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EVALUATION OF FISH FEEDING HABITS AND TROPHIC POSITION IN BUKIT MERAH RESERVOIR, PERAK, MALAYSIA: PRELIMINARY ANALYSIS USING STABLE ISOTOPE ANALYSIS AND GUT CONTENT ANALYSIS

INDAHAYU AB RAHMAN[1], KAMINI SIVAJOTHY[1], AMIR SHAH RUDDIN MD SAH[2], ZARUL HAZRIN HASHIM[2], SYAHIDAH AKMAL MUHAMMAD[1,3,4], MOHD HARIZAL SENIK @ NAWI[5], ROGER DOLOROSA[6] AND WIDAD FADHULLAH[1,3]*

[1]Environmental Technology, School of Industrial Technology, Universiti Sains Malaysia, 11800 Gelugor, Pulau Pinang, Malaysia. [2]School of Biological Sciences, Universiti Sains Malaysia, 11800 Gelugor, Pulau Pinang, Malaysia. [3]Renewable Biomass Transformation Cluster, School of Industrial Technology, Universiti Sains Malaysia, 11800 Gelugor, Pulau Pinang, Malaysia. [4]Analytical Biochemistry Research Centre, Universiti Sains Malaysia, 11800 Gelugor, Pulau Pinang, Malaysia. [5]Department of Neurosciences, School of Medical Sciences, Health Campus, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia. [6]College of Fisheries and Aquatic Sciences, Western Philippines University-Puerto Princesa Campus, 5300 Palawan, Philippines.

**Corresponding author: widad@usm.my*

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Abstract: Monitoring feeding habits and trophic position (TP) is crucial for the timely response to ecological predicaments like agricultural intensification at upper runoffs of Bukit Merah Reservoir (BMR). Selected fish samples were evaluated for feeding habits and TP using stable isotopes analysis (SIA) ($\delta^{13}C$ and $\delta^{15}N$) and gut content analysis (GCA). Cyprinidae comprised 91% of the total samples. No significant differences in δ13C among fish feeding habits ($p > 0.05$) showed that fish consumed similar basal carbon sources derived from C3 photosynthesis pathways. Significant differences were found between $\delta^{15}N$ values and the interactive effect of fish feeding habits ($p < 0.05$) with carnivorous fish recording the highest median score (*Md* = 2.62). *Hampala macrolepidota* (TP = 2.73) and *Mytus singaringan* (TP = 2.50) occupied the highest position in the food web. Detritus was the main food source for BMR fish (44%), showing a high degree of omnivory. The gut content of *Barbonymous gonionotus* and *Larbiobabus leptopcheilus* was the most diverse, indicating a wide range of food consumption. Fish samples that inhabit the BMR food web are favoured as detritivores or omnivores. This study provides the baseline data on the fish diet composition and their TP, which will be useful for future modelling uses in BMR fish management and conservation.

Keywords: Stable isotope signatures, freshwater ecosystem, food items, trophic level, tropical freshwater.

Introduction

Dietary interaction studies among the coexisting fish in the waterbody like lakes, reservoirs, and rivers can reveal the different niche dimensions in the community. Studies on diet composition are vital in community ecology as organisms' use of resources significantly influences population interaction (Cheka *et al.*, 2020). Plant matter like leaf litter and algal biofilms in upper reaches attached to the stony substrate are major food sources for aquatic macro and microorganisms in the waterbody. The upper, middle, and lower streams are the most important freshwater resources that support the reservoir's biological productivity and species richness.

An accurate description of a fish species' diet and feeding habits is essential to understanding the trophic positions in aquatic food webs. Trophic position (TP) is an important parameter in the characterisation of food webs as it can provide a quantitative measure of the species' energetic interaction and has been a widely used descriptor of the role of different species in freshwater food webs (Carscallen *et al.*, 2012; Abdullah *et al.*, 2023). Abdullah *et al.* (2023)

also added that TP in the food web can be used to interpret the resilience of the ecosystem and provide insights into potential anthropogenic effects on freshwater ecosystems. Failure to recognise and address such trophic interactions early may lead to disruptions in the fish food web.

Traditionally, to determine the trophic interaction and feeding habits, most studies have relied upon gut content analysis (GCA) to identify fish food items in any food web structure (Hyslop, 1980; Hanjavanit & Sangpradub, 2012; Baker *et al.*, 2013; Costa & Angelini, 2020). GCA is a useful standard tool for identifying fish feeding ecology, trophic interactions, food web structures, and seasonal variations. This tool can describe taxonomic information about the food items ingested over a short period (Davis *et al.*, 2012; Baker *et al*., 2013; Zacharia, 2014).

On the other hand, stable isotope analysis (SIA) of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) is a complementary approach to identify assimilated diet fractions over an extended timeframe. Valuable diet sources and trophic structure information are commonly integrated into these stable isotopic values of consumers, respectively (Peterson & Fry, 1987; Post, 2002). The stable isotope compositions of organic matter may provide insight into the source sinks of nitrogen and carbon in biota that interact with their physical and chemical environments (Peterson & Fry, 1987; Hong & Gu, 2020) nitrogen stable isotopes (δ15N. Carbon ($\delta^{13}C$) isotopic compositions can give information about the carbon sources of production and energy flow through the food web as ^{13}C is the heavier isotope than ^{12}C, which acts as tracers of ecological processes (Zulkifli *et al*., 2014; Yap *et al*., 2016).

Meanwhile, nitrogen ($\delta^{15}N$) isotopic compositions are useful in providing information about the trophic position in a food web, whereas any animal with the highest concentration of this stable isotope becomes enriched in $\delta^{15}N$ relative to their food with top predators (Yap *et al.*, 2016; Rosli *et al.*, 2017). The availability of resources influences the TP of consumers. It helps to describe the relative positions of the ecosystem's population, species, or functional group (Janjua & Gerdeaux, 2011). In lentic systems like lakes, ponds, and reservoirs for example, if the food quality of algae declines following several factors such as a switch from the uptake of nitrate to nitrogen fixation, this may lead to a negative correlation in both the $\delta^{15}N$ and abundance of the zooplankton community (Matthews & Mazumder, 2005). Without stable isotope analysis as the standard tool in environmental sciences, understanding nutrient cycling in terrestrial and marine systems and tracing pollution sources would be complicated and challenging to implement appropriate management, conservation, and restoration efforts (Yap *et al.*, 2016).

A key challenge in providing robust predictions for developing ecosystem models that apply to sustainable management is accurately representing the structure and dynamics of fish food webs. At present, the use of GCA and SIA approaches largely links to consumers feeding behaviour and trophic interactions (Tripp-Valdez & Arreguin-Sanchez, 2009; Flinders, 2012; Wang *et al.*, 2018; Uğurlu *et al.*, 2020; Choi *et al.*, 2021). These approaches can be explored as tools to evaluate the comparison of modelling food web attributes with the measure of stable isotope composition of taxa (McCormack *et al.*, 2019). Thus, this study applies both approaches to study the feeding behaviours and trophic interactions in one of the tropical shallow reservoirs in Malaysia, Bukit Merah Reservoir (BMR).

BMR is exposed to dynamic changes between terrestrial and aquatic induced sources as agricultural expansion and intensification at upper runoffs become human-predominant drivers that may disrupt the reservoir's purposes in providing water supplies to agricultural, domestic, industrial, and sources of income for the local fisherman. Enrichment of surface water through runoff and leaching of nutrients from agricultural lands is considered to have major implications for the trophic transfer in fish food webs (DerLee *et al.*, 2021). Multiple research was conducted at BMR focusing mainly on fish diversity (Mohd Fadzil *et al.*, 2016; Zakeyuddin *et al.*, 2016; Syaiful Mohammad *et al.*, 2018; Jaya-Ram *et al.*, 2018). Scarcely, there is limited information regarding fish feeding habits in BMR (Yap, 1988). Not many studies have integrated simultaneous GCA and SIA approaches of different fish species from tropical freshwaters (Lugendo *et al.*, 2006; Harrod & Grey, 2006; Janjua & Gerdeaux, 2011; McClain-Counts *et al.*, 2017; Choi *et al.*, 2021). Therefore, this study will be the first study in this country to highlight isotopic signature values for several freshwater fish species listed as the least concerning in the IUCN Red List. Knowing these can provide a useful complementary understanding of effective fisheries management by emphasising the specific feeding habits in this tropical reservoir, BMR.

On this basis, a combination of GCA and SIA data was analysed to portray better fish feeding habits and TP of fish in BMR. Therefore, the aims of this study were (i) to characterise the fish feeding habit using a combination of GCA and SIA ($\delta^{13}C$ and $\delta^{15}N$) and (ii) to determine the TP of fish samples in BMR. Combining both approaches may present important information in assessing the changes in fish food webs, which is essential in fish conservation management (Yap *et al.*, 2016; Mustakim *et al.*, 2020).

Materials and Methods

Study Site

Bukit Merah Reservoir, BMR (Figure 1) is the oldest reservoir in Malaysia (100°39'14.7" E and 5°01'06.8" N), being built in 1902 and completely operated in 1906 (Ismail & Najib, 2011) with three major tributaries flowing into it; Kurau River, which has the largest catchment area of 323 km^2, Jelutong River (7.1 km^2 catchment area), and Merah River (4.25 km^2 catchment area). This reservoir was formed by constructing a dam in the middle of the Kurau River. Water from the reservoir is channelled out by gravity flow through six gates to two outlet canals, the Selinsing Canal and Main Canal, for double cropping of 24,000 ha paddy irrigation.

With a capacity of 70 million m^3 at 28.50 feet (Yaccob, 2019), it serves as a multifunctional reservoir by providing water for irrigation, recreational fishing activities, and water supply (Zakeyuddin *et al.*, 2016). BMR is a multipurpose reservoir as it also serves as a tourism attraction spot through the water theme park (Bukit Merah Reservoir Laketown Resort) and water source used for Arowana fish culture in the downstream area after the outlet canals (Fadhullah *et al.,* 2020).

Samples Collection

Fish were purchased from the local fishermen of BMR immediately after being captured by gillnets with standard mesh size (3.81 cm) over two different years between March 2019 and 2022. The nets were set in the afternoon (about 5:00 p.m.) and lifted in the following morning (about 8:00 a.m.). No sampling was carried out in 2020 and 2021 due to COVID-19 movement restriction orders by the government of Malaysia (Tang, 2022). Only similar fish species in March 2019 were selected for March 2022. All individuals of selected fish were immediately kept in a cool box (Bashir *et al.,* 2020). Fish identification up to the species level was carried out in the field according to the description represented by the web database FishBase (Froese & Pauly, 2023) and Fish of Malaysia (2nd edition) (Ambak *et al*., 2012). Fish species were selected in such a way that they represented commercially important fish species found abundantly in BMR.

Laboratory Analysis

Fish Fillet Collection and Preparation for SIA

For fish samples with standard length (SL) measurement > 4 cm, a fillet of dorsal muscle (Lugendo *et al.*, 2006) was dissected and collected for SIA as this tissue typically has lower lipid and inorganic carbonate content than other tissues and yields lower variability in $\delta^{15}N$ and $\delta^{13}C$ values (Pinnegar & Polunin, 1999). The soft muscle of fish reflects the isotopic signature of their diet and estimates the assimilation of alternative basal carbon sources

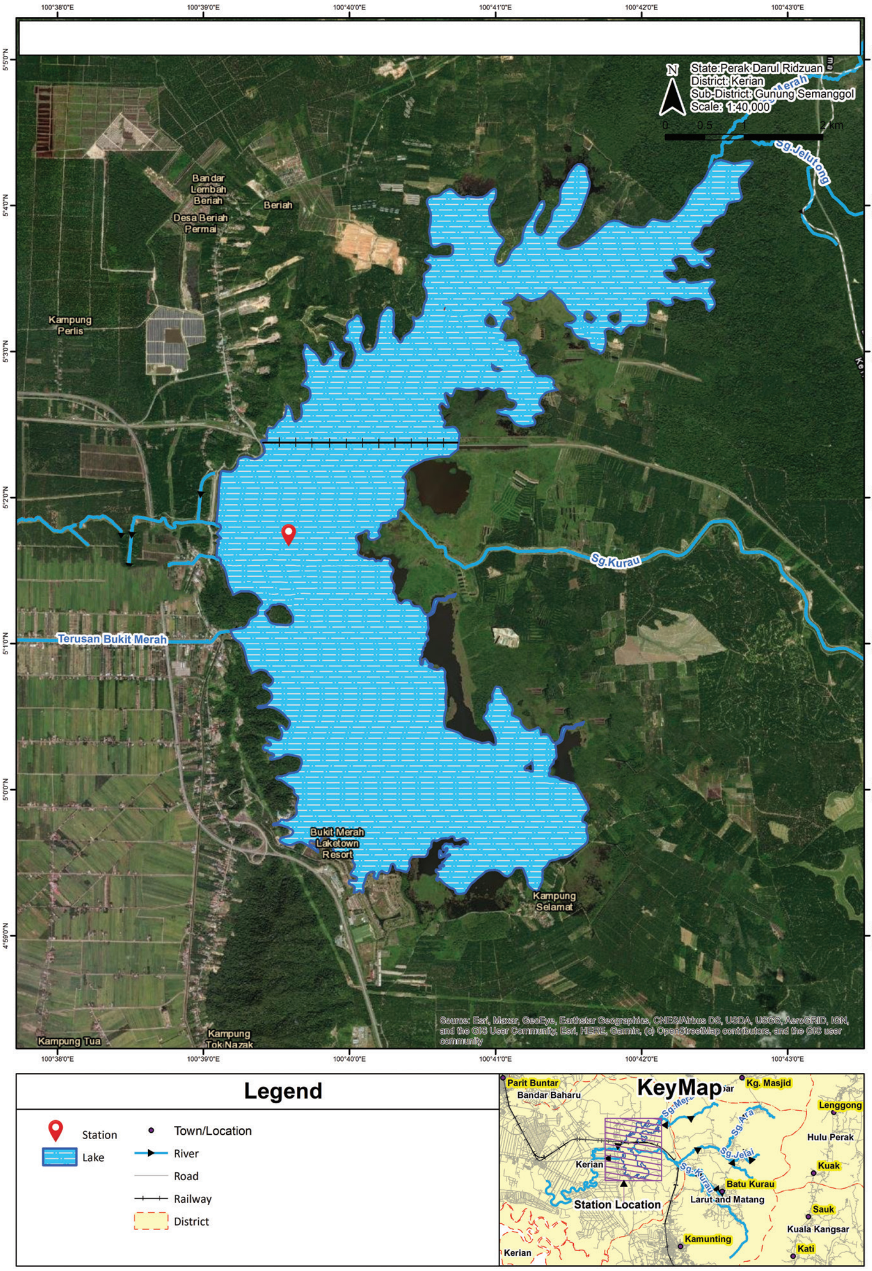

Figure 1: Bukit Merah Reservoir (BMR), the study area showing the main river inlet, the Kurau River. The pinned point in the middle of the reservoir represents the gill net placement for the fish trap

(Kaymak *et al*., 2015). Meanwhile, for fish < 4 cm SL, the whole fish, including head, tail, and viscera were used for analysis. These samples were collected in some number and generally pooled to create a representative composite sample where possible. After that, the collected fillets were dried in an oven for dehydration at 60ºC for 48 hour before grinding and keeping in the desiccator until further analysis.

SIA ($\delta^{13}C$ and $\delta^{15}N$) were performed at the Analytical Biochemistry Research Centre (ABrC), Universiti Sains Malaysia. Powdered fish fillets (~0.4 mg to 0.5 mg) were weighed in duplicates into clean tin capsules (8 x 5 mm) using ultra-microbalance (Metler Toledo, Polaris Parkway, Colombus) scaled to the nearest 1 x 10^{-7} g. Then, the pressed and folded tin capsules were analysed using Flash EA 2,000 elemental analyser (Thermo Science, Waltham, MA) coupled to a Delta V Advantage isotope ratio mass spectrometer (Thermo, Milan, Italy).

Raw isotope ratios from the analysis were then normalised to international scales. USGS-40 and USGS-41 reference materials (~0.5 mg) were assayed with the unknown samples. In addition, urea (IVA-Analysentechnik GmbH & Co., Germany) was used as a quality control material to account for drift and was measured for every sample with known values of $\delta^{13}C$ (–40.81‰) and $\delta^{15}N$ (–0.49‰).

Gut Sample Collection and Preparation for GCA

The standard length (SL) was measured from the anterior mouth part to the end of the caudal peduncle, while the total length (TL) from the tip of the head to the tip of the caudal fin rays. Then, the wet weight (W) of the fish samples was measured using an electronic scale and averagely calculated before dissection to obtain the gut and intestine straightway in the field. The method used for GCA is suggested by Hanjavanit and Sangpradub (2012), Yamagishi *et al.* (2005), and Zakeyuddin *et al.* (2017). The gut length (GL) together with gut-weight (w) were measured and weighed (g) together with its content by using a ruler and an electronic weighing scale (0.1 g accuracy), accordingly, before preserving them in ethanol until further analysis (Choi *et al.*, 2021).

The preserved specimens were observed in a petri dish to visually classify and estimate the value of fullness of the guts and intestine (Tippets & Moyle, 1978; Hyslop, 1980; Flinders, 2012). Scales 0 to 3 were used to indicate the level of fullness; 0 (empty), 1 (full < 25%), 2 (25% > full < 75%), and 3 (full > 75%) (Sajeevan & Kurup, 2013). The stomach samples having > 25% full were examined for food item contribution using the indirect volumetric method of Hyslop (1980) and Zakeyuddin *et al.* (2017) with some modifications. The food items were observed under a dissecting microscope (Olympus SZ51, Binocular Zoom Stereo Microscope). The contribution of food items was categorised into four (detritus, algae, plant matter, and animal) and assigned percentages based on visual observation.

Data Analysis

Analysis of Fish Stable Isotopic Signatures

In this preliminary analysis, the SIA and GCA data of year-wise samples (2019 and 2022) were presented in pooled data due to the scarcity of fish samples for statistical analysis. The $\delta^{13}C$ and $\delta^{15}N$ values were reported as the relative difference between ratios of a sample and standards. The typical precision for the duplicated samples was ± 0.3‰ for $\delta^{13}C$ and ± 0.2‰ for $\delta^{15}N$. The standards used in this study were internationally accepted standards; V-PDB (Vienna-PeeDee Belemnite) for carbon (Perkins *et al.*, 2014) and air (atmospheric nitrogen) for nitrogen (Vander Zanden & Rasmussen, 1999). From the SIA laboratory analysis, the value obtained was the ratio of $^{13}C/^{12}C$ and $^{15}N/^{14}N$ in the sample. To get the value of $\delta^{13}C$ and $\delta^{15}N$, the sample ratio was then calculated by using the formula:

$$\delta\ (‰) = \left[\left(\frac{R_{sample}}{R_{standard}}\right) - 1\right] x\ 1000$$

where:

R = Isotope ratio of heavy to light ($^{13}C/^{12}C$ or $^{15}N/^{14}N$)

δ = Isotopic composition ($\delta^{13}C$ or $\delta^{15}N$) in per mille (‰) deviation of samples based on internationally accepted standard materials

For the TP analysis of fish samples, $\delta^{15}N$ values were used as following the method proposed in Cabana and Rasmussen's (1996) study:

$$Trophic\ Position\ (TP) = [(\delta^{15}N_{mean\ consumer} - \delta^{15}N_{ref}) / 3.4] + \lambda$$

where:

$\delta^{15}N_{mean\ consumer}$ = $\delta^{15}N$ of the consumer whose TP is estimated here

$\delta^{15}N_{ref}$ = $\delta^{15}N$ of the primary consumer was calculated as the mean collected during the sampling event

3.4 = $\delta^{15}N$ expected isotopic discrimination per trophic level

λ = the trophic level of the baseline indicator, set at two since we used a primary consumer baseline (primary producers-trophic level 1, primary consumers-trophic level 2, and so on) (Post, 2002)

For this preliminary analysis, shrimp was used as the baseline organism ($\delta^{15}N_{ref}$) to measure trophic levels as this organism can help estimate the littoral and pelagic bases (Post, 2002). The study calculated $\delta^{15}N_{ref}$ from primary consumers to make baseline corrections because a suitable ubiquitous and abundant taxon was lacking (Rybczynski *et al.*, 2008). Besides, primary consumers provide the best baseline for estimating trophic position using $\delta^{15}N$ values as they integrate temporal and spatial variation in isotopic signatures of basal resources. Trophic value range between two (herbivores/detritivores) to five (piscivores or carnivores) (Pauly *et al.*, 1998; Hashim *et al.*, 2017) and were cross-checked with data from gut content analysis and existing literature.

The extent of fish samples' isotopic signatures across the family groups, the interactive effect of feeding habits and TP result were calculated using non-parametric ANOVA analysis (Kruskal-Wallis Test, *H*) (Pallant, 2011) as the data set fell under abnormality after being examined by using the Shapiro-Wilk statistic test (Le Loc'h & Hily, 2005; Hamid *et al.*, 2015; Qin *et al.*, 2021). This test was applied to compare the median score for the derivation of isotopic signatures in the TP calculation of different fish species (by family group) collected and the variation of their feeding habits in BMR. All analyses were performed using the software SPSS version 27 using $p < 0.05$ as a significant criterion.

Analysis of Fish Gut Content

The fish feeding habit was calculated using the frequency of occurrence of food items (FOFI) in the following categories; detritus, algae, plant matter, and animal (Hyslop, 1980; Zacharia, 2014):

$$\text{FOFI (\%)} = \frac{Total\ number\ of\ fish\ containing\ prey\ (i)}{Total\ number\ of\ fish\ with\ food\ in\ their\ stomach}\ X\ 100$$

This formula can demonstrate what the fish fed on as the food items were examined, identified, and sorted as suggested by Cheka *et al.* (2020). The number of occurrences of food items found in the stomach is expressed as a percentage of the total number of identified stomachs.

For a better understanding of fish species feeding behaviour in different gut lengths, the relative gut length (RGL) (Montgomery, 1977; Hamid *et al.*, 2015) was calculated as follows:

$$RGL = \frac{Gut\ length\ (cm)}{Standard\ length\ (cm)}$$

RGL is used to obtain information on fish diets, as herbivores need longer guts (> 3 cm) for digestion. In comparison, mainly carnivorous fish have relatively much shorter (< 1 cm) guts than omnivorous fish (1 to 3 cm RGL) (Rapita *et al.*, 2021). The value of RGL can be considered low in smaller fish and tends to increase as the fish gain weight until they reach the maximum size.

Results and Discussions

The differences in fish assemblages and distribution depend on food availability, waterbody structures, land use surrounding the catchment area, physical and morphological adaption of fishes to the environment (Vieira & Tejerina-Garro, 2020), and complexity of habitat (Syaiful Mohammad *et al.*, 2018; Devine *et al.*, 2020). Dominant fish families obtained in both years were Cyprinidae (91%), followed by Bagridae and Pristolepididae, five per cent each (Table 1). Previous studies in BMR also recorded Cyprinidae as the dominant family ranging from 38% to 60% (Mohd Shafiq *et al.*, 2014; Mohd Fadzil *et al.*, 2016; Syaiful Mohammad *et al.*, 2018). Our findings are consistent with the recorded fish family assemblage commonly recorded in freshwater tropical regions (Beamish *et al.*, 2006; Muchlisin & Siti-Azizah, 2009; Tran & Thuy, 2014; Ridzuan *et al.*, 2017; Nyanti *et al.*, 2021). Apart from fish, shrimps (n = 2) were also collected in BMR as baseline correction since they are primary consumers, showing a slightly wide range of isotopic signatures, having depleted $\delta^{13}C$ (-30.4‰ to -29.3‰) and enriched $\delta^{15}N$ (7.2‰ to 8.2‰) in both years, accordingly.

Overall, $\delta^{13}C$ values among all fish species ranged from -33.1 ± 2.4‰ to -27.5 ± 0.4‰, of which *Cyclocheilichthys apagon* expressed the highest $\delta^{13}C$ depletion (range from -34.8‰ to -31.4‰), and *Hampala macrolepidota* showed the highest $\delta^{13}C$ enrichment (range from -27.8‰ to -27.3‰). However, two fish from the same genus, *Barbonymus gonionotus* and *Barbonymus schwanenfeldii* recorded differences in the average $\delta^{13}C$ values, indicating that the $\delta^{13}C$ values varied widely among species.

The narrow range in data obtained for the $\delta^{13}C$ values in fishes reflects the carbon source in the diet of these fish species, which can be derived or assimilated from the primary producers or consumers. As reported by Garcia *et al.* (2007) and Li *et al.* (2006), C3 and C4 freshwater plants have $\delta^{13}C$ values ranging

Table 1: Range of carbon and nitrogen stable isotope values (mean ± standard deviation) of collected fish species in BMR during March 2019 and 2022

Family	**Species**	**N**	**$\delta^{13}C$ (‰)**		**$\delta^{15}N$ (‰)**	
			Range	**Mean ± SD**	**Range**	**Mean ± SD**
	Shrimp	2	-30.4 to -29.3	-29.8 ± 0.8	7.2 to 8.2	7.7 ± 0.7
Bagridae	*Mystus singaringan*	2	-33.7 to -30.7	-32.2 ± 2.1	8.5 to 10.3	9.4 ± 1.2
Cyprinidae	*Barbonymus gonionotus*	13	-33.1 to -23.8	-28.4 ± 6.6	6.3 to 7.5	6.9 ± 0.9
	Barbonymus schwanenfeldii	2	-29.7 to -28.9	-29.3 ± 0.6	7.4 to 9.1	8.2 ± 1.2
	Cyclocheilichthys apogon	7	-34.8 to -31.4	-33.1 ± 2.4	5.6 to 6.9	6.3 ± 0.9
	Hampala macrolepidota	2	-27.8 to -27.3	-27.5 ± 0.4	10.0 to 10.4	10.2 ± 0.3
	Labiobarbus leptocheilus	10	-33.3 to -32.6	-33.0 ± 0.5	3.9 to 5.1	4.5 ± 0.9
	Osteochilus vittatus	7	-33.6 to -29.6	-31.5 ± 2.0	5.1 to 7.4	6.7 ± 1.3
Pristolepididae	*Pristolepis fasciatus*	2	-30.9 to -29.4	-30.2 ± 1.1	7.8 to 9.5	8.6 ± 1.2

from -26.1‰ to -30.4‰ and -10.8‰ to -17.0‰, respectively. If it is more enriched, the fish consumes something with a lesser negative value, while a fish with the most depleted value consumes something more negative. Kruskal-Wallis analysis revealed no significant difference in $\delta^{13}C$ values among the fish feeding habits, $p > 0.05$. Therefore, the observed $\delta^{13}C$ values in the fishes in both years are derived from C3 plants of the reservoir. This reservoir has a large number of macrophytes especially C3 vegetation compared to C4 vegetation such as submerged species (*Cabomba furcate* and *Cabomba caroliniana*) (Sharip *et al.*, 2018) and riparian species (*Hanguana malayana*) (Zakeyuddin *et al.*, 2016) due to the nutrient fluxes into lake systems (Ismail & Najib, 2011).

Generally, the fish samples showed a wide range of $\delta^{15}N$ values starting from 4.5 ± 0.9‰ to 10.2 ± 0.3‰ in BMR. This wide range shows an enrichment in $\delta^{15}N$ during the study period. The result in Table 1 also showed that *Labiobarbus leptocheilus* (4.5 ± 0.9‰) was the most $\delta^{15}N$ depleted; meanwhile, the most enriched $\delta^{15}N$ values were found in *H. macrolepidota* (10.2 ± 0.3‰). From the Kruskal-Wallis analysis, the $\delta^{15}N$ values of fish family groups showed significant differences toward the interactive effect of fish feeding habits ($p < 0.05$).

Comparison between the values of $\delta^{15}N$ for *H. macrolepidota* in this study and the other tropical lentic freshwater (Temenggor Lake) in the same country, which was conducted by Yap *et al.* (2016) indicates that this species at BMR has more enriched $\delta^{15}N$ values than at Temenggor Lake. They suggested different food sources would result in different TPs of this species in these two lakes. Similar fish species may have different isotopic compositions depending on their habitat, feeding behavioural, and ecological requirements (Middelburg, 2014; Carvalho *et al.*, 2015; Rossi *et al.*, 2015; Ridzuan *et al.*, 2017; Zorica *et al.*, 2021).

Based on Table 2, there are two trophic levels in this study, and it is comprised of three feeding guilds: Herbivores, omnivores, and carnivores. The average TP values of all fish species ranged from 1.05 to 2.73 throughout both years. Two fish species, *C. apagon* and *O. vittatus* are consistent with recorded TP < 2 values and literature data (Table 3), suggesting they are herbivorous feeding guilds in the BMR. Across all fish assemblages, the TP value range is considered low and moderate trophic in fish caught in BMR. The study findings showed that the TP values of top species in the respective years; *H. macrolepidota* (TP = 2.73 ± 0.28) and *M. singaringan* (TP = 2.50 ± 0.17) were different as in Cipeles River (Herawati *et al.*, 2022), whereas *H. macrolepidota* was recorded lower (TP = 2.13) and *M. singaringan* was found higher (2.85) when compared to these species.

The depletion range of $\delta^{15}N$ values of *H. macrolepidota* (Table 1) during the study period (become less positive) does not affect its TP in BMR. Besides, the second most enriched

Table 2: Trophic position (TP) of collected fish species in BMR

Family	Species	Trophic Position	Feeding Habit Groups
Bagridae	*Mystus singaringan*	2.50 ± 0.17	Carnivores
Cyprinidae	*Barbonymus gonionotus*	1.76 ± 0.06	Herbivores
	Barbonymus schwanenfeldii	2.15 ± 0.16	Omnivores
	Cyclocheilichthys apogon	1.58 ± 0.08	Herbivores
	Hampala macrolepidota	2.73 ± 0.28	Carnivores
	Labiobarbus leptocheilus	1.05 ± 0.46	Detritivores
	Osteochilus vittatus	1.70 ± 0.09	Herbivores
Pristolepididae	*Pristolepis fasciatus*	2.27 ± 0.17	Omnivores

Table 3: Feeding habits of the collected fish species based on the literature-based data

Family	Species	Feeding Habits	Diet	References
Bagridae	*Mystus singaringan*	Carnivore	Animal fraction, crustaceans, insects	Rao, (2017); Herawati *et al*. (2022)
Cyprinidae	*Barbonymus gonionotus*	Omnivore/herbivore	Algae, animal fraction, plant matter	Siaw-Yang, (1988); Kurnia *et al.* (2017); Ain *et al.* (2021); Saowakoon *et al.* (2021)
	Barbonymus schwanenfeldii	Omnivore/herbivore/detritivore	Algae, animal fraction, plant matter, insects, detritus	Siaw-Yang, (1988); Mustafa-Kamal *et al.* (2012); Saowakoon *et al.* (2021); Desrita *et al.* (2021); Nyanti *et al*. (2021)
	Cyclocheilichthys apogon	Detritivore/omnivore	Algae, oligochaeta, plant matter, detritus, crustaceans	Siaw-Yang, (1988); Hamid *et al.* (2015)
	Hampala macrolepidota	Carnivore/omnivore	Animal fraction, algae, plant matter, detritus, crustaceans	Siaw-Yang, (1988); Herawati *et al*. (2022); Rao, (2017); Mustafa-Kamal *et al*. (2012); Nyanti *et al*. (2021)
	Labiobarbus leptocheilus	Detritivore/herbivore	Detritus, algae, plant matter	Ridho *et al.* (2017)
	Osteochilus vittatus	Herbivore	Algae, macrophyte, crustaceans, detritus	Kaban *et al*. (2019); Natkritta *et al*. (2020); Mohd Rosli *et al.* (2021)
Pristolepididae	*Pristolepis fasciatus*	Omnivore	Insect, algae, animal fraction, detritus	Siaw-Yang, (1988); Mustafa-Kamal *et al.* (2012); Saba *et al.* (2021)

(most positive) δ^{15}N value, *Mystus singaringan*, suggested this species might occupy the same trophic level as *H. macrolepidota.* Moreover, this depletion of δ^{15}N values of *H. macrolepidota* can be suspected that this fish is shifting from a carnivore fish but tend to be an omnivore fish (Susilo *et al.*, 2021) as they might have relied on different trophic resources (Davis *et al.*, 2012). Significant differences (Kruskal-Wallis analysis, *H*) were found between TP and fish feeding habits (H = 10.595, p = 0.005). Carnivorous fishes recorded the highest median score (*Md* = 2.62) than the other two groups, herbivores (*Md* = 1.61) and omnivores (*Md* = 1.76), respectively.

Dietary information regarding fish was retrieved from various sources as shown in Table 3, mainly from the original literature to complement the findings of this study and to aid in interpreting the results of stable isotope analysis. The literature provides useful information regarding the variability in the feeding behaviour of tropical freshwater species. Dietary data highlight the importance of differences in the diets of some species between distinct tropical areas. Yet, it cannot provide an insight into the specific diets of local species in a particular area such as this study in, BMR. Thus, this study recognised the importance of evaluating TP patterns of local species in specific areas rather than relying solely on the data provided.

Food is the main energy source and plays an important role in determining the growth rate and condition of fish. GCA results such as fish body and gut measurement (weight and length) and fullness index (IF) were obtained from both years and presented in Table 4. Only fish species of *H. macrolepidota* could not do GCA due to their small size, which caused difficulties in identifying their digestive tracts. Generally, most fish species were small in size ranging from W = 70.50 g to 27.0 g with 17.10 cm to 8.5 cm SL, respectively. The *GW* among specimens was considered Δ 2.39 g (2.65 g ± 1.8 to 0.26 g ± 0.0). *L. leptocheilus* (177.9 cm) and *O. vittatus* (131.40 cm) are exclusively associated with herbivory as they recorded the longest *GL.* The long gut is thus adapted for herbivory and related to a diet mainly of plant materials (Duque-Correa *et al.*, 2024).

Meanwhile, Figure 2 portrayed those four species (*B. gonionotus*, *P. fasciatus*, *O. vittatus,* and *L. leptocheilus*) were intermediate between herbivory and omnivory because of the RGL (Figure 2) values ranging from 4.70 cm to 12.38 cm. These fish use their long gut to assimilate the non-protein amino acid encased in fibrous cell walls like detritus, digested algae, and plant materials (German & Horn, 2006). Only *M. singaringan* (0.93 cm) had < 1 cm RGL, suggesting this fish species was a carnivore. Their RGL values aligned with the mean measurement of *GL* as they had among the shortest mean total gut length for each, 14.0 cm and 7.50 cm respectively.

In this study, the feeding intensity of all fish species had been achieved at least 25% of the fullness index. *M. singaringan* (family Bagridae) and *L. leptocheilus* (family Cyprinidae) had the highest mean relative IF (value = 3). This indicates that most fishes were found in either actively or moderately fed states (Sajeevan & Kurup, 2013). The variety of food habits by fish can be categorised into a monophagous group (fish whose diet consists of one item of food), stenophagous (several items of food) and eurifagous (variety of food items) (Rahardjo *et al.*, 2011). Referring to Figure 3, the fish species in this study can be classified as a monophagous group (consisting of *M. singaringan* and *B. schwanenfeldii*) and a stenophagous group (consisting of *B. gonionotus*, *C. apagagon*, and *L. leptocheilus*). In short, The GCA data were in line with the available previous studies performed on similar fish species as shown in Table 4.

Detritus was likely important for most of this lentic freshwater fish and corroborated with FOFI results (Figure 3) and previous literature database (Table 3). The observed diet in all fish species by GCA can successfully reflect the true situation as the SIA result suggested. According to the interpretation results between GCA and SIA, 45.4% of total fish samples showed a high

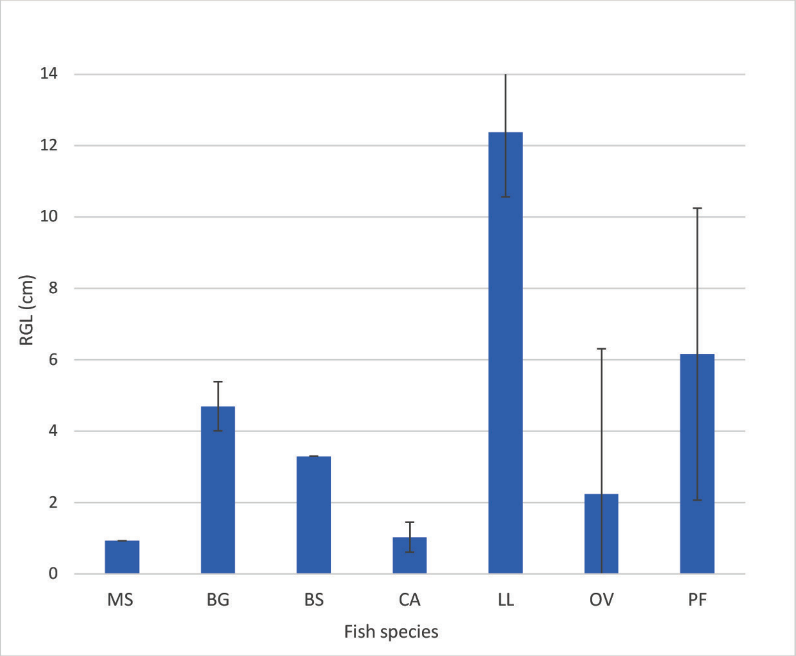

Figure 2: Mean ± standard deviation of relative gut length (RGL) for each fish species; *M. singaringan* (MS), *B. gonionotus* (BG), *B. schwanenfeldii* (BS), *C. apagon* (CA), *L. leptocheilus* (LL), *O. vittatus* (OV), and *P. fasciatus* (PF) in BMR

Table 4: Mean ± standard deviation of GCA information from fish samples collected in BMR

Family	Species	W (g)	*GW* (g)	SL (cm)	*GL* (cm)	IF
Bagridae	*Mystus singaringan*	52.00 ± 0.0	2.10 ± 0.0	15.00 ± 0.0	14.00 ± 0.0	3 ± 0.0
Cyprinidae	*Barbonymus gonionotus*	42.75 ± 6.5	1.33 ± 0.7	11.67 ± 0.7	55.00 ± 7.4	2 ± 1.0
	Barbonymus schwanenfeldii	45.00 ± 0.0	0.52 ± 0.0	11.50 ± 0.0	38.00 ± 0.0	1 ± 0.0
	Cyclocheilichthys apogon	39.67 ± 12.2	0.26 ± 0.0	11.32 ± 1.0	11.58 ± 4.6	2 ± 0.5
	Hampala macrolepidota	nil	nil	nil	nil	nil
	Labiobarbus leptocheilus	70.50 ± 19.5	2.65 ± 1.8	14.45 ± 2.1	177.90 ± 29.5	3 ± 0.5
	Osteochilus vittatus	50.14 ± 12.4	2.24 ± 1.5	12.40 ± 1.1	131.4 ± 55.1	2 ± 0.8
Pristolepididae	*Pristolepis fasciatus*	38.5 ± 27.6	1.17 ± 0.9	9.45 ± 1.3	55.5 ± 30.4	2 ± 1.4

W: weight of species; *GW*: gut and intestine weight; SL: Standard length; TL: Total length; *GL*: gut and intestine length; Index of fullness: 0 (empty), 1 (full < 25%), 2 (25% > full < 75%), and 3 (full > 75%); *nil: no data obtained due to small fish size degree of omnivory feeding guild due to RGL > 3 cm. Coat *et al.* (2009) explained that the high degree of omnivory feeding behaviour is because of freshwater tropical food webs as a detritus-based system. This finding supports the statement by Yap (1988) as he stated that due to biogeographic and trophic dynamics at BMR, this factor leads to the preference for detritivore fish over planktivorous. Besides, most of them were found to be in the Cyprinidae family group as they have a high degree of plasticity and inhabit a broad range of environments due to their adaptive stomach, genetic modification, and intestine adaption (Beamish *et al.*, 2006).

One exception is the species of *C. apagon*, which recorded RGL = 1.03 cm (Figure 2), among the shortest in the Cyprinidae family. The study finding was consistent with the previous study (Hamid *et al.*, 2015) as they described *C. apagon* as omnivorous and the stomach content consisted of oligochaeta, Chironomidae, and detritus. This species together with the other species (*B. gonionotus,* and *L. leptopcheilus*) also had the most diverse food items observed during analysis, indicating that they have wider feeding habits (Nyanti *et al.*, 2021) which eventually could explain the higher distribution and abundance in BMR. They can utilise diverse kinds of food, making them natural omnivores.

Figure 3 summarises the information on food items fed by each fish species. From the total guts collected, 66.7% digested more than three food items, while 33.3% were less than three or empty food items. Among the four food items, detritus (44%) was found to be the most abundant in the stomach and intestine of the fish. Algae (30%) was the second highest food item including phytoplankton, filamentous algae, and seston with a median quantity of 61.8% represented in the gut of five fish species counted (*B.gonionotus, C. apagon, L. leptocheilus, O. vittatus,* and *P. fasciatus*). They also ingested almost predominantly detritus as shown below (Figure 3).

Animal fraction was the least abundant food item (14%). However, the diets of *M. singaringan* mainly involved animal matters such as small fish and insects. On the other hand, the

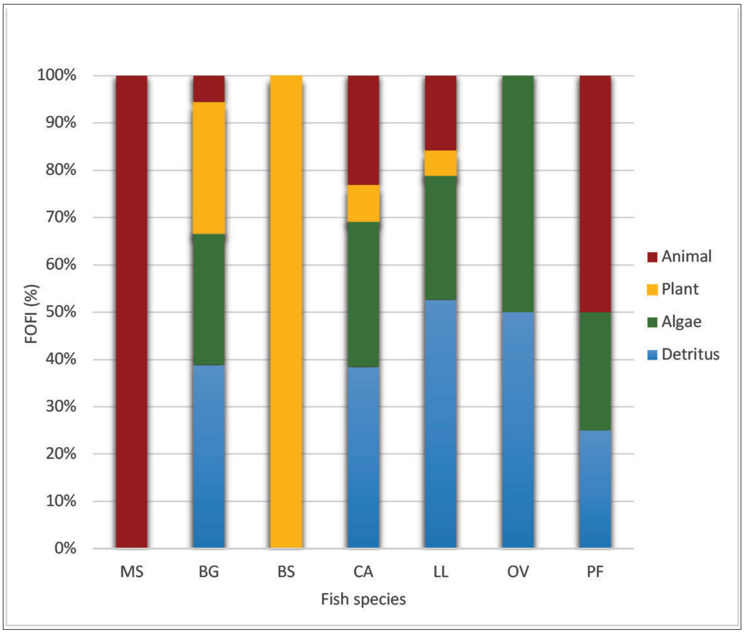

Figure 3: Percentage of relative frequency and distribution of four categories of food items from each fish species; *M. singaringan* (MS), *B. gonionotus* (BG), *B. schwanenfeldii* (BS), *C. apagon* (CA), *L. leptocheilus* (LL), *O. vittatus* (OV), and *P. fasciatus* (PF)

additional food items for *L. leptocheilus* (10%) and *C. apagon* (17%) are likely plant matters, in contrast to the species like *B. schwanenfeldii* which fed mainly the plants as their main food sources (100%), respectively. The stomach that contained distinctive plant matter was related to visible leaves and stems under the dissecting microscope. Only three species comprised all four food item groups: *B. gonionotus*, *C. apagon*, and *L. leptopcheilus*. The highest FOFI of food items in these three species are detritus (80.3%), algae (53.0%), animal fraction (29.3%), and plant matter (23.0%).

As aforementioned, both same genus fishes (*B. gonionotus* and *B. schwanenfeldii*) exhibited different values of $\delta^{13}C$ even though they are from the same family group, respectively, which can probably be attributed to the difference between the compositions of prey items and the position at the base of the lentic freshwater reservoir (Zhou *et al.*, 2009). According to Figure 3, the contribution of food items varied for each species. For instance, a higher proportion of plant matter was identified in the *B. schwanenfeldii* gut than in the *B. gonionotus* gut. Isotopic composition changes in consumers can result from the trophic level at which they feed, as well as temporal changes at the base of the food web that are transferred through the food web (Choi *et al.*, 2021).

Although *H. macrolepidota* was unable to obtain complete GCA information, the results from SIA and database from previous studies (Table 3) point towards the possibility of this species as carnivorous and omnivorous fish respectively (Nyanti *et al.*, 2021; Herawati *et al.*, 2022) and it usually occupied the highest TP in food webs (Yap *et al.*, 2016; Ridzuan *et al.*, 2017). Makmur *et al.* (2014) documented that the gut content of *H. macrolepidota* was high in crustaceans, which agrees with the SIA result where the TP value indicated the fish as a carnivorous fish species. This species tends to be macro-carnivores that actively seek out certain large prey fauna like molluscs, shrimp, and fish (Mustafa-Kamal *et al.*, 2012). Nevertheless, plant parts were also found in this species' stomach as reported by Herawati *et al.* (2022) and Nyanti *et al.* (2021), suggesting that these species can modify their diet by feeding on plant materials during periods of animal scarcity (Beamish *et al.*, 2006; Mérona & Vigouroux, 2006; Makmur *et al.*, 2014).

The GCA can provide insights into diet preferences but does not account for long-term mass transfer patterns (Amundsen & Sánchez-Hernández, 2019). This factor justifies the requirement of complementing SIA with GCA (Twining *et al.*, 2020). The major factors of the significance can be identified from SIA and GCA data obtained when comparing three different species in the same family (Cyprinidae): *H. macrolepidota*, *C. apagon*, and *L. leptocheilus* are *H. macrolepidota* can be labelled as specialised feeder while the other two species are more generalised feeders and unlikely to be limited by food (Yap, 1988). This could be the reason for *L. leptocheilus* (n = 10) and *C. apagon* (n = 7) (Table 1) become the dominant generalist feeder fish compared to *H. macrolepidota* (n = 2) in this reservoir section as they exploit other types of food and have a better chance to become widely distributed in an aquatic ecosystem (Mustafa-Kamal *et al.*, 2012).

Understanding the composition of fish dietary is essential for effective fisheries management since it is one of the most representative survival strategies for organisms (Choi *et al.*, 2021). BMR is famous among fish anglers for recreational fishing. To maintain fish stock for this activity, stocking strategies may emphasise using herbivores and omnivores adapted to the area. By examining the variation of fish feeding behaviour and TP in BMR, this study could suggest that the specific interaction information of trophic links in fish food web should not be generalised especially that can be used as the applications in fish management and conservation such as re-alignment the habitat (Stamp *et al.*, 2023). This application is advocated when a range of fish species exploits the habitats to provide a complete biologically equivalent habitat to those inhabiting the community.

BMR has flourished with aquatic vegetation such as *H. malayana*, *Eichhornia crassipes*, and *C. furcata* (Zakeyuddin *et al.*, 2016; Sharip *et al.*, 2018; Ismail *et al.*, 2019; Mokhtar *et al.*, 2020). Herbivorous fish play a key role in maintaining the health of the aquatic ecosystem by balancing the amount of aquatic plant growth. The habitat requirements of these herbivores can also be considered such as enhancing the plant preferred by the herbivores. On the other hand, omnivorous fish species may compete with herbivorous ones for food resources. Department of Fisheries can monitor population dynamics and adjust stocking densities to maintain a balanced fish community.

Anglers can be educated about the feeding habits of fish in the reservoir to choose appropriate bait, which can help manage fish populations' sustainability. Fisheries regulations can be tailored to protect and manage herbivorous and omnivorous species differently such as size and bag limits to promote conservation and sustainable harvest. Continuous monitoring of fish populations and research on their feeding habits can provide valuable data for adaptive management strategies.

Although SIA and GCA approaches are not commonly employed in the tropical freshwater region, it is important to accurately determine the composition of prey types and the variation of feeding guilds. Thus far, this can provide the most robust approaches to quantifying the true structure of fish food webs in shallow freshwater reservoirs. In summary, this allows managers to make informed decisions regarding stocking, population control, ecosystem health, angler education, habitat management, regulations, and research, all of which contribute to effective reservoir management.

Limitation of the Study

Research on the fish food web particularly using stable isotopes in Malaysia is very sparse compared to other countries. To improve research on the fish food web in surface water ecosystems in Malaysia, more research on fish feeding habits regarding species distribution using this approach must be done. However, because there was insufficient sampling effort in this preliminary analysis study to collect more fish species from this man-made reservoir and primary producer samples were not included as basal carbon sources, the evaluation of fish feeding habit data was impeded. The precise TP and the proportional carbon contribution of local primary producers to fish nutrition were also hindered. This is because the contribution variability of consumer food sources will affect the chosen $\delta^{15}N_{ref}$ (Cabana & Rasmussen, 1996; Post, 2002; Matthews & Mazumder, 2005). For example, measuring the TP of fish in reservoirs may require a different baseline for both the pelagic (mussels, zooplankton) and benthic (snails, crustaceans) food chains since fish use both.

Besides, the limited access to fishing gear and the absence of expertise in catching the fish of the research team for this study have prevented the gathering of an adequate number of fish samples. Thus, further analysis must be performed with well-planned strategies, especially on collecting extended variation of basal carbon and food source samples from study areas to fill the gaps of this study by having an insight into the BMR complete fish food web.

Since only a few basic records on baseline studies of stable isotopes in aquatic ecosystems are available, they are not adequate for understanding the biology of the fish food web and the distribution of isotope signatures in surface water ecosystems. Analysis of isotopes for C and N in organic matters like sediments beneath the lake basin is required in future work to identify the origin of organic matters that sustain productivity at higher trophic levels and help in tracing the mobility of the fish. This approach can help with the assumption that consumers are relying upon temporal, spatial, or compositional isotopic equilibrium with different organic matter and baselines. Therefore, it is possible to determine precisely the measures needed to conserve and preserve fish in BMR.

Conclusions

SIA and GCA indicated that feeding across several TP with an abundance of basal sources is important in the diets of BMR fishes. However, there were spatial differences in what each species consumed, their isotopic signatures values, and TP values and each species fed at a similar TP across locations, indicating similar ecological function. The study findings support the perspective that omnivory is an adaptive response to seasonal variation in water level and trophic resources that characterise the ecological variable of tropical reservoirs. The flexibility of dietary niche can be seen in all fish samples as they can utilise any available detritus in this shallow lentic freshwater reservoir.

From this study's $\delta^{13}C$ signatures data, it can be concluded that C3 aquatic plants are the most important carbon sources to most fish species cau-ght during the study period in BMR. Information on such fish feeding habits and trophic position can also help with resource partitioning, which is useful in estimating the likely size of biotic or fish resources supporting harvests in an ecosystem or tropical reservoir. Here, the study has focused on eight different fish species, but further testing should extend to other taxa like invertebrate taxa and consumers' food sources, in particular, to understand food web studies that include multiple trophic levels. Thus, these data are critical for the integration modelling approach for linking isotopic variation individuals to individual differences in diet. Such limitations in the number of fish samples and sampling effort for this preliminary study cannot be concluded for the whole reservoir. However, from the data obtained (SIA and GCA), the consumers in the BMR food web, which are fish during the study period, are presumed to be detritivores or omnivores. The category detritivore could be necessary for the trophic scaffolding of BMR fish as they mostly utilise detritus. In essence, both results (SIA and GCA) indicated a similar conclusion for the present study. The result of the current study provides insight into fish sample feeding ecology relevant to understanding existing trophic relationships and interactions among co-occurring predators in BMR.

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Conflict of Interest Statement

The authors declare that they have no conflict of interest.

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