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Digestive proteases and carbohydrases along the alimentary tract of the stargazer, *Uranoscopus scaber* Linnaeus, 1758

E. S. PAPOUTSOGLOU and A. R. LYNDON

School of Life Sciences, Heriot-Watt University, Riccarton, EH14 4AS, Scotland, U.K.

e-mail: stratospap@aua.gr

Abstract

Digestive enzyme activity and capacity (activity x tissue weight) for protein (total protease assay, 25° C) and carbohydrates (total carbohydrase and alpha-glucosidase assay at 5, 18 and 25° C) was investigated for the carnivorous stargazer, <u>Uranoscopus scaber</u> along its digestive tract. Results indicated that whole gut total protease activity was highest at pH 1.5 (P<0.05) (25° C) in <u>U. scaber</u> (6.64 ± 2.55 mg tyrosine per g digestive tract per minute, pH 1.5). Total protease activity was apparent mainly in the stomach at pH 1.5 (9.73 ± 3.3), and to a lesser degree in the anterior intestine (11.15 ± 1.5 , pH 10.0) and pyloric caeca (4.92 ± 2.06 , pH 10.0), especially at pH 9.0 and 10.0. Furthermore, 60% of total capacity for protein digestion derives from the stomach region, which takes up 65% of the digestive tract. Total carbohydrase activity and capacity levels were very low compared to other carnivorous teleosts, indicating very low tendency for complex, large molecular weight carbohydrate digestion. However, alpha-glucosidase levels were higher, a fact which combined with relevant data for other marine carnivorous teleosts suggests a possible role of disaccharides in relation to marine carnivorous fish dietary carbohydrate inclusion.

Keywords: Digestive Enzymes; Fish; Digestion; *Uranoscopus scaber*; Proteases; Carbohydrases; Alpha Glucosidase; Temperature.

Introduction

Uranoscopus scaber (Teleostei, Perciformes, Uranoscopidae) is not only a species of some importance for local fisheries in the Mediterranean (TORTONESE, 1975; HUREAU, 1986; RELINI et al., 2000) but also shares many characteristics with species that are currently reared intensively in the same region, such as gilthead seabream (Sparus aurata) and European sea bass (Dicentra-

rchus labrax). These shared characteristics relate to the marine environment, warm water adaptation, the carnivorous feeding habit and similarities of the digestive tract morphology, (muscular stomach, relatively short intestine and relatively few pyloric caeca) (SANZ, 1985; GENTILE et al., 1989; VITTURI et al., 1991; GERKING, 1994).

The ever increasing demand for fishmeal in aquaculture fish feeds, its increasing price and decreasing availability has led research towards alternative protein sources, mainly of plant origin (e.g. soy bean meal), as well as the enhancement of fish feed digestibility and optimum performance of the digestive enzymes of reared species (BLIER et al., 2002). Therefore, all relevant information regarding the improvement of the digestive enzyme activity of marine carnivorous fish, the utilisation of lipid- and carbohydrate-derived energy for metabolic requirements and the utilisation of the protein element of diet solely for growth is considered beneficial (BLIER et al., 2002).

Recent studies concerning the digestive enzyme performance of *U. scaber* (PA-POUTSOGLOU & LYNDON, 1998, 2003) have shown a low affinity for digestion of complex, large molecular weight carbohydrates.

There is particular interest concerning the role of the pyloric caeca since although there have been recent attempts to define their exact function, relating to an increase of digestive and absorptive surface area for amino acids, dipeptides and sugars (BUDDINGTON & DIAMOND, 1986, 1987; BUDDINGTON & HILTON, 1987; GENTILE et al., 1989; BUDDINGTON et al., 1987, 1997; PAPOUTSOGLOU & LYNDON, 2004; ZIMMERMAN et al., 2005), there is still a lack of definite information.

The present study aims to contribute to the biology of *U. scaber*, by considering which digestive enzymes are present, where in the gut protein and carbohydrate digestion take place and where this is most predominant, as well as which of these processes are most important. The extrapolation of relevant information from this species, such as the determination of digestive protease and carbohydrase activity and capacity, can be useful towards further understanding and improving the processes of digestion and utilisation of nutrients in reared marine carnivorous fishes.

Materials and Methods

Experimental animals

The animals (n=10) were collected off the coast of the island of Astypalaia (Dodecanese, Greece) in August 1997 by trawling, were stored in ice after capture and dissected the same day as described by PAPOUT-SOGLOU & LYNDON (2003). The digestive tracts were stored in dry ice and analysed for protease and carbohydrase activity. *U. scaber* individuals ranged from 212±133 g in weight and 23±4 cm in standard length (PAPOUTSOGLOU & LYNDON, 2003).

Determination of total protease activity

Total proteolytic activity was determined using the method of casein hydrolysis developed by KUNITZ (1947) and modified by WALTER (1984). The assay was performed at pH values of 1.5, 7.0, 9.0, and 10.0 and the buffers used were 0.1M KCl-HCl (pH 1.5), 0.1M citrate-0.2M phosphate (pH 7.0), 0.1M Tris-HCl (pH 9.0), and 0.1M glycine-NaOH (pH 10.0), at 25° C.

The values for each pH level present an indication for the activity of more specific proteases, as they correspond to their optima or near-optimal pH levels; pH 1.5 corresponds to pepsin, pH 7.0 for trypsin/chymotrypsin, pH 9.0 and 10.0 for carboxypeptidase, elastase and collagenase.

Enzyme extract (0.1 ml), 1 % (w/v) casein in water (0.25 ml) and buffer (0.25 ml) were incubated for one hour at 25° C, and the reaction was stopped by addition of 0.6 ml 8 % (w/v) trichloroacetic acid (TCA). After keeping samples for one hour at 2° C, they were centrifuged at 1800 g for 10 minutes, and the absorbance of the supernatant was measured at 280 nm. All samples were assayed in triplicate using the appropriate blanks. Tyrosine was used as standard (HIDALGO *et al.*, 1999; PAPOUTSOGLOU & LYNDON, 2006 a,b).

Hydrolysis of starch

For the hydrolysis of starch, 1% soluble starch (0.5 ml), buffer (1.0 ml, 0.1M citrate- 0.2M phosphate buffer at pH 7.6) and enzyme extract (H/50 sample, 0.3 ml) were placed in 10-ml conical digestion tubes. The mixture was then incubated for four hours at 5, 18, 25 and 37°C and aliquots (0.3 ml) were diluted to 1.0 ml, placed in test tubes, and assayed for reducing sugars according to the Somogyi-Nelson assay (ROBYT & WHE-LAN, 1968). Soluble starch (1 g) was made into a paste with methanol (3 ml) and added to distilled water (150 ml) at 100° C. Boiling was then continued for fifteen to twenty minutes to give a final volume of 100 ml (CLARK et al., 1984).

Determination of α-glucosidase activity: hydrolysis of para nitrophenol α-glucosides

Enzyme extract (1.0ml) was incubated with buffer (1.0ml, 0.1M citrate-0.2M phosphate buffer at 7.6 pH) and substrate solution (para-nitrophenol α -glucoside, Sigma) (3mg/ml, 0.2ml). The period of incubation at 5, 18, 25 and 37° C was twenty-five minutes (CLARK *et al.*, 1984).

Enzyme activity was ceased by transferring the digests to ice and the liberated yellow p-nitrophenol colour was enhanced by the addition of 1M potassium carbonate (1.0 ml). The digests were centrifuged (five minutes at 12000x g) and the absorbance of the supernatant was measured at 420 nm against the appropriate blanks. Calibration was made against a glucose (Sigma) standard curve.

Activity of α -glucosidase in the examined species is mainly expressed in mg of α -para nitrophenol glucosides per g weight of tissue per minute hydrolysis.

Calculations

Apart from activity, digestive enzyme levels are also presented as capacity (i.e. per g tissue activity x the corresponding tissue weight), in order to describe both the rela-

tive importance of each specific gut section towards digestion, as well as the capacity of the digestive tract of a species to digest nutrients (KUZ'MINA, 1996).

Results are finally presented as capacity/g body weight, in order to offer a more practical expression of the potential of *U. scaber* for digestion; presenting a single practical value irrespective of size and indicating quantitatively the potential for macronutrient digestion (KUZ'MINA, 1996; PAPOUT-SOGLOU & LYNDON, 2006a, b).

Data analysis

In terms of statistical analysis, it was necessary to investigate whether the digestive enzyme levels (both in the whole gut and along the digestive tract) of *U. scaber* differed significantly between different pH levels (total protease assay) or different incubation temperatures (total carbohydrase and α-glucosidase assays). Therefore, one-way ANOVA was performed using *STATGRAPH-ICS for Windows 2.1* (Statistical Graphics Corp.) followed by Tuckey's test.

Results

Total protease activity and capacity

Total protease capacity (CA) and per g tissue activity (PGA) were significantly higher at pH 1.5, and so was capacity per g body weight (CAB). Intestinal protease levels (anterior intestine-HPG, HCA and whole intestine-IPG and ICA) were elevated at pH 7.0, 9.0 and 10.0 (P<0.05), but were negligible at pH 1.5. Furthermore, posterior intestinal protease levels (LPG and LCA) were negligible at all examined pH levels. Pyloric caeca protease levels (PPG and PCA) demonstrated relatively increased values at all examined pH levels, while stomach levels (SPG and SCA) were significantly higher at pH 1.5 (P<0.05) and negligible at pH 7.0, 9.0 and 10.0 (Table 1).

Table 1

(Mean ± S.D.) Total protease capacity (CA), activity per g intestine weight (PGA), capacity per g body weight (CAB), activity per g anterior (HPG) and posterior intestine (LPG), capacity in anterior (HCA) and posterior intestine (LCA), whole intestinal activity (IPG) and capacity (ICA), pyloric caecal activity (PPG) and capacity (PCA), and stomach activity (SPG) and capacity (SCA) of stargazer *Uranoscopus scaber* at pH 1.5, 7.0, 9.0 and 10.0 (in mg tyrosine per tissue per min) (n=10).

pH level	PGA		HPG	LPG	IPG	PPG	SPG
1.5	6.64±2.55 a		0.53±0.25 a	0.33±0.27 a	0.42±0.24 a	1.24±0.53 a	9.73±3.3 a*
7.0	0.98±0.24 b		5.09±1.64 b*	0.39±0.37 a	2.50±0.74 b	2.61±0.92 b	0.16±0.08 b
9.0	1.42±0.42 b		8.45±2.29 c*	0.47±0.60 a	4.08±1.20 c	3.46±1.23 b	0.12±0.05 b
10.0	1.98±0.59 b		11.15±1.5 d*	1.11±0.93 b	5.55±1.00 c	4.92±2.06 b, c	0.19±0.13 b
pH level	CA	CAB	HCA	LCA	ICA	PCA	SCA
1.5	44.7±28.0 a	0.225±0.100 a	0.30±0.22 a	0.21±0.19 a	0.51±0.35 a	1.53±1.48 a	42.6±26.6 a*
7.0	6.9±4.6 b	0.033±0.010b	3.21±2.39 b*	0.30±0.31 a	3.50±2.52 b*	2.75±1.97 a*	0.64±0.38 b
9.0	10.1±7.2 b	0.047±0.016 b	5.49±4.03 b*	0.30±0.36 a	5.79±4.00 c*	3.81±3.31 a	0.51±0.26 b
10.0	13.7±9.2 b	0.066±0.022 b	6.77±4.32 b, c*	0.85±0.86 a	7.60±4.90 c*	5.34±4.37 a, b*	0.77±0.61 b

Different letters denote significant difference in each column (one-way ANOVA, *P*<0.05) Asterisk denotes significant difference between columns (one-way ANOVA, *P*<0.05)

Table 2
Significance of the effect of body weight on total protease activity (PGA), capacity (CA), and capacity per g body weight (CAB) in the stargazer *Uranoscopus scaber* measured at pH 1.5, 7.0, 9.0 and 10.0 (n=10).

pH value	P value of regression of total protease activity (PGA) against body weight (BW) at each pH level		
1.5	0.381		
7.0	0.558		
9.0	0.903		
10.0	0.654		
Capacity (CA) (pH 1.5)	0.001*, regression equation: capacity=9.818+0.309BW		
Capacity per g body weight	>0.05, regression equation cab= - 0.395BW ^{0.278}		
(CAB) (pH 1.5)			

Effect of body weight on activity and capacity

Table 2 demonstrates the significance of the effect of body weight on total protease activity and capacity in U. scaber measured at pH 1.5, 7.0, 9.0 and 10.0. There was a significant (P<0.05), positive relationship between capacity at all pH levels and body weight. There was also a trend towards a negative relationship between capacity per g body weight and body weight. There did not seem to be any effect of body weight on activity at all examined pH levels.

Effect of temperature on the performance of digestive carbohydrases: total carbohydrase

Total carbohydrase levels for *U. scaber* at 5, 18 and 25I C were low even when compared to marine carnivores (PAPOUT-SOGLOU & LYNDON, 2004). Activity at 5° C was about 30% (P<0.05), at 18° C approximately 50% (P<0.05) and that of 25° C similar to that at 37° C (P>0.05) (Table 3).

a-Glucosidase

Activity at 5, 18 and 25° C was very sim-

Table 3

(Mean ± S.D.) Total carbohydrase capacity (CA), activity per g intestine weight (PGA), activity per g anterior (HPG) and posterior intestine (LPG), capacity in anterior (HCA) and posterior intestine (LCA) and pyloric caecal activity (PPG) and capacity (PCA) of stargazer *Uranoscopus* scaber at 5, 18, 25 and 37° C (in mg glucose per g tissue per min) (n=10).

T (°C)	PGA	HPG	LPG	PPG
5	0.70±0.26 a	0.85±0.19 a	$0.62\pm0.10a$	0.67±0.15 a
18	0.95±0.29 a	1.01±0.24 a	0.91±0.19a	0.94±0.23 a
25	1.90±0.42 b	2.62±0.56 b	1.85±0.42 b	1.54±0.29 b
37	1.92±0.46 b	2.02±0.49 b	1.93±0.43 b	1.86±0.40 b
T (°C)	CA	HCA	LCA	PCA
5	1.64±1.13 a	0.49±0.33 a	0.47±0.33 a	0.68±0.40 a
18	2.30±1.59 a	0.59±0.39 a	0.72±0.42 a	1.00±0.66 a
25	4.60±3.18 b	1.55±1.06 b	1.48±1.00 b	1.56±0.99 b
37	5.54±3.82 b	1.50±1.01 b	1.87±1.06 b	2.18±1.37 b

Different letters denote significant difference in each column (one-way ANOVA, P<0.05)

Table 4

Alpha-glucosidase capacity (CA), activity per g intestine weight (PGA), activity per g anterior (HPG) and posterior intestine (LPG), capacity in anterior (HCA) and posterior intestine (LCA) and pyloric caecal activity (PPG) and capacity (PCA) of stargazer *Uranoscopus scaber* at 5, 18, 25 and 37° C (mg para-nitrophenol-alpha-glucoside per minute) (n=10).

T (°C)	PGA	HPG	LPG	PPG
5	0.0080±0.0015 a	0.0101±0.0018 a	0.0108±0.0019 a	0.0049±0.0010 a
18	0.0113±0.0021 a	0.0143±0.0027 a	0.0125±0.0021 a	0.0090±0.0017 a
25	0.0102±0.0019 a	0.0108±0.0019 a	0.0126±0.0022 a	0.0081±0.0017 a
37	0.0742±0.0141 b	0.0780±0.0150 b	0.0720±0.014 b	0.0720±0.015 b
T (°C)	CA	HCA	LCA	PCA
5	0.0184±0.0064 a	0.0057±0.0018 a	0.0080±0.0021 a	0.0047±0.0014 a
18	0.0267±0.0093 a	0.0078±0.0024 a	0.0092±0.0031 a	0.0096±0.0031 a
25	0.0195±0.0069 a	0.0053±0.0017 a	0.0074±0.0022 a	0.0068±0.0019 a
37	0.2040±0.0710 b	0.0520±0.0170 b	0.0630±0.0195 b	0.0880±0.0220 b

Different letters denote significant difference in each column (one-way ANOVA, P<0.05)

ilar (P>0.05) and about 10% of the activity at 37° C (P<0.05) (Table 4). Values for each gut section were similar and no specific section of the digestive tract differed at any temperature level from the others. There were no specific regions of the tract that contributed more to total capacity at the examined temperatures: Table 4 demonstrates the significantly higher capacity (P<0.05) observed at all gut sections at 37° C.

Discussion

U. scaber demonstrated an increased capacity for protein digestion, especially in the stomach and upper intestine regions, lower potential for large molecular weight carbohydrate digestion but increased tendency for digestion of disaccharides. Furthermore, there was a general trend towards negative correlation between body weight and digestive enzyme activity.

Total protease activity and capacity

Total protease activity and capacity levels were quite elevated, typical of carnivorous, or even piscivorous species: king salmon (Oncorhynchus tschawytscha); pike (Esox luscious); perch (Perca fluviatilis); Atlantic halibut (Hippoglossus hippoglossus); yellowfin tuna (Thunnus albacares); bluefin tuna (T. thynnus) (VONK & WESTERN, 1984).

U. scaber values were higher than reported values for gilthead seabream (Sparus aurata) and comparable to those reported for rainbow trout (Oncorhynchus mykiss) (HIDALGO et al., 1999) and spotted wolffish (Anarhichas minor) (PAPOUTSOGLOU & LYNDON, 2006b). The demonstrated digestive protease profile of *U. scaber* against pH resembled that of eel (Anguilla anguilla) (HIDALGO et al., 1999). The magnitude of the protease values of *U. scaber* can be also realised by the fact that by far the highest values were encountered at pH 1.5 (Table 1), where most carnivorous species exhibit their highest activity, due to pepsin values. Protein digestion in the stomach is evidently very important for *U. scaber*: the stomach comprises a large part of the digestive tract, about 65% of its total weight (PAPOUTSOGLOU & LYNDON, 2003), a fact that suggests high protease capacity for this tissue as well as activity. About 60% of total protease capacity was evident at pH 1.5, only 9% at neutral pH and 31% at alkaline pH (13% at pH 9.0 and 18% at pH 10.0) (Table 1). Values for activity and capacity were significantly lower at the other examined pH levels: 7.0, 9.0 and $10.0 \ (P < 0.05)$ (Table 1). Clearly most protein digestion is accomplished in the stomach at acidic pH levels. This can be also demonstrated by the feeding behaviour of U. scaber, which is a benthic predator, waiting buried in the sand for its prey, relying on a large muscular stomach to accommodate and digest even whole fish.

Calculated values for total protease capacity per g body weight of *U. scaber* led to a value of at least 0.225±0.100 mg of protein digested for each g body weight per minute

(Table 1). This could be a useful tool in calculating how much protein, for example, is likely to be sufficiently digested when included in artificial diets for a given species of a given weight, or even how much of its natural diet the animal is likely to consume in order to obtain the required amount for its growth, assuming that the protein content of its natural diet could be calculated, i.e. mainly fish for this species (PAPOUTSOGLOU & LYNDON, 2006a,b). It should be noted that obtained values are an approximation as they are calculated in vitro at 25° C, and the process of digestion depends on a number of factors other than the presence and abundance of digestive enzymes, which affect their performance either directly or indirectly.

Measurement of the total protease activity and capacity along the digestive tract of U. scaber (Table 1) indicated that the stomach tissue exhibits its highest observed activity (P<0.05) at pH 1.5, whereas at pH 7.0, 9.0 and 10.0 stomach activity is negligible. The capacity requirements of U. scaber, at least quantitatively, for protein digestion are definitely met at acidic pH levels by the large stomach tissue, which exhibits such high activity.

Significantly higher total protease activity was present in the anterior rather than the posterior part of the intestine at the examined pH levels (P<0.05), apart from values at pH 1.5 where anterior and posterior intestinal activity values were similarly low (Table 1). The same findings concerning the anterior and posterior intestine were demonstrated for another benthic carnivore, A. minor (PA-POUTSOGLOU & LYNDON, 2006b). Anterior intestinal activity dramatically increased at neutral and especially alkaline pH. On the contrary, posterior intestinal protease activity and capacity remained constantly low at all examined pH levels. However, the intestinal tissue comprised less than 20% of the digestive tract, and the anterior intestine, which exhibited elevated activity, only 8.7%, therefore it is likely that its role is rather qualitative.

Pyloric caeca exhibited considerable ac-

tivity at all examined pH levels, which leads to the assumption that they may contain different enzymes (or isoenzymes) that function at different pH levels (TORRISSEN & TORRISSEN, 1984). However, their small relative weight, compared to the stomach (PAPOUTSOGLOU & LYNDON, 2003) limited their effect by means of capacity.

The capacity calculated for anterior and posterior intestine, stomach and pyloric caeca (Table 1) demonstrates that stomach capacity at pH 1.5 comprised more than 95% of the total, while at neutral and alkaline pH levels its contribution is less than 10%, and over 80% of capacity at pH 7.0-10.0 is shared between the anterior intestine and the pyloric caeca.

Due to the small size of the sample, no conclusive evidence was present to indicate whether total protease activity is or is not affected by the size of the animals. However, analysis of data on these ten individuals indicated either no effect of size (Table 2, P values between 0.38-0.90 for the examined pH levels, P>0.05), or even a trend towards a negative effect of size against total protease activity, the latter being possibly anticipated as it has been recorded to be generally the case (VONK & WESTERN, 1984: HIDALGO et al., 1999). It is worth noting that pepsin at pH 1.5 demonstrated more of a negative trend than enzymes at neutral and alkaline pH levels. It is possible that pepsin, active in the stomach, is more profoundly affected by size (age) than other proteases, which are active in the intestine and the pyloric caeca.

With regard to total protease capacity against body weight, there was a positive correlation (P=0.001, r²=0.76), bearing in mind the fact that capacity is calculated by multiplying activity by the weight of the corresponding tissue, which increases as the animal increases in size (Table 2).

Finally, it should be stated that available data on the performance of proteases under realistic environmental temperatures suggested that the effect of lower temperatures is comparatively less significant than it is for carbohydrases (HIDALGO *et al.*, 1999). This fact suggests that the activity of proteases for most species at 18 or 25° C should be around 50-60% of what the available data for most proteases at 35-37° C suggests (VONK & WESTERN, 1984).

Total carbohydrase

Total carbohydrase activity and capacity levels of *U. scaber* are very low. Compared to herbivorous marine and freshwater species: parrot fish (Sparisoma cretense); blue tilapia (Oreochromis aureus); common carp (Cyprinus carpio) (PAPOUTSOGLOU & LYNDON, 1998, 2003, 2004, 2005a,b; 2006a; HIDALGO et al., 1999) the values are approximately 50 times lower; compared to other not highly carnivorous species such as O. mykiss; Atlantic salmon (S. salar); D. labrax; S. aurata; sole (Solea solea); roach (Rutilus rutilus); rudd (Scardinius erythropthalmus) they may be only 5-7 times lower (HOFER, 1979; GLASS et al., 1987; MU-NILLA-MORAN & SABORIDO-REY, 1996; PAPOUTSOGLOU & LYNDON, 1998; 2003; 2004, 2005a,b; HIDALGO et al., 1999) but in general, U. scaber is a species with very low potential for complex carbohydrate digestion and utilisation. The only species with comparable values for total carbohydrase activity and capacity are the benthic species, turbot (Scophthalmus maximus) and A. minor (PAPOUTSOGLOU & LYNDON, 2004; 2006b). Even the pyloric caeca, an area of the digestive tract where usually values are significantly higher, demonstrate similar values to the other parts of the gut; and there is no single gut section to demonstrate particularly increased levels (unusually higher or lower values). The feeding habits and choice of habitat of *U. scaber* justify such observations.

a-Glucosidase

Levels of α -glucosidase for U. scaber were, rather surprisingly, generally comparable to those obtained for species with differ-

ent feeding habits and highly differentiated total carbohydrase and α -amylase levels, such as *O. mykiss*, *A. minor*, *S. aurata*, *D. labrax* and *O. aureus* (PAPOUTSOGLOU & LYNDON, 2005a, 2006b). This fact suggests an important role for disaccharides as a source of dietary carbohydrate even in marine carnivorous fish. Recent experiments have shown that α -glucosidase levels (therefore carbohydrate digestion) may be increased due to diet composition, although α -amylase levels were not affected (PAPOUTSOGLOU & LYNDON, 2006b).

There is no region of the digestive tract exhibiting higher α -glucosidase activity or capacity, and α -glucosidase levels will be overestimated if only estimated at 37° C (Table 4).

To conclude, it could be generally stated that *U. scaber* is primarily a straightforward carnivore with high potential for protein digestion, little capacity for complex carbohydrate digestion but increased potential for disaccharide digestion. U. scaber has a very large and muscular stomach with high protease activity and capacity at acidic pH, relatively few but large and long pyloric caeca and a short intestine that exhibit increased protease activity, very low total carbohydrase and α -amylase activity, but with comparable glucosidase activity to other carnivores and omnivores. The potential for disaccharide digestion exhibited by a carnivore such as U. scaber suggests a possibly important role for low molecular weight carbohydrate inclusion (using low-cost ingredients, PAPOUT-SOGLOU & LYNDON, 2005b) in artificial feeds for reared marine carnivorous species.

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