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STABLE ISOTOPES ($\delta^{13}C$ AND $\delta^{15}N$) ANALYSIS INDICATE RESOURCE PARTITIONING BY ELASMOBRANCHS FROM THE TROPICAL COASTAL WATERS OF KUALA PAHANG, MALAYSIA

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Abstract: Nearshore habitats support high levels of biodiversity and important ecological processes such as trophic dynamics. This study aims to determine the trophic dynamics of elasmobranchs and other marine species from the coastal waters of Kuala Pahang, using stable isotope analysis. Samples of invertebrates, teleosts, and juvenile elasmobranchs were collected at the coastal waters of Kuala Pahang, and the composition of stable isotopes $\delta^{13}C$ and $\delta^{15}N$ were determined. Of all species, teleosts had the narrowest range of $\delta^{13}C$ values, of between -17.5‰ and -16.7‰, indicating their reliance on similar types of food resources. On the contrary, elasmobranchs had the widest range of food sources as indicated by the $\delta^{13}C$ values, of between -26.8‰ and-16.8‰. The banded sicklefish (*Drepane longimana*) occupied the highest trophic level as indicated by a $\delta^{15}N$ value of 12.4‰, whereas the red stingray (*Hemitrygon akajei*) had the lowest trophic level with a $\delta^{15}N$ value of 4.9‰. The depleted $\delta^{13}C$ values indicated resource partitioning among elasmobranchs, presumably due to the utilisation on estuarine-based carbon resources. This study highlights the potential importance of the estuary of Kuala Pahang as a feeding ground for juvenile elasmobranchs.

Keywords: South China Sea, bamboo sharks, rays, monsoon, feeding ground.

Introduction

Food web characterisation is one of the key steps in understanding the ecological functioning of marine ecosystems as it provides a useful framework to assess the magnitude and importance of the trophic relationship between its members (Link, 2002). An important parameter in the characterisation of food webs is the trophic position of species, which provides a quantitative measure of the species' energetic interaction and has become one of the most widely used descriptors of the role of different species in marine food webs (Carscallen *et al*., 2012).

On top of this, the trophic position of food web components can be used to interpret the resilience of the ecosystem and provides insights into potential anthropogenic effects on the marine ecosystem. One such example is the recurring pattern of decline in the mean trophic level of fish landings termed 'fishing down the food web. The failure to recognise such trophic interactions and address them accordingly may lead to disruptions in the food web (Montoya *et al*., 2006). An understanding of the trophic interactions within a food web is therefore important to assist in the management of marine resources in the region (Bachok *et al*., 2004). In addition, knowledge of the dietary patterns of marine food webs is important as it helps researchers to understand the biological interactions occurring within a habitat or an ecosystem (Odum, 1953).

Conventionally, the study of trophic links has relied on stomach or gut content analyses. However, this technique has many shortcomings and does not sufficiently fill in the knowledge gap as it only provides information on the most recent feed of an animal and may not accurately reflect the composition of its general diet. This is because only the tissues of prey that are hard to digest remain in the gut for long periods, whereas the rest of the body is mostly digested soon after ingestion (Hyslop, 1980; Christensen & Moore, 2009). Consequently, the results might be skewed by food availability and thus it would not reflect the actual diet of the species.

A more advanced method, Stable Isotope Analysis (SIA) has been widely used to examine the dietary intake and feeding dynamics of marine organisms such as elasmobranchs (MacNeil *et al.*, 2005; Carlisle *et al*., 2012; Burgess *et al*., 2016). The $\delta^{13}C$ of aquatic consumers can provide information on the sources of energy because $\delta^{13}C$ values are conserved ‘up the food chain’ and vary at the base and thus, it has proven useful in identifying where particular organisms feed (Fry, 2008). The $\delta^{13}C$ values are typically lower (more depleted in $\delta^{13}C$) in fluvial and estuarine ecosystems than in marine ecosystems (Hobson *et al*., 1997). Meanwhile, the measurement of stable nitrogen isotopes is based on the relative abundance of ^{15}N to ^{14}N ($\delta^{15}N$) in animal tissues, which shows an enrichment of ^{15}N in consumers over their prey (Fry, 2008).

There is an emerging trend in the application of SIA in food web studies in Malaysia, such as by Zulkifli *et al*. (2014) who studied the food web structure for the Merambong seagrass bed area by analysing specific $\delta^{13}C$ and $\delta^{15}N$ stable isotope ratios of each collected species; Bashir *et al*. (2020) and Abdullah *et al.* (2022) studied the methodological applications of stable isotopes in sharks and rays; Azim *et al.* (2021) studied the feeding habits of five dominant fish species from Matang mangrove estuaries; and Syazwan *et al*. (2021) used stable isotope analysis to understand the trophic ecology of a tropical scyphozoan community in Malaysian coastal waters. Nevertheless, not much is known about the feeding ecology of local elasmobranchs, including sharks and rays (Nur Farhana *et al*., 2013; Lim *et al*., 2019; Bashir *et al*., 2020). The aim of this study is to determine the trophic dynamics of elasmobranchs and other marine species from the coastal waters of Kuala Pahang, using stable isotope analysis of $\delta^{13}C$ and $\delta^{15}N$.

Materials and Methods

Sampling Method

Sampling was conducted on the 6th and 7th November 2018 near the river mouth of Pahang River in the coastal area of Kuala Pahang, Malaysia, in the South China Sea (Figure 1) during the Northeast (NE) monsoon season. All samples were collected via trawling during a research expedition by the Southeast Asian

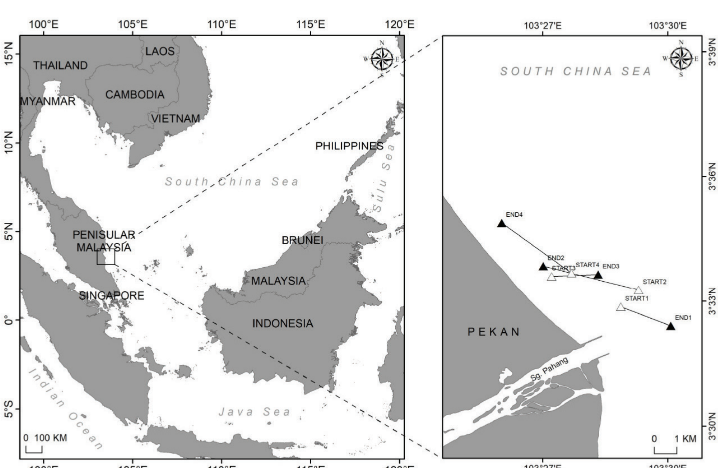


Figure 1: Sampling locations at the Kuala Pahang coastal area in the state of Pahang. START and END indicate the trawling locations at four stations (Stations 1 - 4)

Fisheries Development Centre (SEAFDEC). Table 1 shows the coordinates of the start and end points of the trawling activities at each station.

The four sampling stations were regarded as replicates for the sampling effort. Shrimp bottom trawl nets with 25.6 m net length, 9.14 m net opening width, 36.6 m warp wire, 4.6 m sweep line, 10 m headline and 25 mm cod-end mesh size were used as sampling gear. The duration of the trawl operations at each station was about one hour at a trawling speed of approximately 2.5 knots. The study was conducted in waters between 4.5 - 8.3 m depth located between 0.9 - 2.4 km from the coastline. The trawl distance at each station was about 1.7 nm with a swept area of about 0.29 km^2 (0.091 km × 3.15 km).

Identification and Size Measurement of Samples

In total, 48 samples comprising of 19 species was collected from four sampling stations, and all samples were identified, weighed, and measured. The identification of specimens was performed following Yano *et al.* (2005), Ahmad *et al.* (2014), Last *et al.* (2016) and Lim *et al.* (2018). For the teleosts, measurements were taken of the Standard Length (SL) which is the length from the tip of the snout to the posterior end of the last vertebra, and the Total Length (TL) which is the whole length of fish from its snout to the caudal fin. As for sharks, only its TL was measured. The size of the rays was noted by measuring their Disc Width (DW), which is the maximum distance between wingtips, and the Disc Length (DL) taken from the tip of the snout to the posterior edge of the disc. Carapace Length (CL) for crabs was taken by measuring the length along the anterior to its posterior axis. For cephalopods, Mantle Length (ML) was taken by measuring the length from the midpoint between the eyes to the posterior end of the mantle.

Sample preparation

All samples were kept on ice during its transportation (Bashir *et al.,* 2020) to the laboratory at Universiti Malaysia Terengganu (UMT), and subsequently kept frozen at -20°C until analysis. Whenever possible, three individuals of each species were prepared for SIA as replicates. In this study, the whole animal was sacrificed for the stable isotope analysis. Using scissors and a scalpel blade, a small portion of clean muscle tissues of about 1 to 3 cm^2 was collected for analysis by removing the skin and bones from the teleosts, the cartilage from the sharks and rays as well as the skin and gladius from the cephalopods. Muscle tissues were carefully retrieved from the crabs so that no shell was included in the samples.

For elasmobranch samples, the urea removal was performed following Burgess *et al.* (2016) method by soaking each sample in deionised water for up to 24 hours. This step was necessary to remove the urea content that may affect the stable isotope values (Hussey *et al*., 2012), Subsequently, all samples were rinsed

Table 1: Detailed information on the locations and dates of the trawling activities. Distance was measured from the coastline to the starting point of each trawl in nautical miles (nm), and water depth was measured in meters (m)

Date	Trawl Station	Distance from Coastline (nm)	Water Depth (m)	Coordinate at Start Point of Trawling		Coordinate at End Point of Trawling	
				Latitude	Longitude	Latitude	Longitude
06/11/2018	1	1.0	7.5	3°32.863′	103°28.875′	3°32.404′	103°30.072′
	2	1.3	8.3	3°33.272′	103°29.301′	3°33.845′	103°27.014′
	3	0.5	4.5	3°33.600′	103°27.215′	3°33.644′	103°28.334′
07/11/2018	4	0.9	6.5	3°33.677′	103°27.691′	3°34.886′	103°26.019′

thoroughly with deionized water and oven-dried at 60°C for 48 hours. Each sample was then finely ground manually using mortar and pestle and kept in a 2 ml microcentrifuge tube. To ensure there was no cross-contamination at this stage, the mortar and pestle were wiped thoroughly using tissue paper each time before processing a new sample .

Stable Isotope Analysis (SIA)

SIA was conducted at the Malaysian Nuclear Agency following the method from Peterson & Fry (1987). Approximately 0.5 - 1.0 mg of the dried and homogenised sample was weighed into tin capsules, sealed and combusted at 1000°C using a SerCon ANCA GSL elemental analyser interfaced via continuous flow to a SerCon GEO20-20 isotope-ratio mass spectrometer. Stable isotope abundances was measured in triplicates for each sample by comparing the ratio of the two most abundant isotopes, $^{13}C/^{12}C$ and $^{15}N/^{14}N$, in the sample to the international standard. Results were expressed in terms of parts per thousand (‰) deviation from the standard using the following equation:

$$\delta X = [(R_{sample} / R_{standard}) - 1] \times 1000‰ \qquad (1)$$

where X is ^{13}C or ^{15}N and R is the isotopic ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$ (Peterson & Fry, 1987). The standards used for carbon and nitrogen were secondary standards referenced to a known international standard, Vienna Pee Dee Belemnite (VPDB) and atmospheric nitrogen (air), respectively.

Results and Discussion

Maturity Stage of Elasmobranch Samples

According to Yano *et al*. (2005), the Indonesian bamboo shark *Chiloscyllium hasseltii* is an oviparous with hatchlings measuring between 9.4-12.0 cm when they emerge from the eggs during the monsoon season, and males and females mature at 44 - 54 cm and 54 - 59 cm, respectively. During the monsoon season, juvenile *C. hasseltii* are commonly caught as bycatch in shrimp trawl nets in the inshore waters along the East Coast of Peninsular Malaysia (Nadia *et al*., 2021). Juvenile and adult *C. hasseltii* with mean total lengths over 60 cm comprised one-third of the sharks landed at a commercial fishing port in Kuantan, Pahang, Malaysia (Arai & Azri, 2019). These individuals were larger than the individuals sampled near Kuala Pahang, which is located about 50 km away, with a mean total length of 27.1 cm.

All shark and ray specimens collected in this study were considered to be immature (Yano *et al*., 2005; Ahmad *et al*., 2014; Furumitsu *et al*., 2019). The four species of rays caught in this study, namely *Brevitrygon imbricata*, *Maculabatis gerrardi*, *Telatrygon biasa* and *Hemitrygon akajei*, were all viviparous (Last *et al*., 2016). At the time of birth, *M. gerrardi* has an average disc width of 18 - 21 cm (Ahmad *et al*., 2014) and *T. biasa* has an average disc width of 14 cm (Yano *et al*., 2005). At maximum size, adult *B. imbricata* females and males can have a disc width of 23 cm and 15 cm respectively (Yano *et al*., 2005).

Stable isotopes δ ^{13}C and δ ^{15}N

A summary of the results is described in Table 2. Teleosts generally had the narrowest range of $\delta^{13}C$ values, ranging from -17.5‰ to -16.7‰, indicating their reliance on similar types of food resources. The two species of cephalopods showed slightly different $\delta^{13}C$ values, whereby the common squid *Loligo chinensis* was slightly more depleted (-18.9 ± 2.9‰) than the pharaoh cuttlefish *Sepia pharaonis* (-17.8 ± 1.5‰). As for the crustaceans, the crucifix crab *Portunus pelagicus* had the most depleted $\delta^{13}C$ values, -17.4 ± 1.6‰, while the three-spot swimming crab *Portunus sanguinolentus* had the most enriched $\delta^{13}C$ value, -16.5‰. Generally, teleosts, cephalopods, and crustaceans, had relatively enriched $\delta^{13}C$ values as compared with elasmobranchs, ranging between -19.0‰ and -16.0‰ (Figures 2A and 2B). This indicates that the animals utilized coastal marine food resources such as plankton (-21.8‰ to -19.6‰) or algae (-18.2‰ to -13.6‰) (Thimdee *et al*., 2004; Zulkifli *et al*., 2014).

Sharks consumed the widest range of food resources as indicated by their $\delta^{13}C$ values ranging from -26.8 ± 2.3‰ for juvenile bamboo sharks it ranged from -16.8 ± 0.9‰ for neonate bamboo sharks. Juvenile bamboo sharks have been reported to favour benthic invertebrates such as small crustaceans while bigger bamboo sharks eat larger prey such as teleosts and cephalopods (Nur Farhana *et al*., 2013). It should be noted that the $\delta^{13}C$ values of neonate bamboo sharks were significantly enriched as compared with those of the juveniles. This was likely to be indicative of the $\delta^{13}C$ values of their mothers which were retained in the eggs' yolk sacs because young neonates do not eat anything during the first few days after they are born. For example, a newly born zebra shark with an external yolk refused to eat until all the yolk had been used up (Smith *et al*., 2017). This is supported by Pretorius (2012), where evidence for the absorption of the yolk mass was seen in the swollen stomach of the shark neonates and a thicker yolk stalk was observed. The yolk volume then eventually depleted across the stages, due to the use of the lipid reserves for tissue development in the greater spotted catshark (Musa *et al*., 2019), grey carpet shark (Walton, 2020), and white-spotted bamboo shark (Tullis & Peterson, 2000).

The $\delta^{13}C$ values of the four species of ray ranged from -24.1 ± 4.0 to -22.1‰. Although specific dietary information for each ray species included in this study is scarce, benthic invertebrates such as penaeid shrimps, amphipods, brachyuran crabs, and calanoids are known to be important food items, along with fishes for some of the stingray species. Nevertheless, this depends on whether they are generalists or specialized feeders (Lim *et al*., 2019). The utilization of food resources among sharks and rays was clearly segregated as compared with the other species collected in this study. This segregation forms a distinct cluster on the dual isotope plot of the elasmobranch group, in which the sharks and rays utilized relatively more depleted food resources than the benthic invertebrates that inhabit the mangroves and estuarine areas, as indicated by the most depleted values of $\delta^{13}C$ shown in Figure 2C.

Teleosts had higher $\delta^{15}N$ values than other groups, with $\delta^{15}N$ values ranging from 10.0‰ for *Cynoglossus arel* to 12.4‰ for *Drepane longimana*. As for the cephalopods and crustaceans, the $\delta^{15}N$ values ranged from 9.4 ± 0.8‰ for *Portunus pelagicus* to 11.9 ± 0.4‰ for *Loligo chinensis*. The $\delta^{15}N$ values of elasmobranchs were the lowest, ranging from 4.9 ± 1.0‰ for *Brevitrygon imbricata* to 9.6‰ for *Hemitrygon akajei.* Previous studies reported that most of the teleost species collected in this study, including *Otolithes ruber*, *Nibea soldado*, and *Parascorpaena picta*, feed on fishes, prawns, and other invertebrates (Fischer *et al*., 1990; Sasaki, 2001), while *Coilia dussumieri* and *Sardinella albella* feed on planktonic copepod and fish larvae (Coppola *et al*., 1994; Sommer *et al*., 1996). Species such as *Drepane longimana*, *Gnathophis nystromi* and *Cynoglossus arel* survive on benthic invertebrates (Munroe, 2001; Mundy, 2005). In this study, the $\delta^{15}N$ values of elasmobranchs were lower than those of the other groups, including those of the teleost species that feed on benthic invertebrates. This observation might indicate a higher reliance of the elasmobranchs on benthic-based food resources which have lower $\delta^{15}N$ values as compared with prey items from higher trophic levels.

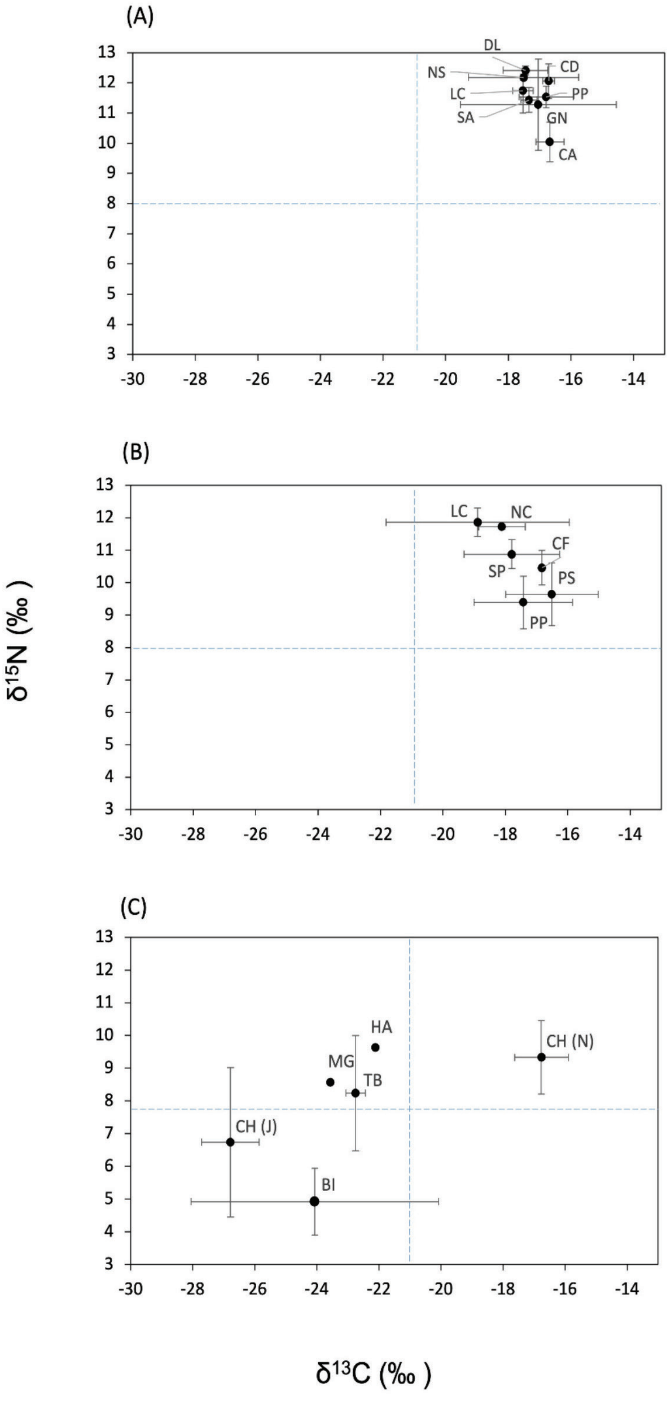
Food Web Dynamics of Elasmobranchs in Kuala Pahang

Many series of previous trawl surveys were conducted during the monsoon season from November until March by the SEAFDEC/MFRDMD. Since 2018, a total of 97.9% of sharks and 68.4% of rays caught were recorded to be at their juvenile stage (Nadia *et al*., 2021). As bottom dwellers, dietary changes among elasmobranchs may occur following ontogenetic dietary shifts or movements between habitats, which are common among this group. During ontogenetic shifts, individual species of sharks and rays reform their movement patterns to increase the availability of resources and reduce

Table 2: Detailed description of the marine coastal food web components of Kuala Pahang. The measurements taken of the specimens were total length (TL) for teleosts and sharks; standard length (SL) for teleosts; disc width (DW) and disc length (DL) for rays; carapace length (CL) for crustaceans and mantle length (ML) for cephalopods. The weight was reported in grammes (g), all length measurements are reported in centimetres (cm), and stable isotopes $\delta^{13}C$ and $\delta^{15}N$ are reported in per mil units (‰). All values were reported as mean ± standard deviation. N represents the number of samples

Species	**Label**	**Common Name/ Local Name**	**Weight (g)**	**TL/ CL/ DL (cm)**	**SL/ DW/ ML (cm)**	**$\delta^{13}C$ (‰)**	**$\delta^{15}N$ (‰)**	**n**
Teleosts								
Otolithes ruber	OR	Tiger-toothed croaker (Gelama jarang gigi)	79.0 ±12	19.1 ±0.4	16.5 ±0.3	-17.5 ±0.7	11.7 ±0.3	3
Nibea soldado	NS	Soldier croaker (Gelama papan)	48.0 ±5.3	16.2 ±0.7	13.5 ±0.6	-17.5 ±1.8	12.2 ±0.1	3
Drepane longimana	DL	Banded sicklefish (Daun baru belang)	65.8 ±22.9	13.1 ±1.3	10.5 ±1.2	-17.5 ±0.7	12.4 ±0.2	3
Sardinella albella	SA	White sardinella (Tamban sisik putih)	24.6 ±2.1	14.1 ±0.4	11.8 ±0.5	-17.3 ±0.3	11.4 ±0.4	3
Gnathophis nystromi	GN	Conger eel (Malong)	605.4 ±169.2	75.6 ±8.3	74.9 ±7.9	-17.0 ±2.5	11.3 ±1.5	3
Parascorpaena picta	PP	Painted scorpionfish (Depu kerapu karang)	96.9	17.3	14.0	-16.8	11.5	1
Coilia dussumieri	CD	Goldspotted grenadier anchovy (Bulu ayam tanda emas)	14.2 ±0.6	14.4 ±1.0	13.1 ±1.5	-16.7 ±0.2	12.1 ±0.6	3 3
Cynoglossus arel	CA	Largescale tonguesole (Lidah sisik besar)	27.2 ±2.2	17.7 ±0.7	16.8 ±0.5	-16.7 ±0.5	10.0 ±0.7	3
Elasmobranchs								
*Chiloscyllium hasseltii****	CH (N)	Indonesian bamboo shark (Yu bodoh) - Neonate	10.5 ±1.7	14.4 ±0.7	-	-16.8 ±0.9	9.3 ±1.1	2 6 2
Chiloscyllium hasseltii	CH (J)	Indonesian bamboo shark (Yu bodoh) - Juvenile	94.5 ±14.8	33.8 ±1.6	-	-26.8 ±2.3	6.7 ±0.9	1
*Brevitrygon imbricata***	BI	Scaly whipray (Pari ketuka lalat)	131.0 ±53.3	15.6 ±2.0	13.9 ±1.9	-24.1 ±4.0	4.9 ±1.0	1
*Telatrygon biasa***	TB	Sharpnose stingray (Pari ketuka)	122.5 ±88.4	16.8 ±4.9	15.2 ±5.5	-22.8 ±0.3	8.2 ±1.8	3
*Maculabatis gerrardi****	MG	Whitespotted whipray (Pari bintik)	202.0	18.2	19.0	-23.6	8.6	3
*Hemitrygon akajei**	HA	Red stingray (Pari merah)	240.0	18.7	19.5	-22.1	9.6	3
Cephalopods								
Loligo chinensis	LC	Common squid (Sotong jarum)	27.7 ±2.2	-	4.4 ±0.6	-18.9 ±2.9	11.9 ±0.4	1
Sepia pharaonis	SP	Pharaoh cuttlefish (Sotong katak)	41.8 ±4.7	-	4.6 ±1.3	-17.8 ±1.5	10.9 ±0.5	1
Crustaceans								
Portunus pelagicus	PP	Flower crab	63.2 ±34.7	10.0 ±1.7	-	-17.4 ±1.6	9.4 ±0.8	
Charybdis feriatus	CF	Crucifix crab	48.5	12.2	-	-16.8	10.5	
Portunus sanguinolentus	PS	Three-spot swimming crab	101.4	6.6	-	-16.5	9.6	

Asterisks indicate the IUCN status *Near Threatened **Vulnerable and ***Endangered. All other species collected in this study fall into the category of Least Concerned.



*Labels denote species names as described in Table 2

Figure 2: Dual isotope plots of the species collected in Kuala Pahang as indicated by $\delta^{13}C$ and $\delta^{15}N$ values, the data is for (A) teleosts, (B) invertebrates, including cephalopods and crabs, and (C) elasmobranchs

competition and the risk of predation (Chin *et al*., 2013).

In this study, although the bamboo sharks and rays were caught outside the river mouth, the $\delta^{13}C$ values were found to be depleted in all species except for neonate bamboo sharks *C. hasseltii*. This observation may indicate a potential influence of mangroves or estuarine input which normally has more depleted $\delta^{13}C$ values than those of a purely marine food web (Gladyshev, 2009). In addition, this result echoes the observations of Wai *et al.* (2012) who reported that, based on its carbon isotopes, the white-spotted bamboo shark *Chiloscyllium plagiosum* obtains most of its detrital food from terrestrial-based sources. Furthermore, juvenile lemon sharks *Negaprion brevirostris* uses shallow mangrove areas and seagrass flats as habitat and moves to deeper offshore habitats in their adult stage (Wetherbee *et al*., 2007). Ontogenetic habitat shifts have also been observed in bluntnose sixgill *Hexanchus griseus*, pigeye or Java *Carcharhinus amboinensis* and lemon sharks *N. brevirostris* and many other elasmobranch species (Andrews *et al*., 2010; Knip *et al*., 2011; Tavares *et al*., 2016).

Conclusion

This study has shown the potential importance of the nearshore ecosystem of the Kuala Pahang estuary to the juveniles of elasmobranchs as indicated by their isotope values. This data, although preliminary, is beneficial in supporting estuarine habitat and species conservation programs, particularly towards the protection of the feeding grounds of sharks and rays in the region. Nonetheless, it is advised that future research should include information on resource discrimination between estuarine and coastal habitats based on the isotopic compositions and investigate temporal variations of the resource and habitat utilisation of elasmobranchs.

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References

Abdullah, M. M., Lua, W. Y., Mostapa, R., Bashir, Z., Naimullah, M., Hajisamae, S., & Hisam, F. (2022). Effects of urea removal on the stable isotopes $\delta^{13}C$ and $\delta^{15}N$ in rays from the coastal waters of Peninsular Malaysia. *Jurnal Ilmiah Perikanan dan Kelautan, 14*, 340-348.

Ahmad, A., Lim, A. P. K., Fahmi, Dharmadi, & Krajangdara, T. (2014). *Field guide to rays, skates and chimaeras of the Southeast Asian Region*. Perpustakaan Negara Malaysia. Malaysia.

Andrews, K., Williams, G., & Levin, P. (2010). Seasonal and ontogenetic changes in movement patterns of sixgill sharks. *PLoS One*, *5*, e12549.

Arai, T., & Azri, A. (2019). Diversity, occurrence and conservation of sharks in the southern South China Sea. *PloS One, 14*(3), e0213864.

Azim, M. M., Amin, S. N., Mazumder, D., Arshad, A., Yusoff, F. M., & Saintilan, N. (2021). Feeding habits of five dominant fish species from Matang Mangrove Estuaries, Malaysia based on stomach contents and stable isotope analyses. *Bulletin of Marine Science*, *96*, 550-565.

Bachok, Z., Mansor, M. I., & Noordin, R. M. (2004). Diet composition and food habits of demersal and pelagic marine fishes from Terengganu waters, East Coast of Peninsular Malaysia. *NAGA, WorldFish Center Quarterly*, *27*, 41-47.

Bashir, Z., Abdullah, M. M., & Rusli, M. U. (2020). Comparisons between tissues, preservation, and desiccation methods on stable isotopes $\delta^{13}C$ and $\delta^{15}N$ of spot-tail sharks (*Carcharhinus sorrah*) from the South China Sea. *Turkish Journal of Fisheries and Aquatic Science, 20*, 659-664.

Burgess, K. B., Couturier, L. I., Marshall, A. D., Richardson, A. J., Weeks, S. J., & Bennett, M. B. (2016). Manta birostris, predator of the deep? Insight into the diet of the giant manta ray through stable isotope analysis. *Royal Society open science, 3*(11), 160717.

Carlisle, A. B., Kim, S. L., Semmens, B. X., Madigan, D. J., Jorgensen, S. J., Perle, C. R., Anderson, S. D., Chapple, T. K., Kanive, P. E., & Block, B. A. (2012). Using stable isotope analysis to understand the migration and trophic ecology of northeastern Pacific white sharks (*Carcharodon carcharias*). *PloS One, 7*(2), e30492.

Carscallen, W. M. A., Vandenberg, K., Lawson, J. M., Martinez, N. D., & Romanuk, T. N. (2012). Estimating trophic position in marine and estuarine food webs. *Ecosphere, 3*, 1-20.

Chin, A., Heupel, M. R., Simpfendorfer, C. A., & Tobin, A. J. (2013). Ontogenetic movements of juvenile blacktip reef sharks: Evidence of dispersal and connectivity between coastal habitats and coral reefs. *Aquatic Conservation: Marine and Freshwater Ecosystems, 23*, 468-474.

Christensen, D. R., & Moore, B. C. (2009). Using stable isotopes and a multiple-source mixing model to evaluate fish dietary niches in a mesotrophic lake. *Lake and Reservoir Management, 25*, 167-175.

Coppola, S.R., Fischer, W., Garibaldi, L., Scialabba, N., & Carpenter, K. E. (1994). SPECIESDAB: Global species database for fishery purposes. User's manual. *FAO Computerized Information Series (Fisheries)*, *9*.

Fischer, W., Sousa, I., Silva, C., de Freitas, A., Poutiers, J. M., Schneider, W., Borges, T. C., Feral, J. P., & Massinga, A. J. (1990). *Field guide to commercial marine and brackish water species of Mozambique.*

Fry, B. (2006). *Stable isotope ecology*. Springer.

Furumitsu, K., Wyffels, J. T., & Yamaguchi, A. (2019). Reproduction and embryonic development of the red stingray *Hemitrygon akajei* from Ariake Bay, Japan. *Ichthyological Research, 66*, 419-436.

Gladyshev, I. (2009). Stable isotope analyses in aquatic ecology (A review). *Journal of Siberian Federal University Biology, 2,* 381- 402.

Hobson, K. A., Sease, J. L., Merrick, R. L., & Piatt, J. F. (1997). Investigating trophic relationships of pinnipeds in Alaska and Washington using stable isotope ratios of nitrogen and carbon. *Marine Mammal Science*, *13*, 114-132.

Hussey, N. E., MacNeil, M. A., Olin, J. A., McMeans, B. C., Kinney, M. J., Chapman, D. D., & Fisk, A. T. (2012). Stable isotopes and elasmobranchs: Tissue types, methods, applications and assumptions. *Journal of Fish Biology*, *80*, 1449-1484.

Hyslop, E. J. (1980). Stomach contents analysis—a review of methods and their application. *Journal of Fish Biology, 17*, 411-429. https://doi.org/10.1111/j.1095-8649.1980.tb02775.x

Knip, D. M., Heupel, M. R., Simpfendorfer, C. A., Tobin, A. J., & Moloney, J. (2011). Ontogenetic shifts in movement and habitat use of juvenile pigeye sharks *Carcharhinus amboinensis* in a tropical nearshore region. *Marine Ecology Progress Series, 425,* 233-246.

Last, P. R., White, W. T., de Carvalho, M. R., Seret, B., Stehmann, M., & Naylor, G. (2016). *Rays of the world*. Australia: CSIRO Publishing.

Lim, A. P. K., Ahmad, A., Nor Azman, Z., & Noor, M. S. (2018). *Field guide to fishes and crustaceans of the Southeast Asian Region*. SEAFDEC/MFRDMD/39.

https://repository.seafdec.org.my/handle/20.500.12561/692

Lim, K.C., Chong, V.C., Lim, P-E., Yurimoto, T., & Loh, K.H. (2019). Feeding ecology of three sympatric species of stingrays on a tropical mudflat. *Journal of the Marine Biological Association of the United Kingdom, 99*, 999-1007.

Link, J. (2002). Does food web theory work for marine ecosystems? *Marine Ecology Progress Series, 230,* 1-9.

MacNeil, M. A., Skomal, G. B., & Fisk, A. T. (2005). Stable isotopes from multiple tissues reveal diet switching in sharks. *Marine Ecology Progress Series, 302*, 199-206.

Montoya, J. M., Pimm, S. L., & Solé, R. V. (2006). Ecological networks and their fragility. *Nature, 442*, 259-264.

Mundy, B. C. (2005). Checklist of the fishes of the Hawaiian Archipelago. *Bishop Museum Bulletin in Zoology, 6*, 1-704.

Munroe, T.A. (2001). *Cynoglossidae. Tonguesoles*. In K. E. Carpenter & V. Niem (Eds.), FAO species identification guide for fishery purposes. *The living marine resources of the Western Central Pacific*, *6*(4). (Labridae to Latimeriidae), estuarine crocodiles. FAO, Rome.

Musa, S., Czachur, M., & Sheils, H. (2018). Oviparous elasmobranch development inside the egg case in 7 key stages. *Plos One, 13*, 29.

Nadia, H. A., Rosdi, M. N., & Muhammad Amirullah, A. A. A. (2021). Species composition of sharks and rays caught in the east coast of Peninsular Malaysia. *Malaysian Fisheries Journal*, *20*, 50-63.

Nur Farhana, A., Samat, A., Zaidi, C. C., & Mazlan, A. G. (2013). Stomach content and trophic level position of two bamboo shark species *Chiloscyllium indicum* and *C. Hasseltii* (Hemiscylliidae) from South Eastern waters of Peninsular Malaysia. *Journal of Sustainability Science and Management*, *8*, 113-120.

Odum, E. P. (1953). *Fundamentals of ecology*. W. B. Saunders Co.

Peterson, B. J., & Fry, B. (1987). Stable isotopes in ecosystem studies. *Annual review of ecology and systematics*, *18*, 293-320.

Pretorius, C. A. (2012). *Factors influencing the development and mortality rate of shy and cat shark embryos in South African waters* [Master's thesis, University of Cape Town, South Africa].

Sasaki, K. (2001). *Sciaenidae: Croakers* (drums). In K. E. Carpenter and V. H. Niem (Eds.) *FAO species identification guide for fishery purposes. The living marine resources of the Western Central Pacific*. (Volume 5. Bony fishes part 3) (Menidae to Pomacentridae). FAO, Rome.

Smith, M., Warmolts, D., Thoney, D., Hueter, R., Murray, M., & Ezcurra, J. (2017). *Elasmobranch husbandry manual II: recent advances in the care of sharks, rays and their relatives*. Columbus, Ohio Biological Survey, 482.

Sommer, C., Schneider, W., & Poutiers, J. M. (1996). *FAO species identification field guide for fishery purposes. The living marine resources of Somalia*. FAO, Rome.

Syazwan, W. M., Then, A. Y. H., Chong, V. C., & Rizman-Idid, M. (2021). Trophic ecology of a tropical scyphozoan community in coastal waters: Insights from stomach content and stable isotope analyses. *Continental Shelf Research*, *225,* 104481.

Tavares, R., Rodriguez, J.P., & Morales, M. (2016). Nursery area and size structure of the lemon shark population, *Negaprion brevirostris* (Poey, 1868), in Los Roques Archipelago National Park, Venezuela. *Universitas Scientiarum, 21*, 33-52.

Thimdee, W., Deein, G., Sangrungruang, C., & Matsunaga, K. (2004). Analysis of primary food sources and trophic relationships of aquatic animals in a mangrove-fringed estuary, Khung Krabaen Bay (Thailand) using dual stable isotope techniques. *Wetlands Ecology and Management, 12*, 135-144.

Tullis, A., & Peterson, G. (2000). Growth and metabolism in the embryonic white-spotted bamboo shark, *Chiloscyllium plagiosum*: Comparison between embryonic birds and reptiles. *Physiological and Biochemical Zoology*, *73*, 271-282.

Wai, T. C., Yeung, J. W. Y., Lam, V. Y. Y., & Leung, K. M. Y. (2012). Monsoons and habitat influence trophic pathways and the importance of terrestrial-marine linkages for estuary sharks. *Ecosphere, 3,* 1-31.

Walton, J. (2020). *The effects of cyclic thermal stress on oviparous shark development* [Master's Thesis, Laurentian University, Sudbury].

Wetherbee, B. M., Gruber, S. H., & Rosa, R. S. (2007). Movement patterns of juvenile lemon sharks *Negaprion brevirostris* within Atol das Rocas, Brazil: A nursery characterized by tidal extremes. *Marine Ecology Progress Series, 343,* 283-293.

Yano, K., Ahmad, A., Gambang, A. C., Idris, A. H., Solahuddin, A. R., & Aznan, Z. (2005). *Sharks and rays of Malaysia and Brunei Darussalam.* SEAFDEC/MFRDMD/SP/12. Kuala Terengganu.

Zulkifli, S.Z., Mohamat-Yusuff, F., Mukhtar, A., & Ismail, A. (2014). Determination of food web in intertidal mudflat of tropical mangrove ecosystem using stable isotope markers: A preliminary study. *Life Science Journal, 11,* 427-43.