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Diet Composition and Digestive Enzymes Activity in Carnivorous Fishes Inhabiting Mudflats of Indian Sundarban Estuaries

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Abstract

Intertidal mudflats occupy a significant component of the total estuarine habitat available to fishes as nursery and foraging grounds. In this study, fifteen sites were randomly explored along three estuarine rivers in Indian Sundarbans and 27 fish species, were recorded. Upon analysis of prey preferences, they were categorized into different trophic types. A comparative study of the digestive physiology of 10 carnivorous species as functional analogues was carried out in order to find out relationship between digestive enzyme activity and trophic niche segregation among them. Rarefaction curves for stomach content analysis indicated diverse nature of prey preferences among different species. A dendrogram based on prey diversity was constructed through cluster analysis. Another dendrogram was constructed based on enzymes (i.e. α-amylase, invertase, cellulose, alkaline protease and pepsin) which were measured from liver, stomach and intestine of ten carnivorous species. A comparison of the two dendrograms did not reflect any positive relationship between prey preferences and digestive enzymes. It was, therefore, concluded that enzyme patterns were more affected by phylogeny rather than adaptability. No clear predominance among digestive enzymes was observed in relation to food, suggesting that the organic matter of animal origin was utilized non-selectively by these fishes since the quality and variety of available food were subjected to change and over time in such a dynamic environment.

Keywords: Stomach content, teleosts, amylolytic enzymes, proteolytic enzymes, dendrogram.

Introduction

Studies of resource requirements by various species have been used in attempts to understand factors controlling the distribution and abundance of organisms (Ross, 1986). In addition, studies on food habits of organisms utilizing each habitat help to illustrate the role of the latter in the ecology of several organisms. Therefore, food resources have received by far the most attention (Simberloff and Dayan, 1991); many studies on feeding ecology having been conducted for different fish communities (Pausey *et al*., 1995; Piet *et al*., 1999; Garrison and Link, 2000).

Tidal mudflats occupy a significant component of the total estuarine habitat available to fishes and play important roles as nursery and foraging grounds (Edgar and Shaw, 1995; Horinouchi and Sano, 2000). Few studies on the feeding habits of each species within such assemblages have been conducted, although most have been made in temperate regions (Edgar and Shaw, 1995; Horinouchi *et al*., 1996).

The Sundarban (India) mudflats (Banerjee, 1998; Bose 2004) are found at the estuary and on the deltaic islands where low velocity of river and tidal current occurs. The flats are exposed in low tides and submerged in high tides, thus being changed morphologically even in one tidal cycle. The interior parts of the mudflats are magnificent home of luxuriant mangroves. The Sundarban mudflats control the food chain in the estuarine ecosystem.

The biodiversity associated with a diverse and dynamic environment makes the study of feeding habits of fishes from the mudflats of Sundarbans unique, since the environmental changes require continuous adjustments at all levels of the biological organization (Val and Almeida-Val, 1995; López-Vásquez *et al*., 2009). These adjustments undoubtedly affect how fishes acquire their food as well as how they metabolize them. Most vertebrates, including fishes, possess digestive enzymes that allow them to digest the food that they consume, but variation exists among species in the activity of individual enzymes (Chakrabarti *et al*., 1995; Kuźmina, 1996a; Alarcón *et al*., 1998). Digestive enzymes, however, may be a complementary tool useful for determining which dietary components are most effectively metabolized

(Brêthes *et al*., 1994). By understanding the digestion and assimilation of specific dietary components, the type of prey that the animals prefer and those that they are best equipped to digest could be identified. In fact, carnivorous fish influence and are influenced by the behavior and abundance of their fish and invertebrate prey species (Hobson and Chess, 1986; Laprise and Blaber, 1992; Sackley and Kaufman, 1996; Silvano, 2001).

The present study investigates the digestive physiology of ten species of fish from the inundated mudflat habitats of Sundarbans with similar nutritional habits (categorized as carnivores). This study is aimed at (1) determining dietary preferences for each of the fish species, using stomach content analysis, and (2) quantifying the activities of a range of digestive enzymes in each fish species to determine the utilization of various food sources available to the fishes.

Materials and Methods

Study Site and Fish Samples Collection

Fifteen study sites were selected randomly along the adjacent mudflats of Matla river, Bidya river and Boro Herobhanga rivulet in Sundarban (22°10′N, 88°40′E) on the Indian territory (Figure 1). Adult fishes belonging to 27 species under 9 orders were collected during high tide with gill nets of 20 m length with 1 cm spacing between adjacent knots and during low tide by hand net. The specimens were retrieved from the net, identified (Day, 1958; Talwar and Jhingran, 1991) and measured for total length (L_T, cm) and weighed for total mass (M, g) (Table 1).

Stomach Content Verification

The fishes were anaesthetized with MS222 (15 specimens per each species) and each stomach was visually assessed for fullness (1=empty, 2=25%, 3=50%, 4=75%, 5=100% full), and those with a score of 3 to 5 were dissected. The contents of the stomach were collected separately in 70% ethanol and observed under microscope. Prey items were identified to the lowest possible taxon and each individual item was counted.

Categorization of Carnivorous Fishes and Stomach Content Analysis

Amongst 27 species, 10 teleosts were categorized into carnivorous habit, since more than 50% of the stomach contents were animal prey items (Figure 2). The carnivorous teleosts were: *Ophisternon bengalense* McClelland 1844, *Uroconger lepturus* Richardson 1845, *Congresox telabon* Cuvier 1829, *Terapon jarbua* Forsskål 1775, *Pisodonophis boro* Hamilton 1822, *Trichiurus gangeticus* Gupta 1966, *Muraenesox bagio* Hamilton 1822, *Scatophagus argus* Linnaeus 1766, *Pseudapocryptes elongates* Cuvier 1816 and *Butis butis* Hamilton 1822 (two orders and six families). To measure the trophic diversity, rarefaction curves (Hurlbert, 1971) were used for the prey populations predated by 10 carnivorous fishes. The total number of food items consumed by each stage gives the richness of the prey consumed. Rarefaction is given by the calculation of *E(S)* for a sequence of *n*,

$$E(S) = \sum 1 - \left[\left(\frac{N - N_i}{n} \right) / \left(\frac{N}{n} \right) \right],$$

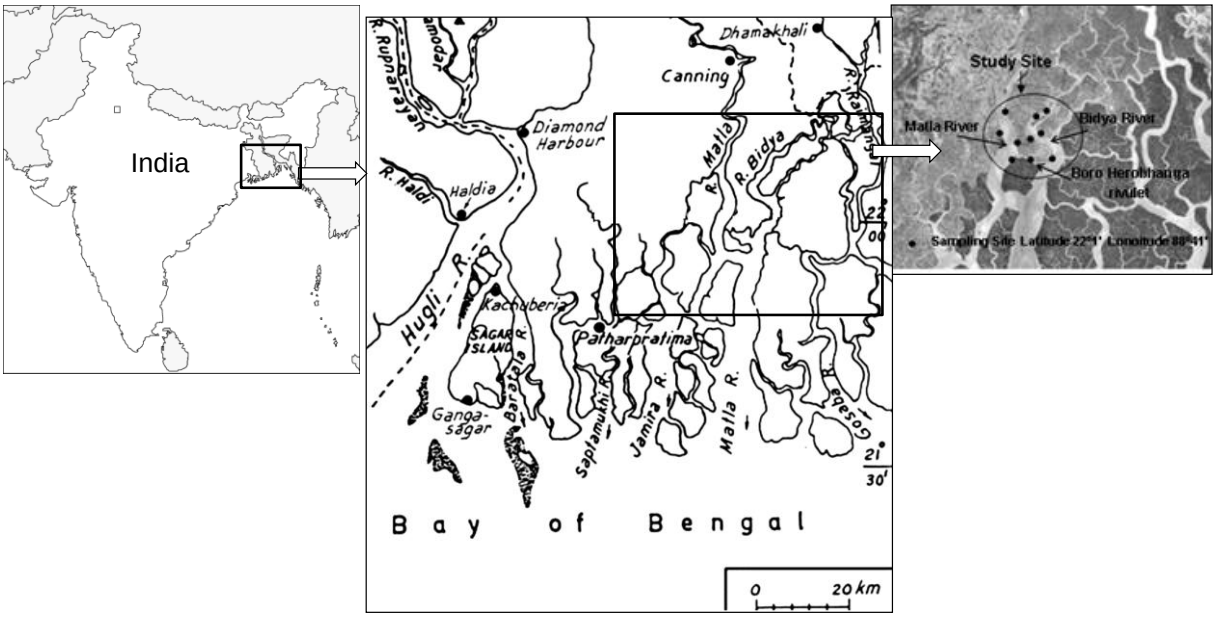

Figure 1. Location of study area in Sundarbans. India. Inset: Location of sampling sites in the mudflats of Matla river, Bidya river and Boro Herobhanga rivulet.

Table 1. Fish species analyzed with mean±SE body mass (M) and total length (L_T) (n = 15)

	Scientific name	Order	Family	M (g)	L_T (mm)
1	*Ophisternon bengalense* (McClelland 1844)	Synbranchiformes	Synbranchidae	2500.0± 12.55	970.00±7.35
2	*Uroconger lepturus* (Richardson 1845)	Anguilliformes	Congridae	69.0±5.71	360.0±5.50
3	*Congresox telabon* (Cuvier 1829)	Anguilliformes	Muraenesocidae	208.0±7.23	570.40±2.08
4	*Muraenesox bagio* (Hamilton 1822)	Anguilliformes	Muraenesocidae	2805.0± 14.05	600.70±3.09
5	*Pisodonophis boro* (Hamilton 1822)	Anguilliformes	Muraenesocidae	55.5±3.17	380.50±2.56
6	*Strongylura strongylura* (van Hasselt 1823)	Beloniformes	Belonidae	65.9±7.44	320.50±0.50
7	*Hyporhamphus limbatus* (Valenciennes 1847)	Beloniformes	Hemiramphidae	70.8±9.20	100.90±0.72
8	*Gudusia chapra* (Hamilton 1822)	Clupeiformes	Clupeidae	14.2±2.62	100.10±1.86
9	*Setipinna taty* (Valenciennes 1848)	Clupeiformes	Engraulidae	13.3±2.07	140.90±3.52
10	*Bregmaceros mcclellandi* (Thompson 1840)	Gadiformes	Bregmacerotidae	2.1±0.05	80.05±0.05
11	*Liza parsia* (Hamilton 1822)	Mugiliformes	Mugilidae	12.5±3.33	150.50±0.55
12	*Uropterygius marmoratus* (Lacepède 1803)	Muraenidae	Anguilliformes	677.6±10.20	470.70±5.10
13	*Butis butis* (Hamilton 1822)	Perciformes	Eleotridae	17.3±3.16	130.20±1.10
14	*Boleophthalmus boddarti* (Pallas 1770)	Perciformes	Gobiidae	11.5±2.07	110.76±2.22
15	*Odontamblyopus rubicundus* (Hamilton 1822)	Perciformes	Gobiidae	5.6±1.11	120.90±2.78
16	*Periophthalmus novemradiatus* (Hamilton 1822)	Perciformes	Gobiidae	1.6±0.67	50.51±0.17
17	*Pseudapocryptes elongates* (Cuvier 1816)	Perciformes	Gobiidae	11.3±2.85	120.61±2.78
18	*Trypauchen vagina* (Bloch & Schneider 1801)	Perciformes	Gobiidae	8.6±1.74	140.80±0.50
19	*Taenioides anguillaris* (Linnaeus 1758)	Perciformes	Gobiidae	9.4±2.95	170.01±1.08
20	*Scatophagus argus* (Linnaeus 1766)	Perciformes	Scatophagidae	29.0±2.58	150.60±3.93
21	*Sillaginopsis panijus* (Hamilton 1822)	Perciformes	Sillaginidae	126.5±8.15	220.56±2.50
22	*Terapon jarbua* (Forsskål 1775)	Perciformes	Terapontidae	26.7±2.25	90.40±0.65
23	*Toxotes chatareus* (Hamilton 1822)	Perciformes	Toxotidae	135.1±6.47	190.79±2.33
24	*Trichiurus gangeticus* (Gupta 1966)	Perciformes	Trichiuridae	18.0±3.19	420.75±4.25
25	*Cynoglossus lingua* (Hamilton 1822)	Pleuronectiformes	Cynoglossidae	12.5±2.81	280.42±2.15
26	*Mystus gulio* (Hamilton 1822)	Siluriformes	Bagridae	66.4±4.12	200.07±3.12
27	*Cephalocassis jatia* (Hamilton 1822)	Siluriformes	Ariidae	71.3±2.58	220.50±2.58

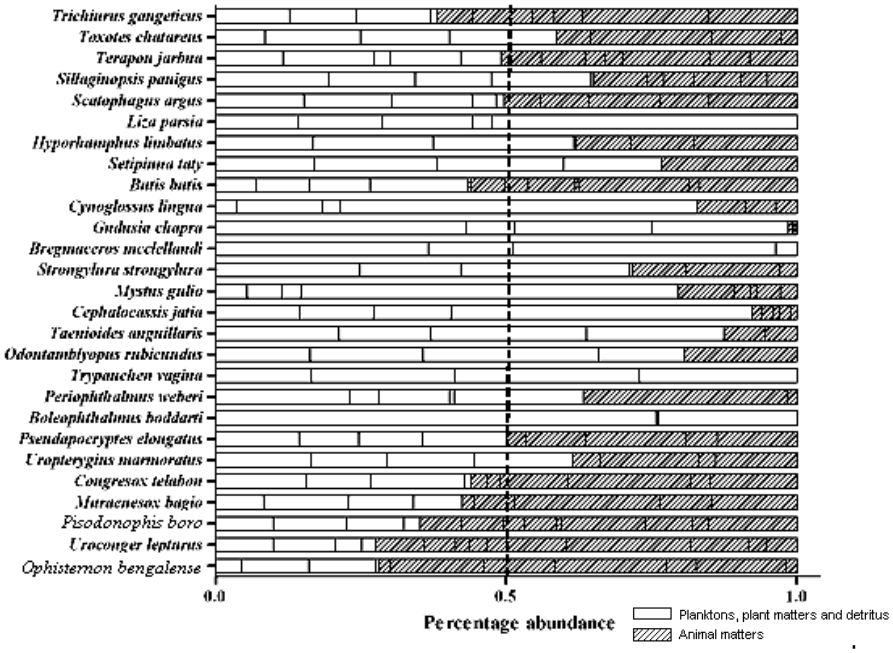

Figure 2. Percentage abundance of seventeen prey items among stomachs of twenty seven teleost species in intertidal mudflats of Sundarbans.

where *E(S)* = expected richness in the rarefacted sample with a given *n*, *n* = standard size of the sample, *N* = total number of quotations of each kind of food, and N_i = number of meals with the i^{th} food item. The computation was performed using Estimates software.

Digestive Enzyme Analysis

After collection of the stomach content, liver, stomach and intestine of ten carnivorous fishes previously anaesthetized, were dissected out, weighed, kept in liquid nitrogen during transportation to the laboratory and frozen at -70°C until assay of the enzymes.

The preparation of tissue extracts was carried out at 4°C. The digestive organs of each fish (liver, stomach and intestine) were thoroughly washed with chilled glass-distilled water and homogenized in 0.02 M phosphate buffer pH 7.0 (1:5 w/v) for 3 min at 5500 G, 4°C. Tissue homogenates were centrifuged in a Hermule Z323K refrigerated centrifuge at 10,000 G for 25 min at 4°C. The supernatant was separated and preserved for enzyme assays. The soluble protein content of each extract was determined against bovine serum albumin as reference (Lowry *et al*., 1951). Five digestive enzymes were assayed at the optimum temperature in all the samples.

α-Amylase activity was assayed as Bernfeld (1955), using starch (1%) [Sigma, U.S.A.] as substrate, phosphate (Na_2HPO_4 + NaH_2PO_4) buffer (pH 6.9) and maltose as standard. One unity (U) of amylase was defined as the amount of enzyme needed to hydrolyze 1 mg of starch per min at 37°C. The amylase activity was expressed per mg of protein. Cellulase activity was determined following Kesler and Tulou (1980) using carboxy-methyl-cellulose (1%) [Sigma, U.S.A.] as substrate, phosphate buffer (pH 5.5) and glucose as standard. A unit of cellulase was defined as the amount of enzyme needed to hydrolyze 1 mg 1% CMC per min at 37°C. Invertase activity was estimated following Pal *et al*. (1980**)** using (2.5%) sucrose [Sigma, U.S.A.] as substrate, phosphate buffer (pH 5.5) and glucose as a standard (Bacon, 1955). A unit of invertase was defined as the amount of the enzyme needed to hydrolyze 1 mg of substrate per min at 37°C. Alkaline protease was measured following Ichishima (1970) using (1%) bovine serum albumin [Sigma, U.S.A] as substrate (pH 10.0). One unit of alkaline protease activity was calculated as the amount of enzyme needed to hydrolyze 1 mg BSA per min at 37°C. Pepsin was measured following Ragyanszky (1980) using casein (1%) [Sigma, U.S.A.] as substrate at pH 1.5 using 60 mM HCL. For alkaline proteases as well as for pepsin, tyrosine was used as standard. Enzyme assays were performed with a Shimadzu UV-1700 PharmaSpec, UV/visible spectrophotometer. Activity of all enzymes was expressed in units per mg of protein (U mg^{-1} protein).

Statistical Analysis

Multivariate Analysis of Variance (MANOVA) (Zar, 1999) was applied using SPSS 7.0. The mean value of fifteen repetitions of each enzyme from each tissue evaluated for each fish species was used to interpret the variations among the species. The homogeneity between mean values of the different fish species was tested using Post Hoc Duncan test; values were considered statistically different at the $P<0{\cdot}05$ level. Results are reported as means ± SE. Dendograms were constructed for hierarchical cluster analysis among the carnivorous teleosts for stomach contents as well as for digestive enzymes using Ward method and Euclidean distance (SPSS 7.5).

Results

Stomach Contents Analysis

Seventeen different prey categories were recorded in the stomach of 27 species apart from some unidentified material and faecal pellets. About eight prey species were found per stomach as majority of the individuals had a more diverse diet (>5 prey types consumed).

As mentioned earlier, ten species were found to be carnivorous based on prevalence of animal matter (>50%) in their stomach content. The trophic diversity of these ten carnivorous fish species was reflected by the rarefaction curves for stomach content analysis, (Figure 3) which indicated differences in prey diversity. Stomach content was most varied (14 prey species) in *U. lepturus* and *Pi. boro* and least diverse (9 prey species) in *M. bagio* and *Ps. elongatus*.

Among all prey items, decapods crabs, decapods shrimps and juvenile fishes were found to be common and consisting the major portions of the stomachs irrespective of species. Gastropods were found frequently in the stomachs of *M. bagio, Te. jarbua* and a little in case of *Pi. boro, U. lepturus* and *O. bengalense*. The stomach contents of *S. argus*, *U. lepturus* and *O. bengalense* were also contributed by decapods crabs prominently along with the other food items. Ophidian group was only found in the stomachs of *O. bengalense* (Table 2).

Dendogram of 10 carnivorous fish species on the basis of their stomach contents showed a clustering between *B. butis, Ps. elongatus* and *M. bagio*, on the other hand *C. telabon*, *Tr. gangeticus*, *Pi. boro* and *O. bengalense* formed another cluster if 0.1 Square Euclidean distance was considered (Figure 4).

Digestive Enzymes

Negligible α-amylase activity was recorded from the digestive organs of *U. lepturus*, *Te. jarbua*, *M. bagio*, *O. bengalense*, *C. telabon*, *Pi. boro* and *Tr. gangeticus*. Alfa-amylase activity was significantly high ($P<0.05$, df = 14) in *Ps. elongatus* (Figure 5a). Most of the fish presented moderate cellulase activity in the gut and higher enzyme activity in liver. In *Te. jarbua*, the liver showed maximum cellulase activity compared to other fishes (Figure 5b). *B. butis* exhibited maximum ($P<0.05$, *df* = 14) invertase activity in gut. *U. lepturus* exhibits lowest invertase activity irrespective of digestive organs (Figure 5c). Alkaline protease activity was at maximum levels in *O. bengalense* and *Te. jarbua*. In *B. butis* (all three tissues) minimum activity of alkaline protease was found (Figure 5d). Maximum and minimum pepsin activity was recorded in the stomach of *Pi. boro* and *B. butis* respectively (Figure 5e) (Table 3).

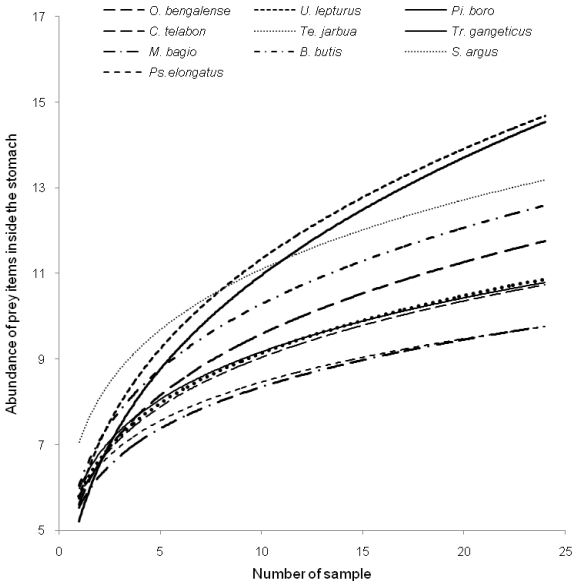

Figure 3. Rarefaction curve of ten carnivorous fishes showing the abundance of prey items inside their stomachs.

Table 2. Percentage abundance of 17 prey items among stomachs of selected ten carnivorous teleost species of inundated mudflats of Indian Sundarbans

	Species	Different prey items																
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	*O. bengalense*																	
2	*U. lepturus*																	
3	*C. telabon*																	
4	*Te. jarbua*																	
5	*Pi. boro*																	
6	*Tr. gangeticus*																	
7	*M. bagio*																	
8	*B. butis*																	
9	*S. argus*																	
10	*Ps. elongatus*																	

0% | **0.1 - 10%** | **10.1 - 20%** | **20.1 - 30%**

Note: 1. Phytoplankton, 2. Copepod zooplankton, 3. Cladoceran zooplankton, 4. Macroalgae, 5. Cnidarians, 6. Amphipods, 7. Polychaete, 8. Oligochaetes, 9. Aquatic insects, 10. Decapod crabs, 11. Decapod shrimps, 12. Isopods, 13. Gastropods, 14. Bivalves, 15. Teleosts, 16. Ophidia, 17. Detritus

Dendogram of 10 carnivorous fish species on the basis of their digestive enzymes showed a single clustering between *C. telabon*, *M. bagio*, *Tr. gangeticus*, *S. argus*, *Te. jarbua*, *U. lepturus*, *B. butis*, and *O. bengalense* when 0.01 Square Euclidean distance was considered (Figure 4).

Discussion

The dietary preference of ten carnivorous species of fish was investigated to determine which dietary components were most likely being assimilated. Although fish did not always occupy separate ecological niche with regard to their food, there might be some kind of preferences or affinity based on which the food habit of fishes could be designated. Prey selectivity of predator fishes was controlled by the apparent size, number and type of prey item consumed (Luo *et al*., 1996; Reiss *et al*., 2002). In this study, *U. lepturus* and *Pi. boro* exhibited more diverse prey preference in comparison to others. The selectivity, however, might change with the prey concentration, distribution and abundance in predictable or food-rich environments (Munk, 1997).

Digestive enzyme activities had been an effective tool for identifying particular components of

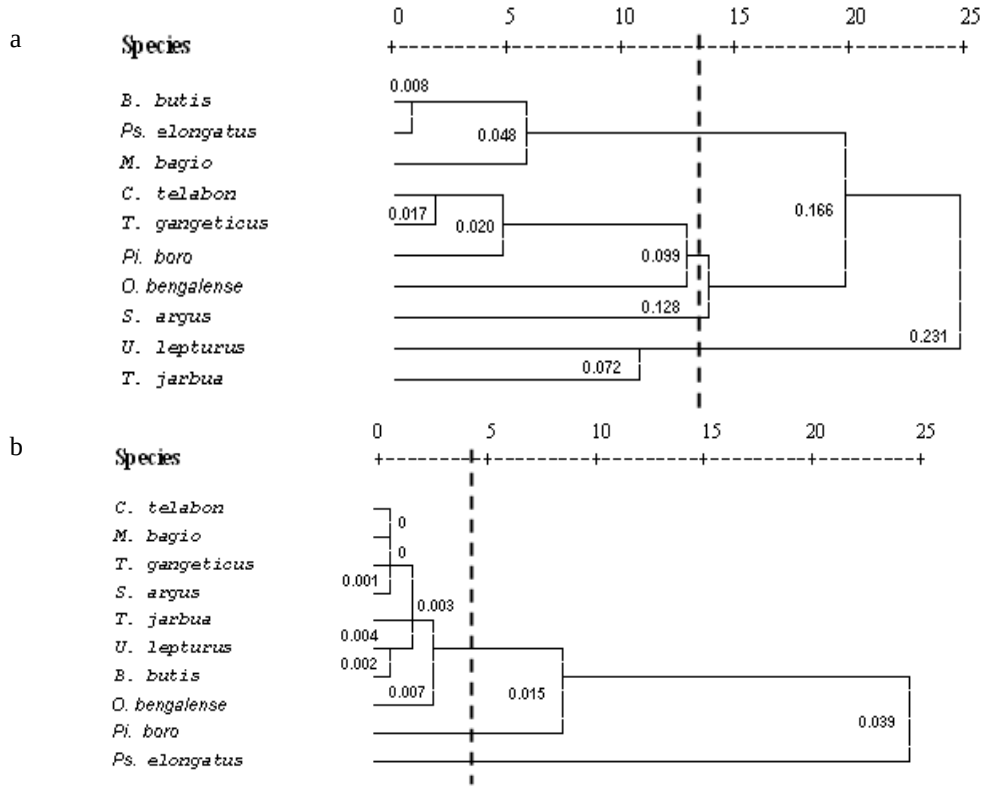

Figure 4. Dendogram of ten carnivorous fishes on the basis of their stomach content (a) and digestive enzymes (b).

an animal's diet (van der Veer, 1986; Kanou *et al*., 2000). Digestive processes in fish aren't well known as in mammals, although the data obtained in fish so far show that the digestive enzymes studied are qualitatively similar to those observed in other vertebrates. Fish may adapt their metabolic functions to the dietary substrates, through a regulation in enzyme secretion, in order to improve the utilization of feed ingredients (Caruso *et al*., 2009). A comparative study of the activity of digestive proteolytic enzymes and amylase can reveal the capacity of different species to use protein and carbohydrates (Hidalgo *et al*., 1999). Chan *et al*. (2004) mentioned that the activity of α-amylase follows a pattern influenced more by phylogeny than by diet in prickleback fishes. On contrary, Fernandez *et al*. (2001) pointed out that the adaptations of the digestive system of different species exhibit closer correlation with their diet rather than on their taxonomic category. This view was also confirmed by the results of Kuźmina (1996) who indicated that changes in digestive enzyme activity could be affected by feeding behaviour and biochemical composition of food.

Most reports on α-amylase in fishes conclude that herbivorous or omnivorous fishes have higher α-amylase activities than carnivorous fishes (Kapoor *et al*., 1975; Sabapathy and Teo, 1993; Hidalgo *et al*., 1999; Fernandez *et al*., 2001; Chan *et al*., 2004; Drewe *et al*., 2004; Horn *et al*., 2006). In the present study, significantly low levels of α-amylase, cellulose and invertase activities were detected in the digestive tract in *U. lepturus* (87.7% animal matter in stomach) and *Pi. boro* (83.4% animal matter in stomach) compared to the other carnivorous fishes studied, indicating that these fishes had a lesser ability to utilize carbohydrates. Munilla-Morán and Saborido-Rey (1996) noted that digestion of carbohydrates was at low rates in three carnivorous fish species, and α-amylase was not considered fundamental in their digestive processes. On the other hand all the three carbohydrases studied showed significantly higher activity in *S. argus* that had comparatively higher plant matter in stomach (19.3%) and in *Ps. elongatus* (14.4% of both plant matter and detritus in stomach) and *B. butis* (6.9% of plant matter and 16.8% of detritus in stomach).

It had earlier been reported (López-Vásquez *et al*., 2009) that carbohydrases and proteolytic activities were higher in the detritivores compared to the omnivorous and carnivorous fishes.This view is supportive of the enzyme pattern obtained in *Ps. elongatus* from the current study. In general, detritivorous fishes consume large amounts of coarse vegetable detritus in the form of fine amorphous material of undetermined origin. Much of the fine particulate organic matter taken up by detritivorous fishes is derived from algae, even in systems in which aquatic macrophytes dominate aquatic primary production (Winemiller and Jepsen, 1998). Higher digestive enzyme activity in detritivorous fishes is an adaptation to extract high nutrient levels from

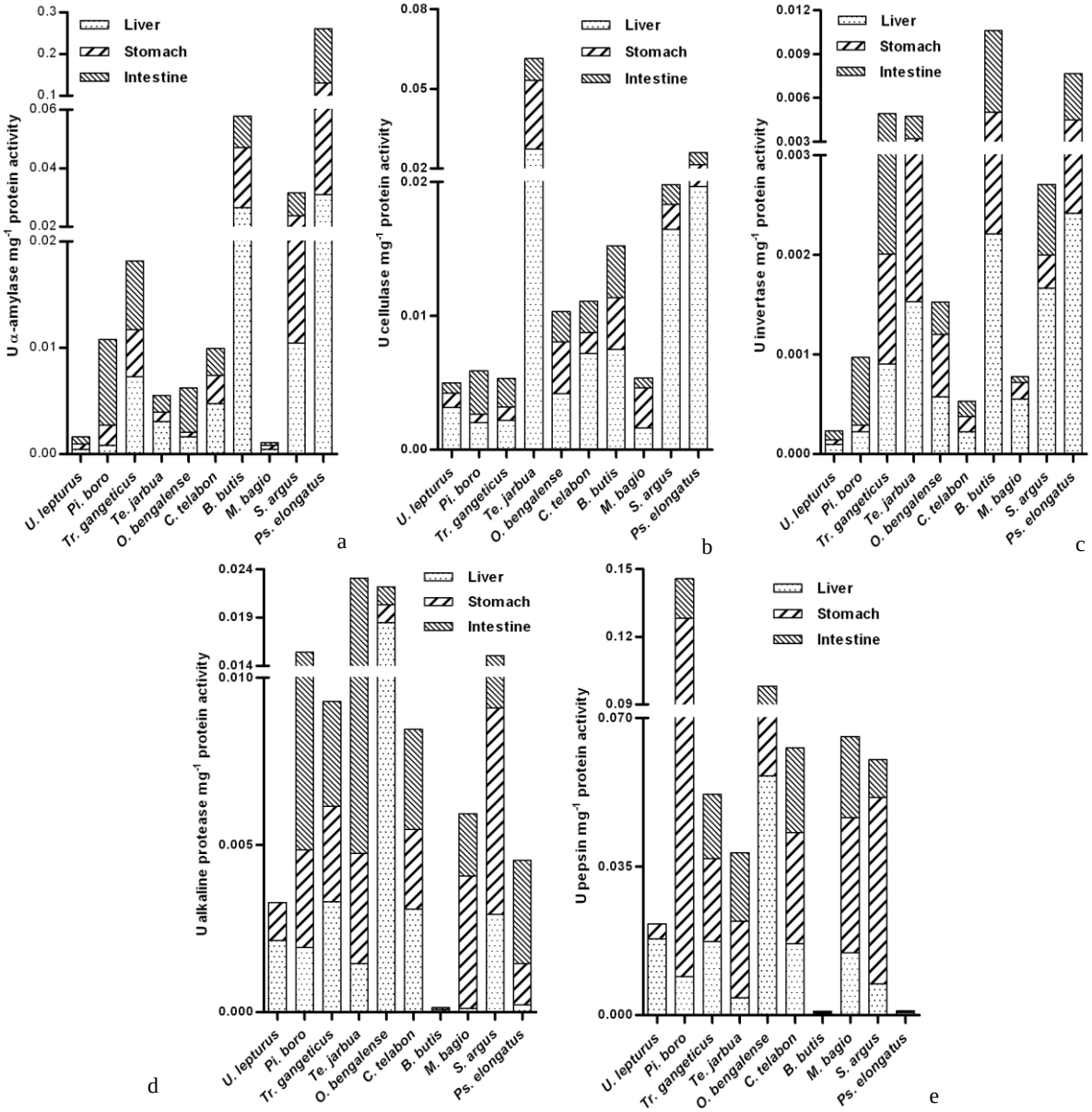

Figure 5. α-amylase (a), cellulase (b), invertase (c), alkaline protease (d) and pepsin (e) activity in seven carnivorous fishes of inundated estuarine mudflats of Sundarbans.

detritus, which represents a poor nutrient source. This adaptation may be species specific and be used extensively by fishes to survive specific environmental conditions. Contrastingly, in spite of having higher proportion of detritus in the stomach, *B. butis* showed the lowest activity of proteolytic enzymes (alkaline proteases and pepsin) in this study.

In fishes, protein is digested initially in the stomach by pepsin and acid, and then further degraded into smaller peptides and free amino acids in the intestine by the combined actions of various alkaline proteases (Hirji and Courtney, 1982). It has been reported that carnivorous fish species possess higher protease activities than herbivorous and omnivorous species (Kapoor *et al*., 1975; Sabapathy and Teo, 1993). In the present study, highest activity of pepsin was observed in *Pi. boro* stomach (animal prey in stomach: 87.4%) followed by *O. bengalense* (liver and stomach) (animal prey in stomach: 94.9%). Pepsin is probably responsible for the earliest stage of protein digestion in breaking down large-chain polypeptides chains in the stomach with the help of secreted hydrochloric acid (Tengjaroenkul *et al*., 2000; Natalia *et al*., 2004). Species such as those of Tilapia with thin stomach walls require a highly acidic medium to enable biochemical digestion of protein compared with those with muscular stomachs such as African catfish, which rely more on the mechanical breakdown of food or chyme and secrete less pepsin (Maier and Tullis, 1984; Uys and Hecht, 1987). The activity of alkaline protease was maximum in *O. bengalense* and *T. jarbua* followed by *Pi. boro* and *S. argus*. The alkaline protease activity was significantly higher in *T. jarbua* though it

Table 3. Digestive enzymes activities in teleost fish speices from the mudflats of Indian Sundarbans

	α-Amylase			Cellulase			Invertase			Alkaline Protease			Pepsin		
	L	S	I	L	S	I	L	S	I	L	S	I	L	S	I
U. lepturus	0.0004^{a} ± 6.3E-06	0.0005^{a} ± 3.8E-05	0.0007^{a} ± 4.9E-05	0.0032^{a} ± 2.4E-04	0.0011^{a} ± 1.0E-04	0.0008^{a} ± 1.2E-04	0.0001^{a} ± 9.9E-06	0.0001^{a} ± 5.5E-06	0.0001^{a} ± 2.2E-05	0.0021^{ab} ± 1.3E-04	0.0011^{ab} ± 1.2E-04	0.0000^{ab} ± 0.0E+00	0.0179^{ab} ± 1.0E-03	0.0035^{ab} ± 6.6E-04	0.0000^{ab} ± 0.0E+00
Pi. boro	0.0008^{ab} ± 9.6E-05	0.0019^{ab} ± 5.2E-04	0.0081^{ab} ± 2.7E-03	0.0020^{a} ± 2.0E-04	0.0006^{a} ± 1.1E-04	0.0032^{a} ± 1.3E-03	0.0002^{ab} ± 5.0E-05	0.0001^{ab} ± 1.3E-05	0.0007^{ab} ± 2.8E-04	0.0019^{c} ± 2.9E-04	0.0029^{c} ± 5.5E-04	0.0106^{c} ± 3.1E-03	0.0091^{e} ± 2.0E-03	0.1192^{e} ± 2.6E-02	0.0176^{e} ± 5.9E-03
T. gangeticus	0.0073^{b} ± 9.5E-04	0.0044^{b} ± 6.8E-04	0.0065^{b} ± 8.1E-04	0.0022^{a} ± 3.1E-04	0.0010^{a} ± 1.2E-04	0.0021^{a} ± 2.7E-04	0.0009^{d} ± 1.2E-04	0.0011^{d} ± 1.7E-04	0.0029^{d} ± 3.6E-04	0.0033^{bc} ± 9.3E-04	0.0029^{bc} ± 6.6E-04	0.0031^{bc} ± 4.1E-04	0.0174^{bc} ± 5.2E-03	0.0195^{bc} ± 4.9E-03	0.0151^{bc} ± 4.8E-03
T. jarbua	0.0030^{ab} ± 3.5E-04	0.0009^{ab} ± 9.1E-05	0.0016^{ab} ± 2.0E-04	0.0274^{d} ± 4.5E-03	0.0258^{d} ± 9.1E-03	0.0082^{d} ± 2.3E-03	0.0015^{d} ± 2.4E-04	0.0017^{d} ± 2.9E-04	0.0016^{d} ± 3.3E-04	0.0015^{d} ± 2.9E-04	0.0033^{d} ± 8.4E-04	0.0183^{d} ± 2.5E-03	0.0041^{bc} ± 8.2E-04	0.0181^{bc} ± 4.0E-03	0.0161^{bc} ± 2.6E-03
O. bengalense	0.0016^{ab} ± 1.4E-04	0.0005^{ab} ± 5.0E-05	0.0042^{ab} ± 8.2E-04	0.0042^{ab} ± 3.3E-04	0.0039^{ab} ± 5.5E-04	0.0023^{ab} ± 3.5E-04	0.0006^{b} ± 9.1E-05	0.0006^{b} ± 1.1E-04	0.0003^{b} ± 6.2E-05	0.0185^{d} ± 4.5E-03	0.0019^{d} ± 2.7E-04	0.0019^{d} ± 2.5E-04	0.0563^{d} ± 1.3E-02	0.0327^{d} ± 6.2E-03	0.0091^{d} ± 1.1E-03
C. telabon	0.0047^{ab} ± 8.0E-04	0.0027^{ab} ± 5.5E-04	0.0025^{ab} ± 4.8E-04	0.0072^{ab} ± 1.6E-03	0.0016^{ab} ± 1.7E-04	0.0023^{ab} ± 3.3E-04	0.0002^{a} ± 3.7E-05	0.0002^{a} ± 1.9E-05	0.0002^{a} ± 2.5E-05	0.0031^{b} ± 7.5E-04	0.0024^{b} ± 5.3E-04	0.0030^{b} ± 5.7E-04	0.0168^{c} ± 4.5E-03	0.0262^{c} ± 4.0E-03	0.0198^{c} ± 3.2E-03
B. butis	0.0265^{d} ± 4.6E-04	0.0206^{d} ± 1.2E-03	0.0108^{d} ± 8.3E-05	0.0075^{ab} ± 1.4E-03	0.0039^{ab} ± 3.3E-04	0.0039^{ab} ± 2.3E-04	0.0022^{f} ± 2.5E-04	0.0028^{f} ± 2.4E-05	0.0056^{f} ± 1.5E-04	0.0000^{a} ± 4.5E-06	0.0001^{a} ± 9.6E-06	0.0001^{a} ± 1.4E-06	0.0003^{a} ± 2.9E-05	0.0003^{a} ± 3.1E-05	0.0003^{a} ± 4.2E-05
M. bagio	0.0004^{a} ± 4.0E-05	0.0004^{a} ± 8.9E-06	0.0002^{a} ± 7.9E-06	0.0016^{a} ± 2.0E-04	0.0030^{a} ± 3.6E-04	0.0007^{a} ± 4.3E-05	0.0006^{ab} ± 8.6E-05	0.0002^{ab} ± 2.0E-05	0.0001^{ab} ± 6.2E-06	0.0001^{ab} ± 2.1E-05	0.0040^{ab} ± 5.7E-04	0.0019^{ab} ± 2.2E-04	0.0147^{c} ± 2.2E-03	0.0318^{c} ± 3.4E-04	0.0191^{c} ± 3.8E-04
S. argus	0.0105^{c} ± 1.8E-03	0.0134^{c} ± 2.1E-03	0.0077^{c} ± 1.2E-03	0.0165^{bc} ± 3.1E-03	0.0019^{bc} ± 2.2E-04	0.0015^{bc} ± 2.0E-04	0.0017^{c} ± 2.7E-04	0.0003^{c} ± 7.1E-05	0.0007^{c} ± 8.9E-05	0.0029^{c} ± 6.4E-04	0.0062^{c} ± 1.4E-03	0.0060^{c} ± 1.3E-03	0.0073^{c} ± 1.6E-03	0.0440^{c} ± 9.4E-03	0.0089^{c} ± 1.9E-03
Ps. elongatus	0.0311^{e} ± 2.1E-03	0.1003^{e} ± 8.8E-03	0.1289^{e} ± 1.1E-02	0.0197^{c} ± 1.7E-03	0.0018^{c} ± 1.3E-04	0.0044^{c} ± 5.7E-04	0.0024^{e} ± 1.8E-04	0.0021^{e} ± 2.1E-04	0.0032^{e} ± 2.9E-04	0.0002^{ab} ± 9.6E-05	0.0012^{ab} ± 2.4E-04	0.0031^{ab} ± 1.1E-03	0.0006^{a} ± 2.6E-04	0.0003^{a} ± 3.4E-05	0.0001^{a} ± 7.7E-06

Note: Values are means (±SEM, n =15). Within-species comparisons of the activities for each enzyme within a feeding category were analyzed with one-way ANOVA and Duncans' Post Hoc with a family error rate of P = 0.05. Values for a specific enzyme that share a letter are not significantly different. Enzymes activities are expressed in mg min^{-1} mg^{-1} protein (U mg^{-1} protein). Different super script letters means enzyme values with significant differences at 5% level of significance. L=Liver; S=Stomach; I=Intestine.

possessed higher plant matter in its stomach (23.7%). This could be a digestive strategy adopted by *T. jarbua* to maximally utilize the low protein content in its natural diet. Hidalgo *et al*. (1999) pointed out that no differences existed in proteolytic activities to classify fishes as either omnivorous or carnivorous. It was also suggested that to make up for the lower amount of protein available in their diet, herbivorous fishes appeared to increase consumption rate and enzyme production (Hofer, 1982). Moreover, as the vegetal proteins are more difficult to digest than animal proteins (Hidalgo *et al*., 1999), the same amount of protein consumed requires a 10 times higher proteolytic activity in fish feeding on grass than in fish feeding on meal worms (Hofer, 1982). This argument probably explains why protease activity is observed in the herbivorous or omnivorous fishes. In contrast, *U. lepturus* showed lower activity of alkaline protease and pepsin, despite having higher percentage (87.7%) of animal matter in its stomach. Each species of the ten carnivorous fishes in this study, however, showed species specific responses towards diet in its proteolytic activities. Chakrabarti *et al*. (1995) noted that the types of diet did not have any bearing on the production of digestive enzymes in eleven confined-water teleost fishes. Chan *et al*. (2004) and German *et al*. (2004) investigated the digestive enzyme activities in four closely related prickleback fishes, including two herbivorous and two carnivorous species. Their results showed that the activities of digestive enzymes correlated more strongly with phylogeny rather than with the fish's natural diets. Influence of the genetic strains on the activities of brush border enzymes was demonstrated in the crosses of *Oreochromis mossambicus* and *O. aureus* (Hakim *et al*., 2006) and in the silver perch *Bidyanus bidyanus* (Hakim *et al*., 2007). Furthermore, the activities of digestive enzymes were also influenced by many other factors such as the ages of the fishes (Kuźmina, 1996), temperature and season (Kuźmina *et al*., 1996b) and the composition of their diets (Zambonino Infante and Cahu, 2001). Thus, the relationship between digestive enzyme activities and feeding habits in fishes is still not very clear.

Generally, the food and feeding relationship had been used to describe trophic niche of a species. But this relationship does not always coincide with the concept of digestive physiology and proves that such a specification is not always necessary as fish can consume and digest different types of food particularly when in competition. The specific nature of the enzymes in some of the mudflat carnivorous fishes considered here appeared to possess a specific feeding behavior and dietary preference. The fact that species like *T. jarbua, M. bagio, C. telabon* and *S. argus* did not have any dominant enzyme was suggestive of their generalist predatory behavior. They utilized a broad range of dietary items, which explained their incredible success in optimal utilization of estuarine habitats. Thus, it might be summarized from this study that the food preference and digestive physiology was always incomplete in fish communities. No such relationship could be established in the carnivorous fishes in the mudflat through present study possibly because of incomplete segregation of food niches in fishes. It is, therefore, concluded that phylogeny rather than adaptation to trophic resources played a determinant role for their digestive physiology.

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