ZOOLOGIA 35: e13286
ISSN 1984-4689 (online)
zoologia.pensoft.net

RESEARCH ARTICLE

Composition of gastrointestinal content, protease and lipase activities in summer and winter of four freshwater siluriforms (Teleostei: Actinopterygii) with two different feeding habits

Ana Paula Gottlieb Almeida[1], Everton Luis Zardo[1], Candida Toni[2], Everton Rodolfo Behr[3], Leila Picolli da Silva[3], João Paes Vieira[4], Vania Lúcia Loro[5], Bernardo Baldisserotto[2]

[1]*Programa de Pós-Graduação em Biodiversidade Animal, Universidade Federal de Santa Maria. 97105-900 Santa Maria, RS, Brazil.*
[2]*Departamento de Fisiologia e Farmacologia, Universidade Federal de Santa Maria. 97105-900 Santa Maria, RS, Brazil.*
[3]*Departamento de Zootecnia, Universidade Federal de Santa Maria. 97105-900 Santa Maria, RS, Brazil.*
[4]*Instituto de Oceanografia, Fundação Universidade Federal do Rio Grande. 96203-900 Rio Grande, RS, Brazil.*
[5]*Departamento de Química, Universidade Federal de Santa Maria. 97105-900 Santa Maria, RS, Brazil.*
Corresponding author: Bernardo Baldisserotto (bbaldisserotto@hotmail.com)

http://zoobank.org/001233EB-A34B-4FB1-B25A-8AE3ECFB6C7F

ABSTRACT. The aim of this study was to determine the composition of gastrointestinal content and protease and lipase activities in summer and winter as well as to evaluate the relationship between digestive enzyme activity and centesimal composition of gastrointestinal content and feeding habits of two omnivorous species, *Rhamdia quelen* (Quoy & Gaimard, 1824) and *Pimelodus maculatus* (LaCèpede, 1803), and of two detritivorous species, *Loricariichthys anus* (Valenciennes, 1835) and *Hypostomus commersoni* (Valenciennes, 1836). The activities of pepsin, trypsin, chymotrypsin, and lipase, and the levels of proteins and lipids in the gastrointestinal tract, were evaluated. The enzyme activities were not related to the centesimal composition of gastrointestinal content or feeding habits. This finding could be associated with the variations of nutrient availability over time in the environment, as was observed in the centesimal composition of food ingested by the fish in summer and winter. The analyzed enzymes exhibited a constitutive character in these species; that is, the digestive enzymes are always available in the gastrointestinal tract to digest any food that the fish may find as an adaptation to better utilize the nutrients available in the environment in winter and summer.

KEY WORDS. Chymotrypsin, fish, lipase, pepsin, trypsin.

INTRODUCTION

The digestive enzymes in vertebrates are synthetized and secreted throughout the gastrointestinal tract. Fish have a high versatility in their digestive processes, which may vary according to species, size, age, stage of maturity, temperature, type of food ingested and feeding history. The appropriate synthesis and levels of digestive enzymes may be affected by environmental factors that vary over time (Garcia Carreño et al. 2002, López-Vásquez et al. 2009).

Fish are usually classified according to feeding habits, and it is expected that digestive enzyme activities reflect the feeding habits and the diet of the fish (Fernández et al. 2001, Langeland et al. 2013). Usually, herbivorous fish show higher carbohydrase activities than carnivorous fish, which exhibit the highest levels of proteolytic activities due to higher dietary carbohydrate and protein levels, respectively (Chan et al. 2004). However, most studies dealing with digestive enzyme activities were performed with species raised in fish culture conditions and fed the same diet for at least one month (García Carreño et al. 2002, Ren et al. 2011, Aguilera et al. 2012, Azarm et al. 2012, Leef et al. 2012).

López-Vásquez et al. (2009) studied eight species with different feeding habits collected in the environment and observed that the enzymatic activity differed from the expected result: higher alkaline protease activities were observed in the omnivorous *Osteoglossum bicirrhosum* (Cuvier, 1829) (Osteoglossidae) and the lowest activity was observed in the carnivorous *Cichla monoculus* Agassiz, 1831(Cichlidae). The detritivorous fish

presented higher amylase, maltase, and alkaline protease activities. In contrast, Ushakova and Kuz'Mina (2010) found higher proteolytic activity in typical ichthyophagous species compared to benthophagous species. Solovyev et al. (2014) also found a correlation between feeding habits and digestive enzyme activity.

Understanding the relationship between digestive enzyme activities and centesimal composition of the ingested food by the fish in the environment is important to the comprehension of the feeding biology of species. In addition, previous studies of fish collected in the environment analyzed only the activities of digestive enzymes without evaluating the centesimal composition of ingested food. Four teleost species belonging to two trophic guilds, omnivorous and detritivorous, were chosen for this study. The omnivorous *Rhamdia quelen* (Quoy & Gaimard, 1824) (Heptapteridae) (jundiá) feeds mainly on fish, crustaceans, insects, plant remains, and organic detritus (Gomes et al. 2000); *Pimelodus maculatus* (LaCèpede, 1803) (Pimelodidae) (mandi-amarelo) feeds on insect larvae, micro-crustaceans, algae, and sand grains (Lolis and Andrian 1996). The detritivorous *Hypostomus commersoni* (Valenciennes, 1836) (Loricariidae) (cascudo) feeds mainly on algae, zooplankton, and sediments (Burgess 1989); *Loricariichthys anus* (Valenciennes, 1835) (Loricariidae) (cascudo-viola) feeds on sediments and plant remains (Albrecht and Silveira 2001). The aim of this study was to determine the composition of gastrointestinal content and protease and lipase activities in summer and winter of two omnivorous species, *R. quelen* and *P. maculatus*, and of two detritivorous species, *L. anus* and *H. commersoni*. Different seasons were chosen because food availability and the activities of digestive enzymes can change with the season (Duarte et al. 2013). Based on the results, it was also evaluated if there are relationships between digestive enzyme activity, centesimal composition of gastrointestinal content and feeding habits.

MATERIAL AND METHODS

Adults of four fish species: *R. quelen* (40.7 ± 0.6 cm, 779.3 ± 73.4 g, voucher number: 16-1901) (Fig. 1); *P. maculatus* (29.7 ± 0.2 cm, 234.6 ± 7.0 g, voucher number: 16-1902) (Fig. 2); *L. anus* (31.7 ± 0.7 cm, 165.1 ± 9.7 g, voucher number: 16-1903) (Fig. 3); *H. commersoni* (36.3 ± 2.5 cm, 403.4 ± 70.7 g, voucher number: 16-1904) (Fig. 4) (n = 15 from each species at each season, total n = 30 each species) were collected between 9 am and 4 pm in March 4th, 2013 (summer) and August 5th, 2013 (winter) at two different places in the freshwater site of the São Gonçalo channel, Pelotas, in southern Brazil (IBAMA license number for collection: 10125-2). They were collected using a shrimp trawl deployed for five minutes at a depth between 5 and 8 m. Immediately after collection, fishermen euthanized the fish by severing the spinal cord, and then fish were weighed and measured. The gastrointestinal tract was removed and divided into the following segments: stomach, anterior intestine, and posterior intestine according to Hernández et al. (2009) (*R. quelen*), Santos et al. (2007) (*P. maculatus*) and German (2009) (*L. anus* and *H. commersoni*). The anterior intestine was the 5-6-cm segment after the stomach and the posterior intestine was the final 5–6-cm segment of the intestine. The contents of the gastrointestinal tract segments were collected by gently stripping each section separately, placing them in plastic tubes, and keeping them refrigerated with ice pack sheets. The segments were then cut longitudinally, washed with 0.7% NaCl and placed in liquid nitrogen. The samples were taken to the Fish Physiology Laboratory at the Federal University of Santa Maria.

Samples from the stomach, anterior intestine, and posterior intestine were homogenized in an ice bath (wet weight 0.05 g: 1 mL homogenization buffer) with an Ultraturrax. The homogenization buffer solution was 20 mM Tris and 10 mM phosphate, pH 7.0 in 50% (v/v) glycerin. The extract was centrifuged (1000 g; 4 °C; 5 min) and the supernatant was utilized in assays as an enzyme source (Goulart et al. 2013).

Pepsin activity was assayed by the specific methods of Hidalgo et al. (1999), as follows. The pepsin substrate was 1.5% casein in 0.2 M KCl (pH 1.8 adjusted with HCl). Reactions were carried out at 30 °C for 40 min, stopped with 15% TCA, centrifuged for 10 min at 1000 g and the optical density of the supernatant recorded at 280 nm against tyrosine as a standard. Specific activity was expressed in µmol hydrolyzed substrate min^{-1} mg $protein^{-1}$ (U mg $protein^{-1}$). Trypsin and chymotrypsin were assayed by the specific methods of Hummel (1959). The trypsin substrate was 1.04 mM TAME-HCl (αp-toluene-sulfonyl-L-arginine methyl ester hydrochloride) in 0.01 M CaCl/0.2 M Tris-HCl (pH 8.1), incubated at 25 °C and optical density followed at 247 nm for 60 s. The chymotrypsin substrate was 1 mM BTEE (N-benzoyl-L-tyrosine ethyl ester) in methanol 2:3 (v/v), assayed in 0.1 M CaCl/0.1 M Tris-HCl (pH 7.8) at 30 °C, and the optical density of the supernatant was followed at 256 nm for 60 s. Activities were expressed in µmol arginine min^{-1} mg $protein^{-1}$ (U mg $protein^{-1}$) and nmol tyrosine min^{-1} mg $protein^{-1}$ (U mg $protein^{-1}$), respectively.

Lipase activity was assayed by the specific method of Gawlicka et al. (2000), as follows. The reaction was incubated with 0.4 mM p-nitrophenyl myristate in 24 nM ammonium bicarbonate (pH 7.8) with 0.5% Triton X-100 at 30 °C for 30 min. The reaction was stopped with 10 mM NaOH and the optical density was followed at 405 nm. One unit was defined as µmol substrate hydrolyzed per min and expressed per milligram protein (U mg $protein^{-1}$).

To establish the specific activities of the enzymes, protein concentrations were determined in the enzyme extracts by the methods of Lowry et al. (1951) with bovine albumin as a standard.

Protein and lipid concentrations were determined in the stomach and intestinal contents. The protein concentration was determined according to the micro Kjeldahl method (method 920.52) according to AOAC (1995). The lipid concentration was extracted and quantified according to Bligh and Dyer (1959).

Data are presented as the mean ± S.E.M. Levene's test was used to verify the homogeneity of variance. If the data were ho-

Figures 1–4. Species used in the analysis: (1) *Rhamdia quelen*; (2) *Pimelodus maculatus*; (3) *Loricariichthys anus*; (4) *Hypostomus commersoni*. Figures 1, 2 and 4 kindly provided by Alexssandro G. Becker and figure 3 by Luiz R. Malabarba.

moscedastic, comparisons between digestive enzymatic activities were assessed by two-way ANOVA (species vs. seasons) followed by Tukey's test. The conditions for parametric ANOVA were not satisfied in the activities of pepsin and trypsin in the posterior intestine; thus, the non-parametric Scheirer-Ray-Hare extension of the Kruskal-Wallis test was used, followed by the Nemenyi test (Zar 1999). The comparisons of centesimal composition between seasons (within the same species) or between species (within the same season) were assessed by one-way ANOVA followed by Tukey's test. These analyses were performed with Statistica 7.0 software. The correlations between the activity of proteolytic enzymes (pepsin, trypsin and chymotrypsin) vs. protein content in each segment and lipase vs. lipid content in both portions of the intestine were assessed by Pearson's correlation using Sigma Plot 11.0 software. Differences were considered significant at $p < 0.05$.

RESULTS

Enzymatic activity

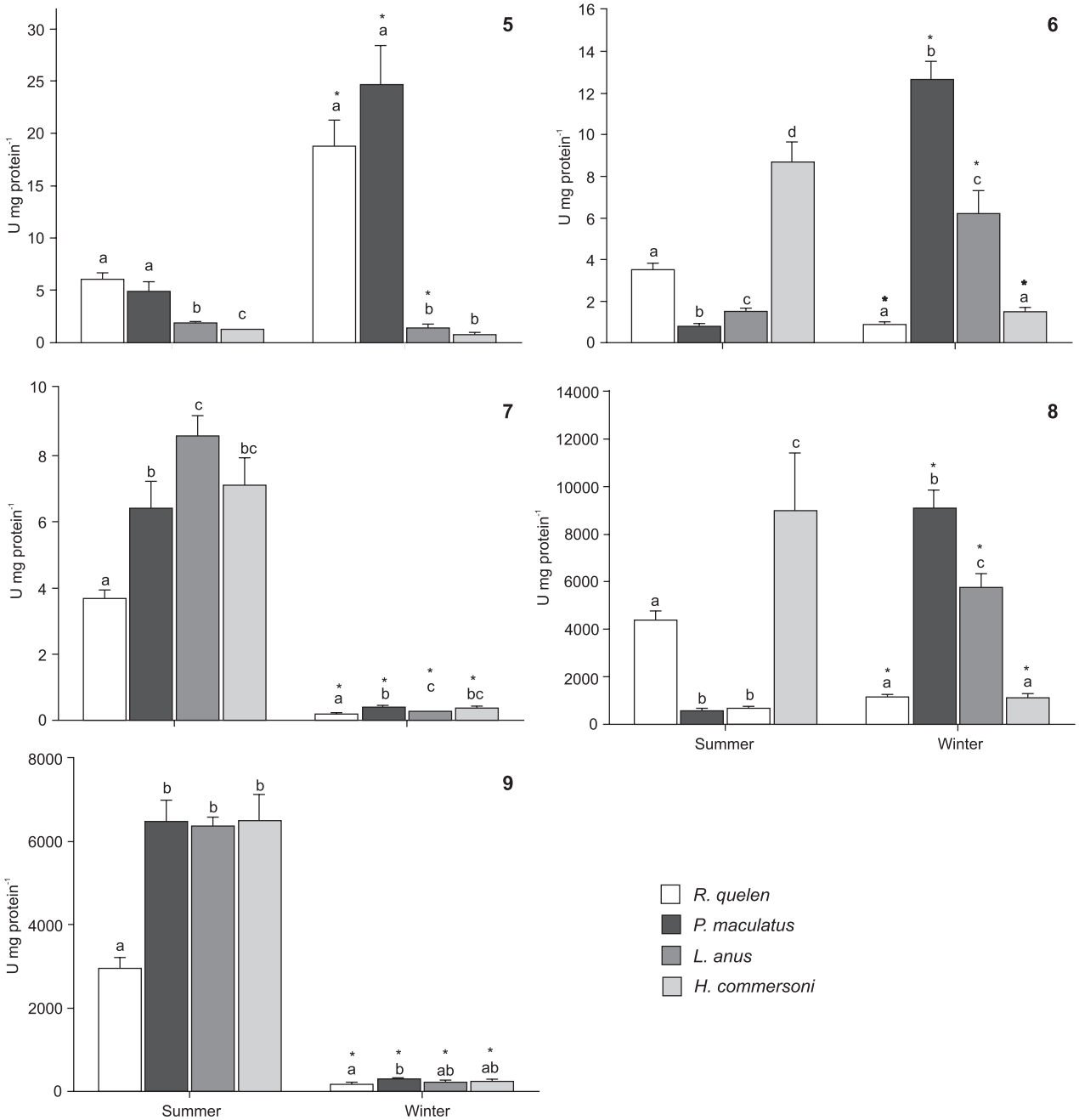
The highest pepsin activity rate per mg protein was detected in the stomach of the omnivorous species (*R. quelen* and *P. maculatus*) in both seasons, and for both species, the higher activity of this enzyme occurred in the winter (Fig. 5). The highest trypsin activity rate per mg protein in the anterior intestine was detected in *H. commersoni*, followed by *R. quelen*, in the summer. On the other hand, in the winter, the highest enzymatic activity of this enzyme in the anterior intestine was found in *P. maculatus* followed by *L. anus*. The trypsin activity rate per mg protein in the anterior intestine of *P. maculatus* and *L. anus* was higher in the winter than in the summer, but the opposite was observed for the other two species (Fig. 6). In the posterior intestine, the highest trypsin activity rate per mg protein in both seasons was detected in *L. anus*, *H. commersoni* and *P. maculatus* (Fig. 7).

The highest chymotrypsin activity rate per mg protein in the anterior intestine in the summer was detected in *H. commersoni* followed by *R. quelen*. On the other hand, in the winter, the highest activity rate per milligram protein was observed in *P. maculatus*, followed by *L. anus*. The activity of this enzyme in the anterior intestine of *P. maculatus* and *L. anus* was higher in the winter than in the summer, but the opposite was observed for the other two species (Fig. 8). The highest chymotrypsin activity rate per milligram protein in the posterior intestine in the summer was detected in *H. commersoni*, *P. maculatus*, and *L. anus*. The chymotrypsin activity rate per mg protein in the winter was higher in *P. maculatus* than in *R. quelen* (Fig. 9). The activity of trypsin and chymotrypsin in the posterior intestine was much lower in the winter than in the summer for all species (Figs 7, 9).

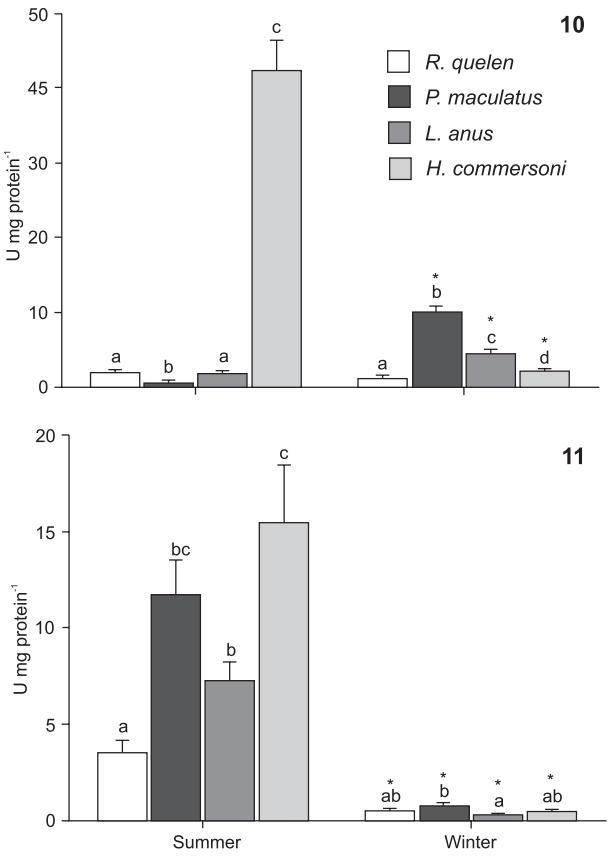
The lipase activity rate per mg protein was highest in the anterior intestine of *H. commersoni* and *R. quelen* in summer. In the winter, the highest lipase activity rate per mg protein was found in *P. maculatus* followed by *L. anus*. The activity of this enzyme in the anterior intestine of *P. maculatus* and *L. anus* was higher in the winter than in the summer, but the opposite was observed for the other two species (Fig. 10). The lipase activity rate per mg protein of the posterior intestine in the summer was higher in *H. commersoni*, followed by *P. maculatus* and *L. anus*. In the winter, the lipase activity rate per mg protein was higher in *P. maculatus* than *L. anus*, with no significant difference with the other species. The activity of this enzyme in the posterior intestine was much lower in the winter than in the summer for all species (Fig. 11).

Centesimal composition of the content of the gastrointestinal tract

The *H. commersoni* specimens collected did not have any content in the stomach in either season, whereas *L. anus* exhibited stomach and intestinal contents in the summer, but not in the winter. *Rhamdia quelen* and *P. maculatus* presented stomach and intestinal content in both seasons.



Figures 5–9. Proteolytic enzymatic activities in the omnivorous *R. quelen* and *P. maculatus* and detritivorous *L. anus* and *H. commersoni* in the summer and winter: (5) pepsin in the stomach; (6) trypsin in the anterior intestine; (7) trypsin in the posterior intestine; (8) chymotrypsin in the anterior intestine; (9) chymotrypsin in the posterior intestine. Different letters indicate significant differences between species in the same season. * Indicates a significant difference from summer in the same segment ($p < 0.05$). (U, a Caraway unit) (n = 15 from each species at each season).



Figures 10–11. Lipase activity in the omnivorous *R. quelen* and *P. maculatus* and detritivorous *L. anus* and *H. commersoni* in the summer and winter: (10) anterior intestine; (11) posterior intestine. Different letters indicate significant differences between species in the same season. * Indicates a significant difference from the summer in the same segment ($p < 0.05$). (U, a Caraway unit) (n = 15 from each species at each season).

The highest protein concentration in the stomach content in the summer was found in *R. quelen*. In the winter, the highest protein concentration was found in *P. maculatus*. *Rhamdia quelen* presented a higher protein concentration in the stomach content in the summer, but for *P. maculatus*, this concentration was higher in the winter. In the anterior intestinal content, the highest protein concentration in the summer was found in *H. commersoni*. In the winter, this species showed a comparatively lower protein concentration than in the summer, and the highest concentrations were observed in *R. quelen* and *P. maculatus*. The highest protein concentration in the posterior intestinal content was found in *R. quelen* in both seasons. The protein concentration of the posterior intestinal content was higher in the winter than in the summer in *P. maculatus*, but the opposite was observed in *R. quelen* (Table 1).

The highest lipid concentration in the stomach content in summer was found in *R. quelen*; in the winter, the highest concentration was observed in *P. maculatus*. In the anterior intestinal content, the highest lipid concentration was found in *H. commersoni* in both seasons. In the posterior intestinal content, the highest lipid concentration in summer was found in *H. commersoni*. In the winter, there was no difference in lipid concentrations in the posterior intestinal content of all species that presented any content. The lipid concentrations in the stomach and posterior intestinal content were lower in the winter than in the summer in *R. quelen*, but for *P. maculatus*, the lipid concentrations in the content of the entire digestive tract were higher in the winter than in the summer (Table 1).

Enzymatic activity x centesimal composition of the gastrointestinal tract

There was no relationship between the enzyme activities and the centesimal composition of the different segments. It was not possible to determine the relationship between the digestive enzymatic activity and the centesimal composition of ingested food in the stomach of *H. commersoni* in both seasons due to the absence of stomach content. In winter, this relationship could not be determined in *L. anus* due to the absence of content in the three segments of the gastrointestinal tract used in this study.

DISCUSSION

Several studies on the activity of digestive enzymes in fish suggested that enzymatic activity is influenced by the diet ingested or by feeding habits (Fernández et al. 2001, Chan et al. 2004, Drewe et al. 2004, Langeland et al. 2013, Solovyev et al. 2014). The results of this study did not fully support the hypothesis that the activity of the studied digestive enzymes was determined by the centesimal composition of the food. Similar results were found in cultured juveniles of *Pseudoplatystoma corruscans* (Spix & Agassiz, 1829) (Pimelodidae), in which the enzyme activity was not influenced by the diet (Lundstedt et al. 2004). The diet also did not influence most digestive enzymes in *R. quelen*, except for the intestinal protease (Moro et al. 2010). However, most studies demonstrating a relationship between diet and digestive enzyme activity were performed in cultivated species. The cultured *Colossoma macropomum* (Cuvier, 1816) (Serralmidae) showed higher lipase activity in the anterior intestine when fed with higher lipid concentrations (De Almeida et al. 2006), and *Monopterus albus* (Zuiew, 1793) (Synbranchidae) presented higher trypsin activity when fed with higher protein concentrations (Ma et al. 2014).

In the present study, the protein and lipid concentrations observed in the gastrointestinal tract did not show any relationship with proteolytic and lipase activities. This absence can be associated with the variation of nutrient availability over time in the environment, as it was observed in the centesimal composition of food ingested by the fish in the summer and winter.

Table 1. Percentage and range (between parentheses) of protein and lipids (dry matter) in the content of the gastrointestinal tract of four teleost species collected in two seasons.

Species	Stomach		Anterior intestine		Posterior intestine	
	Summer	Winter	Summer	Winter	Summer	Winter
Protein						
Rhamdia quelen	61.77 ± 10.81^{a} (50.95 – 72.58)	18.37 ± 6.49^{a*} (11.89 – 24.86)	11.21 ± 0.03^{a} (11. 18 – 11.25)	21.59 ± 12.69^{a*} (8.90 – 34.28)	52.92 ± 4.35^{a} (48.57 – 57.28)	25.87 ± 0.17^{a*} (25.70 – 26.04)
Pimelodus maculatus	9.05 ± 1.16^{b} (7.89 – 10.205)	44.86 ± 0.65^{b*} (44.21 – 45.51)	12.04 ± 0.95^{a} (11.09 – 12.99)	24.35 ± 8.54^{a*} (15.81 – 32.89)	9.45 ± 0.26^{b} (9.19 – 9.72)	17.66 ± 1.41^{b*} (16.26 – 19.07)
Loricariichthys anus	11.04 ± 0.04^{b} (10.99 – 11.08)	N	10.47 ± 0.19^{a} (10.28 – 10.66)	N	13.77 ± 1.45^{bc} (12.31 – 15.22)	N
Hypostomus commersoni	N	N	15.29 ± 2.94^{b} (12.35 – 18.23)	9.57 ± 0.14^{b*} (9.43 – 9.70)	7.05 ± 0.72^{c} (6.34 – 7.77)	7.78 ± 3.01^{c} (4.76 – 10.79)
Lipids						
Rhamdia quelen	7.07 ± 0.08^{a} (6.98 – 7.15)	0.91 ± 0.07^{a*} (0.84 – 0.97)	0.71 ± 0.09^{a} (0.62 – 0.80)	0.51 ± 0.22^{a} (0.29 – 0.73)	1.94 ± 0.23^{a} (1.71 – 2.16)	0.64 ± 0.07^{a*} (0.56 – 0.71)
Pimelodus maculatus	0.41 ± 0.01^{b} (0.39 – 0.42)	1.96 ± 0.29^{b*} (1.68 – 2.25)	0.29 ± 0.02^{a} (0.27 – 0.31)	0.56 ± 0.15^{a*} (0.41 – 0.71)	0.27 ± 0.03^{a} (0.24 – 0.29)	1.67 ± 0.51^{a*} (1.15 – 2.18)
Loricariichthys anus	N	N	0.75 ± 0.08^{a} (0.67 – 0.83)	N	2.34 ± 2.28^{a} (0.06 – 4.62)	N
Hypostomus commersoni	N	N	4.81 ± 1.41^{b} (3.40 – 6.22)	7.36 ± 0.82^{b} (6.54 – 8.17)	3.82 ± 0.01^{b} (3.81 – 3.83)	2.72 ± 1.34^{a} (0.52 – 3.83)

N: not determined because specimens did not have any food content in this segment. Different letters in the columns indicate a significant difference between species. An * indicates a significant difference from the summer in the same segment.

Pepsin was the only digestive enzyme that had higher activity in the omnivorous than in the detritivorous species analyzed in the present study, but protein concentrations in the stomach content were not related to the activity of this enzyme. Similar results were found by López-Vásquez et al. (2009), who observed higher alkaline proteolytic activity in two detritivorous species and one omnivorous species than in carnivorous species. The same authors supposed that the higher proteolytic activity in detritivorous species was due to feeding with detritus, which represents a poor source of nutrients, as observed in *P. nigricans* (10–20% organic matter and 2–5% crude protein), but other detritivorous, *L. pardalis*, has 35–55% organic matter and 10–19% crude protein in the food ingested (Yossa and Araújo-Lima 1998). The higher activity of trypsin and chymotrypsin in the posterior intestine of *P. maculatus*, *L. anus* and *H. commersoni*, in spite of the lower protein content of this portion in both seasons could be related to an increase of the activities of digestive enzymes to completely utilize the low concentrations of protein in the food. Analysis of the digestive enzyme activities in 11 species distributed in three trophic categories (Chakrabarti et al. 1995) and four phylogenetically related species with two feeding habits (Chan et al. 2004) and the studies of López-Vázques et al. (2009) and Duarte et al. (2013) also led to the same conclusion.

Overlapping diets may occur in the wild, such as detritus in *R. quelen* and sand grains in *P. maculatus* (Gomes et al. 2000), and omnivory in *L. anus* (Petry and Schulz 2000), resulting in overlapping digestive enzyme activity between species. The enzymatic activity may also vary according to the specific characteristics of each species in the production of digestive enzymes. Duarte et al. (2013) found increased trypsin, chymotrypsin, and β-glucosidase activities in the winter and spring for the detritivorous/herbivorous *Hypostomus auroguttatus* Kner, 1854 (Loricariidae), and increased peptidase and β-glucosidase activities during the summer in *P. maculatus*. The authors associated this variability with the food availability in *H. auroguttatus* and the reproductive migration that takes place at this time in *P. maculatus*. In the present study, the activities of digestive enzymes in *P. maculatus* varied with the season, with some having higher activity in the summer and others in the winter. Overall, *H. commersoni* and *L. anus* presented higher enzyme activity in the intestine than in the stomach, as observed by Duarte et al. (2013) in *H. auroguttatus*. This is in agreement with the fact the stomach is an accessory respiratory organ in *H. commersoni* and in species of *Loricariichthys*, and consequently the role of the stomach in digestion is very small (Silva et al. 1997, Podkowa and Goniakoska-Witalinska 2003).

In conclusion, the activity of pepsin in the stomach was higher in the omnivorous than in the detritivorous species, but the other studied digestive enzymes could not be used as indicators of feeding habits because this relationship is usually not found in fish collected in the wild.

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Submitted: 18 April 2017
Received in revised form: 9 October 2017
Accepted: 13 October 2017
Editorial responsibility: Carolina Arruda Freire

Author Contributions: APGA, ERB, and BB designed the experiments; APGA, ELZ, ERB, JPV, and BB conducted the samplings; APGA and CT performed the laboratory techniques; LPS provide and taught techniques for determining the content composition of gastrointestinal tract; VLL provided and taught techniques for determination of digestive enzyme activity; APGA and BB analyzed the data and wrote the paper.

Competing Interests: The authors have declared that no competing interests exist.