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Identification of Indian Major Carps (*Catla catla, Labeo rohita* and *Cirrhinus cirrhosus*) and Their Hybrids by Phenotypic Traits, Allozymes and Food Habits

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Abstract

A study was carried out to identify pure and hybrid fingerlings of Indian major carps, catla, rohu and mrigal by analysing phenotypic traits, allozymes and food habits. Pure (catla, rohu, mrigal) and hybrid (rohu x catla, mrigal x catla and rohu x mrigal) fry were produced by artificial insemination. A total of eight phenotypic traits (body shape, body colour, fin colour, head size, mouth shape, eye colour, lateral line, scale shape) were examined where five were found to be intermediate in hybrids relative to the parental species. Four allozyme markers, lactate dehydrogenase (LDH), malate dehydrogenase (MDH), phosphoglucomutase (PGM) and glucosephosphate isomerase (GPI) were used to identify them genetically. GPI was found to be diagnostic at one of two loci revealing three alleles. Gut content was analysed to estimate feeding relationship between pure and hybrids. Among the pure fingerlings, catla and rohu were found to consume a wide variety of planktons while mrigal consumed both planktons and benthos. On the other hand, all hybrids consumed both planktonic and benthic groups. The extent of dietary overlap between hybrid and their parental species revealed a very high competition for food ranging from 0.96 between mrigal x catla and both parental species to 0.98 between rohu x catla and rohu.

Introduction

Indian major carps, *Catla catla* (catla), *Labeo rohita* (rohu) and *Cirrhinus cirrhosus* (mrigal) are of prime importance in food and nutrition security in Bangladesh. Semi-intensive culture of these three species in communal ponds has been carried out for many years. A large number of hatcheries are operating to provide farmers with required seeds. Hatchery managers are concerned merely with the quantity of the seed for the sake of their profit only while the quality has been allowed to decline. Quality deterioration relates largely to inbreeding and unplanned hybridisation (Hansen et al. 2006; Simonsen et al. 2004, 2005). Inbreeding can occur when there is a lack of knowledge on genetic management of broodstock. Hybridisation in hatcheries may be an intentional or unintentional process. Hatchery owners practise hybridisation when they have no appropriate male or female of the same species ready to spawn. They also hybridise in accordance with some nursery owners' demand as hybrids of Indian major carps grow fast during the early stages (Sarder and Simonsen, 2006). Dishonest nursery owners or fry traders sell hybrid fingerlings mingled with pure ones easily to the farmers since

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identification of these is very difficult at that stage. Ultimately farmers suffer from a loss in production as fish growth is stunted at a certain stage of culture before they can be marketed (Sarder and Simonsen, 2006). On the other hand, there is every possibility of escape of hybrids from culture ponds to natural water bodies and thereby threatening carp genetic resources through gene introgression.

Pure fingerlings of Indian major carps possess some distinguishing external morphological features by which they can be identified. But identification of their hybrid fingerlings is very difficult as there is no clear-cut distinguishing character (Sarder and Simonsen, 2006). So development of identification technique of pure and hybrid fingerlings is required to avoid hybrid while purchasing for stocking.

Allozymes have long been used as molecular markers for fish population studies and fishery management (Utter, 1991). Allozymes may be used to detect hybrid individuals by comparing zymograms of the hybrids with the parental species (Senanan et al. 2004). Allozyme markers have also been used for the identification of natural populations of koi, *Anabas testudineus* (Sekino and Hara, 2000) in Thailand, and the crossed *A. testudineus* originated from crossing of Thai and Bangladesh local koi (Alam et al. 2006).

Among the Indian major carps, catla is a surface feeder (Mookerjee, 1945; Misra, 1953), rohu is a column-surface feeder (Das and Motira, 1955) and mrigal is a bottom feeder (Alikunhi, 1957). Azim et al. (2002) compared the production of three Indian major carps, catla (*Catla catla)*, rohu (*Labeo rohita)* and kalbaush (*Labeo calbasu)*, in a periphyton-based polyculture system and found that rohu grazed on periphyton, whereas catla depended on planktonic food organisms. Gut content analysis of catla and rohu showed dominance of phytoplankton in the fry rearing stage (Dewan et al. 1991). As the hybrids may not have any specific feeding behaviour of their own, they are supposed to exploit both of the two feeding layers of their respective parents. Therefore, differentiation between pure and hybrids by food habit was an added objective of the study.

Materials and Methods

Fry production and rearing

Pure and hybrid fry of Indian major carps were produced by hypophysation in a government fish seed multiplication farm in Mymensingh, Bangladesh. Hybrid fry were produced by carrying out the following crosses:

rohu (♂) x catla (♀) producing rohu-catla hybrids
mrigal (♂) x catla (♀) producing mrigal-catla hybrids
rohu (♂) x mrigal (♀) producing rohu-mrigal hybrids

Pure fry were produced by mating male and female of the respective species. Pure and hybrid larvae were stocked in separate ponds, 0.008 ha each, at a stocking density of 12.5 $kg.ha^{-1}$. A communal stocking of pure and hybrids was done in another pond, 0.024 ha in size, for

studying their food habit. The ponds were prepared following the standard procedure of nursery pond. The fry were reared for four months from June to September 2009.

Phenotypic identification

Fish of each group was collected from the rearing pond and brought to the laboratory for studying different phenotypic traits. The traits examined were body shape, body colour, head size, mouth shape, eye, lip structure, lateral line, scale pattern, and fin colour. A comparison was made through a close observation among pure and hybrid fingerlings.

Allozyme analysis

Four allozymes were tested (glucosephosphate isomerise (GPI), lactate dehydrogenase (LDH), malate dehydrogenase (MDH) and phosphoglucomutase (PGM)) using samples of muscle tissue from the four-month old fry. The method applied was horizontal starch (12%) gel electrophoresis and a 0.04 M citric acid buffer adjusted to pH = 6.1 with 10 N NaOH used as electrode buffer. The buffer used for preparation of the gel was a 1/20 dilution of electrode buffer. The initial electrophoresis was performed for 30 minutes at 100 V and the final for 5 hours at 150 V. Locus designation was the abbreviation of the enzyme in italics and alleles were named alphabetically according to the migration rate from the origin of the application.

Gut content analysis

Ten fish of each pure species and of the hybrids were collected from the communal rearing pond for gut content analysis. A solution of 10% formalin was injected inside the abdomen of each fish just after catching in order to retard the digestion of the ingested food materials and then the whole fish was preserved in 10% formalin in a plastic container until analysis. A suspension of stomach content was prepared and food organisms (planktonic and benthic) were examined both qualitatively and quantitatively using a Sedgwick-Rafter cell and a light microscope. Gut content was enumerated by numerical method (Hynes, 1950). The dietary overlap between hybrid and pure fingerling was determined using Schoener's index (Schoener, 1970). The equation of the index is as follows:

$$a = 1 - 0.5 \sum_{i=1}^{n} \left| P_{xi} - P_{yi} \right|$$

where a is the overlap index, P_{xi} is the proportion of food category i in the diet of species x, while P_{yi} is the proportion of food category i in the diet of species y. The calculated highest value indicates greatest overlap between two species.

Results

Phenotypic traits

Eight phenotypic traits namely body shape, mouth, eye, fin colour, lateral line, head size/shape, scale shape and body colour were identified as to have distinguishing nature among pure and hybrid fingerlings of Indian major carps. The characteristics among the pure species fingerlings are presented in Table 1 and that of hybrids used in this study in Table 2.

Mouth, eye and fin colour were found to be the most promising characters in identification. Mouth gap/opening of catla was found to be circular and large and that of rohu was square or somewhat circular and small, and their hybrid, rohu x catla, had a circular and intermediate size mouth opening. Lower lip of catla was found to have an inner fold with no fringe while that of rohu showed fringed character in addition to inner fold. Their hybrid, rohu x catla, exhibited the inner fold only in its lower lip and no fringe there. It might be noted that the fringed lower lip was found only in rohu and it is the most typical character of rohu. Mouth opening of mrigal looked 'D' shaped/rectangular and its lower lip showed no inner fold. Mrigal x catla was noticed to possess a square mouth opening with no inner fold in the lower lip. Rohu x mrigal was seen to have a 'D' shaped/rectangular mouth opening with no inner fold in its lower lip.

A small red spot was noticed on the lower portion of the eye ball in catla. Neither rohu nor mrigal exhibited such spot. This trait was also found in rohu x catla and mrigal x catla hybrids. All fins in catla were found darkish in colour. In case of mrigal, pectoral, pelvic and anal fins were found to be orange and rest of the fins darkish in colour. The only coloured fin in rohu was the pelvic fin which was reddish in appearance. Red coloured pelvic fin of rohu and orange pelvic fin of mrigal were seen in rohu x catla and in mrigal x catla hybrids, respectively. Coloured fins of mrigal were seen in rohu x mrigal hybrid.

Allozymes

Among the four enzymes used for allozyme electrophoresis, three were expressed (LDH, MDH and GPI) of which two (LDH and GPI) were readable. The readable enzymes represented three loci viz. *Ldh-1*, Gpi-1** and *Gpi-2**. Both *Ldh-1** and *Gpi-2** loci revealed a single allele (**a*) (not shown) whereas, *Gpi-1** locus revealed three alleles (**a, *b* and **c*). The pure species, mrigal, catla and rohu showed the genotypes **aa, *bb* and **cc* respectively whereas the hybrids showed heterozygous genotypes i.e. **bc* for rohu x catla, **ab* for mrigal x catla and **ac* for rohu x mrigal hybrids. The *Gpi-1** was found as a diagnostic locus for the three pure species of Indian major carps and their hybrids (Fig. 1).

Gut content

Gut content analysis of the pure and hybrid fingerlings of Indian major carps revealed a total of 44 genera of phytoplankton belonging to different groups viz. Chlorophyceae, Bacillariophyceae, Cyanophyceae, Dinophyceae and Euglenophyceae and ten genera of

zooplankton belonging to Copepoda, Cladocera and Rotifera and two major groups (Oligochaete and Chironomid) of benthos. Percentage composition of three broad categories of food organism (phytoplankton, zooplankton and benthos) in diet of the pure and hybrid fingerlings of Indian major carps was estimated. It might be noted that percentage composition was estimated in relation to the total identified food organism. However, phytoplankton was found to be the major food category ranging from 91.35% in mrigal x catla hybrid to 94.99% in catla. Zooplankton, next to phytoplankton in magnitude, varied from 1.45% in mrigal to 7.18% in rohu x catla hybrid. Benthos, the smallest category in magnitude, made no contribution to the diet of catla and rohu. The lowest value (0.48%) was observed in rohu x catla and the highest (5.32%) in mrigal. Mrigal x catla and rohu x mrigal hybrids took a substantial amount of benthos, 3.13% and 3.21% respectively, in their diet, reflecting the food habit of mrigal. Fig. 2 depicts the percentage composition of the food according to the three major categories.

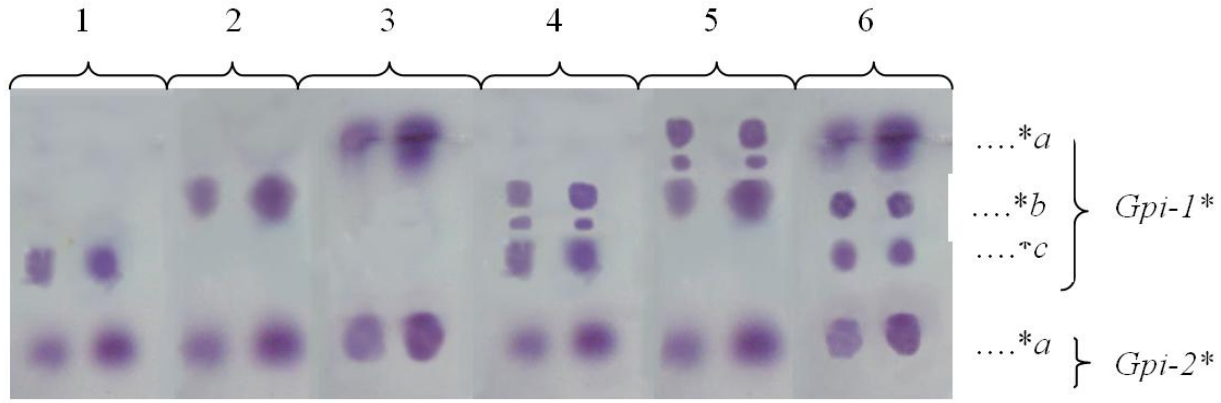

Fig. 1. Zymogram of glucosephosphate isomerase of pure and hybrid fingerlings of Indian major carps, 2 individuals of each, 1 is rohu, 2 catla, 3 mrigal, 4 rohu x catla, 5 mrigal x catla and 6 rohu x mrigal.

Table 1. Characteristic phenotypic traits of pure fingerlings of Indian major carps (*Catla catla, Labeo rohita* and *Cirrhinus cirrhosus*)

Characteristics	Catla	Mrigal	Rohu
Body shape	Wider body depth	Slender body	Moderate
Mouth	• Upturned mouth • Circular mouth gap/ opening • Lower lip folded but not fringed • Lower lip and upper lip terminates at the same point	• Mouth inferior • D-shaped / rectangle mouth gap • Lower lip neither folded nor fringed • Extending upper lip which covers the lower lip	• Terminal mouth • Square mouth gap • Lower lip fringed and folded • Extending upper lip which covers the lower lip
Eye	A red spot on lower part of eye ball	No such red spot	No such red spot
Fin colour	All fins are darkish in colour	Pectoral, pelvic and anal fins are orange in colour	Pelvic fins are red in colour and other fins are darkish in colour
Lateral line	Curved	Clearly visible and almost straight	Slightly curved
Head size/ shape	Larger head	Isosceles head	Equilateral head
Scale size/shape	Larger scale	Exposed portion of scale is rectangular shaped	Exposed portion of scale is diamond shaped
Body colour	Dorsal side blackish in colour while ventral region silvery	Silvery	Slight golden appearance with darker dorsal side

Table 2. Characteristic phenotypic traits of hybrid fingerlings of Indian major carps (rohu x catla, mrigal x catla, rohu x mrigal)

Characteristics	Rohu x catla	Mrigal x catla	Rohu x mrigal
Body shape	Body depth wider than rohu but not so as catla	• Slender as mrigal • More slender than catla-rohu hybrid	Similar to rohu
Mouth	• Somewhat upturned • Circular mouth gap • Lower lip thick and folded but not fringed • Upper lip extended and covers the lower lip	• Slightly upturned • Square mouth gap • Lower lip neither folded nor fringed • Upper lip extended and covers the lower lip	• Terminal mouth • D-shaped/ rectangle mouth gap • Lower lip neither folded nor fringed
Eye	A red spot on lower part of eye ball	A red spot on lower part of eye ball	No such red spot
Fin colour	Pelvic fins are red coloured while other fins are darkish in colour	Pelvic fins are orange while other fins are darkish in colour	Dorsal and caudal fins are darkish in colour while other fins orange
Lateral line	Curved as catla	Curved as catla	Same as mrigal
Head size/shape	Smaller than catla	Isosceles	Isosceles
Scale shape	Exposed portion of scale is diamond shaped i.e. scale like as rohu	Exposed portion of scale is hexagonal	Exposed portion of scale is hexagonal
Body colour	Colour of dorsal region is same as catla while ventral region is same as rohu	Colour same as mrigal	Colour same as rohu

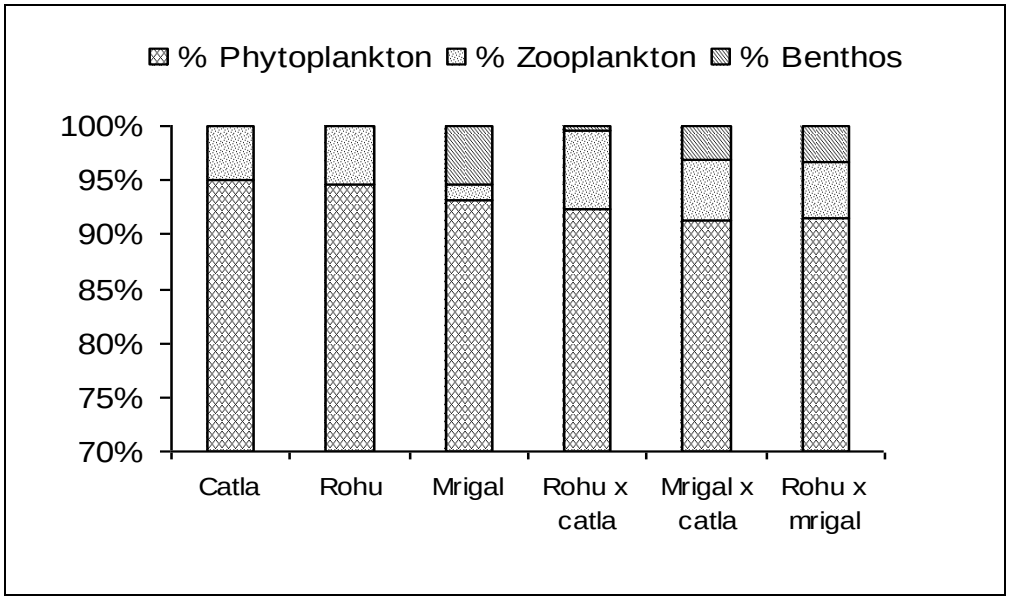

Fig. 2. Percentage composition of phytoplankton, zooplankton and benthos in the diet of pure and hybrid fingerlings of Indian major carps.

The degree of dietary overlap between rohu x catla and catla, rohu x catla and rohu, mrigal x catla and catla, mrigal x catla and mrigal, rohu x mrigal and rohu, and rohu x mrigal and mrigal was estimated separately using Schoener's index and the results are shown in Table 3. High similarity between parental species and their hybrids were found.

Discussion

External morphological characteristics always occupy the first priority when question of identification arises. Several researchers have worked on hybridisation of Indian major carps and observed inheritance of phenotypic traits. Bhowmick et al. (1981) and Jana (1993) reported that *C. catla* (female) x *L. rohita* (male) hybrid exhibited intermediate traits of the parents, but tending more towards the paternal parent (*L. rohita*) with regard to body proportions and fin ray counts. Colouration of the body of the hybrid was near to catla while the shape of the mouth was like rohu. Ibrahim (1977) produced hybrid of *C. mrigala* (female) x *C. catla* (male) and Basavaraju and Varghese (1981) produced hybrid of *L. rohita* (female) x *C. mrigala* (male) and found that both the hybrids expressed intermediate traits between the parents.

The present study also recovered intermediate traits of hybrids to their parents especially for body shape, body colour, fin colour, head size and mouth shape, and noticed the inheritance of some special characters. The presence of a red spot on the lower part of the eyeball distinguishes purebred catla and its hybrids with mrigal and rohu from purebred and hybrids of mrigal and rohu which do not have this spot. Purebred mrigal is distinguished by rectangular scales from purebred rohu and rohu x mrigal hybrids which have diamond shaped scales. Purebred rohu has a fringed and folded lower lip while mrigral and rohu x mrigral hybrids have

lower lips that are neither fringed nor folded. Similarly, purebred catla and its hybrid rohu x catla have thick and folded lower lips but not fringed.

Among the allozyme loci studied in the present study, one locus (*Gpi-1*)* was found to reveal discernible trait with three alleles (*a*, b** and *c**). The three alleles *a*, b** and *c** were found in mrigal (genotype=**aa*), catla (genotype=**bb*) and rohu (genotype=**cc*) respectively with a frequency of 1.000. Among hybrids, they expressed heterozygous genotypes such as **bc* for rohu x catla, **ab* for mrigal x catla and **ac* for rohu x mrigal. The present result attributed the hybrid carps by the diagnostic loci of *Gpi-1** having three different alleles. Similar result was found for identification of crossed koi (*Anabas testudineus*) obtained from crossing between Thai and Bangladesh local koi (Alam et al. 2006). In the diagnostic *Gpi-1** locus, the female parent showed allele **b* dominant (**b*=1.000) and the male parent showed two alleles, **a* dominant with the presence of **b* (**a*=0.967 and **b*=0.033) but the crossed one showed both alleles **a* and **b* (**a*=0.517 and **b*=0.483).

Foremost approach to understand feeding behaviour of fish is to analyse their gut content as adopted by many researchers, for example, Saha and Dewan (1979), Rana et al. (1982), Quddus et al. (1991), Dewan et al. (1991) and Bais et al. (1994) used gut content analysis method in their study. Occurrence of benthic organism in diet of mrigal x catla and rohu x mrigal might be an explanation of inheritance of feeding behaviour of mrigal in these two hybrids. However, benthic organisms, though small in quantity, were also found in diet of rohu x catla hybrid. This phenomenon could hardly be explained since neither of its parent is a bottom feeder.

However, it could be hypothesised that feeding behaviour of the other two bottom feeding hybrids may seduce it to go to bottom for feeding. This speculation needs to be tested by rearing hybrids in separate ponds as well as in a communal pond.

A few studies on dietary overlap among Indian major carps were reported. By using Schoener's index of dietary overlap, Wahab et al. (1991) observed a greater competition for food between silver carp and bighead carp than between catla and rohu. Dewan et al. (1991) reported a low dietary overlap between rohu and catla (0.18). They, however, observed higher dietary overlaps between rohu and silver carp (0.40), rohu and bighead carp (0.35), catla and silver carp (0.52) and catla and bighead carp (0.48). Schoener's indices obtained from the present study varied from 0.76 for Cyanophyceae between mrigal x catla and catla to 1 for many food categories among the fishes. These values could be considered as very high since an index of 0.60 is regarded as high (Schoener 1970).

The present study revealed that rohu x catla hybrid competed more with rohu for phytoplankton while for zooplankton it competed equally with both of the parental species. The overall index values indicated that it competed a little more for its total food organisms with rohu (0.98) than with catla (0.97). Zooplankton consumption by mrigal x catla was overlapped by 99% with catla and by 98% with mrigal while phytoplankton was found to overlap equally with both of the parental species. The index for benthos with mrigal was 0.99 which indicated that mrigal x catla hybrid competed with mrigal for 99% of total benthic organisms and the

remaining 1% was consumed as per their own choice. Overall index was the same with both of the parental species (0.96). The indices yielded by rohu x mrigal hybrid explained more competition with rohu for both phytoplankton and zooplankton than with mrigal. Benthic organism in diet of this hybrid highly overlapped with mrigal (by 99%). It was found to go for competition for total food organism with rohu (0.97) a little more than with mrigal (0.96).

Dietary overlap index values for different groups of food organism as well as for total food organisms among hybrid and pure fingerlings of Indian major carps explored by the present study as stated above strongly supported the hypothesis that food habit of hybrids of Indian major carps overlaps largely to that of parental species.

Table 3. Schoener's indices of dietary overlap between pure and hybrid fingerlings of Indian major carps calculated for different food organism

Food items	Rohu x catla versus catla	Rohu x catla versus rohu	Mrigal x catla versus catla	Mrigal x catla versus mrigal	Rohu x mrigal versus rohu	Rohu x mrigal versus mrigal
Chlorophyceae	0.96	0.94	0.94	0.92	0.96	0.90
Bacillariophyceae	0.98	0.98	0.97	0.98	0.99	0.98
Cyanophyceae	0.77	0.80	0.76	0.85	0.94	0.95
Euglenophyceae	1	0.98	1	0.99	0.99	0.99
Total Phytoplankton	0.90	0.96	0.92	0.92	0.96	0.92
Copepoda	1	1	1	1	1	1
Cladocera	1	1	1	1	1	*
Rotifera	0.99	0.99	0.99	0.98	1	0.98
Total Zooplankton	0.99	0.99	0.99	0.98	1	0.98
Benthos	*	*	*	0.99	*	0.99
Overall	0.97	0.98	0.96	0.96	0.97	0.96

* Value could not be estimated on account of absence of the food item in either the hybrid or the parental species.

Conclusion

Indian major carps and their inter-generic hybrids are morphologically very close to each other. However, pure and hybrid fingerlings of Indian major carps could be identified unequivocally using some morphological traits, allozymes, and distinctive differences of feeding behavior. Farmers could apply the developed identifying traits when purchasing the fingerlings from middlemen or vendors to avoid hybrids and thus ensure the selection of pure fingerlings and eventually their sustainable production.

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