

Article

Stable Isotope Analysis of Two Filter-Feeding Sharks in the Northwestern Pacific Ocean

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Abstract: Understanding the feeding ecology and habitat use of vulnerable shark species is crucial for effective conservation. This study focuses on two large filter-feeding sharks, the megamouth shark (*Megachasma pelagios*) and whale shark (*Rhincodon typus*), in Northwestern Pacific waters. Stable isotope analysis ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) was conducted on white muscle samples ($n = 91$) of *M. pelagios* and fin clips ($n = 90$) of *R. typus*, collected via large-mesh drift nets and set nets in Taiwanese waters. In this study, we investigated feeding strategies, ontogenetic dietary shifts, habitat use, and isotopic niche variation in both species. For *R. typus*, the observed positive correlation between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ supports the previously proposed active suction filter feeding, as well as implying both a diet with an increasing proportion of higher trophic level prey and an ontogenetic shift. In contrast, *M. pelagios* displayed a negative correlation, consistent with a previous study associating such patterns with primary or secondary consumers, further aligning with its reported planktonic prey dominance. Both species had increasing $\delta^{13}\text{C}$ with growth, signifying a shift to nutrient-rich habitats. Only *R. typus* exhibited ontogenetic diet changes ($\delta^{15}\text{N}$). SIBER (Stable Isotope Bayesian Ellipses in R) analysis revealed distinct feeding strategies and habitat use between the two species, potential sexual segregation, and wider isotopic niche widths for males in both species. The findings underscore the importance of considering species-specific behaviors and sex-based differences in conservation strategies.



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Keywords: whale shark; megamouth shark; ecology; trophic position

Key Contribution: This study provides the first comparative stable isotope analysis of megamouth and whale sharks in the northwestern Pacific. Distinct feeding strategies and ontogenetic habitat shifts were identified, including sex-based isotopic niche differences. These findings highlight the need for species- and sex-specific considerations in shark conservation planning.

1. Introduction

Elasmobranchs, comprising approximately 1200 extant species, exhibit diverse trophic niches and play fundamental roles in marine ecosystems by regulating organisms across

various trophic levels [1–3]. Among elasmobranchs, large shark species are usually known as K-selected animals, and they exhibit late maturity, small litter size, and slow growth rate, making their population more vulnerable [4,5]. The global population of sharks has decreased due to the high fishing pressure over the past few decades [6,7]. Acquiring detailed biological information on large shark species is necessary for sustainable management decision-making. Feeding ecology is one of the research projects that helps elucidate the role of animals across and within sub-regions of the ecosystems and provides more information for management strategy [8,9].

There are several methods to study feeding ecology, such as feeding behavior observation, stomach content analysis (SCA), and stable isotope analysis (SIA). Historically, stomach composition research was the typical method, which provides detailed insights into recent feeding events and short-term dietary patterns [10]. However, the SCA method was usually lethal and limited by sample size, high rates of empty stomachs or prey digestive level, especially on large shark species [11–13]. To complement the limitation of SCA and the broader understanding of long-term dietary trends, the stable isotope is employed as a reliable tool for feeding ecology research, and it has been used broadly for marine animals, such as mammals, teleost, and elasmobranchs [14–17].

Stable isotopes are non-radioactive forms of elements that do not decay over time; by examining the ratio of heavy to light isotopes of elements of animals, researchers can infer long-term dietary sources, trophic relationships, and/or habitat use, offering a more comprehensive picture of feeding ecology [18]. Various isotope values provide different information, e.g., the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ from individuals reflect the inhabited environment and assimilated food habits over time, respectively [18–20]. Typically, the gradient $\delta^{13}\text{C}$ could be found from nearshore to offshore, or benthic to pelagic food webs; therefore, $\delta^{13}\text{C}$ is commonly used to study dietary sources or productivity of the environment [21,22]. $\delta^{15}\text{N}$ is often used to determine the relative trophic position of animals in a food web; higher $\delta^{15}\text{N}$ are positively correlated with higher trophic levels, likewise, animals from lower trophic positions usually exhibit lower $\delta^{15}\text{N}$.

Additionally, the potential ontogenetic shifts and sexual segregation of many vulnerable shark species have been described by using stable isotope research. For example, the male white sharks (*Carcharodon carcharias*) prefer to forage either further offshore or closer to western coastal waters as they grow in South Africa [23]. Significant differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were detected among neonate, juvenile, and adult silky sharks (*Carcharhinus falciformis*), indicating an ontogenetic shift in trophic ecology in the Eastern Tropical Pacific Ocean [24]. The majority of previous SIA research focused on predators in the ecosystem, such as scalloped hammerhead shark (*Sphyrna lewini*), blue shark (*Prionace glauca*), bull shark (*Carcharhinus leucas*), tiger shark (*Galeocerdo cuvier*), etc. [25–27]. However, the filter feeders also received attention from several fronts because they are more vulnerable to environmental threats such as pollution, fishing pressure, or coastal human activities [28–30]. Among the marine filter-feeding sharks, there were two well-known species in the Northwestern Pacific Ocean, the whale shark (*Rhincodon typus*) and the megamouth shark (*Megachasma pelagios*).

Whale sharks are the world's largest fish, reaching a maximum total length (TL) of approximately 20 m [31–34]. This filter-feeding species feeds on various plankton and small bony fishes, but the prey items change in different spatiotemporal waters [35–38]. Whitehead et al. indicated that different food webs lead to various stable isotopic values for whale sharks, the La Paz Bay (eastern Pacific) group occupied a wider isotopic niche than in the Yucatan Peninsula (western Atlantic) [14]. Stable isotopes have been widely used in whale shark research, with numerous studies reporting increases in trophic levels with body size in various regions, such as La Paz Bay, Ningaloo, Veraval, and the Gulf of Goubet,

most of which are located in the Indian Ocean [37–40]. One study using stable isotope analysis on whale sharks from the western Pacific Ocean suggested that the turnover time of different tissues (plasma and fin cartilage) was approximately 9 months to 3 years based on feeding experiments of three captive individuals [41]. However, the stable isotope information of the field population from the western Pacific Ocean is still limited [41].

Megamouth sharks are seldom recorded globally, with fewer than 300 individuals being reported in the last 40 years [42,43]. Researchers described this species as viviparous with oophagy, which can reach ~700 cm TL, the size at maturity was ~400 cm TL for males but ~500 cm TL for females [44,45]. The specific vertical movement behavior during dawn and dusk has been verified for megamouth shark according to the tracking and capture records [43,46,47]. The megamouth shark may attract prey by reflecting external light sources, such as the light produced by bioluminescent prey, on the white band (a highly reflective structure on the upper jaw) during feeding [48]. Zooplanktonic prey, mainly *Euphausia pacifica* was found in the diet of megamouth shark, but *Nematoscelis difficilis* has also been identified in the stomach [49,50]. Only one previous study suggested the preference for the pelagic habitat and the zooplanktonic prey-feeding behavior of the megamouth based on stable isotope analysis ($\delta^{13}\text{C} = -22.5\text{‰}$; $\delta^{15}\text{N} = 10.4\text{‰}$) from only one stranded individual in Brazil [51]. Nevertheless, detailed research on megamouth shark ecology is still missing, leaving a knowledge gap on habitat use, and feeding habitats between male and female life history stages.

Therefore, this study aims to address this general knowledge gap by evaluating the stable isotope of the whale shark and megamouth shark in the western North Pacific Ocean. We hypothesized that the isotopic composition would differ between *R. typus* and *M. pelagios*, and that the value would be influenced by size and sex. Overall, this study aims to provide useful information on the feeding and habitat use of *R. typus* and *M. pelagios* in different sex and life history stages.

2. Materials and Methods

2.1. Sample Collection

The tissue samples were collected from *R. typus*, caught as bycatch in set nets in coastal waters off Taiwan from 2008 to 2018 (Figure 1). In Taiwan *R. typus* was consumed greatly before the 1990s, after which its population dramatically declined; therefore, the total allowable catch (TAC) system of *R. typus* was established in 2002, completely banned fishing in 2008, and listed as a conservation species in 2020 [52]. According to the project requirement of the Taiwan Fisheries Agency, the individual photos, body length (cm TL), and sex information were recorded during the tagging processes. A small piece of fin clip (skin with muscle tissue) was collected from the caudal fin of *R. typus* before release, the samples were preserved in 95% ethanol immediately for analysis later.

On the other hand, *M. pelagios* was categorized as of least concerned species on the IUCN red list because of limited research and population information [53]. Nevertheless, in order to preserve the highly biologically diverse waters surrounding Taiwan, the Taiwan Fisheries Agency implemented a mandatory catch report system for this species in 2013 and banned its fishing in 2020 [43]. The white muscle samples of *M. pelagios* were collected during 2013 and 2019 from bycatch. All *M. pelagios* were the bycatch of large-mesh (mesh size 90 cm) drift net vessels, which operated in the eastern waters of Taiwan (Figure 1). After the shark landing in the port or fish market, the TL (cm), weight (kg), and sex were recorded. The white muscle specimens and some prey items from stomach were collected right after the sharks were dissected and preserved in 95% ethanol for further analysis.

Previous study had suggested that the different preservation methods, such as salt and formalin-ethanol could have effects on stable isotopic signatures, but the magnitude of

the difference was found to be small and directionally consistent, suggesting the method may be suitable for stable isotope analysis [54].

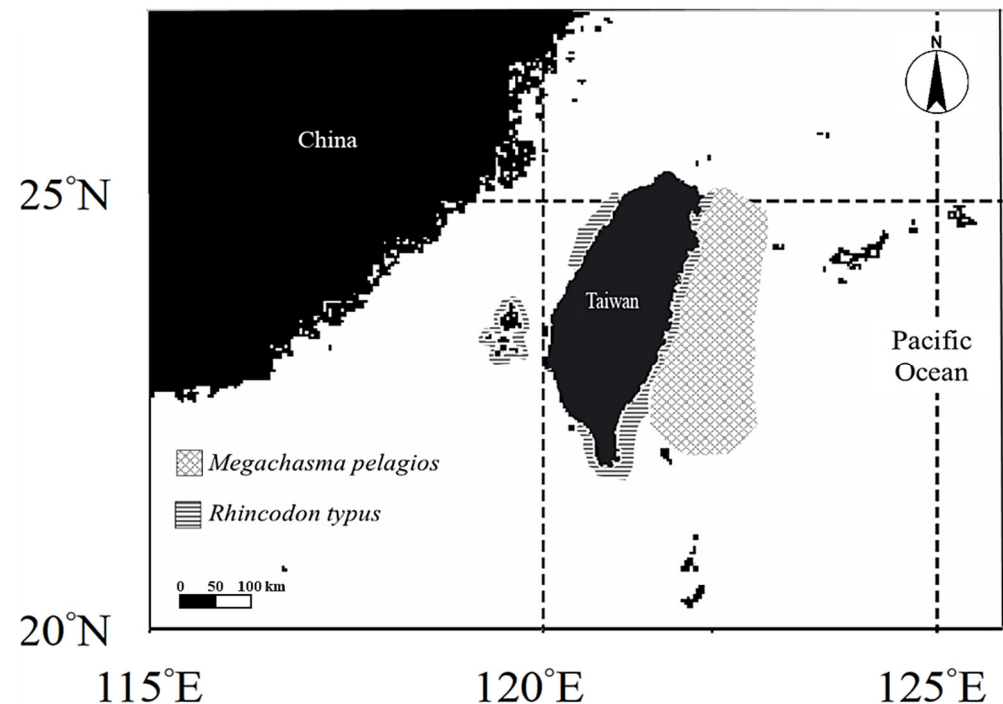


Figure 1. Fishing ground of *M. pelagios*, in the eastern Taiwan waters (the operation area of large-mesh drift net fishery) and fishing ground of *R. typus*, around Taiwan waters (the operation area of set net fishery).

2.2. Life History Stages

Ontogenetic shifts and sexual segregation are commonly gradually found in large shark species in different life history stages due to various purposes, for instance, nutrition, refuge, mating, and reproductive requirements. Therefore, the life history stages were identified into three stages (groups) in this study; the reproductive organs' development was observed for *M. pelagios* (C. J. Yu, personal observation): group I (immature): males and females have undeveloped gonads; group II (maturing): developing reproductive organs, such as slightly rotated clasper from males and inflating ovaries or uteri from females; group III (mature): developed claspers with semen from males or inflated ovaries and uteri with mating scars from females. Additionally, *R. typus* were separated into three groups based on sampling size range due to almost no mature individual in this study. The individuals ≤ 400 cm TL were in group I, from 401 to 600 cm TL were in group II, and >600 cm TL were in group III, respectively.

2.3. Pretreatment of SIA

The SIA samples of *M. pelagios* and *R. typus* were dried for 2–4 days at 60 °C and grinded into homogeneous powder. The tissue powder samples were weighed ~3 mg, transferred into tin cups, and sent to the Société Générale de Surveillance (SGS), Switzerland Taiwan Ltd. for further $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analysis. The steps for analysis were as follow: powder samples were wrapped in a tin cup, placed into the elemental analyzer (EA), and burned at 1000–1050 °C. After the carbon and nitrogen from the sample were converted to CO_2 and N_2 under the high temperature and were separated by the chromatography column. The gas was sent into the Finnigan DELTAplus XP stable isotope ratio mass spectrometer through the ConFlo IV or ConFlo III for analysis.

2.4. Stable Isotope and Trophic Position Analysis

2.4.1. Stable Isotope

The results were expressed in delta (δ) notation, and the specimens were analyzed through continuous flow isotope-ratio mass spectrometer [18]. These values are calculated as:

$$\delta X = \left[\left(\frac{R_{\text{sample}}}{R_{\text{standard}}} \right) - 1 \right] \times 10^3 \quad (1)$$

where X is ^{13}C or ^{15}N , and R is the isotope ratio $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$, respectively. The value is related to Pee Dee Belemnite (PDB) and atmospheric N_2 for ^{13}C and $\delta^{15}\text{N}$. This $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ study also calculated the sample C:N ratio, indicating satisfactory lipid removal efficiency.

2.4.2. Trophic Position

The relative trophic position (TP) values of *M. pelagios* and *R. typus* were estimated from ^{15}N [55]. The equation is as below:

$$TP = \lambda + \frac{\delta^{15}\text{N}_{\text{secondary consumer}} - \delta^{15}\text{N}_{\text{base}}}{\Delta_n} \quad (2)$$

where λ is the trophic position of reference organism, $\delta^{15}\text{N}_{\text{secondary consumer}}$ is the stable isotope of consumer, $\delta^{15}\text{N}_{\text{base}}$ is the stable isotope of reference organism, and Δ_n is the trophic enrichment factor represents a best estimate of isotopic enrichment between consumer and its prey [55]. According to previous study, the species used for estimating $\delta^{15}\text{N}_{\text{base}}$ should come from the same habitat as the consumer [55]. Therefore, $\delta^{15}\text{N}$ of euphotic POM (1.1‰) from the North-Eastern Taiwan water was chosen to be the baseline for *R. typus*, in this case λ is 1 [56]. On the other hand, $\delta^{15}\text{N}$ of identifiable stomach composition ($\delta^{15}\text{N}_{\text{Euphausiacea}} = 6.95\text{‰}$) of *M. pelagios* was used for calculating trophic position in this study, as Euphausiacea is the primary consumer, it was assigned a trophic position of 2. The trophic enrichment factor (TEF) was assumed as 3.4‰ per trophic level, based on the empirical observation that $\delta^{15}\text{N}$ values typically increase by this amount with each trophic level [55,57].

2.5. Data Analysis

The Shapiro–Wilk (S–W) normality test was used to test normality of isotope value for *M. pelagios* and *R. typus*, respectively. When normal distribution was found, Student's t -test was used to test the differences between sexes, otherwise Kruskal–Wallis (K–W) test was used in non-normal conditions. The analysis of variance (ANOVA) was used to test the difference between sex and size groups. *M. pelagios* and *R. typus* specimens used in this study were both separated into three size groups (I, II, and III) for discussing the shift in trophic position. Statistically, significant level (α) was 0.05. Stable Isotope Bayesian Ellipses in R (SIBER) was used to calculate the small sample size corrected SIBER ellipse area (SEAc) for the niche breadth and niche overlap [58]. Moreover, the overlap percentage (Overlap%) of both shark between sexes and different size groups were calculated. The Overlap% value was identified as low ($\leq 35\%$), medium (36–70%), and high ($> 70\%$), respectively [58]. Simple linear regression analyses were carried out between $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values across species and sexes within species.

3. Results

3.1. SIA for *M. pelagios* and *R. typus*: Interspecific Comparison

In total, 91 *M. pelagios* (37 males and 54 females) and 90 *R. typus* (58 males and 32 females) tissue samples were used in SIA (Table 1). The body size of *M. pelagios* ranged from 290 to 710 cm TL, and that of *R. typus* from 284 to 1190 cm TL. The values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in *M. pelagios* were -18.14 to -14.99‰ (mean $\pm$ SD: $-17.2 \pm 0.69\text{‰}$, $n = 91$) and 6.83 to 11.67‰ ($9.04 \pm 0.9\text{‰}$, $n = 91$), respectively (Table 1). The values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in *R. typus* were -18.89 to -13.68‰ ($-15.67 \pm 0.78\text{‰}$, $n = 90$) and 5.17 to 13.01‰ ($9.02 \pm 1.79\text{‰}$, $n = 90$), respectively (Table 1). The C:N ratio of both sharks was <3.5 , indicating that the samples were free from the effects of lipid extraction on the stable isotope composition, as lipids are suggested to be removed from samples when their C:N ratios exceed 3.5 (presumed to be lipid-rich) [55,59].

Table 1. $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and trophic position (TP) value of *M. pelagios* and *R. typus* in different sex and size groups from Taiwan waters.

Species	Groups	n	$\delta^{13}\text{C}$ (‰)			$\delta^{15}\text{N}$ (‰)			TP		
			Min	Max	Mean $\pm$ SD	Min	Max	Mean $\pm$ SD	Min	Max	Mean $\pm$ SD
<i>M. pelagios</i>	all	91	-18.14	-14.99	-17.20 ± 0.69	6.83	11.67	9.04 ± 0.90	1.97	3.39	2.62 ± 0.27
	female	54	-18.14	-14.99	-17.14 ± 0.67	6.83	11.67	9.19 ± 0.87	1.97	3.39	2.66 ± 0.26
	male	37	-18.46	-15.62	-17.27 ± 0.71	6.92	11.05	8.84 ± 0.91	1.99	3.21	2.55 ± 0.27
	group I	19	-18.27	-14.99	-17.12 ± 0.69	7.54	11.67	9.45 ± 0.86	2.17	3.39	2.74 ± 0.29
	group II	39	-18.46	-15.77	-17.26 ± 0.62	7.36	11.05	9.09 ± 0.92	2.12	3.21	2.63 ± 0.26
	group III	33	-18.36	-15.28	-17.18 ± 0.65	6.84	10.14	8.76 ± 1.03	1.99	2.94	2.55 ± 0.26
<i>R. typus</i>	all	90	-18.89	-13.68	-15.67 ± 0.78	5.17	13.01	9.02 ± 1.79	2.2	4.5	3.33 ± 0.53
	female	32	-16.77	-13.68	-15.50 ± 0.61	5.17	11.91	9.33 ± 1.66	2.2	4.18	3.36 ± 0.49
	male	58	-18.89	-14.36	-15.70 ± 0.85	5.52	13.01	8.85 ± 1.83	2.3	4.5	3.28 ± 0.54
	group I	33	-17.12	-14.73	-15.57 ± 0.60	5.17	13.01	9.12 ± 2.25	2.2	4.5	3.36 ± 0.66
	group II	47	-18.89	-14.69	-15.75 ± 0.79	5.75	12.02	8.81 ± 1.43	2.37	4.21	3.27 ± 0.42
	group III	10	-17.87	-13.68	-15.29 ± 1.04	6.54	11.07	9.68 ± 1.34	2.60	3.93	3.52 ± 0.39

The S–W normality test for the $\delta^{13}\text{C}$ values were asymmetrical for both *M. pelagios* (Shapiro–Wilk normality test, $W = 0.96$, $p < 0.05$) and *R. typus* (Shapiro–Wilk normality test, $W = 0.89$, $p < 0.05$), but $\delta^{15}\text{N}$ values were symmetrical for both *M. pelagios* (Shapiro–Wilk normality test, $W = 0.99$, $p = 0.98$) and *R. typus* (Shapiro–Wilk normality test, $W = 0.99$, $p = 0.75$). No significant difference was observed between male and female individuals in $\delta^{13}\text{C}$ for *M. pelagios* (Kruskal–Wallis test, $H = 0.54$, $p = 0.46$), *R. typus* (Kruskal–Wallis test, $H = 0.23$, $p = 0.63$), and in $\delta^{15}\text{N}$ for *R. typus* (Student's t -test, $t = 1.23$, $p = 0.11$); however, $\delta^{15}\text{N}$ was significantly different between female and male *M. pelagios* (Student's t -test, $t = 1.83$, $p < 0.05$). No significant difference was observed between body size and sex for both *M. pelagios* in terms of $\delta^{13}\text{C}$ (ANOVA, $F = 1.45$, $p = 0.24$) and $\delta^{15}\text{N}$ (ANOVA, $F = 2.53$, $p = 0.09$), as well as for *R. typus* in terms of $\delta^{13}\text{C}$ (ANOVA, $F = 0.65$, $p = 0.52$) and $\delta^{15}\text{N}$ (ANOVA, $F = 0.96$, $p = 0.39$).

Figure 2 presents the result of SIBER analysis for two species and sexes; the niche width of *R. typus* was broader than that of *M. pelagios*. Their isotopic niches were separated into two groups (overlap% = 0), but medium to high overlap was found between male and female individuals for both species (Figure 2). The $\delta^{13}\text{C}$ of *M. pelagios* (-17.20 ± 0.69) was lower than that of *R. typus* (-15.67 ± 0.78), indicating different habitats (Table 1). Although $\delta^{15}\text{N}$ was comparable between the two species, the TP of *R. typus* (3.33 ± 0.53) was higher than that of *M. pelagios* (2.62 ± 0.27), implying that *R. typus* was distributed around Taiwan waters (Table 1). Furthermore, the isotopic niche width of males was larger than that of females for both species (Table 2).

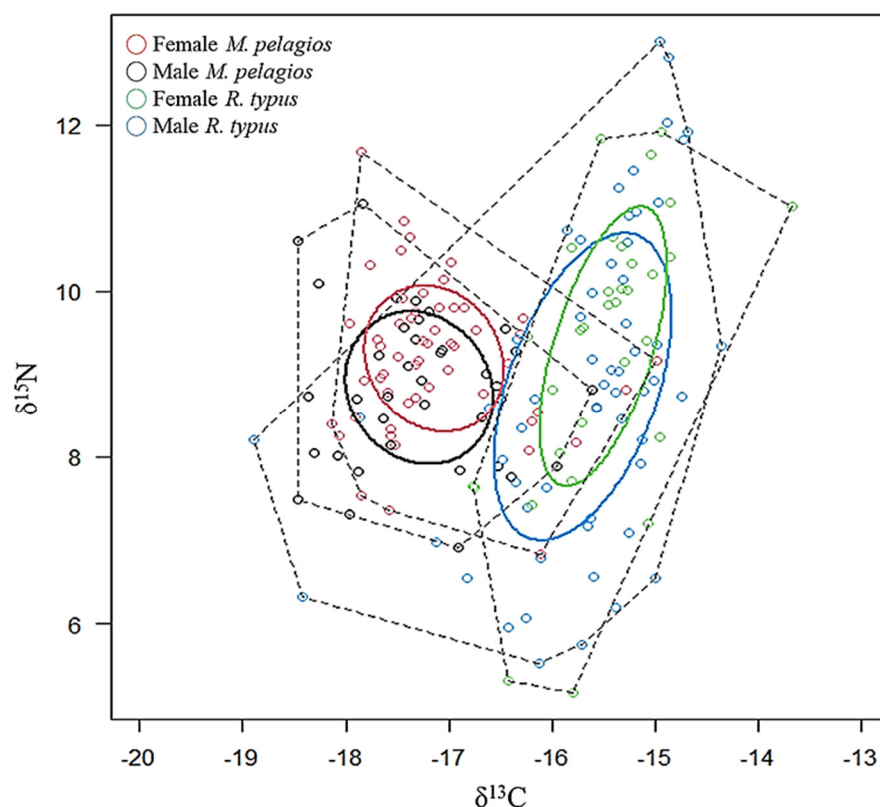


Figure 2. Individual stable isotope value ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) bio-plot, SEAc (small sample size corrected SIBER ellipse area), and TA (convex hull area) of *M. pelagios* and *R. typus*. (Solid line: SEAc, dash line: TA).

Table 2. The convex hull area (TA), standard ellipse area (SEA), and small sample size corrected SIBER ellipse area (SEAc) of *M. pelagios* and *R. typus* in different sex and size groups (♀: female; ♂: male).

Species	Groups	TA	SEA	SEAc
<i>M. pelagios</i>	female	9.00	1.85	1.88
	male	7.74	2.05	2.11
	group I ♀	5.91	2.92	3.17
	group I ♂	1.37	1.22	1.63
	group II ♀	4.33	1.25	1.30
	group II ♂	5.31	2.02	2.19
	group III ♀	5.15	1.86	2.00
	group III ♂	4.56	1.83	1.94
<i>R. typus</i>	female	10.87	2.64	2.72
	male	19.77	4.41	4.49
	group I ♀	5.87	2.71	3.01
	group I ♂	7.59	3.10	3.25
	group II ♀	4.27	1.69	1.80
	group II ♂	15.76	4.12	4.27
	group III ♀	0.82	0.94	1.41
	group III ♂	7.72	5.53	6.91

3.2. SIA for *M. pelagios* and *R. typus*: Intraspecific Comparison

3.2.1. *M. pelagios*

For *M. pelagios*, the stable isotopic niche width was similar across different sizes and maturity stages for both sexes (SEAc: 1.63, 2.19, and 1.94 for Groups I, II, and III in males; 3.17, 1.30, and 2.00 for Groups I, II, and III in females, respectively) (Table 2). The niche of female and male sharks displayed medium to high overlap in Groups I and II but only medium overlap in Group III, indicating potential sexual segregation (Figure 3). The $\delta^{13}\text{C}$ value increased slightly, but the $\delta^{15}\text{N}$ value decreased slightly with the increase in the body size of *M. pelagios* (Figure 4).

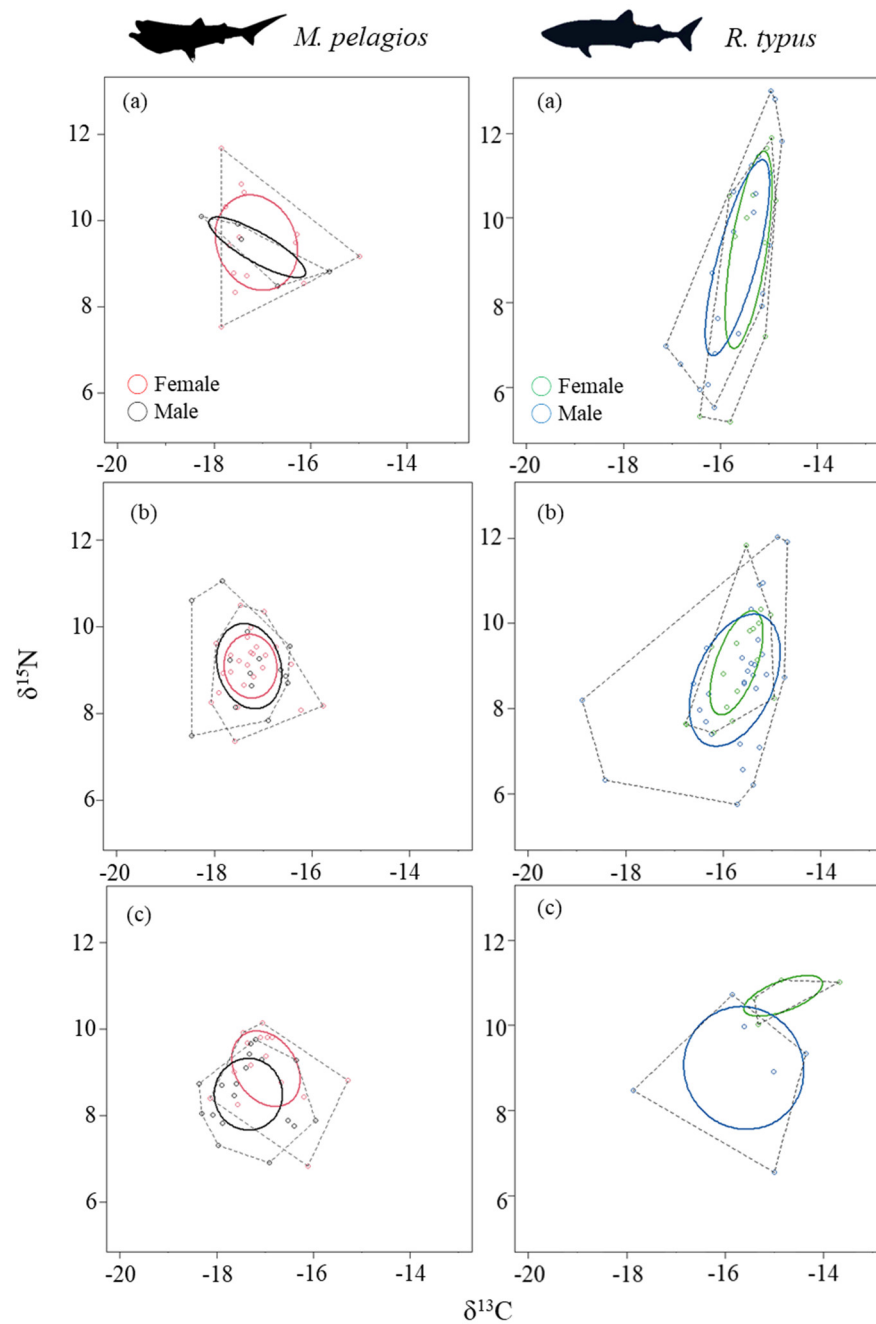


Figure 3. The SEAc (small sample size corrected SIBER ellipse area) and TA (convex hull area) difference between (a) group I; (b) group II; and (c) group III of female and male *M. pelagios* and *R. typus*. (Solid line: SEAc, dash line: TA).

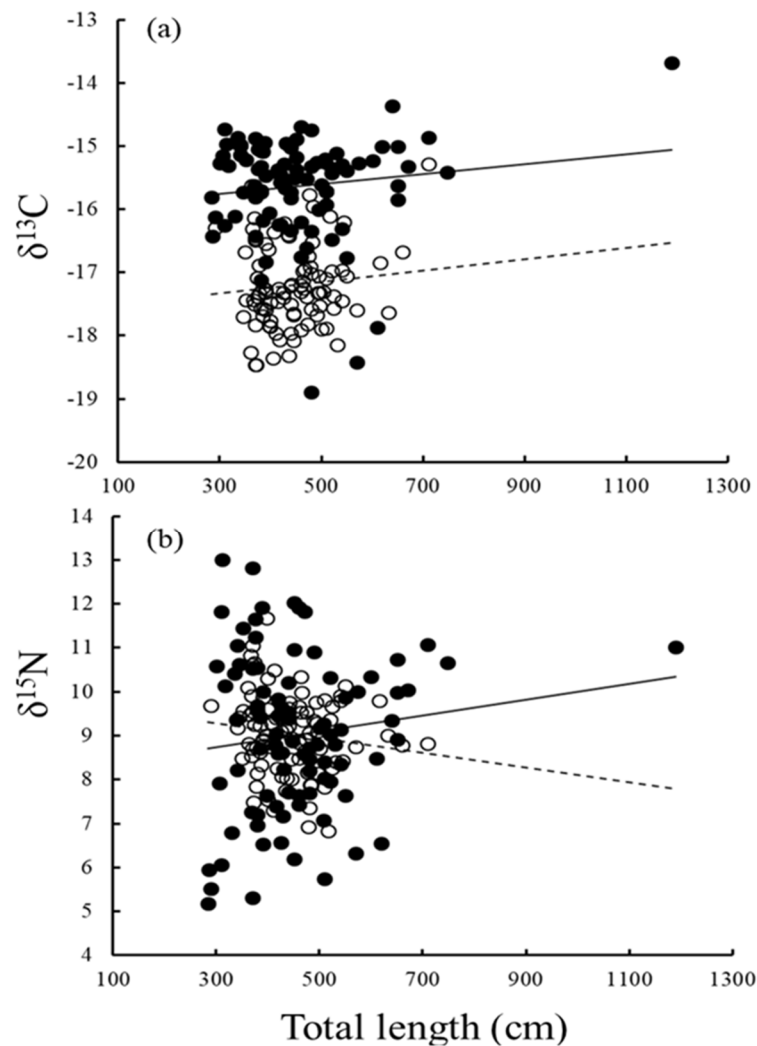


Figure 4. Relationship between (a) $\delta^{13}\text{C}$ and (b) $\delta^{15}\text{N}$ value and total length of *M. pelagios* ($\circ$, ---) and *R. typus* ($\bullet$, —).

3.2.2. *R. typus*

The stable isotopic niche width shifted between different size groups of *R. typus*. The niche width increased with an increase in the body size of male individuals (SEAc: 3.25, 4.27, and 6.91, respectively), but not in female individuals (SEAc: 3.01, 1.80, and 1.41, respectively), suggesting sexual segregation (Table 2). The niche overlap between male and female *R. typus* was medium or high in Groups I and II and low in Group III (Figure 3). Both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values increased slightly with body size in *R. typus*, indicating ontogenetic changes (Figure 4).

4. Discussion

Sample collection of large shark species, especially filter-feeding sharks, is challenging due to their body size and unpredictable migration/movement. This study obtained tissue specimens through cooperation with fishers and observers participating in the catch reporting system of the Taiwan Fisheries Agency. We also attempted to collect stomach content samples from *M. pelagios*. Euphausiacea and Medusozoa (jellyfish) were the most commonly observed prey; however, most planktonic prey items were too fragile to be reliably identified. In most cases, the stomach contents were digested into small pieces, which prevented us from determining the number of prey, which is consistent with the observation of a previous study [50]. Additionally, there was only one mature *R. typus*

individual (>10 m TL) in this study and over 90% of the individuals were immature or juveniles, the bias towards immature individuals is not unusual in other studies [40,60,61]. Furthermore, juveniles, immature, and mature *M. pelagios* individuals were included in this study, with 36% of the specimens being mature, providing a relatively complete size spectrum.

In this study, ethanol storage was chosen as a reasonable method for preservation because the tissue samples were originally collected for molecular research. Before the fishery ban, *M. pelagios* were sold in the market, and only small pieces of tissue such as skin or white muscle were available for scientific use. Alongside this, fin clips were the only sample we took from *R. typus* because of the Animal Conservation Act. Differences in the species, tissue type, sample storage method, and experimental treatment lead to differences in the metabolic rate estimated and can affect the SIA results [62,63]. However, a study has demonstrated that the relatively high lipid content in the muscles and livers of certain large-bodied terrestrial omnivores does not significantly affect stable isotope estimates and that ethanol preservation offers a viable alternative for storing tissues when freezing or drying is impractical [64]. Arrington and Winemiller also indicated that different preservation methods such as formalin-ethanol had minimal and consistent directional effects on stable isotopic signatures for fish, implying that ethanol is acceptable for use in stable isotope analysis [54].

According to the previous study, tissue turnover rates in large, long-lived fishes like elasmobranchs are primarily influenced by catabolism, with younger animals experiencing more pronounced effects [65]. Also, fin clips were suggested as composed of several structural components such as cartilage, skin, and small amounts of muscle, providing a mixed representation of the feeding and environment of the study animal [65]. Previous study indicated the turnover rate of plasma for *R. typus* is approximately 70–200 days, but the fin cartilage appeared to have longer turnover rates (230–851 days) [41]. In addition, different methods for preservation and preparation of *R. typus* tissue were examined for isotopic analysis. Hussey et al. compared the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values between non-lipid extracted (BULK) and lipid extracted (LE) frozen white muscle samples of *R. typus* (without rinsed), indicating that both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ increased (+3.3‰; +0.4–0.6‰) after LE process [66]. Nevertheless, Marcus et al. revealed that the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of *R. typus* dermal tissue changed through LE (+1.7‰; +0.9‰), deionized water rinsed (DIW) (−1.1‰; +1.7‰), and LE + DIW (+1.3‰; +1.3‰) [63]. Additionally, numerous studies have mentioned that urea may affect SIA results; however, after conducting experiments on 14 elasmobranch species, Carlisle et al. ultimately concluded that while urea generally has a direct influence on $\delta^{15}\text{N}$ values, the effects of urea and lipids on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ vary depending on the relative concentration of these compounds between species [67]. Different treatment experiments were not taken in this study, all the tissue samples were originally collected and preserved in ethanol for analysis. The previous studies have shown various results on different tissue types or treatments but are unable to find an explicit method for SIA data correction for our results [63,66]. However, the outcomes of this study are still likely to reflect dietary and habitat use tendencies integrated over long-term scales from several months to years of *M. pelagios* and *R. typus*. Future research is suggested to validate the results of SIA by conducting different species, tissue samples, and treatments.

SIA revealed differences in the feeding strategies and trophic niches between *M. pelagios* and *R. typus* (Figure 2). Abrantes and Sheaves observed a linear negative relationship between $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, suggesting this is the feature of primary producers, primary consumers, or secondary consumers; this finding not only aligns with our SIA results for *M. pelagios* but also agrees with our observation that *M. pelagios* feeds mainly on zooplanktonic preys [68]. However, there is a significant difference in $\delta^{15}\text{N}$ between male and female

M. pelagios, which may be caused by sexual segregation, different foraging grounds, or metabolic rates; further evidence from SCA is required to clarify this observation. SIA demonstrated a positive relationship between $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ for *R. typus*, revealing that for *R. typus* has a more complex diet than filter feeding. Studies have implied that *R. typus* can feed on a broad spectrum of prey, which is consistent with our finding of a wide trophic niche of *R. typus*. Nakaya et al. stated that *M. pelagios* is an engulfment feeder and has special morphological characteristics, such as a large mouth, long jaw cartilages, and flexible skin around the pharynx, which are similar to those of engulfment-feeding whale species, such as humpback whale (*Megaptera novaeangliae*) [69]. This foraging strategy seems beneficial for slow-swimming species, such as *M. pelagios*, which has a swim speed of 1.5 km h^{-1} [46]. By contrast, *R. typus* displays a more active suction-filtering behavior, allowing more opportunities to prey on small fishes and squids, which have better swimming ability [35,37]. There was even some evidence showing the rapid mouth-gulping behavior of *R. typus* to consume prey close to the seabed in shallow water if the prey densities are large enough [70,71]. This study indicates that as the filtering feeders inhabiting the western North Pacific Ocean, *M. pelagios* and *R. typus* have distinct feeding strategies.

The lack of trophic niche overlaps between *M. pelagios* and *R. typus* suggests different habitat use (Figure 2). Thums et al. remarked that *R. typus* usually spends time gaining energy from the sun at the sea surface to regulate body temperature [72]. Moreover, it aggregates and forages at or near the sea surface, implying that it mainly inhabits shallow waters [73–76]. By contrast, *M. pelagios* are distributed in deeper waters, usually around 100 m, and rarely appear near the surface, making their discovery challenging [43,46,77]. The two filter feeders were also found to be distributed in different parts of Taiwan waters: *R. typus* was collected from coastal set-net fishery around Taiwan waters, whereas *M. pelagios* was mainly caught in large-mesh driftnet fishery from the eastern waters of Taiwan (Figure 1).

The trophic niche of male sharks was larger, implying a wider distribution, for both *M. pelagios* and *R. typus* (SEAc: 2.11 and 4.49, respectively; Table 2). In many cases, male sharks have been reported to display more active movement than females. Kock et al. described that female great white sharks (*Carcharodon carcharias*) were frequently found offshore areas, whereas males were rarely discovered in False Bay [78]. Female bull sharks (*Carcharhinus leucas*) were caught and monitored more frequently in coastal waters; however, male sharks were only found in isolated waters far from the coast in New Caledonia [79]. A significant difference was also found in $\delta^{15}\text{N}$ between male and female *M. pelagios* in this study, which may be caused by sexual segregation, metabolic rate, or prey items from different habitats during their life history. Yu et al. reported a widespread distribution of male *M. pelagios* in different waters, including the western and eastern Pacific Ocean, but female sharks were spotted mainly in the western North Pacific Ocean [43]. Male sharks are likely to move in a wide area to increase their chances of meeting females and mating, whereas females tend to focus on coastal areas, which have abundant food, and may stay at a particular place to give birth [80,81].

Trophic position (TP) estimation is helpful for ecosystem-based fisheries management strategies; however, there is a wide spectrum of opinions on the estimation methods, especially the empirically determined trophic enrichment. Minagawa and Wada provided critical evidence for the stable isotope enrichment of $\delta^{15}\text{N}$ along food chains, demonstrating a consistent TEF of approximately 3.4‰ (ranging from 1.3 to 5.3‰) by measuring the animals from the East China Sea, The Bering Sea, Lake Ashinoko and Usujiri intertidal zone [57]. This was followed by a previous study, which suggested the simplest model for estimating the trophic position of secondary consumers, with the mean trophic fractionation of $\delta^{15}\text{N}$ is 3.4‰ (1 SD = 1‰), which was widely applicable [55]. Additionally,

the compound-specific isotope analysis of amino acids (AA-CSIA) was proposed as an unbiased approach to evaluating trophic dynamics in food webs by distinguishing between “source” and “trophic” amino acids (the difference between $\delta^{15}\text{N}_{\text{glutamic acid}}$ and $\delta^{15}\text{N}_{\text{phenylalanine}}$). Some research demonstrated that isotopic fractionation from processes like deamination and transamination results in a $\delta^{15}\text{N}$ enrichment with a TEF of 7.6‰ between glutamic acid and phenylalanine, while dietary protein quality further influences TEFs, with herbivores showing a TEF of ~7.6‰ and epibenthic carnivores (sand tiger shark, *Carcharias taurus*; lemon shark, *Negaprion brevirostris*; and leopard shark, *Triakis semifasciata*) exhibiting a lower TEF (<7.6‰) [82,83]. However, several studies have highlighted considerable variation in TEFs (even from AA-CSIA calculation) due to factors such as species, tissue type, environment, etc. [84–86]. As options vary, this study follows the widely adopted TP calculation method and TEF proposed by Post to provide insights into *M. pelagios* and *R. typus* [55]. Thus, it is recommended that future experimental research focus on examining TEFs in indicator species, such as filter-feeding elasmobranchs.

Based on the mean TP values, both *M. pelagios* and *R. typus* were inferred to be lower trophic level consumers, but SIA provided different information on their life history and feeding habits. Both *M. pelagios* and *R. typus* exhibited sexual segregation and ontogenetic changes, and the niche overlap between male and female sharks in Group III was medium for *M. pelagios* but low for *R. typus* (Figure 3c, Table 2). Sexual segregation may result from various factors, such as forced mating behavior from male sharks, reduced resource competition, and finding an appropriate area for offspring [87]. Ontogenetic shifts are common in elasmobranchs throughout their different life stages, different diet preferences between juveniles and adults have been suggested for *R. typus* [88]. Greenland sharks (*Somniosus microcephalus*) exhibited an ontogenetic dietary shift from low-trophic-level prey, such as squids, to high-trophic-level prey, such as seals and benthic fishes, implying that the larger sharks can prey on fast-swimming species [89]. The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of blue shark (*Prionace glauca*) indicated ontogenetic changes in prey consumption and habitats between life stages, with small and large individuals preferring coastal waters, whereas large juveniles preferred oceans [90].

We observed a slight positive correlation between $\delta^{13}\text{C}$ and body length for both *M. pelagios* and *R. typus*, indicating that they move to a more productive or inshore environment as they grow, with *R. typus* preferring a more productive environment (sea surface) than *M. pelagios*. *R. typus* caught in set nets around Taiwan were all from coastal waters no deeper than 50 m, which were enrich producers, whereas *M. pelagios* mainly inhabits deeper waters, usually approximately 100 m, according to the operation depth of a gillnet and tracking study [46]. Together, data from previous studies indicate that *M. pelagios* may be born in warm tropical waters, which is a lower productive environment, and these sharks may then move toward the zooplankton-rich current as they grow [45,50,51]. Furthermore, the findings of Borrell et al. on $\delta^{13}\text{C}$ and body length for *R. typus* were similar to those in our study [37]. Together, these results imply that whale sharks are born in open or deeper waters, where they grow for a period, and then move toward productive coastal waters to feed on planktonic prey [91–93]. In other words, a large dietary component of juvenile and submature *R. typus* is derived from benthic and coastal habitats [38]. These sharks give birth in low-productive waters, likely to protect their young from other predators [93].

The relationship between $\delta^{15}\text{N}$ and body length differed between *M. pelagios* and *R. typus*: as the body size increased, the $\delta^{15}\text{N}$ values of *M. pelagios* decreased slightly, whereas those of *R. typus* increased, although we lack large and mature *R. typus* individuals (Figure 4, Table 1). Our data indicated that *M. pelagios* is a secondary consumer, engaging in engulfment feeding of mainly small zooplanktonic prey, such as Euphausiacea and Medusozoa, in all life stages. However, how changes in the metabolic rate with growth

affect $\delta^{15}\text{N}$ remains unclear. The $\delta^{15}\text{N}$ data of *R. typus* in this study suggested that it feeds on higher-trophic-level prey. A study implied that *R. typus* may change their habitat and prey preferences as they grow; juveniles may cruise various waters for feeding and growth, which is consistent with the wide range of their $\delta^{15}\text{N}$ values, whereas larger individuals tend to feed on the higher-trophic-level prey because of better swimming ability and well-developed filtering structures on the gills [14,37,94–96].

5. Conclusions

In summary, our analysis of *M. pelagios* and *R. typus* around Taiwan waters revealed distinct feeding strategies and habitat use despite both species inhabiting the western North Pacific. *M. pelagios* is an engulfment feeder targeting small zooplankton across all life stages, with sexual segregation suggesting different habitat use by mature males and females. In contrast, *R. typus* shows ontogenetic shifts in diet and habitat, with larger individuals capable of active suction feeding. Due to limited stomach content samples, further SCA and SIA across regions, sexes, and sizes are needed. This study provides key biological insights to inform regional fisheries management and support species-specific conservation strategies.

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Informed Consent Statement: Not applicable.

Data Availability Statement: The raw data and materials used in this research are not publicly available due to confidentiality but can be made available by the authors upon reasonable request.

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