





# Trophic ecology of juvenile bull sharks (*Carcharhinus leucas*) in the Coyote estuary, Costa Rica

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## Abstract

Bull shark (*Carcharhinus leucas*) is a near-threatened elasmobranch species capable of moving between the fresh and salty waters of tropical and subtropical coastal areas, for which we still lack important ecological information. During their first years of life, bull sharks use estuarine systems as nursery areas, making them highly susceptible to environmental and anthropogenic pressures. We studied the trophic ecology of juveniles found in the Coyote estuary, a potential nursery area in Costa Rica, to understand the potential impact of further bull shark declines and gain knowledge that could aid in their conservation. We analysed the trophic ecology of juvenile bull sharks [81–103 cm total length (TL)] in the Coyote estuary, Costa Rica, using stable isotopes of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ . Since one problem using this technique in juveniles is the confounding effect of the maternal signature, we sampled different tissues (muscle and plasma), verified the status of the shark's umbilical scar and identified the size at which the isotope signature is a result of the animal's current diet. The isotopic values of the muscle tissue reflected the maternal isotopic signature. In contrast, plasma values reflected the diet of juvenile bull sharks >95 cm TL and with a closed umbilical scar. Juvenile bull sharks fed primarily on teleost fishes of the order Anguilliformes and Siluriformes, and have a high trophic position ( $\geq 4.0$ ) in the Coyote estuary. Our findings suggest that this estuary is an important feeding site for juvenile bull sharks of the Pacific of Costa Rica. Thus, the protection of essential habitats such as the Coyote estuary will benefit not only bull shark conservation, but also the conservation of an array of fish species that also use this habitat as a rookery, many of which are of commercial interest.

## KEYWORDS

elasmobranch, isotopic niche, Nicoya Peninsula, prey, stable isotopes, trophic position

## 1 | INTRODUCTION

The bull shark (*Carcharhinus leucas*: Müller & Henle 1839) is a placental viviparous species capable of living in both fresh and saltwater environments in tropical and subtropical areas thanks to their

osmoregulatory capacity (Compagno *et al.*, 2006; Heupel *et al.*, 2015; Jensen, 1976; Martin, 2005; Myers, 1952; Simpfendorfer & Burgess, 2009).

In most of its distribution range, females give birth near the mouth of estuaries and rivers, where the offspring tend to remain

during their first years of life (Heupel *et al.*, 2010; Heupel & Simpfendorfer, 2011; Simpfendorfer *et al.*, 2005). Adult bull sharks are considered an important apex predator in many coastal habits. Juvenile bull sharks may play an important role in regulating and structuring estuarine communities through their feeding habits and by staying within these estuarine systems for prolonged periods.

Information about the trophic ecology of predatory species is essential to understand its role within an ecosystem, the interactions between the members of the community, as well as to infer its behaviour and movements (Estupiñán-Montaño *et al.*, 2009; Jaime-Rivera *et al.*, 2014; Payán *et al.*, 2011). Sharks are capable of regulating populations and structuring marine communities through different direct (e.g., predation) and indirect (e.g., competence for food) mechanisms, contributing to the maintenance and stability of the trophic webs (Bornatowski *et al.*, 2014; Stevens *et al.*, 2000).

Despite the ecological importance of sharks in marine and coastal ecosystems, a considerable global population decline has been observed, mainly due to overfishing, pollution and habitat degradation (Kitchell *et al.*, 2002; Roff *et al.*, 2018; Stevens *et al.*, 2000). Shark population declines produce changes that threaten their own survival and the abundance and distribution of their prey items (Bornatowski *et al.*, 2014; O'Connell *et al.*, 2007; Stevens *et al.*, 2000), which, in contrast, can lead to significant increases in the population of species lower down the food chain, also called the trophic cascade effects (Bornatowski *et al.*, 2014; Heithaus *et al.*, 2008, 2014; O'Connell *et al.*, 2007; Rizzari *et al.*, 2014; Ward & Myers, 2005).

Trophic ecology studies performed in the Western Gulf of México, San Juan Lake, Nicaragua, Florida, United States, and along the east coast of South Africa have all reported that the diet of adult and juvenile bull sharks consisted mainly of teleost fishes (Cliff & Dudley, 1991; Cottrant *et al.*, 2021; Snelson *et al.*, 1984; Tuma, 1976). Studies of juvenile bull sharks using stomach content analysis across their geographic range have shown that they feed primarily on the families Ariidae, Mugilidae, Clupeidae, Carangidae and Sciaenidae (Cottrant *et al.*, 2021; Snelson *et al.*, 1984; TinHan & Wells, 2021).

Juvenile bull sharks are particularly susceptible to the impact of anthropogenic alterations of their habits, and it is difficult to predict the ecosystem's level effects of the alterations without understanding how juvenile bull sharks interact with surrounding ecological communities. Studies of top predator trophic interaction are fundamental for the management efforts of the ecosystems (Crowder & Norse, 2008), nevertheless few studies have been carried out to investigate the trophic ecology of juvenile bull sharks in estuarine systems, and there are no studies regarding the trophic dynamics of juvenile bull sharks in relation to the estuarine community in Costa Rica. There is also uncertainty related to how foraging behaviour may vary within a population. Matich *et al.* (2011) suggested that juvenile bull sharks exhibit some level of individual dietary specialization.

Stable isotope analysis (SIA) is a cost-effective method that provides diet information throughout different time-lapses, depending on the tissue analysed, which can be a complementary tool to stomach content analysis (Kim *et al.*, 2012b; Macneil *et al.*, 2005; Rosas &

Andrade, 2015). In sharks, SIA has been used mainly to understand their feeding habits, thus allowing for the prediction of their trophic position (TP) and niche breadth (Matich *et al.*, 2011; Sánchez, 2017; Tillett *et al.*, 2014; Trystram *et al.*, 2016).

In this research, we studied the contribution of prey items to the diet of juvenile bull sharks in the Coyote estuary, Guanacaste, Costa Rica, using SIA of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ . We investigated the bull shark juvenile diet to understand their functional role in the estuarine food web. This study generated information about the confounding effects of maternal influence on juvenile bull sharks of the Coyote estuary. In addition, we verified the size at which SIA can be used to help design future juvenile diet studies in similar environments. This represents the first study on the trophic ecology of bull sharks in the estuarine systems of Costa Rica and contributes to improving the understanding of this species in the Eastern Tropical Pacific coastal region.

## 2 | MATERIALS AND METHODS

The care and use of experimental animals complied with Costa Rican animal welfare laws, guidelines and policies as approved by the Costa Rican Fisheries Institute (INCOPESCA) under permits AJDIP/330-2019 and AJDIP/495-2019.

### 2.1 | Study area

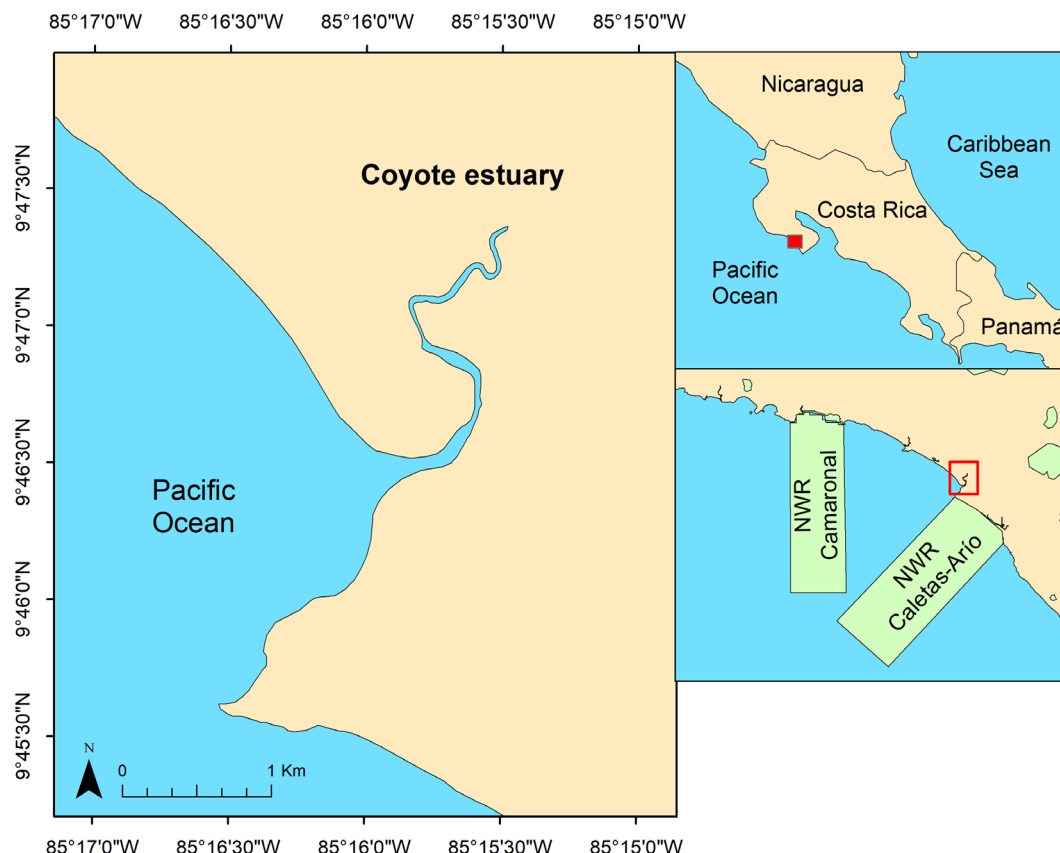
The Coyote estuary is in the south-western region of the Nicoya Peninsula, Guanacaste, Costa Rica (9°46'50"N, 85°16'02"W), between the marine protected areas of the Caletas-Arío and Camaronal National Wildlife Refuges (Figure 1).

The climate is seasonal, with marked dry and rainy seasons (December to May and June to November, respectively) (Carrión-Cortez *et al.*, 2013). The Coyote estuary is approximately 3 km long and 2–4 m deep, although its depth varies significantly with the tides (Chávez, 2017). Important artisanal commercial fisheries are held in surrounding waters, as well as subsistence and sport fishing (Carrión-Cortez *et al.*, 2013). Mangroves constitute the predominant vegetation along the edge of the estuary, although in some areas teak plantations (*Tectona grandis*) and paddocks border the estuary.

### 2.2 | Sample collection

Sample collection was carried out during the 2019 rainy season. We sampled the most representative species of the estuary (more frequent and abundant), as well as species that had been previously reported in the literature as potential bull shark prey (Cliff & Dudley, 1991; Estupiñán-Montaño *et al.*, 2017; Snelson *et al.*, 1984; Sánchez, 2017; Tuma, 1976).

Bottom longlines and gillnets were deployed across the estuary to capture medium-sized and large fish, including juvenile bull sharks.



**FIGURE 1** Coyote estuary, Guanacaste, Costa Rica.  Coyote estuary;  protected areas;  study area

We used a 3 m deep by 30 m long net gillnet with a stretched knot mesh size of 11.43 cm. To avoid mortality and minimize the impact on captured individuals, we monitored and checked the gillnet every 30 min or when the movement of a surface buoy gave away the capture of a large specimen.

The bottom longline consisted of a twisted nylon twine mainline equipped with 12/0, 13/0 and 14/0 circle hooks, baited with sardines (*Clupeidae*), skipjack (*Auxis rochei*) and mackerel (*Scomber japonicus*). The number of hooks used in each set ranged from 37 to 47, with an average of 45 hooks per set. The longline was placed across the channel of the estuary in places with little variable depth to avoid the hooks being left out in the dry at low tide. We checked the bottom longline every 1–1.5 h to minimize mortality and impact on captured specimens (Chávez, 2017; Curtis, 2008; Heupel *et al.*, 2010). A cast net was used to catch small fish and crustaceans.

All captured specimens were measured and identified to the lowest taxon possible using available identification guides (Allen & Robertson, 1998; Fischer *et al.*, 1995; Froese & Pauly, 1999). We used a biopsy punch to obtain 40 mm muscle tissue samples. The date, time, fishing method and site of capture (geographic coordinates) for each sampled specimen were recorded.

To minimize stress and facilitate manipulation, captured bull sharks were boarded and placed upside-down in a tub of water to induce a state of tonic immobility for data collection (Henningson, 1994). We recorded the total length (TL), sex (presence

or absence of myxopterygium) and umbilical scar status (closed or open) of each shark captured.

A visual tag (SDD Hallprint, Hindmarsh Valley, Australia) was placed at the base of the dorsal fin of captured specimens to avoid sampling the same shark twice, and a 4 mm muscle tissue sample was obtained from the area behind the dorsal fin using a biopsy punch, which was then preserved in a 2 ml Eppendorf vial (Jaime-Rivera & Hoyos-Padilla, 2013).

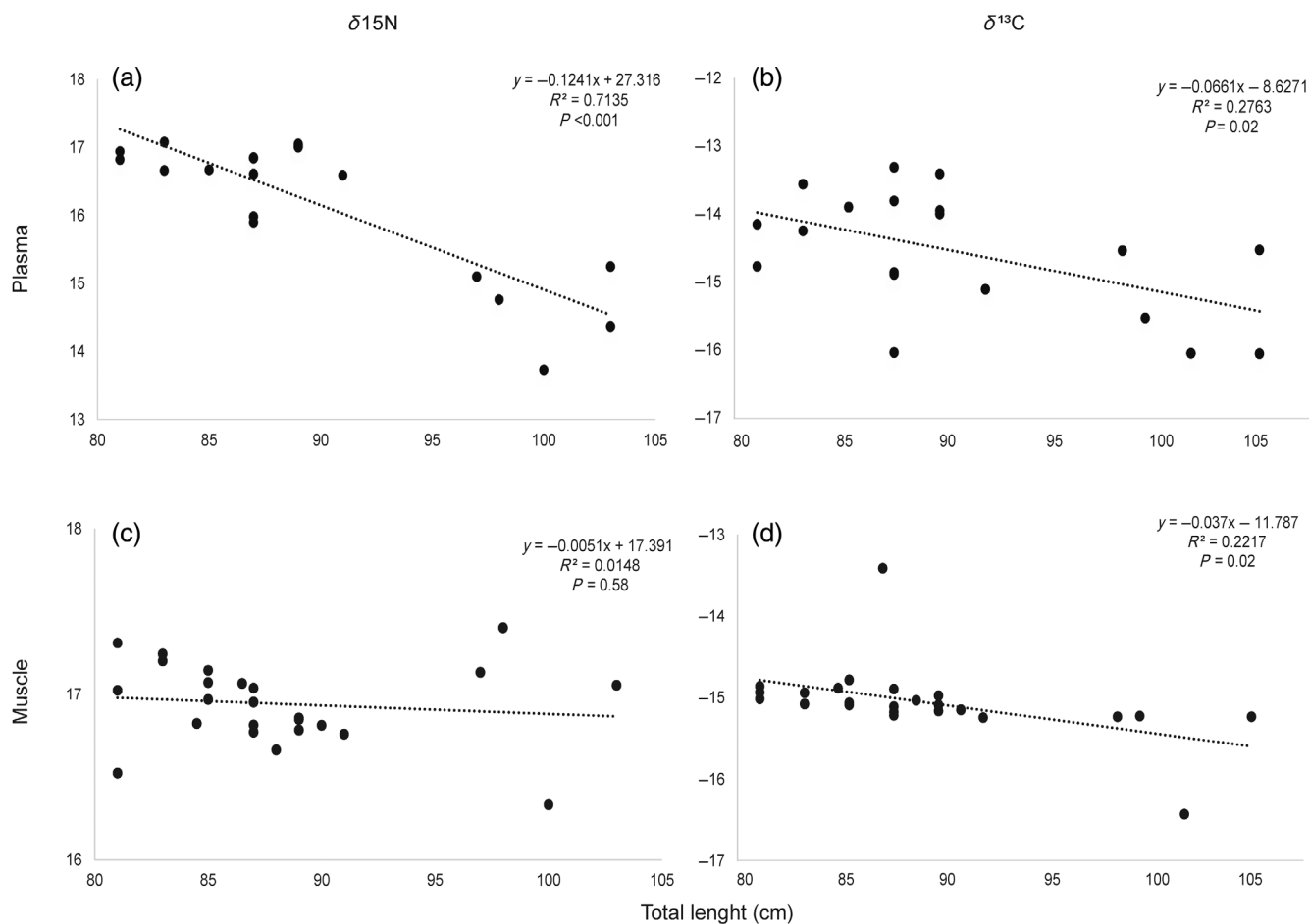
Bull shark blood samples were obtained by means of a caudal vein venepuncture. A 21G  $\times$  1 $\frac{1}{4}$ " needle was used to collect a sample of 4 ml of blood, which was then stored in 6 ml tubes with lithium heparin anticoagulant, and subsequently centrifuged (Unico C829 Power-Spin, 500–3800 rpm, Hong Kong, China) at 3000 rpm for 1 min to separate the plasma from the red blood cells (Kim & Koch, 2012). All collected samples were kept on ice during fieldwork and later stored in a freezer at  $-20^{\circ}\text{C}$ . Samples were frozen at  $-50^{\circ}\text{C}$  for further processing once they arrived at the laboratory (Campbell, 2015; Matich & Heithaus, 2014).

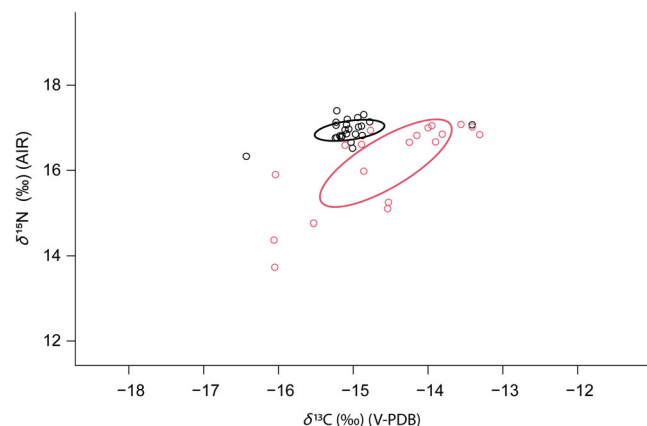
## 2.3 | Sample processing

All samples were lyophilized at an average temperature of  $-50^{\circ}\text{C}$  and a pressure of 85 mTorr (SP Scientific, Sentry 2.0, Warminster, PA, United States) and then ground to a fine powder using an agate

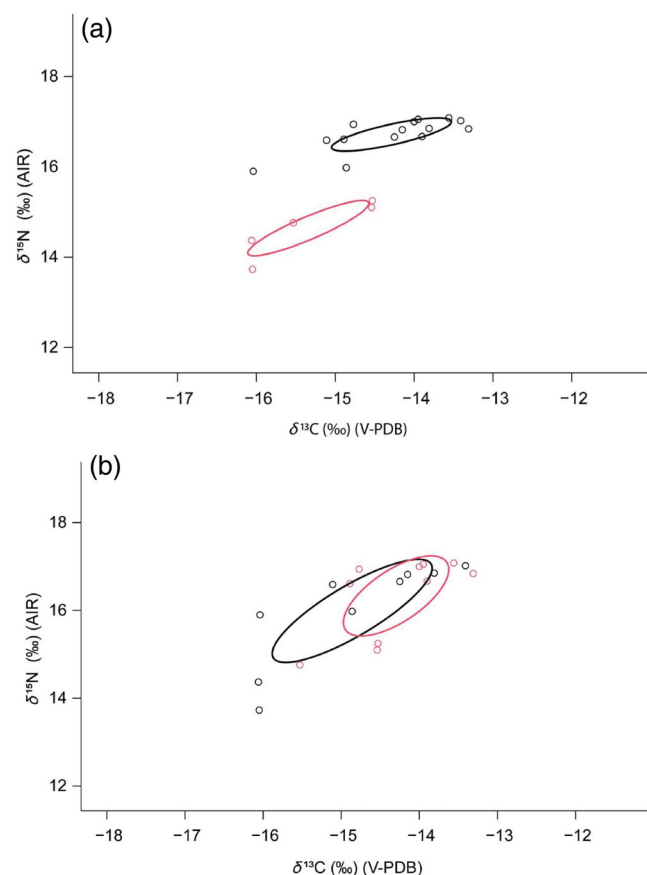
**TABLE 1** Isotopic values of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  (mean  $\pm$  standard deviation) of the groups of potential prey for juvenile bull sharks of the Coyote estuary

Group	Trophic guild	Species	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)
Teleostei-Perciformes	Ichthyophagous-Carcinophagous	<i>Caranx caninus</i> <i>Centropomus</i> spp. <i>Cynoscion</i> spp. <i>Lutjanus</i> spp. <i>Pomadasys branickii</i>	$13.7 \pm 0.7$	$-18.9 \pm 1.7$
Teleostei-Siluriformes	Carcinophagous-Detritivore	<i>Arius</i> sp. <i>Mycteroperca xenarcha</i>	$15.2 \pm 0.8$	$-16.9 \pm 1.2$
Teleostei-Anguilliformes	Malacophagous	<i>Ophichthus zophochir</i>	$12.5 \pm 0.6$	$-17.8 \pm 0.2$
Actinopterygii	Carcinophagous-Malacophagous	<i>Epinephelus</i> sp. <i>Eucinostomus</i> spp. <i>Genyatremus pacifici</i> <i>Selene brevoortii</i>	$12.5 \pm 1.2$	$-21.1 \pm 1.5$
Bivalvia	Filter feeding bivalve	<i>Andara</i> spp. <i>Polymesoda inflata</i> <i>Saccostrea palmula</i>	$9.1 \pm 0.5$	$-21.5 \pm 2.1$
Malacostraca	Herbivores-Detritivores	<i>Goniopsis pulchra</i> <i>Litopenaeus</i> spp.	$8.4 \pm 1.0$	$-21.9 \pm 1.8$
Chondrichthyes	Malacophagous	<i>Styracura pacifica</i>	$12.8 \pm 0.8$	$-18.4 \pm 1.7$

**FIGURE 2** Comparisons of  $\delta^{15}\text{N}$  and total length (TL) for (a) plasma and (c) muscle and of  $\delta^{13}\text{C}$  and TL for (b) plasma and (d) muscle for juvenile bull sharks (*Carcharhinus leucas*)

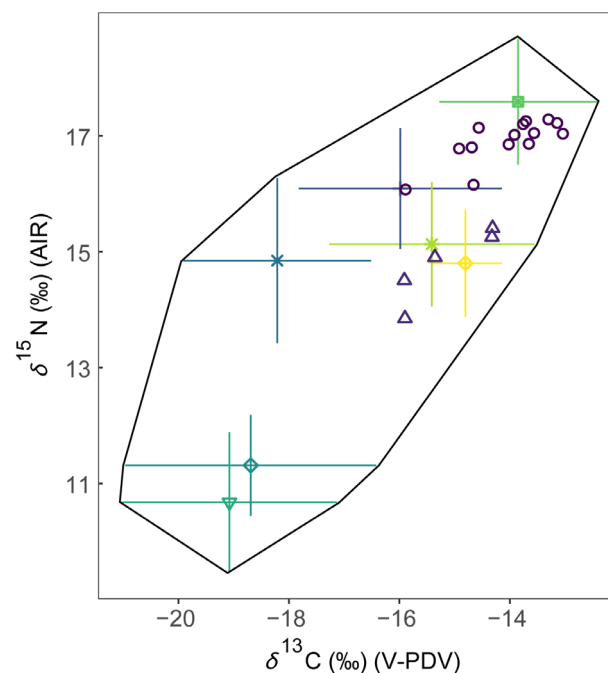


**FIGURE 3** Isotopic overlap between tissues of juvenile bull sharks (*Carcharhinus leucas*) of the Coyote estuary. ○, muscle; ●, plasma



**FIGURE 4** Isotopic overlap observed in the plasma of the juvenile bull sharks from the Coyote estuary: (a) between umbilical scar status, (b) between sex. (a) ○, open; ●, closed and (b) ○, males; ●, females

mortar and stored in 8 × 5 mm tin capsules (0.4–0.6 mg) for later analysis. Urea was extracted from shark muscle samples following the methodology described by Kim and Koch (2012). Lipid extraction was not necessary as the samples' C:N ratio after urea extraction was less than 3.5 (Post, 2002).



**FIGURE 5** Isotopic values of *Carcharhinus leucas* within the mixing polygon, adjusted according to seven groups of prey and the specific trophic discrimination factor ( $\delta^{13}\text{C} = 2.8 \pm 0.6\text{‰}$ ,  $\delta^{15}\text{N} = 2.2 \pm 0.7\text{‰}$ , Kim et al., 2012a). ○, *C. leucas* OUS; △, *C. leucas* CUS; +, Teleostei-Perciformes; \*, actinopterygii; ◆, Bivalvia; ▽, Malacostraca; ■, Teleostei-Siluriformes; ★, Chondrichthyes; ◆, Teleostei-Anguiliformes

## 2.4 | Laboratory analysis

Processed samples were submitted to the Stable Isotope Laboratory at the University of California, Santa Cruz, United States. The equation proposed by Deniro and Epstein (1980), in which the isotopic composition was expressed as  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in parts per thousand (‰), was used to obtain  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values.

## 2.5 | Data analysis

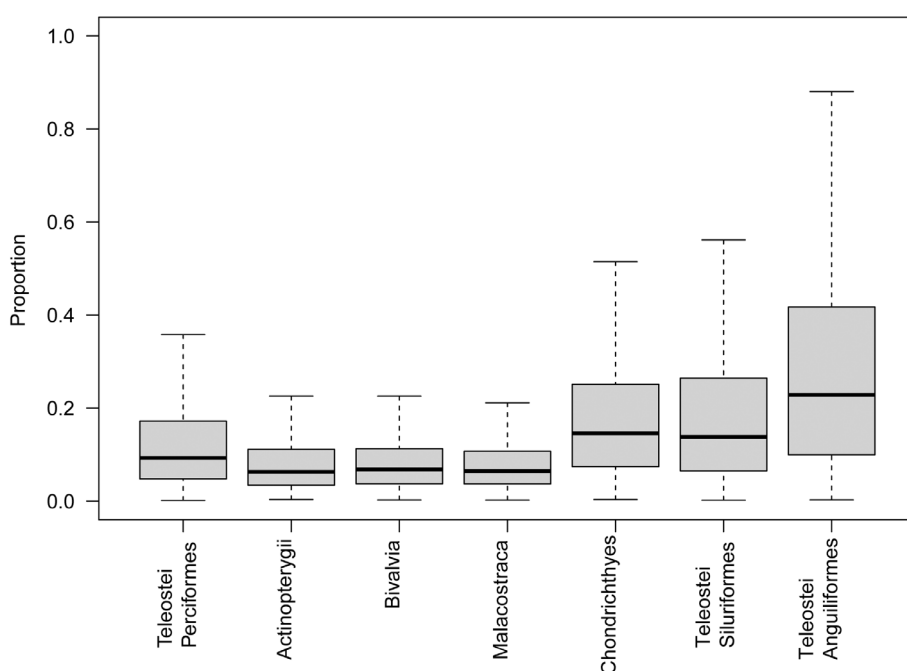
We applied a Bayesian mixing model (SIMMR v0, 4.1) (Parnell, 2019) based on the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of plasma and muscle to determine a consumed prey's degree of contribution to a bull shark's diet. Tissues with a high metabolic turnover rate (i.e., plasma, 22–23 days) reflect dietary changes sooner than tissues with a lower metabolic turnover rate (i.e., muscle, 250–488 days) (Caut et al., 2013; Kim et al., 2012b; Macneil et al., 2005; Matich et al., 2015).

The contribution of prey items was analysed for each tissue sampled, and a comparison was held by sex and umbilical scar status. The mixing model used three steps: (1) selection of the potential prey of *C. leucas* based on potential prey within estuarine systems (Cliff & Dudley, 1991; Estupiñán-Montañón et al., 2017; Sánchez, 2017; Snelson et al., 1984; Tuma, 1976); (2) grouping the potential prey into seven categories according to their trophic habits and stable isotope

Tissue	Category		$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)	TP
Muscle	Mean		16.9 ( $\pm 0.3$ )	-15.1 ( $\pm 0.5$ )	4.5
	Sex	Female	17.0 ( $\pm 0.2$ )	-14.9 ( $\pm 0.5$ )	4.5
		Male	16.9 ( $\pm 0.3$ )	-15.2 ( $\pm 0.4$ )	4.5
	Umbilical scar	Closed ( $\geq 98$ cm LT)	17.0 ( $\pm 0.5$ )	-15.5 ( $\pm 0.6$ )	4.5
		Open ( $< 98$ cm LT)	16.9 ( $\pm 0.2$ )	-15.0 ( $\pm 0.4$ )	4.5
Plasma	Mean		16.2 ( $\pm 1.0$ )	-14.6 ( $\pm 0.9$ )	4.2
	Sex	Female	16.3 ( $\pm 0.9$ )	-14.3 ( $\pm 0.7$ )	4.2
		Male	16.0 ( $\pm 1.2$ )	-14.9 ( $\pm 1.0$ )	4.2
	Umbilical scar	Closed ( $\geq 98$ cm LT)	14.6 ( $\pm 0.6$ )	-15.3 ( $\pm 0.8$ )	4.0
		Open ( $< 98$ cm LT)	16.7 ( $\pm 0.3$ )	-14.3 ( $\pm 0.8$ )	4.3

Note: The values are represented in mean ( $\pm$  standard deviation). LT (total length).

**TABLE 2** Isotopic values of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  and trophic position (TP) values of the juvenile bull sharks (*Carcharhinus leucas*) of the Coyote estuary, corresponding to the different tissues analysed for the different categories



**FIGURE 6** Contribution of the potential prey to the diet of the juvenile bull sharks with closed umbilical scar of Coyote estuary when analysing the plasma using the mixing model

values (Phillips *et al.*, 2014) (rays were grouped and separated due to the possible effect urea could have in the assimilation compared to finfish) (Table 1); and (3) selection of the trophic discrimination factors (TDFs) for the analysis. We used the TDFs reported by Kim *et al.* (2012a) for plasma ( $\delta^{13}\text{C} = 2.8 \pm 0.6\text{‰}$ ,  $\delta^{15}\text{N} = 2.2 \pm 0.7\text{‰}$ ) as this is the only long-term study carried out in captive sharks where the TDF for plasma is determined. For the muscle, we used the TDF reported by Hussey *et al.* (2010) ( $\delta^{13}\text{C} = 0.90 \pm 0.33\text{‰}$ ,  $\delta^{15}\text{N} = 2.29 \pm 0.22\text{‰}$ ). As there are no species-specific TDFs for *C. leucas*, we selected estimated TDFs for other shark species phylogenetically similar to *C. leucas* (Dosay-Akbulut, 2008).

To run the model, we used the mean and standard deviation ( $\pm$ s.d.) of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of juvenile *C. leucas* as a mixture, and the mean  $\pm$  s.d. of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of the potential prey species (Table 1) as sources, the TDFs were used for correction factors. The model was run with  $10^3$  iterations, 1000 burn-in, 10 thinning periods and four Markov Chain Monte Carlo.

Mixed linear models were adjusted considering the individuals as a random effect, taking the values of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  as the response variable, and umbilical scar status and sex of the juvenile bull sharks as explanatory variables. Simple linear regressions of bull shark stable isotope ( $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ ) values versus bull shark TL were adjusted to determine if an asymptote has been reached for  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values with shark size.

Samples to determine the isotopic niche width of juvenile bull sharks were analysed by tissue type, umbilical scar status, and sex. We used a stable isotopic Bayesian ellipses method in R (SIBER; Jackson *et al.*, 2011) to estimate the width of the isotopic niches [standard ellipse corrected area (SEAc)].

The overlap between two ellipses was calculated based on the maximum likelihood of fitted ellipses, using the maxLikOverlap function in the SIBER package. This function uses the maximum likelihood estimated means and covariances matrices of two specified groups to calculate the area of overlap. Values ranging from 0.66 to 1 indicate

**TABLE 3** Proportion of the contribution of prey to the diet of juvenile bull sharks of the Coyote estuary found in the plasma

Prey	Umbilical scar status		Sex	
	Open	Closed	Female	Male
Teleostei-Perciformes	0.067	0.127	0.062	0.096
Actinopterygii	0.036	0.083	0.040	0.058
Bivalvia	0.026	0.083	0.036	0.050
Malacostraca	0.024	0.078	0.035	0.048
Teleostei-Siluriformes	0.718 <sup>a</sup>	0.184	0.676 <sup>a</sup>	0.537 <sup>a</sup>
Chondrichthyes	0.060	0.173	0.068	0.102
Teleostei-Anguilliformes	0.070	0.271 <sup>a</sup>	0.084	0.109

Note: The values are presented as means and by category.

<sup>a</sup>Prey that presented the highest proportion in each category.

high overlap, values ranging from 0.30 to 0.65 indicate medium overlap and values below 0.29 indicate low overlap (Jackson *et al.*, 2011).

We calculated bull shark TP using the R package 'tRophicPosition' (Quezada-Romegialli *et al.*, 2018). Zooplankton and guppy fish (*Poeciliopsis turrubarensis*) sampled within the Coyote estuary were used as baselines, both of which have a TP of 2 (Froese & Pauly, 1999; Post, 2002; Wischnath, 1993).

### 3 | RESULTS

We analysed a total of 166 samples from teleost fishes, 53 from bivalves, 46 from crustaceans, 40 from cartilaginous fishes (including bull sharks), as well as 16 mangrove leaves and phytoplankton and zooplankton samples. Catfish (*Arius* sp.) was the most abundantly caught species in the estuary, both during gillnet and bottom longline operations, with relative abundance percentages of 33.5% and 58.2%, respectively. The silverside (*Atherinella argentea*) was the most abundantly caught species during cast net operations, reporting a relative abundance of 30.8%.

#### 3.1 | Isotopic characterization of juvenile bull sharks

We sampled 24 juvenile bull sharks (14 female, 10 male) with TL ranging from 81 to 103 cm TL, from which we obtained 24 muscle samples and 19 plasma samples. Most of the captured bull sharks that showed an open umbilical scar ( $n = 14$ ) were smaller than 98 cm TL. In contrast, sharks with a closed umbilical scar showed sizes greater than 98 cm TL.

For plasma, we found a strong relationship between the bull shark size (TL) and the  $\delta^{15}\text{N}$  ( $R^2 = 0.71$ ), but a relatively poor relationship between TL and  $\delta^{13}\text{C}$  ( $R^2 = 0.28$ ) (Figure 2). In the muscle, we found poor relationships between the bull shark TL and  $\delta^{15}\text{N}$  ( $R^2 = 0.15$ ) and between the size and  $\delta^{13}\text{C}$  ( $R^2 = 0.22$ ) (Figure 2).

Plasma presented the broadest isotopic niche, with a total area (TA) of  $4.45\text{‰}^2$ , an area of the ellipse (SEA) of  $1.82\text{‰}^2$  and a corrected ellipse area (SEAc) of  $1.93\text{‰}^2$ . Muscle presented an isotopic niche size with a TA of  $1.41\text{‰}^2$ , SEAc of  $0.34\text{‰}^2$ , and SEA of  $0.33\text{‰}^2$  (Figure 3). A low isotopic overlap SEA (0.3) was found when comparing muscle and plasma tissues, with muscle presenting more depleted  $\delta^{13}\text{C}$  values ( $-0.54\text{‰}$ , Confidence interval (IC) 95%  $-0.04$ ,  $-0.93$ ) and more enriched  $\delta^{15}\text{N}$  values ( $0.77\text{‰}$ , IC 95%  $0.35$ ,  $1.19$ ) than plasma.

A significant difference was observed when comparing the isotopic values of muscle and plasma, which suggests that muscle reflects a maternal isotopic signature, while plasma reflects the diet of the juvenile sharks. No significant differences were detected in plasma values when comparing the sex ( $t = 1.80$ ,  $P = 0.18$ ), with high overlap (0.61) (Figure 4).

Sharks with an open umbilical scar presented more enriched  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values in their plasma than sharks with a closed umbilical scar, with a difference of  $1.04\text{‰}$   $\delta^{13}\text{C}$  (IC 95%  $0.17$ ,  $1.89$ ) and  $2.00\text{‰}$   $\delta^{15}\text{N}$  (IC 95%  $1.49$ ,  $2.51$ ) (Table 1). A larger niche width was found for individuals with a closed umbilical scar (TA  $0.67\text{‰}^2$ , SEA  $0.66\text{‰}^2$ , SEAc  $0.88\text{‰}^2$ ) than in individuals with an open umbilical scar (TA  $1.45\text{‰}^2$ , SEA  $0.55\text{‰}^2$ , SEAc  $0.60\text{‰}^2$ ) with a low overlap (0.04) (Figure 4).

#### 3.2 | Contribution of prey to the diet of juvenile bull sharks

The fitting model (i.e., mixing polygon, Figure 5) suggested that these results explained the uncertainty of the TDFs and of the isotopic values of the seven groups of potential prey (Table 2). Thus, the application of the mixing model was adequate to estimate the selection of the potential feeding habits of juvenile *C. leucas*. In addition, the mixing model produced a total of 3600 posterior distributions and Gelman–Rubin (Rhat) convergence diagnostic statistics of 1.0 for all parameters, suggesting that there was convergence.

The degree of importance to which a particular prey item contributes to the diet of juvenile bull sharks varies when comparing the plasma of bull sharks with open umbilical scars to closed umbilical scars. In sharks with an open umbilical scar, prey item Teleostei-Siluriformes provided the greatest contribution to their diet (mean = 71%, s.d. = 6.0%), followed by the group of Teleostei-Anguilliformes (mean = 7.1%, s.d. = 5.3%). In sharks with a closed umbilical scar, prey item Teleostei-Anguilliformes provided the greatest contribution to their diet (mean = 27%, s.d. = 19%), followed by Teleostei-Siluriformes (mean = 18%, s.d. = 16%) and Chondrichthyes (mean = 17%, s.d. = 12%) (Figure 6 and Table 3). Plasma values showed no variation in the contribution of particular prey items with respect to the sex of the specimens, where the prey item Teleostei-Siluriformes contributed the most to their diet (mean = 53%, s.d. = 8.4%) (Table 3).



### 3.3 | Trophic position

Juvenile bull sharks presented a high TP (TP  $\geq 4.0$ ). There was a difference between the TP of closed umbilical scar sharks (4.0 TP) and open umbilical scar sharks (4.3 TP) (Table 2).

## 4 | DISCUSSION

In the present study, the TL estimated for bull sharks with open and closed umbilical scars (91 and 103 cm, respectively) was greater than the TL for neonate bull sharks reported in the literature (56–81 cm TL) (Compagno *et al.*, 2006; Thorson & Lacy, 1982). In Nicaragua, the size at birth reported for *C. leucas* is 65–75 cm TL (Jensen, 1976). Thorson and Lacy (1982) estimated an annual growth rate for the bull sharks of the San Juan River and Lake Nicaragua (Nicaragua) of 11–12 cm year<sup>-1</sup> during their first 2 years of life and from 9 to 10 cm year<sup>-1</sup> the following years. The fast growth of the bull sharks from the Coyote estuary may be due to the high availability of food resources since the rate of consumption directly influences the growth rate (Freitas *et al.*, 2006).

Teleost fishes from the orders Anguilliformes (*Ophichthus zophochir*), Siluriformes (*Arius* sp.) and Chondrichthyans (*Styracura pacifica*) made the greatest contribution to the diet of juvenile bull sharks. Other studies reported similar results (Cortés, 1999; Daly & Smale, 2013; Sánchez, 2017; Tuma, 1976). Tillett *et al.* (2014) reported that bull sharks from Australia fed on teleost fishes, principally of the order Siluriformes. Likewise, Estupiñán-Montaña *et al.* (2017) found that bull sharks from Ecuador fed on teleost fishes from the order Anguilliformes, mainly worm eels (Ophichthidae).

The teleost fishes of the order Siluriformes (*Arius* sp.) were the most caught species during this research using the different fishing methods, indicating that bull sharks are feeding on the probably most available prey item inside the estuary (Estupiñán-Montaña *et al.*, 2017; Sánchez, 2017; Tuma, 1976).

According to the  $\delta^{13}\text{C}$  and the  $\delta^{15}\text{N}$  values found in the plasma of male and female juvenile bull sharks, a similar trophic habitat use exists with a medium and considerable overlap, which indicates that both sexes are using the same feeding zones and sources. Our results are similar to those reported by Daly *et al.* (2013) in the south-east of Mozambique, Estupiñán-Montaña *et al.* (2017) in Ecuador, and Espinoza *et al.* (2019) in Australia, where no differences were found when comparing the diet of males and females.

For stable isotope studies of juvenile sharks, care should be taken in using slow turnover tissues like muscle because they may retain a maternal signature for an extended time (Matich *et al.*, 2015). In sharks, tissues with high turnover rates (such as plasma) have an incorporation time to the diet of 22–33 days, while tissues that are metabolically less active, like muscle, have an incorporation time to the diet of 250–488 days (Caut *et al.*, 2013; Kim *et al.*, 2012b; Macneil *et al.*, 2005; Matich *et al.*, 2015). Olin *et al.* (2011) suggested that tissues with fast turnover rates stopped being influenced by the maternal diet when the sharks reached sizes of  $\sim 90$  cm TL and when the

umbilical scar was barely visible. Thus, the high turnover rates of the plasma make this tissue ideal for the study of young of the year bull sharks (70–90 cm TL) (Bearhop *et al.*, 2004; Matich *et al.*, 2015).

In this study, bull sharks with  $>95$  cm TL had more depleted  $\delta^{15}\text{N}$  values, indicating that sharks that reach sizes of  $>95$  cm TL stopped being influenced by the maternal diet. Although for the Carcharhinidae family the plasma presents a fast turnover rate ( $-0.82\text{‰}$   $\delta^{13}\text{C}$  cm<sup>-1</sup> LT and  $-0.24\text{‰}$   $\delta^{15}\text{N}$  cm<sup>-1</sup> LT) (Matich *et al.*, 2010), the bull sharks that have  $<95$  cm TL still reflect the maternal diet. Some authors have reported that the isotopic values in tissues with a low turnover rate for sharks with open umbilical scars are partly influenced by the maternal diet (Belicka *et al.*, 2012; Olin *et al.*, 2011). In this regard, our findings slightly differ from the size proposed by Olin *et al.* (2011), that the plasma could be used in sharks with  $\sim 90$  cm TL.

In contrast, there was no correlation between the TL and the  $\delta^{15}\text{N}$  in the muscle, indicating that this tissue is still being influenced by the maternal diet, even in individuals of  $>95$  cm TL. Similar to our results, Niella *et al.* (2021) found that the maternal provisioning in the muscle of the bull sharks is detectable up to 3.5 years after birth. Therefore, the muscle does not reflect the diet of juvenile *C. leucas* in the Coyote estuary and should not be used to analyse their diet. For analysing the diet of the juvenile bull sharks of Coyote estuary using Stable Isotopes is important to use the stable isotopes in the plasma and in bull sharks  $>95$  cm TL and with a closed umbilical scar.

The juvenile bull sharks of the Coyote estuary presented a high TP (4.0), acting as apex predators and tertiary consumers, as described by Cortés (1999), Estupiñán-Montaña *et al.* (2017) and Sánchez (2017) (who found a TP of 4.3). Similarly, Daly *et al.* (2013) established a TP for adult bull sharks of 4.6 and 4.4 for subadults. Furthermore, juvenile bull sharks of the Coyote estuary presented TP values similar to other top predators in their juvenile stages, such as tiger sharks (*Galeocerdo cuvier*), shortfin makos (*Isurus oxyrinchus*) and white sharks (*Carcharodon carcharias*) (Cortés, 1999; Dicken *et al.*, 2017; Estupiñán-Montaña *et al.*, 2017; Hussey *et al.*, 2012; Tamburin *et al.*, 2019).

Estuarine systems are important sites for coastal and deep-sea fisheries (Holguin & Bashan, 2007; Primavera, 2000; Rönnbäck *et al.*, 1999), acting as nurseries that provide refuge to young and feeding areas for commercially important species of fish and crustaceans (Loneragan *et al.*, 1997), which then venture to sea, where they are caught by fishers'.

A decline in the population of juvenile bull sharks within the Coyote estuary could cause a population increase of their main prey item and structural effects in the trophic network. Since the teleost fishes of the order Siluriformes feed mainly on crustaceans, small fish, detritus and fish eggs (Benítez-Mondragón *et al.*, 2019), an unchecked population could cause further trickle-down ecosystem effects, affecting prey abundance and availability for commercial species that inhabit these systems and causing a reduction in the economic benefits obtained by the fishermen, as well as inter- and intraspecific interactions. Previous studies have shown that declines in shark populations have had negative effects on commercial fisheries (Bornatowski *et al.*, 2014; Heithaus *et al.*, 2008, 2014; O'Connell *et al.*, 2007; Rizzari *et al.*, 2014).



However, it is necessary to analyse the diet of other possible predators in the area, such as crocodiles, to prove this statement. Bull sharks of the Coyote estuary could be helping to maintain the ecosystem balance by reducing competition for food and space among fish (Bornatowski et al., 2014; Myers et al., 2007).

We thus recommend the implementation of measures for the conservation of bull sharks in the Coyote estuary due to the site's importance as a breeding and feeding habitat for juvenile bull sharks. The protection of the juvenile bull sharks of the Coyote estuary would ensure not only the conservation of the bull shark population but also of the essential habitats needed by an array of marine species, many of commercial interest.

It is important to create awareness in the local population, organizations and authorities, and highlight the importance that bull sharks have for proper ecosystem functioning and productivity, and thus promote actions for their conservation.

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## DATA AVAILABILITY STATEMENT

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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