

Original Article

Feeding ecology of the endemic freshwater puffer fish *Carinotetradon travancoricus* (Hora & Nair, 1941) in Western Ghats hotspot, India

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Abstract: The study aimed to investigate the feeding ecology of a vulnerable freshwater puffer fish, *Carinotetradon travancoricus* from the Chalakudy river of Kerala, Western Ghats, a biodiversity hotspot of India. Fishes were sampled from October 2018 to September 2019. Stomach condition of the fishes showed the percentage of empty gut to be significantly higher during all seasons ($P < 0.01$). Feeding intensity depicted the fish to follow an 'active' feeding strategy ($31.59 \pm 10.32\%$) during pre-monsoon season. Diet composition and relative length of gut analysis indicated that the fish during its early stages relied on an omnivorous diet however preferring autochthonous food materials such as insects (27.91%) and crustaceans (25.30%) during its adult stages. A perceptible variation in the feeding strategy associated with the spawning season of *C. travancoricus* was also noticed. During their spawning season (May-August), a greater preponderance towards animal matter (52.18%) was noticed in their diet. The results of gastrosomatic index indicated that feeding activity of *C. travancoricus* is considerably reduced (2.99) during the spawning period. The present study provides the baseline information on the feeding ecology of *C. travancoricus* which could be helpful to aquarists for breeding and rearing of this species in captivity and thereby reducing their fishing pressure in wild.

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Introduction

Puffer fishes of the family Tetradontidae are one of the most diverse groups in tropical seas and freshwater areas of the world. Among them, only four genera (27 species) are known to adapt to freshwater environments, occurring in three tropical regions of the world (Southeast Asia, Central Africa and South America) (Ebert, 2001; Nelson, 2006). In India, three species of freshwater puffer fishes are reported viz. *Carinotetradon travancoricus*, *C. imitator* and *Tetradon cutcutia* (Yamanoue et al., 2011). *Carinotetradon travancoricus* (Hora & Nair, 1941) is commonly known as Malabar puffer fish, an endemic fish inhabiting the rivers and lakes of Western Ghats, a biodiversity hotspot in Southern India (Remadevi et al., 2000; Dahanukar, 2011). Freshwater puffer fish trade from India substantially increased since 1990's, ever since they were marked as aquarium fish due to its yellowish body colour, ovoid body shape and

puppy dog eye (Prasad et al., 2012; Liya and Ramachandran, 2013). The species in their native range is presently being impacted by the severe modifications in their habitats due to damming, deforestation, conversion of forest area in to agriculture and rubber plantation and also due to over harvesting for aquarium pet trade (Raghavan et al., 2008). The population of puffer fishes have since then declined and is now listed as 'Vulnerable' in the IUCN Red list of Threatened Species due to the over exploitation and habitat loss (Dahanukar, 2011). The global aquarium fish trade industry is large, diverse and involving nearly 5300 freshwater and 1802 marine fishes (Hensen et al., 2010; Rhyne et al., 2012). Nearly 90% of the export market involve tropical freshwater fishes and among them only 10% are captive bred and the remaining are wild caught (Olivier, 2001). In India, the hub for the collection of wild caught ornamental fishes is the Western Ghats

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and Eastern Himalayan biodiversity hotspots known for their exceptional freshwater biodiversity and endemism (Allen et al., 2010; Raghavan et al., 2013). The export market of freshwater ornamental fishes from India accounts to over 1.5 million of fishes belonging to 30 threatened species mostly contributed by *Botia striata*, *C. travancoricus*, *Sahyadria denisonii* and *S. chalakkudiensis* (Raghavan et al., 2013).

Being mostly gregarious, bright coloured and small, the Malabar puffer fish, *C. travancoricus* are easily caught and hence they outnumber other indigenous species exported from India (Raghavan et al., 2013). Indiscriminate collection of this threatened species from natural waters for export trade resulted in the severe population decline (Dahanukar, 2011). In order to reduce their fishing pressure, knowledge regarding the nutritional requirements of *C. travancoricus* in wild is essential to develop technologies for their captive breeding and rearing. Information on the feeding habits of fishes in a given ecosystem have considerable importance in fisheries conservation and is a key factor to determine their growth rate, condition and population level (Begum et al., 2008; Saikia, 2016). The analysis of diet of fishes is also important to better understand the behavior of the organisms and permits a comprehensive understanding of ecosystem functioning that is required for its *in-situ* conservation (Braga et al., 2012; Tonella et al., 2018). Till date any comprehensive study on the feeding ecology of *C. travancoricus* especially from the wild is lacking. On this background, the present study was undertaken to examine the feeding intensity, gastroscopic index (GaSI), relative length of gut (RLG), index of relative importance (IRI) and gut content analysis of *C. travancoricus* in Chalakudy River, Western Ghats of India.

Materials and Methods

Sample collection: Sampling was done monthly from the Chalakudy river (10°18'40"N, 76°38'10"E), a perennial river system in Kerala state of India from October 2018 to September 2019 using seine nets (mesh size 5-8 mm) and scoop nets (mesh size 2-4

mm). The region receives an annual rainfall of 3000 mm during the monsoon season extending from June-September followed by post-monsoon (October-January) and pre-monsoon season (February-May). After collection, the fishes were preserved in ice and brought to the laboratory where total length (TL) was measured to the nearest 0.01 cm and total weight (TW) was taken to an accuracy of 0.01 g. A total of 278 specimen (10.5 to 30.0 mm TL and 0.12 to 0.62 g TW) were analyzed for the study. Healthy fishes without any sign of injury or infection were dissected out for stomach content analysis. The weight and volume of the gut contents of each stomach were measured and preserved in 4% neutral formaldehyde solution for further analysis. Prey items were removed from the dissected stomach and the contents were examined under a stereomicroscope (Motic SMZ 168, China) (X10) and individual food items were identified to lowest possible taxonomic level.

Feeding intensity, gastroscopic index (GaSI) and relative length of gut (RLG): The intensity of feeding was determined based on the degree of fullness and points were allotted to each gut. Depending on the fullness of stomachs, points were assigned as 20, 15, 10, 5 and 0 for full, 3/4 full, 1/2 full, 1/4 full and empty stomachs (Pillay, 1952). Fishes with stomach full and 3/4 full were considered as 'active' feeders, 1/2 full as 'moderate' feeders and 1/4 and empty stomach are 'poor' feeder. The sum of allotted points based on different degrees of fullness of stomach was divided by the number of stomach samples for that month. Monthly gastroscopic index (GaSI) was calculated following Desai (1970) using the formula:

$$\text{GaSI} = \frac{\text{Weight of the gut}}{\text{Total weight of the fish}} \times 100.$$

Fishes were classified based on their body length as juveniles (<20 mm) and adults (>20 mm) and their relative length of gut (RLG) was calculated following Al-Hussaini (1949):

$$\text{RLG} = \frac{\text{Length of the gut}}{\text{Total length of the fish}}$$

Gut content analysis: Gut content analysis was calculated using frequency of occurrence following the formula after Hynes (1950):

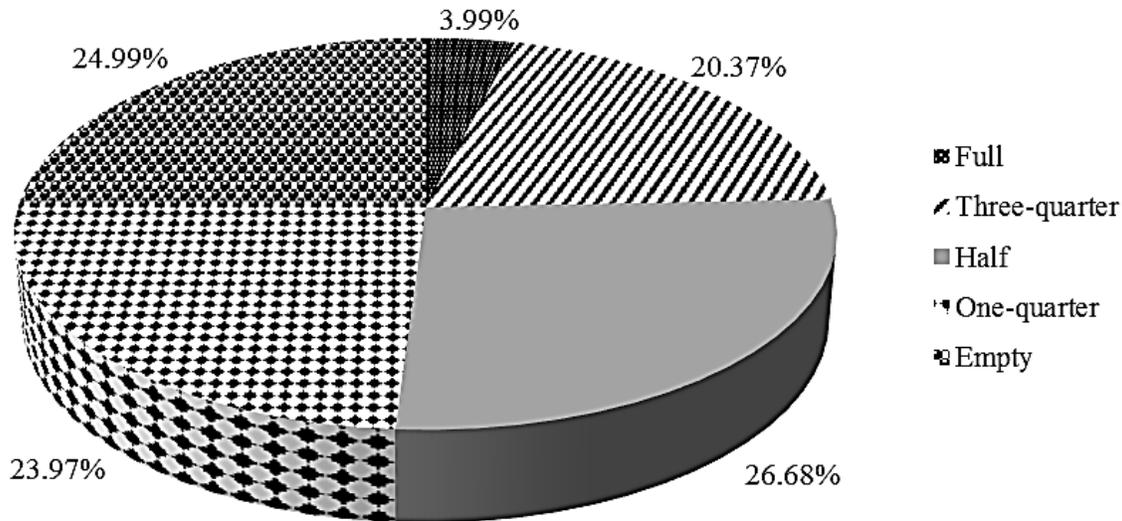


Figure 1. Feeding intensity of *Carinotetradon travancoricus* in Chalakudy River.

$$F_i = 100 * N_i / N$$

Where, F_i is the frequency of occurrence of the i^{th} food item in the sample; N_i = number of stomachs in which the i^{th} item was found and N = total number of stomachs (with food) examined. In order to ascertain the seasonal variations in the gut contents, monthly data pertaining to a particular season were grouped together and analysed.

Index of relative importance (IRI): The contribution of each prey items to the diet of fishes was determined following three relative metrics of prey quantity: percentage frequency of occurrence ($\%O_i$ = number of guts containing prey i /total number of guts containing prey*100), percentage composition by number ($\%N_i$ = number of prey i /total number of prey*100) and percentage composition by volume ($\%V_i$ = volume of prey i /total volume of prey*100) (Hynes, 1950; Pillay, 1952; Bowen, 1996). The index of relative importance (IRI) of each prey taxon (Pinkas et al., 1971) was calculated using the following formula:

$$IRI_i = (\% N_i + \% V_i) \% O_i$$

Statistical Analysis: Seasonal variations in the gut contents were analysed statistically through one-way ANOVA followed by Duncan's Multiple Range Test (DMRT) using SPSS 20.0 software of IBM to determine the significant difference of a food item between seasons (Gomez and Gomez, 1984). Significance was measured at $P < 0.05$ level.

Results

Feeding intensity: An assessment of the feeding intensity of *C. travancoricus* indicated that the percentage of empty and quarter full guts were considerably high (48.96 ± 6.98) throughout the year, while only very few percentages of guts (3.99 ± 3.80) were found to be full. Further assessment on the stomach fullness condition, that relates to the feeding intensity showed that although the proportion of 'poor feeders' were significantly higher ($F = 48.55$, $P < 0.01$) during most part of the year, the percentage of 'active feeders' relatively improved (31.59 ± 10.32) during the pre-monsoon season (Fig. 1). An in-depth analysis on the monthly variation of the feeding intensity depicted that a greater percentage of fishes with full stomach were observed during February to April (Fig. 2). Likewise, a very high percentage of the empty stomach was observed during June to August (monsoon season).

Gastrostomatic index and Relative Length of Gut: Mean values of GaSI for *C. travancoricus* ranged from 2.89 (July) to 4.66 (April). Monthly variation in gastrostomatic index (GaSI) of *C. travancoricus* is depicted in Figure 3. The variations in GaSI values coincided with the patterns of feeding intensity with highest mean values observed during pre-monsoon season (4.38). While corroborating these observations with the spawning season of the species, it is

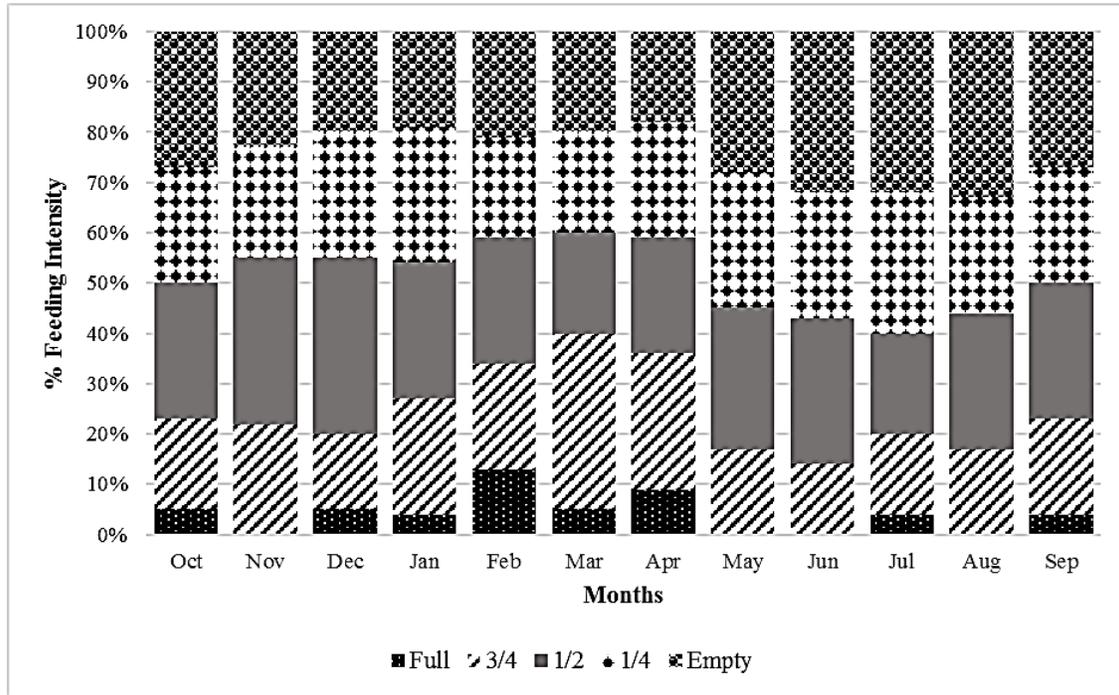


Figure 2. Monthly variations in the feeding intensity of *Carinotetradon travancoricus* in Chalakudy River.

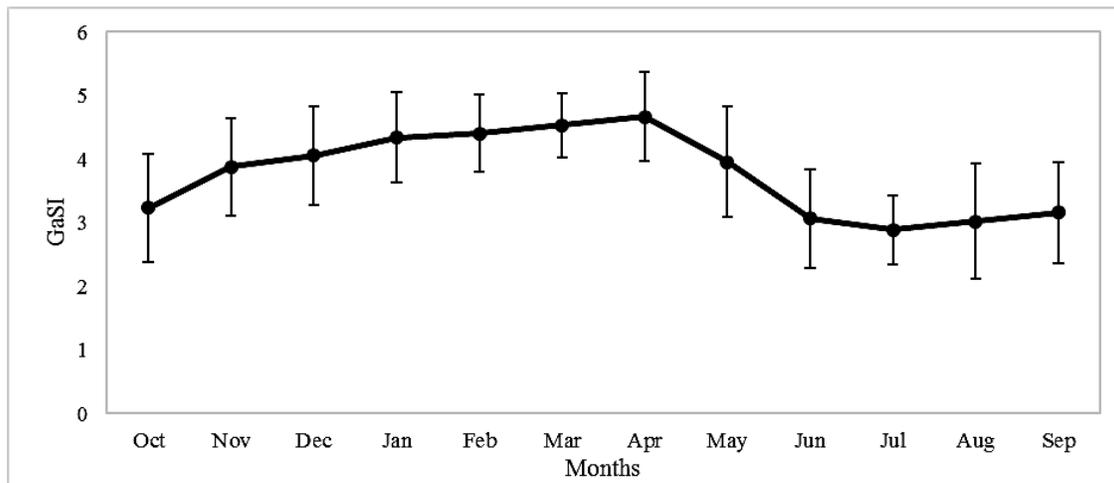


Figure 3. Monthly variations in gastro-somatic index of *Carinotetradon travancoricus* in Chalakudy River.

understood that the gastro-somatic index was high during post-spawning season (December-April) and the reduction in the GaSI during May to November were in tune with the broader spawning season, which indicates that the fish follows voracious feeding during post spawning season amassing required energy reserves for the next spawning period. The average RLG of *C. travancoricus* were found to range from 0.67-1.57 in juvenile fishes and 0.70-1.32 in adult fishes. It was seen that 55.35% of the juvenile fishes and 25.64% of the adult fishes had an RLG

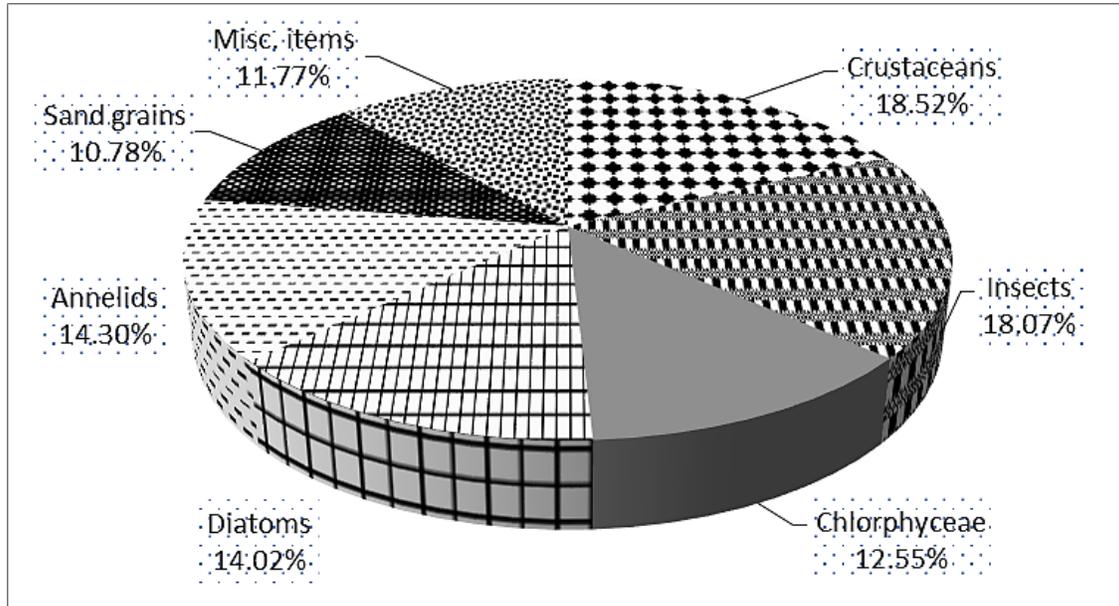
value greater than 1, indicating with age *C. travancoricus* shifts its food preference from a predominantly omnivorous diet to a preferably carnivorous diet.

Gut content analysis: The percentage composition of food items in the gut of *C. travancoricus* were categorized in to seven groups (Fig. 4). Insects (18.52%) and Crustaceans (18.07%) were the most dominant food items in the gut. Insects were represented by the larvae of Odonata (dragonflies and damselflies), Ephemeroptera (Mayflies), Hemiptera

Table 1. Seasonal variation of different food items in the gut of *Carinotetradon travancoricus* from Chalakudy River.

Season	Food items						
	Crustaceans	Insects	Chlorophyceae	Diatom	Annelids	Sand	Others
Pre-monsoon	18.733 ^a	20.010 ^c	9.790 ^a	13.158 ^a	13.185 ^a	12.240 ^a	12.888 ^b
Monsoon	18.305 ^a	16.708 ^a	13.848 ^b	13.293 ^a	16.043 ^b	10.115 ^a	11.688 ^a
Post Monsoon	17.170 ^a	18.833 ^b	14.003 ^b	15.600 ^b	13.668 ^a	9.988 ^a	10.735 ^a
F value	2.086	21.945	52.389	16.137	4.360	2.755	10.607
Significance	0.18	0.001	0.001	0.001	0.047	0.117	0.004

a, b and c - Means with different superscript alphabets in a column indicate significant difference them are homogeneous (DMRT; $P < 0.05$).

Figure 4. Percentage composition of food items in the diet of *Carinotetradon travancoricus*.

(aquatic bugs) and Diptera (Flies), while Copepods, Cladocerans (*Moina* sp. and *Daphnia* sp.) and Ostracods formed the major group of crustaceans in the diet. This was followed by Annelids (14.30%) represented mainly by Chironomid larvae. The fish also devoured a wide array of phytoplankton's namely the diatoms (14.02%) and green algae (12.55%) such as *Navicula* sp., *Cymbella* sp., *Syndera* sp., *Cocconeis* sp., *Pinnularia* sp., *Fragillaria* sp., *Nitzschia* sp., *Spirogyra* sp., *Ulothrix* sp., *Shizogonium* sp., *Pleurodiscus* sp., *Uronema* sp. and *Oedogonium* sp.. The remaining fraction in the diet included sand (10.78%) and miscellaneous items (11.77%), that were mostly composed of detritus.

Seasonal variations in diet composition: The diet of *C. travancoricus* composed predominantly of invertebrates, followed by algal matter in all the periods, but with significant seasonal difference in the

diet (Fig. 5). The results indicate that *C. travancoricus* is a specialistic feeder favouring insect larvae, however, during monsoon season when the availability of such prey decreases, they rely upon crustaceans and annelids as alternate feed. This was further supported by the results of DMRT analysis indicating that the preference for insects significantly varied ($F=21.945$, $P < 0.001$) between seasons (Table 1). The fish consumed more insects during pre-monsoon than any other season. In contrast, the occurrence of annelids in the diet was more during monsoon especially in June (18.39%). The preference for Crustaceans however, did not vary significantly ($F=2.086$; $P > 0.05$), but was found to be higher during June to September. An interesting observation noticed in the diet preference was the greater availability of phytoplankton in the diet of *C. travancoricus* during the post monsoon months. Hence our study suggests

Table 2. Monthly changes in the index of relative importance (IRI) of different food items in *Carinotetradon travancoricus* from Chalakudy River.

Months	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep
Crustaceans	23.38	19.64	22.09	24.53	23.02	26.19	26.07	27.68	29.87	26.40	27.80	26.96
Insects	28.31	28.99	29.92	29.92	30.27	31.93	30.88	29.31	24.32	22.69	24.38	24.05
Chlorophyceae	12.63	12.50	12.18	10.79	9.34	7.69	7.95	7.27	11.46	14.15	14.10	12.43
Diatoms	13.19	15.21	16.89	15.80	14.83	13.23	11.43	10.35	11.94	12.23	11.06	14.55
Annelids	13.56	9.37	8.88	6.68	7.70	6.45	6.42	9.02	15.28	14.58	14.18	11.36
Sand grains	2.62	5.71	3.55	6.17	6.42	6.38	7.22	8.02	2.78	4.63	3.47	4.16
Miscellaneous items	6.31	8.57	6.49	6.10	8.43	8.13	10.03	8.35	4.34	5.32	5.00	6.48

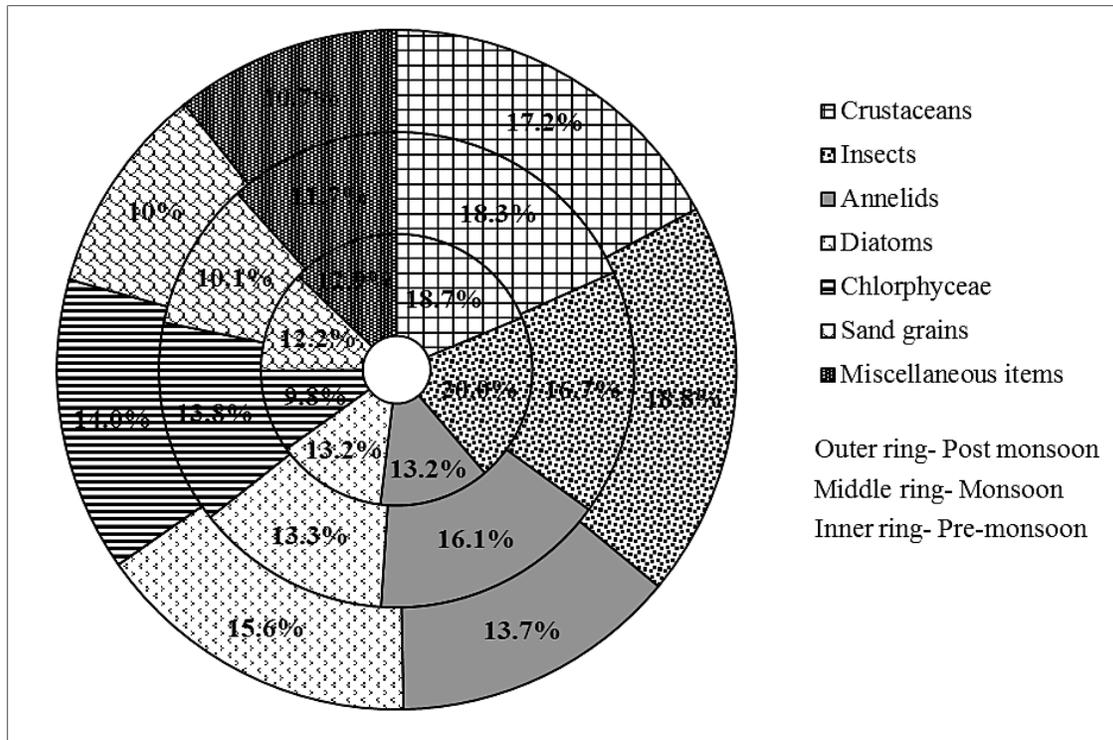


Figure 5. Seasonal variation in the frequency of occurrence of different food items in *Carinotetradon travancoricus*.

that the fish has a preference for specific food items during certain period of the year.

Index of relative importance: Monthly variation in the percentage of IRI for different food items is indicated in Table 2. Month-wise percentage of IRI for different food items indicated that insects were the dominant food item in every month with maximum values observed during March (31.93%). The occurrence of crustaceans was observed round the year with maximum value during June (29.87%) and minimum in November (19.64%). The composition of diatoms in the gut varied from 10.35 to 16.89% in May and December, respectively while for green algae it was 7.27% in May to 14.15% in July. Peak index value for annelids was recorded in July (15.28%) and lowest

value in April (6.42%).

Discussions

Chalakudy River, the fifth largest river in Kerala state, India originates in the Western Ghats, one of the 34 global biodiversity hotspots (Myers et al., 2000). The river system harbors a rich and diverse fish fauna of 98 species and many of them are endemic and threatened (Ajithkumar et al., 1999). The river also faces serious threats in the form of habitat degradation, overexploitation, and invasion of alien fish species (Raghavan et al., 2008). Knowledge on the feeding biology of endemic and threatened fish species in an ecosystem is important to link scientific knowledge with biodiversity conservation issues to

prepare suitable management measures (Huo et al., 2014).

Freshwater fishes have a broad range of feeding characteristics and adaptations, consuming a variety of food items and improving their diet by using the more energetic or easily available food resources (de Oliveira et al., 2019). Limited knowledge is available on the feeding profile and ecology of the Malabar puffer. Our study shows the feeding intensity of *C. travancoricus* to follow a distinct seasonal pattern whereby the fish was relatively a 'poor feeder' during the monsoon season, which on a closer observation coincided with the gonadal development of *C. travancoricus*. In the present study, most the fishes during May–August were either mature or ripe, indicating the period to be the peak spawning season for this species. Feeding intensity of fish can be influenced by maturity, spawning and the availability of food items in its environment (Ikusemiju and Olaniyan, 1977). The feeding habits of fishes are greatly decreased during the reproductive season and a high feeding activity reported during pre-spawning period should therefore be to store food as energy reserves during the spawning period. The ripe gonads occupying more space in the peritoneal cavity compresses the gut during the spawning season leading the fish to follow a low feeding regime (Serrajuddin et al., 1998). Feeding pattern of fishes is determined by a number of other factors such as intensity of light, temperature, pH, salinity, time of day, season, and any internal rhythm that may exist (Lagler, 1956).

The gastrosomatic index (GaSI) encountered in our study were relatively lower during the months of June–October indicating the poor feeding activity of *C. travancoricus* which in turn corresponds to their spawning period (Anupama et al., 2019). The low GaSI value during the spawning season has been observed in many tropical freshwater species (Sarkar and Deepak, 2009; Mondal and Kaviraj, 2010; Gupta and Banerjee, 2014; Alam et al., 2016; Roshni et al., 2016; Manorama and Ramanujam, 2017). The GaSI thereafter increased gradually along with their feeding activity. Availability of preferred food item is also a

factor that may influence the GaSI in omnivorous fishes. Our observations showed that for meeting its pre-spawning nutritional requirements, *C. travancoricus* preferentially feeds on larvae and juveniles of insects and crustaceans. Since these developmental stages of insects are not available throughout the year, they shift to a more phytoplankton rich diet during their post spawning phase. Following the classification of Kramer and Bryant (1995) reporting the RLG values for carnivorous, omnivorous and herbivorous fish species to be 0.6–0.8, 0.8–1.0 and 2.5–16.4, respectively, our results suggest *C. travancoricus* to be an omnivore. The average RLG of *C. travancoricus* in this study was close to 1.00 indicating that this species is an omnivore preferring more of animal matter in their diet. The results are comparable to the results of Karmaker and Biswas (2015) in *T. cutcutia* (0.67–0.86). Our study also suggests that the juvenile individuals prefer phytoplankton or zooplankton-based diet while the adults favour insects and annelids. Hence there is a discernable shift in the feeding strategy with development in this fish.

The present study revealed that *C. travancoricus* in Chalakudy River feeds only a few types of food items and therefore can be categorized as a stenophagic feeder. The main food constituents of the gut contents were aquatic invertebrates and algal matter. Seasonal changes in the availability of prey items was found to significantly affect the diet composition in this species. Hence the changes in the feeding habits of fishes are closely related to the changes in the food availability due to the changes in the environmental parameters and physiological variation (Wootton, 1990). Littoral zones in the river systems are occupied by extensive macrophytes that provide suitable habitat for small sized fishes which have distinct behavior pattern, such as low swimming activity (Priyadarshana et al., 2001; Thomaz et al., 2008; Dibble and Thomaz, 2009; Grzybkowska et al., 2018). The macrophytes habitat is a favorable environment for these fishes because of the high availability of food resources like invertebrates and algae (Quirino et al., 2015; Grzybkowska et al., 2018). The favorable

macrophyte habitat of *C. travancoricus* is *Cabomba* in riverine areas of Western Ghats region (Anupama and Harikrishnan, 2015). Medium densities of aquatic macrophytes, including *C. caroliniana* enhance fish diversity, feeding, growth and spawning (Dibble et al., 1996; Grzybkowska et al., 2018). This plant is abundantly found in the downstream floodplains of Chalakudy River which also provided good protection to this fish. The higher consumption of microcrustaceans during the monsoon period has been reported in various riverine environments (Nandy and Mandal, 2020). The increase in the content of copepods and cladocerans in the gut of *C. travancoricus* is a strong indication of the increased abundance of these microcrustaceans in the river during the monsoon season. Cladocerans (*Daphnia* and *Moina*) may be preferred over copepods and rotifers due to their lower swimming speed in aquatic ecosystem and slow prey avoidance response (Lazzaro, 1987). The thick overhanging vegetation of the forest in Western Ghats brings in a wide array of allochthonous organisms, including insects in their larval forms and other invertebrates into the aquatic environment, most of them are microcrustaceans and aquatic insects that utilize this wet spell for their development (Bonato et al., 2012; Prasad et al., 2012). Chironomid larvae, the main insect items found in the gut of puffer fish, are frequently associated with the submerged macrophytes which use as support, shelter and food source (Pinder, 1995). In dry season, with a decrease in the volume of water inflow and increase of water temperature favours a higher productivity in the ecosystem and growth of microalgae mainly diatoms (Johnson and Arunachalam, 2012; Lopes et al., 2016). The presence of sand grains and small amount of detritus indicate the bottom feeding habit of the fishes. Sand grains and detritus could be accidentally ingested along with other food items such as insects and crustaceans.

Our observations are in tune with the earlier studies of Joshy (2004) from Puzhakkal river and Prasad et al. (2012) from Kallar River, India, indicating the most preferred food item of *C. travancoricus* as crustaceans (cladocera, rotifers, copepods) and insects. A

comparison on the feeding habits of *C. travancoricus* with that of other freshwater puffer species revealed similarities as well as differences in the diet. Krumme et al. (2007) observed that freshwater banded puffer *Colomesu spittacus* to be carnivorous, preferring molluscs and crustaceans. The emerald puffer, *T. cutcutia* from Assam, India mainly feeds on insects, and mollusks (Karmaker and Biswas, 2015). The present finding therefore provides a salient understanding on the feeding biology of *C. travancoricus* in a lesser studied Chalakudy River with impetus on their preferential feeding strategy. Our study also suggests that this species has established well to the lotic ecology of Chalakudy River by modifying its feeding habits, which includes diversifying its prey preference, adjusting to seasonal fluctuations in food availability and synchronizing its spawning period in such a manner that their young ones has ample phytoplankton or zooplankton based diet during their early developmental stages. Later in the reproductive phase they selectively feed on insects especially their larvae which have 10-60% fat in their dry matter (Kouřimská and Adámková, 2016), required for the gonadal development and spawning.

Conclusion

Study of feeding ecology is vital to understand the ecological adaptation of the species to the environment. It also provides knowledge on the feed preference, seasonal variabilities in feed availability associated with the environment and also relates them to other biological factors impacting the fishes. Our result provides the first account on the feeding ecology of the Malabar puffer fish from a tropical river in a biodiversity hotspot. *Carinotetradon travancoricus* is an omnivore, feeding mainly on insects, crustaceans and algal matter. The variations in the feeding intensity, diet composition and other food indices indicate that environment plays an important role in altering the feeding biology of this fish. Being a widely traded ornamental aquarium fish, we hope the inputs on its feeding strategy will help develop an artificial diet for its breeding and rearing in captivity thereby reducing their capture pressure from the wild.

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