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Original Article

Prey identification of invasive peacock bass from Telabak Lake Malaysia using DNA barcoding technique

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Abstract: Invasive peacock bass *Cichla* spp. have recently invaded freshwater habitats across Malaysia. Stomach contents of 135 peacock bass captured from the Telabak Lake of East Coast of Peninsular Malaysia were analysed. The preys were examined using visual identification method and mitochondrial DNA barcoding technique to identify the partial digested and decaying preys in the stomach. The current study identified 7 prey species (6 fishes 43.0% and 1 shrimp 5.1%) belongs to 5 families in fishes' stomach. The results revealed that peacock bass is highly predator and generalist feeder with an opportunistic feeding behaviour. It is highly important to reduce and monitor the abundance of this species for future survival of native species in the lake.

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Introduction

The existence of an invasive fish species especially in inland waters is regarded as a crucial challenge in the conservation of tropical fish biodiversity (Clavero and Garcia-Berthou, 2005; Agostinho et al., 2005; Cucherousset and Olden, 2011; Matsuzaki et al., 2016). Non-native fish species are intentionally or accidentally introduced to a new habitat by human activities (Radkhah et al., 2016; Mousavi-Sabet and Eagderi, 2016; Eagderi et al., 2018). Peacock bass *Cichla* spp. are highly predatory fishes originated from the Amazon and introduced to many countries (Fugi et al., 2008; Kovalenko et al., 2009; Marques et al., 2016). These fishes were intentionally introduced into Malaysian freshwater by anglers in early 1990s (Rahim et al., 2013). Since then, it spreads to many freshwater bodies such as Temengor Reservoir and Lake, Raban Lake, Kapal Tujuh Lake, Kampar River (Hamid and Mansor, 2013; Desa and Aidi, 2013; Saat et al., 2014; Tan and Sze, 2017; Yap et al., 2016; Ng et al., 2018). Peacock bass exert high predation on prey fish population which may lead to the decreasing of the prey fish abundances and diversity in a particular area (Zaret and Paine, 1973; Santos et al., 2001; Pelicice and Agostinho, 2008; Franco et al., 2017). These fishes are daytime active piscivorous that consume a wide range of prey and tend to ingest the whole prey (Zhao et al., 2014). To date, there is no documentation regarding their prey species across Malaysian freshwater bodies. Thus, diet composition study of this invasive fishes is necessary for better understanding of their ecological impacts on native biodiversity (Garvey and Chipps, 2012).

Study on piscivorous fish diet composition is traditionally based on stomach contents analysis. Visual identification methods have been widely used in taxonomic identification of fish diet content (Morris and Akins, 2009; Layman and Allgeier, 2012; Côté et al., 2013). However, this method has failed to identify 70% prey content to the lowest taxonomic species level in the stomach content due to high digestion effect and prey degradation (Morris and Akins, 2009; Côté et al., 2013). This weakness, especially at low sample sizes may bias the ecological impact predictions since the detected prey might not represent the unknown percentage (Côté et al., 2013).

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Therefore, DNA barcoding technique is used for high taxonomic resolution of fish diet as a supplement to traditional method (Leray et al., 2011).

Analysis of mitochondrial DNA is proven to be a useful tool for the study of genetic diversity (Ahmad-Syazni et al., 2017; Ha et al., 2017; Khaleel et al., 2019) and species identification (Li et al., 2019; Golani et al., 2019). Matching of a short DNA sequences from unknown samples to known sequences in global databases such as National Centre for Biotechnology Information (NCBI) and Barcode of Life Database (BOLD) is known as barcoding (Ratnasingham and Hebert, 2007; Moran et al., 2015). This approach has been used effectively to classify dietary components in fishes (Côté et al., 2013; Moran et al., 2015), small body sized larval fish (Riemann et al., 2010), rare deep-water sharks (Dunn et al., 2010), and coral reef fish with rich generalist diet (Leray et al., 2011). Telabak Lake is a man-made freshwater lake which play a pivotal socio-economic and ecosystem role for the people living in the surrounding area (Khaleel et al., 2020). Freshwater lakes in Malaysia are known for the vast diversity of the aquatic live and fishes (Shahabudin and Musa, 2018). However, the introduction of invasive species such as peacock bass which preying on native fishes might give a threatening effect on the fish diversity. In this regard, current study aimed to provide first information concerning the prey identification and feeding habit of peacock bass in Telabak Lake, Malaysia using DNA barcoding technique.

Materials and Methods

Sampling: A total of 135 peacock bass samples with average total body length of 24±2.1 cm and body weight of 244±2.3 g were collected from the Telabak Lake (5°37'56.9"N, 102°28'24.5"E), East Coast of Peninsular Malaysia from October 2018 to January 2019. The samples were immediately transferred to the Aquatic Laboratory, Faculty of Bioresources and Food Industry, University Sultan Zainal Abidin Malaysia for further analyses.

Taxonomic classification and feeding habit: Fish were dissected to remove the stomach content based on Barbato et al. (2019). Following the protocol of Côté et al. (2013) with some modification, all prey items in the stomach were identified to the minimum taxonomic level. The highly digested preys with difficulty to identify were classified as fish and invertebrates, labelled separately and frozen. The feeding regime of *Cichla* spp. was measured in qualitative and quantitative methods based on Hynes (1950) and Sahtout et al. (2018). The following indices were used to evaluate the importance of different prey items in the diets of *Cichla* spp.

$VC\ (\%) = No.of\ empty\ stomach \times \frac{100}{No.\ of\ full\ stomachs}$ (Peyami et al., 2018)

$FO\ (\%) = No.of\ stomach\ containing\ prey \times \frac{100}{No.\ of\ full\ stomachs}$ (Ashelby et al., 2016)

$NI\ (\%) = No.of\ individual\ prey\ items \times \frac{100}{Total\ No.\ of\ preys}$ (Karimi et al., 2019)

$VI\ (\%) = Weight\ of\ prey\ items \times \frac{100}{Total\ weight\ of\ stomach\ content}$ (Karimi et al., 2019)

$IRI = (\%N + \%V) \times \%F$ (Barbato et al., 2019)

Where VC is vacuity coefficient, FO = frequency of occurrence, NI = number of individuals, VI = volume of individuals and IRI = index of relative importance.

Barcoding sample preparations: A small piece of the muscle tissue (2-3 mm^3) was used from every frozen prey item identified as fish and invertebrates, respectively. Then, all samples were carefully taken from each prey (preferably from dorsal muscle). To minimize the sample contamination by peacock bass cells, approximately 1 mm top layer of the tissue muscle of the prey that has direct contact to stomach fluids were removed prior to sampling for barcoding. All tools were sterilized using 95% ethanol and Bunsen burner flame between each sample removing to avoid any possible contamination.

Prey DNA extraction, amplification and sequencing: The total genomic DNA of each prey item was isolated using Favorgen DNA extraction Kit (Favorgen Biotech Corp., Ping-Tung 908, Taiwan) by following manufacturer's protocol. The partial COI gene of mitochondrial DNA was amplified by PCR using the universal primers COI-Fish2 F (5'TCGACTAATCA

Table 1. Monthly variations of peacock bass dietary items with respect to their percentage frequency of occurrence (%FO), percentage number of individual (%NI), percentage volume of individuals (%VI) and percentage index of relative importance (%IRI).

Variables	Prey	Oct.	Nov.	Dec.	Jan.	Male	Female
FO (%)	Fish	68.4	85.7	66.7	78.6	42.2	04.7
	Invertebrates	42.1	25.0	33.3	42.9	26.6	32.8
NI (%)	Fish	64.5	80.5	20.0	65.2	38.0	31.0
	Invertebrates	35.5	19.5	80.0	34.8	05.0	26.0
VI (%)	Fish	67.1	42.1	62.7	57.5	23.5	31.1
	Invertebrates	04.1	01.1	04.4	02.5	01.9	00.7
IRI (%)	Fish	84.4	87.2	32.8	85.8	92.6	96.3
	Invertebrates	15.6	12.8	67.2	14.2	07.4	04.7

TAAAGATATCGGCAC3') and COI-Fish2 R (5'ACTTCAGGGTGACCGAAGAATCAGAA3') (Ward et al., 2005) for unidentified fish samples and LCO1490: 5'-GGTCAACAAATCATAAAGATATTGG-3' and HCO2198: 5'-TAAACTTCAGGGTGACCAAAAAATCA-3' (Folmer et al., 1994) for unidentified invertebrates. For both fish and invertebrates preys, the PCR was carried out in a 25 µl reaction volume containing 18.2 µl sterile distilled water, 2.5 µl Taq buffer, 2.0 µl dNTP Mix (2.5mM), 0.5 µl of each primer (10 µM), 0.3 µl of 5 unit/µl Taq polymerase (TaKaRa) and 1 µl template DNA (1-50 ng/µl) on a thermal cycler PCR machine Veriti 96 Well Thermal Cycler (Applied Biosystem, California, USA), under the following thermal cycling conditions. Initial denaturation at 95°C for 5 min, 35 cycles including denaturation at 95°C for 30s, annealing at 50°C for 30s and elongation at 72°C for 10 min, followed by final extension for 10 min at 72°C and the PCR product was maintained at 4°C. Sequencing was succeeded using BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems) following manufacturer's instructions, performed on an ABI Prism 3730xl Genetic Analyser (Applied Biosystems).

Data analysis: Unknown sequences from fish and invertebrates were aligned and edited using ClustalW multiple sequence alignment program in MEGA 7 (Kumar et al., 2016). DnaSP software was used to determine the variable sites among the sequence (Librado and Rozas, 2009). To discover the taxonomy of each prey species, the obtained haplotypes were queried using basic local alignment search tool (BLAST) against National Center for Biotechnology (NCBI) nucleotide database. A top species match was identified with a sequence similarity of at least >94% to avoid false positives. Number of observed and detected prey species in the stomach of peacock bass was computed in percentages using Minitab 16 software.

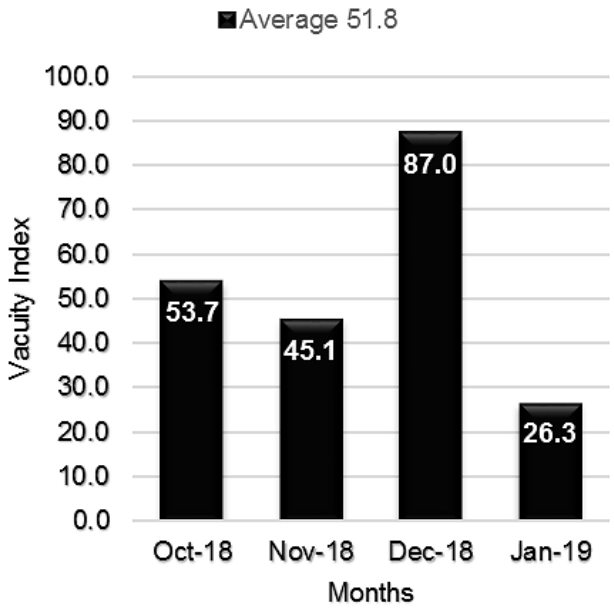

Figure 1. Monthly variations of vacuity index of peacock bass stomachs examined between October 2018 and January 2019.

Results

Feeding intensity of peacock bass: Among 135 examined stomach contents monthly from October 2018 till January 2019, 70 were empty (average 51.8%) with high value in December (87%) and sudden decline in January (26%) (Fig. 1). Using visual identification method, the remaining prey samples were successfully identified as fish and invertebrates with their percentages (Fig. 2B, C, respectively).

Table 2. BLAST sequence match showing percentage identity of prey in peacock bass using barcode.

Sequence	Family	Species	Accession No.	% Identity	% N_0
PSC01	Cichlidae	*Cichla ocellaris*	KU878410	99.20	5.1
PSC02	Pristolepididae	*Pristolepis fasciata*	MK049486	99.07	15.2
PSC03	Ambassidae	*Parambassis ranga*	MK448145	94.87	10.1
PSC04	Cyprinidae	*Rasbora trilineata*	KU569018	99.99	2.5
PSC05		*Cyprinus carpio*	LN591958	94.03	2.5
PSC06		*Cyclocheilichthys enoplos*	KU692459	99.68	7.6
PSC07	Palaemonidae	*Macrobrachium lanchesteri*	KP759429	98.18	5.1

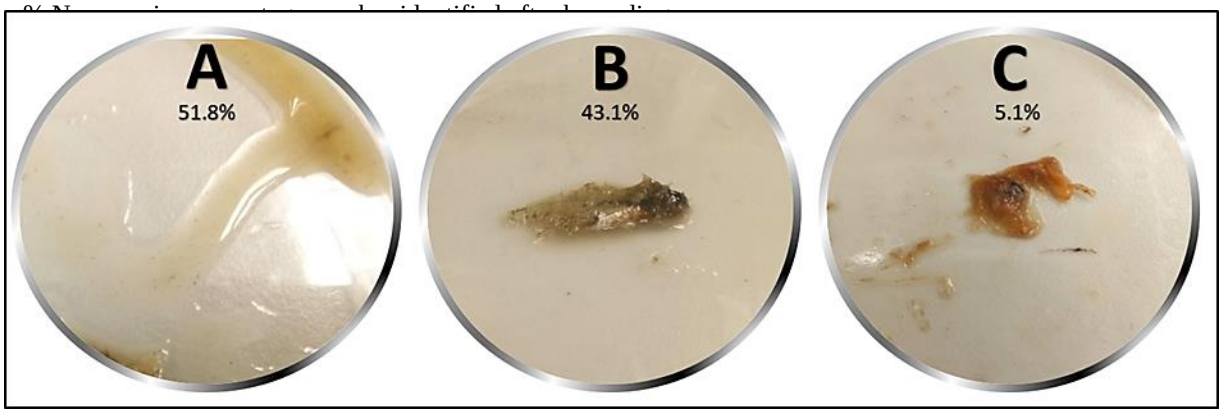

Figure 2. Visual identification of 135 peacock bass stomach content from October 2018 to January 2019 captured in Telabak Lake, (A) 51.8% Empty stomach, (B) 43.1% unidentified fish species and (C) 5.1% unidentified invertebrate species.

However, it failed to classify prey to their lowest taxonomy due to high ingestion effect and degradation. Table 1 provides the overall results of the classification and diet composition on prey of the examined peacock bass between October 2018 and January 2019. The fish preys dominated the entire diet with the exception of December at which high number values of invertebrates were recorded.

Mitochondrial DNA barcode: A total of 656 base pair (bp) of COI for fish species and 679 bp of COI for invertebrates were obtained after deletion of low-quality nucleotides at the 5' and 3' ends. Six different sequences were obtained from COI of fishes (PSC01, PSC02, PSC03, PSC04, PSC05, and PSC06) and one sequence for invertebrate (PSC07). After blasting, in NCBI, following our criterion of >94% sequence similarities, 7 prey species belong to five families were *Cichla ocellaris, Pristolepis fasciata, Parambassis ranga, Rasbora trilineata, Cyprinus carpio* and *Cyclocheilichthys enoplos* (Table 2). The percentage of each prey species identified (% N_0) using DNA barcode were also recorded with high value of 15.2% in *P. fasciata*.

Discussions

The introduction of peacock bass into Telabak Lake was accompanied by a sharp and gradual decline of small-sized fishes (personal communication). This study showed that the decrease in fish diversity might be associated with feeding habit of peacock bass in the Lake, since the observed prey items in the peacock bass stomach confirm its piscivorous feeding habit on targeted native prey species. We used traditional visual identification method and further DNA barcoding technique to identify its preys. The highest vacuity coefficient was observed in December indicating their breeding and spawning season (Gomiero et al., 2009) which limits their hunting time. High vacuity coefficient during breeding season was also reported from other fish species such as *Diplodus vulgaris* (Pallaoro et al., 2006), *Caranx rhonchus* (Sley et al., 2008), *Pagellus erythrinus* (Šantić et al., 2011).

The lowest vacuity coefficient observed in January (26.3%) revealed its feeding initiation after breeding period. Full recovery to feeding activity helps fishes to compensate the energy used during breeding (Derbal et al., 2007). Fish dominates the entire diet of the peacock bass in all months, except in December where invertebrates (prawn) were dominated. *Macrobrachium lanchesteri* (Prawn) spawns throughout the year with a peak at November (Phone et al., 2005). Therefore, it could be more available in December for peacock bass. Another explanation for high predation of peacock bass on fish prey species might be related to naturally clear transparency of Telabak Lake. It is reported that peacock bass thrive well in clear freshwater for excellent predation (Kovalenko et al., 2010).

Visual identification has failed to identify the prey items to the lowest species level due to the degradation of essential features such as fin ray shape and body coloration. However, only 48.2% of the ingested prey into fish and invertebrates were visually distinguished. This percentage is closer to the results of other similar studies (Côté et al., 2013; Dahl et al., 2017). Only few species were successfully identified to the lowest species taxonomic level using visual method similar to other works (Morris and Akins, 2009; Côté et al., 2013; Moran et al., 2015; Mzaki et al., 2017; Sahtout et al., 2018). All identified prey species were native to Malaysia freshwater except for *C. carpio* and *Cichla* spp. Opportunistic feeding habit of *Cichla* spp. is one of the serious aspects that helped them adapt to a new environmental condition. The presence of *Cichla* spp. in the stomach as a prey item might be due to cannibalism, and as proof of its opportunistic feeding habit in nature. It was previously examined that cannibalism is more pronounced during the spawning periods with scarcity of alternative foods, like small indigenous species of fish (Junior and Gomiero, 2010). In addition, low rates of cannibalism observed in this study might be due to native prey abundance (Carvalho et al., 2014). Once *Cichla* spp. is introduced into a lake, they prey on variety of available fish species, shrimps and cichlids (Pereira et al., 2015; Mendonça et al., 2018). All native species found in the stomachs are of least concern (IUCN Red List, 2012) but they contribute largely in aquaculture, and as a source of income for the local community e.g. *M. lanchesteri* is used as food by locals (Phone et al., 2005; Aznan et al., 2017).

The studies on introduction peacock bass have indicated a negative effect on local fish species (Zaret and Paine, 1973; Molina et al., 1996; Pinto-Coelho et al., 2008; Pelicice and Agostinho, 2008; Rahim et al., 2013). Previous works of the fish population in Lake Redonda of Cuba from 1989 to 1990, documented that many local fish species have been extinct after the introduction of peacock bass (Molina et al., 1996). Recently, Menezes et al. (2012) reported that the introduction of peacock bass in the coastal Lakes of Rio Grande do Norte Brazil had reduced native fish abundantly with a negative impact on their diversity. The invasion and adaptation of peacock bass in Telabak Lake might likely lead to the reduction of native fish species. The existence of these highly adaptive and fast-growing piscivorous fish may cause severe damages to the local aquatic populations through competition, predation and cascade effects across the trophic chain. Although peacock bass attracts recreational anglers (Mendonça et al., 2018), but local people depends on native aquatic species in the Telabak Lake. The lake plays a significant role for their daily needs and incomes. As our finding, 48.2% of the prey items submitted for barcoding were 100% identified to species level. Other studies identified less than 70% when submitted for barcoding (Morris and Akins, 2009; Côté et al., 2013), which is due to species differences. Without using DNA barcoding technique, most of the prey items could have been labelled as partially digested unidentified prey, leading to missing information, misidentification and less understanding of invasive peacock bass impact in the lake.

Conclusion

This study provided useful information about feeding habits of *Cichla* spp. for better understanding of the relationship between fish species and other living organisms in Telabak Lake. The presence of this invasive species may affect the government effort on

boosting and promoting the lake as recreational and aquaculture centre. The technique of DNA barcoding has proved to be a useful tool in discovering diet of the *Cichla* spp. in the lake. The information gathered in this recent study is important for stakeholders and policy makers in considering the management of biodiversity of the lake in the future.

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References

Agostinho A.A., Thomaz S.M., Gomes L.C. (2005). Conservation of the biodiversity of Brazil's inland waters. Conservation Biology, 19: 646-652.

Ahmad-Syazni K., Khaleel A.G., Norshida I., Connie F.K., Nguang S.I., Ha H.C. (2017). Population structure of swamp eel *Monopterus albus* in East Coast of Peninsular Malaysia inferred from 16S mitochondrial DNA. World Applied Sciences Journal, 35: 1392-1399.

Ashelby C.W., De Grave S., Johnson M.L. (2016). Diet analysis indicates seasonal fluctuation in trophic overlap and separation between a native and an introduced shrimp species (Decapoda, Palaemonidae) in the tidal river Thames (UK). Crustaceana, 89: 701-719.

Aznan A.S., Iberahim N.A., Rahman N.I., Zakaria K., Leong L.K., Ibrahim W.N.W. (2017). Health surveillance of freshwater prawn, *Macrobrachium lanchesteri* in Setiu wetland, Terengganu, Malaysia. Journal of Sustainability Science and Management, 12: 167-175.

Barbato M., Kovacs T., Coleman M.A., Broadhurst M.K., de Bruyn M. (2019). Metabarcoding for stomach-content analyses of Pygmy devil ray (*Mobula kuhlii cf. eregoodootenkee*): Comparing tissue and ethanol preservative-derived DNA. Ecology and Evolution, 9: 2678-2687.

Carvalho D.C., Oliveira D.A.A., Sampaio I., Beheregaray L.B. (2014). Analysis of propagule pressure and genetic diversity in the invisibility of a freshwater apex predator: the peacock bass (genus *Cichla*). Neotropical Ichthyology, 12: 105-116.

Clavero M., Garcia-Berthou E. (2005). Invasive species are a leading cause of animal extinctions. Trends in Ecology and Evolution, 20: 110-110.

Côté I., Green S., Morris J., Akins J., Steinke D. (2013). Diet richness of invasive Indo-Pacific lionfish revealed by DNA barcoding. Marine Ecology Progress Series, 472: 249-256.

Cucherousset J., Olden J.D. (2011). Ecological impacts of non-native freshwater fishes. Fisheries, 36: 215-231.

Dahl K.A., Patterson III W.F., Robertson A., Ortmann A.C. (2017). DNA barcoding significantly improves resolution of invasive lionfish diet in the Northern Gulf of Mexico. Biological Invasions, 19: 1917-1933.

Derbal F., Nouacer S., Kara M.H. (2007). [Composition and variations of dietary regime of sparaillon *Diplodus annularis* (Sparidae) in Annaba Golf (East of Algeria)]. Cybium, 31: 443-450. (In French)

Desa S., Aidi D. (2013). A proposed model of fish distribution in shallow lake based on feeding behaviour of fishes in Raban Lake. Conference: UMTAS 2013: The 12th International UMT Annual Symposium, At Terengganu, Malaysia.

Dunn M.R., Szabo A. McVeagh M.S., Smith P.J. (2010). The diet of deep-water sharks and the benefits of using DNA identification of prey. Deep Sea Research Part I: Oceanographic Research Papers, 57: 923-930.

Eagderi S., Nasri M., Çiçek E. (2018). First record of the Amur goby *Rhinogobius lindbergi* Berg 1933 (Gobiidae) from the Tigris River drainage, Iran. International Journal of Aquatic Biology, 6(4): 202-207.

Folmer O., Black M., Hoeh W., Lutz R., Vrijenhoek R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase sub-unit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology, 3: 294-299.

Franco A.C.S., dos Santos L.N., Petry A.C., García-Berthou E. (2017). Abundance of invasive peacock bass increases with water residence time of reservoirs in South-eastern Brazil. Hydrobiologia, 817: 155-166.

Fugi R., Luz-Agostinho K.D.G., Agostinho A.A. (2008). Trophic interaction between an introduced peacock bass and a native dogfish piscivorous fish in a Neotropical impounded river. Hydrobiologia, 607: 143-150.

Garvey J.E., Chipps S.R. (2012). Diets and energy flow. In: A.V. Zale, D.L. Parrish, T.M. Sutton (Eds.). Fisheries techniques, 3rd edition. American Fisheries Society,

Bethesda, Maryland. 7 pp: 33-779.

Golani D., Sonin O., Snovsky G., David L., Tadmor-Levi R. (2019). The occurrence of the peacock bass (*Cichla kelberi* Kullander and Ferreira 2006) in Lake Kinneret (Sea of Galilee), Israel. BioInvasions Records, 8: 706-711.

Gomiero L., Villares J.G., Naous F. (2009). Reproduction of *Cichla kelberi* Kullander and Ferreira, 2006 introduced into an artificial lake in South-eastern Brazil. Brazilian Journal of Biology, 69: 175-183.

Ha H.C., Nguang S.I., Zarizal S., Komilus C.F., Norshida I., Ahmad-Syazni K. (2017): Genetic diversity of kampung chicken (*Gallus gallus domesticus*) from selected areas in East Coast Peninsular Malaysia inferred from partial control region of mitochondrial DNA. Malaysian Journal of Applied Biology, 46: 63-70.

Hamid M.A., Mansor M. (2013). The inland fisheries with special reference to Temengor and Bersia Reservoirs, Perak. Malaysian Journal of Applied Biology, 42: 73-76.

Hynes H.B.N. (1950). 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, 19: 36.

Junior G.A., Gomiero L.M. (2010). Feeding dynamics of *Cichla kelberi* Kullander & Ferreira, 2006 introduced into an artificial lake in South-eastern Brazil. Neotropical Ichthyology, 8: 819-824.

Karimi S., Katiraei E., Soofiani N.M., Taghavimotlagh S.A., Vazirizadeh, A. (2019). Feeding habits of striped piggy, *Pomadasys stridens* (Forsskal, 1775) (Haemulidae) in northern part of the Persian Gulf. International Journal of Aquatic Biology, 7: 85-92.

Khaleel A.G., Ha H.C., Ahmad-Syazni K. (2019). Different population of Malaysia swamp eel in East and Southeast Asia inferred from partial 16s mitochondrial DNA. Bioscience Research, 16: 01-09.

Khaleel A.G., Nasir S.A.M, Norshida I., Ahmad-Syazni K. (2020). Origin of invasive fish species, peacock bass *Cichla species* in Lake Telabak Malaysia revealed by mitochondrial DNA barcoding. Egyptian Journal of Aquatic Biology and Fisheries, 24: 311-322.

Kovalenko K.E., Dibble E.D., Agostinho A.A., Cantanhêde G., Fugi R. (2009). Direct and indirect effects of an introduced piscivore, *Cichla kelberi* and their modification by aquatic plants. Hydrobiologia, 638: 245-253.

Kovalenko K.E., Dibble E.D., Agostinho A.A., Pelicice F.M. (2010). Recognition of non-native peacock bass, *Cichla kelberi* by native prey: testing the naivete´ hypothesis. Biological Invasions, 12: 3071-3080.

Kumar S., Stecher G., Tamura K. (2016). MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution, 33: 1870-1874.

Layman C., Allgeier J. (2012). Characterizing trophic ecology of generalist consumers: a case study of the invasive lionfish in The Bahamas. Marine Ecology Progress Series, 448: 131–141.

Leray M., Boehm J.T., Mills S.C., Meyer C.P. (2011). Moorea BIOCODE barcode library as a tool for understanding predator–prey interactions: insights into the diet of common predatory coral reef fishes. Coral Reefs, 31: 383-388.

Li Q., Deng J., Chen C., Zeng L., Lin X., Cheng Z., Huang X. (2019). DNA barcoding subtropical aphids and implications for population differentiation. Insects, 11: 11.

Librado P., Rozas J. (2009). DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics, 25: 1451-1452.

Marques A.C.P.B., Franco A.C.S., Salgueiro F., García-Berthou E., Santos L.N. (2016). Genetic divergence among invasive and native populations of the yellow peacock cichlid *Cichla kelberi.* Journal of Fish Biology, 89: 2595-2606.

Matsuzaki S.S., Sasaki T., Akasaka M. (2016). Invasion of exotic piscivorous causes losses of functional diversity and functionally unique species in Japanese lakes. Freshwater Biology, 61: 1128-1142.

Mendonça H.S., Santos A.C.A., Martins M.M., Araújo F.G. (2018). Size-related and seasonal changes in the diet of the non-native *Cichla kelberi* Kullander & Ferreira, 2006 in a lowland reservoir in the South-eastern Brazil. Biota Neotropica, 18: 1-8.

Menezes R.F., Attayde J.L., Lacerot G., Kosten S., Souza L.C., Costa L.S., Van Nes E.H., Jeppesen E. (2012). Lower biodiversity of native fish but only marginally altered plankton biomass in tropical lakes hosting introduced piscivorous *Cichla cf. ocellaris*. Biological Invasions, 14: 1353-1363.

Molina W.F., Gurgel H.C.B., Vieira L.J.S., Canan B. (1996). Acao de um predador exogeno sobre um ecossistema aquatico equilibrado, I Extincoes locais e medidas de conservacao genetica. UNIMAR, 18: 335-

345.

Moran Z., Orth D.J., Schmitt J.D., Hallerman E.M., Aguilar R. (2015). Effectiveness of DNA barcoding for identifying piscine prey items in stomach contents of piscivorous catfishes. Environmental Biology of Fishes, 99: 161-167.

Morris J.A., Akins J.L. (2009). Feeding ecology of invasive lionfish (*Pterois volitans*) in the Bahamian archipelago. Environmental Biology of Fishes, 86: 389-398.

Mousavi-Sabet H., Eagderi S. (2016). First record of the convict cichlid, Amatitlania nigrofasciata (Günther, 1867) (Teleostei: Cichlidae) from the Namak Lake basin, Iran. Iranian Journal of Ichthyology, 3(1): 25-30.

Mzaki F., Manchih K., Idrissi H.F., Boumaaz A., Haddouch A.B., Tazi O. (2017). Diet of the common cuttlefish *Sepia officinalis* (Linnaeus, 1758) (Cephalopoda: Sepiidae) in the Southern Moroccan Atlantic waters, Cap Boujdour, Cap Blanc. AACL Bioflux, 10: 1692-1710.

Ng C.K.C., Ooi P.A., Wong W., Khoo G. (2018). Ichthyofauna checklist (Chordata: Actinopterygii) for indicating water quality in Kampar River catchment, Malaysia. Biodiversitas, 19: 2252-2274.

Pallaoro A., Santic M., Jardas I. (2006). Feeding habits of the common two-banded sea bream, *Diplodus vulgaris* (Sparidae), in the eastern Adriatic Sea. *Cybium,* 30: 19-25.

Pelicice F.M., Agostinho A.A. (2008). Fish fauna destruction after the introduction of a non-native predator (*Cichla kelberi*) in a Neotropical reservoir. Biological Invasions, 11: 1789-1801.

Pereira L.S., Agostinho A.A., Gomes L.C. (2015). Eating the competitor: a mechanism of invasion. Hydrobiologia, 746: 223-231.

Peyami F.Y. (2018). Food and feeding habit of a Teleostean fish *Salmostoma bacaila* (Ham.) at Partapur dam, Makhdumpur Jehanabad, Bihar. International Journal of Fisheries and Aquatic Studies, 6: 388-391.

Phone H., Ohtomi J., Suzuki H. (2005). Reproductive biology of the freshwater palaemonid prawn, *Macrobrachium lanchesteri* (De Man, 1911) from Myanmar. Crustaceana, 78: 201-213.

Pinto-Coelho R.M., Bezerra-Neto J.F., Miranda F., Mota T.G., Resck R., Santos A.M. (2008). The inverted trophic cascade in tropical plankton communities: Impacts of exotic fish in the Middle Rio Doce Lake district, Minas Gerais, Brazil. Brazil Journal of Biology, 68: 1025-1037.

Radkhah A., Eagderi S., Mousavi-Sabet H. (2016). First record of the exotic species *Hemiculter leucisculus* (Pisces: Cyprinidae) in southern Iran. Limnetica, 35: 175-178.

Rahim K.A.A., Esa Y., Arshad A. (2013). The influence of alien fish species on native fish community structure in Malaysian waters. Kuroshio Science, 7: 81-93.

Ratnasingham S., Hebert P. (2007). BOLD: the barcode of life data system. Molecular Ecology Notes, 7: 355-364.

Riemann L., Alfredsson H., Hansen M.M., Als T.D., Nielsen T.G., Munk P., Castonguay M. (2010). Qualitative assessment of the diet of European eel larvae in the Sargasso Sea resolved by DNA barcoding. Biology Letters, 6: 819-822.

Saat A., Isak N.M., Hamzah Z., Wood A.K. (2014). Study of radionuclides linkages between fish, water and sediment in former tin mining lake in Kampung Gajah, Perak, Malaysia. The Malaysian Journal of Analytical Sciences, 18: 170-177.

Sahtout F., Boualleg C., Kaouachi N., Khelifi N., Menasria A., Bensouilah M. (2018). Feeding habits of *Cyprinus carpio* in Foum El-Khanga Dam, Souk-Ahras, Algeria. AACL Bioflux, 11: 554-564.

Šantić M., PAlAdin A., Rađa B. (2011). Feeding habits of common pandora *Pagellus erythrinus* (Sparidae) from eastern central Adriatic Sea. Cybium, 35: 83-90.

Santos L.N., Gonzales A.F., Araujo, F.G. (2001). Dieta do tucunare´-amarelo *Cichla monoculus* (Bloch & Schneider) (Osteichthyes, Cichlidae), no reservato´rio de Lajes, Rio de Janeiro, Brasil. Revista Brasileira de Zoologia, 18: 191-204.

Shahabudin M.M., Musa S. (2018). An overview on water quality trending for lake water classification in Malaysia. International Journal of Engineering and Technology, 7: 5-10.

Sley A., Jarboui O., Ghorbel M., Bouain A. (2008). Diet composition and food habits of *Caranx rhonchus* (Carangidae) from the Gulf of Gabes (central Mediterranean). Journal of the Marine Biological Association of the UK, 88: 00-00.

Tan K.W., Sze K.Z. (2017). Determination of heavy metal concentration in fish species in Kampar mining lake, Perak state of Malaysia. Fresenius Environmental Bulletin, 26: 4202-4207.

Ward R.D., Zemlak T.S., Innes B.H., Last P.R., Hebert P.D. (2005). DNA barcoding Australia's fish species. Philosophical Transactions of the Royal Society of London B: Biological Sciences, 360: 1847-857.

Yap S.K., Muneera I., Syakir M.I., Zarul H.H., Widad F. (2016). Stable isotopes approach to infer the feeding habit and trophic position of freshwater fishes in tropical lakes. Iranica Journal of Energy and Environment, 7: 177-183.

Zaret T.M., Paine R.T. (1973). Species introduction in a tropical lake. Science, 182: 449–455.

Zhao T., Villéger S., Lek S., Cucherousset J. (2014). High intraspecific variability in the functional niche of a predator is associated with ontogenetic shift and individual specialization. Ecology and Evolution, 4: 4649-4657.