

1 STOMACH CONTENT ANALYSIS (SCA) AND STABLE ISOTOPE ANALYSIS (SIA): A  
2 DUAL APPROACH TO ASSESS DIET AND TROPHIC POSITION (TP) OF *H. macrolepidota*  
3 AND *C. ocellaris* FROM CHENDEROH RESERVOIR, MALAYSIA  
4

## 18 ABSTRACT

19 The present study assessed and compared the diet and trophic positions (TP) of two carnivorous  
20 fish *H. macrolepidota* and *C. ocellaris* from Chenderoh Reservoir, Malaysia. One of the few  
21 reasons for the study was to understand the effects of invasive non-indigenous species (NIS), *C.*  
22 *ocellaris*, on the native indigenous (IS) fish species, *H. macrolepidota*. The assessment was  
23 grounded in stomach content analysis (SCA) and stable isotope analysis (SIA), which  
24 collectively clarified the feeding habits and trophic positions (TP) of these selected fish. The  
25 mean RGL values for *H. macrolepidota* and *C. ocellaris* were  $0.98 \pm 0.18$  and  $1.10 \pm 0.15$  (Mean  
26  $\pm$  SD), respectively, aligning with known ranges for carnivorous fish. These values also clarified  
27 that both species occupy higher TP in the food web as tertiary or quaternary consumers. SCA  
28 findings also revealed that fish and crustaceans were the predominant food categories for *H.*  
29 *macrolepidota*, while *C. ocellaris* predominantly fed on fish. The mean stomach fullness index  
30 (MSF) and the gastroscopic index (GSI) corroborated the differences in the foraging  
31 performance of the fishes, with *C. ocellaris* having a higher MSF (2.03) compared to *H.*  
32 *macrolepidota* (0.65). These implied that *C. ocellaris* had plentiful of food and encountered  
33 fewer diet-related challenges in the ecosystem. From SIA,  $\delta^{13}\text{C}$  values indicated that the primary  
34 carbon sources for both species are C3 plants, particularly aquatic vegetation. Further,  $\delta^{15}\text{N}$   
35 values further ensured that both *H. macrolepidota* and *C. ocellaris* are carnivorous in nature and  
36 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*.

37

## 38 1. INTRODUCTION

39 Unintentional and purposeful introductions of fish species to our freshwater ecosystems have  
40 been a repeated phenomenon since the distant past (Hughes, 2003; Sultana & Hashim, 2016). It  
41 is estimated that approximately 20% of the freshwater fish species of the world are already  
42 extinct or endangered due to non-indigenous (NIS) fish introduction (Hanif *et al.*, 2016). Biotic  
43 homogenization, in other words, the replacement of specific indigenous species (IS) by NIS  
44 (Tabarelli *et al.*, 2012), results in freshwater ecosystems with lower diversity and species  
45 extinction (Rahel, 2002; Drake & Lodge, 2004). Hence, it has become a top priority in the  
46 present era to evaluate the introduction, diversity, distribution, magnitude, and impacts of non-  
47 indigenous (NIS) and invasive alien (IAS) fish species (Sala *et al.*, 2000; Vörösmarty *et al.*,  
48 2010) in freshwater ecosystems.

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

56 Stomach content analysis (SCA) and stable isotope analysis (SIA) are both valuable tools in fish  
57 ecology and food-web research for assessing the diets and trophic positions (TP) of freshwater  
58 fish. SCA offers insights into diet preferences and selections. Therefore, it provides only a  
59 snapshot of a fish's diet over a short period and doesn't account for long-term dietary patterns. In  
60 contrast, SIA is a strategic method that reveals the assimilated diet fraction over a more extended  
61 time frame and also identifies carbon and nitrogen sources in the ecosystem. However, SIA has  
62 its own limitations, as it can't directly pinpoint the specific prey items consumed by fish.  
63 Therefore, combining these methods can offer a more comprehensive understanding of a fish's  
64 trophic role and the larger ecological picture (Woodward & Hildrew, 2002; Renones *et al.*,  
65 2002).

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

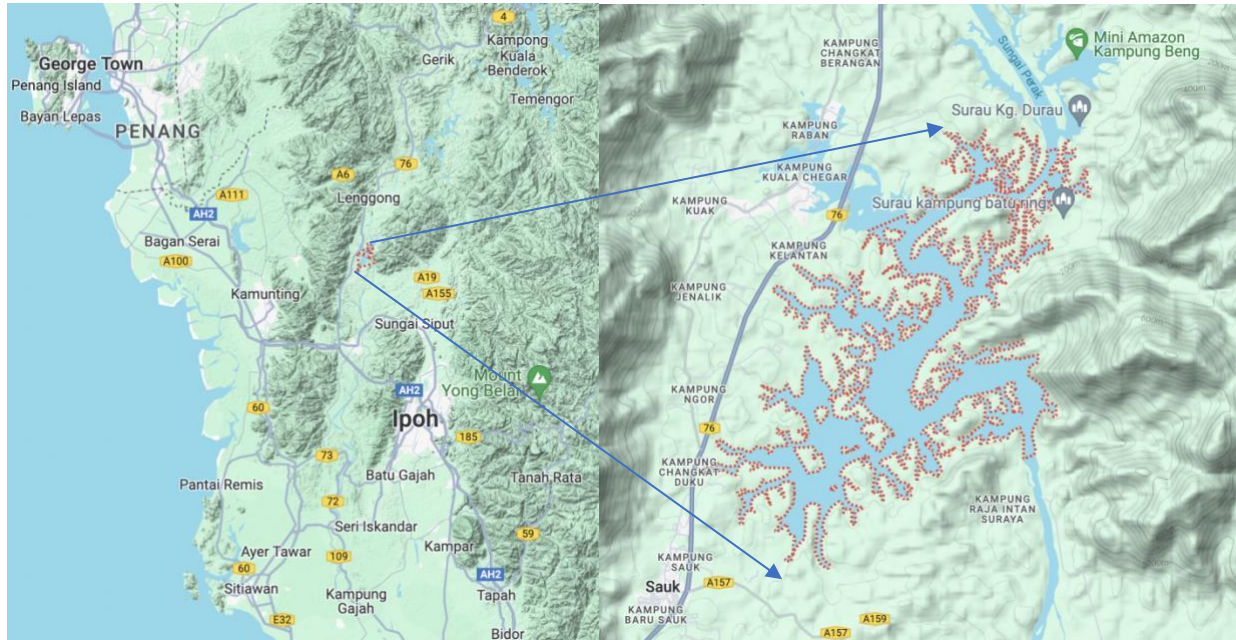
72 This research paper was centered around addressing three specific questions. 1) what does SCA  
73 reveal regarding the diet preference of *H. macrolepidota* and *C. ocellaris* (*i.e.*, identity, quantity,  
74 and size of prey items)? 2) what is the trajectory of isotopic signatures about the food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

77

## 78 **2. MATERIALS AND METHODS**

### 79 **2.1. Location and General Features of Study Area, Chenderoh Reservoir:**

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



84  
 85 **Figure 1:** Study area; Chenderoh Reservoir, Penang, Malaysia. Source: Google Map

86  
 87 **2.2. Fish Sample Collection:**

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

93  
 94 **2.3. Stomach Sample Collection and Preparation For SCA:**

95 The selected fishes for SCA were preserved in formalin straightaway in the field to prevent food  
 96 digestion. Afterward, they were washed off thoroughly before further analysis. Total length (TL),  
 97 standard length (SL), and weight (W) of fish were measured to the nearest 0.1 cm/g. Fishes were  
 98 cautiously dissected to obtain the gut. Gut length (GL) was measured from the esophagus until  
 99 the tip of the anus. Gut weight (GW) was taken with and without its content, while the contents

100 of the stomachs were also measured using an electronic scale to the nearest 0.1 g. Each stomach  
 101 was placed into a sample bottle containing 10% formalin for further observation and analysis.

102

#### 103 **2.4. Fish Fillet Collection and Sample Preparation for SIA:**

104 Fish samples were dissected and filleted at the laboratory to get the white swimming muscle  
 105 (Sweeting *et al.*, 2006) and stored in a frozen state in a deep freezer ( $-20^{\circ}\text{C}$ ) (Kim *et al.*, 2014).  
 106 All fillets were dried in the oven for dehydration at 60 degrees Celcius temperature, ground to a  
 107 fine powder, and divided into two sub-samples. Thus, a total of 54 samples were used for SIA  
 108 which comprised 27 acidified sub-samples and 27 non-acidified sub-samples where all the sub-  
 109 samples were weighted from  $400\mu\text{g}$  to  $500\mu\text{g}$ .

110

#### 111 **2.5. Data Analysis for Stomach Content Analysis (SCA):**

112 **2.5.1. Relative Gut Length (RGL):** The gut length was measured with an accuracy of 0.5 cm in  
 113 order to obtain the relative gut length. RGL was calculated by using the formula given by  
 114 Montgomery (1997):

$$115 \quad RGL = \frac{\text{Gut Length (cm)}}{\text{Standard Length (cm)}}$$

116

117 **2.5.2. Frequency Of Occurrence (%FOC):** To identify diet category and the usage of prey  
 118 resource, frequency of occurrence (%FOC) were calculated for each food item in each selected  
 119 fish species as outlined by Bowen (1996) as:

$$120 \quad [\%] Fi = \frac{Mi}{M\Sigma} \times 100$$

121 Where,  $M_i$  = number of stomachs containing prey component  $i$  and  $M_{\Sigma}$  = number of stomachs  
 122 containing food

123

124 **2.5.3. Mean Stomach Fullness (MSF) Index:** In this study, stomachs were visually assessed  
 125 (Sarkar & Deepak, 2009) for the degree of SF (stomach fullness) using the following numerical  
 126 scale (Carniatto *et al.*, 2014): 0 = empty stomach; 1 = up to 25% SF; 2 = 25% to 75% SF; 3  $\geq$   
 127 75% SF. The value of MSF was calculated as following calculation by Santos (1978):

$$128 \quad MSF = \frac{(N0 \times 0) + (N1 \times 1) + (N2 \times 2) + (N3 \times 3)}{N}$$

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

131

132 **2.5.4. Gastro-Somatic Index (GSI):** The gastro-somatic index indicates the feeding activities  
 133 and foraging performances of fish (Sarkar & Deepak, 2009). In the present study, the gastro-  
 134 somatic index of selected fishes was calculated as:

$$135 \quad \text{GSI} = \frac{GW}{BW} \times 100$$

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

137

## 138 **2.6. Data Interpretation and Analysis for Stable Isotope Analysis (SIA):**

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

$$145 \quad \delta^{13}\text{C} = \left\{ \left( \frac{^{13}\text{C}/^{12}\text{C}_{\text{sample}}}{^{13}\text{C}/^{12}\text{C}_{\text{standard}}} \right) - 1 \right\} \times 1000 \text{ (‰)}$$

$$146 \quad \delta^{15}\text{N} = \left\{ \left( \frac{^{15}\text{N}/^{14}\text{N}_{\text{sample}}}{^{15}\text{N}/^{14}\text{N}_{\text{standard}}} \right) - 1 \right\} \times 1000 \text{ (‰)}$$

147

## 148 **2.7. Trophic Position (TP) of Fish Analysis from $\delta^{15}\text{N}$ :**

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

$$152 \quad TP_{\text{selected consumer}} = \left[ \left( \delta^{15}\text{N}_{\text{selected consumer}} - \delta^{15}\text{N}_{\text{primary consumer}} \right) / 3.4 \right] + 2$$

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

155

## 156 **2.8. Statistical Analysis:**

157 All data were subjected to a normality test by using SPSS (version 19.0). Subsequently, based on  
 158 low  $P$  values ( $P < 0.05$ ) from the test statistics, parametric analysis with permutation was

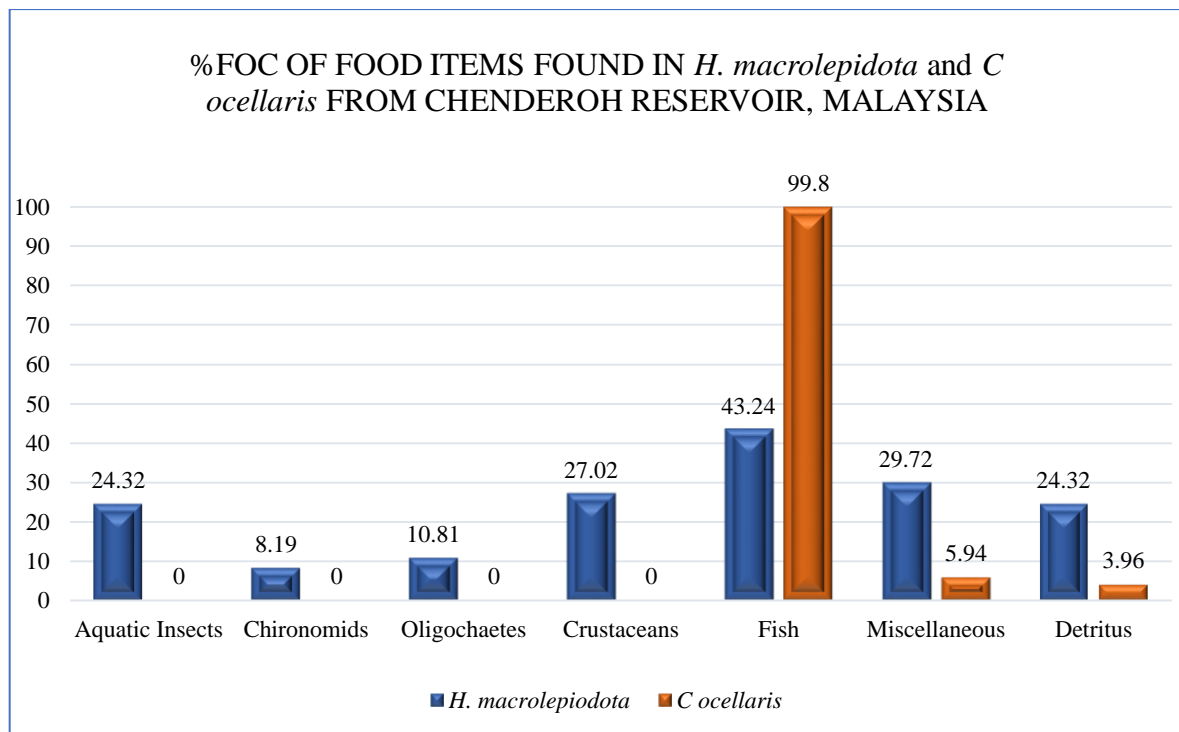
159 performed. Besides, descriptive statistics, Student's t-test, one-way ANOVA, and post-hoc  
 160 analysis were done.

161

### 162 3. RESULTS

#### 163 3.1 Diet Composition of *H. macrolepidota* and *C. ocellaris*:

164 Out of 64 stomachs analyzed for *H. macrolepidota*, 37 had food in them (57.81%) and 27 were  
 165 empty (42.18%) whereas, out of 120 stomachs observed for *C. ocellaris*, 101 were with food  
 166 (84.16%), and the remaining 19 were (15.83%) empty. In the present study, out of seven food  
 167 categories identified for *H. macrolepidota*, fish and crustaceans were the most common items  
 168 with 43.24% and 27.02% occurrence respectively (Fig. 2), while fish fingerlings were the only  
 169 perceived food item in the stomachs of *C. ocellaris* with nearly 100% occurrence. The other  
 170 significant food items of *H. macrolepidota* include aquatic insects (24.32%), Oligochaetes  
 171 (10.81%), and Chironomids (8.19%). Similar findings were observed in a study conducted by  
 172 Makmur (2014) in Indonesia, where *H. macrolepidota* was characterized as a carnivorous fish  
 173 primarily preying on other fish. It was also found to feed on a variety of other organisms,  
 174 including shrimp, crabs, insects, and mollusks (Makmur, 2014).



175

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

177

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

182

### 183 3.2. Categorization of Fish According to RGL:

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

191

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

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

195

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

198

### 199 3.3. Diet Consumption Frequency and Foraging Performance:

200 Subjective methods “mean stomach fullness index” (MSF index) and gastro somatic index (GSI)  
 201 were used in the present study to quantify the diet consumption frequency and foraging

202 performance of selected fishes (Phelps *et al.*, 2007). In this study, the NIS fish *C. ocellaris* had a  
 203 higher MSF value (2.03) than that of *H. macrolepidota* (0.65), which means *C. ocellaris* had  
 204 plenty of food in the system and it could consume its prey without any competition with similar  
 205 species (i.e., *H. macrolepidota*). Garrido *et al.* (2008) also supported this statement while  
 206 working on stomach fullness of Horse Mackerel in Portugal. According to Kihlberg *et al.* (2023),  
 207 the greater MSF value also ensures the establishment and necessary adaptation and spread of any  
 208 NIS fish species in the wild.

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

217

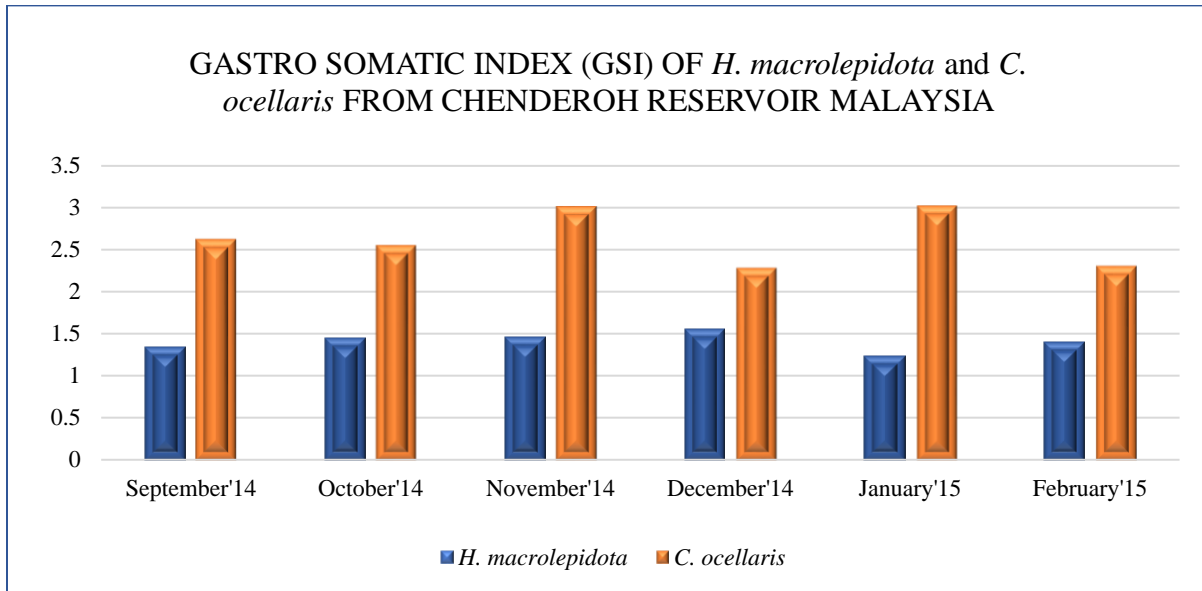
218 **Table 2:** Level of foraging activities of *H. macrolepidota* and *C. ocellaris* according to their  
 219 mean stomach fullness (mean  $\pm$  SD).

Species	Mean stomach fullness (mean $\pm$ SD)						Foraging performance
	Sep'20	Oct'2	Nov'20	Dec'20	Jan'	Feb'20	
<i>C. ocellaris</i>	14	014	14	14	2015	15	Very High
	1.90 $\pm$ 1.33	2.25 $\pm$ 1.06	1.85 $\pm$ 1.13	2.15 $\pm$ 1.03	2.00 $\pm$ 1.13	2.05 $\pm$ 1.14	
<i>H. macrolepidota</i>	0.10 $\pm$ 0.31	0.70 $\pm$ 1.05	0.60 $\pm$ 0.96	0.80 $\pm$ 1.03	0.90 $\pm$ 1.10	0.85 $\pm$ 0.94	Medium

220

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

224 was proved to be true while the GSI index of *C. ocellaris* was compared to the GSI index of *H.*  
 225 *macrolepidota*.



226

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

228

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

236

### 237 **3.4. Trophic Positioning (TP) of Fish According to RGL:**

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

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

247

### 248 **3.5. Carbon Sources of the Reservoirs According to SIA:**

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

253 All the values of  $\delta^{13}\text{C}$  of fishes ranged from (mean  $\pm$  SD)  $-34.3 \pm 0.49\text{‰}$  to  $-23.0 \pm 0.05\text{‰}$   
254 (Table 3). According to Garcia et al. (2007), the isotopic values of carbon ranged from  $-25\text{‰}$  to  $-$   
255  $19\text{‰}$  indicating that the carbon sources of the water body are C3 plants. Similar studies suggest  
256 alike clarifications of carbon sources of water bodies (Larsen *et al.*, 2009; Daniele *et al.*, 2012)  
257 for tropical lakes and reservoirs. Here, the  $\delta^{13}\text{C}$  values for *H. macrolepidota* ranged from  $-35.318$   
258  $\pm 0.088\text{‰}$  to  $-34.096 \pm 0.250\text{‰}$ ; whereas,  $\delta^{13}\text{C}$  values for *C. ocellaris* ranged from  $-33.624 \pm$   
259  $0.466\text{‰}$  to  $-32.182 \pm 1.376\text{‰}$ . The values of isotopic carbon from the SIA interpretation  
260 indicated that the main energy source of the reservoirs is emergent, submerged, floating, or  
261 exotic C3 plants using the C3 photosynthetic pathway (diatoms, cyanobacteria, freshwater algae,  
262 macrophytes). Yap et al. (2016) also made a similar statement while working on Chenderoh  
263 Reservoir. However, to affirm the species diversity, abundance, and distribution of the primary  
264 producers, further and detailed research is required.

265

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

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

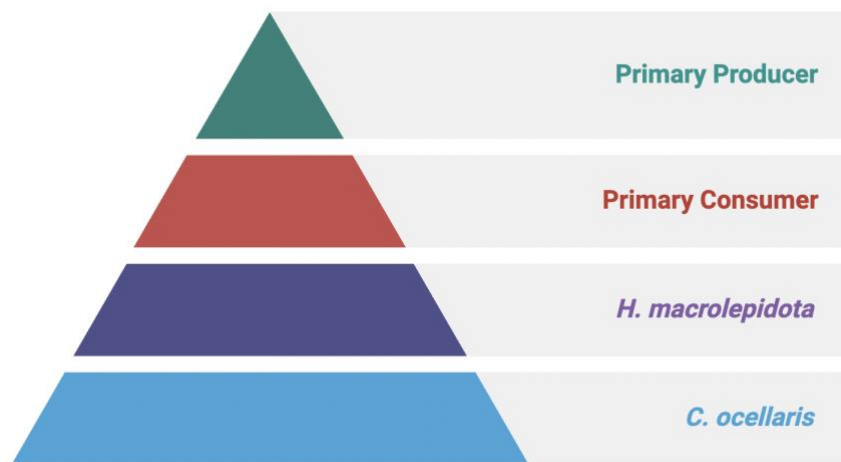
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### 268 **3.6. Trophic Positioning (TP) of Fish According to $\delta^{15}\text{N}$ :**

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

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

274 In present study, the  $\delta^{15}\text{N}$  values for *H. macrolepidota* and *C. ocellaris* ranged from  $9.202 \pm$   
 275  $0.173\text{‰}$  to  $11.385 \pm 1.380\text{‰}$  and from  $12.690 \pm 0.231\text{‰}$  to  $13.356 \pm 0.396\text{‰}$  respectively  
 276 (Table 3), and the mean values calculated of  $\delta^{15}\text{N}$  of *H. macrolepidota* and *C. ocellaris* are  
 277  $10.157 \pm 1.125\text{‰}$  and  $13.044 \pm 0.29\text{‰}$  respectively. Moreover, the trophic level calculated for *C.*  
 278 *ocellaris* (TP = 4.2) (Fig. 4) suggested that this species occupies an upper TP (*i.e.*, tertiary or  
 279 quaternary consumer level) and are high-order carnivore, more specifically defined as a  
 280 piscivore. TP identified for *H. macrolepidota* was 3.4, which means this species also occupies an  
 281 upper TP in the ecosystem (*i.e.*, secondary consumer level) and are carnivore. A similar study  
 282 conducted by Yap et al. (2016) also suggested that *H. macrolepidota* and *C. ocellaris* from the  
 283 Chenderoh Reservoir occupy higher trophic positions (TP) as secondary or tertiary consumers  
 284 within the food web of the reservoir.



285  
 286 **Figure 4:** Schematic Trophic Positioning (TP) of *H. macrolepidota* and *C. ocellaris*.

287  
 288 **4. DISCUSSION**

289 *Cicla ocellaris* is a ravenous predator and feeds extensively on fish; therefore, it has the potential  
 290 to modify the diversity and distribution of a habitat to which it is introduced (Gomiero & Braga  
 291 2004; Novaes *et al.*, 2004). Moreover, it can generate negative environmental impacts by  
 292 competing with similar native species for food and space, preying on juveniles and eggs, and  
 293 disrupting the habitat by grazing on detritus and benthic algae (Canonico *et al.*, 2005; Ansa *et*

294 *al.*, 2014). These threats include flow modification, habitat alteration (Johnson *et al.*, 2008),  
295 overexploitation, pollution (Dudgeon *et al.*, 2006), and overall environmental modification  
296 (Kiruba-Sankar *et al.*, 2018), which all can lead to biodiversity degradation of a particular  
297 ecosystem. Several similar studies have demonstrated a significant decline in fish densities over  
298 the past two decades, with a particular impact on species like *Hampala macrolepidota* in riverine  
299 systems, especially the Perak River system of Malaysia (Department of Fisheries, 2005; Radhi *et*  
300 *al.*, 2017; Ambak & Mohd Zaidi, 2010).

301 In a recent study conducted in Perlis, Malaysia, the Peacock Bass was reported to exert  
302 significant predation pressure on the fry of Tinfoil Barb (*B. schwanefeldii*) (Saba *et al.*, 2021).  
303 Notably, it was observed that the Peacock Bass heavily preyed upon approximately 50,000 fry of  
304 Tinfoil Barb, which had been intentionally released into Timah Tasoh Lake, Perlis by the  
305 Department of Fisheries (DOF) Malaysia with the aim of enhancing the fish population within  
306 the lake. This predation highlights the potential impact of Peacock Bass on native fish  
307 populations and the challenges posed by NIS in local ecosystems (Isa *et al.*, 2012; Zulkefli,  
308 2017).

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

324 Physical barriers, such as dams, are known to enhance the potential for the establishment of NIS  
325 in ecosystems (Rahel & Olden, 2008). Within the context of the Perak River system in Malaysia,  
326 a series of cascading hydroelectric dams can be observed (Mohd Sidek *et al.*, 2020), namely,  
327 Temengor Dam, Bersia Dam, Kenering Dam, and Chenderoh Dam. Chenderoh Reservoir, being  
328 the terminal reservoir in this series of dams from upstream to downstream along the Perak River  
329 system, is particularly susceptible to increased water turbidity originating from inflows from the  
330 upstream reservoirs (Zarul, 2013). This elevated turbidity as well as mineralization of the water  
331 may significantly raise the likelihood of NIS fish invasion in Chenderoh reservoir. Nyanti *et al.*  
332 (2021) have also demonstrated the impact of cascading dams on the development of complex  
333 ecosystems, such as those observed in the Murum River, Malaysia. This study indicates a  
334 transitional zone in the Murum River, displayed reduced fish species diversity, richness, and  
335 evenness, particularly degraded water quality with high turbidity, which may facilitate the  
336 establishment of NIS species (Tan & Rohasliney, 2013).

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

343

## 344 **5. CONCLUSION**

345 Based on the findings of the present study, it is evident that both *H. macrolepidota* and *C.*  
346 *ocellaris* in Chenderoh Reservoir, Malaysia, are carnivorous fish occupying higher trophic  
347 positions in the ecosystem. Through a combination of stomach content analysis (SCA) and stable  
348 isotope analysis (SIA), the feeding habits and trophic positions of these species were elucidated.  
349 The mean relative gut length (RGL) values indicated that both species are tertiary or quaternary  
350 consumers, with *C. ocellaris* exhibiting a slightly higher RGL compared to *H. macrolepidota*.  
351 SCA results revealed distinct differences in foraging preferences, with *H. macrolepidota*  
352 predominantly feeding on fish and crustaceans, while *C. ocellaris* primarily consumed fish.  
353 Additionally, the mean stomach fullness index (MSF) and gastrosomatic index (GSI) highlighted  
354 variations in the foraging performance between the two species, with *C. ocellaris* exhibiting

355 higher MSF values, suggesting a more abundant food supply and potentially fewer diet-related  
356 challenges. Furthermore, stable isotope analysis (SIA) results indicated that both species  
357 primarily derive their carbon sources from C3 plants, particularly aquatic vegetation, while  $\delta^{15}\text{N}$   
358 values confirmed their carnivorous nature and higher trophic positions in the ecosystem. Overall,  
359 these findings contribute to a better understanding of the dietary dynamics and trophic  
360 interactions between native and non-indigenous fish species in Chenderoh Reservoir, providing  
361 valuable insights for the conservation and management of aquatic ecosystems facing the  
362 challenges of invasive species.

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

377 Future research potential includes Research on biological invasions particularly freshwater  
378 invasions by NIS and IAS fish needed to be documented for each and every state of Malaysia.  
379 Besides, existing statistical data of NIS and IAS should be updated on a periodic basis to get a  
380 clear picture of their quantity, diversity, distribution, and effects. We should look through every  
381 angle to understand the threat posed by IAS. Only a few basic records are not adequate for  
382 understanding the biology of invasion and the impacts of IAS in the freshwater ecosystems of  
383 this country. Therefore, comprehensive research and documentation are mostly needed. Also,  
384 primary and secondary assessments are needed for all freshwater ecosystems, such as lakes,  
385 rivers, reservoirs, and inland water bodies of Malaysia. These assessments include water quality,

386 ecological condition, ecosystem evaluation, anthropogenic activities in and out of the water  
387 bodies, and so on. There is an indication in invasion ecology that beyond the biological invasions  
388 in any ecosystem, there are unswerving stimuli of native species diversity and abundance. As can  
389 be grasped from the worldwide NIS distribution, where there is a high abundance of freshwater  
390 fish present, the NIS and IAS fish species also emerged therein. Therefore, together with the  
391 information on introduced species, vigorous documents about the diversity and distribution of  
392 native fish species of the broad aquatic networks are crucial. Moreover, while molecular and  
393 genetic technologies can significantly enhance our ability to assess and manage the threat posed  
394 by IAS, baseline molecular data are remarkably limited for both NIS and IAS fish in Malaysia.  
395 This inadequacy includes genetic diversity, genome sequencing, stable isotope signatures, and  
396 similar molecular studies. The shortage of baseline molecular data highlights the necessity for  
397 comprehensive research in these areas to improve our understanding of these species and their  
398 impacts on native biodiversity. Finally, elaborate research on NIS fish is required for Chenderoh  
399 Reservoir which comprises a multi-step schema: 1) Inclusive explorations on NIS fish which  
400 include their habitat preference, habitat constraints, biological profile, ecological traits,  
401 interaction nature with other species, and their genetic variations within the ecosystems. 2) A  
402 continuous periodical monitoring which includes abundance and distribution information on  
403 NIS, their dispersal method, identifying species posing the highest threat, identifying species  
404 under the highest predation condition, identifying areas within the highest cumulative risk of  
405 invasion, and disclosing genetic interactions (if any) with the natives. 3) To make a socio-  
406 temporal background which includes mode of continuous introduction, areas and time-frame of  
407 introduction, spread velocity of introduced NIS, and making complimentary maps showing  
408 significant socio-bio-geographical circumstances.

409

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