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Assessing Diet & Trophic Position of Fish in Chenderoh Reservoir, Malaysia: SCA & SIA Approach

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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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. The focal goal of the study was to understand the effects of invasive non-indigenous species (NIS), *C. ocellaris,* on the native indigenous (IS) fish species, *H. macrolepidota*. Data were acquired from September 2014 to February 2015 within the study area. The assessment was grounded in stomach content analysis (SCA) and stable isotope analysis (SIA), which collectively clarified the feeding habits and trophic

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positions (TP) of these selected fish. In total, 184 fish samples (comprising 64 individuals of *H. macrolepidota* and 120 individuals of *C. ocellaris*) underwent stomach content analysis (SCA). Additionally, 24 individuals (12 of *H. macrolepidota* and 12 of *C. ocellaris*), sampled from December 2014 to February 2015, were selected for stable isotope analysis (SIA). 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 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 [1,2]. 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 [3]. Biotic homogenization, in other words, the replacement of specific indigenous species (IS) by NIS [4], results in freshwater ecosystems with lower diversity and species extinction [5,6]. 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 [7,8] 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 [9]. 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 [10]. 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 [11,12].

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 from September 2014 to February 2015 in Chenderoh Reservoir, a man-made reservoir on the Perak River, Malaysia (4^{o}58'N, 100^{o}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 [2] (Fig. 1).

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

The white dorsal muscle tissue of fish was collected from each selected fish for analysis (Thomas & Cahoon, 1993; Chipps & Garvey, 2007). This is because muscle- turnover rate for that part is longer than those of other parts as well as liver, and blood (Tieszen et. al., 1983). Besides, in temperate fishes, lipid concentration in white muscle is generally low and this tissue was demonstrated to be the most suitable for stable isotope analysis (Pinnegar & Polunin, 1999; Cresson et al., 2014). Therefore fish samples were dissected cautiously and filleted at laboratory to get the white dorsal muscle tissue and stored in a frozen state in the deep freezer (–20oC) [13] with its remaining body parts until isotopic analysis.

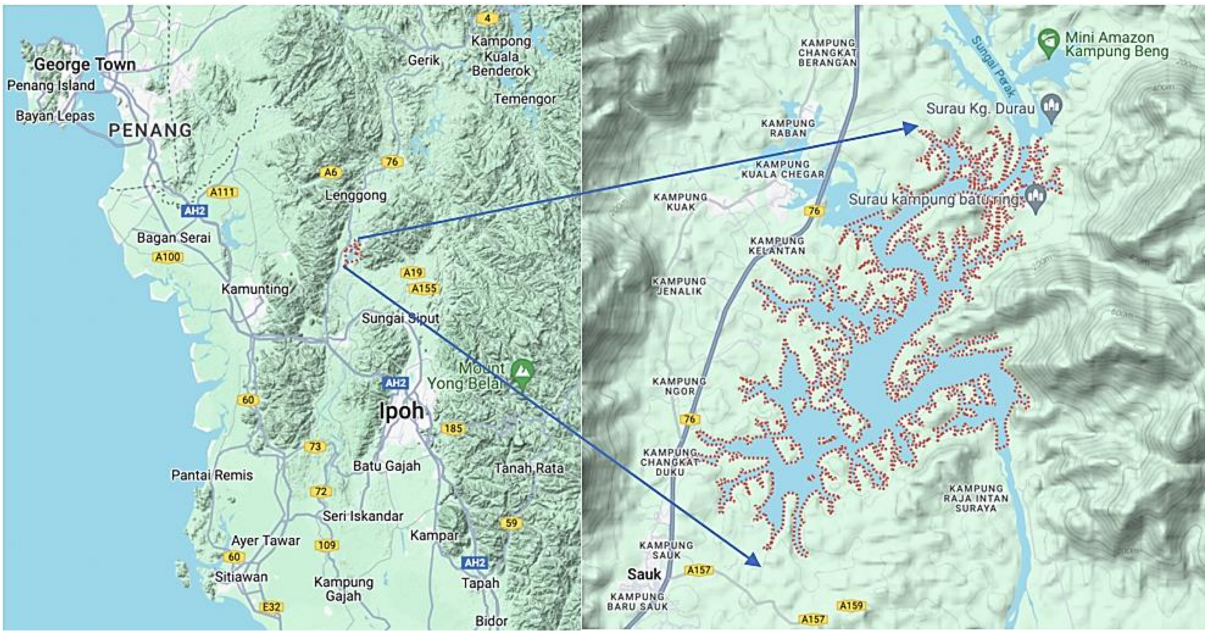

Fig. 1. Study area; Chenderoh Reservoir, Penang, Malaysia. Source: Google Map

All fillets were dried in an oven for dehydration at 60oC until a constant weight (Chipps & Garvey, 2007). Afterwards, the samples were grounded to a fine powder with an agate mortar and pestle. Two replicates of every classified (species × month × location) sample were planned to use for SIA. Therefore, the sample powders were divided equally into two subsamples (Carabel et al., 2006), where all the subsamples were weighted from 400 µg to 500 µg. Prior to stable isotope analysis, about 0.8 to 10 mg of samples was filled into small tin capsules (8 x 5 mm) in triplicates (Chipps & Garvey, 2007). These samples were then folded and compressed before being loaded into an auto-sampler.

Stable isotope analysis was performed at the laboratory of ABRC (Analytical Biochemistry Research Centre) of Universiti Sains Malaysia. For the analysis of stable carbon and nitrogen isotopic composition (δ13C and δ15N), Flash EA 2000 elemental analyzer (Thermo Scientific, Waltham, MA) coupled to a Delta V Advantage isotope ratio mass spectrometer (Thermo, Milan, Italy) was used in laboratory. Raw isotope ratios from the analysis were then normalized to the international scales using USGS-40 and USGS-41 reference materials (~0.5 mg, respectively) assayed with the unknown samples. Urea (IVA-Analysentechnik GmbH & Co., Germany) was used as a quality control material to correct for drift and was measured for every 12 samples with known values of δ13C = -40.81‰ and δ15N = -0.49‰. The typical precision for the triplicate samples was ±0.2‰ for δ13C and ±0.3‰ for δ15N.

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 [14]:

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

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 [15] as:

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

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 [16] for the degree of SF (stomach fullness) using the following numerical scale [17]: 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 [16]. In the present study, the gastro-somatic index of selected fishes was calculated as:

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

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 [18] described by Hobson et al. [19]:

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

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 [20] 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 [20].

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 [63]. 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 [21] for carnivorous fish (0.2 to 2.5) and the RGL values for *C. ocellaris* were close to those found by Pouilly et al. [22] for neotropical piscivores (0.93 to 1.23). Therefore, this can be concluded from the study that both of the species are carnivores.

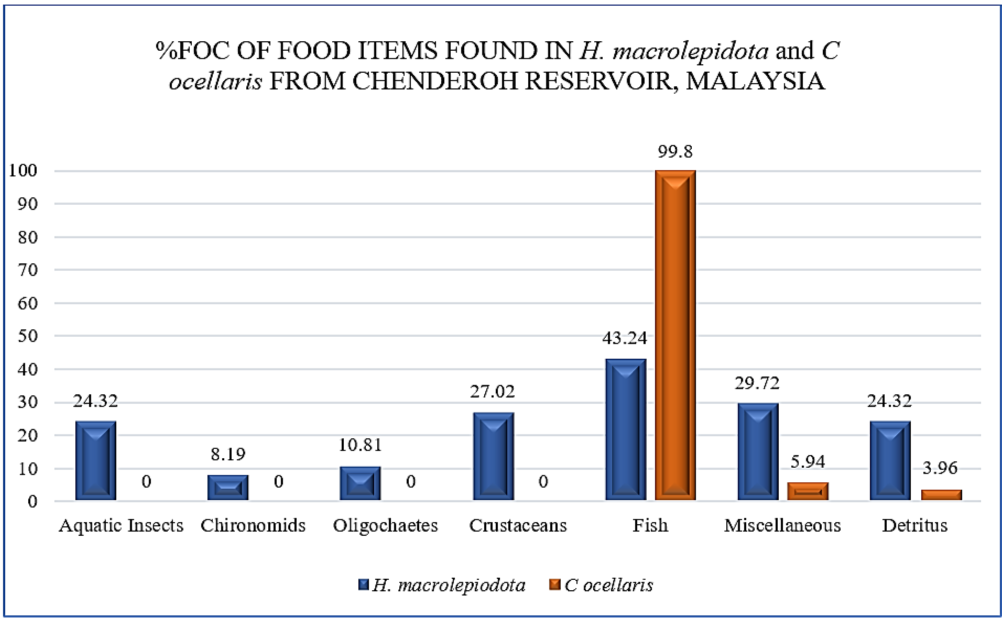

Fig. 2. Percentage of identified food items observed in the stomachs of *H. macrolepidota.*

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 [23,24]. 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. [25] also supported this statement while working on stomach fullness of Horse Mackerel in Portugal. According to Kihlberg et al. [26], 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.

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) [27]. This proclamation was proved to be true while the GSI index of *C ocellaris* was compared to the GSI index of *H. macrolepidota.*

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 [28], 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 [29,30]. 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. [31], conducted in the Chenderoh reservoir.

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

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

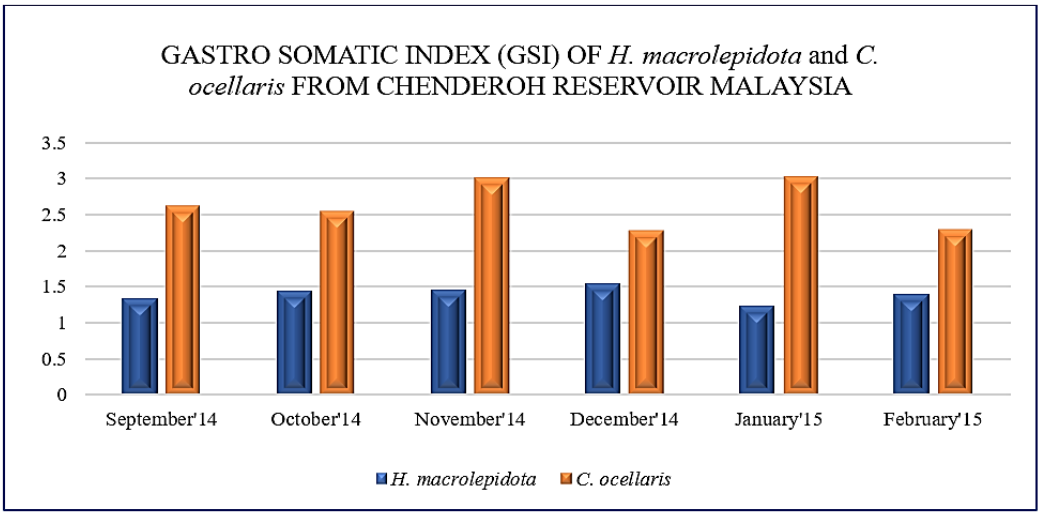

Fig. 3. Monthly Gastro Somatic Index (GSI) of *H. macrolepidota* and *C. ocellaris*

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 [32,33]. 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. [34], 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 [35,36] 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. [31] 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.

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 [13]. 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 $\delta^{15}N$ 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 $\delta^{15}N$ 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. [31] 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.

Table 3. Monthly δ^{13}C and δ^{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

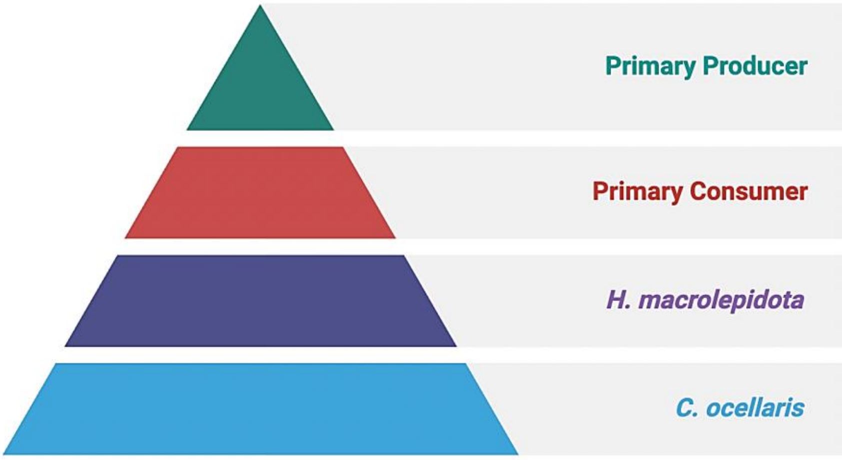

Fig. 4. Schematic Trophic Positioning (TP) of *H. macrolepidota* and *C. ocellaris*.

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 [27,28]. 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 [37,38]. These threats include flow modification, habitat alteration [39], overexploitation, pollution [40], and overall environmental modification [41], 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 [42,43,44].

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*) [45]. 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 [46,47].

The potential for the establishment of NIS is strongly influenced by environmental factors also, [48,49,50] and is often associated with disturbances [51,52]. 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 [53]. For instance, degraded water quality in Chenderoh Reservoir, as evidenced by Ismail et al. [54], 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 [54], 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 [55,56,57,58].

Physical barriers, such as dams, are known to enhance the potential for the establishment of NIS in ecosystems [59]. 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 [60]. 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. [61] 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 [62,63].

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 and both species primarily derive their carbon sources from C3 plants. 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 $\delta^{15}N$ 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 $\delta^{15}N$ 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. [19], and Pauly [64]. And, finally, the study excluded considerations of seasonal variation, age, sex of fishes, and spatial influences in the stomach content analysis [65-68]. As a result, the precise feeding capacity and feeding behaviors of the selected fishes could not be precisely determined.

Research on freshwater invasions by non-indigenous and invasive fish in Malaysia is crucial. Regular updates to statistical data are necessary to understand the extent and impact of these invasions. Comprehensive documentation and research are essential to grasp the biology of invasion and the effects of invasive species on freshwater ecosystems. Assessments of various freshwater bodies, including lakes, rivers, and reservoirs, are needed to evaluate water quality, ecological conditions, and anthropogenic activities [69-70]. Understanding the distribution and diversity of both native and introduced fish species is vital for effective management.

Molecular and genetic technologies can enhance our ability to manage invasive species, but baseline data for Malaysian fish species are limited. More research is needed to gather genetic information and understand the impacts of invasive species on native biodiversity. In particular, detailed research on non-indigenous fish in Chenderoh Reservoir should include habitat preferences, ecological traits, and genetic variations. Continuous monitoring is necessary to track abundance, distribution, and interactions with native species. Socio-temporal background information, such as introduction methods and spread velocity, is also crucial for effective management strategies.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Hughes JD. Europe as consumer of exotic biodiversity: Greek and Roman times. Landscape Research. 2003;28(1):21-31.
2. Sultana M, Hashim ZH. Invasive alien fish species in freshwater of the continents. Journal of Environmental Science and Natural Resources. 2015;8(2):63-74.
3. Hanif MA, Siddik MAB, Nahar A, Chaklader MR, Rumpa RJ, Alam MJ, Mahmud S. The current status of small indigenous fish species (SIS) of River Gorai, a distributary of the river Ganges. Bangladesh. J Biodivers Endanger Species. 2016;4(2).
4. Tabarelli M, Peres CA, Melo FP. The ‘few winners and many losers’ paradigm revisited: Emerging prospects for tropical forest biodiversity. Biological Conservation. 2012;155:136-140.
5. Rahel FJ. Homogenization of freshwater faunas. Annual review of ecology and systematics. 2002 Nov;33(1):291-315.
6. Drake JM, Lodge DM. Global hot spots of biological invasions: Evaluating options for ballast–water management. Proceedings of the Royal Society of London. Series B: Biological Sciences. 2004;271(1539):575-580.
7. Sala OE, Stuart Chapin FIII, Armesto JJ, Berlow E, Bloomfield J, Dirzo R, Wall DH. Global biodiversity scenarios for the year 2100. Science. 2000;287(5459):1770-1774.
8. Vörösmarty CJ, McIntyre PB, Gessner MO, Dudgeon D, Prusevich A, Green P, Davies PM. Rivers in crisis: Global water insecurity for humans and biodiversity. Nature. 2010;467(7315):555-561.
9. Rahim KAA, Esa Y, Arshad A. The influence of alien fish species on native fish community structure in Malaysian waters. Kuroshio Science. 2013;7(1):81-93.
10. Davis AM, Blanchette ML, Pusey BJ, Jardine TD, Pearson RG. Gut content and stable isotope analyses provide complementary understanding of ontogenetic dietary shifts and trophic relationships among fishes in a tropical river. Freshwater Biology. 2012;57(10): 2156-2172.
11. Woodward GUY, Hildrew AG. Food web structure in riverine landscapes. Freshwater Biology. 2002;47(4):777-798.
12. Renones O, Polunin NVC, Goni R. Size related dietary shifts of *Epinephelus marginatus* in a western Mediterranean littoral ecosystem: An isotope and stomach content analysis. Journal of Fish Biology. 2002;61(1):122-137.
13. Kim SJ, Park SJ, Cha IT, Min D, Kim JS, Chung WH, Rhee SK. Metabolic versatility of toluene-degrading, iron-reducing bacteria in tidal flat sediment, characterized by stable isotope probing-based metagenomic analysis. Environmental Microbiology. 2014;16(1): 189-204.
14. Montgomery JC, Hamilton AR. Sensory contributions to nocturnal prey capture in the dwarf scorpion fish (*Scorpaena papillosus*). Marine and Freshwater Behaviour and Phy. 1997;30(4):209-223.
15. Bowen SH. Quantitative description of the diet. 513–532. Fisheries techniques. BR Murphy and DW Willis. Second edition. American Fisheries Society. Bethesda, Maryland; 1996.
16. Sarkar UK, Deepak PK, Negi RS. Length–weight relationship of clown knifefish Chitala chitala (Hamilton 1822) from the River Ganga basin, India. Journal of Applied Ichthyology. 2009;25(2):232-233.
17. Carniatto N, Fugi R, Thomaz SM, Cunha ER. The invasive submerged macrophyte Hydrilla verticillata is a foraging habitat for small-sized fish. Natureza and Conservação. 2014;12(1):30-35.
18. Brasso RL, Polito MJ. Trophic calculations reveal the mechanism of population-level variation in mercury concentrations between marine ecosystems: Case studies

of two polar seabirds. Marine Pollution Bulletin. 2013;75(1-2):244-249.
19. Hobson KA, Piatt JF, Pitocchelli J. Using stable isotopes to determine seabird trophic relationships. Journal of Animal Ecology. 1994;786-798.
20. Makmur S, Arfiati D, Bintoro G, Ekawati AW. Food habit of hampala (*Hampala macrolepidota* Kuhl and Van Hasselt 1823) and its position in food web, food pyramid and population equilibrium of ranau lake, Indonesia. Journal of Biodiversity and Environmental Sciences (JBES). 2014; 4(6):167-177.
21. Bertin L. Appareil digestif. Traité de Zoologie. 1958;13(2):1249-1301.
22. Pouilly M, Miranda G. Morphology and reproduction of the cavefish Trichomycterus chaberti and the related epigean Trichomycterus cf. barbouri. Journal of Fish Biology. 2003;63(2):490-505.
23. Phelps QE, Willis DW, Powell KA, Chipps SR. A method for determining stomach fullness for planktivorous fishes. North American Journal of Fisheries Management. 2007;27(3):932-935.
24. Suvarna Devi S, Sivan A. Diet diversity in tetraodontid fish, *Lagocephalus spadiceus* (Richardson) using Conventional and DNA barcoding approaches. Journal of Aquatic Biology and Fisheries. 2017;5:203-209.
25. Garrido S, Ben-Hamadou R, Oliveira PB, Cunha ME, Chícharo MA, Van der Lingen CD. Diet and feeding intensity of sardine Sardina pilchardus: Correlation with satellite-derived chlorophyll data. Marine Ecology Progress Series. 2008;354:245-256.
26. Kihlberg IW, Florin AB, Lundström K, Östman Ö. Detection of multiple fish species in the diet of the invasive round goby reveals new trophic interactions in the Baltic Sea. Aquatic Invasions. 2023;18(2):141-162.
27. Gomiero LM, Braga FMDS. Reproduction of species of the genus Cichla in a reservoir in southeastern Brazil. Brazilian Journal of Biology. 2004;64:613-624.
28. Novaes JLC, Caramaschi EP, Winemiller KO. Feeding of Cichla monoculus Spix, 1829 (Teleostei: Cichlidae) during and after reservoir formation in the Tocantins River, Central Brazil. Acta Limnologica Brasiliensia. 2004;16(1):41-49.
29. Elliott JP, Bellwood DR. Alimentary tract morphology and diet in three coral reef fish families. Journal of Fish Biology. 2003; 63(6):1598-1609.
30. Karachle PK, Stergiou KI. Food and feeding habits of nine elasmobranch species in the N Aegean Sea. Rapp Comm int Mer Médit. 2010;39:553.
31. Yap SK, Muneera I, Syakir MI, Zarul HH, Widad FJIJ. Stable isotopes approach to infer the feeding habit and trophic position of freshwater fishes in tropical lakes. Iranian (Iranica) Journal of Energy and Environment. 2016;7(2):177-183.
32. Finlay JC. Stable-carbon-isotope ratios of river biota: Implications for energy flow in lotic food webs. Ecology. 2001;82(4):1052-1064.
33. Syväranta J, Högmander P, Keskinen T, Karjalainen J, Jones RI. Altered energy flow pathways in a lake ecosystem following manipulation of fish community structure. Aquatic Sciences. 2011;73:79-89.
34. Garcia AM, Hoeinghaus DJ, Vieira JP, Winemiller KO. Isotopic variation of fishes in freshwater and estuarine zones of a large subtropical coastal lagoon. Estuarine, Coastal and Shelf Science. 2007;73(3-4):399-408.
35. Larsen T, Taylor DL, Leigh MB, O'Brien DM. Stable isotope fingerprinting: A novel method for identifying plant, fungal, or bacterial origins of amino acids. Ecology. 2009;90(12):3526-3535.
36. Daniele G, Maitre D, Casabianca H. Identification, quantification, and carbon stable isotopes determinations of organic acids in monofloral honeys. A powerful tool for botanical and authenticity control. Rapid Communications in Mass Spectrometry. 2012;26(17):1993-1998.
37. Canonico GC, Arthington A, McCrary JK, Thieme ML. The effects of introduced tilapias on native biodiversity. Aquatic Conservation: Marine and Freshwater Ecosystems. 2005;15(5):463-483.
38. Ansah YB, Frimpong EA, Hallerman EM. Genetically-improved tilapia strains in Africa: Potential benefits and negative impacts. Sustainability. 2014;6(6):3697-3721.
39. Johnson BM, Martinez PJ, Hawkins JA, Bestgen KR. Ranking predatory threats by nonnative fishes in the Yampa River, Colorado, via bioenergetics modeling. North American Journal of Fisheries Management. 2008;28(6):1941-1953.

40. Dudgeon D, Arthington AH, Gessner MO, Kawabata ZI, Knowler DJ, Lévêque C, Sullivan CA. Freshwater biodiversity: Importance, threats, status and conservation challenges. Biological Reviews. 2006;81(2):163-182.
41. Kiruba-Sankar R, Raj JP, Saravanan K, Kumar KL, Angel JRJ, Velmurugan A, Roy SD. Invasive species in freshwater ecosystems–threats to ecosystem services. In Biodiversity and climate change adaptation in tropical Islands. Academic Press. 2018;257-296.
42. Department of Fisheries, – Annual Fisheries Statistics; 2005.
43. Radhi AM, Rohasliney H, Zarul H. Fish composition and diversity in Perak, Galas and Kelantan rivers (Malaysia) after the major flood of 2014. Transylvanian Review of Systematical and Ecological Research. 2017;19(3):41-56.
44. Ambak MA, Zakaria MZ. Freshwater fish diversity in Sungai Kelantan. Journal of Sustainability Science and Management. 2010;5(1):13-20.
45. Saba AO, Ismail A, Zulkifli SZ, Shohaimi S, Amal MNA. Public knowledge and perceptions of the impacts and importance of alien fish species in Malaysia: Implications for freshwater biodiversity and conservation. Management of Biological Invasions. 2021;12(2):441.
46. Isa MM, Basri MNA, Zawawi MZM, Yahya K, Nor SAM. Length-weight relationships of some important estuarine fish species from Merbok Estuary, Kedah. Journal of Natural Sciences Research. 2012;2(2):8-19.
47. Zulkefli AF. Alien fish invasion: Peacock bass taking over. New Strait Times; 2017.
48. Herborg LM, Jerde CL, Lodge DM, Ruiz GM, MacIsaac HJ. Predicting invasion risk using measures of introduction effort and environmental niche models. Ecological Applications. 2007;17(3):663-674.
49. Kilroy C, Snelder TH, Floerl O, Vieglais CC, Dey KL. A rapid technique for assessing the suitability of areas for invasive species applied to New Zealand's rivers. Diversity and Distributions. 2008; 14(2):262-272.
50. Kulhanek SA, Ricciardi A, Leung B. Is invasion history a useful tool for predicting the impacts of the world's worst aquatic invasive species? Ecological Applications. 2011;21(1):189-202.
51. Minchinton TE. Disturbance by wrack facilitates spread of Phragmites australis in a coastal marsh. Journal of Experimental Marine Biology and Ecology. 2002;281(1-2):89-107.
52. Marchetti MP, Light T, Moyle PB, Viers JH. Fish invasions in California watersheds: Testing hypotheses using landscape patterns. Ecological Applications. 2004; 14(5):1507-1525.
53. Diagne C, Leroy B, Vaissière AC, Gozlan RE, Roiz D, Jarić I, Courchamp F. High and rising economic costs of biological invasions worldwide. Nature. 2021;592(7855):571-576.
54. Ismail SN, Subehi L, Mansor A, Mashhor M. Invasive Aquatic Plant Species of Chenderoh Reservoir, Malaysia and Jatiluhur Reservoir, Indonesia. In IOP Conference Series: Earth and Environmental Science. IOP Publishing. 2019;380(1):012004.
55. Kolar CS, Lodge DM. Progress in invasion biology: Predicting invaders. Trends in Ecology and Evolution. 2001;16(4):199-204.
56. Marchetti MP, Moyle PB, Levine R. Invasive species profiling? Exploring the characteristics of non-native fishes across invasion stages in California. Freshwater Biology. 2004;49(5):646-661.
57. Funk JL, Cleland EE, Suding KN, Zavaleta ES. Restoration through reassembly: Plant traits and invasion resistance. Trends in Ecology and Evolution. 2008;23(12):695-703.
58. Barrett SC. Why reproductive systems matter for the invasion biology of plants. Fifty years of invasion ecology: The legacy of Charles Elton. 2011;1:195-210.
59. Rahel FJ, Olden JD. Assessing the effects of climate change on aquatic invasive species. Conservation Biology. 2008;22(3): 521-533.
60. Zarul H. Limnological influence of dams placed in series along the Perak River, Malaysia, Mississippi State University. 2013;108.
61. Nyanti L, Soo CL, Chundi AY, Lambat ECD, Tram A, Ling TY, Lee KSP. Patterns of Fish Assemblage, Growth, and Diet Composition in a Tropical River between Two Cascading Hydropower Dams. International Journal of Ecology, 2021;1-10.

62. Tan PY, Rohasliney H. Status of water quality subject to sand mining in the Kelantan River, Kelantan, Tropical Life Sciences Research. Search in. 2013;24: 19-34.
63. Ahmed I, Azra BANO, Siddique S. Relative gut length and gastro-somatic index of Acanthopagrus arabicus (Iwatsuki, 2013) from the Offshore Waters of Pakistan. Natural and Engineering Sciences. 2022; 7(1):67-79.
64. Pauly D. Tropical fishes: Patterns and propensities. Journal of Fish Biology. 1998; 53:1-17.
65. Cortés E. Standardized diet compositions and trophic levels of sharks. ICES Journal of Marine Science. 1999;56(5):707-717.
66. DeNiro MJ, Epstein S. Influence of diet on the distribution of nitrogen isotopes in animals. Geochimica Et Cosmochimica Acta. 1981;45(3):341-351.
67. Eduardo Lima Junior S, Goitein R. A new method for the analysis of fish stomach contents. Acta Sci. Biol. Sci. 2001;421-424.
68. Hynes HBN. The food of fresh-water sticklebacks (Gasterosteus aculeatus and Pygosteus pungitius), with a review of methods used in studies of the food of fishes. The Journal of Animal Ecology. 1950;36-58.
69. Sajeevan MK, Kurup BM. Evaluation of feeding indices of cobia Rachycentron canadum (Linnaeus. 1766) from northwest coast of India. J. Mar. Biol. Ass. India. 2013;55(2):5-10.
70. Sweeting CJ, Polunin NVC, Jennings S. Effects of chemical lipid extraction and arithmetic lipid correction on stable isotope ratios of fish tissues. Rapid Communications in Mass Spectrometry: An International Journal Devoted to the Rapid Dissemination of Up-to-the-Minute Research in Mass Spectrometry. 2006; 20(4):595-601.systematics. 2006;33(1): 291-315.

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