UNDER PEER REVIEW

STOMACH CONTENT ANALYSIS (SCA) AND STABLE ISOTOPE ANALYSIS (SIA): A DUAL APPROACH TO ASSESS DIET AND TROPHIC POSITION (TP) OF *H. macrolepidota* AND *C. ocellaris* FROM CHENDEROH RESERVOIR, MALAYSIA

ABSTRACT

The present study assessed and compared the diet and trophic positions (TP) of two carnivorous fish *H. macrolepidota* and *C. ocellaris* from Chenderoh Reservoir, Malaysia. One of the few reasons for the study was to understand the effects of invasive non-indigenous species (NIS), *C. ocellaris*, on the native indigenous (IS) fish species, *H. macrolepidota*. The assessment was grounded in stomach content analysis (SCA) and stable isotope analysis (SIA), which collectively clarified the feeding habits and trophic positions (TP) of these selected fish. The mean RGL values for *H. macrolepidota* and *C ocellaris* were 0.98 ± 0.18 and 1.10 ± 0.15 (Mean ± SD), respectively, aligning with known ranges for carnivorous fish. These values also clarified that both species occupy higher TP in the food web as tertiary or quaternary consumers. SCA findings also revealed that fish and crustaceans were the predominant food categories for *H. macrolepidota*, while *C. ocellaris* predominantly fed on fish. The mean stomach fullness index (MSF) and the gastrosomatic index (GSI) corroborated the differences in the foraging performance of the fishes, with *C. ocellaris* having a higher MSF (2.03) compared to *H. macrolepidota* (0.65). These implied that *C. ocellaris* had plentiful of food and encountered fewer diet-related challenges in the ecosystem. From SIA, $\delta^{13}C$ values indicated that the primary carbon sources for both species are C3 plants, particularly aquatic vegetation. Further, $\delta^{15}N$ values further ensured that both *H. macrolepidota* and *C. ocellaris* are carnivorous in nature and occupy higher TP in the ecosystem.

Keywords: Non-indigenous Species, NIS, IAS, Stomach Content Analysis, Stable Isotope 17 Analysis, SCA, SIA, Hampala macrolepidota, Cicla ocellaris.

1. INTRODUCTION

Unintentional and purposeful introductions of fish species to our freshwater ecosystems have been a repeated phenomenon since the distant past (Hughes, 2003; Sultana & Hashim, 2016). It is estimated that approximately 20% of the freshwater fish species of the world are already extinct or endangered due to non-indigenous (NIS) fish introduction (Hanif *et al.*, 2016). Biotic homogenization, in other words, the replacement of specific indigenous species (IS) by NIS (Tabarelli *et al.*, 2012), results in freshwater ecosystems with lower diversity and species extinction (Rahel, 2002; Drake & Lodge, 2004). Hence, it has become a top priority in the present era to evaluate the introduction, diversity, distribution, magnitude, and impacts of non-indigenous (NIS) and invasive alien (IAS) fish species (Sala *et al.*, 2000; Vörösmarty *et al.*, 2010) in freshwater ecosystems.

Alike some other reservoirs in Malaysia, Chenderoh Reservoir is also comprised of indigenous (IS) fish and non-indigenous (NIS) fish. In Chenderoh, Bass fish, *i.e., Cicla ocellaris,* were introduced by the Department of Fisheries, Malaysia, mainly for sport-fishing or entertainment purposes (Rahim *et al.,* 2013). This intentional introduction of *C. ocellaris* has traditionally been viewed as a form of fishery enhancement in Chenderoh, and, until now, there have been little concerns about their ecological consequences. Therefore, this study was a pioneer which is exploring the preliminary conditions of the invasive Bass species in the reservoir.

Stomach content analysis (SCA) and stable isotope analysis (SIA) are both valuable tools in fish ecology and food-web research for assessing the diets and trophic positions (TP) of freshwater fish. SCA offers insights into diet preferences and selections. Therefore, it provides only a snapshot of a fish's diet over a short period and doesn't account for long-term dietary patterns. In contrast, SIA is a strategic method that reveals the assimilated diet fraction over a more extended time frame and also identifies carbon and nitrogen sources in the ecosystem. However, SIA has its own limitations, as it can't directly pinpoint the specific prey items consumed by fish. Therefore, combining these methods can offer a more comprehensive understanding of a fish's trophic role and the larger ecological picture (Woodward & Hildrew, 2002; Renones *et al.*, 2002).

We selected two fish species, one indigenous (IS) and one non-indigenous (NIS) from Chenderoh Reservoir, *Hampala macrolepidota*, and *Cicla ocellaris*, respectively. The reasons behind the selection were: 1) These two were the most abundant IS (indigenous) and NIS (non-indigenous) fish caught on that time frame of fish sampling. 2) They had similar diet patterns. 3) Assessment of similar diet patterns is crucial to understanding diet overlap, trophic position (TP) overlap, and overall invasiveness posed by the NIS fish (if there is any).

This research paper was centered around addressing three specific questions. 1) what does SCA reveal regarding the diet preference of *H. macrolepidota* and *C. ocellaris* (*i.e.,* identity, quantity, and size of prey items)? 2) what is the trajectory of isotopic signatures about the food consumption of the selected fish species? 3) similarities and/or dissimilarities in food preferences and trophic positions (TP) between the two species that may impact each other in the ecosystem.

2. MATERIALS AND METHODS

2.1. Location and General Features of Study Area, Chenderoh Reservoir:

The study was conducted in Chenderoh Reservoir, a man-made reservoir on the Perak River, Malaysia (4°58’N, 100°57’E). With an elevation of 68 meters above sea level, the Chenderoh Reservoir covers a surface area of 25,910,000 meters square with an average mean depth of nine meters (Fig. 1).

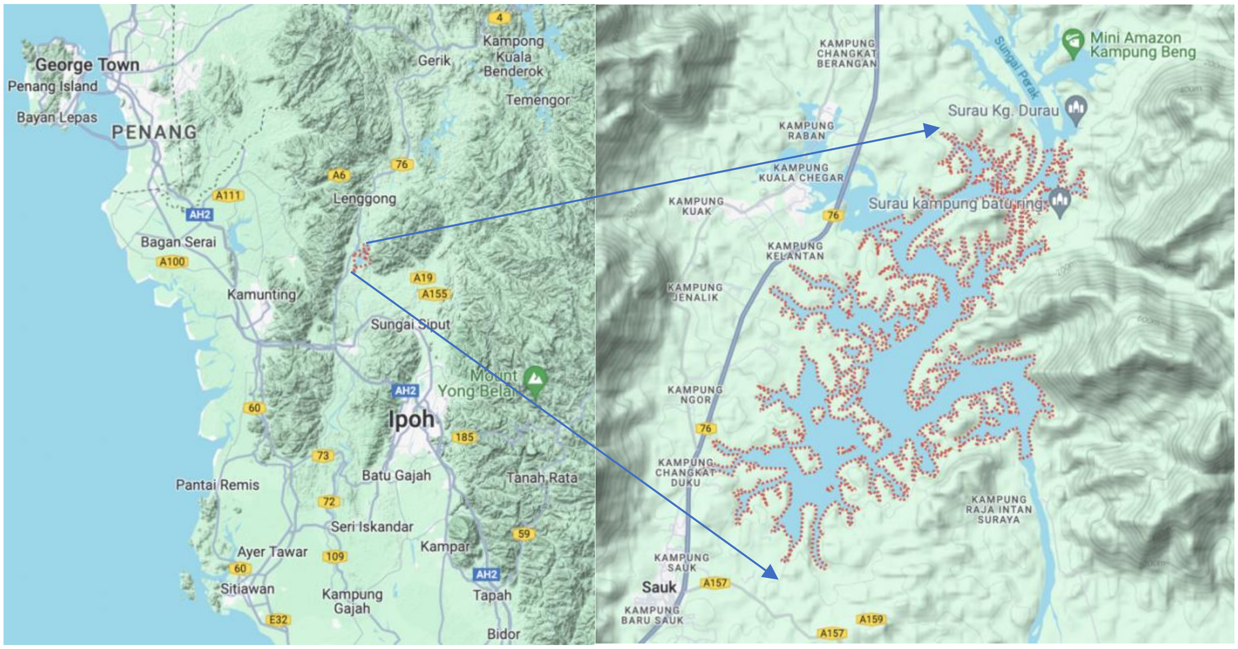

Figure 1: Study area; Chenderoh Reservoir, Penang, Malaysia. Source: Google Map

2.2. Fish Sample Collection:

Three sets of experimental gill nets (250 cm vertical length and 2,976 cm total width) with five different stretch mesh sizes (10 cm; 7.5 cm; 6.5 cm; 5 cm; and 3.7 cm) were deployed overnight randomly to capture fish from the reservoir. SCA was conducted from fish samples captured between September 2014 to February 2015 while SIA was carried out using fish samples collected from December 2014 to February 2015.

2.3. Stomach Sample Collection and Preparation For SCA:

The selected fishes for SCA were preserved in formalin straightaway in the field to prevent food digestion. Afterward, they were washed off thoroughly before further analysis. Total length (TL), standard length (SL), and weight (W) of fish were measured to the nearest 0.1 cm/g. Fishes were cautiously dissected to obtain the gut. Gut length (GL) was measured from the esophagus until the tip of the anus. Gut weight (GW) was taken with and without its content, while the contents of the stomachs were also measured using an electronic scale to the nearest 0.1 g. Each stomach was placed into a sample bottle containing 10% formalin for further observation and analysis.

2.4. Fish Fillet Collection and Sample Preparation for SIA:

Fish samples were dissected and filleted at the laboratory to get the white swimming muscle (Sweeting *et al.*, 2006) and stored in a frozen state in a deep freezer (–20ºC) (Kim *et al.*, 2014). All fillets were dried in the oven for dehydration at 60 degrees Celcius temperature, ground to a fine powder, and divided into two sub-samples. Thus, a total of 54 samples were used for SIA which comprised 27 acidified sub-samples and 27 non-acidified sub-samples where all the sub-samples were weighted from 400µg to 500µg.

2.5. Data Analysis for Stomach Content Analysis (SCA):

2.5.1. Relative Gut Length (RGL): The gut length was measured with an accuracy of 0.5 cm in order to obtain the relative gut length. RGL was calculated by using the formula given by Montgomery (1997):

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

2.5.2. Frequency Of Occurrence (%FOC): To identify diet category and the usage of prey resource, frequency of occurrence (%FOC) were calculated for each food item in each selected fish species as outlined by Bowen (1996) as:

$$[\%]\ \boldsymbol{Fi} = \frac{\boldsymbol{Mi}}{\boldsymbol{M\Sigma}} \times \mathbf{100}$$

Where, Mi = number of stomachs containing prey component i and M_Σ = number of stomachs containing food

2.5.3. Mean Stomach Fullness (MSF) Index: In this study, stomachs were visually assessed (Sarkar & Deepak, 2009) for the degree of SF (stomach fullness) using the following numerical scale (Carniatto *et al.*, 2014): 0 = empty stomach; 1 = up to 25% SF; 2 = 25% to75% SF; 3 ≥ 75% SF. The value of MSF was calculated as following calculation by Santos (1978):

$$\mathbf{MSF} = \frac{(\mathbf{N0} \times \mathbf{0}) + (\mathbf{N1} \times \mathbf{1}) + (\mathbf{N2} \times \mathbf{2}) + (\mathbf{N3} \times \mathbf{3})}{\mathbf{N}}$$

Where N_0, N_1, N_2, and N_3 are the number of stomachs with SF values of 0, 1, 2, and 3, respectively, and N is the number of individuals.

2.5.4. Gastro-Somatic Index (GSI): The gastro-somatic index indicates the feeding activities and foraging performances of fish (Sarkar & Deepak, 2009). In the present study, the gastro-somatic index of selected fishes was calculated as:

$$\mathbf{GSI} = \frac{GW}{BW} \times 100$$

Where, GW = gut weight in grams, and BW = body weight in grams.

2.6. Data Interpretation and Analysis for Stable Isotope Analysis (SIA):

SIA was performed at the Doping Control Centre (DCC) of University Sains Malaysia (USM), using an elemental analyzer Thermo Finnigan Flash EA2000 connected to Finningan DELTA V-AVANTAGE plus isotope ratio mass spectrometry by a Con Flo II interface with an analytical precision of ±0.2‰. Standards considered were VPDB (Pee Dee Belemnite) for Carbon and atmospheric Nitrogen for Nitrogen (Carabel *et al.,* 2006). In this study, isotopic ratios for Carbon ($\delta^{13}C$) and for Nitrogen ($\delta^{15}N$) were calculated as:

$$\delta^{13}C = \{(^{13}C/^{12}C_{sample} / ^{13}C/^{12}C_{standard})-1\} \times 1000\ (‰)$$

$$\delta^{15}N = \{(^{15}N/^{14}N_{sample} / ^{15}N/^{14}N_{standard})-1\} \times 1000\ (‰)$$

2.7. Trophic Position (TP) of Fish Analysis from $\delta^{15}N$:

To estimate the TP of selected fish species from SIA, the $\delta^{15}N$ values were converted into relative trophic positions using a modification of the model (Brasso & Polito, 2013) described by Hobson et al., (1994):

$$TP_{selected\ consumer} = [\ (\delta^{15} N_{selected\ consumer} - \delta^{15} N_{primary\ consumer)} / 3.4\ ] + 2$$

Where 3.4 represents a ‘1.0 Trophic Level’ increment in $\delta^{15}N$ and 2 represents the trophic position (TP) for the primary consumers in the ecosystem.

2.8. Statistical Analysis:

All data were subjected to a normality test by using SPSS (version 19.0). Subsequently, based on low *P* values ($P < 0.05$) from the test statistics, parametric analysis with permutation was performed. Besides, descriptive statistics, Student's t-test, one-way ANOVA, and post-hoc analysis were done.

3. RESULTS

3.1 Diet Composition of *H macrolepidota* and *C ocellaris:*

Out of 64 stomachs analyzed for *H. macrolepidota*, 37 had food in them (57.81%) and 27 were empty (42.18%) whereas, out of 120 stomachs observed for *C. ocellaris*, 101 were with food (84.16%), and the remaining 19 were (15.83%) empty. In the present study, out of seven food categories identified for *H. macrolepidota*, fish and crustaceans were the most common items with 43.24% and 27.02% occurrence respectively (Fig. 2), while fish fingerlings were the only perceived food item in the stomachs of *C. ocellaris* with nearly 100% occurrence. The other significant food items of *H macrolepidota* include aquatic insects (24.32%), Oligochaetes (10.81%), and Chironomids (8.19%). Similar findings were observed in a study conducted by Makmur (2014) in Indonesia, where *H. macrolepidota* was characterized as a carnivorous fish primarily preying on other fish. It was also found to feed on a variety of other organisms, including shrimp, crabs, insects, and mollusks (Makmur, 2014).

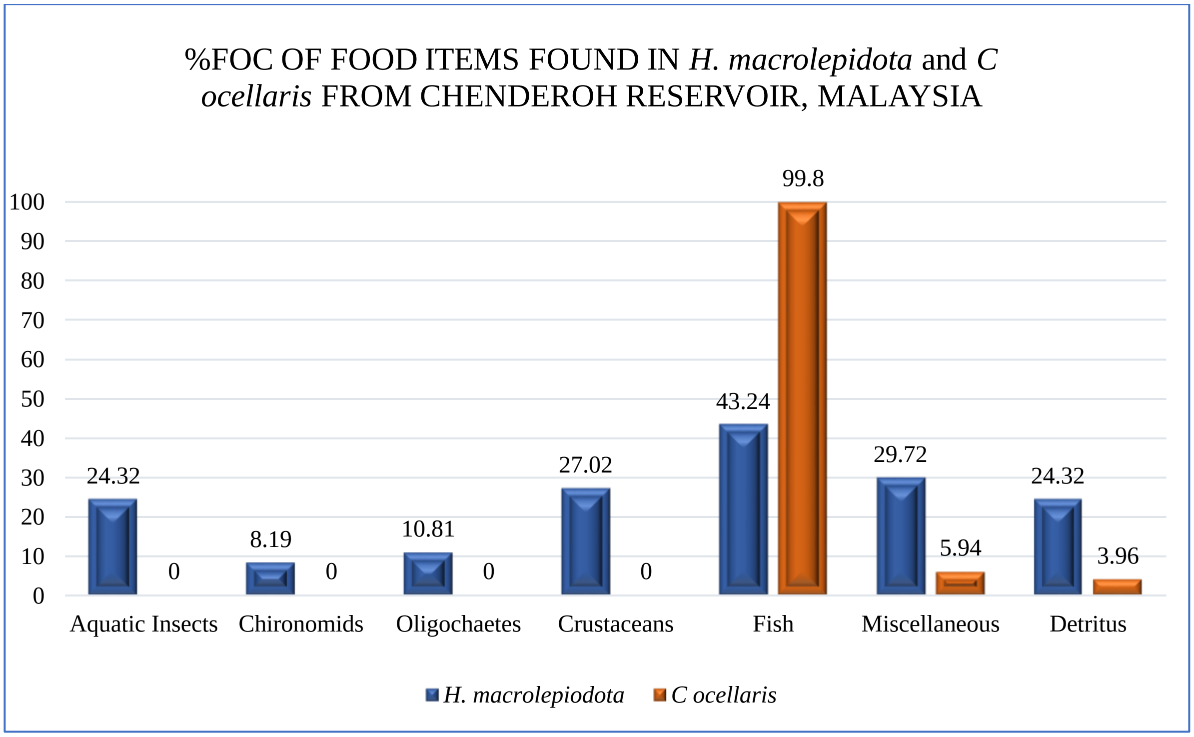

Figure 2: Percentage of identified food items observed in the stomachs of *H. macrolepidota.*

From this study, it can be perceived that a significant proportion of *H. macrolepidota* had empty stomachs, indicating potential challenges in finding food. Among those with food in their stomachs, fish and crustaceans were the primary food items, but their occurrence was notably lower compared to *C. ocellaris*, which exclusively fed on fish.

3.2. Categorization of Fish According to RGL:

The relative gut length (RGL) of fish facilitates comparisons among fishes with varying diets, such as herbivores, carnivores, and omnivores (Ahmed *et al.*, 2022). In this study, *H. macrolepidota* had a RGL value (mean ± SD) of 0.98 ± 0.18. Besides, *C. ocellaris* had an RGL value (mean ± SD) of 1.10 ± 0.15. The RGL values of *H. macrolepidota* were similar to those proposed by Bertin (1958) for carnivorous fish (0.2 to 2.5) and the RGL values for *C. ocellaris* were close to those found by Pouilly et al. (2003) for neotropical piscivores (0.93 to 1.23). Therefore, this can be concluded from the study that both of the species are carnivores.

Table 1: The (mean ± SD) values of Standard Length, Weight, Gut Length, Gut Weight, Relative Gut Length, and category of fishes according to their relative gut length of *H. macrolepidota* and *C. ocellaris.*

Species	SL (cm)(Mean ± SD)	W (g) (Mean ± SD)	GL (cm) (Mean ± SD)	GW (g) (Mean ± SD)	RGL (cm) (Mean ± SD)	Category of fish
H. macrolepidota	16.94 ± 2.84	128.21 ± 69.05	16.41 ± 3.06	1.75 ± 0.58	0.98 ± 0.17	Carnivore
C. ocellaris	15.73 ± 2.10	97.91 ± 46.60	17.26 ± 3.03	2.47 ± 1.71	1.10 ± 0.15	Carnivore

Note: SD = standard deviation; SL = standard length; W = weight; GL = gut length; GW = gut weight; RGL = relative gut length; cm = centimeter; g = gram

3.3. Diet Consumption Frequency and Foraging Performance:

Subjective methods “mean stomach fullness index” (MSF index) and gastro somatic index (GSI) were used in the present study to quantify the diet consumption frequency and foraging performance of selected fishes (Phelps *et al.*, 2007). In this study, the NIS fish *C. ocellaris* had a higher MSF value (2.03) than that of *H. macrolepidota* (0.65), which means *C. ocellaris* had plenty of food in the system and it could consume its prey without any competition with similar species (i.e., *H. macrolepidota*). Garrido et al. (2008) also supported this statement while working on stomach fullness of Horse Mackerel in Portugal. According to Kihlberg et al. (2023), the greater MSF value also ensures the establishment and necessary adaptation and spread of any NIS fish species in the wild.

Bass fishes are voracious predators and tend to consume their diet by encountering any species of similar feeding habits. This assertion was corroborated by the findings of the present research, while comparing the MSF value of *C. ocellaris* with that of *H. macrolepidota*, it becomes evident that *H. macrolepidota* faced challenges in obtaining adequate diet in the reservoir. However, the low MSF value observed from *H. macrolepidota* can further be attributed to various causes including the strong predatory behavior of *C. ocellaris*, the limited availability of preferred prey, adverse ecosystem conditions, and other contributing factors, which need further and extensive research.

Table 2: Level of foraging activities of *H. macrolepidota* and *C. ocellaris* according to their mean stomach fullness (mean ± SD)**.**

Species	Mean stomach fullness (mean ± SD)						Foraging performance
	Sep’2014	Oct’2014	Nov’2014	Dec’2014	Jan’2015	Feb’2015	
C. ocellaris	1.90 ± 1.33	2.25 ± 1.06	1.85 ± 1.13	2.15 ± 1.03	2.00 ± 1.13	2.05 ± 1.14	Very High
H. macrolepidota	0.10 ± 0.31	0.70 ± 1.05	0.60 ± 0.96	0.80 ± 1.03	0.90 ± 1.10	0.85 ± 0.94	Medium

Like MSF, the GSI of *C. ocellaris* was higher than that of *H. macrolepidota* (Fig. 3). The foraging activities of Bass fish are always harsh and voracious compared to the other species in any community, as it is an invasive species (IAS) (Gomiero & Braga, 2004). This proclamation was proved to be true while the GSI index of *C ocellaris* was compared to the GSI index of *H. macrolepidota.*

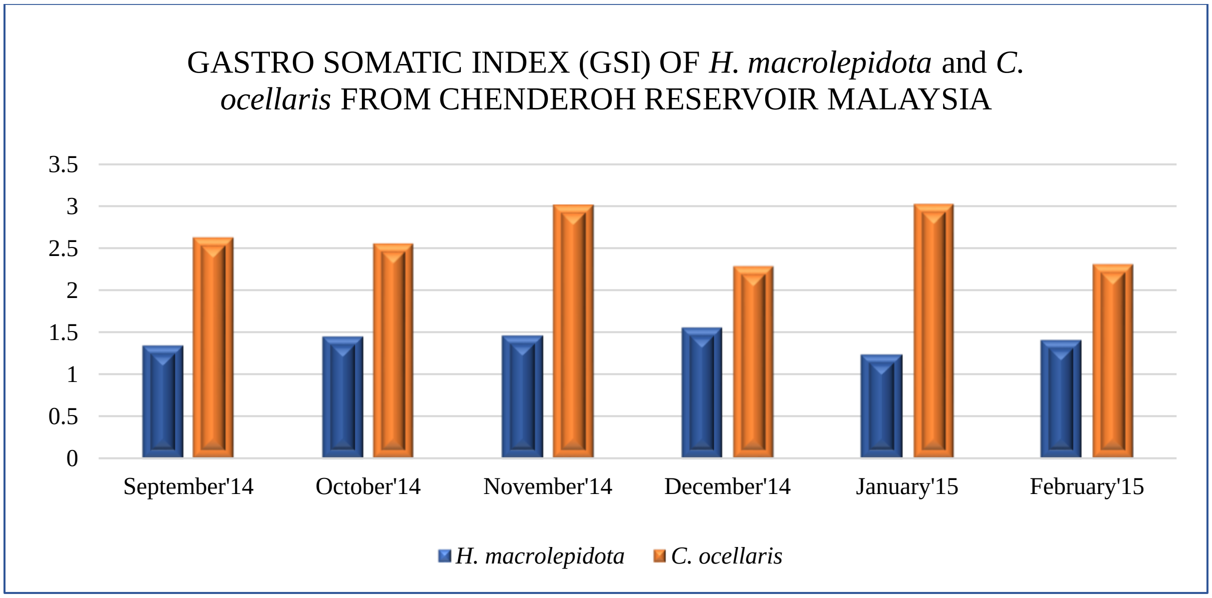

Figure 3: Monthly Gastro Somatic Index (GSI) of *H. macrolepidota* and *C. ocellaris.*

According to the above results and discussions from stomach content analysis (SCA) of *H. macrolepidota* and *C. ocellaris* it can be hypothesized that 1) the foraging activities of the NIS species *C. ocellaris* may cause diet inadequacy in the ecosystem for *H. macrolepidota;* 2) the predatory and violent behavior of *C. ocellaris* may resist *H. macrolepidota* to become more dynamic and lively to catch the prey. However, both of the hypotheses were preliminary predictions and expectations from an ecosystem consisting of predatory NIS fishes, especially Bass fishes (Novaes *et al.*, 2004), and need to be assessed further.

3.4. Trophic Positioning (TP) of Fish According to RGL:

The trophic position (TP) of fish is closely related to their gut morphology. Relative gut length (RGL) is a common method used to correlate a fish's TP with its diet (Elliott & Bellwood, 2003; Karachle & Stergiou, 2010). Generally, carnivorous and predatory fish have shorter and simpler gut structures, while omnivores and herbivores exhibit longer and more complex digestive tracts. In this study, both indigenous (IS) fish *H. macrolepidota* and non-indigenous (NIS) fish *C. ocellaris* displayed similar gut structures characterized by short digestive tracts, muscular and elastic stomachs, and short intestines, aligning with the typical features of carnivorous fishes.

These findings are consistent with similar results reported by Yap et al. (2016), conducted in the Chenderoh reservoir.

3.5. Carbon Sources of the Reservoirs According to SIA:

$\delta^{13}C$ values are often used to track energy flows and energy sources of fish in a community (Finlay, 2001; Syväranta *et al.,* 2011). Therefore, in this research, the $\delta^{13}C$ values of the fish were used to understand the core energy source of the reservoir. It also helped us make baseline data of isotopic signatures from selected fishes from Chenderoh Reservoir.

All the values of $\delta^{13}C$ of fishes ranged from (mean ± SD) -34.3 ± 0.49‰ to - 23.0 ± 0.05‰ (Table 3). According to Garcia et al. (2007), the isotopic values of carbon ranged from -25‰ to -19‰ indicating that the carbon sources of the water body are C3 plants. Similar studies suggest alike clarifications of carbon sources of water bodies (Larsen *et al.,* 2009; Daniele *et al.,* 2012) for tropical lakes and reservoirs. Here, the $\delta^{13}C$ values for *H. macrolepidota* ranged from -35.318 ± 0.088‰ to -34.096 ± 0.250‰; whereas, $\delta^{13}C$ values for *C. ocellaris* ranged from -33.624 ± 0.466‰ to -32.182 ± 1.376‰. The values of isotopic carbon from the SIA interpretation indicated that the main energy source of the reservoirs is emergent, submerged, floating, or exotic C3 plants using the C3 photosynthetic pathway (diatoms, cyanobacteria, freshwater algae, macrophytes). Yap et al. (2016) also made a similar statement while working on Chenderoh Reservoir. However, to affirm the species diversity, abundance, and distribution of the primary producers, further and detailed research is required.

Table 3: Monthly $\delta^{13}C$ and $\delta^{15}N$ values of *H. macrolepidota and C. ocellaris*.

Month	*H. macrolepidota*		*C. ocellaris*	
	Carbon (‰)	Nitrogen (‰)	Carbon (‰)	Nitrogen (‰)
December	-34.311 ± 0.477	9.202 ± 0.173	-32.182 ± 1.376	12.690 ± 0.231
January	-34.096 ± 0.250	10.837 ± 0.409	-33.624 ± 0.466	13.356 ± 0.396
February	-35.318 ± 0.088	11.385 ± 1.380	-32.265 ± 0.058	13.085 ± 0.091

3.6. Trophic Positioning (TP) of Fish According to $\delta^{15}N$:

$\delta^{15}N$ values of fishes are considered worthwhile categorizing TP of fish in an ecosystem (Kim *et al.,* 2014). The higher the $\delta^{15}N$ value is, the higher the TP of that species is supposed to be.

However, it is considered to be relatively difficult to identify the TP of upper-level consumers, such as *C. ocellaris*, within a freshwater community. The main reason is logistical limitations, such as the difficulty of long-term sampling.

In present study, the δ^{15}N values for *H. macrolepidota* and *C. ocellaris* ranged from 9.202 ± 0.173‰ to 11.385 ± 1.380‰ and from 12.690 ± 0.231‰ to 13.356 ± 0.396‰ respectively (Table 3), and the mean values calculated of δ^{15}N of *H. macrolepidota* and *C. ocellaris* are 10.157 ± 1.125‰ and 13.044 ± 0.29‰ respectively. Moreover, the trophic level calculated for *C. ocellaris* (TP = 4.2) (Fig. 4) suggested that this species occupies an upper TP (*i.e.,* tertiary or quaternary consumer level) and are high-order carnivore, more specifically defined as a piscivore. TP identified for *H. macrolepidota* was 3.4, which means this species also occupies an upper TP in the ecosystem (*i.e.,* secondary consumer level) and are carnivore. A similar study conducted by Yap et al. (2016) also suggested that *H. macrolepidota* and *C. ocellaris* from the Chenderoh Reservoir occupy higher trophic positions (TP) as secondary or tertiary consumers within the food web of the reservoir.

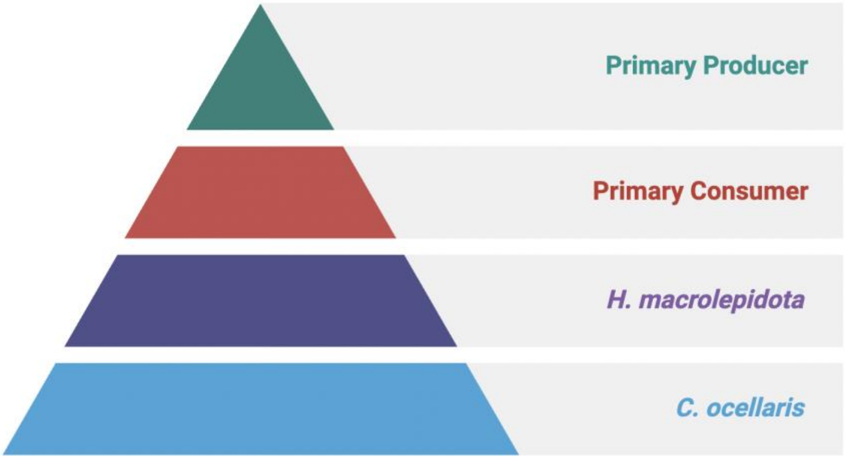

Figure 4: Schematic Trophic Positioning (TP) of *H. macrolepidota* and *C. ocellaris*.

4. DISCUSSION

Cicla ocellaris is a ravenous predator and feeds extensively on fish; therefore, it has the potential to modify the diversity and distribution of a habitat to which it is introduced (Gomiero & Braga 2004; Novaes *et al.,* 2004). Moreover, it can generate negative environmental impacts by competing with similar native species for food and space, predating on juveniles and eggs, and disrupting the habitat by grazing on detritus and benthic algae (Canonico *et al.,* 2005; Ansah *et* *al.*, 2014). These threats include flow modification, habitat alteration (Johnson *et al.*, 2008), overexploitation, pollution (Dudgeon *et al.*, 2006), and overall environmental modification (Kiruba-Sankar *et al.*, 2018), which all can lead to biodiversity degradation of a particular ecosystem. Several similar studies have demonstrated a significant decline in fish densities over the past two decades, with a particular impact on species like *Hampala macrolepidota* in riverine systems, especially the Perak River system of Malaysia (Department of Fisheries, 2005; Radhi *et al.*, 2017; Ambak & Mohd Zaidi, 2010).

In a recent study conducted in Perlis, Malaysia, the Peacock Bass was reported to exert significant predation pressure on the fry of Tinfoil Barb (*B. schwanefeldii*) (Saba *et al.*, 2021). Notably, it was observed that the Peacock Bass heavily preyed upon approximately 50,000 fry of Tinfoil Barb, which had been intentionally released into Timah Tasoh Lake, Perlis by the Department of Fisheries (DOF) Malaysia with the aim of enhancing the fish population within the lake. This predation highlights the potential impact of Peacock Bass on native fish populations and the challenges posed by NIS in local ecosystems (Isa *et al.*, 2012; Zulkefli, 2017).

The potential for the establishment of NIS is strongly influenced by environmental factors also, (e.g., Herborg *et al.*, 2007; Kilroy *et al.*, 2008; Kulhanek *et al.*, 2011) and is often associated with disturbances (Minchinton, 2002; Marchetti *et al.*, 2004a). Degraded water quality, for example, plays an important role in the establishment of NIS in aquatic ecosystems. Factors such as turbidity and water disturbances, coupled with the presence of invasive aquatic plants and fauna, can significantly influence the successful establishment of NIS (Diagne *et al.*, 2017). For instance, degraded water quality in Chenderoh Reservoir, as evidenced by Ismail et al. (2019), has created an environment that may be conducive to the establishment of species like *C. ocellaris*. The combination of water turbidity and disturbances, along with invasive aquatic plants (Ismail *et al.*, 2019), can provide favorable conditions for the successful colonization of NIS, contributing to their persistence in this ecosystem. Additional characteristics that increase the invasive potential of NIS include high reproductive rates, extended lifespans, the ability for long-distance dispersal, a high degree of physiological tolerance, a generalist lifestyle, and significant trophic adaptability (Kolar & Lodge, 2001; Marchetti *et al.*, 2004b; Funk, 2008; Barrett, 2011).

Physical barriers, such as dams, are known to enhance the potential for the establishment of NIS in ecosystems (Rahel & Olden, 2008). Within the context of the Perak River system in Malaysia, a series of cascading hydroelectric dams can be observed (Mohd Sidek *et al.*, 2020), namely, Temengor Dam, Bersia Dam, Kenering Dam, and Chenderoh Dam. Chenderoh Reservoir, being the terminal reservoir in this series of dams from upstream to downstream along the Perak River system, is particularly susceptible to increased water turbidity originating from inflows from the upstream reservoirs (Zarul, 2013). This elevated turbidity as well as mineralization of the water may significantly raise the likelihood of NIS fish invasion in Chenderoh reservoir. Nyanti et al. (2021) have also demonstrated the impact of cascading dams on the development of complex ecosystems, such as those observed in the Murum River, Malaysia. This study indicates a transitional zone in the Murum River, displayed reduced fish species diversity, richness, and evenness, particularly degraded water quality with high turbidity, which may facilitate the establishment of NIS species (Tan & Rohasliney, 2013).

The significance of this study lies in its contribution to understanding the potential impact of the introduced Bass fish, *C. ocellaris,* in Chenderoh Reservoir. Assessing trophic position, feeding habits, and foraging activities are essential components of this understanding, complementing other ongoing studies in Chenderoh Reservoir, which were mentioned in this paper. While our research provides valuable insights, further investigations are required to comprehensively assess the impact of invasive species on the food web and the overall ecosystem health.

5. CONCLUSION

Based on the findings of the present study, it is evident that both *H. macrolepidota* and *C. ocellaris* in Chenderoh Reservoir, Malaysia, are carnivorous fish occupying higher trophic positions in the ecosystem. Through a combination of stomach content analysis (SCA) and stable isotope analysis (SIA), the feeding habits and trophic positions of these species were elucidated. The mean relative gut length (RGL) values indicated that both species are tertiary or quaternary consumers, with *C. ocellaris* exhibiting a slightly higher RGL compared to *H. macrolepidota*. SCA results revealed distinct differences in foraging preferences, with *H. macrolepidota* predominantly feeding on fish and crustaceans, while *C. ocellaris* primarily consumed fish. Additionally, the mean stomach fullness index (MSF) and gastrosomatic index (GSI) highlighted variations in the foraging performance between the two species, with *C. ocellaris* exhibiting higher MSF values, suggesting a more abundant food supply and potentially fewer diet-related challenges. Furthermore, stable isotope analysis (SIA) results indicated that both species primarily derive their carbon sources from C3 plants, particularly aquatic vegetation, while δ15N values confirmed their carnivorous nature and higher trophic positions in the ecosystem. Overall, these findings contribute to a better understanding of the dietary dynamics and trophic interactions between native and non-indigenous fish species in Chenderoh Reservoir, providing valuable insights for the conservation and management of aquatic ecosystems facing the challenges of invasive species.

However, there are several limitations associated with the study. Firstly, only the isotopic value of nitrogen of a fish species alone cannot be considered to represent the trophic position (TP) of that particular fish, since the δ^{15}N of primary producers (defined as organisms that convert inorganic N to organic N) are highly involved in the systems (Kling *et al.*, 1992). It necessitates that the TP of fish should be measured considering a lake-specific “baseline” of δ^{15}N signature of primary producers/ primary consumers. However, there were no baseline data available for Chenderoh Reservoir. Secondly, the TP analyses of fish were calculated excluding the considerations of fish age, sex, and TL (total length) of fish which may influence the result (Cortes, 1999). However, the outcome obtained from the calculation of this study was an average trophic positioning of *H. macrolepidota* and *C. ocellaris* which is supported by several authors such as Hobson et al. (1994), and Pauly (1998). And, finally, the study excluded considerations of seasonal variation, age, sex of fishes, and spatial influences in the stomach content analysis. As a result, the precise feeding capacity and feeding behaviors of the selected fishes could not be precisely determined.

Future research potential includes Research on biological invasions particularly freshwater invasions by NIS and IAS fish needed to be documented for each and every state of Malaysia. Besides, existing statistical data of NIS and IAS should be updated on a periodic basis to get a clear picture of their quantity, diversity, distribution, and effects. We should look through every angle to understand the threat posed by IAS. Only a few basic records are not adequate for understanding the biology of invasion and the impacts of IAS in the freshwater ecosystems of this country. Therefore, comprehensive research and documentation are mostly needed. Also, primary and secondary assessments are needed for all freshwater ecosystems, such as lakes, rivers, reservoirs, and inland water bodies of Malaysia. These assessments include water quality, ecological condition, ecosystem evaluation, anthropogenic activities in and out of the water bodies, and so on. There is an indication in invasion ecology that beyond the biological invasions in any ecosystem, there are unswerving stimuli of native species diversity and abundance. As can be grasped from the worldwide NIS distribution, where there is a high abundance of freshwater fish present, the NIS and IAS fish species also emerged therein. Therefore, together with the information on introduced species, vigorous documents about the diversity and distribution of native fish species of the broad aquatic networks are crucial. Moreover, while molecular and genetic technologies can significantly enhance our ability to assess and manage the threat posed by IAS, baseline molecular data are remarkably limited for both NIS and IAS fish in Malaysia. This inadequacy includes genetic diversity, genome sequencing, stable isotope signatures, and similar molecular studies. The shortage of baseline molecular data highlights the necessity for comprehensive research in these areas to improve our understanding of these species and their impacts on native biodiversity. Finally, elaborate research on NIS fish is required for Chenderoh Reservoir which comprises a multi-step schema: 1) Inclusive explorations on NIS fish which include their habitat preference, habitat constraints, biological profile, ecological traits, interaction nature with other species, and their genetic variations within the ecosystems. 2) A continuous periodical monitoring which includes abundance and distribution information on NIS, their dispersal method, identifying species posing the highest threat, identifying species under the highest predation condition, identifying areas within the highest cumulative risk of invasion, and disclosing genetic interactions (if any) with the natives. 3) To make a socio-temporal background which includes mode of continuous introduction, areas and time-frame of introduction, spread velocity of introduced NIS, and making complimentary maps showing significant socio-bio-geographical circumstances.

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