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Comparative Study on Amylase Activity in Three Freshwater Fish Larvae during Early Development

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Abstract

An experiment was conducted to quantitatively compare amylase activity during post embryonic developmental period of three bundh-bred Indian major carps, *Labeo rohita* (Ham), *Catla catla* (Ham) and *Cirrhinus mrigala* (Ham). For this purpose, amylase activ-ity in fed and starved fish larvae was measured. In all three species and in larvae starved for 24 h, amylase activity was depressed although significant changes in activity in starved and fed larvae were observed after day 17. The result showed a rhythmic pattern of amy-lase activity during the study period (5-68 days) starting from low activity (5-26 days) accel-erating to a peak (29-56 days) and very low activity thereafter. Changes of enzymatic pat-terns were found to be more prominent in *L. rohita* followed by *C.catla* and *C. mrigala*. The activity of this enzyme was found to be low in *C.mrigala* compared to *L. rohita* and *C.catla*. These changes may be related to different digestive systems and of carps' species specific food habits. The results are discussed in the text.

Introduction

Amylase, a widely distributed enzyme in the plant and animal kingdom was chosen for study (King 1957). Investigations of amylase activity of different fishes in relation to different food habits and preferences to ontogenic development are still limited (Barrington 1957; Burt 1966; Fraisse *et al.* 1981; Hofer 1979a; Hofer and Nasiruddin 1985; Kawai and Ikeda 1971, 1973a,b; Lauff and Hofer 1984; Mc Greachin *et al.* 1960; Moitra and Bhattacharya 1975; Moyano *et al.* 1996; Seshadri 1967; Tanaka *et al.* 1972). Reports on the activity of this enzyme during early developmental period of Indian major carps *L. rohita* (Ham), *C. catla* (Ham) and *C. mrigala* (Ham) are non-existent. The present communication compares the quantitative changes of amylase activity in the early life of three commercially important bundh-bred Indian common cyprinids.

Materials and Methods

Spawnings of *L. rohita*, *C. catla* and *C. mrigala* (average weight of 2.0-2.5 kg) were hormonally induced and the released eggs were artificially fertilized. Three-day-old larvae of *L. rohita* , *C. Catla* and *C. mrigala* were collected from a fish farm in West Bengal. About 5000 fish larvae of these three species were reared in experimental hoopnets (150cm x 140cm x 90cm) with 2 mm mesh size installed in the same pond. Zooplankton in the pond, the preferred principal food of carp larvae (Chakraborty and Kowtal 1973) was counted on a weekly basis using a Sedgewick Rafter counting cell with a thousand squares. Amylase activity in the larvae of three carp species fed with zooplankton available in the pond and in the larvae starved for 24h in aquaria filled with plankton free pond water was assayed. The fish larvae in the hoopnets were treated as a control group (nature fed). The pond water was monitored for temperature, pH and dissolved oxygen, and ranged at 29^0C-31^0C, 6.9-7.2 and 6.8-10.0 ppm respectively. On the following day, 20 samples of each species were collected separately at 10.00h from the glass aquarium (starved larvae) and from the experimental hoopnets (nature fed). The assay of enzyme activities in both fed and starved larvae was conducted at 10.00h every third day starting from the 5th day and continuing up to the 68th day. The surface water of each larva was absorbed through Whatman Filter paper No.1. The length (cm) and weight (mg) were measured. The size range of the fishes *L. rohita*, *C. catla* and *C. mrigala* during the period of study were 0.6-3.6 cm, 0.6-2.3 cm, and 0.6-5.3 cm larvae respectively. The enzymatic assay with pooled samples of five of nature fed and starved larvae were homogenized separately at 4^0C with neutral glass powder and centrifuged immediately at 3500 rpm at 4^0C for 5 min. The supernatant was diluted with normal saline and was used for the enzyme assay.

Assay of enzyme activity

Amylase activity for each species was assayed in triplicate following the method described by Wootton (1974). The activities were expressed in Somogyi Units/100 mg (w/w of fish). Somogyi units are the International unit of enzyme activity and was defined as the amount of enzyme digesting 5 mg of starch under the defined conditions (15 min. incubation at 37^0C).

Zooplankton count

Zooplankton samples were collected weekly from subsurface water of the pond around the hoopnets for a period of two months. Zooplankton samples were collected weekly from different zones of the pond at 0400h (early dawn) by filtering 50L of pond water through plankton nets. The organisms were identified by genera and quantity was expressed as Unit/L of pond water. The range of the zooplankton number varied from 100-142 unit/L during experimental period. Among zooplanktons, cladoceran dominated followed by copepoda and rotifer. The average percentage of zooplankton/L of pond water was *Moina*,

44.4%; *Diaptomous*, 25.3%; *Cyclops*, 11.3%; 'unidentified' *Naupli*, 13.4% and *Keratella*, 5.6%.

Analysis of variance test and the follow-up 'Studentized range test' was performed according to Goldstein (1965).

Results

Amylase activities in both fed and starved larvae of three species during the 5 to 68-day post-hatch period are shown in Table 1. Remarkable changes in amylase activity with increasing age in the larvae of *L. rohita*, *C. catla* and *C. mrigala* during the first 68-day post hatch period are clearly indicated. Amylase activity in all three species gradually increased up to day 18 and then declined to a minimum at day 26. Significant elevation of amylase activity was observed in *L. rohita* and *C. catla* from the 29th to the 56th day. A similar trend in amylase activity changes were also noted in *C. mrigala* and to a lesser extent in the other two species. In all the species, amylase activity of starved larvae was depressed from day 5 to day 68 but significant differences in the activities of fed and starved larvae were observed after day 17 (Fig.1). Overall, amylase activities in the fish larvae of the three species varied from 4.38-29.49 S.U/100mg BW. *C. mrigala* was found to contain a fairly low amount of amylase activity compared to *C. catla* and *L. rohita* (Fig.1).

Differences in amylase activities between fed and starved larvae of all three species were not significant (t-test, $p<0.05$) up to the age group (5-17 days) but significant difference was observed afterwards (Table 1). A significant variation (1% level) among the species and also within age was noted by two-way ANOVA. Follow-up "studentized range test" showed the supremacy of *L.*

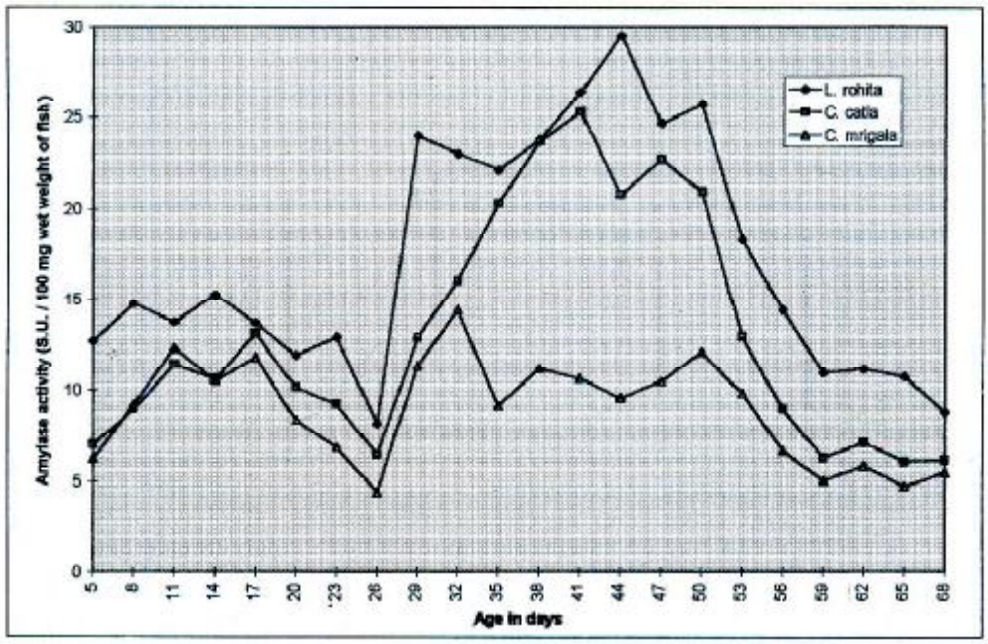

Fig. 1. Comparative results of amylase activities in the fed spawns of *L. rohita*, *C. Catla and C. mrigala*. Each dot represents the average value of three observations on every third day.

Table 1. Amylase activity (S.U/100 mg wet weight of fish) in the fed and starved larvae of *L.rohita*, *C. catla* and *C. mrigala* during early period of development.

Aged in days	*L. rohita*		*C. catla*		*C. mrigala*	
	Fed	Starved	Fed	Starved	Fed	Starved
5	12.72±0.36	12.36±0.08	6.98±0.15	6.66±0.26	6.19±0.24	5.81±0.38
8	14.74±0.51	13.53±0.22	8.95±0.36	7.82±0.41	9.21±0.37	8.90±0.28
11	13.72±0.38	12.90±0.40	11.40±0.34	11.24±0.09	12.32±0.76	11.94±0.17
14	15.24±0.44	14.94±0.36	10.63±0.69	10.26±0.26	10.52±0.61	10.09±0.22
17	13.65±0.29	13.30±0.18	13.10±0.12	12.47±0.24	11.18±0.46	11.05±0.22
20	11.93***±0.20	10.60±0.23	10.14***±0.33	8.63±0.19	8.33***±0.22	7.32±0.18
23	12.92***±0.29	11.06±0.37	9.23***±0.37	7.51±0.24	6.83***±0.12	5.94±0.17
26	8.16*±0.24	4.81±0.21	6.42**±0.24	5.11±0.12	4.38**±0.21	2.72±0.20
29	23.97*±0.19	20.07±0.23	12.88**±0.15	11.41±0.24	11.33***±0.23	9.74±0.26
32	22.98**±0.25	19.30±0.51	16.04**±0.30	13.91±0.17	14.39**±0.39	11.93±0.22
35	22.10*±0.22	18.50±0.28	20.28**±0.25	18.60±0.22	9.11***±0.21	8.12±0.13
38	23.74***±0.49	21.61±0.24	23.64**±0.29	20.85±0.48	11.19**±0.33	9.01±0.17
41	26.38***±0.54	24.09±0.24	25.28*±0.23	22.51±0.12	10.62**±0.26	8.55±0.13
44	29.49**±0.60	26.53±0.20	20.78**±0.29	18.53±0.25	9.56**±0.28	7.89±0.14
47	24.64**±0.53	21.92±0.26	22.66**±0.23	20.99±0.23	10.46**±0.28	8.47±0.10
50	25.71**±0.54	22.61±0.21	20.92*±0.13	17.80±0.22	12.10*±0.11	10.54±0.07
53	18.27**±0.31	15.28±0.39	12.92**±0.39	10.06±0.40	9.80**±0.17	8.32±0.17
56	14.36**±0.28	12.20±0.14	8.94***±0.49	6.85±0.20	6.63*±0.20	4.45±0.05
59	10.95**±0.17	9.17±0.16	6.24**±0.17	4.42±0.17	5.01**±0.08	4.53±0.06
62	11.14*±0.13	9.47±0.09	7.06*±0.11	4.60±0.12	5.78***±0.44	3.96±0.07
65	10.73***±0.36	8.89±0.11	6.01*±0.09	4.26±0.14	4.69**±0.17	3.65±0.08
68	8.76*±0.20	6.59±0.11	6.10**±0.22	3.34±0.27	5.44**±0.24	3.40±0.22

*P<0.001
**P<0.01
***P<0.025.
Each value represents mean ± S.E. (N = 3).

rohita over the other species (Table 1). The mean amylase activity of *L. rohita* differs from that of both *C. catla* and *C. mrigala* at 1% level (Table 2).

Discussion

Three stanzas or stages with respect to the amylase acitity correlated with their natural food and feeding habits were observed during development up to the 68-day post-hatch period.

Stage 1: A low level of amylase activity in the fish larvae in the 5-26 day age group. Amylase activity was found to be high at the earliest embryonic stage of *Erimyzon sucette* and *Lepomis cyanellus* with a subsequent decline

Table 2. Two-way analysis of variance test to find out the significance of variation within species and also within their ages and the follow-up Studentized range test to compare the degree of superiority of species mean in terms of amylase activity.

Source of variation	Sum of squares	Degree of freedom	Mean squares	F value	Minimum significance range (K) at 1 % level
Within species	2225.85	2	1112.92	3273.29*	
Within age	4597.01	21	218.90	643.82*	
Species x age (interaction)	1182.03	42	28.14	82.76*	
Error	44.99	132	0.34		0.53
Comparative enzymatic response			*L. rohita* 17.10	*C. catla* 13.03	*C. mrigala* 8.89
Total	8049.88	197			

*Significant at 1 % level

(Champion *et al.* 1975). Das (1965) observed a linear trend of amylase activity with age in the serum of adult *C. catla*. During metamorphosis, larvae of rainbow trout, *Coregonus* and *Roach* experienced several changes of enzyme activity with age (Lauff and Hofer 1984). This has been attributed to low enzyme production due to the non-functioning of digestive tract activity in the early larval stages of *Coregonus* or due to the short and simple intestinal tract of the fish larvae during early developments, which allows the larvae to feed on an animal diet composed mainly of zooplankton (Dabrowski 1979; Hofer and Nasiruddin 1985; Stroband and Dabrowski 1979).

Stage 2: A high level of enzyme activity occurred from day 29-56. Shaklee *et al.* (1974) reported low amylase activity at 240h after fertilization of the teleost *E. sucette*. Subsequently, a sharp increase in activity was observed at about 280h of development followed by highly persistent amylase activity up to 480h after the post-embryonic stages of development of the species. Shaklee *et al.* (1974) assumed the rapid changes of enzyme activity in *E. sucette* as the first attempt of the fry to derive energy from exogenous food sources. Similar drastic increases in enzyme activity during larval development have also been observed in different fishes (Kawai and Ikeda 1973a, b; Lauff and Hofer 1984; Tanaka *et al.* 1972). The fishes change slowly to their species-specific food habits from their normal zooplankton diet, as the differentiation of the digestive tract progressed. Michael and Banerjee (1995) also found low amylase activity in the digestive tract of *Aristichthys nobilis* (Richardson) in their early stages and highly persistent amylase activity in advanced stages (above 10 cm) of the species. Rae (1967) also reported that a change in diet brought about changes in different enzymes in the digestive tract of *Gadus morhua*. Under natural conditions, the *Roach* (but not the *Rudd*) experienced a higher or lower amylolytic activity subject to feeding on animals or detritus (Hofer 1979a). The high amylase activity in the gut of different fish species (*C. punctatus* and carps) and also in sea bass larvae feeding on a carbohydrate-rich diet suggest extensive amylase synthesis (Peres *et al.* 1996) or exogenous feeding associated

with the development of a digestive tract. Decreased a-amylase activity at early stage of predatory pike and fluctuating activity with age in benthophages (bream and roach) has also been reported by Kuz'mina (1996). The activity of this enzyme in the gut of *C. mrigala* may be correlated with diet and is subject to adaptation as a result of changes in food habits during the different developmental stages, i.e., fry stage (carnivorous), fingerlings (omnivorous) and adult (harbivorous) (Sinha 1978). The low enzyme activity during the first stage of development (5-26 days) of the fish larvae may be correlated with a carnivorous feeding habit and the under-developed gut of the species. However, the fish larvae generally fed on zooplankton during the early developmental period (Chakraborty and Kowtal 1973). These groups of fishes take animal food with a minor proportion of phytoplankton in their fry stage (Mukherjee 1944). With respect to their feeding habits, Das and Moitra (1955) categorized *C. catla* as a 'surface feeder,' *L. rohita* as a 'mid-layer feeder' and *C. mrigala* as a 'bottom feeder,' with all being omnivorous. Thus, it may be possible that the high species-specific amylase activity in the fish larvae during development (29-56 days) is correlated with diet and is subject to adaptation because of changes in food habit (Mukherjee 1944) as well as differentiation of the digestive gut in different life-cycle stages. The reduction in amylase activity in all the starved larvae of the species may have resulted from decreased endogenous enzyme production due to starvation.

Stage 3: Fluctuating and very low enzyme activity from day 59 onwards indicate the rapid utilization of enzyme to maintain the normal digestive process associated with the functional maturation of the digestive system.It is difficult to interpret from this preliminary investigation the reason for species-specific quantitative variation in enzyme activity in the fish larvae during development. But it may be suggested that the overall low enzyme activity in *C. mrigala* (bottom feeder) compared to other species may be due to increased intake of detritus as well as low carbohydrate content in the diet as observed in the omnivorous species, *Roach* (Hofer 1979a). Though *L. rohita* is herbivorous and carnivorous, we presume that its higher amylase activity throughout the experimental period (day 5-68) may be a result of its preference for carbohydrate-rich food as it increases in age (Mukherjee 1944). The overall medium level of enzyme activity observed in *C. catla*, a surface feeder, might be related to its equal preference for zooplankton and phytoplankton. The ontogenic changes in amylase activity is genetically determined and to some extent reflect the food availability at that time.

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