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Enzymatic Profiling and Feeding Preferences of Catla: *Catla Catla*, Rohu: *Labeo rohita* and Mrigala: *Cirrhinus mrigala* in Rural Polyculture Ponds

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Abstract

This study was aimed at understanding the enzymatic profile of three major Indian carps in managed and unmanaged polyculture systems in the Hisar district, Haryana India. The three species were catla (*Catla calta*), rohu (*Labeo rohita*) and mrigala (*Cirrhinus mrigala*). Analysis of gut contents of *C. mrigala* from both the ponds was significantly (*p*<0.05) dominated by phytoplankton. *L. rohita* gut had similar values of both phytoplankton and zooplankton while *C. catla* gut was significantly dominated by zooplanktons. The specific cellulase and amylase activities were higher in *C. mrigala*. It was further observed that these activities are higher in managed ponds as compared to the unmanaged ponds. *L. rohita* revealed elevated levels of protease and amylase activities which supported the omniplanktivorous nature of the fish. Analysis of digestive enzymes from the gut of *C. mrigala* revealed more lipase, cellulase and amylase in comparison to other enzymes. It can be concluded that *C. mrigala* was phytoplanktivorous, *L. rohita* was omniplanktivorous and *C. catla* was zooplanktivorous. Fishes reared in managed ponds seemed to have higher enzymatic activities in the gut advocating higher growth. The results of the study enhance our understanding of the feeding patterns at different stratus levels within the available nutrition in polyculture systems.

Keywords: Polyculture system; Digestive enzymes; Gut contents; Exogenous enzyme; Enzyme producing bacteria; Trophic level

Introduction

Fish culturing in small water bodies is quite commonly practiced in the Asia region as well as parts of Africa where culture fisheries are established in reservoirs, communal lakes and village ponds / private farm ponds [1-8]. For pond productions based primarily on natural food, achieving a good yield requires sufficient understanding of the plankton production system and limnetic ecology.

The abundance of the species composition and condition of the aquatic organisms in the natural community are related directly with the water quality. The effect of stress due to anthropogenic activities manifests themselves in the pond biota *viz.* changes in the pattern of distribution, elimination of the sensitive species, changes in diversity and subtle morphological and physiological changes [5,9-11]. The productivity level of a fish pond is dependent upon suitable pond ecological conditions, growth and abundance of fish food organisms.

Digestion depends on the physical state of food, as well as the kind and quality of enzymes secreted. Activity of digestive enzymes has been reported to change with the feeding habits of fish or availability of fish food organisms [12-14]. Improved understanding of pond fish digestive enzyme profiling can provide a better insight to explaining the digestive processes of the pond fishes that operate under variable conditions of feeding. From such understanding, the incorporation of appropriate enzymes can help in increasing the digestibility and improving the growth performance of fish.

Various works have been carried out on endogenous fish digestive enzymes by a host of workers [13-17]. Despite this, there is shortage of material on the subjects of enzyme production, the sources of these enzymes and their significance in fishes. Previous works have studied the native microflora of aquaculture fish for various objectives. These include microbial spoilage mechanisms, the environmental relationship with fish microflora [18-20] and profiling of the indigenous microflora antibiotic resistance [21-23].

Fish tend to continuously intake large quantities of microbes in their gut system from the surrounding water, food and sediments populated with bacteria [24]. Fish gastrointestinal tract is usually colonized by various types of heterotrophic bacteria in large numbers which include both aerobic and anaerobic bacteria. Despite various ecological research works on the gut bacterial flora in fish being previously available [25-27], reports concerning their functions have only appeared recently. There are some reports on microbiological enzymatic production in fish intestinal tract [13,14,17,28-33], however studies on the enzyme producing bacteria distribution in the gut of fish is quite limited. But it is confirmed that there are variations in the quality and quantity of these enzyme producing microorganisms the amounts of enzymes present in fish gastrointestinal tracts also vary.

Catla *Catla catla,* mrigala *Cirrhinus mrigala* and rohu *Labeo rohita* are three widely utilized and significantly important polyculture species in India [34,35], however there is insufficient information on the digestive physiology and feeding preferences of these three species. This study aims to provide a deeper insight on the digestive enzymatic activities and whether the three fish species in polyculture arrangement are occupying different trophic levels in terms of preferred foods for permitting feeding and good production.

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Materials and Methods

Study area

Four polyculture village ponds were selected as study sites in the Hisar district, state of Haryana, India with the geo-referenced location (Longitude, 75°-72' E and Latitude, 29°-15' N) as shown in Figure 1. Two of the ponds were unmanaged pond but with cattle and domestic effluent intakes. The other two ponds were managed type pond with control of household effluent intake and cattle movement. The four ponds are geographically evenly located with similar soil characteristics and exposed to sunlight. The physicochemical and biological characteristics of the unmanaged and managed ponds are shown in Table 1.

Analysis of gut contents and forage ratio

Specimens of live and mature freshwater fishes of the species *Labeo rohita*, *Catla catla* and *Cirrhinus mrigala* from each of the selected ponds were sampled using cast net on fortnightly between the months of March 2011 and February 2012. 120 mature fish of each species (360 fish) were taken for analysis from each pond (total 1440 fish) with a weight range of 248.00g to 298.00g. Fish were dissected in ice surface and the digestive tracts were removed together with the contents and preserved in 5% formalin. The gut contents were then analysed both quantitatively and qualitatively following the procedure outlined in Garg [36].

Forage ratio

Plankton samples were collected from each of the ponds and analysed. They were classified up to the genus level. Equation 1 shown below was used for the forage ratio.

$$F = \frac{g}{w} \tag{1}$$

Where *F* represents the forage ratio, *g* represents the percentage of organisms that are present in gut contents and *w* represents the percentage of organisms present in the water/environment sample. When the value of $F = 1$, it indicates random food selection from the surroundings, $F > 1$ indicates active preference of the item by the fish, $F < 1$ is an indication that the fish avoids the food item. *F* value is directly proportional to the level of food preference.

Digestive physiology analysis

The extracted alimentary tracts of fish were homogenized with 10 parts cold 0.89% NaCl solution [17]. The homogenate was used for different measurements. Bovine Serum Albumin (BSA) was used as 1% substrate to measure the proteolytic enzyme activity using the methods presented in Walter [37]. Estimation of proteins was done using the methods of Lowery [38]. The methods of Bernfeld [39] were used for measurement of the amylase activity in the intestinal tract using starch solution as 1% substrate. Microcrystalline cellulose was used as 1% substrate to estimate the intestinal cellulase activity.

Estimation of intestine lipase enzyme activity

Two volumes of ice-cold acetone were added to intestinal tissue which was homogenized in a homogenizer. Filtration of the homogenate was done and washed in sequence with acetone, acetone-ether mixture and ether. The obtained residue was air dried and one gram was added to ice-cold water and stirred for 15 minutes before use. The contents were subjected to centrifugation at 15,000 rpm for 10 minutes. The supernatant was utilized as a crude enzyme extract and measurement of lipase activity was done using the procedure outlined in Colowick and Kaplan [40].

Estimation of liver glycogen

The liver was taken out from the fish and the excess of blood was immediately removed with filter paper. The liver was then transferred to a test-tube which contained 2.0 mL of 30% KOH solution/gram of liver. It was then placed in a water bath at boiling temperature for 90 minutes to digest the tissue and allowed to cool. Following this procedure, two volumes of 95% ethanol was added to the sample and placed in a water bath at boiling temperature. The samples were then left overnight, under cold conditions and centrifuged at 3000 rpm for 20 minutes. After this, the obtained precipitate was dissolved in 5-10 mL of lukewarm water. For glycogen, the precipitate was precipitated again by addition of two volumes of 95% ethanol. The obtained precipitate was collected through centrifugation at 5000 rpm for 15-20 minutes at a temperature of 4°C followed by several washings using 60% (v/v) ethanol. The obtained precipitate was further transferred with 2N H_2SO_4/gram of liver. The contents were hydrolyzed in a water bath at boiling temperature for 3-4 hours. After cooling the contents were neutralized with 6N NaOH (phenol red was used as an indicator). The volume was made to and followed by filtration. An appropriate aliquot was used for determination of glucose content. The factor of 0.93 was utilized in the conversion of glucose to glycogen. Sugar level was determined following the methods in Dubios [41].

Statistical analysis

ANOVA variance analysis and Duncan Multiple range statistical test was carried out to determine if significant differences existed between the different ponds for each parameter. Coefficient of correlation was also calculated. All statistical analysis was carried out using the SPSS package.

Results

Gut content analysis and forage ratio of Indian major carps

Managed ponds of district Hisar

***Cirrhinus mrigala*:** Analysis of gut contents of *C. mrigala* samples collected from managed ponds of district Hisar indicated the presence of members of Bacillariophyceae (31.17%) along with some unidentified matter and debris (40.48%) (Table 1). Forage ratio indicating the preferred food items were calculated and results showed significantly ($p < 0.05$) high value of forage ratio for *Cyclotella* (2.45), *Navicula* (1.83) and *Synedra* (1.63) for *C. mrigala*. The value for zooplankton was much lower indicating that *C. mrigala* is a phytoplanktivorous and *Cyclotella, Navicula* and *Synedra* are its preferred food items (Tables 2 and 3).

***Labeo rohita*:** Analysis of gut contents of *L. rohita* collected from managed ponds of district Hisar indicated the presence of member of Bacillariophyceae (14.20%), Chlorophyceae (13.20%), Cladocera (12.84%) and Copepoda (9.47%) along with some unidentified matter and debris (43.18%) (Table 2). The preferred food items that were foraged appear in Table 3. The results showed significant ($p < 0.05$) value of *Microspora* (3.21), *Moina* (3.20) and *Diaptomous* (2.56) indicating that *L. rohita* is omnivorous and *Microspora*, *Moina* and *Diaptomous* are preferred food items.

***Catla catla*:** Analysis of gut contents of *C. catla* collected from managed ponds of district Hisar indicated the presence of member of Copepoda (15.20%) and Cladocera (11.40%) along with some unidentified matter and debris (46.80%) (Table 1). Forage ratio

Parameters	H1	H2	H3	H4
Water Temp. (°C)	22.12 ± 1.40	22.24 ± 1.36	23.57 ± 1.06	23.92 ±. 90
pH	9.08 ± 0.09	8.19 ± 0.02	8.17 ± 0.08	8.12 ± 0.13
Conductivity ($\mu S\ cm^{-1}$)	4201.94 ± 56.60	1618.33 ± 10.45	1023.44 ± 84.40	427.66 ± 15.97
Salinity ppt	2.0 ± 0.02	0.0	0.0	0.0
Free CO_2 ($mg\ L^{-1}$)	Absent	22.55 ± 1.42	11.50 ±. 95	13.10 ± 1.55
Dissolved oxygen ($mg\ L^{-1}$)	3.93 ± 0.08	6.26 ± 0.03	7.21 ± 0.05	7.51 ± 0.06
BOD ($mg\ L^{-1}$)	2.70 ± 0.13	3.04 ± 0.10	2.45 ± 0.05	2.38 ± 0.10
Carbonate Alkalinity ($mg\ L^{-1}$)	57.21 ± 4.25	Nil	Nil	Nil
Biocarbonate Alkalinity ($mg\ L^{-1}$)	429.77 ± 25.64	382.83 ± 14.67	240.55 ± 9.16	168.16 ± 2.34
Total alkalinity $mg\ L^{-1}$	486.98 ± 22.82	382.83 ± 14.67	240.55 ± 9.16	168.16 ± 2.34
Chloride ($mg\ L^{-1}$)	221.26 ± 11.16	193.18 ± 13.04	115.65 ± 22.55	9.42 ±. 38
Total Hardness ($mg\ L^{-1}$)	543.72 ± 14.60	206.33 ± 6.6	234.83 ± 28.21	172.11 ± 3.24
Calcium ($mg\ L^{-1}$)	73.26 ± 10.65	41.57 ± 1.69	43.54 ± 11.76	26.45 ± 4.06
Magnesium ($mg\ L^{-1}$)	87.96 ± 8.88	25.04 ± 2.40	30.75 ± 1.05	25.87 ± 2.79
O-phosphate ($mg\ L^{-1}$)	2.3 ± 0.06	1.93 ± 0.04	0.74 ± 0.02	0.46 ± 0.02
Total phosphate ($mg\ L^{-1}$)	2.87 ± 0.10	2.54 ± 0.03	1.15 ± 0.05	0.63 ± 0.05
Total ammonia ($mg\ L^{-1}$)	2.05 ± 0.56	1.36 ± 0.35	0.52 ± 0.04	0.59 ± 0.11
Plankton Population (L^{-1})	3780.00 ± 193.21	3893.33 ± 238.62	3520 ± 208.65	3333.33 ± 156.86
Phytoplankton (L^{-1})	2153.33 ± 143.35	2076.66 ± 172.74	2206.66 ± 157.73	2083.33 ± 113.02
Zooplankton (L^{-1})	1626.66 ± 83.94	1816.66 ± 151.17	1313.33 ± 98.55	1037.33 ± 183.00
All values are mean ± S.E. of mean				

Table 1: Physicochemical and biological characteristics of the unmanaged ponds (H1 and H2) and managed ponds (H3 - H4) of Hisar district.

Hisar managed ponds			
Variables	*Catla catla*	*Labeo rohita*	*Cirrhinus mrigala*
Phytoplankton			
Debris	40.48	43.18	46.80
Bacillriophyceae	31.17	14.20	9.5
Chlorophyceae	5.66	13.20	9.5
Zooplankton			
Rotifera	11.33	7.10	7.6
Cladocera	5.66	12.84	11.4
Copepoda	5.66	9.47	15.2
Hisar unmanaged ponds			
Variables	*Catla catla*	*Labeo rohita*	*Cirrhinus mrigala*
Phytoplanton			
Debris	42.39	45.66	47.72
Bacillriophyceae	24.69	19.55	6.83
Chlorophyceae	12.32	6.52	4.54
Zooplankton			
Rotifera	6.16	4.34	11.36
Cladocera	8.22	8.69	13.62
Copepoda	6.16	15.21	15.90

Table 2: Gut content analysis of *Labeo rohita*, *Cirrhinus mrigala* and *Catla catla* from the managed and unmanaged ponds of the Hisar district. All values are in percentage of total contents.

indicating the preferred food items were calculated (Table 3) and results exhibited significantly ($p<0.05$) high value of forage ratio for *Cyclops* (2.75) and *Branchionus* (1.83). This indicated that *C. catla* is zooplaktovorous and *Cyclops* and *Branchionus* are its preferred food items.

Unmanaged ponds of district Hisar

Cirrhinus mrigala: Analysis of gut contents of *C. mrigala* collected from unmanaged ponds of district Hisar indicated the presence of member of Bacillariophyceae (24.69%) and Chlorophyceae (12.32%) along with some unidentified matter and debris (42.39%) (Table 2). Forage ratio indicating the preferred food items were calculated (Table 4) and results showed significantly ($p<0.05$) high value of forage ratio

for *Microspora* (3.64), *Synedra* (2.73) and *Navicula* (2.27) for *C. mrigala* whereas the value for zooplankton was much lower indicating that *C. mrigala* is a phytoplanktivorous and *Microspora*, *Synedra* and *Navicula* are its preferred food items.

Labeo rohita: Analysis of gut contents of *L. rohita* collected from unmanaged ponds of district Hisar indicated the presence of member of Bacillariophyceae (19.55%) and Copepoda (15.21%) along with some unidentified matter and debris (45.66%) (Table 2). Forage ratio indicating the preferred food items were calculated (Table 4) and results showed significantly ($p<0.05$) high value of forage ratio for *Cyclops* (2.20), *Synedra* (1.85), *Closterium* (1.54) and *Daphnia* (1.54) indicating that *L. rohita* is omnivorous and *Cyclops*, *Synedra*, *Closterium* and *Daphnia* are its preferred food items.

Variables	*Cirrhinus mrigala*	*Labeo rohita*	*Catla catla*
Phytoplankton			
Synedra sp.	1.63	1.07	1.22
Navicula sp.	1.83	1.20	0.68
Cyclotella sp.	2.45	-	0.91
Closterium sp.	0.91	0.80	1.03
Microspora sp.	-	3.21	-
Total Phytoplankton	6.82	6.28	3.84
Zooplankton			
Branchionus sp.	1.22	1.60	1.83
Diaptomous sp.	0.73	2.56	1.65
Daphnia sp.	0.91	-	1.37
Moina sp.	-	3.20	-
Cyclops sp.	-	-	2.75
Total Zooplankton	2.86	7.36	7.60

Table 3: Forage ratio of *Labeo rohita*, *Cirrhinus mrigala* and *Catla catla* from the managed ponds of the Hisar district.

Variables	*Cirrhinus mrigala*	*Labeo rohita*	*Catla catla*
Phytoplankton			
Synedra sp.	2.73	1.85	0.62
Navicula sp.	2.27	1.02	0.51
Cyclotella sp.	-	1.23	1.23
Closterium sp.	-	1.54	1.02
Microspora sp.	3.64	-	-
Total Phytoplankton	8.64	5.64	3.38
Zooplankton			
Branchionus sp.	1.17	-	2.20
Diaptomous sp.	1.36	1.02	2.57
Daphnia sp.	1.36	1.54	1.54
Keretella sp.	-	1.23	-
Cyclops sp.	0.78	2.20	1.32
Total Zooplankton	4.67	5.99	7.63

Table 4: Forage ratio of *Labeo rohita*, *Cirrhinus mrigala* and *Catla catla* from the unmanaged ponds of the Hisar district.

Catla catla: Analysis of gut contents of *C. catla* collected from unmanaged ponds of district Hisar indicated the presence of member of Copepoda (15.90%) and Cladocera (13.62%) along with some unidentified matter and debris (47.72%) (Table 2). Forage ratio indicating the preferred food items were calculated (Table 4) and results showed significantly ($p<0.05$) high value of forage ratio for *Diaptomous* (2.57) and *Branchionus* (2.20). This indicated that *C. catla* is zooplanktivorous and that *Diaptomous* and *Branchionus* are its preferred food items.

Intestinal enzymes and liver glycogen of Indian major carps

Managed pond of district Hisar: Total and specific protease and liver glycogen activity were high but total and specific lipase activity, amylase and cellulase were low in *C. catla* in comparison to *L. rohita* and *C. mrigala*. For *L. rohita* total and specific protease, amylase and cellulase activity, lipase activity and liver glycogen were in moderate quantity. In the case of *C. mrigala* total and specific protease and live glycogen activity were much lower but total and specific lipase activity; amylase and cellulase were much higher in comparison to *L. rohita* and *C. catla* (Figures 2, 3 and 4).

Unmanaged pond of District Hisar: The activity of digestive enzymes and liver glycogen followed the same trend as that in managed pond (Figures 2, 3 and 4).

Discussion

The natural food of the fishes under this work has been studied in various research works by examination of the gastrointestinal contents [42-47] and it was revealed that they were planktivorous. Analysis of gut contents of *C. mrigala* indicated that apart from debris and decaying matter, Bacillariophyceae and Chlorophyceae were the dominant group. Although detritus/debris was high, the preferred food items as determined by calculating the forage ratios were *Cyclotella*, *Spirogyra*, *Synedra* and *Closterium* indicating that fish was a herbivore i.e., phytoplankton feeder or phytoplanktivorous. Forage ratio for zooplanktons was always lower than phytoplanktons in case of *C. mrigala*. Khabade [46] has also reported similar results.

Analysis of the gut contents of *L. rohita* from all the ponds indicated large quantities of members of Bacillariophyceae, Chlorophyceae, Copepoda and Cladocera. High forage ratio was observed for *Spirogyra*, *Cyclotella*, *Cyclops* & *Diaptomus* and sometimes *Daphnia*. No significant difference was observed in the gut content data for unmanaged and managed ponds indicating that *L. rohita* was Omniplanktivorus fish. Similar results have been reported in Mohanty, [48] and Majumder [49]. Similarly, for *C. catla*, irrespective of the culture practice, the analysis of gut contents revealed the dominance of Cladocera, Copepoda or Rotifera groups i.e., zooplankton along with large quantities of mud debris. Similar results have been reported in Khan and Siddiqui [50]. Here forage ratio was low for phytoplanktons indicating that the fish
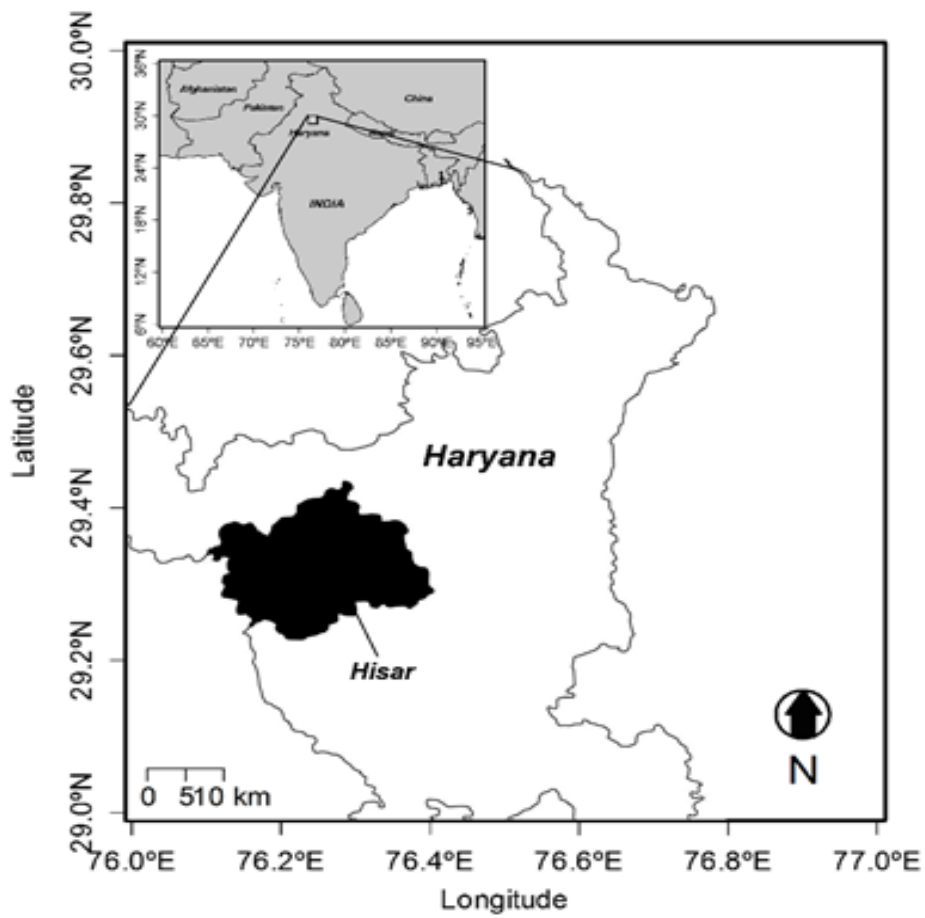
Figure 1: Four polyculture village ponds, in the Hisar district, state of Haryana, India with the geo-referenced location (Longitude, 75°-72' E and Latitude, 29°-15' N).
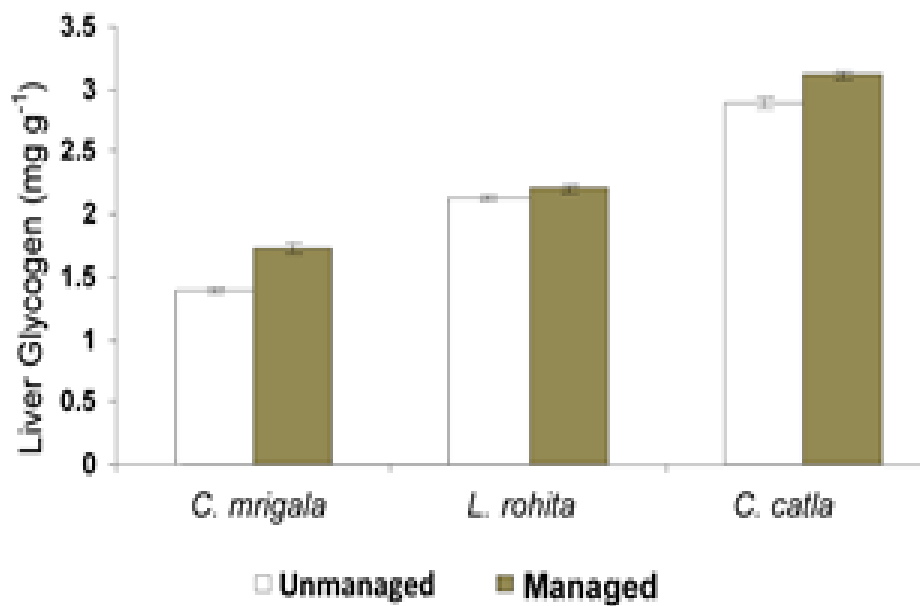

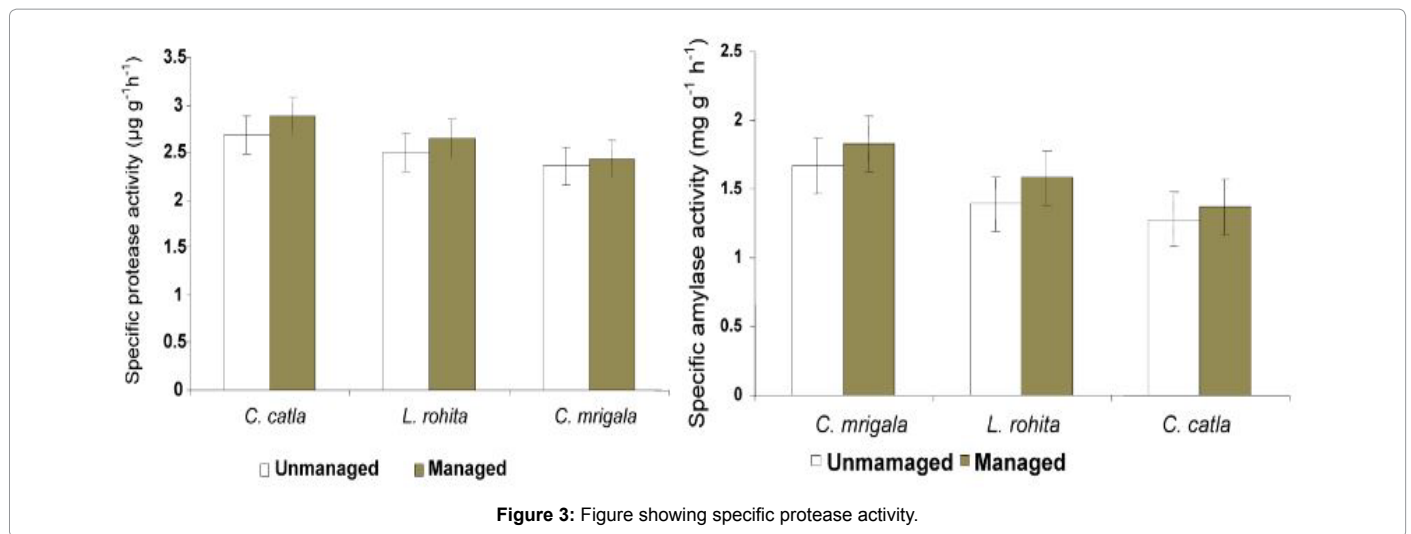**Figure 2:** Figure showing intestinal enzymes and liver glycogen of Indian major carps.

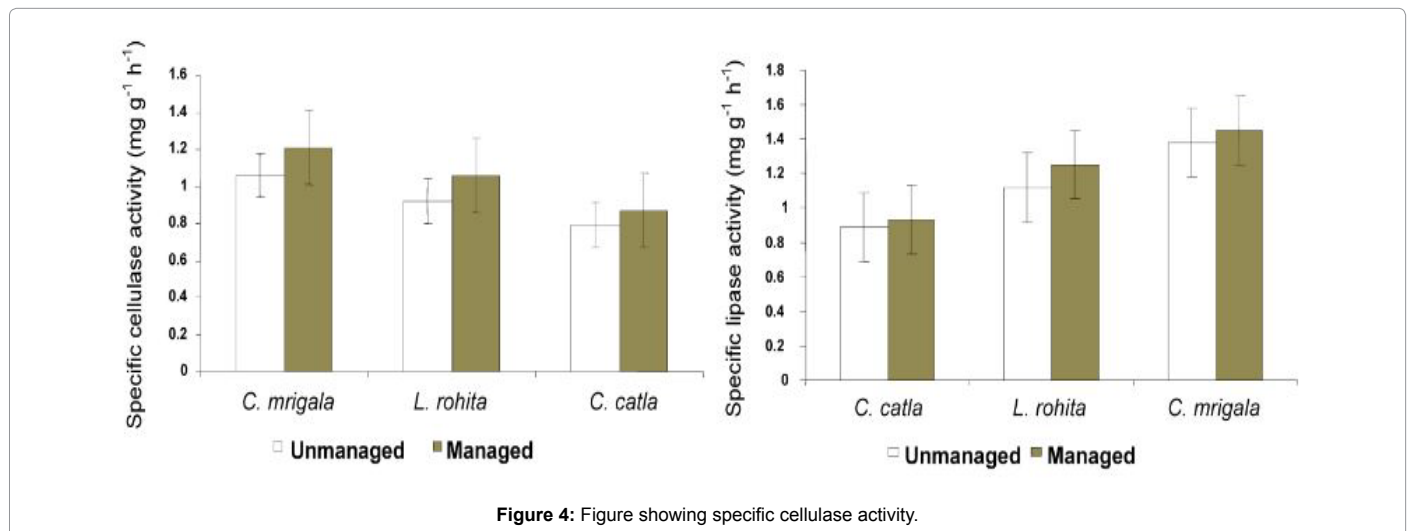**Figure 3:** Figure showing specific protease activity.

Figure 4: Figure showing specific cellulase activity.

was zooplanktivorous. These results provide a clearer distinction of the occupation of different stratus by each fish species for feeding purposes among the food that was made available in the polyculture village pond system. This allows for a deeper understanding of how good production tends to occur.

The ability of fish to use nutrients from food depends upon several factors and digestive enzyme is one of them. It was observed that specific cellulase and amylase activities were higher in *C. mrigala* which also advocate the phytoplanktivorous feeding habit. It was further observed that these activities are higher in the managed ponds as compared to the unmanaged ponds. The amylase activity in the gastrointestinal tract of herbivorous carps is more intense compared to carnivorous fishes as suggested in Dhage [15], Sarbahi [51] and Philips [52]. Bairagi [30] reported the presence of amylolytic bacteria (responsible for exogenous amylase production) from the gut of *C. mrigala* and *C. catla* even after 24 hrs of starvation.

Cellulase activity was high in case of *C. mrigala*. Barrington [42], Fish [53] and Yokoe and Yasumasu [54] suggested that there is no cellulase available in fish. Shcherbina and Kazlawlene [55] explained that there is endogenous secretion of cellulase in the anterior section

of the carp digestive tract. Lesel [28] estimated cellulase activity for grass carp and gold fish. Bairagi [30] made observations of cellulolytic bacteria in considerable amount from gut of *C. catla*. Both *C. catla* and *L. rohita* had lower celullase activity than *C. mrigala*. Cellulase producing bacteria have been previously islolated from *C. mrigala* and *L. rohita* [33,56].

Detection of elevated levels of protease and amylase in *L. rohita* further support the omniplanktivorous nature of the fish. Das and Tripathi [17] have also determined that fish having omnivorous feeding habits have high levels of amylase activity in their guts. Bairagi [30] reported the presence of considerable amylolytic and proteolytic bacterial load in the gastrointestinal tract of *L. rohita* and suggested that along with endogenous there is also exogenous source of protease and amylase.

Analysis of digestive enzymes from the gut of *C. mrigala* revealed more lipase in comparison to other enzymes. Dhage [15] also reported that lipase activity is more concentrated in the intestine of *C. mrigala*. Liver glycogen was high in *C. catla*, supporting the zooplanktivorous nature of the fish as revealed by gut content analysis. Hepatic glycogen was low in *C. mrigala* and *L. rohita*. Bhattacharya [57] reported that

hepatic glycogen is hydrolyzed by α-amylase and β-amylase and later metabolized in the glycolytic sequence. In the present study amylase was high in *C. mrigala* and *L. rohita* and thus the low glycogen level. Fishes reared in managed ponds had higher enzymatic activities in the gut advocating higher growth. Glycogen levels are also affected by the population densities as shown in Chatterjee [58].

Digestion depends on the physical state of food, as well as the kind and quantity of enzymes secreted. It is reported that in addition to endogenous digestive enzymes [15-17] there is distinct microbial origin for the digestive enzymes available in the gastrointestinal tracts of fish [28,30,33,56,59]. The growth of these microbes is dependent upon the ingested food by the fish, scaled off fragments from the mucosal epithelium and digestive secretions [29,60,61]. Fish tend to frequently take in large loads of microbes in their gut from the environment in which fish live and thereafter, some colonize in the gut of the fish forming persistent population adhering to intestinal mucosa and assist in the production of intestinal enzymes as exogenous source.

Conclusion

The results of the study enhance our understanding of the feeding patterns at different stratus levels within the available nutrition for the three fish species in polyculture systems. We are able to better understand how production results with each species targeting a particular stratus.

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