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RESEARCH PAPER

Phytoplankton-periphyton orientation influences feeding behavior of cultivable species: A case study on rohu, *Labeo rohita* (Hamilton, 1822)

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Abstract

The Indian Major Carp, *Labeo rohita* was stocked in four different resource environments namely, Periphyton monoculture, Periphyton polyculture, Plankton monoculture, and Plankton polyculture. Bamboo substrates were used as periphyton colonizers in culture ponds. In polyculture stocks, the *Catla catla* and *Cirhinus mrigala* were used as companion crop. The on-farm experiment was conducted for 210 days, and gut contents of rohu were collected and analyzed throughout the stocking period. The gut abundances of rohu showed an inclination towards algal food organisms, especially Cyanophyceae, Chlorophyceae, Baccillariophyceae, and Eugelophyceae, towards the later period of stocking when the fish attains a considerable total length. Results from the Shanon Diversity index supported higher algal diversity in the fish gut in periphytic conditions. The Diet breadth index also clearly indicated that rohu has a strong preferences towards periphyton than plankton. Such preferences are more prevalent in polyculture than in monoculture conditions. In conclusion, it can be accepted that rohu has clear preference towards periphyton over plankton when it is available in the environment. Stocking of other planktonic feeders in polyculture conditions may have no impact on the feeding pattern of rohu if periphytic resources are made available in the environment.

Introduction

Labeo rohita (rohu) is an important freshwater fish species normally cultured in Asia particularly in the Indian subcontinent (Khan et al., 2004). This Indo-Gangetic riverine species is distributed throughout South Asia, South-East Asia, Sri Lanka, the former USSR, Japan, China, Philippines, Malaysia, Nepal and some countries of Africa. Its compatibility for resource utilization with other freshwater carps, mainly catla (*Catla catla*) and mrigal (*Cirrhinus mrigala*) made it an ideal candidate for polyculture (Jhingran, 1991).

During the last decade, voluminous studies have been done on the feeding habit of rohu with regard to aquaculture management (Rahman et al., 2006; Majumder et al., 2018; Saikia et al., 2013; Mishra, 2020; Biswas and Mandal, 2021). Few of these studies on its feeding habit has forwarded interesting outcomes in the field of aquaculture research in rohu. For example, Majumder et al.

(2018) has reviewed that it follows ontogenic shifting of food habits from zooplanktivorous to phytoplanktivorous during its growth. Further, Saikia et al. (2013) reported its periphytophagous feeding habit under the availability of periphytic resources. These outcomes are important since fish meal nowadays have become the most expensive protein ingredient in aquaculture research. Several animal protein sources were evaluated to formulate the diets for fish including rohu to accomodate the objective of low input and high produce in aquaculture (De Silva and Gunasekera, 1991; Rangacharyulu et al. 2003; Asimi et al., 2017).

As an alternative way, a concern of growing fish in periphyton-based conditions has been popularized by a group of aquaculturists (Wahab et al., 1999; Azim et al., 2001a; Gangadhara et al., 2004; Azim et al., 2004a). Their studies have shown that rohu attained a profitable growth in periphytic condition when stocked in combination with other cultivable major carps. Subsequently, similar fish culture practices of common carp (*Cyprinus carpio*) has also been reported in India (Saikia and Das, 2009; 2014). Very recently, Biswas et al. (2022) proposed utilization of the periphytic biomass as a replacement of artificial feed in brackishwater polyculture and suggested that periphyton grown on 75% surface area provides a cost-effective production in polyculture. In view of the available reports of feeding habit of rohu and its potential use as a candidate in periphyton based aquaculture conditions, this is necessary to evaluate its comparative performance in feeding under periphyton-free and periphyton-based conditions. The present study has been design to understand such choices by rohu. In most of the periphyton-based fish culture experiments with carps, rohu constituted major part of the composition of fishes stocked (Wahab, 1999; Azim et al., 2001a; Gangadhara et al., 2004; Azim et al., 2004a). Being planktonic feeder, the synergistic effect that helps rohu grow under periphytic condition is not known. These studies performed in periphytic condition concluded that rohu feeds on periphyton under substrate-based condition and hence growth is accelerated. However, such shifting of food from plankton to periphyton by rohu were not based on direct evidence from the gut content and other feeding ecological tools. The present study, therefore, is based on gut content analysis as direct evidence of feeding on available resources under the periphytic monoculture as well as in polyculture conditions.

Materials and Methods

Pond preparation and experimental set up

The present study has been performed in two conventional fish ponds for 210 days separated by an earthen embankment at Jaydeb, Birbhum, West Bengal (23° 38' 0" N, 87° 26' 0" E, Fig 1).

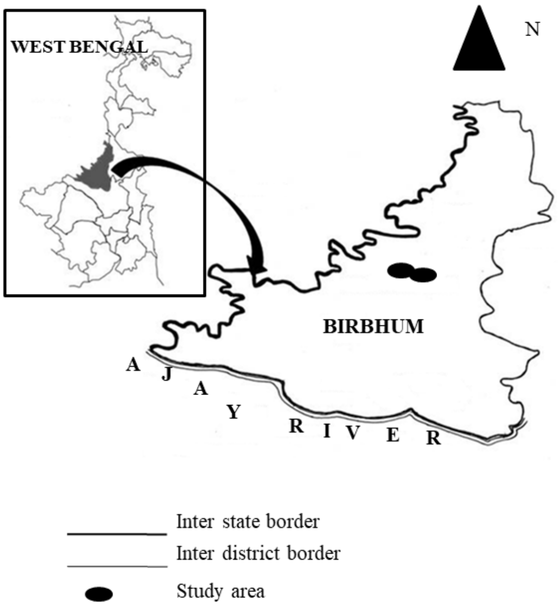

Figure 1. Study area in Birbhum, West Bengal, India. (Not to scale)

Each pond was divided into two areas with the help of fine nylon nets (mesh size <10mm). Along with the rohu, two other fishes (Catla, *Catla catla* (Hamilton, 1822), Mrigal, *Cirrhinus mrigala* (Hamilton, 1822)) were selected because they are compatible for polyculture with rohu (FAO, 2009). For the growth of periphytic organism, bamboo poles (lengths 2.53±0.21 m and diameters 5.2±0.37 cm) were implanted in the two areas of the first pond (depth 1.87 ± 0.25m) vertically at a distance of 1m from each other (4 poles/ m^2) (Fig. 2A and B). Both the areas partitioned in the second pond (depth 1.89 ± 0.28m) remained free i.e. without any bamboo substrates. In one of the bamboo substrate implanted areas of the first (Size: 903 sq. ft.) pond, fingerling of rohu (average wt. 8.15 ±1.75 g, average length 7.3 ± 0.81 cm) were introduced and this area was considered as the periphytic monoculture (**PR-M**) area. In the second area (Size: 1058 sq. ft.) of the first pond, along with rohu, fingerlings of catla (average wt. 8.91 ±1.89 g, average length 7.6 ± 0.75 cm) and mrigal (average wt. 4.53 ± 0.76 g, average length 5.2 ± 0.62 cm) were introduced and this area was considered as the periphytic polyculture (**PR-P**) area. In the second pond, the first area (Size: 923 sq. ft.) was stocked with fingerlings of rohu (average wt. 8.15 ±1.75 g, average length 7.3 ± 0.81 cm) and was considered as the planktonic monoculture (**PL-M**) area. In its second area (Size: 1025 sq. ft.), along with rohu, *Catla catla* (average wt. 8.91 ±1.89 g, average length 7.6 ± 0.75 cm) and *Cirrhinus mrigala* (average wt. 4.53 ± 0.76 g, average length 5.2 ± 0.62 cm) were introduced and this area was considered as the planktonic polyculture (**PL-P**) area (Fig 2C and D). All fishes were released in May 2014. Stocking density in both the monoculture areas was 400 fingerlings of rohu and in polyculture area, 200 fingerlings of rohu, 150 fingerlings of catla and 100 fingerlings of mrigal. Fishes were released 15 days before first sampling.

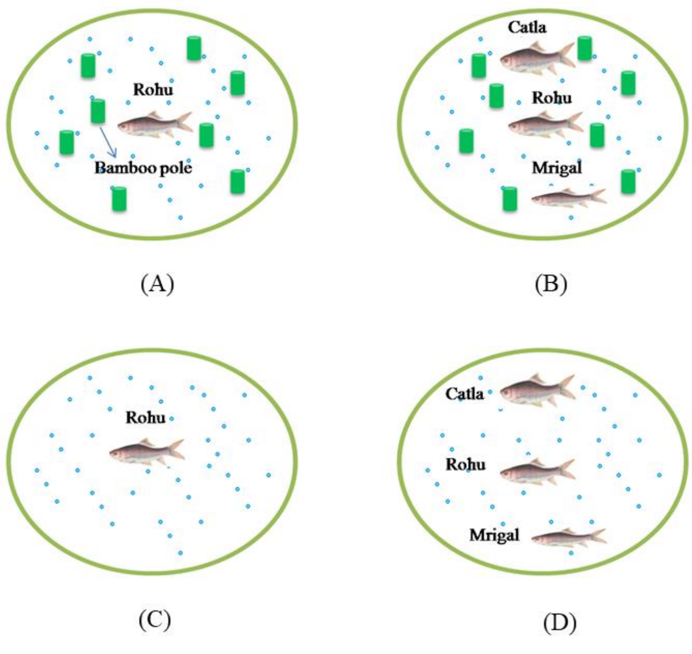

Figure 2. Experimental design. (A) Periphytic monoculture (PR-M) in first pond, (B)) Periphytic polyculture (PR-P) in first pond, (C)) Planktonic monoculture (PL-M) in second pond, (D) Planktonic ployculture (PL-P) in second pond. (Figures are not to scale)

Fish capture technique and collection of gut content

In every sampling, 15-20 fishes (rohu) were captured using fish net from the four experimental plots in 30-day intervals starting from June 2014 to November 2014. All fishes were collected before 9:00 AM Ethical procedures for experiment with animals were maintained throughout the period of research work.

The fishes (n=15-20) were sacrificed and guts were cut from the oesophagus region to first major constriction of the alimentary canal to obtain identifiable food items ingested by the fish (Haroon and Pittman, 1998). This length is around 4.5-12.6 cm (proportionately to TL 11.7-36.5 cm). Before gut collection, every fish was weighed and the total length was recorded. Immediately after collection, guts were transferred to 10% formalin. In the laboratory, each gut along with the content was blotted uniformly with tissue paper and weighed accordingly. In spite of measuring gut weight, guts were cut longitudinally and the fullness index was measured (Haroon 1998). Gut contents from samples with considerable food (Gut fullness index > 0.5) were removed with the help of a fine scalpel visible to the naked eyes. These were then preserved in 4% formalin in 10 ml glass vials (Borosil) for further analysis. After that, empty guts were again weighed to obtain gut content weight.

Identification, quantification, abundance, diversity and dominance of gut samples

Gut-content organisms were identified up to generic level using standard manuals (Pentecost, 1984; Edmondson, 1992; Perumal and Anand, 2009) and online resource (https://www.algaebase.com) and wherever possible, identified up to species level. The whole gut content collected from each gut were analyzed. These were estimated following Lackey's (1938) drop count methods under an inverted microscope (Victory plus, Dewinter, Italy). Abundance was expressed as L/gut sampled for analysis. Shanon's Species diversity measure was performed for documenting taxonomic diversity of gut contents. In addition to relative abundance and Shanon diversity, dominance measure was computed as follows:

Dominance Index (Odum 1971) $C = (ni / N)^2$

Where, ni is the total number of individuals of species.

N is the total number of individuals of all species in hand.

Diet breadth analysis

Three indices of diet breadths were considered. The selection of diet breadth indices was made to obtain a clear understanding of the resource use and mode of selection of resources. The first index was the popularly used Levin's (1968) diet breadth measure. It is calculated as-

$$B_A = \frac{\left(\frac{1}{\Sigma P_i^2}\right) - 1}{n-1}$$

where, B_A is Levin's diet breadth, p_i is the fraction of items in the diet that are of food category j and n is the number of resource states. As Levin's diet measure does not take resource availability in the environment into consideration while determining diet breadth, the second index i.e. Hulbert (1978) diet measure was calculated. It is-

$$B_A = \frac{1}{(\Sigma P_i^2 / aj)}$$

Where B´ is Hulbert's standardized diet breadth, pi is the fraction of items in the diet that are of food category i (∑(pi =1.0), aj is the proportion of total available resources consisting of resource j (∑aj=1.0). B´ ranges from 1/n to 1.0.

The third index considered was $Db(\chi^2)$ (Saikia, 2012). Statistically, this measure not only considers resource utilization along with diet breadth, but also sensitive to variation within resources and resource utilized by the organism. As Levin's and Hulbert diet measures are based on proportional availability of food item to the total foods and Hulbert is a product of fraction of resource and gut content, they are insensitive to variations within the sample. The $Db(\chi^2)$ is computed as follows-

$$DB(\chi 2) = \sum_{i=1}^{n} \frac{(\log Oj - \log Ej)^2}{\log Ej}$$

Here, the $DB(\chi^2)$ is the diet breadth, $\log O_i$ and $\log E_i$ are the log value of observed and expected food abundances of i^{th} category respectively. The expected food abundance in $DB(\chi^2)$ is constituted of available food resources in the environment. The $DB(\chi^2)$ value '0' indicates a complete overlap of gut content abundance on resource abundance from environment. This value is termed as Resource Db(x) or $R_{DB(\chi 2)}$. Theoretically, $R_{DB(\chi 2)}$ =0.0.

Determination of factors influencing food preference of rohu

To examine which factor is responsible for such preference of food organisms by rohu, three primary factors viz. resource type (Plankton and Periphyton), season (across months) and culture type (monoculture and polyculture) and two secondary factors viz. mono-poly resource and mono-poly season were analyzed.

Statistical analysis

One-way ANOVA was computed to analyze differences among means of samples. Homogeneity measure was tested before computing ANOVA. If ANOVA showed any difference among the means, Tukey's post hoc test was used to identify means with difference. The p value of 0.05 or smaller is considered as significant. The Software **Minitab, version 17.0** was used for all statistical analysis.

Results

Community composition of food organisms in the gut of rohu

In the periphytic culture condition of rohu, a total 51 genera of food organisms including 17 genera of Chlorophyceae followed by 13 genera of Bacillariophyceae, 6 genera of Cyanophyceae, 3 genera of Euglenophyceae, 5 genera of and 7 genera of Cladocera-Copepoda and other zooplanktonic organisms (CCO) were recorded (Table 1). From the planktonic culture condition, a total of 48 genera of food organisms which includes 16 genera of Chlorophyceae followed by 12 genera of Bacillariophyceae, 5 genera of Cyanophyceae, 3 genera of Euglenophyceae, 5 genera of Rotifera and 7 genera of CCO were recorded. The fraction of these food items used by the fish showed great variation with the progress of the season.

Abundance of food organisms in the gut content of rohu from the culture pond

The abundances of Cyanophyceae organisms in PR-M conditions are shown in Fig 3a. In the initial months i.e. in June and July, Cyanophycean abundance did not show any significant difference ($p>0.05$). But with the progression of time starting from September onwards the abundance of Cyanophyceae was significantly increased($p<0.05$) in the gut of rohu as compared to the initial months. In the case of PR-P conditions there was a significant increase in abundance during the later period of months compared to June and July. In planktonic culture conditions (PL-M, PL-P) also there was no significant difference ($p>0.05$) in abundance during the initial months, but from August onwards there was a sharp increase in number. When the abundance of Cyanophyceae was analyzed among the four culture conditions, in the initial months starting from June to August, the abundance did not show any significant difference ($p>0.05$). among all the four culture conditions. But from September onwards, both the PR-M and PR-P conditions showed significant difference($p<0.05$) with the PL-M and PL-P conditions showing significantly higher abundances in the periphytic culture conditions in comparison to the planktonic culture conditions. Although statistically not significant ($p>0.05$), Cyanophyceae showed higher abundances in the PR-P condition compared to the PR-M condition with the progression of time.

Abundance studies of Chlorophyceae showed great variations of periphytic and planktonic food organisms in the gut of rohu (Fig 3b). In the month of June and July in PR-M conditions, the abundance of Chlorophyceae organisms did not show significant difference ($p>0.05$). But from August onwards, significantly ($p<0.05$) higher abundance of Chlorophyceae organisms were observed compared to the initial months. Similar abundance pattern of Chlorophyceae was observed in the other three culture conditions viz. PR-P, PL-M and PL-P. From August onwards, guts of rohu from all these three areas showed significantly higher ($p<0.05$). abundance of Chlorophyceae organisms. When abundances of Chlorophyceae were analyzed among the four culture conditions, in the initial months of June to July, the abundances did not show any significant difference ($p>0.05$) among the four culture conditions. But from August onwards the PR-M and PR-P showed significant difference ($p<0.05$) with the PL-M and PL-P conditions, indicating significantly highest ($p<0.05$) abundances of gut Chlorophyceae from the periphytic culture conditions in comparison to the planktonic culture conditions. Here too, although statistically not significant ($p>0.05$), the abundance of Chlorophyceae organisms from the gut of rohu were higher in the PR-P conditions compared to the PR-M conditions with the progression of time. Similar pattern was observed for the abundances of gut Bacillariophyceae too (Fig 3c) and Euglenophyceae (Fig 3d). Like the gut abundances of Cyanophyceae and Chlorophyceae, the

Bacillophyceae and Euglenophyceae also showed significantly higher abundances in periphytic in comparison to planktonic conditions.

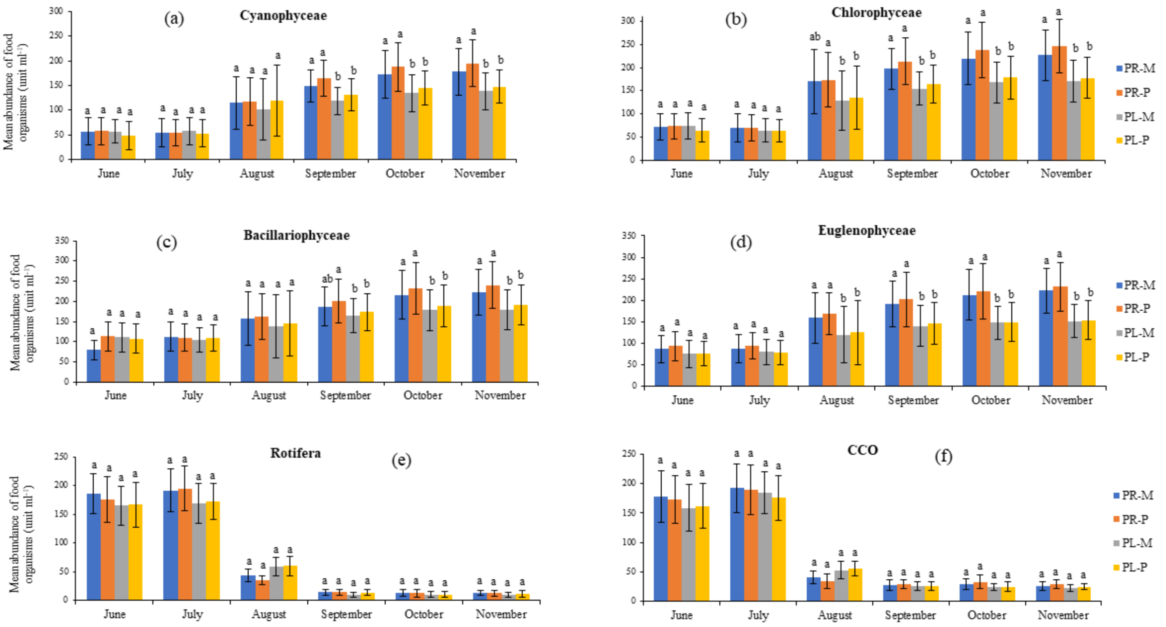

Figure 3. Mean abundance of gut contents of *Labeo rohita*. Abundances of Cyanophyceae (a), Chlorophyceae (b), Bacillariophyceae (c), Euglenophyceae (d), Rotifer (e) and Cladocera, Copepoda and other zooplanktonic organisms (CCO) (f) in Periphytic Monoculture (PR-M), Planktonic Monoculture (PL-M), Periphytic Polyculture (PL-P) and Planktonic culture (PL-P) conditions. Mean±SD, one way ANOVA followed by Tukey's test ($p<0.05$), n=9, Means that do not share any letter are significantly different.

In case of Rotifera, in the initial months i.e. in June and July there was no significant difference ($p>0.05$) in abundance in all the four culture conditions (Fig 3e). But with the progression of time starting from August onwards the abundance of Rotifera significantly decreased ($p<0.05$) in the gut of rohu as compared to the initial months in all the four culture conditions (PR-M, PR-P, PL-M, PL-P). When the abundance of Rotifera was analyzed among the four culture conditions, the abundance did not show any significant difference ($p>0.05$) among all the four culture conditions throughout the months, although there was a sharp decrease in abundance during the later period of months.

Similar to Rotifera, abundances of Cladocera, Copepoda and other zooplanktonic organisms (CCO) showed insignificant differences ($p>0.05$) among all the four culture conditions during June and July (Fig. 3f). But with the progression of time starting from August onwards the abundance of CCO was significantly decreased ($p<0.05$) in the gut of rohu as compared to the initial months in all the four culture conditions. Like Rotifera, when abundance of CCO was analyzed among the four culture conditions, the abundance did not show any significant difference among all the four culture conditions throughout the months, although there was a sharp decrease in abundance starting from August onwards.

Dominance index of food organisms from gut content of rohu

As found in Table 1, a total of 51 species in the gut content of rohu from the periphytic culture conditions and a total of 48 species of gut content organisms of rohu from the planktonic culture conditions were recorded. Among all food organism, only the most dominant species from both periphytic as well as planktonic conditions were identified through species dominance index for analysis and presented in Fig 4. From the dominance analysis in periphytic culture conditions, 12 genera viz. *Anabaena*, *Aphanocapsa*, and *Chroococcus* from the

Cyanophyceae, *Chlorella*, *Closterium*, *Scenedesmus* and *Oedogonium* from Chlorophyceae, *Navicula* and *Diatoma* from Bacillariophyceae, *Phacus* and *Euglena* from Euglenophyceae and *Arcella*, the only representative from the zooplanktonic organisms were identified which showed highest abundance among all the gut content organisms of rohu throughout the study period. Except *Oedogonium* all the other 11 dominant species from the periphytic conditions also showed highest dominance in case of planktonic culture conditions throughout the months.

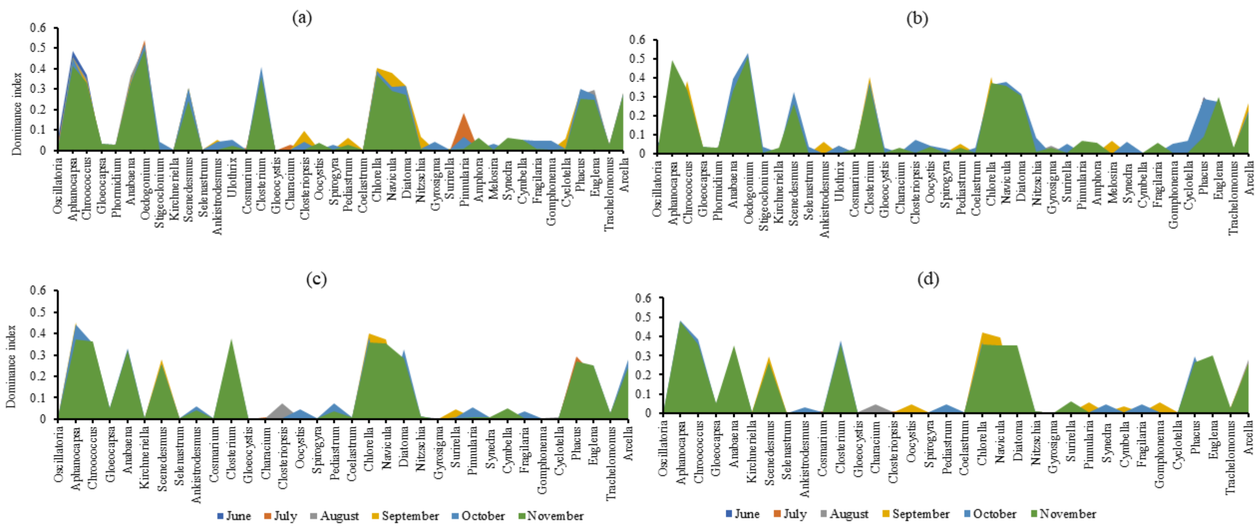

Figure 4. Dominance index of gut content of *Labeo rohita* from Periphytic Monoculture (PR-M) (a), Periphytic Polyculture (PL-P) (b), Planktonic Monoculture (PL-M) (c), and Planktonic Monoculture (PL-P) (d) conditions.

Factors influencing the food preference of rohu

When resource was considered as a factor (Table 2), most of the dominant organisms except *Diatoma* and *Arcella* showed statistically insignificant (p>0.05) results. Similarly, when season was considered as a factor, all of the dominant organisms except *Arcella* showed statistically significant (p<0.05) results. Whereas, when culture condition (monoculture/polyculture) was considered as a factor, none of the dominant organisms showed statistically significant results. But interestingly, when resource was considered as the combined factor with culture conditions, most of the dominant organism showed statistically significant (p<0.05) results. When season was considered as the combined factor with culture type, all of the dominant organisms except *Arcella* showed statistically significant results.

Diversity of food organisms and diet breadths in the gut of rohu

Shanon-Wiener diversity

The diversity (H′) values were low during the initial months but increased gradually with the progression of time in the gut of fish (Fig 5a). In the month of June and July, the diversity (H′) of the food organisms showed no significant difference(p>0.05) among all the four culture conditions. With the progression of time, starting from August onwards, the diversity of the food organisms in the gut content of rohu showed significantly higher (p<0.05) values in both the periphytic monoculture and polyculture conditions over planktonic culture conditions. The diversity of the gut food organisms in the gut of rohu from periphytic polyculture condition was significantly higher (p<0.05) than the periphytic monoculture condition during the later period of months. However, for planktonic conditions, the diversity of food organisms in the fish gut was significantly higher (p<0.05) in polyculture compared to the monoculture condition during the month of August and September only. In all other months there was no significant difference (p>0.05) in diversity of gut content organisms between planktonic monoculture and polyculture condition.

Diet breadth

The Levin's diet breadths (B_A) are presented in Fig 5b. The B_A values were low during the initial months but increased gradually with the progression of time in the gut of fish. In the month of June and July, the B_A values of the food organisms showed no significant difference ($p>0.05$) among all the four culture condition. Whereas, with the progression of time, starting from August onwards, the increment of diet breadth of rohu was significantly higher ($p<0.05$) in both the periphytic monoculture and polyculture condition in comparison to both the planktonic culture condition. The Hulbert diet breadths (B′) are also presented in Fig 5c. The B′ values also were low during the initial months just like the Levin's diet breadth, but increased gradually with the progression of time in the gut of rohu. The Hulbert's diet breadth behaves more or less similar to the Levin's diet breadth throughout the period of culture of the fish. In general, both the Levin's and Hulbert diet breadth showed higher values of diet breadth in periphytic polyculture condition over periphytic monoculture condition during the later part of the study (in months).

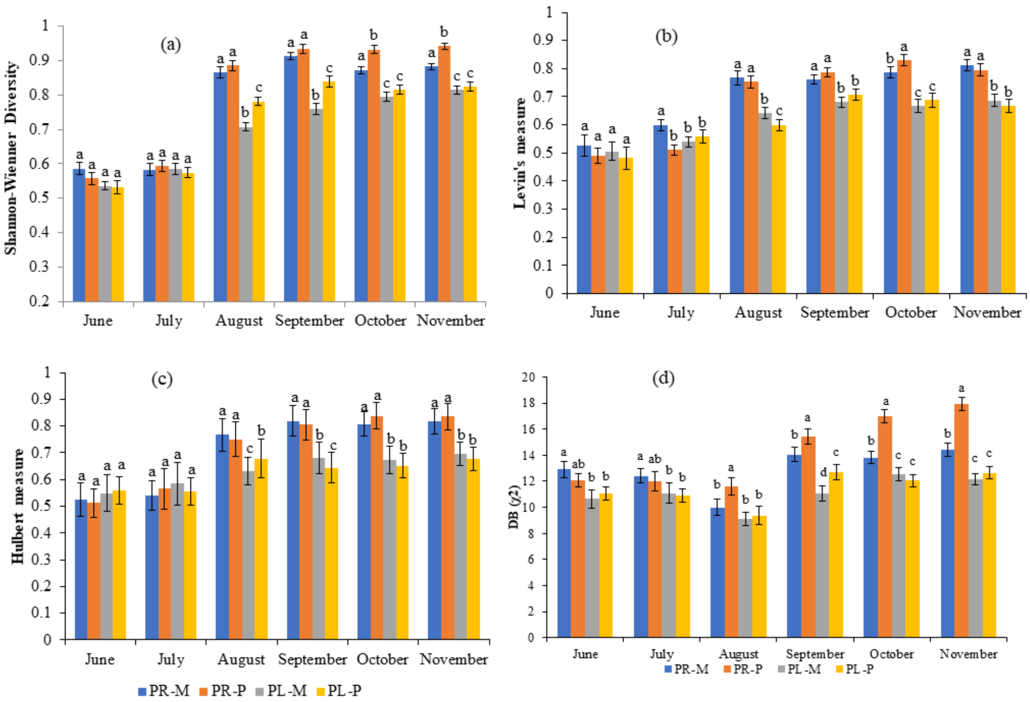

Figure 5. Diversity and diet breadth measures of gut contents of *Labeo rohita*under different culture conditions, (a) Shanon-Wiener diversity measure, (b) Levin's diet breadth , (c) Hulbert's diet breadth and (d) DB (χ2). Mean±SD, one way ANOVA followed by Tukey's test ($p<0.05$), n=9, Means that do not share any letter are significantly different.

Fig 5d represents DB(χ^2) values from all the culture condition. When plankton were considered as food resource, the DB(χ^2) values of PR-M and PR-P were significantly higher ($p>0.05$) in comparison with the PL-M and PL-P during the later months. In the month of June and July, the DB(χ^2) values in the periphytic PR-M showed indifference with PR-P condition but was significantly higher($p<0.05$) compared to the PL-M and PL-P condition. However, from August onward the DB(χ^2) value in PR-P condition was significantly higher ($p<0.05$) compared to the PR-M, PL-M and PL-P area. The PR-M area again showed significantly higher ($p<0.05$) DB(χ^2) value compared to the PL-M and PL-P condition from September onward. In case of the planktonic culture condition, there was no significant difference ($p>0.05$) among the monoculture and polyculture condition throughout the months except in the month of September.

Discussion

The earlier reports with rohu as an active periphyton feeder (Gangadhara et al., 2004; Azim et al., 2004a) did not consider gut analysis to confirm the diet shift of rohu from plankton to periphyton. The present report is the first of its kind to understand resource utilization by rohu under a substrate-based condition. In June and July rohu fed more zooplankton irrespective of monoculture and polyculture conditions, however, in the later months, it inclined mostly towards plankton of plant origin (four major algal groups). This situation happened in both periphytic and planktonic resources. From August onward, abundances of food organisms of plant origin in the gut of rohu increased in PR-M and PR-P conditions. Although statistically not significant, the abundance of food organism in the gut of rohu in PR-P condition is relatively higher compared to PR-M condition. Also, in comparison to the other two plankton-only condition, the gut content of rohu showed significantly higher ($p<0.05$) plant-origin food in the periphyton based condition. These results indicate that rohu has the preference for periphytic resources in substrate-based condition.

During the experiment, two resource types, i.e. plankton and periphyton in the pond conditions, were available for the fish. Even, as discussed for the ontogenic shifting of food habit of rohu, its feeding nature was repeated from the zooplanktivorous to phytoplanktivorous nature all the culture conditions.

Rohu showed an overall narrow diet breadth in the case of plankton throughout the months. The greater the diet breadth, the greater the possibility for fish to access the resource types. A smaller diet breadth is the indication of either resource partitioning (Haroon and Pittman, 2000) or less affinity of the fish towards the resource type on which diet breadth was measured. Small-sized fishes are generally less opportunistic and occasional feeder of different resource types (Haroon and Pittman, 2000), exhibiting feeding activity in a limited zone of the environment, resulting in insignificant narrow diet breadth. Less periphytic productivity during the early months on the bamboo substrates might have some effect on such values of diet breadth. Moreover, it is also evident that the smaller forms of the carp mainly fed on zooplankton.

The Levin’s diet breadth was compared for two different resource types, namely, plankton and periphyton in the fish culture condition to draw a more meaningful conclusion about the food accessibility by rohu. The lower diet breadth resulting from plankton compared to periphyton indicated the fish's grazing nature considerably on periphyton biomass. From August onward, Levin’s diet breadth of rohu showed a greater value when periphyton was ingested as a food source. This difference increased with the progression of the season showing a maximum diet breadth during October-November.

With the progression of time, Levin’s and Hulbert diet breadth increased for the periphytic food organisms, indicating its nature of accessibility to brows on the attached organisms (Fig 4A, B). Simultaneously, the shift towards periphyton resource caused a gradual decline in feeding activity on plankton. The increasing resource availability in the form of periphyton might have positive effect for such findings. Therefore, during later period of months the fish consumed maximum number of available food items through random selection.

Under substrate-based conditions, it was repeatedly reported that the rohu shows faster growth compared to substrate-free conditions. Azim et al. (2001a) reported that its growth was 77% higher in substrate-based conditions than substrate-free conditions. Azim et al. (2001b) observed that periphyton biomass significantly decreased with increasing biomass of rohu in a substrate-based condition. Although rohu has been reported as an exclusive plankton feeder, these observations suggest that rohu is an opportunistic periphyton feeder because when rohu was subjected to a periphytic condition, it preferred periphyton over plankton. In periphytic conditions, from August onwards, Levin’s diet breadths for rohu were significantly higher ($p<0.05$) compared to planktonic conditions, indicating rohu as a successful feeder on both resources i.e., periphytic and planktonic. Thus, there is an condition-specific effect on the feeding behavior of rohu when gut contents were considered. However, Levin’s measure of diet breadth has been criticized for excluding resources in the environment while enumerating the diet breadth of an organism (Saikia, 2012). Significantly higher ($p<0.05$) Hulbert diet breadth under periphytic condition than plankton condition indicates its exclusive preference for periphyton.

Such result concurs with the findings of Azim et al. (2004b). Earlier, Das and Moitra (1955) reported it as a phytoplankton feeder. Probably for the same reason, in the following stage of growth (during later months) the Hulbert values from the periphytic area for both resources (plankton and periphyton) remained higher, suggesting increased accessibility of the fish on periphytic and phytoplanktonic resources when substrates are installed. From Hulbert's values, it is difficult to conclude that the fish exploited only one resource or both since plankton and periphyton have common algal members in them (Saikia and Das, 2009). Though Hulbert diet breadth suggests increased feeding preference of rohu in substrate-based condition, its specific selection towards any of the both resources could hardly be ascertained from this index. Considering such variations existed within resource data to gut content data, the newly reported diet breadth Db(χ^2) is discussed to understand the real situation.

Similar to Hulbert, Db(χ^2) values also reflected that, initially, there was no difference in diet breadth in both periphytic and planktonic conditions. In August, the fish successfully fed on plankton and periphyton from the periphytic polyculture condition, and as a result it showed significantly higher ($p<0.05$) Db(χ^2) value than other culture conditions. However, the preference was more from periphytic than planktonic resources as evident from the abundance study. One reason behind such preference may be the rate of colonization of periphyton on the substrates which increased during the later period of months. Alikunhi (1958) observed that the structure of gill rakers in rohu is such that they are not adapted to filter minute planktonic organisms. For this reason, the early periphytic colonizers, which are mainly bacteria and blue-green algae, might have escaped from the mouth cavity and could not be retained in the fish gut. Therefore, initially, the Db(χ^2) values were more or less similar in the periphyton and plankton based condition. With the precedence of the stocking period, the successional progression of colonizing communities was occupied with algal forms like diatoms, filamentous algae, etc. This has enhanced its preference towards periphytic food rather than plankton. The fish, being basically column feeders, favored maximally to brows probably on substrate at this period and hence received maximum food items from the substrate than an actual planktonic resource. When periphyton arrives at late successional stage, the colonization rate reduces with the occurrence of self-shading in periphytic layers. This might have enhanced rohu's preference on periphytic communities leading to significantly higher ($p<0.05$) Db(χ2) values in substrate-based condition. Thus, it is evident that the fish actually prefers periphyton in substrate-based condition. This could be the reason why Azim et al. (2001a, 2001b) and Keshavanath et al. (2001) observed a correlation of increased biomass of rohu with periphytic condition. Azim et al. (2004a) also reported that lower periphyton biomass in ponds with lower amounts of substrate indicated a higher grazing pressure on the substrates. This is probably because rohu, being opportunistic in nature, exploits the periphytic resource available on the substrate. Hence, rohu's feeding rate increases, affecting reduced colonization of algae on the substrate.

Rahman et al. (2008) observed that rohu spends 65-85% of swimming time grazing in water column. Such a longer grazing time could be explained by the dependency of the rohu on planktonic food (Rahman et al., 2006). Compared to plankton, periphyton is a static type of community on substrate, enhancing feeding rate of fish through two-dimensional exploration of food (Horne and Goldman, 1994). It is reported that the feeding of rohu is deliberate and selective in nature (Alikunhi, 1958).

Studies on the feeding habit of rohu always suggests it to be an opportunistic feeder. It was reported as a plankton feeder (Das and Moitra, 1955), phytoplankton and zooplankton feeder (Khan and Siddique, 1973), zooplankton feeder under fed and fertilized ponds at fry stage (Miah et al. 1984) and detritus feeder in shallow ponds (Das and Chakrabarty, 2006). Ramesh et al. (1999), Wahab et al. (1999) and Azim et al. (2001a) reported that under fed and polyculture condition, rohu is a very active periphyton grazer. In the present study, all diet breadths measures suggest that in most of the cases the fish explored resources common in both the water column and substrate in periphyton based condition thereby increasing in diet breadth measure in periphytic based condition as compared to the plankton based conditions where only one resource i.e. only plankton is present. As observed in Table 2, the seasonal influence on the significance abundances of resources (plankton and periphyton) in periphytic and planktonic culture conditions may also have led to wider diet breadth of rohu. The probability of occurring common food items in periphytic condition could be possible if planktonic organisms with periphytic intensity appear in close association with the substrate. When colonization of periphyton was initiated on substrate, the fish started feeding on such colonizing resources. Earlier, Saikia et al. (2013) proposed a 'sub-periphytic' zone from which rohu explores the periphytic resources. With precedence of colonizing event, the successional stages of periphyton varied and algae with different colonizing ability started to attach on the substrates that facilitates the increased feeding of rohu on periphytic organisms.

Conclusions

The present study confirmed that the rohu is a potential candidate for culture in planktonic and periphytic ambiances. When both resources are available, it was observed to be inclined towards periphytic biomass. Although exploration from such a hypothetical periphytic zone has not been confirmed physically, the present study on feeding ecology through diet breadth measures has reaffirmed its choice of periphytic resources. The fish can be, therefore, featured as a generalist feeder in terms of plankton and periphyton resources and the best fit to such a cultivable system. The area of research needing further attention is the probability of maximum assimilation of nutrients and growth of rohu under such ambiances. Feeding on periphytic and planktonic organisms might induce assimilation of food-dependent nutrients to fish. Since the fish is mainly cultured as a source of animal protein, a confirmation on protein gain compared to the conventional practice through periphyton based rohu culture would additionally credit the fish for polyculture in rice-fish or pond conditions.

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Ethical approval

Although ethical clearance is not mandatory, the author declares that this study complies with research and publication ethics.

Informed consent

The corresponding author on behalf of all authors is responsible for obtaining and providing written consent of any third party, or their legal representatives.

Conflicts of interest

The author(s) declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper."

Data availability statement

The authors declare that data are available from authors upon reasonable request.

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Author contribution

Author1: Writing original draft, Data collection and processing, Review

Author2: Supervision, Validation, Visualization, Project administration, Resources, Review, Editing.

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