Table 1. The location of each shark nursery bay, number of sharks sampled from each bay, whether the bay was also a scalloped hammerhead nursery, and the years each bay was sampled from.

net in Puerto Grande took place at sunrise. The process began with one end of the net being anchored at one side of the beach, while the rest of the net was deployed from a boat to encircle the area. Once the net looped around to the opposite end of the beach, it was anchored there and then pulled ashore, creating a small, confined area where sharks were enclosed. The entire process took approximately 10 min, during which the sharks remained in the water, swimming freely within the enclosed area, until they were individually worked up.

A 100×3.5 m gillnet with 6.35 cm eye-mesh was used in the shallow areas at all bays and soaked for approximately one hour per deployment. To avoid shark mortality, the net was constantly monitored and sharks were immediately removed from the net as soon as they were captured.

Handlines were also used in all bays to target deeper areas that were inaccessible to the gillnet and seine net. Each handline was free-floating and comprised of a #4/0 barbless steel octopus hook attached to 0.7 mm, 25 kg monofilament line, 200 g polypropylene rope, and a foam buoy. We baited the hooks with either squid purchased from a local grocery or fresh mullet. Eight to 14 hooks were dispersed throughout the bay at a time. The buoys were constantly monitored, allowing sharks to be immediately brought onto the boat for the work up once hooked. We checked and re-baited the hooks as needed, approximately every 20–30 min.

Fieldwork and data collection were conducted under research permits PC-13-21, PC-18-22, PC-13-23, and MAATE-DBI-CM-2021-0174 granted by the Galapagos National Park and the Ecuadorian Ministry of Environment. Animal care and use were approved and overseen by UNC-CH's IACUC (23–210.0) and was performed in accordance with relative guidelines and regulations. All sharks were released alive immediately

after a brief workup, which lasted approximately three minutes. No sharks were harmed or killed as a result of this research.

fDNA collection

We followed the protocol outlined by Ryburn (2025)³² and Ryburn et al. (2026)³³ to collect fecal DNA (fDNA) samples. If the shark was caught using a hook, the hook was immediately removed upon capture. The shark was then placed ventral side up on a flat surface, with its pelvic fins spread apart to prevent the swab from making contact with the exterior skin. The cloacal area was sterilized and dried by wiping the exterior with a Kimwipe soaked in 98% ethanol, which was discarded after a single use. Using a sterile, individually packed cotton-tipped swab with a wooden handle (Puritan 25–806 1WC FDNA; length 15.2 cm, tip diameter 0.46 cm) we carefully inserted the swab approximately 2.5 cm into the shark's cloaca, past the sphincter, and rotated it for about 10 s. After removal, the swab was immediately placed into a sterile vial, stored in a cooler with ice, and the shark was released. The swabs were then stored at -20 °C until further processing.

Metabarcoding laboratory protocol and bioinformatics

We used the metabarcoding laboratory protocol from Ryburn (2025)³² and Ryburn et al. (2026)³³. The full protocol is available on protocols.io (<https://doi.org/10.17504/protocols.io.261ge5dnwg47/v2>)³⁴. For DNA extraction, we used the DNeasy Blood and Tissue Kit (www.qiagen.com), adhering to the manufacturer's protocol with two adjustments: samples were digested at 70 °C for 24–48 h, and the DNA was eluted to 100 µl. The extracted DNA was stored at -20 °C until further processing.

We amplified two target regions for all samples: the MiFish-U³⁵ region of the mitochondrial 12S rRNA gene and the Crustacean_16S²⁶ mitochondrial rRNA gene. These primer sets were chosen based on previous diet studies that identified teleost fishes and crustaceans as primary components of the blacktip shark's diet^{11,17}. For both primer sets, Illumina Nextera-style adapters were appended to the 5' ends of both the forward and reverse primer binding regions. PCR reactions were performed in triplicate for each sample and primer combination using the Takara High Fidelity PCR EcoDry™ Premix. DNA extraction and PCR were completed at the GSC for samples collected in 2021, and the resulting PCR products were exported to the United States at room temperature. However, for the 2022 and 2023 samples, DNA extraction took place at the GSC, and the extracted DNA was exported to the United States in a cooler on ice. PCR amplification and all subsequent laboratory procedures for these samples were carried out in the Department of Biology at the University of North Carolina at Chapel Hill (UNC).

We combined 10 µl of PCR product from each reaction (i.e., both MiFish and Crustacean_16S), resulting in a total of 60 µl per sample. Negative controls were pooled separately by primer set, with 20 µl from each PCR reaction combined to create 60 µl of MiFish PCR control and 60 µl of Crustacean_16S PCR control. All pooled PCR products were cleaned using a 1.5x ratio of Ampure XP beads and eluted in 30 µl of Qiagen Buffer EB. In a second PCR step, we applied a unique dual-indexing strategy by adding IDT for Illumina UDI-UMI indexes to 2.5 µl of the combined and cleaned amplicons.

We then combined 10 µl of each indexed sample and cleaned the combined amplicons using a 0.9x ratio of Ampure XP beads. The 10 nM pooled library was sent to the High-Throughput Sequencing Facility (HTSF) at UNC for paired-end sequencing on a single MiSeq lane using 250 cycles. Throughout all laboratory procedures, we used single-use disposable gloves and all equipment and surfaces were sterilized with a 20% bleach solution before and after use.

We used the same bioinformatics protocol as Ryburn (2025)³² and Ryburn et al. (2026)³³. In January of 2024, we downloaded all vertebrate sequences (for fish) and all invertebrate sequences (for crustaceans) from the European Molecular Biology Laboratory (EMBL). Based on the distribution of percent sequence similarity scores, we filtered the MiFish sequences to an increased stringency of 98% similarity and Crustacean_16S to 97% similarity in the final dataset.

Statistical analysis

We conducted all statistical analyses in R (version 4.2.2; www.R-project.org) with RStudio (version 2022.12.0 + 353). The map of the sampling locations (Fig. 1) was also created in R using the Galapagos Archipelago shapefiles obtained from the Instituto Oceanográfico y Antártico de la Armada (INOCAR; <https://www.inocar.mil.ec/web/>). Spatial data were imported and handled using the *sf* package (version 1.0–16). Map visualization and figure layout were produced with *ggplot2* (version 3.5.1), including coastline rendering and spatial layering of geographic features. Sample-based rarefaction curves, which included all identified prey taxa, were created with the *vegan* package (version 2.6-4; ³⁶) for all blacktip sharks (Fig. 2), male and female sharks (Fig. 3), and sharks sampled during El Niño and La Niña (Fig. 4). We calculated the Levin's niche width at each shark nursery bay using the *spaa* package (version 0.2.2; ³⁷; Table 3). To assess dietary differentiation among sharks across the nursery bays, we used a permutational multivariate analysis of variance (perMANOVA) with the *adonis2* function in the *vegan* package³⁶. This analysis used the Jensen-Shannon distance of relative prey read abundance as the metric. Additionally, species richness for each shark was calculated using the *phyloseq* package (version 1.42.0; ³⁸; Table 3). Statistical analysis R Markdown code is available at https://github.com/sryburn95/Galapagos_Blacktip_Diet.

Results

From the 107 cloacal swabs collected, we identified 989,789 reads of amplicon sequence variants (ASVs), with an average count of $9,029 \pm 1,593$ (mean $\pm$ standard error) per individual shark. A total of 25 prey taxa were identified, including 19 to species, five to genus, and one to class. On average, each juvenile blacktip shark had 3.8 ± 0.2 prey taxa, with the number of taxa per individual ranging from one to eight. The diet of the juvenile blacktip

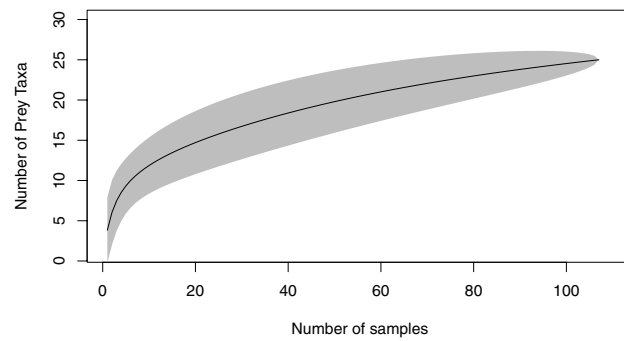


Fig. 2. Rarefaction curve illustrating cumulative prey richness as a function of blacktip sharks sampled. Prey richness includes all 25 classified prey taxa. Figure includes all 107 juvenile blacktips, with the shaded area representing the 95% confidence interval after 100 permutations.

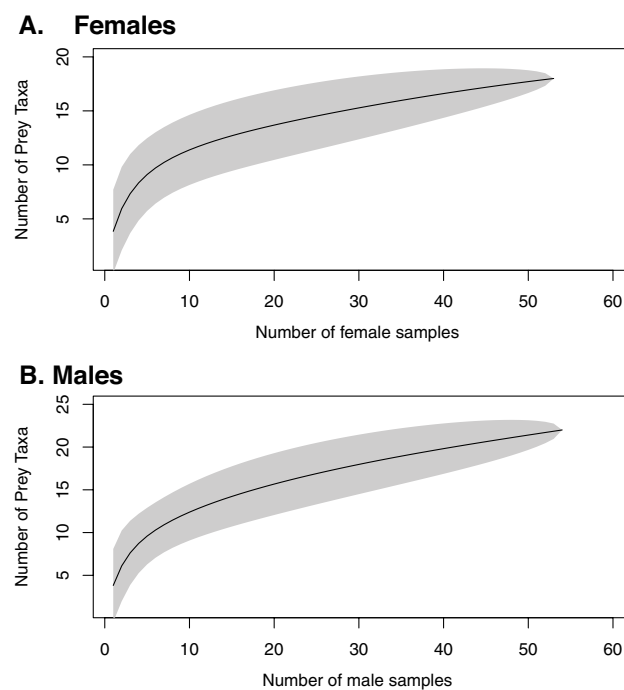


Fig. 3. Rarefaction curves illustrating cumulative prey richness as a function of female (A) and male (B) blacktip sharks sampled. Prey richness includes all 25 classified prey taxa. Figure includes all 107 juvenile blacktips, 53 females and 54 males. The shaded area representing the 95% confidence interval after 100 permutations.

was predominantly composed of fishes (98.8%, based on percent of occurrence), with smaller contributions from crustaceans (0.9%) and a single species of brittle star, *Amphipholis squamata* (dwarf brittle star; 0.2%). The Thoburn's mullet (*Mugil thoburni*) was the most common prey taxon (20.0%) followed by fishes in the genus *Abudefduf* (sergeant-majors; 13.4%), the Galapagos ringtail damselfish (*Stegastes beebei*; 12.7%), the scalloped hammerhead shark (*Sphyrna lewini*; 11.7%), and the yellowtail damselfish (*Stegastes arcifrons*; 10.7%; Table 2). Among all identified prey, the Thoburn's mullet was the only prey taxon endemic to the Galapagos. Scalloped hammerhead shark was identified in the diet of the juvenile blacktip shark in all six nursery bays located on San Cristobal, despite Puerto Grande being the only bay on the island classified as a scalloped hammerhead nursery. However, scalloped hammerhead was absent from the diets of the blacktip sharks from all nursery bays on the other three islands (i.e., Isabela, Santiago, and Fernandina), even though juvenile scalloped hammerheads are present in Cartago Bay. The rarefaction curve (Fig. 2) began to reach a plateau, suggesting that the 107 cloacal swabs provided a comprehensive representation of the dietary richness of juvenile blacktip sharks in the Galapagos. Of the 107 sharks, 54 were male and 53 were female. Sex-specific rarefaction curves (Fig. 3) for both males and females also began to plateau, suggesting that sampling effort was sufficient to capture dietary richness for each sex. In addition, we found a statistical difference between male and female juvenile blacktips when comparing the prey composition between sexes (perMANOVA: pseudo $F = 2.424$, $R^2 = 0.023$, $df = 1$, $P = 0.035$).

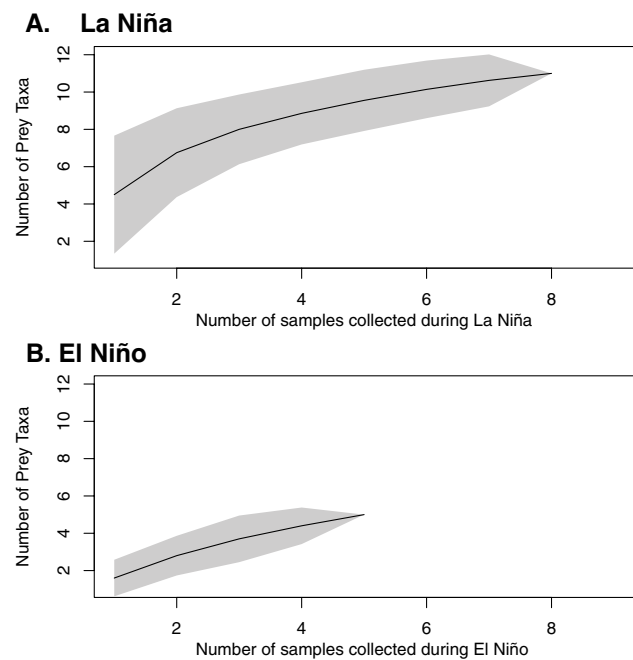


Fig. 4. Rarefaction curves illustrating cumulative prey richness as a function of blacktip sharks sampled from Rosa Blanca 2 during La Niña (**A**) and El Niño (**B**). Prey richness includes 14 classified prey taxa. Figure includes all 13 juvenile blacktips sampled from Rosa Blanca 2, eight during La Niña and five during El Niño. The shaded area representing the 95% confidence interval after 100 permutations.

Class	Order	Family	Genus	Species	Common Name	POO%
Actinopteri					Ray-finned fishes	4.4
		Pomacentridae	<i>Abudefduf</i>		Sergeant-majors	13.4
		Pomacentridae	<i>Stegastes</i>	<i>arcifrons</i>	Yellowtail damselfish	10.7
		Pomacentridae	<i>Stegastes</i>	<i>beebei</i>	Galapagos ringtail damselfish	12.7
Actinopterygii	Mugiliformes	Mugilidae	<i>Mugil</i>	<i>thoburni</i>	Thoburn's mullet	20.0
		Mugilidae	<i>Mugil</i>	<i>cephalus</i>	Flathead grey mullet	7.1
	Carangiformes	Carangidae	<i>Decapterus</i>		Jacks	2.7
		Carangidae	<i>Caranx</i>	<i>crysos</i>	Blue runner	0.2
		Carangidae	<i>Selar</i>	<i>crumenophthalmus</i>	Bigeye scad	0.7
	Beloniformes	Hemiramphidae	<i>Hemiramphus</i>	<i>lutkei</i>	Lutke's halfbeak	6.1
	Tetraodontiformes	Tetraodontidae	<i>Sphoeroides</i>	<i>annulatus</i>	Bullseye puffer	3.9
	Clupeiformes	Clupeidae	<i>Sardinops</i>		Sardines	1.2
	Scombriformes	Scombridae	<i>Thunnus</i>		Tunas	0.2
		Scombridae	<i>Euthynnus</i>	<i>affinis</i>	Mackerel tuna	0.5
	Labriformes	Labridae	<i>Scarus</i>	<i>ghobban</i>	Blue-barred parrotfish	0.7
		Labridae	<i>Semicossyphus</i>	<i>pulcher</i>	California sheephead	0.7
	Lutjaniformes	Lutjanidae	<i>Lutjanus</i>		Snappers	0.2
		Haemulidae	<i>Anisotremus</i>	<i>scapularis</i>	Peruvian grunt	0.5
	Anguilliformes	Muraenidae	<i>Muraena</i>	<i>lentiginosa</i>	Jewel eel	0.7
	Syngnathiformes	Fistulariidae	<i>Fistularia</i>	<i>commersonii</i>	Bluespotted cornetfish	0.2
Chondrichthyes	Carcharhiniformes	Carcharhinidae	<i>Sphyrna</i>	<i>lewini</i>	Scalloped hammerhead shark	11.7
Malacostraca	Decapoda	Mithracidae	<i>Teleophrys</i>	<i>crispulipes</i>	Spider crab	0.5
		Grapsidae	<i>Grapsus</i>	<i>grapsus</i>	Sally lightfoot crab	0.2
		Grapsidae	<i>Pachygrapsus</i>	<i>socius</i>	Pacific mottled shore crab	0.2
Ophiuroidea	Amphilepidida	Amphipruridae	<i>Amphipholis</i>	<i>squamata</i>	Dwarf brittle star	0.2

Table 2. Percent occurrence (POO) of all prey taxa identified in the combined diet of 107 juvenile Blacktip sharks.

A statistically significant difference in prey composition was also found when comparing the diets of juvenile blacktip sharks across the different nursery bays (perMANOVA: pseudo $F = 2.40$, $R^2 = 0.251$, $df = 13$, $P = 0.001$). Prey taxa richness was higher in the nursery bays located on San Cristobal compared to all other nursery bay locations (Figs. 5 and 6; Table 3). Crustaceans were only found in the diets of the sharks sampled from San Cristobal. The Thoburn's mullet was present in the diet of the sharks at every nursery bay except Albemarle (Fig. 5).

Rosa Blanca 2 was the only nursery bay where samples were collected across multiple years (i.e., 2021 and 2023). In 2021, the Galapagos experienced a La Niña event and in 2023 an El Niño event. In total, 13 juvenile blacktips were sampled from Rosa Blanca 2, eight during La Niña and five during El Niño. As shown in Fig. 7, the average prey taxa richness for juvenile blacktips caught in Rosa Blanca 2 was higher in 2021, during La Niña conditions, than in 2023, when the archipelago was experiencing an El Niño event. However, neither of the rarefaction curves (Fig. 4) from the sharks sampled from Rosa Blanca 2 during La Niña and El Niño reached an asymptote, suggesting that shark diet richness may be underestimated.

Discussion

In the Galapagos, juvenile blacktip sharks primarily consumed fishes, particularly Thoburn's mullet. We found that the diet of juvenile blacktip sharks varied by location; prey taxa richness at the population and individual level was significantly higher on San Cristobal than the other three islands (Table 3). Additionally, diet varied between samples collected at the same location during a La Niña event and those collected during an El Niño event (Fig. 7).

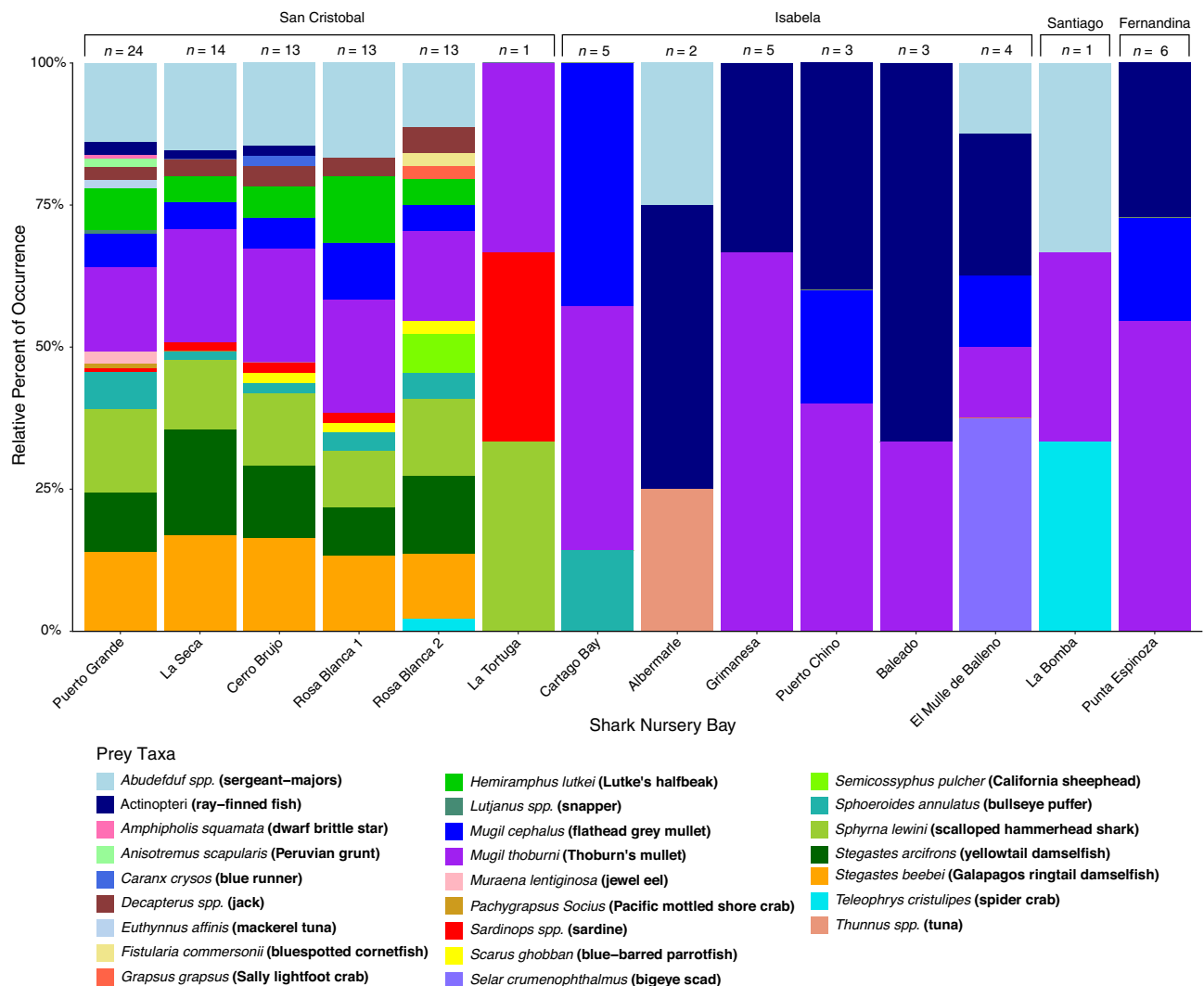


Fig. 5. Relative percent of occurrence (POO) of all prey taxa identified in the diets of the 107 juvenile blacktip sharks sampled across the 14 nursery bays. The x-axis represents the individual nursery bays: Puerto Grande, La Seca, Cerro Brujo, Rosa Blanca 1, Rosa Blanca 2, and La Tortuga are located on San Cristobal; Cartago Bay, Albemarle, Grimanese, Puerto Chino, Baleado, and El Muelle de Balleno are located on Isabela; La Bomba is located on Santiago; and Punta Espinoza is located on Fernandina.

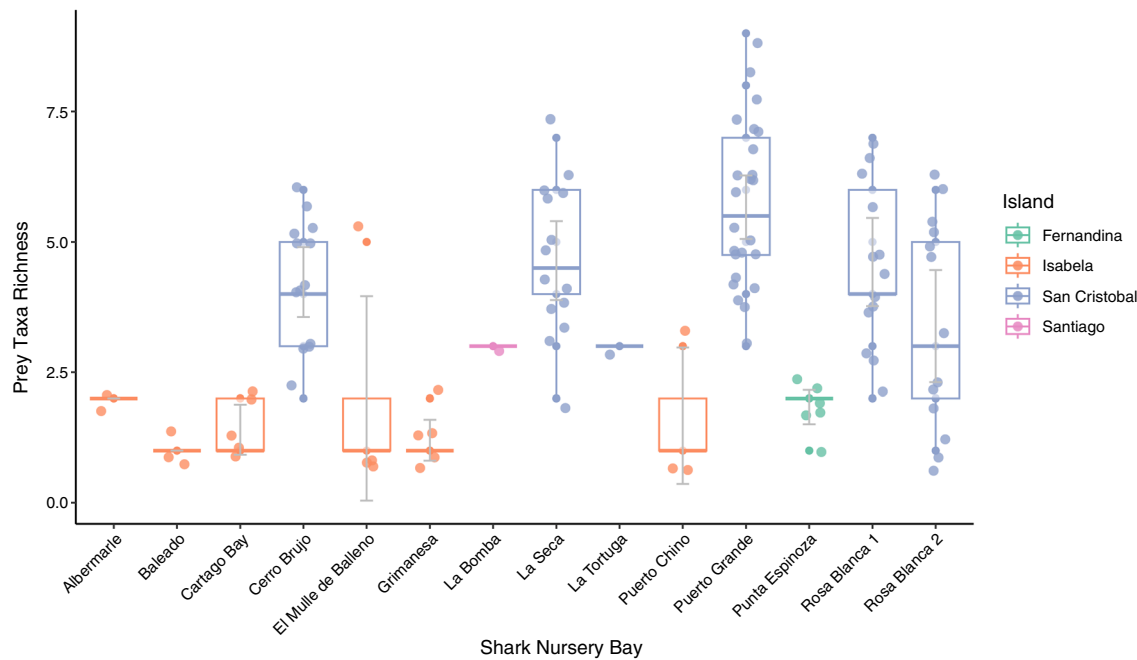


Fig. 6. Prey taxa richness across juvenile blacktip shark nursery bays in the Galapagos Archipelago. Colors indicate the island where each bay is located: San Cristobal, Isabela, Santiago, and Fernandina. Taxa richness encompasses all 25 identified prey taxa, and the figure includes all 107 sampled sharks. Grey error bars indicate a 95% confidence interval.

Shark nursery bay	Island	Sample size	Mean number of prey taxa	Levin's niche width	Richness
Albemarle	Isabela	2	2	1.42	3
Baleado	Isabela	3	1	1.82	2
Cartago Bay	Isabela	5	1.4	1.84	3
Cerro Brujo	San Cristobal	13	4.3	3.67	13
El Muelle de Balleno	Isabela	4	2	1.12	5
Grimanesa	Isabela	5	1.2	1.25	2
La Bomba	Santiago	1	3	1.12	3
La Seca	San Cristobal	14	4.6	2.80	11
La Tortuga	San Cristobal	1	3	1.46	3
Puerto Chino	Isabela	3	1.7	2.00	3
Puerto Grande	San Cristobal	24	5.7	4.13	17
Punta Espinoza	Fernandina	6	1.8	1.02	3
Rosa Blanca 1	San Cristobal	13	4.5	2.00	11
Rosa Blanca 2	San Cristobal	13	3.4	1.19	14

Table 3. The mean number of different prey taxa per individual shark, Levin's niche width, and richness across all replicates for a given shark nursery bay. Table includes results from all 107 sampled sharks.

Previous studies focused on the diet of the juvenile blacktip shark relied on morphological stomach content analysis, which frequently resulted in high proportions of unidentified prey (e.g., 99.3% of the Index of Relative Importance [IRI] consisted of unidentified teleosts)^{11,17,18}. In contrast, using DNA metabarcoding of fecal matter, we were able to identify 19 of the 25 prey taxa to the species level (Table 2). The diet of blacktip sharks is known to vary geographically¹⁷, likely reflecting differences in prey availability. However, Clupeids and Sciaenids are recorded as being the most common prey consumed by blacktip sharks worldwide¹⁷. In our study, we did not detect any Sciaenids in the diet of juvenile blacktips from the Galapagos and observed only a small contribution of Clupeids to their overall diet (1.2% based on percent of occurrence; Table 2). Llerena-Martillo et al. (2018)⁶⁵ reported that blacktip shark and Thoburn's mullet were the two most abundant fish species inhabiting the mangrove bays of Santa Cruz Island in the Galapagos. The ample abundance of Thoburn's mullet could explain why it had the largest contribution to the diet of the juvenile blacktips in our study. Additionally, Puerto Grande

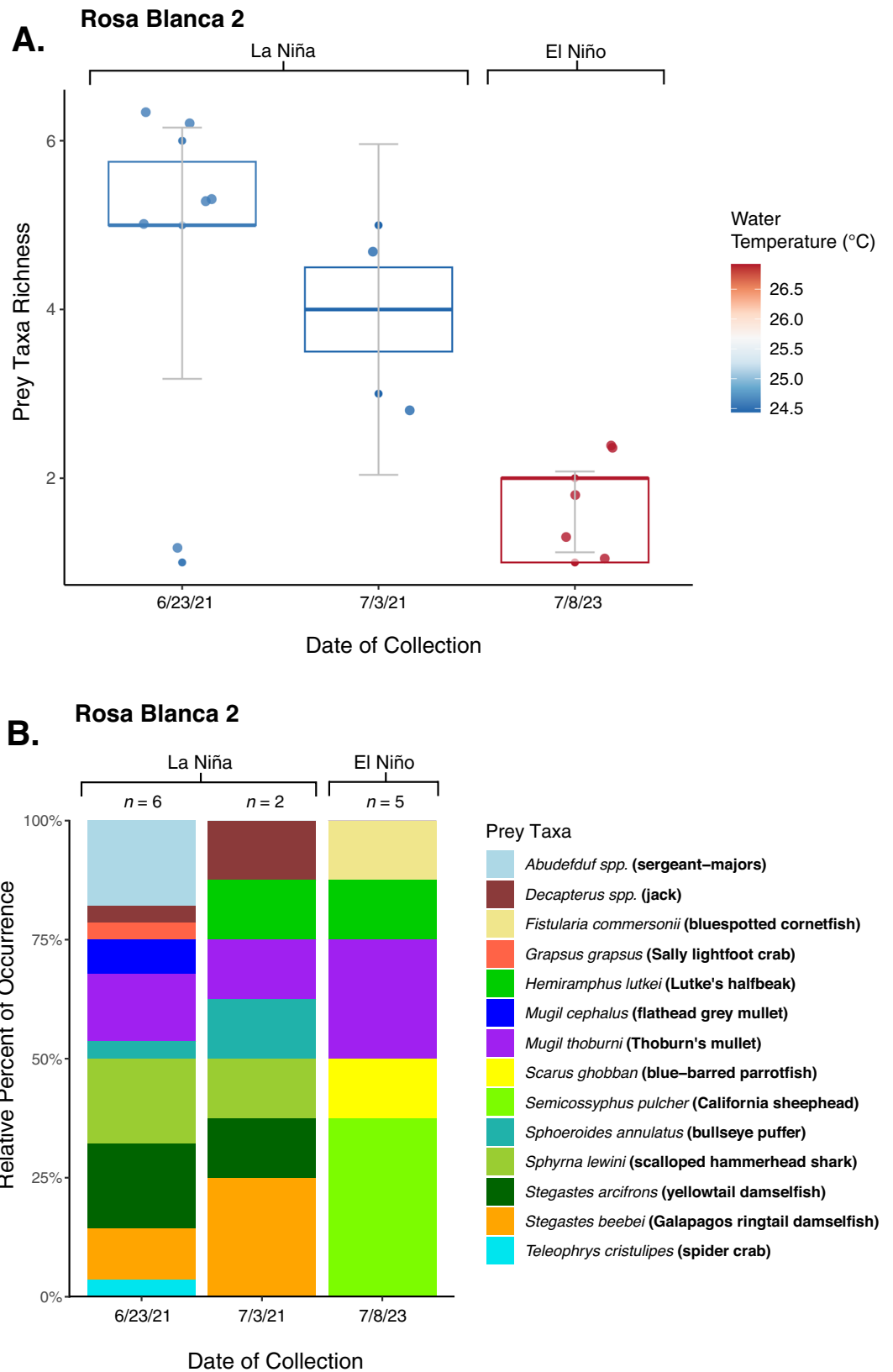


Fig. 7. Relationship between capture date and prey for juvenile blacktip sharks sampled at Rosa Blanca 2 on San Cristobal. Data includes all 13 sharks collected from this location. In 2021 there was a La Niña event and in 2023 there was an El Niño event. **A)** Boxplot comparing capture date, prey taxa richness, and water temperature. Grey error bars indicate a 95% CI. **B)** Relative percent of occurrence (POO) of all prey taxa identified in the diets of juvenile blacktip sharks across the three different sampling dates.

exhibited the largest Levin's niche width among the 14 sampled bays, followed by Cerro Brujo and La Seca (Table 3). Geographically, La Seca and Cerro Brujo are the closest bays to Puerto Grande among the other 13 sampled locations. Further research on prey species abundance and richness is needed to better understand the ecological significance of Puerto Grande and how its prey composition effects the diet of juvenile blacktip sharks.

Overall, scalloped hammerhead shark was the fourth most common prey species (11.7%; Table 2). Our methodology focused on identifying the presence of scalloped hammerheads in the blacktips' diet but did not differentiate the age or size of the individuals consumed, nor whether they were actively hunted or scavenged. While it is implausible that juvenile blacktips were hunting adult scalloped hammerheads, it is possible that they targeted juveniles. Juvenile blacktip sharks are known to be highly mobile³⁹ and are thought to utilize areas much larger than the boundaries of individual nursery bays. For instance, juvenile blacktips originally caught in Puerto Grande were documented traveling up to 46.8 km within 35 h⁹. Puerto Grande was the only bay on San Cristobal where juvenile scalloped hammerheads were reliably observed. However, juvenile blacktips from other nursery bays on San Cristobal could have ventured into Puerto Grande, providing opportunities for them to consume scalloped hammerheads. Further research is needed to better understand the home range and movement patterns of juvenile scalloped hammerheads in the Galapagos⁴⁰. It remains unclear how far and frequently these juvenile scalloped hammerheads travel from their specific nursery bays, but they likely venture into other bays on the island, increasing their risk of predation. Additionally, neonate scalloped hammerheads are known to have high mortality rates due to malnutrition and starvation⁴¹, so it may be more likely that juvenile blacktips scavenged on deceased neonate scalloped hammerheads in or near Puerto Grande.

The role of ENSO

The El Niño Southern Oscillation (ENSO) cycle influences the Galapagos Archipelago in various ways, including shifts in primary productivity and upwelling⁴². During El Niño events, primary productivity declines dramatically due to increased stratification and reduced upwelling^{43,44} which has cascading effects on the food web^{42,45}. For example, during the 1997–1998 El Niño, the Galapagos Archipelago had elevated temperatures for over 12 months, resulting in clearer oceanic waters, declines in dissolved nutrients, and reduced phytoplankton productivity⁴⁶. This prolonged disruption at the base of the marine food chain ultimately led to a decrease in biomass. Most algal species became scarce or declined, leading to mortality and reduced densities of herbivores such as marine iguanas (*Amblyrhynchus cristatus*) and crabs⁴⁵. The impacts extended throughout the food web, leading to population declines in higher trophic-level species, including the Galapagos penguins (*Spheniscus mendiculus*), flightless cormorants (*Phalacrocorax harrisi*), Galapagos fur seals (*Arctocephalus galapagoensis*), and Galapagos sea lions (*Zalophus wollebaeki*)^{46,47}. As food availability declines, many species, such as marine iguanas and Galapagos penguins, face an increased risk of starvation^{45,48}. Given that El Niño events have intensified in the eastern tropical Pacific over the past millennium, with the last 50 years marking an unprecedented period of warming⁴⁹, such disruptions to marine food webs may become more frequent and severe.

The majority of cloacal swabs (98 out of 107) were collected in 2021 and 2022 during La Niña conditions, however, samples were collected from Rosa Blanca 2 in both 2021 (La Niña) and 2023 (El Niño). We found that prey richness was lower in the samples collected from Rosa Blanca 2 during the El Niño event in 2023 compared to those collected in 2021 during a La Niña event (Fig. 7A). However, this result is derived from a very small sample size and therefore should be interpreted with caution. Previous studies have reported that fish biomass and species richness are greater during La Niña, when upwelling and productivity are much greater^{50,51}. For example, the abundance of Thoburn's mullet in the Galapagos artisanal fisheries landings decreased during the 1997–1998 El Niño but increased during the 1998–1999 La Niña⁵². The reduction in prey availability during El Niño events could explain the observed decline in dietary richness among juvenile blacktips in Rosa Blanca 2. Notably, two damselfish species (Galapagos ringtail damselfish and yellowtail damselfish) and scalloped hammerhead sharks were present in the diet of the juvenile blacktips in Rosa Blanca 2 in 2021 but were absent in 2023 (Fig. 7B). Damselfish primarily consume algae⁵³ and since El Niño events reduce algal production and biomass⁴⁵, damselfish populations could have declined during this period. For example, the Galapagos damselfish (*Azurina eupalama*) has not been observed in the Galapagos since the 1982 El Niño event and is currently classified as possibly extinct⁵⁴. Additionally, juvenile scalloped hammerheads are particularly vulnerable to starvation in general⁵⁵, and their abundance could have been similarly affected by the El Niño event.

Prey richness across the archipelago

Due to variation in temperature, nutrient availability, and primary productivity, fish species richness is lower on the western side of the Galapagos Archipelago³. The region's heightened productivity and strong upwelling create a unique environment that supports a smaller number of species, favoring those that are well-adapted to these conditions. Additionally, cooler water temperatures in the west make it difficult for many tropical species to survive. Juvenile blacktip sharks could be consuming few prey species in this region simply because there are fewer prey options compared to the more species-rich eastern region (Fig. 6). Water temperature in the Galapagos varies considerably due to multiple factors, including ENSO events, upwelling, ocean currents, and seasonal fluctuations^{56–58}. However, the western region is characterized by a temperate climate with colder waters that support more Peruvian fish species, while the eastern region has a more tropical climate, favoring Panamic and Indo-Pacific species³. The strong regional divisions in marine fauna are a reflection of local environment as well as connectivity of larval propagules with external source regions³. Fish species with Indo-Pacific distributions likely maintain gene flow across the East Pacific Barrier, contributing to higher species richness in the region³. In contrast, populations of Panamic and Peruvian species in the Galapagos are likely self-sustaining, with Panamic species experiencing intermittent recruitment from the mainland, while Peruvian species persist without reliance on larval input from the continent³. The environmental conditions in the western Galapagos resemble those of central South America, with cooler temperatures and high productivity⁵⁹. As a

result, Peruvian fish species may have a stronger affinity for this distinct region of the archipelago³. Conversely, tropical species could be intolerant of the cooler waters of the western Galapagos, thereby reducing the number of potential prey species available to juvenile blacktips residing in that area.

Conservation and conclusions

Mangrove-fringed bays are not only critical habitats for juvenile blacktip sharks but also serve as vital fishing grounds for artisanal fishermen in the Galapagos, where they catch mullet and bait for offshore fishing⁵². Mullet, specifically Thoburn's mullet and Flathead grey mullet (*Mugil cephalus*), are the most important target species of the artisanal gillnet fisheries operating within the Galapagos Marine Reserve (GMR)^{60,61}. These two species accounted for 30% of the total fish biomass landed in the GMR from 1998 to 2006⁶². Thoburn's mullet, an endemic species to the Galapagos Archipelago, also accounted for the largest percentage of prey found in the blacktip's diet (Table 2). Sustained fishing pressure can lead to declines in species density⁶³ and diversity⁶⁴, making it imperative to prevent overfishing of these mangrove bays and, in particular, Thoburn's mullet. Protecting these ecosystems is crucial to maintaining both the ecological balance and the livelihoods of local fishers.

In conclusion, our findings indicate that location and the ENSO cycle had a considerable effect on the diet of the juvenile blacktip in the Galapagos, likely due to their effects on prey availability. These results are in concordance with previous research identifying water temperature as the most important environmental variable driving variations in fish abundance within the mangrove bays of the Galapagos⁶⁵. For example, Thoburn's mullet, a critical prey species for juvenile blacktips, has been found to be more abundant in the cold season than the warm. With rising sea surface temperatures due to climate change, this could negatively affect the abundance of one of the juvenile blacktip's primary prey species. Many shark species are often categorized as generalist predators⁶⁶. However, using metabarcoding techniques on non-lethal fecal samples to expand our understanding of shark diets can improve our ability to predict how disruptions in food web structure and prey availability, particularly due to overfishing and climate change, may impact shark survival and conservation efforts.

Data availability

The raw data is available at Figshare: 10.6084/m9.figshare.30359203.

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Author contributions

SJR and *EW* designed the study. *SJR* , *EW* , *EE* , *DA* , *HEC* , and *JFB* collected the samples. *DAP* and *AH* provided the permits. *SJR* and *EW* completed the DNA analysis. *SJR* analyzed the data. The manuscript was written by *SJR* with help from *JFB* . All authors revised the manuscript and gave final approval for publication.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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Correspondence and requests for materials should be addressed to S.J.R.

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