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Qualitative algal analysis from the fish-gut: Tested in the rice fish cropping system

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ABSTRACT: Algae constitute a major proportion of fish food in rice-fish culture system. Study of algal preferences and their availability in the natural environment might be important for developing natural fish feed. Preparation of formulated feeds using under-exploited materials such as algae not only reduces production costs, but also increases procurement accessibility of locally available alternative ingredients. Methodological difficulties were being encountered in the processing and identification of ingested algal material from fish guts during the study. A new approach was thus developed to make the analysis easier, simple and accurate. For the purpose, algal ingestion by *Cyprinus carpio*.L stocked in a rice-fish cropping system was undertaken. The intact guts or gut contents of 25 fishes were transferred directly to five different solid nutrient mediums to study the species of algae consumed by the fish. The method adopted was found to be simpler, accurate and more convenient compared to conventional technique for gut analysis. Moreover, the methodology also reduces the number of fishes 'sacrificed' for the study.

Key words: *Algal feed, Cyprinus carpio.L, rice fish, gut analysis*

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INTRODUCTION

In subsistence level aquaculture, artificial feed materials are in short supply and many potential ingredients have competitive uses in other farm enterprises. Matching fish optimal needs with a prepared diet is often impractical or impossible, that too, in the rural areas. Under these conditions, comparing the availability of natural foods in the pond with the food preferences of a species might be of use in making decisions about culture species and management (Brummett, 1996). Therefore, a qualitative estimation of imbalances between food need and availability might be used to design input regime. Fish in rice fields feed on weeds, plankton, and periphyton, and comprise an ecological system that benefits both the fish and the rice (Das *et al.*, 2000). The algal flora (phytoplankton and periphyton) and aquatic vascular plants present in the submerged rice field are the principal primary producers in the aquatic habitat and their role on fish culture is well documented (Das *et al.*, 1994; Attayde and Hansson, 1999; Azim *et al.*, 2002). Besides fishes in rice fields, there are other fishes that depend upon algae for their food namely coldwater fish *Garra pingi pingi, Ancistrus temminckii, Otocinclus vittatus, Hypostomus punctatus, Liposarcus anisitsi, Liposarcus pardalis, Liposarcus multiradiatus, Puntius spp, Ctenopharyngodon spp., Tor spp, Schizothorax spp, Neolissochelius spp.*, Algal supplementation of fish feeds has certain physiological merits since algae are rich in vitamin precursors, growth promoters and essential fatty acids. In spite of their importance in enhancing growth of certain fish such as silver carp research on the use of algae as a protein source for fish-feed formulation is scant (Appler and Jauncey, 1983; Appler, 1985; Nakagawa *et al.*, 1987; Chow and Woo, 1990). Additionally, escalating costs of fish-feed components necessitates identification of alternative raw materials and their local abundance for use in feed production. Till now scientists have tried a single algal species like *Spirulina, Laminaria digitada*, and *Chlorella* extract as a dietary protein source for certain fishes. However a combination of algal species can be tried out as fish feeds. This composition of algal species can be determined from the fish gut itself. Food selection of fish may depend upon the type of natural food present in the field as well as on the agro-climatic conditions. However, under stress they show

complex feeding habits and strategy (Haroon, 1998). Therefore, a study on the feeding behavior and feeding strategy becomes important in order to identify the natural feeds being ingested by the fish. In order to estimate feed intake, stomach contents analysis may be carried out on either live or dead fish. The measurement of feed intake in fish can be considered at different levels depending upon the aims and constraints of particular studies. Test conditions will also differ between studies. Hence techniques used should also depend upon the research application. In the present study, algal species composition in a fish gut was observed using a fresh method. During the study, methodological difficulties were being faced in the processing and identification of consumed algal members from fish gut through conventional techniques. So, a new approach was finally adopted to make the analysis easier and simple. To testify the method, compositions of algae taken up by the fish in rice fields in two different seasons were studied. The disadvantages felt during the gut-analysis as well as the importance of the present method is discussed. The study also serves the purpose of search of an alternative feed preparation or supplement food for local fish in local environment and consequently explores other advantages of the study.

MATERIALS AND METHODS

Studied area: The Ziro area of Arunachal Pradesh (India, altitude of 1800 m) can be regarded as a high altitude rice fish farming system. Consumption of algae by *Cyprinus carpio.L* stocked in the rice-fish cropping system was undertaken. Intact gut or gut content of 50 fishes were analysed in the present study. Sampling was done during May- July (summer) and Sept-Nov (winter). The size of the fishes could not be controlled but it varied from 4-6 inches in length. Fish were not weighed. For identification of algae, temporary mounts were prepared using glycerin jelly. The algae were identified to genus and species or to genus only. Medium for algal growth: Initially five algal mediums were studied; however this could be increased depending on the habitat of the fish. The media selected were Bacillariophycean Medium**,** Desmidiacean Medium**,** Hughes, Chu-10 and BG 11. The algae were grown Allen medium as modified by Hughes (Hughes *et al.,* 1958) with $NaNO_3$ (17.6 mM). The pH (6.8) was maintained with phosphate buffer. The growth medium (Chu-10, Gerloff *et al.*, 1950, pH 6.8) was prepared using double-distilled water and subsequently filter sterilized. For BG11, the pH was maintained at 7.0-7.5.

Water quality

Surface to sub-surface (0.02 m) water samples from 10 fields were analyzed. Air and water temperature, dissolved oxygen, carbon dioxide, pH, alkalinity, hardness and ammonia-nitrogen were monitored every week prior to fish sampling in both the seasons. pH were measured with pH electrode. Temperature and DO were measured with a DO meter (Systronics DO meter 312). Water samples were filtered before the nutrients were analysed.

Stomach content analysis

The live fish was washed properly with sterile water in a tub in the field. An abdominal incision is made from the gills to the anal opening and the stomach removed by cutting at the upper end of the oesophagus and behind the pyloric sphincter. A larger part of the gut is sampled in those fish, which lack a well-defined stomach was made with a sharp knife to remove the gut. The gut of 18-20 inches common carp fish was washed in 150 mL of sterile water for about half-an-hour 2-3 times, soaked in blotting paper, kept in a petri plate, opened the gut, added 2-3 ml water with a syringe into the gut and cleaned with a brush, and pour down the water in one agar plate. Initially the process was repeated for 5 algal mediums. For small fishes, after giving an incision in the gut, intact gut was transferred directly on different solid nutrient mediums to study the species of algae consumed by the fish. No bacterial or fungal infection was found during the experiment. Culturing and identification: The agar plates were kept at 26 °C ± 2 °C under 14.4 W/m^2 of light intensity and 16/8 h light/dark cycle. The results could be obtained within 15 days of incubation. Analyzed after 15 days when growth of the algae were ensured. Visible colonies were transferred to another agar plates for isolation. Standard microbial techniques were employed for selection, isolation and cloning of algae in pure culture. For identification, the cells of the colonies were picked up carefully from the agar plates and transferred to microscopic slides and in-vitro study of actual feed selection is done after immobilizing the algae in alginate beads and noticing the number of beads taken up by the fish.

Table 1: Algal members found in the fish gut in winter

Chlorophyta (including Desmids)	Continued from col 1	Diatoms
Asterococcus spp.	*Pleurotaenium spp.*	*Navicula cuspidata*
Chlamydomonas spp.	*Pseudococcomyxa spp.*	*Pinnularia viridis*
Chlorella pyrenoidosa	*Scenedesmus obliquus*	*Skeletonema*
Chlorella vulgaris	*Scenedesmus quadricauda*	*Thalassiosira*
Chlorogonium elongates	*Staurastrum spp.*	*Phaeodactylum*
Chloromonas muculata	*Ulothrix spp.*	*Chaetoceros*
Closterium acutum	*Xanthidium spp.*	*Cylindrotheca*
Closterium arcutum		*Bellerochea*
Closterium dianae	Cyanophyta	*Actinocyclus*
Closterium dianae	*Nostoc calcicola*	*Nitzchia*
Closterium ehrenbergii	*Anabaena spp.*	*Cyclotella*
Closterium leibleinii	*Oscillotoria spp.*	
Closterium tumidulum	*Gleocapsa spp.*	
Euglena gracilis	*Gleocystis spp.*	
Gonium pectorale	*Chroococcus turgidus*	
Mesotaenium spp.	*Synechococcus elongates*	
Mougeotia spp.		

Table 2: Algal members found in the fish gut in summer

Chlorophyta (including Desmids)		Diatoms
Apatococcus lobatus	*Pediastrum spp.*	*Navicula spp.*
Cosmarium botrytis	*Pleurotenium spp.*	*Pinnularia viridis*
Chlorella vulgaris	*Scenedesmus obliquus*	*Skeletonema*
Chlorogonium spp.	*Scenedesmus quadricauda*	*Thalassiosira*
Chlorococcum spp.	*Spirogyra spp.*	*Phaeodactylum*
Closterium acutum	*Staurastrum inflexum*	*Chaetoceros*
Closterium arcutum	*Tetraspora spp.*	*Cylindrotheca*
Closterium dianae		*Bellerochea*
Closterium ehrenbergii	Cyanophyta	*Actinocyclus*
Closterium leibleinii	*Anabaena spp.*	*Nitzchia*
Closterium tumidulum	*Nostoc spp.*	*Cyclotella*
Euglena gracilis	*Oscillotoria spp.*	
Oedogonium spp.	*Synechocystis spp.*	

Table 3: Seasonal variation in limnological parameters of rice fields at zero

Parameters	Winter (Sept-Nov)	Summer (May-July)
Air temperature	18.0 °C ± 2.5	30.5 °C ± 3.0
Water temperature	27.5 ± 1.0	30.0 ± 2.5
Turbidity (JTU)	25 ± 5	45 ± 5
pH	8.9 ± 0.5	8.0 ± 0.9
Dissolved oxygen (mg/L)	9.6 ± 2.2	8.2 ± 1.6
BOD (mg/L)	2.1 ± 1.3	2.9 ± 1.5
Free CO_2 (mg/L)	2.8 ± 0.8	3.5 ± 1.4
Alkalinity		
Carbonate (mg/L)	0	1.1 ± 1.2
Bicarbonate (mg/L)	220 ± 35	200 ± 40
Hardness (mg/L)	176 ± 22	165 ± 15
Chloride (mg/L)	23 ± 1.0	15 ± 2.5

Table 4: Different groups of phytoplankton found in the field

Phytoplankton Units/L	Season	
	Winter (Sept-Nov)	Summer (May-July)
Diatom	1747.25 ± 45.60	701.50 ± 16.25
Green algae	201.00 ± 15.30	146.40 ± 10.60
Blue green algae	50.25 ± 12.00	38.00 ± 5.55

Table 5: Seasonal variations in the plankton

Plankton (Units/L)	Season	
	Winter	Summer
Phytoplankton	2138.50 ± 152.53	1006.00 ± 18.72
Zooplankton	437.06 ± 7.14	112.25 ± 16.58
Total plankton	2575.56	1118.56

RESULT

It was felt necessary to study the feed especially the algal composition in the fish diet in rice-fish system because raising fish (*Cyprinus carpio.L*) in rice fields doesn't require any artificial diet plus only 3-4 months to grow. The physico-chemical properties of the field (Table 3) is provided to better understand the region. The temperature ranges from 30.5 °C ± 3.0 in summer and 18.0 °C ± 2.5 in winter. The conductivity ranges from 120-130 µmho in both the seasons. DO recorded was 8.2 ± 1.6 in summer and 9.6 ± 2.2 ppm in winter. The quantitative analysis of the phytoplankton (Table 4) shows that the diatom varies from 701.50 to 1747.25 units/L while green algae and blue green algae varies from 146.40 to 201.00 and 38.00 to 50.25 units/L. Total plankton (Table 5) is found to be varying from 1118.56 to 2575.56 units/L. A Table is given showing the composition of algae found in the fish gut during summer and winter (Tables 1 and 2). 50 fishes were studied in each season and the variety of members taken up by the common carp was considered at present. 20 genera/species of Chlorophyta, 11 members of diatoms and 4 members of Cyanophyta was found in summer while it is 23 for Chlorophyta, 11 for Diatoms and 7 for Cyanophyta, which were present in the gut in winter. Out of total 99 members of Chlorophyta, 43 members were present in the field but not found in the gut of fish in summer while in case of winter, the genera/ species were 23. The stress was also given to the methodology. Before discussing the importance of gut analysis followed for the present study, it is essential that we are aware of the conventional method of gut analysis. Several workers have given the summaries of the stomach contents analysis by conventional technique (Hynes, 1950; Windell and Bowen, 1978; Hyslop, 1980; Talbot, 1985 and Bowen, 1996). The stomach of each fish was taken apart after recording the body mass. Guts were weighed and preserved in buffered formalin. In the laboratory, the content of each stomach was rinsed carefully in a small beaker and filled up to 20 or 40 mL depending on the respective quantity. After mixing, three aliquots of 50 µL were taken from each gut sample, transferred to microscopic slides and analyzed. Each slide was scanned completely at low magnification and the relative contribution of natural food and supplemental feed estimated. The average share of both components in each sample was calculated from the 3 replicates and recorded as the percentage contribution to the gut content.

DISCUSSION AND CONCLUSION

Though the conventional technique can produce valuable information in overall dietary intake, it has number of disadvantages when considering the specific intake of algal cells. The disadvantages in using the conventional technique are (i) The material is preserved in formalin, which may lead to structural deformity and color loss (ii) The identification of preserved algae is much difficult compared to live material due to factors like, pigmentation, motility and different stages of growth (iii) It might lose some of the food material taken in small quantity (iv) Fragments of the food material may be of varying size (sometimes structure is very confusing and not clear) as well as the number of cells or filaments available for the study is also very less (v) The study is time consuming and it is labor intensive i.e. removal of gut and removing its content for the microscopic study is very tiring and even difficult in small fishes (when required for study of different stage fish food) (vi) The technique very much depends on the skill and ability of the researcher and need to know the name of the algae before formulating into artificial diet and thus there is always possibility of high error due to the need of transfer and retransfer of the gut content after incision (vi) In conventional technique, the algal composition in a single gut was very difficult to record or keep and not easy to study separately each gut or know the probability or frequency of algal species taken by the fish as feed. On the contrary there are certain advantages of the present technique. First of all, it is very simple to perform i.e. only one incision is required to remove the gut. Afterwards the gut can be put into solid nutrient medium in number of ways as per the condition, age and type of fish under study. When the field is far away from the field, it can be performed in

the field as well (Guts can be collected and transferred on agar plates directly in the field). It is more accurate since whole gut can be inserted into media instead of the gut content, hence very small amount of algal material can also grow well when provided with proper medium. Putting different gut simultaneously in different media specific for different kind of algae can pass over the problem of precise nutrient needed for specific algal group. Moreover the media can be modified to suit the different algal group. It is a very good method for studying algae in juvenile fishes for the reason that they are very difficult to handle being small in size. For big fish, the gut content can be flushed out through the syringe and transferred in the nutrient medium. Moreover, large no of samples can be examined in very short time period. There is no urgency for microscopic examination since the material is not destroyed. Due to its intact pigmentation, motility and different stages of growth, it is better to identify live material and growing colonies.

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