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**Distribution of  $\alpha$ -amylase along the alimentary tract of two Mediterranean fish species, the parrotfish *Sparisoma cretense* L. and the stargazer, *Uranoscopus scaber* L.**

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**Abstract**

*There is very little information available for the only Mediterranean species of parrotfish (*Sparisoma cretense*) and its feeding habits, especially since most other parrotfish species are associated with coral reefs. The same lack of information is true for another fish species, the stargazer (*Uranoscopus scaber*), which is carnivorous, and important for local fisheries in the Mediterranean. Comparative information is presented concerning the digestive activity and capacity for alpha amylase for these species with completely different feeding strategies, as well as main location(s) of carbohydrate digestion along their digestive tract. Alpha-amylase activity and capacity is significantly higher in *S. cretense* than *U. scaber* ( $p < 0.05$ ). Activity in *S. cretense* is very high, comparable to levels reported for carp and tilapia. It is similar in anterior and posterior intestine, however, the posterior intestine comprises a more important role in alpha amylase capacity. In *U. scaber*, activity is present in pyloric caeca and intestine. Levels are very low, comparable to levels reported for other benthic marine carnivores. There is no difference between activities in intestine and pyloric caeca. However, activity is higher in the anterior part of the intestine, lower in the posterior intestine and absent in the stomach.*

*Such information is thought to be beneficial for improving knowledge on the biology of the examined species, and the physiology of nutrition, as well as for assisting towards understanding these processes in other, more valuable species for aquaculture. Also, the possibility of using the stargazer alimentary tract, especially its pyloric caeca as a model system is discussed.*

**Keywords:** Fish, Digestion, Enzymes, Alpha Amylase, *Uranoscopus scaber*, *Sparisoma Cretense*.

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**Introduction**

The main role carbohydrates have in metabolism is that of fuel, which when oxidised offers energy for other metabolic functions. Carbohydrates are used for this purpose in the form of monosaccharides, mainly glucose. Alpha-amylase is a key enzyme for carbohydrate

digestion. It is probably secreted by the pancreas into the intestine and the pyloric caeca (where present), where it may be adsorbed. It acts on complex polysaccharides, such as starch and glycogen, hydrolysing them up into maltose, maltotriose and a combination of branched (1:6) oligosaccharides ( $\alpha$ -limit dextrins) and some glucose (JOBLING, 1995).

*Uranoscopus scaber* (Uranoscopidae) is a benthic, carnivorous species, typical of sand-mud bottoms, and an important species for the commercial fishery of the Mediterranean (Table 1) (RELINI *et al.*, 2000) and Black Sea (LUTHER & FIEDLER, 1976). It is a specialised predator feeding mainly on small crabs and fish (ADAMICKA, 1973, WHITEHEAD *et al.*, 1986, GERKING, 1994, HUET *et al.*, 1999). Its digestive tract is typical of carnivores, with a large, muscular and very distensible stomach, several long pyloric caeca and a relatively short intestine (MIKHAIJENKO, 1973, PROTASOV & KRUMIN', 1973, LUTHER & FIEDLER, 1976).

*Sparisoma cretense* (Teleostei: Perciformes: Scaridae) is a herbivorous scarid associated with coral reefs, with a geographical distribution that extends from the Mediterranean Sea to the west coast of Europe and Africa from Portugal to Senegal, in shallow waters to about 50 m (Table 1) (TORTONESE, 1975, WHITEHEAD *et al.*, 1986, PETRAKIS & PAPAConstantinou, 1990). It is a commercially important species only for the small-scale fisheries in the Canary Islands, the Azores, the Mediterranean, and Kenya (NZIOKA, 1984, WHITEHEAD *et al.*, 1986, GONZALEZ *et al.*, 1995, 1996, FALCON *et al.*, 1996, OTERO & GALEOTE, 1996, THORSEN *et al.*, 2000, MORATO *et al.*, 2001).

*S. cretense* is a specialised herbivore with fused teeth on each jaw forming a sharp-edged plate, a very long intestine, a pharyngeal mill, featuring an extremely complex and unique feeding mechanism (WHITEHEAD *et al.*, 1986, BULLOCK & MONOD, 1997). It is reported to feed on algae and *Posidonia*, as well as on small crustaceans and molluscs (TORTONESE, 1975). Table 1 presents some general information regarding the two examined species.

There is very little information available for *S. cretense* and its feeding habits, especially since most other parrotfish species are associated with coral reefs. The same lack of information is true for *U. scaber*. Furthermore, information on digestive carbohydrases of other marine species (e.g. MACDONALD, 1986, UYS and HECHT, 1987, SABAPATHY & TEO, 1993, MUNILLA-MORAN & SABORIDO-REY, 1996, HIDALGO *et al.*, 1999, PAPOUTSOGLU & LYNDON, 2003, unpublished data) offer contradictory information and use different methodology to exhibit carbohydrase activity, making comparison difficult.

The aim of the present study is to present information concerning the digestive activity and capacity of  $\alpha$ -amylase for these species with completely different feeding strategies, as well as the main location of carbohydrate digestion in their digestive tract. This

**Table 1**  
Descriptive information regarding *S.cretense* and *U.scaber* (from Tortonese, 1975, Luther and Fiedler, 1976, Whitehead *et al.*, 1986, Petrakis and Papaconstantinou, 1990)

Species	<i>Sparisoma cretense</i>	<i>Uranoscopus scaber</i>
<b>Size</b>	to 50 cm SL, usually 20-30 cm	to 35 cm SL, usually 20-25 cm
<b>Habitat</b>	on rocky and sandy shores down to 30 m	Benthic species, on sandy or muddy bottoms, burrowing in the sediment, only the eyes being above the level of the bottom. On the continental shelf and upper slope between 15 and 400 m depth
<b>Reproduction</b>	August-October	Between April and August
<b>Food</b>	Endolithic and crustose coralline algae ( <i>Corallina</i> ), epilithic algae and small invertebrates	Mainly crabs and fishes
<b>Distribution</b>	in the Mediterranean and from Portugal and the Azores southwards to Senegal	Atlantic coasts of Europe and Africa from Bay of Biscay, Portugal to Morocco. Common in the Mediterranean and Black Sea. Elsewhere, rare along the coast of Senegal

information could improve on current knowledge of the biology of two species commercially important for local fisheries along the Mediterranean. Furthermore, it would enhance current knowledge of the physiology of fish nutrition and perhaps assist in understanding these processes in other, more valuable species for aquaculture. *U. scaber* is a marine carnivore that shares many characteristics with marine carnivorous species currently reared intensively. The possibility of using the stargazer alimentary tract, especially its pyloric caeca as a model system is explored, since the role of pyloric caeca has not been fully clarified at present, but there is a general belief it is quite important towards carbohydrate digestion. On the other hand, *S. cretense* is a marine herbivore that is thought to digest and utilise carbohydrates very efficiently, and there has been an effort towards a more extensive use of carbohydrates and production of more cost-effective artificial diets in the last twenty years, in order to benefit from their protein-sparing effect.

## Materials and Methods

### Experimental Animals

The animals (n=10 for each species) 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. The digestive tracts were stored in dry ice and analysed for carbohydrase activity. *S. cretense* individuals ranged from  $64.5 \pm 26.8$  g weight and  $15.90 \pm 1.7$  cm SL. According to the detailed study by PETRAKIS & PAPAConstantinou (1990) the above length corresponds to fish aged (2) two years old. *U. scaber* ranged from  $212.0 \pm 133$  g and  $23.02 \pm 4.1$  cm SL. Most of the animals had a full gut (although there were also starved specimens). Stomach contents for *U. scaber* included whole fish (possibly *Maena maena*).

Tables 2-4 describe the different morphometric characteristics of the two examined species. It is important to note the absence of pyloric caeca and stomach in *S. cretense*.

### Dissection and Analysis

Most animals had a full gut, so tissues were rinsed with saline solution before analysis. Dissection of gut was applied in ice. Gut was distinguished to anterior and posterior intestine (KAPOOR *et al.*, 1975, GLASS *et al.*, 1987, HIDALGO *et al.*, 1999), stomach and pyloric caeca (for *U. scaber*), was weighed, homogenised (all procedures in ice), centrifuged, and the supernatant was used to produce samples diluted ten-fold (H/10), which were

**Table 2**

**Body weight (BW), standard length in cm (SL), anterior, posterior and whole intestine weight (HIW, LIW, IW), pyloric caeca weight, number and dimensions (in cm) (PCW, PCN) stomach and whole gut weight (SW, GW) (all in g), in *U.scaber* and *S.cretense***

(n=10)	BW	SL	HIW	LIW	IW	PCW	PCN	SW	GW
<i>U.scaber</i>	$212.00 \pm 132.6$	$23.02 \pm 4.1$	$0.62 \pm 0.5$	$0.83 \pm 0.8$	$1.45 \pm 1.2$	$1.18 \pm 1.1$	$11.7 \pm 0.7$ range: 11-13 length: $2.53 \pm 0.5$ width: $0.44 \pm 0.1$	$4.46 \pm 2.9$	$7.09 \pm 5.1$
<i>S.cretense</i>	$64.50 \pm 26.8$	$15.90 \pm 1.7$	$1.30 \pm 0.6$	$2.44 \pm 1.5$	$3.74 \pm 2.0$	-	-	-	$3.74 \pm 2.0$

**Table 3**

**Anterior, posterior and whole intestine length, gut length (HIL, LIL, IL, GL, in cm) and intestinal to standard length ratio (IL/SL) in *U.scaber* and *S.cretense***

	HIL	LIL	IL	GL	IL/SL
<i>U.scaber</i>	$5.30 \pm 1.1$	$13.35 \pm 4.7$	$18.65 \pm 5.5$	$21.47 \pm 5.1$	$0.8 \pm 0.1$
<i>S.cretense</i>	$5.90 \pm 1.1$	$25.70 \pm 10.5$	$33.70 \pm 9.0$	$36.30 \pm 9.2$	$2.13 \pm 0.4$

**Table 4**  
**Anterior, posterior and whole intestine, as well as pyloric caeca and stomach % weight proportion (HIWP, LIWP, IWP, PCWP, SWP), and digestive somatic index (DSI:GW/BW) in *U. scaber* and *S. cretense***

	HIWP	LIWP	IWP	PCWP	SWP	DSI
<i>U. scaber</i>	8.7±2.9	11.1±3.1	19.8±5.6	15.6±3.1	64.6±7.9	3.30±0.6
<i>S. cretense</i>	36.3±7.5	63.7±7.4	100			5.94±1.0

stored at -40° C. H/10 samples were further diluted five/ten-fold to make H/50-H/100 samples (whichever appropriate), which were used for the analysis.

#### *Determination of $\alpha$ -amylase activity*

The activity of  $\alpha$ -amylase was determined using Phadebas Amylase Test tablets based on the method of CESKA *et al.* (1969). Activity of  $\alpha$ -amylase was expressed as mmoles glucosidic linkage/g/tissue/min.

#### *Statistical analysis*

The digestive carbohydrase assays were performed in order to investigate whether the examined species differ in  $\alpha$ -amylase activity and capacity, both in whole gut and different gut sections. In statistical terms the null hypothesis is that the values between different gut sections in each species, as well as between different species are equal. One-way analysis of variance (one-way ANOVA) was performed between whole gut activities and capacities using STATGRAPHICS for Windows 2.1. Significance between different species was investigated at the 95% confidence level.

## **Results**

Specific carbohydrase ( $\alpha$ -amylase) activity in the examined species is mainly expressed in mmoles of glucosidic linkage hydrolysed per g tissue per min, as specified by CESKA *et al.* (1969) and as capacity in U/organ or organ section (i.e. per g. activity x the tissue weight), in order to describe the capacity of each section to hydrolyze sugars. Finally, results are presented as activity per g of body weight, in

order to offer a more practical expression of specific carbohydrase activity and indicate the capacity of the animal to assimilate sugars.

#### *Biometric characteristics*

Tables 2-4 indicate that the ratio of intestinal to standard length for *S. cretense* is significantly higher, more than 2.6 times longer than in *U. scaber*. Therefore, the relative length of the intestine for *S. cretense* is significantly higher, reflecting its herbivorous feeding type (KAPOOR *et al.*, 1975). The proportion of different sections of the gut also differs between the two species. The stomach takes up more than 64% of total gut weight for *U. scaber*, and the intestine less than 20%, while in *S. cretense* the stomach is absent and the intestine takes up all of its gut (especially posterior intestine, comprising more than 63% of its gut tissue).

#### *Activity and capacity of $\alpha$ -amylase*

Table 5 demonstrates comparative values for per g digestive tract (intestine for *S. cretense*, intestine and pyloric caeca for *U. scaber*)  $\alpha$ -amylase activity (PGA, in mmoles glucosidic linkage/g digestive tract/min), capacity (per section of the digestive tract, CA), relative capacity per g body weight (CAB), and ratio of PGA to gut length. All values were significantly higher for *S. cretense*.

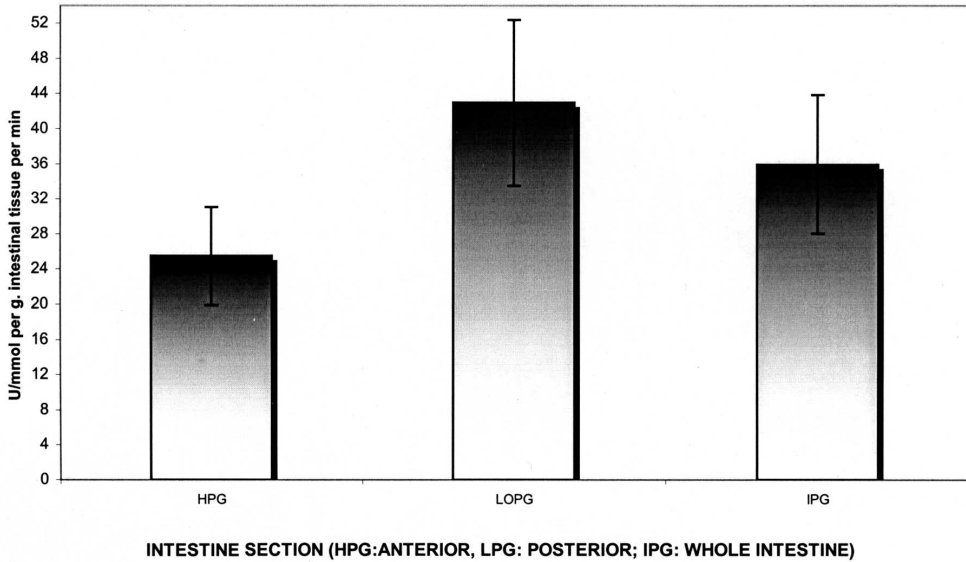
Figure 1 exhibits  $\alpha$ -amylase activity in different portions of the intestine of *S. cretense*. Posterior intestinal activity is higher than anterior intestinal one, but not significantly due to high variance.

Figure 2 presents  $\alpha$ -amylase activity in different gut sections of *U. scaber* (anterior and

**Table 5**

**Comparative values per g digestive tract (intestine for *S.cretense*, intestine and pyloric caeca for *U.scaber*) for  $\alpha$ -amylase activity (PGA, in U/ml), capacity (of the digestive tract, CA) and capacity per g body weight, and ratio of PGA to gut length in *S.cretense* and *U.scaber***

	<i>S.cretense</i>	<i>U.scaber</i>
Per g. digestive tract activity (PGA)	35.9±22.1	0.202±0.04
Capacity of the digestive tract (CA)	125.4±82.3	0.48±0.29
Capacity per g. body weight (CAB)	2.07±1.1	0.002±0.001
Ratio PGA/gut length	0.990±0.49	0.009±0.003



**Fig. 1:** Alpha-amylase activity in anterior, posterior and whole intestine of *S.cretense*.

posterior intestine and pyloric caeca). Anterior intestinal activity is significantly higher than posterior intestinal activity which is lowest, but not from whole intestinal and pyloric activity, which are similar.

Figure 3 demonstrates the activity of  $\alpha$ -amylase in different sections of the pyloric caeca of *U. scaber*: proximal to the pylorus (P1), middle portion (P2), distal portion (P3) and average values (P). There is no significant difference in activity between the different pyloric caeca sections.

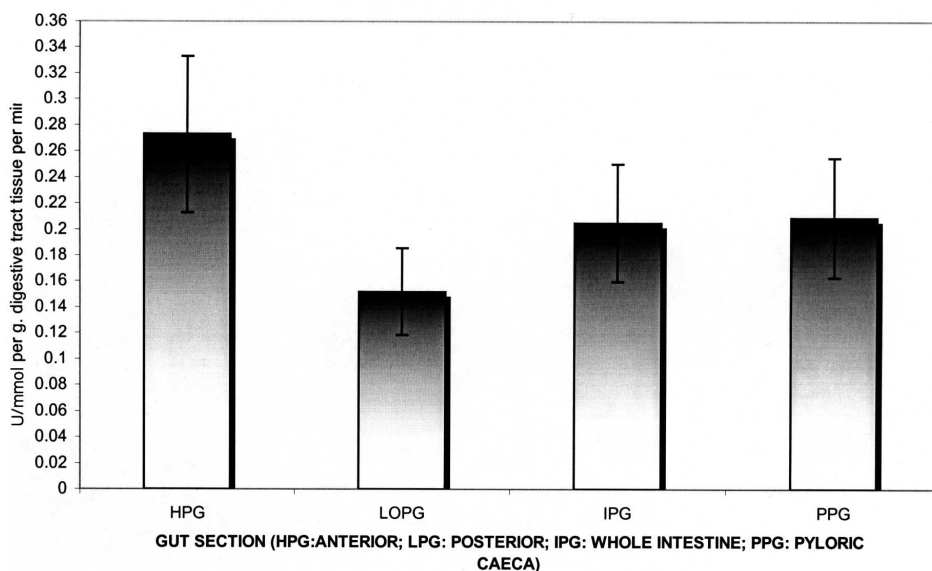
Table 6 presents the capacity of each part of the gut to hydrolyse amylase in proportion to the total gut capacity. Although anterior

**Table 6**

**Relative percent contribution of different gut sections to whole gut  $\alpha$ -amylase capacity in *S.cretense* (HCP: anterior intestine relative capacity, LCP: posterior intestine relative capacity) and *U.scaber* (PCP: pyloric caeca relative capacity)**

	<i>S. cretense</i>	<i>U. scaber</i>
HCP	28%	31%
LCP	72%	25%
PCP	-	44%

intestine proportion is similar, posterior intestine is significantly higher for *S. cretense*, taking up over 72% of total capacity, while pyloric caeca take up more than 44% for *U. scaber* and posterior intestine less than 25%.



**Fig. 2:** Alpha-amylase activity in different gut sections (anterior -HPG, posterior-LPG and whole intestine-IPG, and pyloric caeca-PPG) of *U.scaber*.

#### *Effect of body weight on $\alpha$ -amylase activity and capacity*

Table 7 exhibits the significance of the effect of body weight on  $\alpha$ -amylase activity and capacity in *S. cretense* and *U. scaber* measured at 37°C. Values for both species are significantly important ( $p < 0.05$ ) only for capacity (CA).

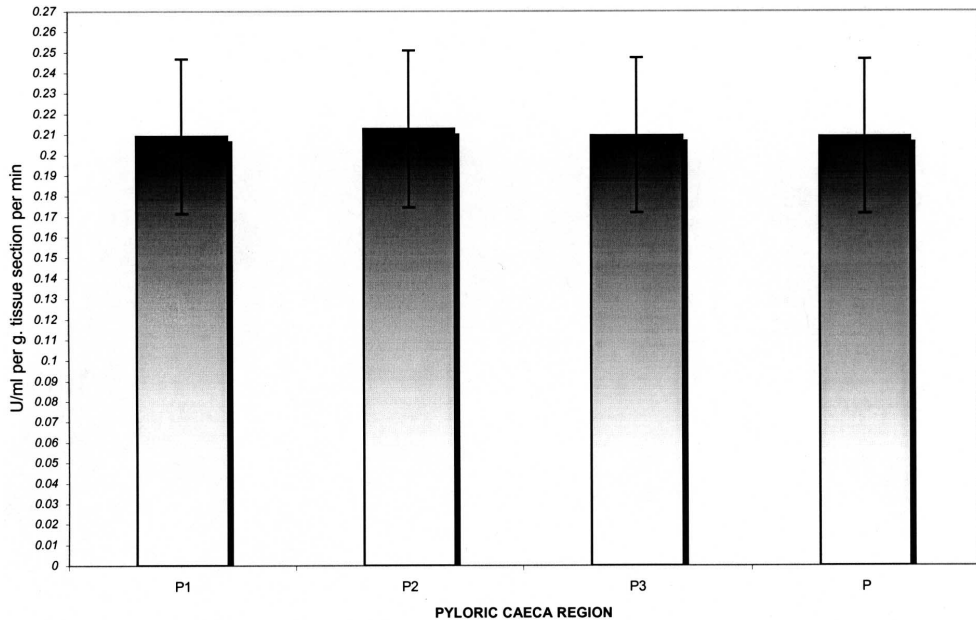
### **Discussion**

In general, it can be said that the reported values for  $\alpha$ -amylase activities of the two examined species confirm that *S. cretense* demonstrates an increased potential for carbohydrate digestion. The low values obtained for *U. scaber*, in conjunction with the anatomy of its digestive tract reflect that it probably mainly relies on protein digestion for its growth.

Values from Tables 2-4 demonstrate the differences in digestive tract anatomy and the different feeding strategies of the two species. *U. scaber*, a typical benthic carnivore (piscivore), has a short intestine, large pyloric caeca and a large muscular stomach, which comprises more

than 64% of total gut weight. The relatively large and long, as well as rigid pyloric caeca make *U. scaber* an ideal species for analysis, in contrast to e.g. salmonids which may have as many as 50-60 delicate and small pyloric caeca. In contrast, *S. cretense* has a long and thin intestine, especially at the posterior part, comprising more than 63% of its gut tissue, no stomach or pyloric caeca. The ratio of intestinal to standard length for *S. cretense* is significantly higher, more than 2.6 times longer than in *U. scaber*. For *U. scaber* the value of 0.82 is typical for carnivorous fish, such as salmon, while the significantly higher value of 2.13 for *S. cretense* is characteristic of herbivorous and detritivorous species (KAPOOR *et al.*, 1975, JOBLING, 1995).

From Figure 1 it becomes evident that *S. cretense* has significantly higher activity than *U. scaber*. This activity is comparable to species such as tilapia or carp (HIDALGO *et al.*, 1999, PAPOUTSOGLU & LYNDON, 2003, unpublished data), and demonstrates the importance of carbohydrates as a food source for this species. On the contrary, *U. scaber* demonstrates very low activity and capacity for



**Fig. 3:** Alpha amylase activity in different regions of pyloric caeca of *U. scaber*. (P1: proximal; P2, middle; P3, distal region to the pylorus; and P, average pyloric caecal activity).

**Table 7**  
**Significance of the effect of body weight (BW) on  $\alpha$ -amylase activity (PGA), capacity (CA) and relative capacity per g. body weight (CAB) in *Sparisoma cretense* and *Uranoscopus scaber* measured at 37°C.**

	P value of regression of $\alpha$ -amylase PGA, CA and CAB against BW	
	<i>Sparisoma cretense</i>	<i>Uranoscopus scaber</i>
PGA at 37°C	0.191-	0.289-
Capacity (CA) at 37°C	0.0010* +	0.0011* +
CAB at 37°C	0.268-	0.242-

\* denotes significance at 0.05 level or more

carbohydrate digestion, even when compared to other (perhaps non-highly) carnivorous species, such as *S. aurata*, *D. labrax*, rainbow trout or salmon (MUNILLA-MORAN and SABORIDO-REY, 1996, HIDALGO *et al.*, 1999, PAPOUTSOGLU and LYNDON, 2003, unpublished data). In fact, the values obtained are comparable to those observed for deep-water, cold-water benthic carnivores such as halibut, *Hippoglossus hippoglossus*, turbot, *Scophthalmus maximus* and spotted wolffish, *Anarhichas minor* (MACDONALD, 1986, MUNILLA-MORAN and SABORIDO-REY,

1996, HIDALGO *et al.*, 1999, PAPOUTSOGLU and LYNDON, 2003, unpublished data). It is obvious that this species relies heavily on protein and lipid rather than carbohydrate digestion for its metabolic needs.

When comparing the ratio of activity to digestive tract length of each species (Table 5), it is apparent that the significantly higher value obtained for *S. cretense* is more than 100-fold higher than that for *U. scaber*.

The calculation of activity is primarily made in order to compare absolute values per g tissue weight/min to the activity of other species



(HIDALGO *et al.*, 1999). However, the calculation of capacity is mainly attempted in order to offer a more practical way to express the digestive enzyme potential of a species, as it can present an indication of the quantity of a given nutrient the animal can cope with by means of digestion. For example, calculated values for  $\alpha$ -amylase capacity per g body weight of *S. cretense* leads to a value of 2.07 and for *U. scaber* it is 0.002 (mmoles of glucosidic linkage broken for each g body weight per min). This could be a useful value to know in order to estimate how much, e.g. starch is likely to be sufficiently digested when included in artificial diets for a given species, or even how much of its natural diet is the animal likely to consume in order to obtain the required amount for its growth, given the fact that one could calculate the protein content of its natural diet, i.e. algae for this species. In any case, it has to be noted that the obtained value is a mere approximation as, firstly, it is calculated *in vitro* at 37°C, and secondly, 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. It has been shown that generally the performance of digestive hydrolases is reduced at realistic temperatures such as the ones encountered by examined species (UYS & HECHT, 1987, KUZ'MINA and KUZ'MINA, 1991, HIDALGO *et al.*, 1999, PAPOUTSOGLOU & LYNDON, 2003, unpublished data).

From Figures 1 and 2 it is apparent that each section of the intestine of *S. cretense* has significantly higher activity than that of *U. scaber*. Furthermore, the highest activity in *S. cretense* occurs in the posterior intestine, while in *U. scaber* in the anterior portion of the intestine. The pyloric caeca of *U. scaber* have an activity comparable to that of the whole intestine.

The capacity of  $\alpha$ -amylase in different gut sections between the two species (Table 6) demonstrates the importance of the posterior intestine for *S. cretense* (72% of total capacity) and the pyloric caeca (44% of total capacity)

for *U. scaber*. The anterior intestine, in both compared species, supplies approximately 30% of total capacity.

There is no conclusive evidence to support that  $\alpha$ -amylase activity (PGA) and CAB is or is not affected by the size of the animals (Table 7). A negative effect of size against activity has been generally reported (HIDALGO *et al.*, 1999). A negative trend is also apparent in the present study. With regard to capacity against body weight, there is a positive correlation, bearing in mind the fact that capacity is calculated by multiplying activity with the weight of the corresponding tissue, which increases as the animal increases in size.

The pyloric caeca of *U. scaber* are relatively few (approx. 12) and large enough to calculate individual pyloric caeca  $\alpha$ -amylase activities, or even activity in different sections of the caeca themselves (Fig. 3), as well as to identify the different carbohydrases or other hydrolases present. Furthermore, they are especially rigid and long (GENTILE *et al.*, 1989). Therefore, *U. scaber* could perhaps be used as a model species in order to obtain important information about the role of pyloric caeca in other, more significant species for aquaculture, where several problems (small sample size, mesentery fat contamination, large number of very delicate pyloric caeca) hinder efforts to evaluate the significance of pyloric caeca towards digestion (and absorption) as well as their role and significance in relation to teleost feeding type/habits (BUDDINGTON & DIAMOND, 1987, BUDDINGTON & HILTON, 1987, BUDDINGTON *et al.*, 1987, BUDDINGTON *et al.*, 1997).

GENTILE *et al.* (1989) reported that *U. scaber* pyloric caeca are very muscular. The *lamina propria* and epithelium form villi which protrude into the lumen and the villi are covered with columnar epithelium and goblet cells, which are more numerous in *U. scaber*. These data support our view that the pyloric caeca of *U. scaber* offer a large surface area for digestion of nutrients.

In *U. scaber*,  $\alpha$ -amylase activity is not significantly higher in the caeca, while in other carnivores (e.g. *O. mykiss*, *S. aurata*, *D. labrax*) the caeca exhibit the highest carbohydrase activity (PAPOUTSOGLU & LYNDON, 2003, unpublished data). Furthermore,  $\alpha$ -amylase activity between pyloric caeca regions in *U. scaber* does not differ significantly, perhaps indicating the lack of a specialised region of attached  $\alpha$ -amylase, and therefore a digestive process taking place in the caeca chyme.

The exhibited potential of *S. cretense* for efficient carbohydrate digestion, could be combined with additional data that demonstrate not only high activity in other specific carbohydrases, but also increased potential for protein digestion for *S. cretense* (PAPOUTSOGLU & LYNDON, 2003, unpublished data). It could be argued, therefore, that omnivorous and herbivorous species may play a more important role in the direction that intensive and semi-intensive marine fish rearing will follow in the future.

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