DIGESTIVE ENZYME ASSAYS IN THE GUT OF *Oreochromis niloticus* LINNAEUS 1757, *Parachanna* (*Channa*) *obscura* GUNTHER 1861 AND *Gymnarchus niloticus* CUVIER 1829

[1]FAGBENRO, Oyedapo, [2]ADEDIRE Olukayode, [1]FATERU, Oluwabukola, [1]OWOLABI, Ibidun, [1]OGUNLANA, Oladipupo, [1]AKANBI, Bukola, [1]FASANMI, Temitope and [1]AYO-AMU Peace
[1]Department of Fisheries and Wildlife, Federal University of Technology, Akure, Nigeria
[2]Department of Biology, Federal University of Technology, Akure, Nigeria

Corresponding Author: Prof. FAGBENRO, O. A., Department of Fisheries and Wildlife, Federal University of Technology, Akure, Nigeria

ABSTRACT

Digestive enzyme assays in the different gut regions (oesophagus, stomach, caecum, duodenum, ileum, and rectum) of three commercial African freshwater fish species: Nile tilapia Oreochromis niloticus, African snakehead fish Parachanna obscura, and African long knife fish, Gymnarchus niloticus, revealed an array of glycosidases (amylase, sucrase, maltase, lactase, and cellulase); proteases (chymotrypsin, pepsin, and trypsin) and lipases. The pattern of distribution and relative activity of the enzymes showed that the fishes are capable of digesting carbohydrates, proteins and lipids such that they complemented the different dietary habits of the three fish species. Enzyme activity was not detected in the oesophagus and rectum of the three fish species. The relative distribution and activity of the various enzymes were possibly induced by the nutritional requirements of the fishes.

Keywords: Digestive enzymes, Fish gut, *Oreochromis niloticus*, *Parachanna obscura*, *Gymnarchus niloticus*

INTRODUCTION

Oreochromis niloticus (Linnaeus 1757) (Family Cichlidae) is the most common species grown in ponds, it is robust, hardy and easy to handle. It is native to Africa, hence found in almost every river and lake. Adult *O. niloticus* are omnivorous but feed predominantly on phytoplankton and can utilize blue-green algae. Juveniles consume a wider range of food items (Jauncey and Ross, 1982). The length of the entire intestine of a tilapia is between five and eight times the length of the fish (Caulton, 1976; Jauncey and Ross, 1982). The intestine is differentiated into an anterior short thin-walled duodenum and a very long posterior section which has a smaller diameter (Bowen, 1982).

The African snakehead fish, *Parachanna* (*Channa*) *obscura* (Gunther 1861) (Family Channidae) is distributed from the Zaire basin through West Africa as far as the Senegal River in the west and the Nile in the east. It is of economic importance as food fish and has great potential for aquaculture in Africa (Fagbenro, 1989; Victor and Akpocha, 1992). *P. obscura* has been described by many authors as a pelagic predator feeding mostly on small fishes and insects (Imevbore and Bakare, 1970; Adebisi, 1981; Elliot, 1986; Victor and Akpocha, 1992; Fagbenro, 1996).

Gymnarchus niloticus (Cuvier 1829) (Family Gymnarchidae) is endemic to Africa and live mainly in swamps and is one of the most important commercial fishes in West Africa notably, Lake Chad, Kainji Lake and River Niger (Sagua, 1983). *G. niloticus* are caught throughout the year but become abundant during the wet season when they are speared by fishermen as they swim around their nests. *G. niloticus* is an electro-navigator; it emits continuous electric charges from its tail which it uses in locating objects and its prey. They are most active at night and according to Sagua (1983), young *G. niloticus* feeds on insects, medium sized on mixed diet of insects and fry of other fishes, while the adults are strictly piscivorous feeding mainly on small fish, *Alestes spp.*

The ability of an organism to digest a given material is dependent on the presence of appropriate enzymes. Enzymes responsible for the digestion of lipids, carbohydrates and proteins all occur in the pyloric caeca or intestinal mucosa of fish (Phillip, 1969). In this paper, the occurrence, distribution and relative activities of glycosidases, proteases and lipases in the different gut regions of *O. niloticus*, *P. obscura* and *G. niloticus* are described.

MATERIALS AND METHODS

Twenty-five *O. niloticus* (SL, 14.40 - 16.50 cm), 25 *P. obscura* specimens (SL, 25.6 - 47.2 cm) and 25 *G. niloticus* specimens (SL, 25.6 - 47.2 cm) were obtained live from catches of artisanal fishermen in Ogbese and Ose rivers in south-western Nigeria. No sexual selection was made. They were transported live to Federal University of Technology Akure fish farm where they were kept unfed for 72 hours inside outdoor concrete tanks in order to bring them to similar physiological state as well as ensure the emptiness of the entire gut. They were anaesthetized with benzocaine (ethyl-p-aminobenzoate) at 100 mg/litre and dissected to remove the entire guts, later separated into the anatomatically distinct regions. The different gut regions were pooled, homogenized and the homogenates were centrifuged

at 1200 rpm for 30 minutes at 4 °C. The supernatants were used as crude enzyme extracts without further purification.

Benedict's qualitative reagents were used for the qualitative assay of glycosidases (carbohydrases) following the methods of Olatunde *et al.* (1988). Glycosidases were assayed in a reaction mixture containing 2.0 ml of phosphate buffer (pH 7.0), 0.4 ml of 1 % of substrate and 0.2 ml of the enzyme extract. The test and control samples were incubated for one hour in a water bath at 37 °C. Hydrolysis of polysaccharides and non-reducing disaccharides were determined in terms of the appearance of reducing properties using Benedict's reagents. An aliquot of 5.0 ml of the alkaline copper reagent of Benedict was added to 1.0 ml of the reaction mixture and heated for 30 minutes in a water bath at 100 °C. The appearance of brick red to cream yellow precipitate was taken as an index of positive reaction. Quantitative assays were conducted using the dinitrosalicyclate (DNS) methods described by Plummer (1978). Each reaction mixture comprised 0.4 ml of 1 % substrate, 0.2 ml phosphate buffer (pH 7.0), 1.6 ml of alkaline 3, 5-dinitrosalicyclic acid reagent (DNSA) and 0.2 ml of the enzyme extract. The reaction mixtures for test and control samples were heated for 30 minutes in a water bath at 100 °C. Each of the mixtures was made to 4.0 ml by diluting with 1.6 ml distilled water. The amount of reducing sugars produced on enzymatic reaction was estimated colorimetrically and the absorbance read at 550 nm on a spectrophotometer.

Qualitative determination of proteases followed the method of Balogun and Fisher (1970). Trypsin and chymotrypsin were estimated in a reaction mixture consisting of 1 % alkaline casein (pH 7.6) and 0.5 ml of the enzyme extract. The test and control samples were incubated simultaneously for one hour in a water bath at 37 °C. After incubation, 1% acetic acid was added drop by drop. Increase in turbidity indicated the presence of the enzyme. Pepsin was estimated in a reaction mixture consisting of 1% acid casein (pH 2.0) and 0.5 ml of the enzyme extract. The test and control samples were incubated simultaneously for one hour in a water bath at 37 °C. After incubation, 1% sodium acetate was added drop by drop. A change in colour indicated the presence of pepsin. Quantitative determination of proteases followed the method of Laskowsky (1955) and Herriott (1955). Trypsin and chymotrypsin were determined in a reaction mixture comprising 10 mg of hide powder azure (HPA), 2.0 ml phosphate buffer (pH 8.0) and 0.5 ml of enzyme extract. The reaction mixture for the determination of pepsin was similar except that the phosphate buffer was at pH 2.0. The test and control samples were incubated for one hour at 37 °C. After incubation, 3.0 ml of ice-cold phosphate buffer was added, the mixture filtered immediately and the absorbance read at 595 nm on a spectrophotometer.

The methods described by Ogunbiyi and Okon (1976) were used to determine the qualitative and quantitative activity of lipases. The reaction mixture comprised equal volumes of 1.0 ml of 25 % olive oil emulsion (ph 7.0) and 1.0 ml of enzyme extract. The test and control samples were incubated simultaneously for one hour in a water bath at 37 °C. After incubation, 3.0 ml of 95 % ethanol and two drops of phenolphthalein were added. The reaction mixture was titrated against 0.05N sodium hydroxide to a similar pink colour. Increase in titre value indicated the presence of lipases.

RESULTS AND DISCUSSION

Oreochromis niloticus: A variety of glycosidases (amylase, sucrase, maltase and cellulase), proteases (trypsin, chymotrypsin and pepsin) and lipases were detected in the stomach, duodenum and ileum of *O. niloticus* gut. Their distribution and activity varied along the entire length of the fish gut, and are presented in Table 1. Lactase was not detected in the entire gut of *O. niloticus*. Akintunde (1985) observed a similar general pattern of enzyme distribution in *Sarotherodon galilaeus* (Table 1), which like *O. niloticus*, has a planktivorous dietary habit (Akintunde, 1976). The variety of glycosidases detected (Table 1) indicate the ability of *O. niloticus* to digest a variety of carbohydrate food components. The occurrence of cellulase in the entire gut regions is suspected to be of exogenous microbial origin. The concentration, specific activity and distribution of trypsin, chymotrypsin, amylase and esterase have been measured from the intestine of *O. mossambicus* (Fish, 1960; Nagase, 1964; Moriarty, 1973). Fish (1960) found that a predominantly herbivorous tilapia had amylase activity distributed throughout the gastro intestinal tract. *O. niloticus* is established as an omnivore, feeding on a variety of food items, therefore it is expected that it will possess the array of enzymes required to digest the food. From the foregoing, it is evident that *O. niloticus* is well equipped to digest carbohydrate, protein and lipid components in its diet.

Parachanna obscura: Various glycosidases (amylase, sucrase, maltase and lactase), proteases (trypsin, chymotrypsin and pepsin) and lipases were detected in the different regions of *P. obscura* gut. Their distribution and activity varied along the entire length of the fish gut, and are presented in Table 2. Cellulase was not detected in the entire gut of *P. obscura*. The variety of glycosidases detected (Table 2) indicate the ability of *P. obscura* to digest a variety of carbohydrate food components. The relatively higher activity levels of proteases, particularly in the pyloric caeca and duodenum (Table 2), was not surprising taking cognizance of the large proportion of protein components (fish, insects) in its natural diet (Fagbenro, 1996). A similar general pattern of protein-hydrolyzing enzymes distribution was detected in *Malapterurus electricus* (Table 4), which like *P. obscura*, has a piscivorous dietary habit (Fagbenro *et al.*, 2001). This is the first record of the digestive enzyme activities in *P. obscura*, which is known to be piscivorous, feeding mainly on insects and small fishes.

Table 1: Assays of digestive enzymes in the gut of *Oreochromis niloticus*

	Stomach	Duodenum	Ileum
GLYCOSIDASES[1]			
α-amylase	0.951 ± 0.069 a	0.758 ± 0.084 b	0.421 ± 0.013 c
Sucrase	1.476 ± 0.189 a	0.784 ± 0.233 b	0.609 ± 0.018 b
Maltase	2.844 ± 0.009	2.777 ± 0.067	2.810 ± 0.015
Lactase	ND	ND	ND
Cellulase	2.879 ± 0.006	2.845 ± 0.048	2.828 ± 0.040
PROTEASES[2]			
Chymotrypsin	0.025 ± 0.006 b	0.172 ± 0.043 a	0.150 ± 0.038 a
Trypsin	0.166 ± 0.048 b	0.261 ± 0.089 a	0.273 ± 0.138 a
Pepsin	0.292 ± 0.040 a	0.118 ± 0.000 b	0.174 ± 0.040 b
LIPASES[3]	34.72 ± 3.15 b	40.15 ± 3.89 b	96.02 ± 6.92 a

Values (Mean ± standard deviation) in the same row with dissimilar alphabets are different (P= 0.05) ND = not detected [1] mg glucose/min/mg protein at 37°C [2] change in optical density at 595 nm/hr/mg of L-tyrosine/hr at 37°C [3] milliequivalents of fatty acids/mg protein/hr at 37°C

Table 2: Assays of digestive enzymes in the gut of *Parachanna obscura*

	Stomach	Caecum	Duodenum	Ileum
GLYCOSIDASES[1]				
α-amylase	0.065 ± 0.069 b	0.225 ± 0.163 a	0.023 ± 0.010 b	0.040 ± 0.025 b
Sucrase	0.120 ± 0.018 b	0.224 ± 0.173 a	0.107 ± 0.057 b	0.211 ± 0.046 a
Maltase	1.408 ± 0.137 b	2.357 ± 0.547 a	0.968 ± 0.330 c	1.145 ± 0.506 b
Lactase	2.695 ± 0.022 a	1.748 ± 0.061 b	1.001 ± 0.043 c	1.628 ± 0.153 b
Cellulase	ND	ND	ND	ND
PROTEASES[2]				
Chymotrypsin	0.025 ± 0.007 b	0.175 ± 0.059 a	0.157 ± 0.073 a	ND
Trypsin	0.040 ± 0.012 b	0.260 ± 0.014 a	0.196 ± 0.035 a	0.084 ± 0.042 b
Pepsin	0.385 ± 0.035 a	0.251 ± 0.057 b	0.176 ± 0.014 c	0.139 ± 0.041 c
LIPASES[3]	73.19 ± 5.33 b	64.30 ± 5.09 b	119.56 ± 9.94 a	88.93 ± 7.26 b

Values (Mean ± standard deviation) in the same row with dissimilar alphabets are different (P= 0.05); ND = not detected, [1] mg glucose/min/mg protein at 37°C, [2] change in optical density at 595 nm/hr/ mg of L-tyrosine/hr at 37°C, [3] milliequivalents of fatty acids/ mg protein/hr at 37°C

Table 3: Assays of digestive enzymes in the gut of *Gymnarchus niloticus*

	Stomach	Caecum	Duodenum	Ileum
GLYCOSIDASES[1]				
α-amalylase	0.39 ± 0.014 b	0.063 ±0.048 a	0.040 ± 0.008 b	0.020 ± 0.000 c
Sucrase	ND	ND	ND	ND
Maltase	0.120 ± 0.071 c	0.077 ± 0.052 b	0.057 ± 0.0047 b	0.037 ± 0.010 c
Lactase	0.420 ± 0.094	0.370 ± 0.035	0.300 ± 0.008	0.330 ± 0.014
Cellulase	ND	ND	ND	ND
PROTEASES[2]				
Chymotrypsin	0.010 ± 0.005 b	0.268 ± 0.086 a	0.251 ± 0.072 a	ND
Trypsin	0.017 ± 0.009 b	0.277 ± 0.076 a	0.200 ± 0.069 a	ND
Pepsin	0.280 ± 0.194 a	0.083 ± 0.068 b	0.030 ± 0.022 b	ND
LIPASES	ND	ND	ND	ND

Values (Mean ± standard deviation) in the same row with dissimilar alphabets are different (P= 0.05), ND = not detected, [1] mg glucose/min/mg protein at 37°C, [2] change in optical density at 595 nm/hr/ mg of L-tyrosine/hr at 37°C, [3] milliequivalents of fatty acids/mg protein/hr at 37°C

Table 4: Digestive enzymes assayed in the guts of selected tropical African fishes

	S. m.	***E. n.***	***P. p.***	***S. g.***	***C. g.***	***C. i.***	***H. b.***	***H. n.***	***M. e.***	***O. n.***	***P. o.***	***G. n.***
GLYCOSIDASES												
Amalylase	+	+	-	+	+	+	+	+	+	+	+	+
Cellulase	-	-	-	-	-	+	+	+	-	+	-	-
Lactase	-	-	-	-	+	-	-	+	-	-	+	+
Maltase	-	-	-	+	+	+	+	+	+	+	+	+
Sucrase	-	-	-	-	+	+	+	+	-	+	+	-
Salicinase	-	-	-	-	-	+	+	-	-	-	-	-
Trehalase	-	-	-	-	-	+	+	-	-	-	-	-
PROTEASES												
Chymotrypsin	-	-	-	+	-	+	+	+	+	+	+	+
Trypsin	+	+	+	+	+	+	+	+	+	+	+	+
Pepsin	+	+	+	+	+	+	+	+	+	+	+	+
LIPASES	-	-	-	+	+	+	+	+	+	+	+	-
References	Olatunde and Ogunbiyi (1977)			Akintunde (1985)	Olatunde *et al.* (1988)	Fagbenro (1990)	Fagbenro *et al.* (1993)	Fagbenro *et al.* (2000)	Fagbenro *et al.* (2001)	This study		

S. m. = Schilbe mystus, E. n. = Eutropius niloticus, P. p. = Physalia pellucida, S. g. = Sarotherodon galilaeus, C. g. = Clarias gariepinus C. i. = Clarias isheriensis, H. b. = Heterobranchus bidorsalis, H. n. = Heterotis niloticus, M. e. = Malapterurus electricus, O. n. = Oreochromis niloticus, P. o. = Parachanna obscura, G. n. = Gymnarchus niloticus

It is expected that high activity of proteases and lipases will be recorded as they are required to digest the major food items in the diet. From the foregoing, it is evident that *P. obscura* is well equipped to digest carbohydrate component in addition to both protein and lipid components in its diet.

Gymnarchus niloticus: The distribution and activities of glycosidases and proteases along the entire length of the *G. niloticus* gut are presented in Table 3. Sucrase and cellulase were not detected in the entire gut of *G. niloticus* Lipases were also not detected in the entire gut of *G. niloticus*. Proteases activities were not detected in the ileum region in the gut of *G. niloticus*. The lower activity levels of glycosidases in the entire gut regions and the relatively higher activity levels of proteases, were not surprising taking cognizance of its strict piscivorous diet in the wild reported by Sagua (1983). Pepsin would hardly be expected to occur in the two distal gut regions since they are active only in strongly acid media found in the stomach. Fagbenro *et al.* (2001) observed a similar general pattern of proteases distribution in the electric catfish, *Malapterurus electricus* (Table 4), which also has a strictly piscivorous dietary habit (Sagua, 1979; Fagbenro *et al.*, 2001). This is the first record of the digestive enzyme activities in *G. niloticus,* which is known to be piscivorous, feeding almost exclusively on fishes. It is expected that high activity of proteases will be recorded as they are required to digest the major food items in the diet. From the foregoing, it is evident that *G. niloticus* is well equipped to digest carbohydrate component in addition to protein component in its diet; and there is no evidence of its ability to digest lipid components of food items.

REFERENCES

ADEBISI, A. A. (1981). Analysis of stomach contents of the piscivorous fishes of the Upper Ogun River, Nigeria. *Hydrobiologia, 79:* 167 – 177.

AKINTUNDE, E. A. (1981). *The biology of Tilapia and Sarotherodon species of Lake Kainji, Nigeria with special reference to Sarotherodon galilaeus.* M.Sc. Thesis, University of Ife, Ile-Ife, Nigeria.

AKINTUNDE, E. A. (1985). Digestive enzymes in the gut of *Sarotherodon galilaeus* (Family Cichlidae) of the Lake Kainji, Nigeria. *Nigerian Journal of Science, 18:* 22 – 25.

BALOGUN, R. A. and FISHER, O. (1970). Studies on the digestive enzymes of the common African Toad, *Bufo, regularis* Boulenger. *Comparative Biochemistry and Physiology 33:* 813 – 820.

BOWEN, S. H. (1982). Feeding, digestion and growth – qualitative considerations. Pages 141 – 156. *In:* PULLIN, R. S. V. and LOWE-MCCONNELL, R. H. (Eds.). *The biology and culture of tilapias.* ICLARM Conference Proceedings, Volume 7, ICLARM, Manila.

CAULTON, M. S. (1976). The importance of pre-digestive food preparation to *Tilapia rendalli* Boulanger when feeding on aquatic macrophytes. *Transactions of the Rhodesian Science Association, 57:* 22 – 28.

ELLIOT, O. O. (1986). *Some aspects of the biology of Asejire Lake.* PhD Thesis, University of Ibadan, Nigeria. 299 pp.

FAGBENRO, O. A. (1989). Recruitment control and production of *Tilapia guineensis* (Dumeril) with the predator, *Channa obscura* (Gunther). *Journal of Aquatic Sciences, 4:* 7 – 10.

FAGBENRO, O. A. (1996). The dietary habits of *Channa obscura* (Gunther) from Owena Reservoir, Nigeria. *Journal of Tropical Forest Resources, 12:* 54 – 61.

FAGBENRO, O. A., ADEDIRE, C. O., OWOSEENI, E. A. and AYOTUNDE, E. O. (1993). Studies on the biology and aquacultural potential of feral catfish, *Heterobranchus bidorsalis* Geoffroy St. Hilaire (1809) (Clariidae). *Tropical Zoology, 6:* 67 – 79.

FAGBENRO, O. A., ADEDIRE, C. O., AYOTUNDE, E. O. and FAMINU, E. O. (2000). Haematological profile, food composition and digestive enzyme assay in the gut of African bony tongue fish, *Heterotis* (*Clupisudis*) *niloticus* (Cuvier 1829) (Osteoglossidae). *Tropical Zoology, 13:* 1 – 9.

FAGBENRO, O. A., ADEDIRE, C. O. and AIYEGBENI, M. L. (2001). Food composition and digestive enzymes in the gut of the African electric catfish, *Malapterurus electricus* (Gmelin 1789) (Malapteruridae). *Tropical Zoology, 14:* 1 – 6.

FISH, G. E. (1960). The comparative activity of some digestive enzymes in the alimentary canal of tilapia and perch. *Hydrobiologia, 15:* 151 – 178.

HERRIOTT, R. M. (1955). Swine pepsin and pepsinogen. Pages 3 – 6. *In:* COLOWICK, S. P. and KAPLAN, N. O. (Eds.). *Methods in enzymology.* Volume 2, Academic Press, London.

IMEVBORE, A. M. A. and BAKARE, O. (1970). The food and feeding habits of non-cichlid fishes of the river Niger in the Kainji reservoir area. Pages 49 – 64. *In:* VISER, S. A. (Ed). *Kainji Lake Studies Volume 1: Ecology* NISER/Ibadan University Press.

JAUNCEY, K. and ROSS, B. (1982). *A guide to tilapia feeds and feeding.* Institute of Aquaculture, University of Stirling, Scotland. 111 pp.

LASKOWSKY, M. (1955). Trypsinogen and trypsin. Pages 26 – 35. *In:* COLOWICK, S. P. and KAPLAN, N. O. (Eds.). *Methods in enzymology,* Volume 2, Academic Press, London.

MORIARTY, D. J. W. (1973). The physiology of digestion of blue-green algae in the cichlid fish *Tilapia nilotica. Journal of Zoology, 171:* 15 – 24.

NAGASE, G. (1964). Contribution of the physiology of digestion in *Tilapia mossambica.* Digestive

enzymes and the effect on their activities. *Z. Vergl. Physio., 49:* 270 – 284.

OGUNBIYI, O. A. and OKON, E. E. (1976). Studies on the digestive enzymes of the African fruit bat, *Eidolon helvum* (Kerr.). *Comparative Biochemistry and Physiology, 55:* 359 – 361.

OLATUNDE, A. A., UKOHA, A. I. and OGUNTAYO, B. F. (1988). Digestive enzymes in the alimentary tract of *Clarias lazera* (Cuvier and Valenciennes) Family Clariidae (Osteichthys: Siluriformes). *Archiv fur Hydrobiologies, 112:* 107 – 113.

PLUMMER, D. T. (1978). *An introduction to practical biochemistry,* 2nd edition. McGraw Hill Book Co., London. 283 pp.

SAGUA, V. O. (1979). Observations on the food and feeding habits of the African electric catfish, *Malapterurus electricus* (Gmelin). *Journal of Fish Biology, 15:* 61 – 69.

SAGUA, V. O. (1983). Preliminary observations on the feeding habits and reproduction biology of *Gymnarchus niloticus* from Lake Chad. Pages 41 – 49. *In: Proceedings of the 3rd Annual Conference of the Fisheries Society of Nigeria* (FISON), Maiduguri.

VICTOR, R. and AKPOCHA, B. O. (1992). The biology of snakehead, *Channa obscura* (Gunther) in a Nigerian pond under monoculture. *Aquaculture, 101:* 17 – 24.