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A study of the pattern of digestive enzymes in *Diplodus puntazzo* (Cetti, 1777) (Osteichthyes, Sparidae): evidence for the definition of nutritional protocols

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Abstract. The digestive enzymes (proteases, carbohydrases and lipases) present along the alimentary tract of juveniles and adults of *Diplodus puntazzo* were studied. The data obtained showed a different distribution of the enzymatic activities in the different parts of the alimentary tract. Levels of enzymatic activity in the adults were higher than those measured in juveniles, suggesting that adults have a greater ability to digest larger pieces of food. The enzymatic pattern of *D. puntazzo* justifies its omnivorous habit and suggests an high potential for digesting vegetable polysaccharides. The results of this study suggest the need to adapt the diet to the digestive potential of this new farming species, thus contributing to the attainment of a product that is qualitatively more similar to the wild one.

Key words: Adults, Digestive enzymes, *Diplodus puntazzo*, Juveniles, Sharpsnout seabream

Introduction

The study of digestive enzymes in fish is of great potential interest. Biochemical information about digestive enzymes in fish constitutes a contribution to the definition of nutritional protocols. Several studies have been made on the digestive enzymes of different fish species (Uys and Hecht 1987; Alarcón et al. 1998; Hidalgo et al. 1999; Chiu and Pan, 2002). In farmed fish, such information may be useful in the selection of feed ingredients (Lan and Pan 1993), particularly for newly farmed species, such as sharpsnout seabream (*Diplodus puntazzo*), a sparid recently adopted in the diversification of Mediterranean fish farming. In this study the main digestive enzyme classes (such as proteases, carbohydrases and lipases) present along the alimentary tract of *D. puntazzo*

were investigated, with the aim of improving our knowledge of its nutritional physiology and digestive capabilities.

Materials and methods

Juveniles (n = 30; SL = 79.12 ± 6.13 mm; WW = 14.37 ± 3.58 g) and adults (n = 30; SL = 187.23 ± 18.12 mm; WW = 265.60 ± 83.32 g) of *D. puntazzo*, obtained from a local fish farm, were captured 3 h after being fed with commercial feeds containing 42% protein and 21% fat. The digestive tract of each fish was dissected and divided into stomach, pyloric caeca, foregut, midgut and hindgut/rectum. Tissues from 10 fish were then pooled to minimize the inter-individual variability. Preparation of the enzyme extracts was carried out according to Savona (1998), and protease, α-amylase, cellulase and lipase activities were determined (Table 1). Enzyme assays were carried out in triplicate. Data were analyzed by analysis of variance (ANOVA) with size (juveniles and adults) and region (stomach, pyloric caeca, foregut, midgut, hindgut/rectum) as sources of variation. The heterogeneity of variances was tested using Cochran's test prior to the analysis of variance.

Results and discussion

Table 2 summarizes the enzymatic activities measured. The data obtained showed, for both juveniles and adults, a different distribution of the enzymatic activities along the alimentary tract (ANOVA $P < 0.001$; Table 2). In the stomach, acidic proteases (pH 1.0–3.0) (Figures 1 and 2) and cellulase (Figure 4) predominated, while in the other regions of the alimentary tract neutral and alkaline proteases (pH 6.0–11.0) (Figures 1 and 2), α-amylase (Figure 3) and lipase (Figure 5) were more abundant.

Table 1. Enzymatic activities detected and relative analytical conditions

Enzyme	Substrate	pH	References
Proteases	Casein	1.0–11.0	Kunitz (1947), Glass et al. (1987)
α-Amylase	Starch	6.9	Bernfeld (1955), Rick (1974)
Cellulase	Carboxymethyl-cellulose	5.0	Pettersson and Porath (1966)
Lipase	Olive oil	8.0	Tietz and Fiereck (1966)

Table 2. Statistics and ANOVA results of the variables [F =: Fisher value; P = p-level; ** =: $P \leq 0.01$; *** =: $P \leq 0.001$); Ns =: non-significant difference ($P > 0.05$)] [§ = data transformed to Sqrt (X + 1); §§ =: data transformed to Ln(X + 1)]

Variables	Mean	± S.D.	Minutes	Maximum	SIZE $F_{(1,20)}/P$	REGION $F_{(4,20)}/P$	SIZExREGION $F_{(4,20)}/P$	Cochran's C
Protease pH 1[1]	54.69	126.87	0.00	433.85	308.54/***	665.36/***	906.11/***	Ns
Protease pH 2[1]	74.93	147.85	1.52	489.23	428.33/***	4199.34/***	8203.75/***	Ns(§)
Protease pH 3[1]	46.30	86.87	0.00	294.56	962.86/***	838.69/***	2083.56/***	Ns (§§)
Protease pH 4[1]	26.58	46.86	0.00	158.11	10.03/**	238.34/***	447.67/***	Ns
Protease pH 5[1]	29.27	34.25	3.42	152.72	1.59/***	386.13/***	54.48/***	Ns (§§)
Protease pH 6[1]	138.68	123.75	16.13	421.83	12.54/***	2279.99/***	181.83/***	Ns (§§)
Protease pH 7[1]	165.66	177.08	0.00	548.91	0.92/***	2668.51/***	264.66/***	Ns
Protease pH 8[1]	223.63	231.29	0.00	703.60	755.79/***	13313.50/***	5724.70/***	Ns (§§)
Protease pH 9[1]	246.49	254.84	0.00	796.02	81.82/***	4275.49/***	482.52/***	Ns (§)
Protease pH 10[1]	287.91	304.53	0.00	968.43	476.81/***	21400.15/***	10335.55/***	Ns (§§)
Protease pH 11[1]	215.64	218.72	0.00	674.33	202.34/***	22743.10/***	10673.41/***	Ns (§§)
α-Amylase[2]	557.80	460.56	0.00	1363.75	1.43/Ns	3215.39/***	318.36/***	Ns
Cellulase[3]	40.10	26.70	7.32	96.26	39.45/***	627.77/***	883.50/***	Ns
Lipase[4]	137.86	154.26	0.00	497.90	16.44/***	266.75/***	333.41/***	Ns

[1] μg tyrosine min^{-1} mg^{-1} of protein;
[2] μg maltose min^{-1} mg^{-1} of protein;
[3] μg glucose min^{-1} mg^{-1} of protein;
[4] μl NaOH 0.05 N mg^{-1} of protein.

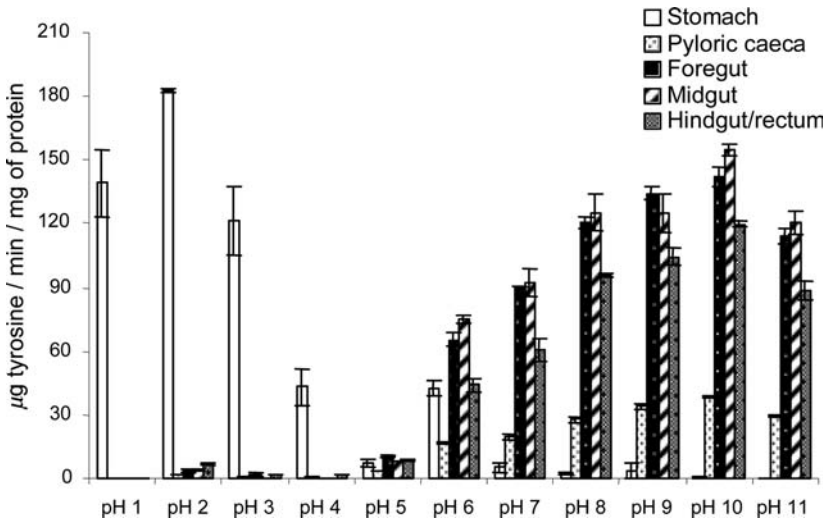

Figure 1. Proteolytic activities in *D. puntazzo* juveniles: pH-dependent profiles of hydrolysis of casein by homogenates from different regions of the alimentary tract (means ± standard deviation).

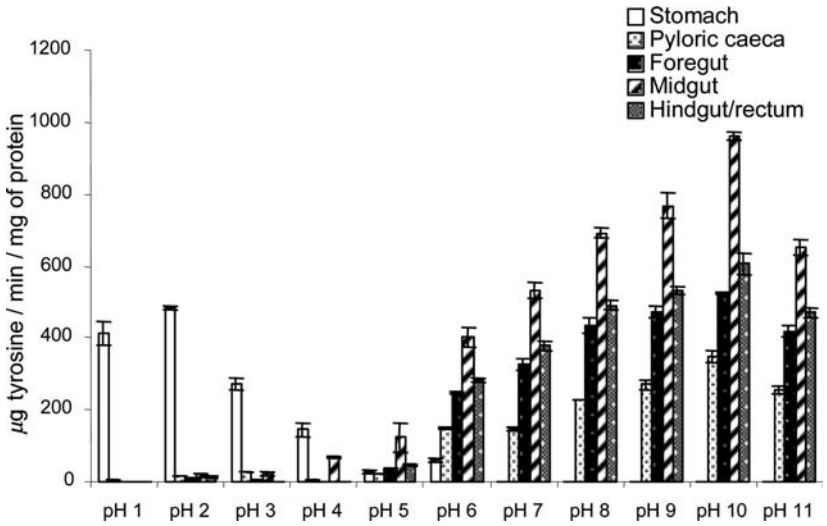

Figure 2. Proteolytic activities in *D. puntazzo* adults: pH-dependent profiles of hydrolysis of casein by homogenates from different regions of the alimentary tract (means ± standard deviation).

Adult *D. puntazzo* showed the same enzymatic pattern as juveniles but with higher enzymatic activity (Figures 1–5) (ANOVA $P < 0.001$; Table 2). In fish, the age and/or stage of development influences the

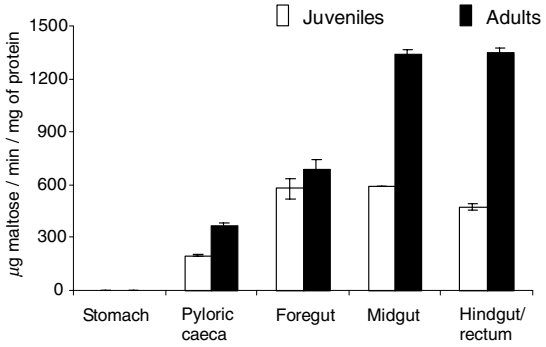

Figure 3. Distribution of α-Amylase activity along the alimentary tract of *D. puntazzo* juveniles and adults (means ± standard deviation).

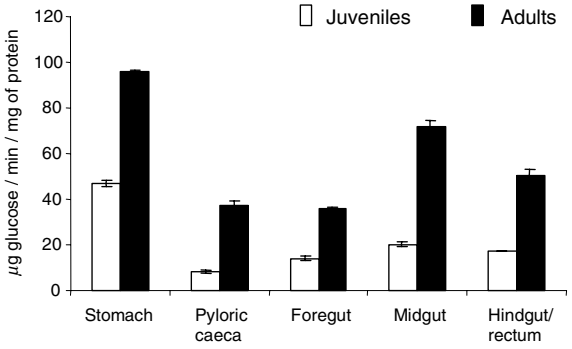

Figure 4. Distribution of Cellulase activity along the alimentary tract of *D. puntazzo* juveniles and adults (means ± standard deviation).

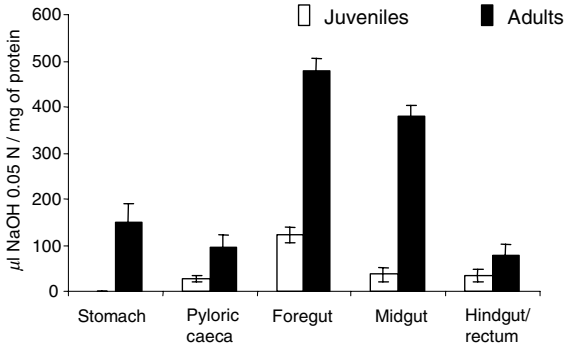

Figure 5. Distribution of Lipase (triglyceride lipase) activity along the alimentary tract of *D. puntazzo* juveniles and adults (means ± standard deviation).

anatomical and physiological development of the digestive organs, justifying the different alimentary habits at various stages of the life cycle (Kuz'mina 1996). The digestive processes are also correlated with the size and composition of food. Under natural conditions, adults tend to capture prey of larger dimensions, which demands a greater digestive effort due to the smaller surface area exposed to enzymatic action.

The results of this study suggest specific conclusions regarding the breeding of this species. *D. puntazzo* showed an enzymatic pattern that is well suited to protein digestion, but also a high potential for digesting vegetable polysaccharides, in agreement with its omnivorous habits (Ceccarelli et al. 1983; Mirto et al. 1994). The need to develop an appropriate diet for intensive farming of this species is evident. This could be achieved from a thorough understanding of its digestive capabilities with regard to various feed constituents, in order to obtain a product that is qualitatively more similar to the wild one. One possible strategy is the breeding in polyculture of *D. puntazzo* with carnivorous species such as seabass *Dicentrarchus labrax*.

References

Alarcón, F.J., Díaz, M., Moyano F.J. and Abellán, E. 1998. Characterization and functional properties of digestive proteases in two sparids; gilthead seabream (*Sparus aurata*) and common dentex (*Dentex dentex*). Fish Physiology and Biochemistry 19: 257–267.

Bernfeld P. 1955. Amylases, α and β. In: (eds) Colowick, S.P. and Kaplan, N.O. Methods in Enzymology. American Press, NY, 1; pp. 149–158.

Ceccarelli, R., Fresi, E., Plastina, N., and Scardi, M. 1983. Comportamento alimentare degli stadi giovanili di *Diplodus puntazzo* (Gm.) dell'Isola d'Ischia. Nova Thalassia 6: 503–509.

Chiu, S.-T. and Pan, B.S. 2002. Digestive protease activities of juvenile and adult eel (*Anguilla japonica*) fed with floating feed. Aquaculture 205: 141–156.

Glass, H.J., MacDonald, N.L. and Stark, J.R. 1987. Metabolism in marine flatfish - IV. Carbohydrate and protein digestion in atlantic halibut (*Hippoglossus hippoglossus* h.). Comparative Biochemistry and Physiology 86B: 281–289.

Hidalgo, M.C., Urea, E. and Sanz, A. 1999. Comparative study of digestive enzymes in fish with different nutritional habits. Proteolytic and amylase activities. Aquaculture 170: 267–283.

Kunitz, M. 1947. Crystalline soybean trypsin inhibitor. II. General properties. Journal of General Physiology 320: 291–310.

Kuz'mina, V.V. 1996. Influence of age on digestive enzyme activity in some freshwater teleosts. Aquaculture 148: 25–37.

Lan, C.C. and Pan, B.S. 1993. *In vitro* digestibility simulating the proteolysis of feed protein in the midgut gland of grass shrimp (*Penaeus monodon*). Aquaculture 109: 59–70.

Mirto, S., Scilipoti, D., Lopiano, L., Badalamenti, F. and Mazzola, A. 1994. Primi dati sul ritmo alimentare giornaliero di tre specie ittiche nello Stagnone di Marsala (Sicilia Occidentale). Biologia Marina Mediterranea 1: 335–336.

Pettersson, G. and Porath, J. 1966. A cellulolytic enzyme from *Penicillum notatum*. In: (eds) Colowick, S.P. and Kaplan, N.O. Methods in Enzymology. American Press, NY, VIII, pp. 603–607.

Rick, W. 1974. α-Amylase. In: Bergmeyer H.U. (ed) Methods of Enzymatic Analysis Verlag Chemie, Weiheim, 2: pp. 885–890.

Savona, B. 1998. Variabilità nei processi enzimatici della digestione in *Diplodus puntazzo* (Pisces, Sparidae) a diverse condizioni di allevamento, PhD dissertation, University of Palermo, p. 72.

Tietz, N.W. and Fiereck, E.A. 1966. A specific method for serum lipase determination. Clinica Chimica Acta 13: 352–358.

Uys, W. and Hecht, T. 1987. Assays on the digestive enzymes of sharptooth catfish, *Clarias gariepinus* (Pisces: Clariidae). Aquaculture 63: 301–313.