Zooplankton as dietary component of selected freshwater fish: network analysis based on gut content

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Abstract. The presence of the zooplankton as the dietary component of the freshwater fish species occurring in the wetlands of India was justified through the gut content analysis. Using the fish *Channa punctata, Channa striata, Glossogobius giuris, Mystus tengara* and *Notopterus notopterus* as model species of the freshwater wetlands, the gut contents were assessed. With regard to the dietary items, considerable extent of similarity was observed for the fish species, reflected through the niche overlap indices. Irrespective of the fish species, in all instances, cladocerans, copepods and rotifers were found to be common dietary elements in the fish gut analysis, along with plant parts, detritus and insects, in different proportions. Significant variations among the fish species were observed with respect to the proportion of cladocerans and copepods found in the gut content. The network analysis revealed the extent of the generality of the fish species, in terms of their consumption pattern as revealed through the gut content analysis. The links with the different zooplankton in the gut content of the fish species reflect the significance as live food and prospective use in the aquaculture. However, the quality of the zooplankton requires further assessment in terms of the nutritive content. Further studies may be initiated to substantiate the specific link of the fish species and the zooplankton in the wetlands of Assam, India.
Key Words: Rotifera, Cladocera, Copepoda, freshwater wetlands, fish, network analysis.

Introduction. The zooplankton constitutes staple food for a wide range of fish species in tropical and subtropical freshwater habitats (Bogard et al 2015). Among other dietary items, the zooplankton like copepods, cladocerans, and rotifers are consumed in varied proportions by almost all the fish species particularly during the juvenile stage. In juvenile and fingerlings, the dependence on the zooplankton is considerably high and the growth of certain species is inevitably determined by the extent of the zooplankton in the diet (Guo et al 2009). Thus many studies have emphasized the significance of the zooplankton in the culture of the fish species of freshwater origin (Ahmad et al 2012). Variation in the species composition and the abundance is a redundant component of the zooplankton species assemblage in freshwaters. On a broader scale the proportional abundance of the zooplankton varies with the water quality parameters, physical features and the seasons, evident from several studies worldwide and from India (Gupta & Devi 2014). Irrespective of the variations in the abundance, the dependence on the zooplankton by the fish is an invariant property. This proposition is being tested in the present instance, where the gut content analysis of five different fish species was accomplished highlighting the differences in the proportional presence of three major groups of zooplankton, namely, Cladocera, Copepoda and Rotifera, along with other components like plant parts, detritus and insects remains (Chakrabarti et al 1995; Datta et al 2013; Singh et al 2013; Ahmed et al 2016).

The fish *Channa punctata* (Bloch, 1793) (Perciformes: Channidae), *Channa striata* (Bloch, 1793) (Perciformes: Channidae), *Glossogobius giuris* (Hamilton, 1822) (Perciformes: Gobiidae), *Mystus tengara* (Hamilton, 1822) (Siluriformes: Bagridae) and

Notopterus notopterus (Pallas, 1769) (Osteoglossiformes: Notopteridae) are common in the wetlands of Assam and West Bengal, India, including the rice fields and allied trap ponds (Aditya et al 2010; Sonawane et al 2012; Saha et al 2017a, b; Zehra & Khan 2018). All these species of fish are consumed as a cheap protein source and bear significance in traditional aquaculture as well as harvest fisheries. The economical and the nutritional value of these fish species are high in Bangladesh, Cambodia, Vietnam and several Asian countries where the consumption of these fish species ensures dietary supplement of the protein (Sagada et al 2017; Hossain et al 2016). In many instances, these species are promoted for the rice fish culture (Rao & Rao 2002) while species like *N. notopterus* is of high demand as a preferred fish for consumption (Kiran & Waghray 1998) in Indian context. Keeping in view the significance of the wetlands of Assam, West Bengal and other states of India the assessment on the gut content of the fish would substantiate the relevance of the zooplankton as a dietary constituent of the five fish species. Application of the network model to the data would also elaborate the links among the different dietary items and the fish species that occupy the same habitat conditions. While most of the studies on the gut content of the fish have aptly provided the dietary requirements of the concerned species, in the present instance an elaborated network would illustrate the extent of complexity observed in the constituent members of the freshwater communities.

Material and Method. The fish species were collected from the oxbow lakes and flood plain lakes in and around Silchar, Assam, India on different dates in a year (between 2015 and 2016). The fish species were collected at different time interval from the different oxbow lakes and the beels in different phases between August and September of 2015 and 2016. Owing to the consideration of multiple individuals of the fish species originating from different space and time, the fish individuals considered for analysis qualified as a true replicate (Hurlbert 1984). Following the capture using the traditional fishing gears, the fish species were placed in plastic bags and brought to the laboratory for identification and segregation to the species levels. Subsequent to the segregation, the fish specimen was considered for the assessment of the gut content (Baker et al 2014). In the present instance five fish species namely *C. punctata, C. striata, G. giuris, M. tengara* and *N. notopterus* were considered. For each fish species, 20 individuals were analyzed and thus a total of 100 individuals were analyzed during the whole study. After noting the total length to the nearest millimeter and weight to the nearest 0.01 g, the abdomen was cut open and stomach removed. The gut content analysis (Hyslop 1980; Winemiller 1990; Tobler 2008) was accomplished employing the standard protocols (Scharnweber et al 2013; Baker et al 2014) with certain modifications as applicable for these fish species. For each of the dietary items retrieved through from the dissected gut, a percent frequency of encounter was recorded and interpreted as a measure of diet composition (Baker et al 2014). The %F technique is dependent on the number of encounter of the specific form of the hard tissue remains of the dietary items and the following diet categories were recognized for this study (Winemiller 1990): detritus (DET), scales of other fish (SCA), rotifers (ROT), cladocerans (CLA), copepods (COP), insects (INS) and algal and plant remains (PHY). Although, the contents characterized as insects varied considerably, including the mosquito larvae, chironomid larvae as well as water striders. However, to avoid the confusion in identification, these were considered as insects. Identification of the above dietary items was made on the basis of the shape of the body parts and confirmed comparing the morphological and anatomical descriptions stated in general fresh water biology identification keys (Edmondson 1959; Battish 1992). For macroinvertebrates, owing to lack of complete body parts of the prey, identification could not be made up to appropriate genus and species level, though the field sampling data provided substantial clue for the taxonomic resolutions of the prey items. Since the algae were inseparable as complete entity, identification of the alga separated from gut was not carried out further. Primary emphasis was given to identify the different groups of the zooplankton to highlight the relevance of the zooplankton in the freshwater food web of the concerned habitats from where the fish species were collected for the study. A single fish individual was considered only once for the purpose of the gut content analysis.

The data on the gut content of the fish species were used for the Discriminant function analysis (DA), as a part of the exploratory data analysis and highlight the differences among the fish species, if any, with reference to the food types (Manly 1994; Legendre & Legendre 1998). A factorial ANOVA was applied to justify the differences among the fish species with reference to a particular group of the zooplankton, as well as detritus, plant parts and the insect remains. The purpose was to substantiate the differences if any among the fish species concerned. The data obtained on the gut content of the fish species were subjected to the niche breadth and niche overlap analyses using the formulae stated below (Ludwig & Reynolds 1988; Krebs 1999):

1. Levene's niche breadth $B = 1/\sum p_i^2$ and the standardized form of niche breadth is $B_A = B-1/(n-1)$, where pi is the proportion of *i*th food item in diet, B_A is standardized niche breadth;

2. Pianka's niche overlap: $O_{ij} = \sum p_{ij}p_{ik} / \sqrt{(\sum p_{ij}^2 \sum p_{ik}^2)}$

Where, O_{ij} is the overlap index between *i* and *j* species in terms of k resources; a value of 1 indicates maximum overlap while a value of 0 indicates no overlap.

Differences in the proportion of diet of animal origin and plant origin were analyzed for each species, using a two tailed t-test (Zar 1999). In addition to the niche overlap a network of the fish and the dietary items was constructed to highlight the complexity of the network and the relative significance of one or the other groups of the zooplankton in the sustenance of the fish populations (Hannon 1973; Fath & Patten 1999; Fath et al 2007; Ings et al 2009).

In addition to the niche overlap a network of the fish and the dietary items was constructed to highlight the complexity of the network and the relative significance of one or the other groups of the zooplankton in the sustenance of the fish populations. The bipartite network of the fishes and dietary constituents were constructed using a data matrix (Warren & Lawton 1987; Blüthgen et al 2006, 2008), where the row elements being the food types (j), and the column elements were fishes (i). To identify the network connectivity between fishes and food items in the weighed or quantitative matrix was constructed considering frequency of interactions of each fish species on zooplankton and other food items. The different indices like connectance, asymmetry, and weighted nestedness and degree of complementary specialization (H_2) were measured using quantitative matrixes. R software version 3.4.0 (2017-04-21) (freeware) and bipartite package was used to calculate different indices and to create the bipartite graph (Table 1). In all other instances of the statistical analysis, *XL*STAT (Addinsoft 2010) was used with the models being chosen appropriate references.

Table 1

Elaboration of the terms used for describing a network: (a) the symbols with the corresponding meaning; (b) matrix of a fish and food type association matrix based on weighted links with c numbers of fish species and r numbers of food types; (c) indices based on weighted links (quantitative webs) (E) (McCoy & Heck 1987; Blüthgen et al 2006; Dormann et al 2008, 2009)

(a)

Symbol	*Meaning*
L	Number of actual links in a network
I	Number of lower trophic level (seven feed types)
J	Number of higher trophic level (fishes)
M	Total number of interactions for all species
a_{ij}	Number of interactions between species *i* from the lower and species *j* from the higher trophic level
A_i	Total number of interactions of species i from the lower trophic level, $A_i = \sum_{j=i}^{J} aij$
A_j	Total number of interactions of species j from the higher trophic level, $A_j = \sum_{i=i}^{I} aij$

(b)

	Fish sp.$_1$	*Fish sp.$_2$*	.....	*Fish sp.$_c$*	*Total*
Food type$_1$	$a_{1,1}$	$a_{1,2}$	.....	$a_{1,c}$	$A_{i=1} = \sum_{j=1}^{c} a_{1j}$
Food type$_2$	$a_{2,1}$	$a_{2,2}$	......	$a_{2,c}$	$A_{i=2} = \sum_{j=1}^{c} a_{2j}$
...	.....	.....	.....	....	....
Food type$_r$	$a_{r,1}$	$a_{r,2}$	....	$a_{r,c}$	$A_{i=r} = \sum_{j=1}^{c} a_{rj}$
Total	$A_{j=1} = \sum_{i=1}^{r} a_{i1}$	$A_{j=2} = \sum_{i=1}^{r} a_{i2}$	....	$A_{j=c} = \sum_{i=1}^{r} a_{ic}$	$m = \sum_{i=1}^{r}\sum_{j=1}^{c} a_{ij}$

(c)

Weighted nestedness	This index measures directly the departure of a matrix from the nested pattern on the basis of abundance data matrix (Corso et al 2008; Araujo et al 2010)
Interaction strength	Interaction strength of species i on species *j* (b_{ij}) portion of interactions between *i* and *j* (a*ij*) of the total interactions recorded for *i*. $b_{ij} = a_{ij} / \sum_{i=1}^{j} aij$ and the reciprocal interactions (Jordano 1987; Bascompte et al 2006; Bascompte & Jordano 2007).
Interaction strength asymmetry	Measure the disparity between the interaction strengths of a species pair. $AS_{ij} = (b_{ij} - b_{ji})/(b_{ij} + b_{ji})$,
Shannon diversity	$H_i = -\sum_{i=1}^{j} [(aij/Ai) Ln(aij/Ai)]$, for species i or for the whole web $H_2 = -\sum_{i=1}^{j} \sum_{i=1}^{j} \left(\frac{aij}{m}\right) Ln\left(\frac{aij}{m}\right)$
Standardized interaction diversity (H_2')	$H_2' = (H_{2max} - H_2)/(H_{2max} - H_{2min})$
Interaction evenness	$E_i = H_i/\ln L_i$, for each species, or for the whole web, $E_2 = H_2/\ln L$

Results. In all instances, the gut contents were found to be positive with the cladocerans, copepods and rotifers in different numbers, along with the detritus, plant parts and the insect remains. The mosquito and chironomid larval heads and the siphons were observed from the gut contents and the chitinous exoskeletal features of the cladocerans and copepods and the lorica of different rotifers were observed. Although species specific confirmation was not made the zooplankton types remained different in the five fish species (Figure 1) substantiated by the significant differences in the ANOVA, followed by post hoc Tukey test (Table 2). The relative abundance of the different food items (Figure 2) was used as explanatory variables to discriminate the fish species (Figure 3) which is shown in the ordination in the biplots and the significant values of the Fisher's distance. The niche overlap and the diet width of the fish species were also different as shown in the Table 3.

Table 2

The results of the ANOVA highlighting the differences among the fish species in terms of the specific food items

Source of variation	*DF*	*Sum of squares*	*F*	*Source of variation*	*DF*	*Sum of squares*	*F*
CLA	4	7.060	1.373	SCL	4	4.760	1.831
Error	95	122.100		Error	95	61.750	
Total	99	129.160		Total	99	66.510	
COP	4	32.360	**6.756**	INS	4	29.660	**3.150**
Error	95	113.750		Error	95	223.650	
Total	99	146.110		Total	99	253.310	
ROT	4	36.640	**3.872**	DET	4	16.500	1.687
Error	95	224.750		Error	95	232.250	
Total	99	261.390		Total	99	248.750	
PHY	4	2.160	0.980				
Error	95	52.350					
Total	99	54.510					

The values in bold indicate significance at $p < 0.001$; CLA - Cladocera, COP - Copepoda, ROT - Rotifera, PHY - Plant remains, SCA - Scales of fishes, INS - Insects larvae and its parts, DET - Detritus.

Table 3

The niche overlaps (Mean±SE) among the fish species along with individual Levene's niche breadth, based on the data on relative number of prey type available

Levene's niche breadth	*Fish pair*	*CST*	*CPU*	*MTE*	*GGI*
4.7	CPU	0.984±0.001			
5.2	MTE	0.991±0.003	0.981±0.004		
5.3	GGI	0.971±0.001	0.947±0.007	0.98±0.004	
5.526	NNO	0.975±0.005	0.956±0.007	0.984±0.0001	0.992±0.003
5.2	CST				

CST - *Channa striata,* CPU - *Channa punctata,* MTE - *Mystus tengara,* GGI - *Glossogobius giuris,* NNO - *Notopterus notopterus.*

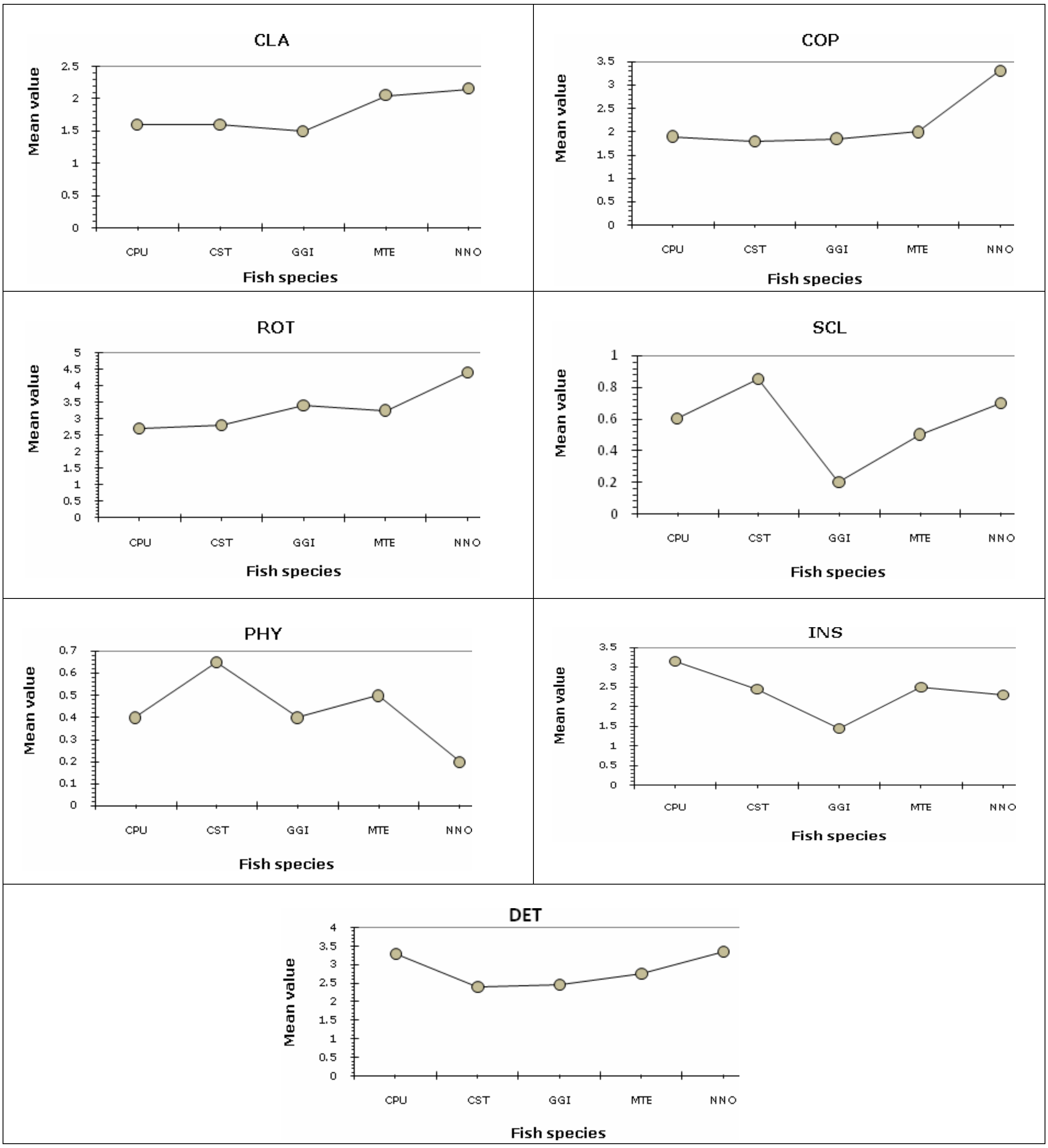


Figure 1. The dietary constituents of the fish species as revealed through the gut contents of the fish species namely *C. punctata, C. striata, G. giuris, M. tengara* and *N. notopterus* (with the number of the fish species being n = 20 for each species). The asterisk against the respective fish species indicate significant ($p < 0.001$) differences following multiple comparison (post hoc Tukey test, Table 2) CST - *Channa striata,* CPU - *Channa punctata,* MTE - *Mystus tengara,* GGI - *Glossogobius giuris,* NNO - *Notopterus notopterus*, CLA - Cladocera, COP - Copepoda, ROT - Rotifera, PHY - Plant remains, SCA - Scales of fishes, INS - Insects larvae and its parts, DET - Detritus.

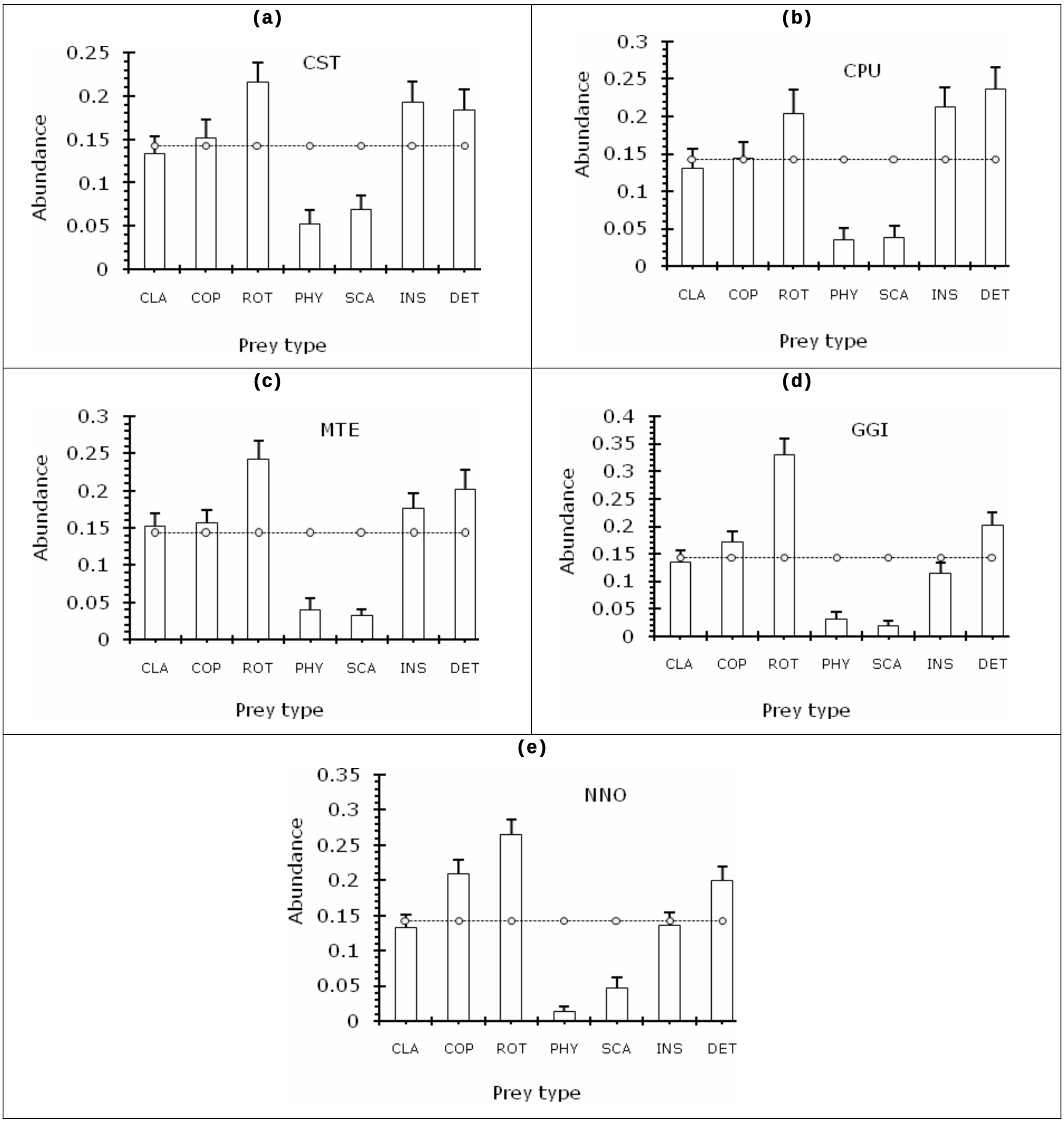


Figure 2. The abundance (Mean±SE) of the different prey type observed during the gut content analysis of six different fish species [(a): CST, (b): CPU, (c): MTE, (d): GGI, (e): NNO]. CST - *Channa striata,* CPU - *Channa punctata,* MTE - *Mystus tengara,* GGI - *Glossogobius giuris,* NNO - *Notopterus notopterus*, CLA - Cladocera, COP - Copepoda, ROT - Rotifera, PHY - Plant remains, SCA - Scales of fishes, INS - Insects larvae and its parts, DET - Detritus.

(a)			
	F1	F2	F3
Eigenvalue	0.563	0.135	0.002
Discriminatio (%)	80.475	19.253	0.271
Cumulative %	80.475	99.729	100.0
Canonical correlations	0.600	0.345	0.044

(b)	Fisher's distance			
	CPU	CST	GGI	MTE
CST	0.704			
GGI	**4.903**	2.078		
MTE	1.193	0.479	1.584	
NNO	**13.66**	**12.30**	**9.065**	**8.250**

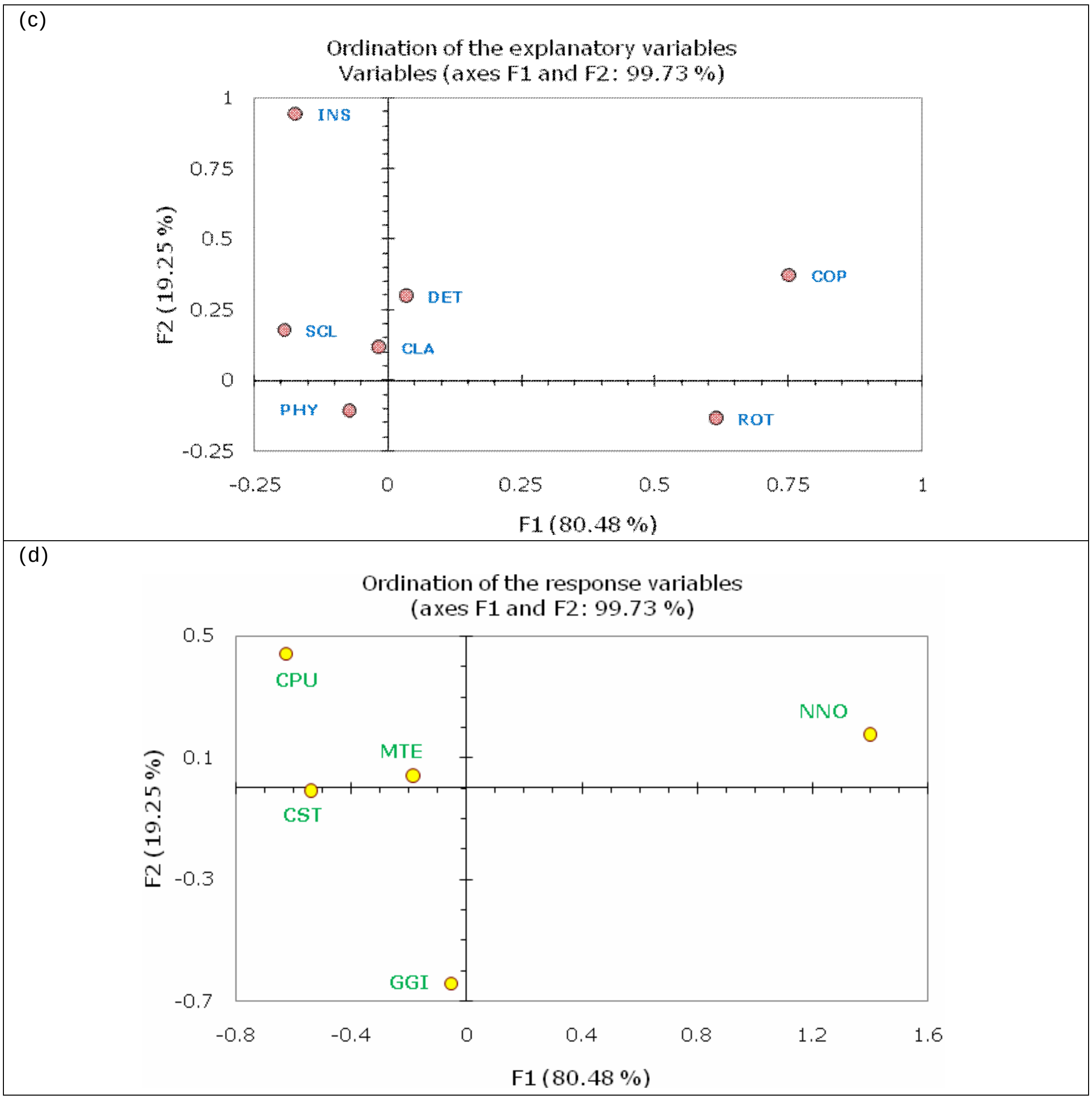
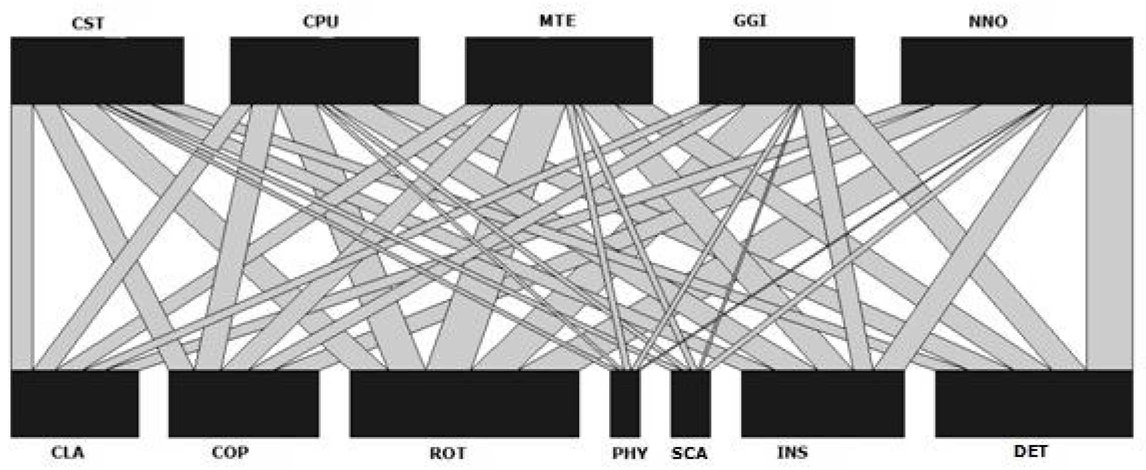


Figure 3. The discriminatory function analysis (DA) of the fish species based on the dietary items observed through gut content analysis. The results include the Eigen values (a), Fisher's distance (b), ordination of the explanatory variables (c), and ordination of the response variables (d). The values in bold indicate significance at $p < 0.05$ level. The Wilks' lambda value was 0.563; $F_{12,\ 246}$ = 4.984 significant at p< 0.001 level. CST - *Channa striata,* CPU - *Channa punctata,* MTE - *Mystus tengara,* GGI - *Glossogobius giuris,* NNO - *Notopterus notopterus*, CLA - Cladocera, COP - Copepoda, ROT - Rotifera, PHY - Plant remains, SCA - Scales of fishes, INS - Insects larvae and its parts, DET - Detritus.

Network*.* The network of present study represented 12 nodes (species) and 35 links among species. During this study period there were five fish species (j) and seven prey species (i) found (Figure 4) and their interacting matrix were presented in Figure 4a and each filled cell (link weights) showed the interaction frequencies between two species, i.e. how many individuals of a particular snail were observed on a particular food type. The maximally packed matrix indicated the highly nested pattern of fish-prey interacting association. Though the number of link was equal in all fishes, the frequency of choice of prey species was not equal and it was measured by weighted bipartite network analysis in R Software using bipartite package (Blüthgen et al 2006, 2008; Dorman et al 2008, 2009). The relative weight of the fish species on the different food types and vice versa is shown in Figure 5.

The maximum weight on PHY was NNO, similarly, on SCL was MTE, on CLA was NNO, on INS was CST, on DET was CPU, on ROT was NNO. On the other hand CST, MTE, GGI, NNO preyed maximum on ROT and CPU on DET. The high value of connectance (1) low value of H_2' indicated that the fish-prey community was highly nested and generalised. Each fish was linked with every prey in different frequency. The niche overlap value was also high (> 0.9), similar to those calculated other than the network.

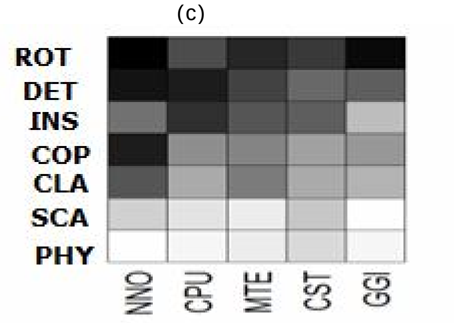
(a)



(b)

	CST	CPU	MTE	GGI	NNO
CLA					
COP					
ROT					
PHY					
SCA					
INS					
DET					

(c)



(d)The different indices of the fish-food type network

Indices	Values	Indices	Values
Connectance	1	H_2	0.002
Links per species	2.917	Interaction evenness	0.948
Shannon diversity	3.371	Interaction strength asymmetry	0.086
Weighted nestedness	0.024	Generality	5.876
Linkage density	5.379	Vulnerability	4.882

Figure 4. The network of the fish and the food types observed in the gut content as represented through the links and nodes. CST - *Channa striata,* CPU - *Channa punctata,* MTE - *Mystus tengara,* GGI - *Glossogobius giuris,* NNO - *Notopterus notopterus*, CLA - Cladocera, COP - Copepoda, ROT - Rotifera, PHY - Plant remains, SCA - Scales of fishes, INS - Insects larvae and its parts, DET - Detritus.

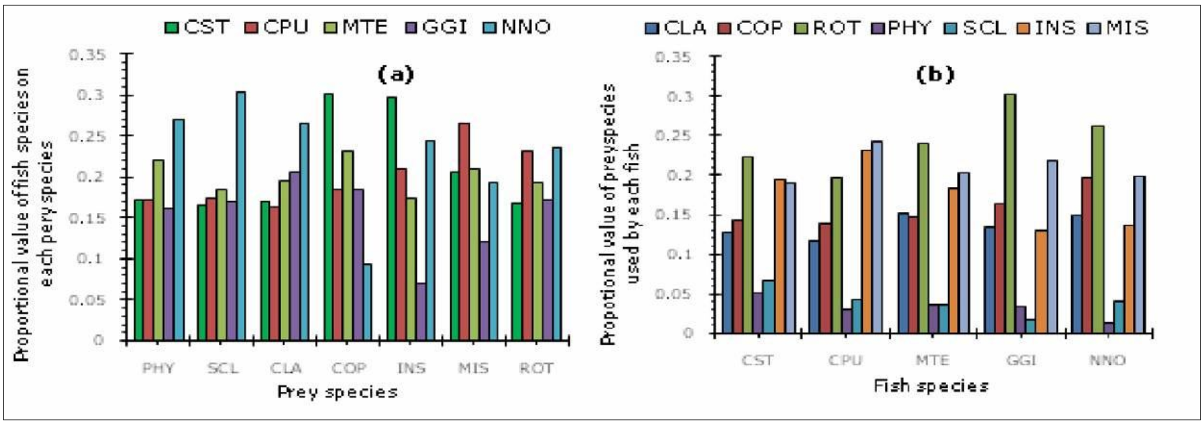


Figure 5. The pattern of interaction between individuals or proportion of preferences of prey by fish species. a. proportion of fish occurrence on each prey, b. proportion of prey species preference for each fish. CST - *Channa striata,* CPU - *Channa punctata,* MTE - *Mystus tengara,* GGI - *Glossogobius giuris,* NNO - *Notopterus notopterus*, CLA - Cladocera, COP - Copepoda, ROT - Rotifera, PHY - Plant remains, SCA - Scales of fishes, INS - Insects larvae and its parts, DET - Detritus.

Discussion. The gut contents of the fish species namely *C. punctata, C. striata, G. giuris, M. tengara* and *N. notopterus*, revealed the presence of the zooplankton consistently along with the detritus, plant parts and the insect remains. Although less specific in terms of predicting the predatory impact of these fish on the entire freshwater community, the gut contents confirm the presence of the zooplankton and thus the significance in sustenance of population of the respective fish species. Earlier studies on the gut content analysis and the feeding pattern of the five species indicate high dependency on the macroinvertebrates and the detritus as dietary items. Of particular relevance are the fish species *C. punctata* and *C. striata,* which consume high amount of animal prey and detritus (Sonawane et al 2012; Singh et al 2013). Plant parts are least preferred among dietary items of the fish *N. notopterus* (Kiran & Waghray 1998), which is also true for the fish *G. giuris* (Hossain et al 2016). In parity with these observations, in the present instance, the plant remains/algae were observed in least amount. In the present instance also, the insect remains and the detritus were observed in high quantity, though variations among the five fish species was prominent (for insects, cladocerans and copepods). Perhaps the differences in the habitat preferences and the resource choice by the five species are reflected through the variations in the proportional presence of the different food types. The morphological and anatomical peculiarities and the physiological features including the enzymes are also significant contributors to the differences in the food preferences. The variations among the fish species are elaborated in the network analysis, which is an important tool in projecting the resource partitioning mechanism and the robustness of the freshwater community. As shown through the analysis, the degree of specialization was too low, in parity with the propositions of Woodward & Hildrew (2002). At the other end the niche overlap among the five species can be considered as an indication of the niche segregation in situations where these species are common (Herder & Freyhof 2006). However, further studies are required to understand the variations in the food choice and feeding pattern of the concerned fish species, including the shifts, if any in the dietary choices.

Irrespective of the dietary requirements and the consumption of the five fish species, including size and ontongenic features and variations in the habitat elements, zooplankton forms a redundant component included in the diet of almost all the fish species (Guo et al 2009). The presence of the cladocerans in the gut content was not significantly different for the five species, though the rotifers and the copepods were found in different proportions. Perhaps, this was correlated with the habitat preferences and the exploitation of the specific zooplankton in the habitats (de Senerpont Domis et al 2013). Although the gut content of the fish species were not assessed in accordance with the species specific presence of different zooplankton, the bipartite network indicated consistent linkage among the two groups. However, the strength and interactions can be further explored using the species specific information of both the groups including temporal scale exploration. Nonetheless, the gut content analysis confirms the presence of the zooplankton as a prominent dietary item suggesting the significance of zooplankton in pisciculture particularly in the wetlands of Assam, West Bengal and similar regions of India and neighbouring countries.

Conclusions. The dietary items of the fish species namely *C. punctata, C. striata, G. giuris, M. tengara* and *N. notopterus* revealed through the gut content analysis included zooplankton as a redundant item. The relative abundance of the cladocerans, the copepods and the rotifers different among the fish species considerably reflected through the statistical analysis. As reflected through the bipartite network, the dietary patterns of the fish species were generalized with consistent links to the zooplankton. An extension of this observation is to enhance the zooplankton of different groups as a food item for all the five fish species considered here, in the intensive aquaculture using the concerned fish species. However, further studies are required to identify the preferences for the specific species of the zooplankton by the fish species. Such studies may enable utilizing the zooplankton species with higher precision and enhancing the productivity of the fish species.

Acknowledgements. The authors are grateful to the respective Heads, Department of Life Science and Bio informatics, Assam University, Silchar, Assam, and Department of Zoology, University of Calcutta, Kolkata, India for the facilities provided, including DST-FIST and UGC UPE-II (University of Calcutta). The authors, namely, Papia Das, Sulata Kar and Uma Das, acknowledge the financial assistance provided by UGC in carrying out the work.

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Received: 27 December 2017. Accepted: 31 January 2018. Published online: 25 February 2018.
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How to cite this article:
Das P., Mukherjee S., Kar S., Das U., Kar D., Aditya G., 2018 Zooplankton as dietary component of selected freshwater fish: network analysis based on gut content. AACL Bioflux 11(1):232-244.