

USING REFERENTIAL VALUES OF $\delta^{13}\text{C}$ AND $\delta^{15}\text{N}$ TO INFER THE FORAGING ECOLOGY OF GALÁPAGOS SEABIRDS

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ABSTRACT

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The Galápagos Penguin *Spheniscus mendiculus*, Flightless Cormorant *Phalacrocorax harrisi*, and Waved Albatross *Phoebastria irrorata* are endemic to Islas Galápagos. They are known to feed on different prey (including crustaceans, cephalopods, and/or several species of epipelagic and benthic fish), in accordance with different foraging strategies. In this work, we used stable-isotope analysis of carbon and nitrogen to corroborate available information on habitat use ($\delta^{13}\text{C}$) and trophic position ($\delta^{15}\text{N}$). Feather samples from the three species were collected in six different areas prior to the 2011 and 2012 breeding seasons. Results showed differences in foraging strategies between Galápagos Penguins and the other two species ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, $P < 0.01$). The Flightless Cormorant and Waved Albatross showed similar proportions of $\delta^{13}\text{C}$ ($P = 0.07$), but they occupied different trophic levels ($\delta^{15}\text{N}$, $P < 0.01$). Isotopic signatures in Galápagos Penguins reflected differences based on their breeding areas ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, $P < 0.01$), which were subject to different environmental conditions. This information could be used to evaluate future ecological conditions among the feeding areas of these species.

Key words: marine birds, Islas Galápagos, isotopic values, foraging strategies, trophic level

INTRODUCTION

The Galápagos Penguin *Spheniscus mendiculus* (GAPE), Flightless Cormorant *Phalacrocorax harrisi* (FLCO), and Waved Albatross *Phoebastria irrorata* (WAAL) are endemic marine birds of Ecuador, breeding in Islas Galápagos (Harris 1973b). More than 90 % of GAPEs and the entire population of FLCOs occur on the western islands of the archipelago (Islas Isabela and Fernandina), while 99.9 % of the WAAL breeding population occurs in the eastern region, on Isla Española (Snow 1966, Harris 1973b). The remaining GAPEs are found on Islas Floreana and Santiago (Wiedenfeld & Jiménez-Uzcátegui 2008), and a few pairs of WAALs are found on Isla La Plata, which is located a few kilometers off the coast of Ecuador (Harris 1973a, Awkerman *et al.* 2014).

The foraging activities of GAPEs and FLCOs are restricted to the western bioregion of the archipelago (Edgar *et al.* 2004); both species are coastal predators and probably use different foraging strategies (Snow 1966, Boersma *et al.* 2013). GAPEs forage up to 23.5 km from their nesting area and usually feed at depths of up to 6 m (Steinfurth *et al.* 2008) in the upwelling system of Bahía Elizabeth. The GAPE is considered to be a generalist predator because its diet includes several species of epipelagic fish and cephalopods (Vargas *et al.* 2006, Boersma *et al.* 2013), among which the larvae or juveniles of anchovies *Engraulis* spp. and sardines *Sardinops* spp. stand out (Steinfurth unpubl. data). FLCOs forage up to 5 km from their nests at depths of up to 6 m (Vargas 2006). The FLCO is seen as a benthic predator because it consumes such prey as crustaceans, octopus, and benthic fish (Snow 1966, Valle 1994). The WAAL is considered to be a pelagic predator

because it consumes squid and pelagic fish mainly outside the Galápagos Marine Reserve on the Peruvian and Ecuadorian coasts (Fernández *et al.* 2001, Awkerman *et al.* 2014), but occasionally in Bahía Elizabeth at Isabela Island (Jiménez-Uzcátegui unpubl. data).

There are currently several techniques used to study the feeding behavior of marine predators. Among these, stable-isotope analysis for carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) provides information on the food assimilated by a consumer (DeNiro & Epstein 1978, 1981). Knowing the isotopic niche makes it possible to infer an individual's habitat type (using $\delta^{13}\text{C}$) or trophic level and breadth (using $\delta^{15}\text{N}$) (Boecklen *et al.* 2011, Kim *et al.* 2012). The isotopic signatures of a predator's tissues can thus be used as natural chemical tracers of ecological processes, allowing us to identify energy flows and to characterize the sources of primary production that support its food web (Newsome *et al.* 2007, Martínez del Río *et al.* 2008). In marine organisms, $\delta^{13}\text{C}$ values reflect the type of habitat (coastal/oceanic or pelagic/benthic) used by their prey (Hobson & Welch 1992). Differences in $\delta^{13}\text{C}$ are determined by physicochemical and biological factors that influence the taxonomic composition of phytoplankton and the concentration of dissolved CO_2 in primary consumers (Goericke & Fry 1994, France 1995). Nitrogen isotopes, on the other hand, are strongly fractionated from prey to predator, resulting in $\delta^{15}\text{N}$ enrichment at higher trophic levels (Post 2002).

Inert tissues, such as feathers, retain their isotopic information once grown and therefore capture mid- and long-term dietary changes (Cherel *et al.* 2000, Jaeger *et al.* 2009). Since feather keratin is metabolically inert after protein synthesis, the isotopic composition

of feathers reflects diet before moult (Hobson & Clark 1992, Bearhop *et al.* 2002). For this reason, isotopic analysis of feathers has become a powerful method to investigate the foraging ecology of adult seabirds (Cherel *et al.* 2002, Quillfeldt *et al.* 2005). Several studies on seabird feathers have focused on wing feathers (primaries and secondaries), but the ethics of sampling wing feathers from live birds is questionable because of the resulting impairment on flying (Weimerskirch *et al.* 1995, Cherel *et al.* 2008). Since body feathers are more readily obtained from live animals, they provide an alternative to the use of wing feathers (Bearhop *et al.* 2000, Norris *et al.* 2007). Body feather moult in GAPEs, FLCOs, and WAALs occurs weeks before the onset of breeding and at an interval of about six months (Boersma 1976, Harris 1993b, Valle 1994). Therefore, the isotopic information from body feather tissue would be limited to the few months before the breeding season (Becker *et al.* 2007, Jaeger *et al.* 2009).

Despite the increasing use of stable isotopes to monitor the trophic ecology of seabirds over recent years, only a few researchers have used this technique on Galápagos marine birds (Lee-Cruz *et al.* 2012, Awkerman *et al.* 2014) and none have focused on using the isotope data to infer or validate the trophic habits of GAPEs and FLCOs. Our main goal was to test the use of body feathers from adult seabirds to provide another perspective on the trophic behavior and feeding strategies of GAPEs, FLCOs, and WAALs in Islas Galápagos. By better understanding the role of these predators in the food web, we can establish baseline knowledge that will contribute to the management and conservation of these species.

METHODS

Study area

The Islas Galápagos are in the eastern Pacific Ocean along the Equator, approximately 960 km west from the continental coast. In total, six sampling areas were selected. GAPEs were sampled on Isla Isabela at Caleta Iguana (00°58.6'S, 091°26.7'W) and Puerto Pajas (00°45.3'S, 091°22.5'W), as well as on Islotes Las Marielas (00°35.8'S, 091°05.4'W). FLCO populations were sampled on Isla Fernandina at Playa Escondida (00°15.7'S, 091°28.1'W) and on Isla Isabela at Punta Albemarle (00°09.2'N, 091°22.0'W). WAALs were

sampled on Isla Española at Punta Suárez (01°22.3'S, 089°44.4'W) (Fig. 1).

Sampling

In 2011 and 2012 we captured 170 adult females (79 GAPEs, 61 FLCOs, and 30 WAALs) in August (one month before the breeding season), as part of an ongoing Marine Birds Project conducted by the Charles Darwin Foundation and the Galápagos National Park. We banded every bird and collected biological samples and clinical data from each. Body feathers were collected near the tail for GAPEs but near the wings for FLCOs and WAALs. Feathers were collected using a common non-destructive sampling protocol (Burger 1993) and were kept in a paper envelope with all pertinent information (e.g., species, date, location, and identification number).

Laboratory analysis

Each feather sample was rinsed with deionized water to remove residues that might interfere with the isotopic signature. The samples were then desiccated in an oven at 80 °C for 24 h, and lipids were extracted following the Microwave Assisted Extraction protocol (Delazar *et al.* 2012), using 25 mL of chloroform/methanol (1:1 v/v). This process was applied to eliminate any bias that may be introduced by lipids in the tissues, which could negatively skew the $\delta^{13}\text{C}$ isotopic signature (Post *et al.* 2007). After lipid extraction, the samples were air-dried, cut into small pieces, and ground to a very fine powder using an agate mortar. A subsample of this powder (~ 1 mg) was sealed in a tin capsule.

Isotope values were measured under continuous-flow conditions in a mass spectrometer (20–20 PDZ Europe, Sercon Ltd., Cheshire, UK) at the Stable Isotope Facility at the University of California, Davis. The results are presented as delta values per mil (‰) using the following equation:

$$\delta^{15}\text{N} \text{ or } \delta^{13}\text{C} = [(R_{\text{sample}} / R_{\text{standard}}) - 1]$$

where R_{sample} and R_{standard} are the values of $^{15}\text{N}/^{14}\text{N}$ or $^{13}\text{C}/^{12}\text{C}$, respectively (DeNiro & Epstein 1978).

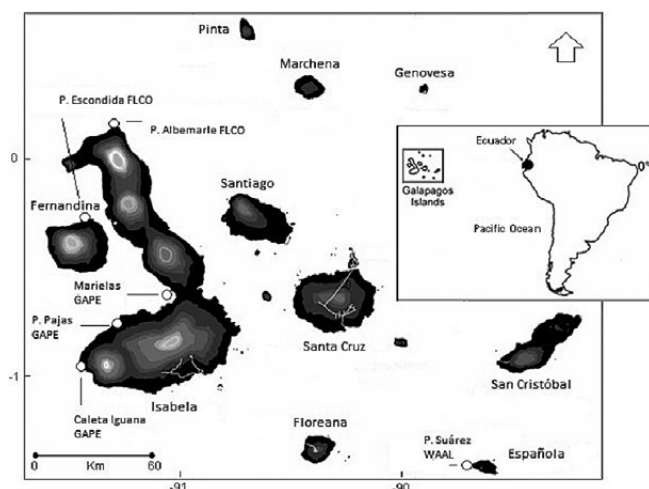


Fig. 1. Breeding colony locations for Galápagos Penguins (GAPE), Flightless Cormorants (FLCO), and Waved Albatross (WAAL), sampled in 2011 and 2012.

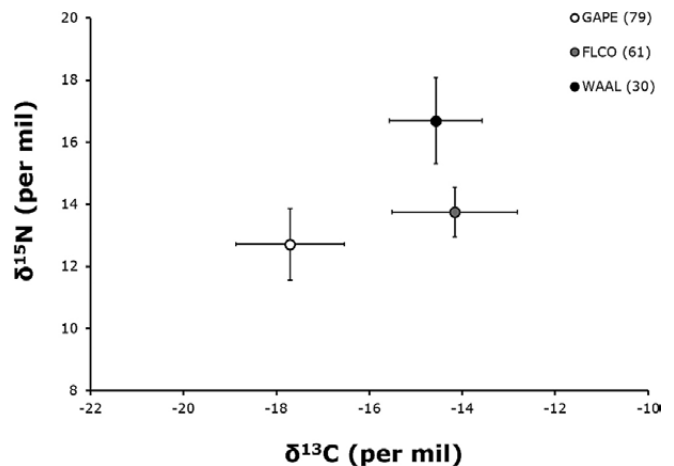


Fig. 2. Values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (expressed in ‰; mean \pm SD) in feather samples from Galápagos Penguin (GAPE), Flightless Cormorant (FLCO), and Waved Albatross (WAAL). The sample size for each species is shown in the parentheses beside the species code.

The standards used were atmospheric nitrogen (N_2) for $\delta^{15}N$ and Pee Dee Belemnite for $\delta^{13}C$. The results were calibrated using international standards (ammonium sulfate for $\delta^{15}N$ and sucrose for $\delta^{13}C$), which generated a standard deviation between the isotopic measurement trials of $< 0.3\text{‰}$ for $\delta^{15}N$ and $< 0.2\text{‰}$ for $\delta^{13}C$.

Data analysis

Statistical tests were performed with Minitab, version 15 (Minitab Inc.) and Statistica 8.0. Data were tested for normality and homoscedasticity using the Shapiro–Wilk and Levene tests, respectively. The statistical significance of differences in $\delta^{13}C$ and $\delta^{15}N$ values were determined using parametric or non-parametric tests and are reported when $P < 0.05$. The graphics were created using SigmaPlot, version 11 (Systat Software, Inc.). Values are reported as mean \pm standard deviation (SD).

RESULTS

Isotopic difference among species

Respectively, the mean estimated $\delta^{13}C$ and $\delta^{15}N$ values were $-17.7\text{‰} \pm 1.2\text{‰}$ and $12.7\text{‰} \pm 1.2\text{‰}$ for GAPE feathers;

$-14.2\text{‰} \pm 1.4\text{‰}$ and $13.7\text{‰} \pm 0.8\text{‰}$ for FLCO feathers; and $-14.5\text{‰} \pm 0.9\text{‰}$ and $16.6\text{‰} \pm 1.3\text{‰}$ for WAAL feathers (Fig. 2). The C/N ratios of the samples ranged from 2.8 to 3.2 and were thus within the theoretical range established for the assimilation of protein from a predator's diet (McConnaughey & McRoy 1979) (Table 1). The $\delta^{13}C$ and $\delta^{15}N$ values were significantly different among species (Kruskal–Wallis test, $P = 0.01$ and $P < 0.01$, respectively): $\delta^{13}C$ values for GAPEs differed from those for FLCOs and WAALs, while $\delta^{15}N$ values differed among all species (multiple comparisons of median ranks, $P < 0.01$; Fig. 2).

Isotopic difference among breeding areas

The mean $\delta^{13}C$ and $\delta^{15}N$ values for GAPEs and FLCOs are shown by breeding areas and seasons in Table 1. The $\delta^{13}C$ and $\delta^{15}N$ values for GAPEs were significantly different between breeding areas (ANOVA: $F = 2.76$ and $P = 0.01$ for $\delta^{13}C$; $F = 2.90$ and $P = 0.01$ for $\delta^{15}N$). There were significant differences in $\delta^{13}C$ values for all populations, and the $\delta^{15}N$ values at Caleta Iguana differed from the other two sites (Tukey multiple comparisons, $P < 0.01$; Fig. 3a). In contrast, there were no significant differences between $\delta^{13}C$ and $\delta^{15}N$ values in the FLCO breeding areas (paired t -test, $P = 0.83$ and 0.55 , respectively; Fig. 3b).

TABLE 1
Isotope values ($\delta^{13}C$ and $\delta^{15}N$) and C/N ratio (mean \pm SD in ‰) in feather samples of Galápagos Penguin (GAPE), Flightless Cormorant (FLCO), and Waved Albatross (WAAL), categorized by breeding area and year

Species	Site	Year	<i>n</i>	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	C/N mass ratio
GAPE	Caleta Iguana	2011	18	-18.09 ± 0.65	12.35 ± 0.72	2.87 ± 0.03
		2012	12	-18.80 ± 0.91	11.61 ± 0.84	2.83 ± 0.14
	Puerto Pajas	2011	10	-17.72 ± 1.11	13.02 ± 0.73	3.20 ± 0.04
		2012	11	-18.03 ± 0.36	13.05 ± 0.99	3.20 ± 0.02
	Las Marielas	2011	18	-16.90 ± 0.84	13.49 ± 1.06	3.18 ± 0.02
		2012	10	-16.39 ± 0.91	13.01 ± 1.29	3.15 ± 0.03
FLCO	Playa Escondida	2011	13	-14.81 ± 1.71	13.33 ± 0.90	3.09 ± 0.06
		2012	19	-13.84 ± 0.98	13.94 ± 0.65	3.19 ± 0.04
	Punta Albemarle	2011	19	-14.24 ± 1.40	13.82 ± 0.80	3.20 ± 0.02
		2012	10	-13.87 ± 1.07	13.81 ± 0.89	3.17 ± 0.02
WAAL	Punta Suárez	2012	30	-14.57 ± 0.99	16.69 ± 1.39	3.18 ± 0.02

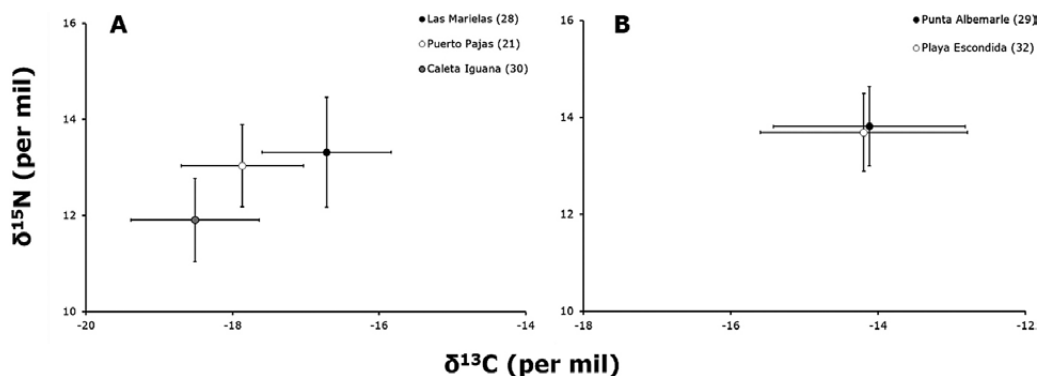


Fig. 3. Values of $\delta^{13}C$ and $\delta^{15}N$ (expressed in ‰; mean \pm SD) in feathers of (A) Galápagos Penguins sampled in three breeding areas: Caleta Iguana, Puerto Pajas (Isla Isabela), and Islotes Las Marielas; and (B) Flightless Cormorants sampled in two breeding areas: Punta Albemarle (Isla Isabela) and Playa Escondida (Isla Fernandina).

Between 2011 and 2012, the GAPE populations did not show significant differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (Mann–Whitney U-test: Las Marielas, $P = 0.15$ and 0.27 , respectively; Puerto Pajas, $P = 0.67$ and 0.52 , respectively; Caleta Iguana, $P = 0.07$ and 0.08 , respectively). These differences between years were not apparent in FLCO populations (paired t -test: Punta Albemarle: $P = 0.50$ and 0.97 , respectively; Playa Escondida, $P = 0.24$ and 0.61 , respectively).

DISCUSSION

Given that stable-isotope analysis is influenced by organic matter sources ($\delta^{13}\text{C}$), and by prey type and trophic level ($\delta^{15}\text{N}$), our results indicate that there is trophic segregation among GAPE and FLCO populations. Displacement patterns were identified in both species with the help of satellite tags (Vargas 2006, Steinfurth *et al.* 2008), and the variation in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values between species supports the hypothesis that GAPEs and FLCOs feed in different ocean areas around the Galápagos. This, in turn, influences trophic level.

Some of the advantages of using feathers for trophic research include the ability to gather long-term information (i.e., over a span of months) and the possibility of monitoring feeding behavior in pre-breeding stages (Cherel *et al.* 2000, Jaeger *et al.* 2009). Thus, the appropriate use of GAPE, FLCO, and WAAL body feathers is crucial, because these species retain these feathers for at least five months, then moult weeks before the breeding season (Harris 1973a, Boersma 1976, Valle 1994). Inaccuracies in isotopic niche estimates may be debated; however, we detected feeding-strategy patterns for all species that were consistent with those previously reported by groups using regurgitations from adult females to determine diet in different areas of the archipelago (Snow 1966, Fernández *et al.* 2001, Boersma *et al.* 2013).

The $\delta^{13}\text{C}$ values for FLCOs were more positive than those for GAPEs, indicating the use of separate environments. Due to differences in carbon sources (benthic–macroalgae vs. pelagic–phytoplankton), food webs in inshore and benthic ecosystems show a stronger enrichment of $\delta^{13}\text{C}$ than those in pelagic ecosystems (Goericke & Fry 1994, France 1995). Therefore, the $\delta^{13}\text{C}$ signature we observed in GAPEs indicates a consistently pelagic foraging strategy. In contrast, FLCOs have a more inshore foraging strategy, which limits their diet to prey associated with rocky bottoms (Snow 1966, Valle 1994). However, Steinfurth *et al.* (2008) mention that, in many instances, GAPEs feed near and parallel to the coast; this pattern does not coincide with the few available diet studies, which suggest that cephalopods and larval or juvenile anchovies and sardines are their main prey (Boersma *et al.* 2013, Steinfurth unpubl. data). The $\delta^{15}\text{N}$ values for FLCOs were higher than those for GAPEs over the entire study period. This is consistent with the preferred foraging zones among cormorants and penguins in Islas Galápagos (Valle 1994, Vargas 2006). The diet of GAPEs, which feed mostly in oceanic waters, is mainly composed of prey from the lower trophic levels (filter fish and squids; Boersma *et al.* 2013, Steinfurth unpubl. data), as opposed to that of FLCOs, which feed mainly near of the coast and consume prey from the higher trophic levels (benthic fish and octopus; Valle 1994).

Although WAALs are not a diving bird, they exhibited ^{13}C -enriched values, as did the FLCOs. This result suggests that WAALs would be foraging in highly productive areas, such as the coast of Peru,

where elevated primary productivity is associated with upwelling in the Humboldt Current System (Banks 2002); this corroborates the pelagic-predator strategy reported for this species (Fernández *et al.* 2001, Awkerman *et al.* 2014). WAALs are primarily teuthophagous, but they also feed on epipelagic fish (Harris 1973a). This is reflected in their nocturnal feeding behavior, in which they take mesopelagic prey that surface at night (e.g., squid; Harris 1973a). The high $\delta^{15}\text{N}$ values in WAALs relative to other species could indicate that they are feeding on prey of a higher trophic level or that they are consuming similar prey (i.e., filter fish and squid) in areas of greater nitrification, such as the coasts of Peru and Ecuador (Farrell *et al.* 1995). The $\delta^{15}\text{N}$ fractionation depends on temporal variation in the number of nitrate sources and on the nitrate consumption rate in the upper ocean (Maslin & Swann 2006). Higher $\delta^{15}\text{N}$ values in coastal–benthic species may be influenced by a combination of factors, including the presence of marine plants that use ammonium and nitrate enriched in ^{15}N (Macko & Estep 1984) or a higher food web complexity that may include several trophic levels (DeNiro & Epstein 1981). This scenario could apply to the isotopic differences in $\delta^{15}\text{N}$ that we observed between GAPEs and WAALs, although the two species consume a large variety of small pelagic fish that are in a similar trophic position (Harris 1973a, Boersma *et al.* 2013).

The isotopic differences found in GAPEs between breeding areas showed that individuals from Caleta Iguana feed further away from the coast than individuals from Las Marielas. Satellite telemetry data from GAPEs at Las Marielas show a coastal behavior (i.e., with few feeding trips and few deep dives in front of the islets) towards the Canal Elizabeth at Isla Isabela (Steinfurth *et al.* 2008). Because the population of Caleta Iguana is farther away from the area of greater productivity (Bahía Elizabeth, where there is greater availability of prey; Ruíz & Wolff 2011), it would be forced to travel long distances to find food (Vargas *et al.* 2006, Steinfurth *et al.* 2008). This may be one of the reasons why there is a constant population of GAPEs in Las Marielas (Jiménez-Uzcátegui pers. obs.). Spatial differences not observed in the FLCO populations can be explained in two ways: (a) the FLCOs of distinct breeding areas consume the same variety of prey species but in different proportions, resulting in similar $\delta^{15}\text{N}$ average values (Newsome *et al.* 2007); or (b) the FLCOs of these sites feed on different prey, but of a similar trophic level (Post 2002). This low degree of trophic overlap was suggested by Valle (1994), who mentioned similarities in both prey and potential feeding zones throughout their entire distribution area. In this study, no temporal differences were observed in GAPE or FLCO feeding strategies. This corroborates other studies that mention that, during normal years, there are no great variations in the primary productivity of the ecosystems where these populations live (Banks 2002, Nims *et al.* 2008, Schaeffer *et al.* 2008).

Our results show that the GAPEs, FLCOs, and WAALs follow different trophic strategies and use different foraging habitats: the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values placed them in foraging zones and trophic positions that coincide with previously published feeding strategies and known prey types. Further research is required on these three species to analyze their trophic variability and to establish their roles in the different ecosystems. Our results also indicate that stable-isotope analysis is a valuable tool to monitor the ecological conditions of the feeding areas occupied by these endangered species and to detect temporal/spatial changes associated with climate variability or human impacts.

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