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DIET DIVERSITY IN TETRAODONTID FISH, *LAGOCEPHALUS SPADICEUS* (RICHARDSON) USING CONVENTIONAL AND DNA BARCODING APPROACHES

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Abstract: Gut content analysis of fishes not only help documenting their food spectrum but also provide an overview of the food web they are involved in. The common methods adopted for the study include morphological identification to the lowest possible taxonomic rank and DNA barcoding of individual prey components. This paper documents the diet content diversity of the half-smooth golden pufferfish *Lagocephalus spadiceus* (Pisces: Tetraodontidae) collected from the Kerala coast. The study revealed that this demersal carnivore feeds largely on benthic teleosts (*Upeneus guttatus*), crustaceans (*Aristeus* sp.) and cephalopods (*Uroteuthis* and *Amphioctopus*). The fish also fed on pelagic teleosts such as *Stolephorous devsii*. Of the 75 stomachs analysed, 50.87% were empty and 49.13% contained food items with maximum feeding intensity reported for the month of November and least in February of which 100% of fish examined had empty stomach. Mean gastrosomatic index (GSI) values clearly denotes high GSI values for the month of March with least value in February. Small unidentifiable pieces from gut content of different fishes of *L. spadiceus* were subjected to DNA barcoding and confirmed the prey item to be *Upeneus guttatus*. Thus the molecular genetic approach improved the ability to identify diet items of fishes in which original visual inspection failed to identify any identifiable prey remains.

Keywords: Puffer, gut content, Tetraodontidae, DNA barcoding, *Lagocephalus*

INTRODUCTION

Puffer fishes belonging to the family Tetraodontidae are a unique group of fishes those inhabit freshwater, marine and estuarine environments in tropical and temperate regions (Nelson, 2016) and due to the presence of neurotoxin called tetrodotoxin (TTX) in its body (Noguchi *et al.*, 2006), which may even cause human moralities due to consumption of fish meat (Arakawa *et al.*, 2010). They are consumed in Southeast Asian countries and considered as a delicacy as "FUGU" in specialized restaurants in Japan (Arakawa *et al.*, 2010) and also along the Gulf of Suez and Red sea region (Sabrah *et al.*, 2006).

The determination of diet relies on indirect evidence such as gut content and their taxonomic identification of partially digested prey fragments which require an exhaustive prior knowledge of prey morphological diversity. A number of researchers carried out studies on the feeding biology of *Lagocephalus* sp.; Mohamad and Isa (2013) on *L. lunaris* from Malaysian waters, Kumar *et al* (2013) on *L. guentheri* from Andaman waters Sabrah *et al.* (2006) on *L. sceleratus* from Gulf of Suez, Aydin (2011) on *L. sceleratus* from Turkey, and Mohamed *et al.* (2013) on *L. inermis* from Kuching, Sarawak. Although traditional diet analysis continues to be a useful component in ecological studies due to its low cost and logistical ease (Andraso, 2005; Roseman *et al.*, 2009), in recent years these data have been complemented with other more technologically advanced approaches, including fatty-acid analysis, stable-isotope analysis and DNA-based diet determination techniques (Schmidt *et al.*, 2009; Corse *et al.*, 2010; Hardy *et al.*, 2010). DNA-based techniques have been successfully implemented to identify prey items in stomach, gut or faeces samples (Symondson, 2002). Some other works showing the effectiveness of DNA barcoding for identifying prey items in stomach contents of include those of Moran *et al*. (2015), Martha *et al.* (2012) and Carreon *et al.* (2011).

Biology of puffer fish *L. spadiceus* attracted no fishery biologist because of their insignificance as food fish which may not justified as each organism has its own significance in the ecosystem. The present study has been undertaken as a preliminary one with a view to know about the feeding biology of *L. spadiceus* from west coast of India. Also in this problem a DNA based approach to validate the fish diet is also studied because most of the gut contents in the fish obtained through by catch were in semi digested condition. According to Teletchea (2009) for successful amplification of DNA which are usually not in optimal condition (i.e. faeces and gut contents) requires a small amount of tissue which is an advantage of DNA-based techniques for prey identification purposes.

MATERIAL AND METHODS

A total of 75 *Lagocephalus spadiceus* (Richardson 1845) were collected for the present study from the commercial trawlers of Neendakara and Sakthikulangara fishing harbours, Kollam district, Kerala. In the lab stomach was dissected and parts of the contents are preserved in 95% ethanol for barcoding analysis and rest of the contents was preserved with 10% formalin.

The preserved stomach contents were then examined under a stereomicroscope and contents were enumerated and identified to the lowest possible taxa (Pillai, 1952). The diet was analysed using point method. Each stomach provided with points Gorged, full, good, moderate, poor, empty and points to the item given as swarm, plenty, few, little, rare, the variation in feeding intensity was calculated. Gastro somatic index was found using formula following Khan *et al*. (1988).

The content of each stomach were collected in a petridish and examined by light microscope. The percentage volume of gut content was determined by point method (Pillai, 1952). The frequency of occurrence method was used to determine the important food items (Hyslop, 1980) and Index of preponderance was also determined following Natarajan and Jhingran (1961).

Several large sized fish pieces present in the gut which could not be identified visually were subjected to DNA analysis to identify the species. Muscle tissues (200–500 mg) were taken from all prey items. Total DNA was extracted from 100-500 mg of tissue using. Standardised salting out protocol was adopted for precise and quick DNA isolation from the tissues (Miller *et al*., 1988).

RESULTS

Gut content analysis revealed the qualitative and quantitative food spectrum of *L. spadiceus*. Traditional morphological characterization led to species-level identification of a number of gut contents and those which are unsure were identified up to genus level with the help of DNA barcoding. Lower size frequency fishes were absent in the catch survey due to the selective nature of the commercial gear used in fishing vessels operated along the south coast of Kerala.

Qualitative gut content analysis

The analyses of stomach contents revealed that these fishes are carnivorous as well as predatory in nature. Items obtained from gut content throughout the study period were categorised into fishes, cephalopods, crustaceans, digested matter and miscellaneous. Among fishes, *Stolepherous devisi, Cyanoglossus zanzibarensis*, and *Leognathus* sps were identified. *Amphioctopus, Uroteuthis* and *Aristeus* were also identified from gut contents of *L. spadiceus.*

Quantitative gut content analysis

The percentage composition for the entire study period was represented in Fig 1. This clearly indicates the higher incidence of fish in the gut except for the month of February during which the gut was empty. Digestive matter was also present in most of the months except February. Cephalopods and crustaceans were the least represented except for the month of empty gut.

Feeding Intensity

Maximum feeding intensity was reported for the month of November and least in February (Table 1) of which 100% of fish examined in February had empty stomach. Similarly 50% of fishes during March also had empty stomach. Feeding intensity showed fluctuating values for rest of the months. Very low feeding intensities were seen in the months of December and March. Despite the low intensities in the month of February and March, there was a sudden increase of feeding intensity during April. Dry season showed low feeding intensity and wet season showed high feeding intensity with good stomach filled condition. The stomach analyses indicated a high incidence of empty stomachs for the months of December, February and March, i.e., a mean monthly value of > 50 % of empty stomachs was recorded with lows records during November and January.

Mean fish weight and mean gut weight was higher for the months of November, January and April and low during December, February and March. Least mean fish and gut weight was recorded for the month of February due to two reasons, primarily only few fish samples were obtained and secondly fishes had empty stomach during February and March. Seasonal analysis showed significant difference in mean fish weight and gut weight for *L. spadiceus* (Table 2). Fish and gut weight were significantly low during dry season where as it consume more food during wet season.

Mean gastrosomatic index (GSI) values were represented in the Fig. 2 which clearly denotes high GSI values for the month of March with least value in February which correlates with the values of the percentage composition of food items for the study period. Statistical analysis of GSI depicted in Table 2 shows that there was no significant seasonal difference in GSI of *L. spadiceus* and moreover dry season registered high GSI (5.82) values than wet season (4.94).

Index of preponderance

The percentage occurrence, percentage volume and Index of Preponderance in the diet of *L. spadiceus* are represented in the Table 3. Mainly 5 types of food items were found in the gut which included fish and fish remains, cephalopods, crustaceans, digestive matter and miscellaneous items. It is evident from the table that the highest index value was reported for fish and fish remains followed by digested matter and crustaceans and cephalopods with the least.

Stomach content analysis using DNA barcoding

Several fish pieces present in the gut which could not be positively identified through conventional methods were subjected to DNA analysis and revealed the presence of *Upeneus guttatus*. All of the prey tissue fragments were barcoded, but only 2 yielded readable sequences. There were no insertions, deletions or stop codons, indication of heteroplasmy or NUMTs in any sequence. The sequences were compared to the reference library of sequences in the Barcode of Life Database (BOLD). Translation of sequences did not result in stop codons indicating that the amplified domains were functional mitochondrial CO1 sequences. Sequence length of CO1 ranged between 307 to 470 bp suggests that NUMTs (nuclear DNA sequences originating from mitochondrial DNA sequences) were not sequenced. GC content in the sequence of CO1 gene was calculated by BioEdit software. Two sequences from the gut from different fish matched with Indo-Pacific *Upeneus guttatus* with 99% similarity to reference sequences on BOLD. The average K2P distance among individuals was 7 %.The mean sequence composition for A1was guanine 18.24%, cytosine 30.62, adenine 22.15%, thyamine 28.99%, GC 48.86%, AT 51.14%. The mean sequence composition for A2was guanine 18.72% cytosine 31.28%, adenine 20.85%, thyamine 29.15%, GC 48.86%, AT 51.14%. The mean sequence composition for the second sample was GC 50%, AT 50%.

Table 1. Monthly feeding intensity (%) of *L. spadiceus*

Number of fishes examined	13	11	22	8	12	9
Condition of stomach	Nov 2015	Dec 2015	Jan 2016	Feb 2016	Mar 2016	Apr 2016
Gorged	7.69	0	9.09	0	0	11.11
Full	15.38	9.09	22.72	0	0	22.22
Good	15.38	18.18	18.18	0	8.38	0
Moderate	30.76	0	13.63	0	16.66	11.11
Poor	15.38	0	13.63	0	25	11.11
Empty	15.38	72.72	22.72	100	50	44.44

Table 2. Seasonal comparison of Fish weight, Gut weight and GSI of *L. spadiceus*

Parameters	**Season**	**Mean**	**+ SD**	**t value**
Fish Weight (mg)	Wet Season	139.43	97.86	4.227**
	Dry Season	50.47	71.72	
Gut Weight (mg)	Wet Season	4	2.95	3.251*
	Dry Season	1.75	2.86	
GSI	Wet Season	4.94	9.81	-0.422
	Dry Season	5.82	5.36	

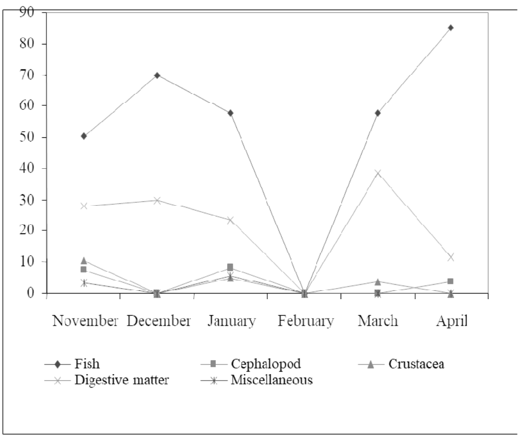

Fig. 1. The percentage composition of various food items in the gut of *L. spadiceus*

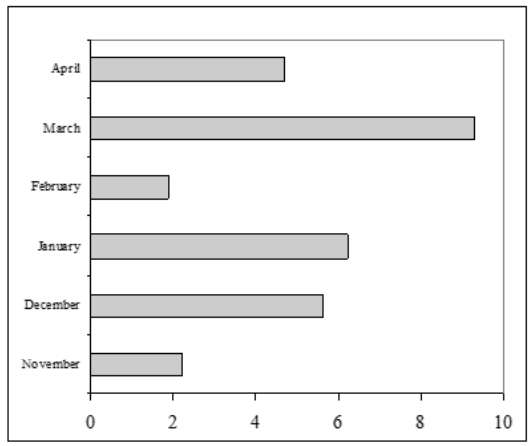

Fig. 2. Mean GSI of *L. spadiceus* during the collection period

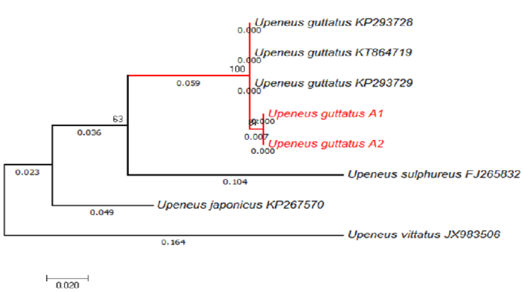

Fig. 3. Neighbour Joining (NJ) phylogenetic tree of *Upenus guttatus* obtained from the gut contents of *Lagocephalus spadiceus* matched with similar species from data bank

Table 3. The Percentage occurrence, percentage volume and index of preponderance in the diet of *L. spadiceus*

No of food items	**% occurrence**	**% volume**	**IP**
Fish and fish remains	51.09	53.53	69.03
Octopus remains	5.4	3.2	0.93
Crustacean remains	4.69	3.13	1
Digestive matter	19.28	21.9	11.97
Miscellaneous	2.79	1.46	0

In A1 Sequence length of CO1 derived was 307 base pairs and the number of Adenine, Cytosine, Guanine, Thymine were 68 (22.15%), 94(30.62%), 56 (18.24%), 89 (28.99%) respectively. The fish sequences matched with greater than 99% similarity to reference sequences in BOLD, allowing identification to the species level. Neighbour Joining (NJ) phylogenetic tree of *U.guttatus* obtained from the gut contents of *L.spadiceus* along with similar species from data bank is represented in the Fig. 3.

DISCUSSION

The analyses of stomach contents revealed that the puffer fish *L. spadiceus* is carnivorous and its gut contents were divided into three major groups, namely, cephalopoda, crustacea and fish because of the invertebrate feeding nature of *L. spadiceus*. Sabrah *et al*. (2006) also suggested that *L. sceleratus* from Gulf of Suez feeds on cephalopods, crustaceans and fishes. According to Aydin (2011) *L. sceleratus* is an invertebrate and fish feeder, which is in accordance with our recent findings. Al-Zibdah and Odat (2007) also reported that puffers like *Katsuwonus pelamis* and *Euthynnus affinis* from the Red Sea are carnivorous. Heterogeneity in the food components of puffer fish species was found in the Andaman Sea (Al-Zibdahh and Odat, 2007). Feeding habits of similar fish species is varied in different environments (Abdu Rahiman, 2006).

However Kumar *et al*., (2013) reported that *L. guentherii* feeds on microalgae, zooplanktons, rock oysters, polychaetes and sea weeds. In the Mediterranean, *L. Scleratus* was found to be carnivorous, feeding mainly on shrimps, but also on crabs, fish including individuals of its own species, squids, and cuttlefish (EastMed 2010; Aydin, 2011). Mohanraj and Prabhu (2012) in their studies on food habits and diet composition of demersal marine fishes from Gulf of Mannar observed that, the most frequently eaten prey items like fish, crustaceans, mollusks, worms and echinoderms are the same in all other studies on the diet of tropical carnivorous coastal fishes.

Mohamed *et al*. (2013) examined the stomach contents of *L. inermis* and found that anchovies and squids were more prevalent in the diet. In *L. scleratus*, an ontogenetic diet that shift to a mollusk such as *Sepia officinalis* and *Octopus vulgaris* is reported with increasing size mainly due to the shift in habitat from sandy bottoms to meadows (Kalogirou 2011). But in our present study there were no shift in diet with increase in size is noticed. The analyses of the food have shown that most of them are predators on actively moving benthic invertebrates and teleost fishes (Suseelan and Nair, 1969). Nekton probably offers the best nutritional input for carnivorous fish and is therefore favored when possible (Domenci and Blake, 1997).

In our present study there was more number of empty stomachs which were attributed to post capture digestion. Matthes (1968) also noted similar results in *H. vittatus* from Lake Kariba and stated that empty stomachs were due to post-capture digestion. The feeding intensity was studied from the data on the degree of fullness of stomach. The condition of stomach fullness related to the irregular feeding habits of the fish. Changes in mean weight of the stomach contents through the study period indicate differences in feeding intensity (Man and Hodgkiss, 1977).

In the wet season mean fish weight as well as mean gut weight was higher than that in the dry season. This may be due to the availability of food items after the monsoon. Similar findings are reported by Hammerschlag *et al*. (2010) stating that feeding intensity was significantly lower during the dry season.

Morphological identification led to species-level identification of two fish prey items, *Stolephorous devsii* and *Cyanoglossis zanzibarensis* from the gut contents, as they were in an identifiable condition and also two genus, *Uroteuthis sp.*and *Apmhioctopus*, whereas DNA barcoding revealed the presence of *Upeneus guttatus*, which may be considered as the preferred food item for *L. spadiceus*. Being demersal, *L. spadiceus* feeds on *U. vittatus* which is also a demersal species. *Urotuethis* and *Amphioctopus*, both are demersal species which proves that *L. spadiceus* feeds on demersal organisms. Predation on these prey items has a significant impact on population's natural mortality. Since natural mortality is an important parameter in ecosystem models, e.g. ECOPATH (Christensen and Pauly, 1993; Moreau *et al*., 1997) as well as in stock assessment models (Sparre and Venema, 1998), a follow-up study investigating such parameters as well as the interactions between the different prey and predator groups is recommended to better understand the long-term effects of the species in Pacific ocean.

This work clearly shows that molecular genetic techniques can identify prey fish species after much longer digestion times than possible with conventional methods. In cases where the proportion of stomachs with unknown material and unidentified fishes is present in most diet studies (Legler, 2009;

Mullowney, 2001), molecular genetic techniques would have proven useful in identifying prey species. DNA barcoding can identify species with fragments as short as 100 bp with at least 90% efficiency Meusnier *et al*. (2008). The development of these mini- barcodes permits the species identification which opens a great possibility to obtain sequences from short DNA fragments, quickly and cheaply Hajibabaei *et al*. (2006). According to Carreon-Martinez *et al*. (2010) molecular techniques were more precise than traditional visual inspection and could provide insight into diet preferences for even highly digested prey that have lost all physical characteristics.

Though our study had limitations with short sampling period and small sample size, present study suggests the food preference of *L. spadiceus*. This may help in future to understand the feeding ecology of these fish species better. More importantly, this study demonstrates the potential application of molecular genetic techniques for identifying semi digested food remains in the gut.

REFERENCES

Abdu Rahiman, 2006. Studies on food and feeding of marine demersal finfishes with special reference to trophic interactions. Ph.D thesis, Cochin University of Science and Technology, Kerala..

Al-Zibdah, M. and Odat, N. 2007. Fishery Status, Growth, Reproduction Biology and Feeding Habit of two Scombrid fish from the Gulf of Aqaba, Red Sea. *Lebanese Science Journal,* 8 (2). PP. 3 – 20.

Andraso, G. M. 2005**.** Summer food habits of pumpkinseeds (*Lepomis gibbosus*) and blue-

gills (*Lepomis macrochirus*) in Presque Isle Bay, Lake Erie. *Journal of Great Lakes*

Research, 31: 397–404.

Arakawa, O., Hwang, D.F., Taniyama, S., and Takatani, T. 2010. Toxins of puffer fish that cause human intoxications. In Coastal Environmental and Ecosystem Issues of the East China Sea, edited by Ishimatsu, A. and H.J. Lie. pp. 227-244

Aydýn, M. 2011. Growth, Reproduction and Diet of Pufferfish (*Lagocephalus sceleratus* Gmelin, (1789) from Turkey's Mediterranean Sea Coast. *Turkish Journal of Fisheries and Aquatic Sciences* 11: 569-576.

Carreon-Martinez, L. and Heath, D.D. 2010. Revolution in food web analysis and trophic ecology: diet analysis by DNA and stable isotope analysis. *Molecular Ecology* 19, 25–27.

Carreon-Martínez, L., Johnson, T., Ludsin, S and Heath, D. 2011. Utilization of stomach content DNA to determine diet diversity in piscivorous fishes. *Journal of Fish Biology* 78: 1170–1182.

Corse, E., Costedoat, C., Chappaz, R., Pech, N., Martin, J.F and Gilles, A.2010. A PCR based method for diet analysis in freshwater organisms using 18S rDNA barcoding on faeces. *Molecular Ecology Resources* 10**,** 96–108.

Christensen, V. and Pauly (eds) 1993. Trophic models of aquatic ecosystems. *ICLARM* conference proceeding No. 26,390pp

Domenci, P. And Blake, R.W. 1997. Review: the kinetics and performance of fish fast-start swimming.*J. Exp. Biol.,* 200: 1165-1178.

EastMed, 2010**.** Report of the Sub-Regional Technical meeting on the Lessepsian migration and its impact on Eastern Mediterranean fishery. GCP/INT/041/EC – GRE –ITA/TD-04

Hardy, C. M., Krull, E.S., Hartley, D.M and Oliver, R. 2010. Carbon source accounting for fish using combined DNA and stable isotope analyses in a regulated lowland river weir pool. *Molecular Ecology* 19, 197–212

Hajibabaei, M. S., Mith, M.A., Janzen, D.H., Rodriguez, J.J and Whitfield, J.B. 2006. A minimalist barcode can identify a specimen whose DNA is degraded. *Molecular Ecology* Notes 6: 959–964.

Hammerschlag, N., Ovando, M.D and Serafy, J.E 2010. Seasonal diet and feeding habits of juvenile fishes foraging along a subtropical marine ecotone. Aquat Biol Vol. 9: 279–290.

Hwang, D.F., Cao, C.F., Yang, H.C., Jeng, S.S., Noguchi, T. and Hashimoto, K.1992. Toxicity of puffer in Taiwan. Nippon Suisan Gakkaishi. 58. Pp.1541-1547.

Hyslop, E.J. 1980. Stomach content analysis –a review of methods and their application. *J. Fish.Biol*.,17:411-429

Kalogirou, S. 2013. Ecological characteristics of the invasive pufferfish *Lagocephalus sceleratus* (Gmelin, 1789) in Rhodes, Eastern Mediterranean Sea. A case study Medit. *Mar. Sci., 14/2, 2013, 251-260Fish.Biol*.,17:411-429

Kumar, P., Mishra, J.K., Samin, Y and Santosh Kumar, C. 2013. Studies on Biology and Feeding Habit of Puffer Fish Species from South Andaman Sea *Jour. Coast. Env*., Vol. 4, No. 1, 2013.

Khan, M. S., Ambak, M.A and Mohsin, A.K.M. 1988. Food and feeding biology of a tropical catfish *Mystus nemurus* (C&V) with reference to its functional morphology. *Indian. J. Fish*., 35(2): 78-74.

Legler, N. D. 2009. Effects of Lake Erie habitat variation and digestion rate on feeding in

freshwater ûshes. Master's Thesis, Great Lakes Institute for Environmental Research,

University of Windsor, Windsor, ON, Canada

Matthes, H. 1968. The food and feeding habits of Tigerfish, *Hydrocynus vittatus* (Cast. 1861), in Lake Kariba. Beaufortia, 15: 143-153.

Man, H.S.H and Hodgkiss, I.J 1977. Studies on the ichthyo-fauna in Plover Cove Reservoir, Hong Kong: feeding and food relation. *J.Fish Biol*., 5;707-736.

Meusnier I., Singer, G., Landry, J.F., Hickey, D.A and Hebert. P. 2008. A universal DNA mini-barcode for biodiversity analysis. BMC *Genomics*. pp. 1–4.

Miller, S.A., Dykes, D.D and Polesky, H.F 1988. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucl. Acids Res*., 1215.

Mohamad and Isa. 2013. Morphology and Stomach Content Analysis of Green Rough-backed Puffer Fish, *Lagocephalus lunaris* from Kuching, Sarawak. *Borneo J. Resour. Sci. Tech*. (2013) 2(2): 67-70

Mohanraj, T and Prabhu K., 2012. Food Habits and Diet Composition of Demersal Marine Fishes from Gulf of Mannar, Southeast Coast of India. Advances in Biological Research 6 (4): 159-164.

Moreau, J., Cronberg, G., Games, I., Hustler, K., Kautsky. N., M. Kiibus, M., Machena, C and Marshall, B. 1997. Biomass flows in Lake Kariba: towards an ecosystem approach. pp. 219-230. *In: J. Moreau* (ed.). Advances in the ecology of Lake Kariba. Harare, Zimbabwe, University of Zimbabwe Publications.

Moran, Z., Orth, D.J., Schmitt, J.D., Hallerman, E.M and Aguilar, R. 2015. Effectiveness of DNA barcoding for identifying piscine prey items in stomach contents of piscivorous catfishes. *Environmental Biology of Fishes*: 1-7.

Mohamad, K. S., Sathianan Kripa, T.V and Zacharia, P.U. 2013. Current Science, vol. 104, no. 4, 25 February 2013.

Mullowny , D. 2001. Food web studies and diet trends in some Lake Erie Predators: .Wheately,ON. Lake Erie Management Unit, Ontario Ministry of Natural Resources.

Natarajan, A.V and Jhingran, A.G., 1961. Index of preponderance - a method of grading the food elements in the stomach analysis of fishes. Indian J. Fish. 8(1):54-59

Nelson., Joseph, S., Grande., Terry, C., Wilson., W.H Mark. 2016. *Fishes of the world*. Jhon wiley & Sons, Inc, New Jersey, 5th ed.,

Noguchi, T. and Arakawa, O. 2008. Tetrodotoxin – distribution and accumulation in aquatic organisms and cases of human intoxication. Marine Drugs 6: 220-242.

Noguchi, T., Arakawa, O. and Takatani., T. 2006. TTX accumulation in pufferfish. Comparative Biochemistry and Physiology, Part D 1: 145-152.

Mohamad and Isa. 2013. Morphology and Stomach Content Analysis of Green Rough-backed Puffer Fish, *Lagocephalus lunaris* from Kuching, Sarawak. *Borneo J. Resour. Sci. Tech.* (2013) 2(2): 67-70.

Pillay, T.V.R. 1952**.** A critique method of study of food of fishes.*J.Zool.Soc*.,4:185-200

Richardson, J. 1845. Ichthyology — Part 1. In Hinds, R. B. (ed.): The zoology of the voyage of H. M. S. Sulphur, under the command of Captain Sir Edward Belcher, R. N., C. B., F. R. G. S., etc., during the years 1836–1842. No. 10, pp. 99–150, pls. 55–64.

Roseman, E. F., Stott, W., 'Brien T.P.O., Riley, S.C. and Schaeffer, J.S. 2009. Heritage strain and diet of wild young of year and yearling lake trout in the main basin of Lake Huron. *Journal of Great Lakes Research* 35, 620–626.

Sabrah, M.M., El-Ganainy, A.A and Zaky, M.A. 2006. Biology and Toxicity of the puffer fish Lagocephalus sceleratus (Gmelin, 1789) from The Gulf of Suez. *Egyptian Journal of Aquatic Research*, 32: 283-297

Schmidt, J. E. U., Almeida, J.R.M., Rosati, C and Arpaia, S. 2009. Identification of trophic interactions between *Macrolophus caliginosus* (Heteroptera: Miridae) and *Myzus persicae* (Homoptera: Aphididae) using real time PCR. Biocontrol 54, 383–391.

Sparre, P., Venema, S.C. 1998. Introduction to tropical fish stock assessment. Part 1. Manual.*FAO Fisheries Technical Paper.* No. 306.1, Rev. 2.Rome, FAO. 407p

Symondson, W. O. C. 2002. Molecular identification of prey in predator diets. Molecular Ecology 11, 627–641.

Teletchea, F. 2009. Molecular identification methods of fish species: reassessment and possible applications.*Rev Fish Biol Fisheries* (2009) 19: 265. doi:10.1007/s11160-009-9107-4

Yasumoto, T., Yasumura, D., Yotsu, M., Michishita, T., Endo, A and .Kotaki, Y. 1986. Bacterial production of tetrodotoxin and anhydrotetrodotoxin. Agricultural and Biological Chemistry 50(3): 793-795