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Influence of season, environment and feeding habits on the enzymatic activity of peptidase and β-glucosidase in the gastrointestinal tract of two Siluriformes fishes (Teleostei)

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ABSTRACT. The enzymatic activities involved in the digestion of proteins and carbohydrates were compared among three organs of the digestive track of two Siluriformes fish species, and between two areas: a reservoir, and an area downriver of it. Our aim was to test the hypothesis that the digestive organs of species with varied feeding habits have different enzymatic activities, and that the enzymatic activity differs among seasons and environmental conditions. The iliophagous/herbivorous species *Hypostomus auroguttatus* Kner, 1854 had higher trypsin-like, chymotrypsin-like peptidase and β-glucosidase activity in the intestine when compared with the omnivorous species *Pimelodus maculatus* Lacepède, 1803, whereas the latter had more hepatic trypsin-like activity than the former. The peak of activity of the three enzymes in *H. auroguttatus* was recorded in the winter and spring. On the other hand, *P. maculatus* tended to have the more prominent peptidase and β-glucosidase activity in the summer, and the smallest in the winter. The intestine of *H. auroguttatus* had higher enzymatic (trypsin, chymotrypsin and β-glucosidase) activity than the stomach and the liver. For *P. maculatus*, the highest β-glucosidase activity was found in the liver. The enzymatic activity of *H. aurogutattus* did not differ between lotic and lentic systems, whereas *P. maculatus* had comparatively higher stomach and hepatic trypsin levels and hepatic chymotrypsin-like activities in the reservoir than down in the river. These findings indicate that, in *H. auroguttatus*, most digestive activity occurs in the intestine, which is long and adapted for the digestion of bottom-river vegetable matter and detritus. The seasons and the type of the system (lentic vs. lotic) seem to affect the enzymatic activity for these two species differently, a likely consequence of their different lifestyles.

KEY WORDS. Enzymatic activity; fishes; physiology; reservoirs; Siluriformes; peptidases; β-glucosidase.

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, Kuz'mina *et al.* 1996, Alarcón *et al.* 2001). A comparative study of the activity of these digestive peptidase and glycosidase enzymes can elucidate how different species use proteins and carbohydrates, and how they deal with variations in the availability of those nutrients among seasons and different habitats (Hidalgo *et al.* 1999).

In general, herbivores have higher levels of glycosidases than carnivores, which in turn have higher peptidase activity; this has been attributed to their need to digest food rich in carbohydrates and proteins, respectively (Kuperman & Kuz'mina 1994, Kuz'mina *et al.* 1996, Chan *et al.* 2004). The food preferences of fishes were established during the course of their adaptive radiation and the colonization of new habitats. This process required a broad array of different digestive mechanisms to successfully exploit the variety of foods available in an ever-changing environment (Dabrowski & Portela 2006, López-Vásquez *et al.* 2009).

The most important enzymes for digestion are peptidases (Chakrabarti *et al.* 1995, Castillo-Yáñez *et al.* 2006, Jun-sheng *et al.* 2006, Klomklao *et al.* 2007, Bougatef *et al.* 2010), the lipases (Natalia *et al.* 2004, Jun-sheng *et al.* 2006) and enzymes that hydrolyze carbohydrates such as amylases, maltases, chitinases and glucosidases (Kuz'mina *et al.* 1996, Papoutsoglou & Lyndon 2006). An important aspect of this is the distribution of enzymes in the organs involved in the biosynthesis of digestive

activities (Buddington & Diamond 1986, Bairagi *et al*. 2002, Bougatef *et al*. 2010), which can change among species, seasons and environmental conditions.

It is currently believed that the activity of digestive enzymes in fishes is strongly correlated with diet. Several studies have found a correlation between feeding habits and α-amylase activity, and have shown that herbivorous and omnivorous fishes have higher amylase activity than carnivorous fishes (Kuz'mina *et al*. 1996, Hidalgo *et al*., 1999, Fernandez *et al*. 2001, Drewe *et al*. 2004, Horn *et al*. 2006, Al-Tameemi *et al*. 2010). Other studies, for instance that of Chakrabarti *et al*. (1995), have failed to find such correlation.

Tropical Siluriform fish species are among the best adapted ones to use the food associated with the bottom of freshwater systems. Lundstedt *et al*. (2004) reported that the peptidase activity in the stomach of the long lived Siluriform *Pseudoplatystoma corruscans* Spix & Agassiz, 1829 is high enough to allow the digestion of proteins from the diet. Corrêa *et al*. (2007) found chymotryopsin and trypsin activity in the intestine and in the pyloric caeca of the black-finned pacu *Colossoma macropomum* (Cuvier, 1816), but such activity was not present in stomachs that had an acid peptidase. Concerning the presence of glycosidases, Nelson *et al*. (1999) found that the wood-eating catfish *Panaqolus maccus* (Schaefer & Stewart, 1993) has enzymes that hydrolyze cellulose and hemicellulose. Also, the intestine of some loricariid species is capable of digesting carbohydrate fibers (Fuji & Hahn 1991, Nelson *et al*. 1999).

Different environmental conditions and feeding habits may affect the activity of the digestive enzymes in fish species of the same family, even if they live in the same freshwater system. Two Siluriform fish species widely distributed in rivers and reservoirs of southeastern Brazil were chosen for this study: the armored catfish *Hypostomus auroguttatus* Kner, 1854 and the long-whiskered catfish *Pimelodus maculatus* Lacepède, 1803. *Hypostomus auroguttatus* is a benthic iliophagous/herbivorous species closely associated with the rocky substrate. Individuals adhere to it using their ventral lips (oral papillae), and feed by grasping the vegetal organic matter and "microalgae film" associated with the rocky substrate. Such characteristics probably enable this species to have one of the largest intestines among the teleosteans, with the intestines of some species reaching about fifteen times their individual length. This suggests that the digestive process takes a long time (Nelson 2002), and might depend on the presence of glycosidase enzymes to digest the carbohydrates. *Pimelodus maculatus* is also a benthic species, but with comparatively lesser dependency on the substratum, being capable to perform limited reproductive seasonal migration to spawn (Dei Tos *et al*. 2002). It is an omnivorous species with a tendency for carnivory, having an intestine of almost 1.5 folds the total length of the specimens (Barbosa *et al*. 1998). The enzymatic activity of *P. maculatus* is probably adapted to digest a wide source of foods, mainly of animal origin; therefore, this species is probably more dependent on peptidase enzymes for digestion (Lundstedt *et al*. 2004, Xiong *et al*. 2011).

In this study, the enzymatic activities involved in the digestion of proteins and carbohydrates of the two catfish species mentioned above were compared between each other, among three organs of the digestive tract of the two species, and between two areas: a reservoir, and downriver of it. In the lentic system (reservoir), the environmental variables change more widely than in the more stable lotic (river) system (Soares *et al*. 2008). The continuous and unidirectional water movement of the lotic system enables more stable environmental conditions and generally results in better water quality (Klapper 1998).

Specifically, the enzymatic activity of the two fish species, *P. maculatus* and *H. auroguttatus* were compared with respect to: 1) interspecific differences, considering their different feeding habits; 2) the three organs involved in digestion (stomach, intestine and liver); 3) the four seasons; and 4) environmental conditions. The hypothesis to be tested is whether the activity of enzymes that digest proteins (peptidases) and carbohydrates (glucosidase) in the gastrointetinal tract (including the epithelium) or in the liver change according to the seasons and environmental constraints.

MATERIAL AND METHODS

Study area. The Funil Reservoir (22°30'-22°38'S, 44°32'-44°42'W, 440 m above sea level), built in 1969 to generate electricity, is located in the middle of the Paraíba do Sul River, within the Atlantic Forest biome of Southeastern Brazil (Fig. 1). This reservoir is the largest artificial impoundment on the river, with an area of 40 km^2, maximum depth of 70 m, and water retention time of 10-50 days. The climate is subtropical with monthly mean water temperatures of 18-24°C. Rainfall is greatest in the summer (December-January; 200-250 mm per month) and lowest in the winter (June-August), with less than 50 mm per month (Marengo & Alves 2005). Approximately 1.8 million people live upriver from the reservoir in houses without sewage or with poorly planned sewage systems. This means that the river receives large amounts of pollutants, mainly from domestic and industrial origins (Pinto *et al*. 2006). The high nutrient loads brought into the reservoir creates euthrophication, which results in algal blooms and high productivity (Soares *et al*. 2008). The soil cover around the reservoir is loose due to agriculture practices and the fluctuating water levels that erodes the shoreline, increasing the amount of suspended sediment in the reservoir (Branco *et al*. 2002). By contrast, downstream the reservoir the habitat and water flow are regulated by the hydroelectric power station. In this part of the river there are a variety of substrate types comprised by sand, cobbles and bedrocks. There are also several shelters favored by some small islands and marginal cover comprised by trees, shrubs and grasses. The fishes were collected from these two systems, the Funil Reservoir (lentic system) and the stretch of the Paraíba do Sul downriver of the Funil Reservoir (lotic system).

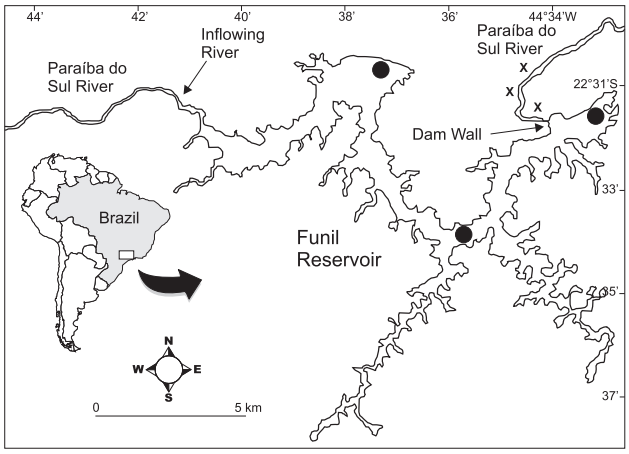


Figure 1. Study area, Funil Reservoir and the main inflowing river (Paraíba do Sul River), indications of the sampling sites in the reservoir (●) and in the stretch downriver of the reservoir (×).

Fish collection and handling. Fishes were collected in three different areas (upper, middle and lower reservoir) encompassing the entire reservoir and along an extension of approximately 5 km in the Paraiba do Sul River immediately downstream of the reservoir (Fig. 1).

Fifty-one adults of *H. auroguttatus* averaging 242.0 ± 4.5 mm s.e. TL (total length) and 152.0 ± 11.6 g s.e. TW (total weight) (23 from the reservoir and 28 from the river) and 49 adults of *P. maculatus* averaging 252 ± 5.4 mm s.e. TL and 184 ± 13.1 g s.e. TW (29 from the reservoir and 20 from the river) were collected in January, April, July and October 2004. In both lentic and lotic systems, we employed four sets of three gill nets (30 m long by 2.5 m height) with different mesh sizes (2.5, 4.5 and 6.5 cm stretched knots) to sample an area of c.a. 900 m^2. The nets were deployed next to the shoreline during the afternoon and retrieved in the following morning, fishing for approximately 15 hours. Voucher specimens of the two species were deposited in the fish collection of the Laboratory of Fish Ecology of the Universidade Federal Rural do Rio de Janeiro.

Tissue preparation for digestive enzyme analysis. Fishes were transported alive to the laboratory, where they were killed by immersion in water at 4°C, and dissected. Their stomachs were removed by cutting it at the end of the esophagus and at the pyloric sphincter. For the intestinal tissue, only the distal part was selected. This corresponded to 200-300 mm from the anus for *H. auroguttatus* (c.a. 1/10 of intestine length) and to 60-150 mm from the anus for *P. maculatus* (c.a. 1/3 intestine length). The liver was also removed and these three organs were washed with Mili-Q™ water and processed in an appropriate manner for the enzymatic analyses.

The internal contents of the intestine and stomach were removed by washing with 0.9% (w/v) saline solution. Then, tissues from the stomach, intestine and liver were weighed and homogenized in saline solution that was kept in ice in a water bath for five minutes. Five grams of tissue were extracted with 1 mL of saline solution. Thus, both tissue and lumen enzymes were extracted. The aqueous extracts were centrifuged at 5,200 g for 60 minutes a 4°C (Skea *et. al*. 2005) in a Sigma 4K15 desktop centrifuge (11156 rotor). Following centrifugation, the supernatant of each tube was gently pipetted into four separate polypropylene vials, and frozen at -80°C with aliquots corresponding to each enzymatic analysis, that is, trypsin-like, chymotrypsin-like and β-D-glucosidase while the fourth aliquot was reserved for the total protein assay.

Chemicals and equipments. The chromogenic substrates used in enzymatic activity assays (benzoyl-DL-arginine-4-nitroanilide, BApNA; benzoyl-L-tyrosine-4-nitroanilide, BTpNA and 4-nitrophenyl-β-D-glucopyranoside, PNPG) were obtained from Sigma-Aldrich (St Louis, USA). The Folin-Ciocalteu reagent used for the protein quantification was a Vetec (Rio de Janeiro, Brazil) product. All other reagents used were of analytic grade and were used without additional purification. Water was always type 1, ultra-pure (Milli-Q™). Records of absorbance for the enzymatic activity assays were performed using a Shimadzu UV 160 A spectrophotometer, with temperature control.

Peptidase activity with trypsin-like specificity. The enzymatic assay was initiated by the addition of 900 µL of a 0.1 mol/L Tris-HCl buffer, pH 8.0, containing 20 mmol/L $CaCl_2$, to a 1.5 mL polypropylene tube. Then, 50 µL of BapNA solution (at 5 mmol/L in dioxane) were added and the mixture was incubated at 37°C during 15 minutes. The tissue samples were then defrosted and the microtubes were centrifuged during 15 minutes at 14,500 g for particulate matter removal. Fifty microliters of the tissue extract were added to the reaction mixture and the enzymes left to work for 10 minutes at 37°C. The reaction was interrupted by the addition of 250 µL of acetic acid solution at 60% (by volume). Then, samples in the microtubes were centrifuged during 15 minutes at 14,500 g and the absorbance values of the supernatants were recorded at 410 nm. After dilution corrections, specific trypsin-like activity was calculated accordingly to the following equation: $V_t = Abs/\epsilon.t.[prot]$, where V_t is the specific trypsin-like activity, *Abs* is the measured absorbance, ϵ is the p-nitroaniline molar absorptivity (7,680 mol/L.cm), *t* is the reaction time and *[prot]* is the protein concentration corresponding to 1 g of tissue extract. To subtract any non enzymatic BApNA hydrolysis, a blank was prepared with water instead of the tissue extract and incubated in the same experimental conditions as the samples containing enzymes.

Peptidase activity with chymotrypsin-like specificity. The enzymatic assay was initiated by the addition of 900 µL of Tris-HCl buffer at 0.1 mol/L, pH 8.0 containing $CaCl_2$ at 20 mmol/L to a 1.5 mL polypropylene tube. Then, 50 µL of the BTpNA solution (at 1.0 mmol/L in dimethyl sulfoxide) were added and the solution was incubated at 37°C for 15 minutes. The tissue samples were then defrosted and the microtubes were centrifuged during 15 minutes at 14,500 g for particulate matter re-

moval. Fifty microliters of the tissue extract were added to the reaction mixture that was incubated for 10 minutes at 37°C. The reaction was interrupted by the addition of 250 µL of acetic acid solution at 60% (by volume). Then, samples in the microtubes were centrifuged during 15 minutes at 14,500 g and the absorbance values were read at 410 nm. After dilution corrections, specific chymotrypsin-like activity was calculated accordingly to the following equation: $V_c = Abs/\epsilon.t.[prot]$, where V_c is the specific chymotrypsin-like activity, *Abs* is the absorbance, ϵ is the p-nitroaniline molar absorptivity (7,680 mol/L.cm), *t* is the reaction time and *[prot]* is the protein concentration corresponding to 1 g of tissue extract. To subtract any non enzymatic BTpNA hydrolysis, a blank was prepared with water instead of the tissue extract, and incubated in the same experimental conditions as the samples containing enzymes.

β-D-glucosidase activity. The enzymatic assay was trigged by the addition of 900 µL of 0.2 mol/L citrate buffer, at pH 6.0, to a 1.5 mL polypropylene tube. Then, fifty microliters of a 4-nitrophenyl-β-D-glucopyranoside solution (30 mmol/L in dioxane) were added and the mixture was incubated at 37°C during 15 minutes. The tissue samples were then defrosted and the microtubes were centrifuged during 15 minutes at 14,500 g for the removal of particulate matter. Fifty microliters of the tissue extract were added to the reaction mixture and the enzymatic action occurred for 10 minutes at 37°C. The reaction was interrupted by the addition of 500 mL of 0.5 mol/L glycine buffer, pH 10.0. Then, samples in the microtubes were centrifuged during 15 minutes at 14,500 g and the absorbance values were recorded at 400 nm. After dilution corrections, specific β-D-glucosidase activity was calculated accordingly to the following equation: $V_g = Abs/\epsilon.t.[prot]$, where V_g is the specific β-D-glucosidade activity, *Abs* is the absorbance, ϵ is the p-nitrophenol molar absorptivity at pH 10 (16,640 mol/L.cm), *t* is the reaction time and *[prot]* is the protein concentration corresponding to 1 g tissue extract, to subtract any non enzymatic 4-nitrophenyl-β-D-glucopyranoside hydrolysis, a blank was prepared with water instead of the tissue extract and incubated in the same experimental conditions as the samples containing enzymes.

Total protein quantity of tissue extracts. The amount of total protein (in micrograms) in the stomach, intestine and liver aqueous extracts was measured by the modified Lowry (1953) methodology. The experiments were conducted with 50 µL of tissue extracts that were incubated with the Lowry reagents for 15 minutes at room temperature. Protein amounts corresponding to 1 g of tissue extracts were calculated. Standard curves of Absorbance as function of protein quantity were obtained by using an aqueous solution of bovine albumin (1.0 mg/mL).

Statistical treatment. The sampling design was planned to test the variation in enzyme activity between the two systems (lentic and lotic environments) as well as between-species, and among seasons and organs. A non-parametric Kruskal-Wallis analysis was used to compare median values followed by a multiple comparisons of mean ranks for all groups to detect the significant ($p < 0.05$) differences.

RESULTS

General remarks

Samples of *H. auroguttatus* had significantly higher peptidase activity, either with trypsin-like specificity or with chymotrypsin-like specificity in the intestine, when compared with samples of *P. maculatus*. Stomachal trypsin-like activity was also higher in *H. auroguttatus* samples. From the liver extracts, only the trypsin-like enzymatic activity differed significantly between the two species, with comparatively higher values obtained for *P. maculatus*. Concerning the β-glucosidase activity, the activities in stomach and intestine were lower for *P. maculatus* than for *H. auroguttatus* (Table I). It is important to point out that both the lumen and the tissue homogenates can contribute to the gastrointestinal enzymatic activity. Nevertheless, the aim of this study was not the investigation of the digestive process, but to assay how the activities of hydrolases vary between the two species and according to abiotic conditions.

Enzymatic activity for *Hypostomus auroguttatus*

The tryptic enzymatic activity in the stomach and intestine was significantly higher than the chymotrypsin-like activ-

Table I. Significant differences for enzymatic activity in organs between *Hypostomus auroguttatus* and *Pimelodus maculatus*, according to non-parametric Kruskal-Wallis test. H and P-significance are shown.

Organ	Enzymatic activity	H (p-significance)	Significant difference
Stomach	Trypsin	6.2 (<0.001)	*H. auroguttatus* > *P. maculatus*
	Chymotrypsin	–	–
	β-glucosidase	44.9 (<0.001)	*H. auroguttatus* > *P. maculatus*
Intestine	Trypsin	44.2 (<0.001)	*H. auroguttatus* > *P. maculatus*
	Chymotrypsin	38.2 (<0.001)	*H. auroguttatus* > *P. maculatus*
	β-glucosidase	38.3 (<0.001)	*H. auroguttatus* > *P. maculatus*
Liver	Trypsin	4.7 (0.03)	*P. maculatus* > *H. aurogutattus*
	Chymotrypsin	–	–
	β-glucosidase	–	

ity in samples of *H. auroguttatus* (Figs 2-4). In the liver, the trypsin-like and the chymotypsin-like activities were of comparable magnitude and significantly lower than the activities for these peptidases observed in the gastrointestinal tract (Fig. 2). The β-glucosidase enzymatic activity was always low in the liver, but it was detectable in the stomach and in the intestine. However, all three enzymatic activities were significantly higher in the intestine when compared with the values obtained for stomach and liver (Table II), apart from a particular case related below.

A peak in the trypsin-like activity in the stomach was observed in the spring ($p < 0.01$) in samples either from the river or from the reservoir (Fig. 2, Table II). The intestinal tryptic activity seems to gradually increase from summer to spring, with significant differences between the higher values in the spring and winter and the lower values in the summer, for fish samples collected both in the river and the reservoir (Fig. 2). On the other hand, in the liver, the tryptic activities varied seasonally only in individuals from the lotic system, with significant higher values in the winter than in the summer and autumn (Table II). Nevertheless, tryptic activities from hepatic extracts were much lower than those from samples of the gastrointestinal tract (Fig. 2, Table II).

The peptidase activity with chymotrypsin-like specificity was comparatively higher in the intestinal extracts than in homogenates from the stomach or liver (Fig. 3, Table II). No significant difference was observed throughout the seasons for the generally low chymotrypsin-like activity from stomach extracts. The intestinal peptidase activity with chymotrypsin-like specificity tended to increase during the winter, when the observed values were comparatively higher than in the summer ($p < 0.05$), both in the lentic and in the lotic environments. The hepatic chymotryptic activity was comparatively higher in the spring for the reservoir and in the winter for the river. Concerning the two different systems, no clear trend was found in the chymotrypsin-like activity between fishes collected in the lentic and in the lotic system (Fig. 3, Table II).

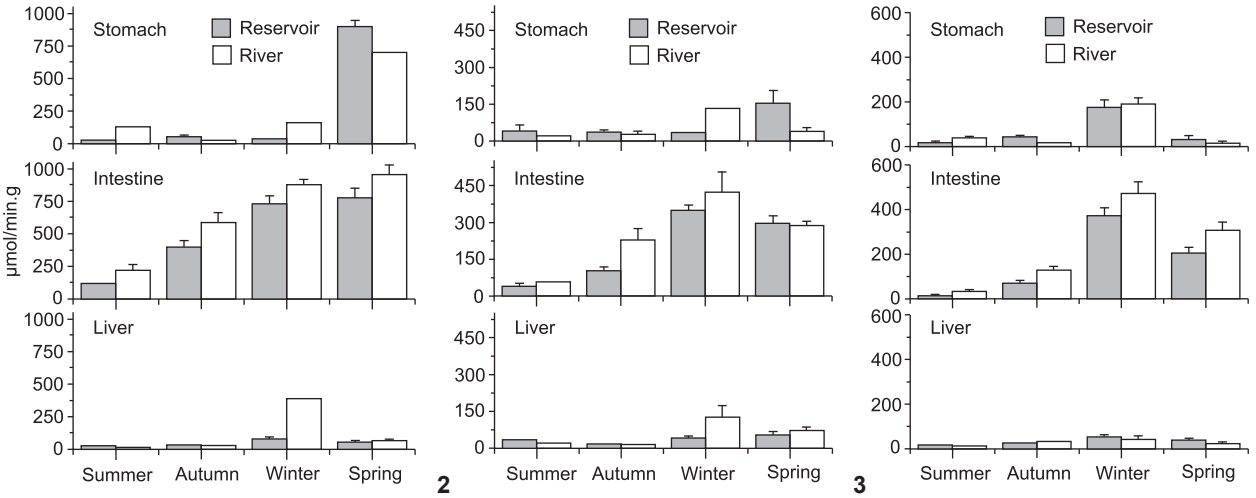
The highest values for the β-glucosidase activity were recorded during the winter for all the three organs, and the lowest values were observed in the summer for the lentic and lotic systems. The only exception was recorded for the hepatic β-glucosidase activity in the river, which did not differ among seasons. No significant difference was found in β-glucosidase activity between the lentic and the lotic systems for any of the three organs surveyed (Fig. 4, Table II).

Enzymatic activity for *Pimelodus maculatus*

The tryptic and chymotryptic enzymatic activities could be detected in all three organs at least in one season, with no overall differences among organs. On the other hand, the β-glucosidase enzymatic activity was significantly higher in the liver and intestine than in the samples from stomach (Table III).

Trypsin-like activities in the stomach, intestine and liver of individuals from the reservoir were significantly higher in the summer than in any other season, apart from a still high stomachal activity in autumn. For individuals from the river, significant seasonal differences were recorded only for hepatic tryptic activities, which had higher values in the summer and lower in the autumn, winter and spring (Fig. 5, Table III). Trypsin activity in stomach and liver extracts were significantly different between the systems, with higher values in the reservoir than in the river in the summer (Fig. 5, Table III), whereas no significant difference between lotic and lentic systems were found for the other seasons.

Seasonal variation for the chymotrypsin-like was observed in some special cases. For samples from stomach, activity dif-



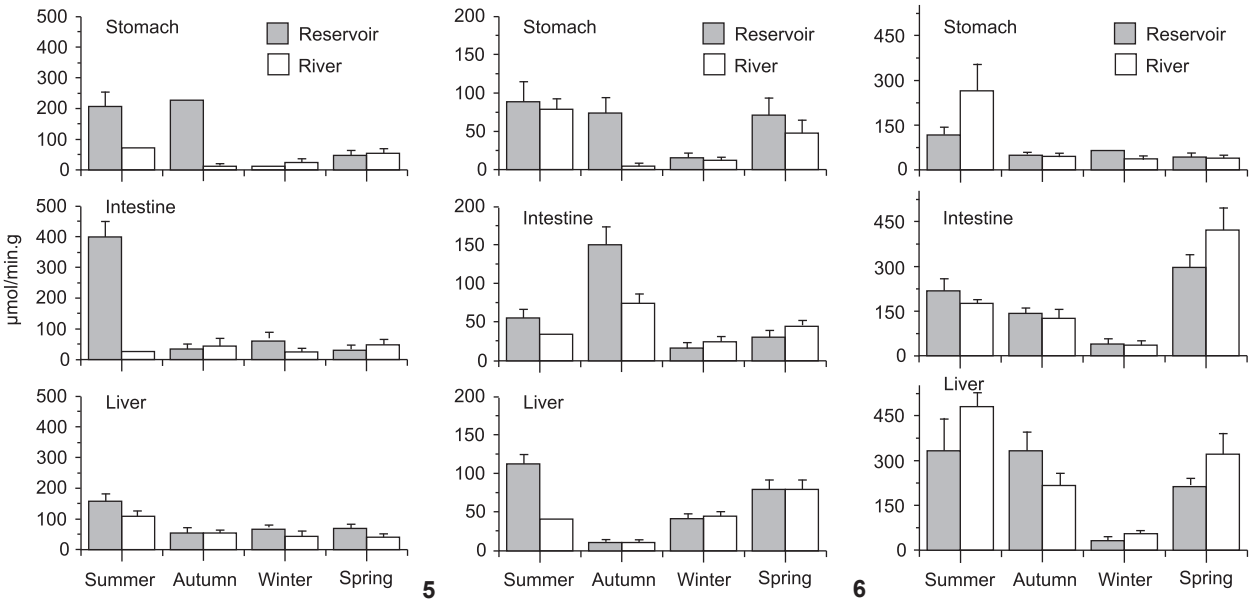
Figures 2-4. Enzymatic activity for trypsin (2), chymotrypsin (3), and (4) β-glucosidase in *Hypostomus auroguttatus* among organs, seasons and the lotic and lentic study systems.

Table II. Significant differences for enzymatic activity of *Hypostomus aurogutattus* among seasons and organs for each system, according to non-parametric Kruskal-Wallis test. H and P-significance are shown.

Enzymatic activity	Organ	Systems	H (p-Significance)	Significant difference
Trypsin	Stomach	Reservoir	17.2 (0.000)	spring > summer
		River	5.9 (0.020)	spring > summer, autumn, winter
	Intestine	Reservoir	21.7 (0.000)	spring, winter > autumn, summer
		River	7.3 (0.050)	spring, winter > autumn > summer
	Liver	Reservoir	19.7 (0.000)	winter > summer, autumn
		River	7.0 (0.060)	–
	Among organs	Pooled systems	47.5 (0.000)	intestine > stomach, liver
Chymotrypsin	Stomach	Reservoir	7.3 (0.060)	–
		River	3.2 (0.360)	–
	Intestine	Reservoir	22.0 (0.000)	winter, spring > autumn, summer
		River	7.3 (0.050)	winter > autumn, spring > summer
	Liver	Reservoir	11.4 (0.000)	spring > autumn
		River	8.2 (0.050)	winter > summer, autumn
	Among organs	Pooled systems	52.0 (0.000)	intestine > stomach, liver
β-glucosidase	Stomach	Reservoir	20.1 (0.002)	winter > spring, summer
		River	7.6 (0.050)	winter > autumn, spring, summer
	Intestine	Reservoir	25.7 (0.000)	winter, spring > autumn, summer
		River	9.3 (0.030)	winter >summer
	Liver	Reservoir	13.7 (0.000)	winter > summer
		River	5.7 (0.100)	–
	Among organs	Pooled systems	33.6 (0.000)	intestine > stomach, liver

Table III. Significant differences for enzymatic activity of *Pimelodus maculatus* among seasons and organs for each system, according to non-parametric Kruskal-Wallis test. H and P-significance are shown.

Enzymatic activity	Organ	Systems	H (p-significance)	Significant difference
Trypsin	Stomach	Reservoir	8.6 (0.03)	summer > winter
		River	7.8 (0.05)	
	Intestine	Reservoir	7.3 (0.05)	summer > autumn, winter, spring
		River	0.6 (0.90)	
	Liver	Reservoir	6.2 (0.05)	summer > autumn, winter, spring
		River	6.3 (0.05)	summer > autumn, winter, springf
	Stomach	Between systems	4.4 (0.04)	reservoir > river
	Liver	Between systems	4.8 (0.03)	reservoir > river
Chymotrypsin	Stomach	Reservoir	6.8 (0.07)	
		River	12.8 (0.01)	summer, spring > autumn, winter
	Intestine	Reservoir	12.6 (0.01)	autumn > winter
		River	11.0 (0.01)	autumn>winter
	Liver	Reservoir	14.9 (0.01)	summer, spring >autumn
		River	12.8 (0.01)	spring > autumn
	Stomach	Between systems	8.4 (0.01)	reservoir > river
β-glucosidase	Stomach	Reservoir	9.2 (0.03)	summer > spring
		River	5.1 (0.05)	summer > autumn, winter, spring
	Intestine	Reservoir	13.4 (0.00)	spring >winter
		River	17.6 (0.00)	spring > winter
	Liver	Reservoir	9.8 (0.02)	summer, autumn > winter
		River	12.9 (0.01)	summer, spring > winter
	Among organs	Pooled systems	24.9 (0.00)	liver, intestine > stomach



Figures 5-7. Enzymatic activity for trypsin (5), chymotrypsin, (6) and β-glucosidase (7) in *Pimelodus maculatus* among organs, seasons and the lotic and lentic systems.

fered only for fishes collected in the river, with comparatively higher values in the summer and spring and lower values in autumn and winter, while intestinal chymotrypsin-like activity was higher in autumn and lower in the other seasons for both the river and the reservoir specimens (Fig. 6, Table III). Chymotrypsin activity in the stomach tended to be higher in the reservoir than in the river, but significant differences were observed only for samples collected in the autumn. The activity of the hepatic chymotrypsin-like enzyme was significantly higher in the summer and spring than in the autumn in the reservoir, whereas in the river a lower enzymatic activity was observed for fishes collected in the autumn when compared with the other seasons.

The β-glucosidase activity in stomach extracts from specimens collected both in the river and in the reservoir was highest in the summer. The activity of the intestinal β-glucosidase, by contrast, was significantly higher in the spring than in the other seasons, with the lowest values in the winter, for both systems (Fig. 7, Table III), while the hepatic β-glucosidase activity was highest in the summer and autumn in the reservoir and in the summer and spring in the river. No significant difference in β-glucosidase between the two systems was observed for any organ extracts.

DISCUSSION

Activities of peptidases with specificity for trypsin and chymotrypsin were consistently higher in stomachs and intestines of the iliophagous/herbivorous species *H. auroguttatus* when compared with the omnivorous *P. maculatus*. These results show that *H. auroguttatus* is capable of efficiently digesting proteins, despite not being a carnivorous fish. In fact, higher levels of peptidase activity in the digestive system of fishes are not necessarily related to their feeding-habits. Also, β-glucosidase activity was higher in the stomach and in the intestine of *H. auroguttatus* than in the gastrointestinal tract of *P. maculatus*. Since β-glucosidase is a component of the cellulolytic complex that catalyses the final step of cellobiose hydrolysis into glucose, its activity is expected to be high in iliophagous/herbivorous species. It is important to point out that the enzymatic activity assayed in this study can be both from the gastrointestinal lumen and from the extracts of epithelial cells. The peptidase activity with chymotrypsin-like and trypsin-like specificities observed at pH 8 in the stomach probably came from the epithelial cells, since these fish species have stomachs with acid digestion in the lumen (Podkowa & Goniakowska-Witaliñska 2003, Santos *et al*. 2007). Nevertheless, the overall enzymatic activity was useful to distinguish two Siluriform species with different feeding habits. The generally higher enzymatic activity of *H. auroguttatus* may partly be due to the larger gastrointestinal tract of this species, which may cause the resting metabolic rate of individuals to be higher than in *P. maculatus* (Cant *et al*. 1996, German *et al*. 2010). However, this must be interpreted with caution since Nelson (2002) reported that the mass-normalized resting metabolism of loricariids does not seem to significantly vary among species.

Enzymatic activities can be either endogenous or come from the associated microbiota. Bairagi *et al*. (2002) suggested that the presence of microorganisms that produce cellulolytic enzymes are associated with the feeding habits of the fish, be-

ing commonly found in herbivorous and more rarely found in carnivorous species. However, until now, little is known about the origin of digestive enzymes in the gastrointestinal tract of fishes. Izvekova (2005) studied peptidases and amylases secreted by bacteria associated with the intestines of fish, and found that the origin of the enzymatic activity in some herbivorous species is rather unclear. German *et al.* (2009) proposed that the low concentration of short-chain fatty acids in either herbivorous or carnivorous Cyprinidae fishes suggest a minor contribution of the microbiota. On the other hand, some workers (Prejs & Blaszezzyk 1977, Lobel 1981, Sugita *et al.* 1997) have found that a great amount of digestive enzymes is produced by the microbiota. Nelson *et al.* (1999) found that species of *Panaque*, known as wood-eating catfish, have digestive cellulase activity produced by the associated microbiota. German & Bittong (2009) reported that both the microbiota and the gut wall contribute to the production of peptidases, glycosidases and lipases.

Wood-eating loricariid species are known to produce high amounts of endogenous trypsin and β-glucosidase, suggesting that such species efficiently hydrolyze proteins and they also digest simple carbohydrates more efficiently than complex polysaccharides such as cellulose and xilanose (German & Bittong 2009). Another important issue is the amount of time it takes for the food to pass through the digestive tract. German & Bitong (2009) suggested that a fast passage favors digestion by endogenous enzymes rather than by the gut's microbiota. The long intestine of *H. auroguttatus* might indicate a fast food transit and that the digestive processes are mainly performed by endogenous enzymes. It has been shown that, in loricacariid catfishes, both the microbiota and the gut wall of the fish contribute to the production of β-glucosidase, but that the enzyme produced by the fish is more efficient (German & Bittong 2009). In the study presented here it was not possible to know the source of the enzyme because of the extraction protocol used. However, the contribution from the microbiota, especially to the digestion of complex carbohydrates, cannot be ruled out.

We found out that the microbiotic fauna of *P. maculatus* is more numerous and has more morphotypes of than that of *H. auroguttatus* (S. Duarte, unpublished data), which could be linked to their different feeding habits, or to the slower food transit in the intestine of *P. maculatus*. This also suggests that the digestion of carbohydrates in *H. auroguttatus* is mainly due to enzymatic activity instead of microbiota, which seems to play a more important hole for the omnivorous species. Furthermore, the intestinal β-glucosidase activity could be underestimated in this study, since we only examined the distal part of the intestine, which might possibly have lower enzymatic activity compared to the proximal part. However, our aim was to find some biochemical marker related to feeding habits or to abiotic conditions instead of performing an investigation of the digestive process. Denovan *et al.* (2010) found that the pattern of β-glucosidase activity oscillated distally in the intestine of the wild-caught *Pterygoplithys disjunctivus* (Weber, 1991), with the gut lumen showing higher activity of this enzyme in the proximal and distal intestine than in the middle intestine, and the gut had higher activity in the middle intestine than in the other parts. Xiong *et al.* (2011) found that peptidase and amylase activities levels in a carnivorous teleost of Tibet, *Glyptosternum maculatumin* (Regan, 1905) were highest in the anterior intestine and stomach and lowest in the middle and posterior intestine. In the study presented here it is clear that protein and carbohydrate digestion begin in the stomach for *H. auroguttatus*. Also, hepatic peptidase and β-glucosidase activities were generally low in this species. In *Pimelodus maculatus* the digestion also begins in the stomach.

Hepatic trypsin-like, chymotrypsin-like and glucosidasic activities are higher in *P. maculatus* than in *H. auroguttatus*. Overall, no significant difference was observed between the lotic and lentic systems ($p > 0.05$), suggesting that the hepatic peptidase and glucosidase activities could not be used as a biochemical environmental marker in opposition to other hepatic enzymes (Ahmad *et al.* 2000). The activities of the digestive enzymes in the gastrointestinal tract can also not be used as a biochemical marker of the environment. Only the activities of trypsin and chymotrypsin-like enzymes in the stomach, and trypsin activity in the liver of *P. maculatus* were significantly different between the two systems, being higher for the reservoir samples. Thus, analysis of *P. maculatus* enzymatic data indicates that stomach peptidase activities and hepatic trypsin activity can be affected by the environment. The enzymatic activity in *H. auroguttatus* samples was unaffected by this parameter. Since only minor differences between individuals from lotic and lentic systems could be observed, further studies are necessary to evaluate those enzymatic activities as biomarkers of environmental quality.

Higher peptidase and glucosidase activity during the winter and spring were found in *H. auroguttatus* samples, especially in intestinal extracts. By contrast, in *P. maculatus* samples, the enzymatic activities peaked in the summer. *H. auroguttatus* is a resident, relatively sessile and territorialist species with restricted capacity for displacement along its distribution area. Such bottom dwelling iliophagous/herbivorous species found better environmental conditions to feed on the algal "film" on rocks of the Funil reservoir during the winter (dry season) when the increased water transparency allows for more intense algal reproduction. Thus, feeding rate and enzymatic activities associated with digestion are expected to increase in the winter, consistent with our data. Conversely, individuals of *P. maculatus* have the ability to perform small range migrations during the summer, when they spawn more intensely (Die Tos *et al.* 2002, Maia *et al.* 2007). This behavior may be associated with the higher enzymatic activity in summer samples of this species.

It is widely known that circadian and seasonal changes in environmental variables, such as dissolved oxygen, pH, temperature and conductivity, vary more widely in reservoirs than

in rivers (Soares *et al*. 2008). Between-habitat differences in enzymatic activity could represent different adaptive digestive and metabolic strategies to cope with variations in environmental conditions. Additionally, biotic interactions are more intense in closed systems, such as lentic systems, than in lotic environments that generally offer more shelters and habitat complexity (Agostinho *et al*. 2004). The reservoir environment could pose more stress to the fish, thereby influencing their enzymatic activity. Consistent, significant differences in enzymatic activity between the reservoir and the stretch of the main river down the reservoir were not observed in samples of of *H. auroguttatus*. There are specific seasonal variations and significant differences between the river and the reservoir, such as a higher chymotryptic activity in samples collected in the reservoir in some cases for *P. maculatus*, but without a general trend. Influences of the environmental condition on fish enzymatic activities have not been yet elucidated. Further studies on this subject are needed to depict patterns and cause-effect relationships.

In conclusion, the activity of three enzyme groups (the peptidases, trypsin and chymotrypsin-like, and the carbohydrases β-glucosidase) were more intense in the stomach and in the intestine of *H. auroguttatus* samples than in *P. maculatus*, but hepatic trypsin-like activity was higher in *P. maculatus* than in *H. auroguttatus*. The most intense enzymatic activity was generally found in the intestine of *H. auroguttatus*, which is probably associated to its iliophagous/herbivorous feeding habits, which require more complex digestion of protein and complex carbohydrates of microalgae. Seasonal variability influences the enzymatic activity of the two species in different ways, with a trend for increasing activity in the winter and spring for *H. auroguttatus*, when this species has better food available, resulting from the increased water transparency and increased production of the "algae film". Conversely, the trend for increasing enzymatic activities during the summer for *P. maculatus* can be associated to more intense reproductive migration in this season.

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LITERATURE CITED

Agostinho, A.A.; L.C. Gomes; S. Veríssimo & E.K.Okada. 2004. Flood regime, dam regulation and fish in the Upper Paraná River: effects on assemblage attributes, reproduction and recruitment. **Reviews of Fish Biology and Fisheries 14**: 11-19.

Ahmad, I.T.; M. Hamid; H. Fatima; S.K. Chand; M. Jain; M. Athar & S. Raisuddin. 2000. Induction of hepatic antioxidants in freshwater catfish (*Channa punctatus* Bloch) is a biomarker of paper mill effluent exposure. **Biochemica et Biophysica Acta 1523**: 37-48.

Al-Tameemi; R.; A. Aldubaikul & N.A. Salman. 2010. Comparative study of á-amylase activity in three Cyprinid species of different feeding habits from Southern Iraq. Turk. **Journal of Fisheries Aquatic Science 10**: 411-414.

Bairagi, A.; K.S. Gosh; S.K. Sen & A.K. Ray. 2002. Enzyme producing bacterial flora isolated from fish digestive tracts. **Aquaculture International 10**: 109-121.

Barbosa, J.M.; M.N. Moraes; A. Ferreira & E.C. Campos. 1988. Aspectos da estrutura populacional da mandiuva *Pimelodus maculatus* Lacepède, 1803 (Osteichthyes, Pimelodidae) na represa Bariri, rio Tietê, Estado de São Paulo. **Boletim do Instituto de Pesca 15**: 123-133.

Bougatef, A.; R. Balti; R. Nasri; K. Jellouli; N. Souissi & M. Nasri. 2010. Biochemical properties of anionic trypsin acting at high concentration of NaCl purified from the intestine of a carnivorous fish: smooth hound (*Mustelus mustelus*). **Journal of Agricultural and Food Chemistry 58**: 5763-5769.

Branco, C.W.C.; M.I.A. Rocha; G.F.S Pinto; G.A. Gômara & R. Filippo. 2002. Limnological features of Funil Reservoir (RJ, Brazil) and indicator properties of rotifers and cladocerans of the zooplankton community. **Lakes and Reservoir Management 7**: 87-92.

Buddington, R.K. & J.M. Diamond. 1986. Aristotle revisited: The function of pyloric caeca in fish. **Proceedings of the National Academy of Science 83**: 8012-8014.

Cant, J.P.; B.W. McBride & W.J. Croom-Jr. 1996. The regulation of intestinal metabolism and its impact in whole animal energetics. **Journal of Animal Science 74**: 2541-2553.

Castillo-Yáñez, F.J.; R. Pacheco-Aguilar; F.L. García-Carreño; M.A. Navarrete-Del Toro & M.F. López. 2006. Purification and biochemical characterization of chymotrypsin from the viscera of Monterey sardine (*Sardinops sagax caeruleus*). **Food Chemistry 99**: 252-259.

Chakrabarti, I.; M.A. Gani; K.K. Chaki; R. Sur & K.K. Misra. 1995. Digestive enzymes in 11 freshwater teleost fish species in relation to food habit and niche segregation. **Comparative Biochemistry and Physiology – Part A 112**: 167-177.

Chan, A.S.; M.H. Horn; K.A. Dickson & A. Gawlicka. 2004. Digestive enzyme activities in carnivores and herbivores: comparisons among four closely related prickleback fishes (Teleostei: Stichaeidae) from a California intertidal habitat. **Journal of Fish Biology 65**: 848-858.

Corrêa, C.F.; L.H. Aguiar; L. Lundstedt & G. Moraes. 2007. Responses of digestive enzymes of tambaqui (*Colossoma macropomum)* to dietary cornstarch changes and metabolic inferences. **Comparative Biochemitry and Physiology – Part A 147**: 857-862.

Dabrowski, K. & M.C. Portela. 2006. Feeding plasticity and nutritional physiology in tropical fishes, p. 155-224. *In*: A.L. Val; V.M.F. Almeida-Val & D.J. Randall (Eds). **The Physiology of Tropical Fishes.** San Diego, Academic Press, 634p.

Dei Tos, C.; G. Barbieri; A.A. Agostinho; L.C. Gomes & H. I. Suzuki. 2002. Ecology of *Pimelodus maculatus* (Siluriformes) in the Corumbá Reservoir, Brazil. **Cybium 26**: 275-282.

Drewe, K.E.; M.H. Horn; K.A. Dickson & A. Gawlicka. 2004. Insectivore to frugivore: ontogenetic changes in gut morphology and digestive enzyme activity in the characid fish *Brycon guatemalensis* from Costa Rican rain forest streams. **Journal of Fish Biology 64**: 890-902.

Fernandez, I; F.J. Moyano; M. Diaz & T. Martinez. 2001. Characterization of á-amylase activity in five species of Mediterranean sparid fishes (Sparidae, Teleostei). **Journal of Experimental Marine Biology and Ecology 262**: 1-12.

Fuji, R. & N.S. Hahn. 1991. Espectro alimentar e relações morfológicas com o aparelho digestivo de três espécies de peixes comedores de fundo do rio Paraná, Brasil. **Revista Brasileira de Biologia 51**: 873-879.

German, D.P. & R.A. Bittong. 2009. Digestive enzyme activities and gastrointestinal fermentation in wood-eating catfishes. **Comparative Bichemistry and Physiology – Part B 179**: 1025-1042.

German, D.P.; D.T. Neuberger; M.N. Callahan; N.R. Lizardo & D.H. Evans. 2010. Feast to famine: The effects of food quality and quantity on the gut structure and function of a detritivorous catfish (Teleostei: Loricariidae). **Comparative Biochemistry and Physiology – Part A 155**: 281-293.

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

Horn, M.H.; A.K. Gawlicka; D.P. German; E.A. Logothetis; J.W. Cavanagh & K.S. Boyle. 2006. Structure and function of the stomachless digestive system in three related species of New World silverside fishes (Aherinopsidae) representing herbivory, omnivory, and carnivory. **Marine Biology 149**: 1237-1245.

Izvekova, G.I. 2005. Hydrolytic activity of enzymes of microflora associated with digestive-transport surfaces of pike intestine and *Triaenophorus nodulosus* (Cestoda, Pseudophyllidea) parasitizing in it. **Journal of Evolutionary Biochemistry and Physiology l41**: 185-193.

Jun-Sheng, L.; L. Jian-Lin & W. Ting-Ting. 2006. Ontogeny of protease, amylase and lipase in the alimentary tract of hybrid Juvenile tilapia (*Oreochromis niloticus* × *Oreochromis aureus*). **Fish Physiology and Biochemistry B 132**: 295-303.

Klapper, H. 1998. Water quality problems in reservoirs of Rio de Janeiro, Minas Gerais and São Paulo. **Interantional Review Hydrobiology 83**: 93-102.

Klomklao, S.; S. Benjakul; W. Visessanguan; H. Kishimura & B. K. Simpson. 2007. Trypsin from the pyloric caeca of Atlantic bonito (*Sarda sarda*): Recovery and characterization. **Journal of Agricultural and Food Chemistry 55**: 45-53.

Kuperman, B.I. & V.V. Kuz'mina. 1994. The ultrastructure of the intestinal epithelium in fish with different types of feeding. **Journal of Fish Biology 44**: 181-193.

Kuz'mina, V.V.; I.L. Golovanova & I.G. Izvekova. 1996. Influence of temperature and season on some characteristics of intestinal mucosa carbohydrases in six freshwater fishes. **Comparative Biochemistry and Physiology – Part B 113**: 255-260.

Lobel, P.S. 1981. Trophic biology of herbivorous reef fishes: alimentary pH and digestive capabities. **Journal of Fish Biology 19**: 365-397.

López-Vásquez, K.; C.A. Castro-Pérez & A.L. Val. 2009. Digestive enzymes of eight Amazonian teleosts with different feeding habits. **Journal of Fish Biology 74**: 1620-1628.

Lowry, O.H.; N.J. Rosbrough; A.L.Farr & R.J. Randall. 1953. Protein measurement with the folin phenol reagent. **The Journal of Biological Chemistry 193**: 265-275.

Lundstedt, L.M.; J.F.B. Melo & G. Moraes. 2004. Digestive enzymes and metabolic profile of *Pseudoplatystoma corruscans* (Teleostei: Siluriformes) in response to diet composition. **Comparative Biochemistry and Physiology – Par B 137**:331-339.

Maia, B.P.; S.M.F. Ribeiro; P.M.Bizzotto; V.Vono & H.P.G Godinho. 2007. Reproductive activity and recruitment of the yellow-mandi *Pimelodus maculatus* (Teleostei: Pimelodidae) in the Igarapava Reservoir, Grande River, Southeast Brazil. **Neotropical Ichthyology 5**: 147-152.

Marengo, J.A. & L.M. Alves. 2005. Hydrological tendencies in the Paraíba do Sul river basin. **Revista Brasileira de Meteorologia 20**: 385-394.

Natalia, Y.; R. Hashim; A. Ali & A. Chong. 2004. Characterization of digestive enzyme in a carnivorous ornamental fish, the Asian bone tongue *Scleropages formosus* (Osteoglossidae). **Aquaculture 233**: 305-320.

Nelson, J.A.; D.A. Wubah; M.E. Whitmer; E.A. Johnson & D. Stewart. 1999. Wood-eating catfishes of the genus *Panaque*: gut microflora and cellulolytic enzyme activities. **Journal of Fish Biology 51**: 1069-1082.

Nelson, J.A. 2002. Metabolism of three species of herbiborous loricariid catfishes: influence of size and diet. **Journal of Fish Biology 61**: 1586-1599.

Papoutsoglou, E.S. & A. R.Lyndon. 2006. Digestive enzymes of *Anarhichas minor* and the effect of diet composition on their performance. **Journal of Fish Biology 69**: 446-460.

Pinto, B.C.T.; F.G. Araújo & R.M. Hughes. 2006. Effects of landscape and riparian condition on a Fish Index of Biotic Integrity in a large Southeastern Brazil river. **Hydrobiology 556**: 69-83.

Podkowa, D. & L. Goniakowska-Witaliñska. 2003. Morphology of the air-breathing stomach of the catfish *Hypostomus plecostomus*. **Journal of Morphology 257**: 147-163.

Prejs, A. & M. Blaszczyk. 1977. Relationship between food and cellulose activity in freshwater fishes. **Journal of Fish Biology 11**: 447-452.

Santos, C.M.S.; T. Duarte; G.L. Souza; T.P. Ribeiro; A. Sales & F.G. Araújo 2007. Histology and histochemical characterization of the digestive tract of *Pimelodus maculatus* (Pimelodidae, Siluriformes) in Funil reservoir, Rio de Janeiro, Brazil. **Iheringia, Serie Zoologia 97**: 411-417.

Skea, G.L.; D.O. Mountfort & K.D. Clement. 2005. Gut carbohydrases from the New Zealand marine herbivorous fishes *Kyphosus sydneyanus* (Kyphosidae), *Aplodactylus arctidens* (Aplodactylidae) and *Odax pullus* (Labridae). **Comparative Biochemistry and Physiology – Part B 140**: 259-269.

Soares, M.C.S.; M.M. Marinho; V.L. M. Huszar; C.W.C. Branco & S.M.F.O. Azevedo. 2008. The effects of water retention time and watershed features on the limnology of two tropical reservoirs in Brazil. **Lakes and Reservoir Management 13**: 257-269.

Sugita, H.; J. Kawasaki & Y. Deguchi. 1997. Production of amylase by the intestinal microflora in cultured freshwater fish. **Letters in Applied Microbiology 24**: 105-108.

Xiong, D.M.; C.X. Xie; H.J. Zhang & H.P. Liu. 2011. Digestive enzymes along digestive tract of a carnivorous fish *Glyptosternum maculatum* (Sisoridae, Siluriformes). **Journal of Animal Physiology and Animal Nutrition 95**: 56-64.

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