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ORIGINAL PAPER

Digestive enzyme activity during early larval development of the Cuban gar *Atractosteus tristoechus*

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Abstract The ontogenesis of digestive enzymes (proteases, amylases, lipases, and phosphatases) in Cuban gar *Atractosteus tristoechus* was determined in larvae between 5 and 18 days after hatching (DAH). Variations in specific activities of most enzymes were related to the transition from endogenous to exogenous feeding and to the transition from the larval to the juvenile stage. Alkaline protease activity was not detected until 8 DAH in contrast to acid protease activity, which was quantifiable at 5 DAH. Acid protease activity was consistently higher than alkaline protease activity, indicating the presence of a functional stomach in the early stages of larval development. The acid protease activities of larvae and adults were compared by means of zymogram analysis. Four acid protease bands were found in adults (two more than in larvae). This result is the first time that more than one band of acid proteolytic activity has been found in Lepisosteidae. High lipase activity indicated the importance of lipid utilization, particularly during yolk-sac absorption. In contrast to the other enzymes studied, amylase activity was consistently low, probably due to the strictly carnivorous diet of gar larvae and their low capacity for carbohydrate digestion. High activities of aminopeptidase and acid and alkaline phosphatases suggest intestinal absorption. This result, together with the existence of a short gut and a lower proteolytic activity in the intestine than in the stomach, suggest that most of the proteolytic activity takes place in the stomach, while the primary function of the intestine is nutrient uptake.

Keywords *Atractosteus tristoechus* · Cuban gar · Digestive enzymes · Larval development

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Introduction

The Cuban gar *Atractosteus tristoechus* is an endemic freshwater carnivorous fish (family: Lepisosteidae) which inhabits rivers, bayous, swamps,

and brackish waters. The largest populations are restricted to southwestern Cuba, primarily in the peninsular region of Zapata. In addition to the natural restricted range of this species, anthropogenic factors such as habitat loss and alteration, overfishing of prey items, and the introduction of exotic species (e.g., *Clarias* spp.) have contributed to the decline of Cuban gar populations. In response to these recent population declines, the Cuban gar was listed as a vulnerable species in 1999 (Pérez et al. 1999).

Within this context, there is increased interest in the culture of this species.It will be necessary to develop effective culture techniques in order to preserve and restore natural populations of the species through the stocking of cultured individuals. Research efforts and the development of culture techniques for the Cuban gar should focus initially on the physiology of the early larval stages. Larval nutritional studies (Divakaran et al. 1999) have been receiving much attention because an understanding of changes associated with the processes of food ingestion, digestion, and assimilation are the first steps in determining the ability of larvae to utilize a given diet (Martínez et al. 1999).

Recent studies have looked at the ontogeny of digestive enzymes in fish larvae. The expression of different digestive enzymes has been used as an indicator of developmental and survival rates in larval fishes (Ueberschär 1993) and as a reference point as to when to initiate inert diets (Moyano and Sarasquete 1993). Similar studies have determined the effect of different diets on digestive enzyme levels (Baglole et al. 1998; Fountoulaki et al. 2005), while other studies have evaluated the effect of feeding frequency on enzyme activity (Divakaran et al. 1999). Moreover, physiological research not only results in the improvement of aquaculture techniques but also provides valuable insight into the biology of the species. This type of research is needed for Cuban gar, a unique and poorly understood species that is a member of a small but important group of ancient fishes that date back to the Cretaceous period.

The objective of this study was to quantify and describe the early development of the primary digestive enzyme activities of Cuban gar larvae fed live prey. To accomplish this objective, we quantified different enzyme activities (proteases, amylases, lipases, phosphatases) and used zymogram analysis to characterize total proteases.

Materials and methods

Animals and diets

Cuban gar (*Atractosteus tristoechus*) eggs were obtained in May, 2005 from the induced spawning of one female and three males maintained in captivity at the Center for Native Ichthyofauna Reproduction located in the peninsular region of Zapata, Cuba.

Breeding adults were placed in a concrete pond measuring 3×2.5 m containing water to a depth of 50 cm. In order to accommodate the spawning behavior of lepisosteids (León et al. 1978; Dean 1985; Simon and Wallus 1989), artificial branches were spread throughout the pond to provide spawning substrate. A first injection of luteinizing hormone-releasing hormone analog (LHRH-A; 25 μg/ml) was administered to the broodstock, with a second injection given 16 h later. Courtship and spawning occurred 9 h after the second injection. Broodstocks were removed from the pond after spawning. The spawned adhesive eggs remained in the pond for 4 days without water replacement.

To assess the development of the primary digestive enzyme activities, larvae 4 days after hatching (DAH) were moved to two rectangular fiberglass tanks measuring 300×40×50 cm, with a water volume of 300 l. The initial stocking density was 2000 larvae/tank (6.7 larvae/l). From this time onwards, larvae were reared at a constant water temperature (28±1°C), under a light regime from 08:00 to 20:00 h, with oxygen provided by a compressor to maintain oxygen levels above 6 ppm. Each morning, 75% of the water was exchanged in each tank after bottom cleaning.

Larvae were fed live *Artemia nauplii* ad libitum three times a day (09:00, 14:00, and 19:00 h) from 4 to 17 DAH according to the following sequence (expressed as *A. nauplii*per larvae per day): 4–5 DAH (400), 6 DAH (600), 7 DAH (800), 8 DAH (1000), 9 DAH (1200), 10 DAH (1500), 11 DAH (2000), 12 DAH (2500), 13 DAH (2800), 14

DAH (3000), 15 DAH (3200),16–17 DAH (3400). *Artemia* cysts (Salt Creek Select, Great Salt Lake, Utah) were hydrated for 1 h in aerated freshwater and later incubated in salt water (28 g/l) for 23 h with continuous aeration and light. The cysts were prepared 24 h prior to each feeding. Just before being fed to the larvae, the *A. nauplii* were washed with freshwater and equally distributed between the tanks.

Sampling and dissection

Between 5 and 18 DAH, 100 larvae were removed from each tank prior to each morning feeding. Following collection, the larvae were maintained in two small tanks without food for at least 1 h to allow the digestive tract to eliminate or assimilate any remaining food. Ten larvae per tank were then randomly selected and individually weighed on an Ohaus balance (±0.1 mg) and measured (total length) using an ocular micrometer or a caliper (±0.1 mm). All larvae were washed with distilled water, and excess water was removed with tissue paper before dissection. Dissection was performed under a stereoscopic microscope on a glass dish maintained at a constant 0–2°C.

Between 5 and 12 DAH, whole larvae, with exception of the head and the tail, were sampled. From 13 DAH onwards a cut was made at the beginning of the esophagus, and the entire intestinal segment, including the liver and the diffuse pancreas embedded on its outer surface, was removed. The dissected gastrointestinal tracts from larvae of each tank were pooled and placed in a plastic Eppendorf tube that was kept on ice. Immediately thereafter, the gut samples were shock-frozen with liquid nitrogen, taking care that the elapsed time between dissection and shock-freezing did not exceed 15 min. The samples were stored at –80°C until freeze drying. Fractions of the digestive tract of an adult (stomach, pyloric caeca, intestine, spiral valve) were also taken and processed as a reference for the analysis.

Enzyme analyses

Samples were weighed and homogenized at a 1:10 ratio (tissue: bi-distilled water) for a few minutes using a Polytron homogenizer (Brinkman Instruments, Westbury, N,Y.) in an ice bath. The homogenates were centrifuged at 10,600 *g* for 15 min at 4°C. The supernatant, free from the lipid layer, was collected and stored at –70°C in 100-µl aliquots until the analyses of enzyme activity and protein content were performed.

To assay the specific activities of the enzymes, we determined the total soluble protein concentrations in the enzyme extracts using the method of Bradford (1976) with bovine serum albumin (BSA) as a standard.

All assays were carried out in triplicate at room temperature (25°C) using a Tecan Sunrise microplate spectrophotometer and Nalge Nunc flat-bottom 96-well microplates (Fisher Scientific, Hampton, N.H.), except for the assays of amylase and total protease activities, where a Spectronic spectrophotometer (Thermo Electronic, Waltham, Mass.)with quartz cells was used. All reactions were run at saturating substrate concentrations and compared to an appropriate blank.

Total protease activity

Acid protease (pepsin) activity in the stomach samples was measured according to Anson (1938): 0.5 ml of hemoglobin was used as the substrate (0.25%) in 0.5 ml of sodium acetate buffer-HCl (0.1 *M*, pH 3) and incubated with 25 µl of the sample homogenate in a micro-centrifuge tube for 30 min at 37°C. The reaction was stopped by adding 0.5 ml of cold trichloroacetic acid (TCA, 12%) and kept at 4°C for 15 min. The samples were centrifuged at 20,800 *g* at 4°C for 5 min and the absorbance of the supernatant was read at 280 nm.

Alkaline protease activity was measured using a protocol similar to that described above, except that 50 m*M* Tris-HCl/20 m*M* $CaCl_2$ (pH 8.5) was used as the buffer, and casein (0.1%) was used as a substrate (Walter 1984).

We also characterized protease activities in native conditions by polyacrylamide gel electrophoresis (PAGE) using 15% acrylamide and 10×11.3×0.75-cm gels. Each well was filled with samples containing 35 µg of total protein. Electrophoresis was performed at a constant current of 60 mA per gel for 90 min at 4°C.

In the case of the alkaline proteases, following electrophoresis the gels were washed with distilled water and incubated in 50 m*M* Tris-HCl buffer, pH 9, containing 1% casein for 30 min at 4°C, after which the temperature was raised to 37°C for 90 min without agitation. Thereafter, the gels were washed, fixed in 12% TCA prior to staining with 0.1% Coomassie Brilliant Blue in a methanol-acetic acid solution (50:20:50) solution. Destaining was carried out in a methanol-acetic acid-water solution (35:10:55) (Garcia-Carreño et al. 1993).

For the acid proteases, the electrophoresis conditions were similar to those of the alkaline proteases, with the exception that the buffer used in the gel preparation was 0.1 *M* Tris-PO_4(pH 5.5)/20% glycerol/0.02% bromophenol blue, and a 0.25% hemoglobin/sodium acetate (pH 3.0) solutionwas used in the incubation (Alarcón 1997).

Specific activity

Aminopeptidase activity was determined by first mixing 30 µl of homogenate samples with 70 µl of 50 m*M* Tris-HCl , 20 m*M* $CaCl_2$ (pH 8.5) buffer. Next, 200 µl of the substrate L-leucine-pNA (LNA, 2 m*M*) was added (Lauff and Hofer 1984). The change in absorbance was continuously registered for 10 min at 405 nm. Alkaline and acid phosphatase activities were measured in a similar way using 2% *p*-nitrophenyl phosphatewith 1.0 *M* diethalonamine/50 m*M* $MgCl_2$ (pH 9.8)/0.1 *M* sodium acetate-HCl(pH 4.8) as buffers, respectively (Moyano et al. 1996).

Lipase (non-specific *β*-esterase) activity was estimated following the method of Brogdon and Dickinson (1983) using potassium phosphate (pH 7.2) as the buffer and 100 µl of *β*-naphthyl acetate as the substrate. The absorbance was read at 540 nm after 10 min of incubation at room temperature.

α-Amylase was assayed in a0.1 *M* sodium acetate-HCl/6 m*M* NaCl (pH 4.8) with a 1% starch solution as the substrate (Bernfeld 1951). In a glass tube, 0.5 ml of the starch solution was combined with 25 µl of homogenate and 0.5 ml of buffer. The reaction was incubated at 25°C for 30 min and was stopped by the addition of 1.5 ml of di-nitrosalicylic acid (DNS), followed by boiling for 15 min. The resulting solution was diluted with 2.5 ml of distilled water and α-amylase activity was determined spectrophotometrically at 540 nm.

For all the assays, one unit of activity (U) was defined as the amount of enzyme that catalyzes the substrate causing a 0.001 change in absorbance per minute (Walter 1984)

Statistical analyses

Homogeneity of variance and normality of the biochemical data were examined before using one-way analysis of variance (ANOVA) to detect differences ($p < 0.05$) in enzyme activity during larval development. When differences were found, multiple comparisons of enzymatic activity were conducted using a Student-Newman-Keuls (SNK) test. The analyses were conducted using STATISTICA ver. 6.0 (StatSoft, Tulsa, Okla.).

Results

Larvae increased 2.8-fold in total length and 6.7-fold in weight from 5 to 18 DAH (Fig. 1). The total protein concentration gradually increased with the age of the larvae from 3.5 mg/ml at 5 DAH to 16.6 mg/ml at 18 DAH (Fig. 2). Significant differences were evident after the fourth day of the experimental period, and were independent of the sampling method.

The activities of the different enzymes assayed are shown in Fig. 3A-F expressed in units per milligram soluble protein ($_1$) and units per larvae ($_2$). The major events taking place during the anatomical differentiation in larvae (unpublished data) and enzymatic activities in the adult are also included in the figure as a reference. In the case of adult enzymatic activities, only the higher values (stomach and pyloric caeca) were selected.

Variations in the total and specific activities of each enzyme assayed were observed during the experimental period. Results expressed in units per larvae revealed a trend of increased activity with age, with the exception of amylase (Fig. 3, C_2).

Both acid and alkaline protease activities showed similar trends, with the exception of the

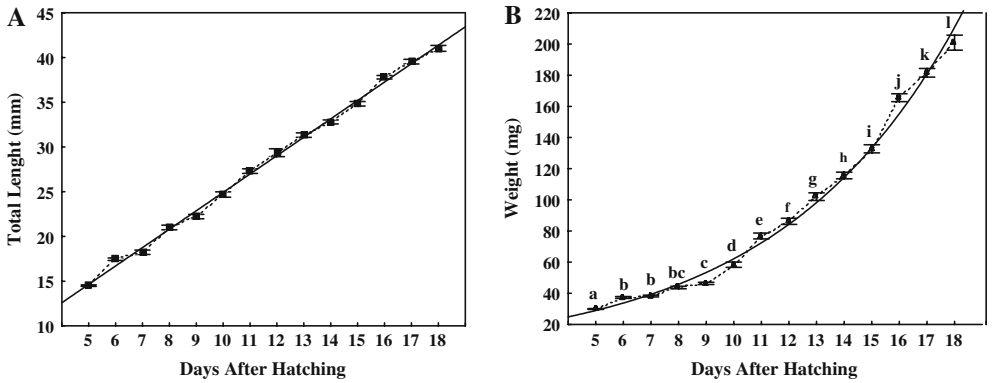

Fig. 1 Growth of Cuban gar (*Atractosteus tristoechus*) larvae expressed as total length (**A**) and wet weight (**B**) during the experiment. Values are means ± standard errors (SE) (n=20). Increase in total length (TL) is based on the equation TL = 4.3717 + 2.0555 × DAH, while the increase in weight is based on the equation WT = 13.4998 × exp(0.1527×DAH)

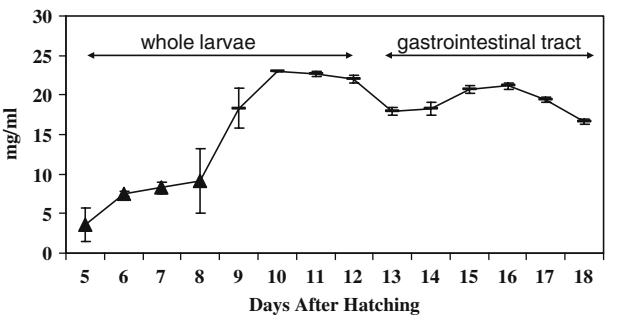

Fig. 2 Total protein concentration in a crude extract of Cuban gar larvae. Points represent the means ± SE (n=6), with different markers (*filled triangle*, *line*) indicating significant differences ($p < 0.05$)

period between 8 and 12 DAH. During this time, increased alkaline protease activity coincided with a steady decrease in acid protease activity (Fig. 3, A_1); however, acid protease activity was consistently higher than alkaline protease activity. Alkaline activity was barely detectable during the first 3 days, but began to increase at 8 DAH. The acid protease activity in the adult stomach sample was 20 U higher than the highest value found in larvae, while the alkaline protease activity in the adult pyloric caeca was similar to larval alkaline protease activity from 8 to 12 DAH.

The development of the activities of the two pancreatic enzymes is summarized in Fig. 3B and C ($_1$ and $_2$). Lipase activity showed a significant increment during the complete period of yolk sac absorption. Larval lipase activity from 5 to 12 DAH was similar to the lipase activity in the adult pyloric caeca. Overall larval amylase activity was low, with an irregular progressive decrease from the highest value found at 5 DAH larvae (4.3 U/mg protein) to a more or less constant value reached from 9 DAH onwards (around 1.5 U/mg protein); this latter value was similar to the activity observed in the adult pyloric caeca.

The intestinal enzyme activities were varied (Fig. 3D–F). Aminopeptidase activity did not show any significant increment during the experimental period, and the activity of this enzyme during the last days of the experiment were similar to values found in the adult. The activities of acid and alkaline phosphatases (Fig. 3E_1 and F_1, respectively) showed opposite trends during the first days of the experiment and up to 12–13 DAH, following which time the activities of both enzymes increased only slightly. Alkaline phosphatase activities were consistently higher than those of acid phosphatase. The alkaline phosphatase activity in the adult was similar to that in the larva during the first days of the experiment. Acid phosphatase activity was significantly lower

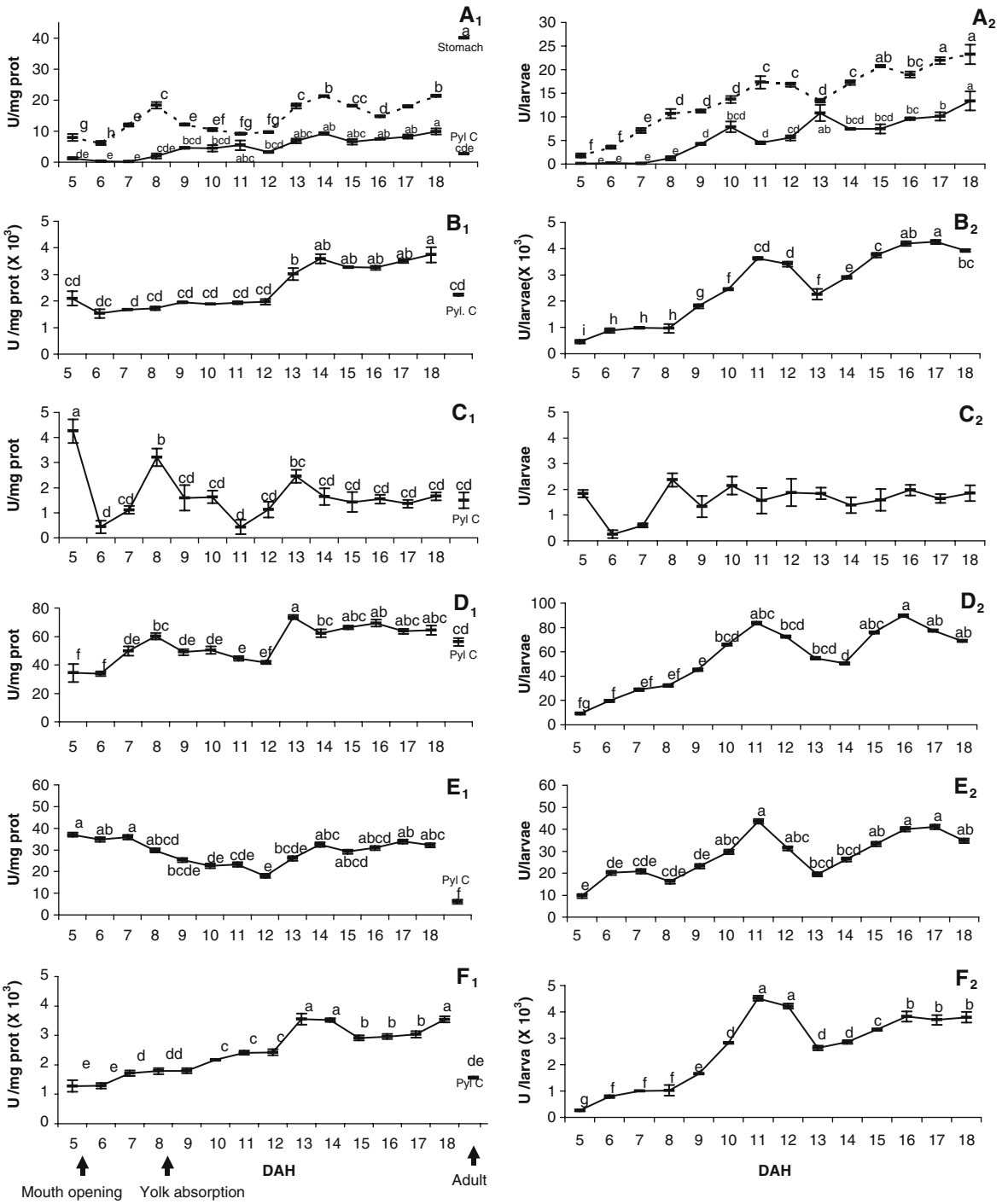

Fig. 3 Digestive enzyme activities in Cuban gar larvae during the early developmental stages expressed in units per milligram soluble protein (*left*) and units per larvae (*right*): **a** acid (- - -) and alkaline (——) proteases, **B** lipase, **C** amylase, **D** aminopeptidase, **E** acid phosphatase, **F** alkaline phosphatase. Each *point* represents the mean of six measures ± SE, *different superscripts (letters)* indicate significant differences ($p < 0.05$). *Arrows* show the main events taking place in larvae that are associated with anatomical differentiation (unpublished data); adult values are indicated as a reference. Note the differences in the scales

in the adult than those values observed throughout larval development.

Caseinolytic activity was first observed at 8 DAH in the form of a clear band and remained until 18 DAH (Fig. 4A). A progressive increase in enzymatic activity in older larvae was evidenced by a set of four bands that were observed from 13 to 17 DAH. The number of bands decreased to three by 18 DAH. The adult pyloric caeca extract produced only one clear and one diffuse band.

Zymogram analysis revealed the presence of one diffuse band of larval acid protease at 5–6 DAH (Fig. 4B). Two bands of larval acid proteases were observed from 7 to 18 DAH and, these were similar to two of the acid protease bands produced by the adult stomach extract. Two additional bands with hemoglobinolytic activity were also observed in the stomach extract of adult fish.

Discussion

Different patterns of larval growth with respect to weight and length were observed during the first days of experiment (6–8 DAH). Although a significant increase in mean length was observed each day, mean weight measurements from 6 to 8 DAH were not different. One possible explanation for this is that from 6 to 8 DAH the larvae may only increase in length due to their utilization of yolk sac elements while weight remains stable because there is no uptake of external nutrients. This explanation is supported by our observation of a significant increase in larval protein concentration starting on 9 DAH, which coincided with the input of new protein sources from exogenous feeding. Based on these observations we consider 6–8 DAH to be the transitional phase from endogenous to exogenous feeding. This should be corroborated in subsequent morphological and histological studies. This interpretation is in agreement with the transition from the lecithoexotrophic stage to the exotrophic stage at the same time that has been reported for *A. spatula* (Mendoza et al. 2002b).

We observed changes in the activities of a variety of digestive enzymes in the early developmental stages of Cuban gar larvae. These changes may be attributed to the growth and development of new organs and tissues and can not be explained as a response to ration levels or by changes in the composition of live food, since the larvae were fed the same type of live foodad libitum during the entire experiment. Different opinions have been expressed concerning the contribution of enzymes provided in the live food.

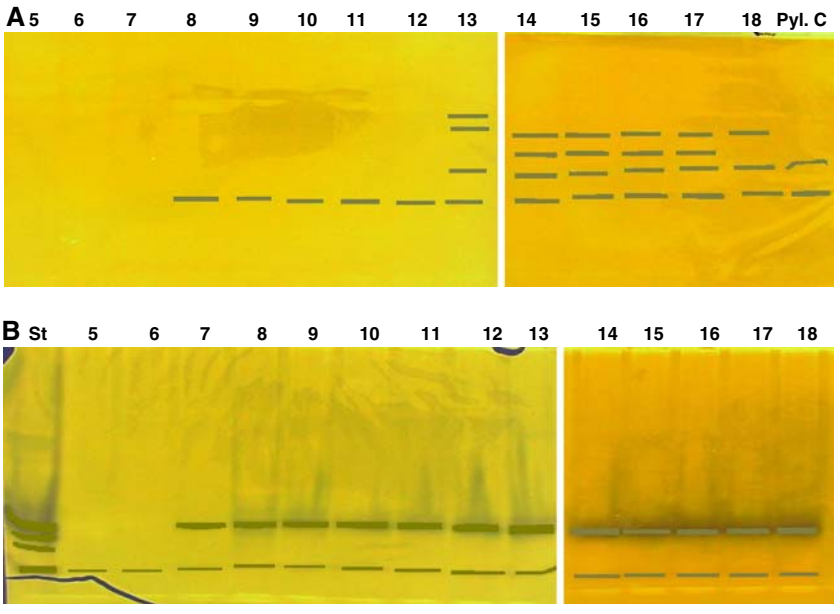

Fig. 4 Zymogram of extracts obtained from Cuban gar larvae at different days after hatching (5–18). **A** Alkaline proteases including the pyloric caeca (*Pyl. C*) from the adult. **B** Acid proteases including the stomach (*St*) from the adult. Gels and samples were prepared and treated as described in the Materials and methods section

Several authors have suggested that, at least in some species, the levels of the primary larval enzymes at the moment of first feeding may be high enough to allow the digestion of either a prey or an artificial diet (Cahu and Zambonino 1994; Moyano et al. 1996). Moreover, Kim and Brown (1995) demonstrated that enzymes were produced prior to the presence of live feed in the gastrointestinal tract of the Pacific threadfin larvae *Polydactylus approximas*. The low contribution of enzymes from live food has been reported for the larvae of sea bass (*Dicentrarchus labrus*; Zambonino and Cahu 1994), sea bream (*Sparus aurata*; Moyano et al. 1996) and sardine (*Sardinops melanotictus*; Kurokawa et al. 1998). García-Ortega et al. (2000) demonstrated that the contribution of digestive enzymes from *Artemia* to the total digestion of food by catfish larvae (*Clarias gariepinus*) was less than 1% of the total proteolytic activity. Kurokawa et al. (1998) estimated that this contribution in Japanese sardine larvae was only 0.6%, concluding that the contribution of enzymes from the prey to the digestive process in the larvae was insignificant. In the same sense, Diaz et al. (1997), who used sodium dodecyl sulfate-PAGE to determine protease activity in sea bream larvae and their prey, found that larval proteases play the most important role in protein digestion. To minimize the potential effects of enzymes from undigested prey in our measurements, larvae were sampled prior to the morning feeding and left in small tanks without food for at least 1 h after collection and before analysis.

The transition from yolk-sac larvae to actively feeding larvae has been considered to be the most critical event during the early life stage of larval fishes. Unlike the gradual development of other organ systems, the development of the alimentary canal from the simple, undifferentiated, straight gut of the yolk-sac larvae to the complex, segmented alimentary canal of the adult occurs by periodic or punctuated rapid changes rather than by continuous development (Govoni et al. 1986). Our results support this assertion, as we found a generalized pattern of two peaks in enzymatic activity (around 8 DAH and 13–14 DAH) that may be associated with important anatomical and physiological modifications related to the transition from endogenous to exogenous feeding and the beginning of metamorphosis from the larval to juvenile stage (Segner et al. 1995; Mendoza et al. 2002b).

Moyano et al. (1996) summarized the developmental stages occurring in the digestive tract of several marine fish species where alkaline protease activity was detected very early (prior to mouth opening) whereas pepsin activity was detected very late. We detected the beginning of alkaline protease activity on 8 DAH, while the activity of acid protease was quantifiable from the beginning of the experiment and was consistently higher than that of alkaline protease. This pattern of early and high acid protease activity is indicative of the presence of a functional stomach and gastric glands early in development. Examples of this pattern have only been observed in few species, such as salmonids and sturgeons (Buddington 1985; Buddington and Dorshov 1986; Gawlicka et al. 1995), pintado (Lundstedt et al. 2004), and alligator gar (*A. spatula*), with the latter possessing a well-developed intestinal tract characterized by a functional stomach and the secretion of pepsin-like enzymes from the beginning of exogenous feeding and before complete absorption of the yolk sac (Mendoza et al 2002a). According to some authors, stomach differentiation is a decisive event in larval fish nutritional physiology, leading to precocious behavior and efficient protein digestion (Segner et al. 1994; Martínez et al. 1999). In Cuban gar, it does not appear that the activity of alkaline protease compensates for the deficiency in acid protease during the first stages of development, as occurs in most fishes, although pepsin does appear to replace the role of alkaline protease in protein digestion. In fact, when zymograms of larvae between 13–18 DAH and adult Cuban gar were compared, a decrease in the number of bands of alkaline proteases was observed in the adults, which could be related to a decrease in alkaline enzyme activation stimuli. The early functionality of the stomach in this species is noteworthy and the results of enzymatic activity in the adult showed a significant and continuous development of this organ. PAGE revealed two clear bands of acid proteases in larvae and two additional bands in adults. This is the first time that more than one band with acid proteolytic activity has been

reported for lepisosteids; however, this is not uncommon in other fish species (Kapoor et al. 1975; Squires et al. 1986; Gildberg et al. 1990). In the case of Cuban gar, further research is needed to explore this finding.

High lipase activity was detected early and continues during all the entire experimental period, as has been found in other species, such as walleye pollock (*Theragra chalcogramma*; Oozeki and Bailey 1995), striped bass (*Morone saxatilis*; Ozkizilcik et al. 1996), haddock (*Melanogrammus aeglefinus*; Perez-Casanova et al. 2004), and grouper *(Epinephelus coioides*; Eusebio et al. 2004). However, this activity seems to be species-dependent; for example, lipolytic activity was not detected in turbot larvae *(Scophthalmus maximus*) until the larvae reached an age of 15–20 days, and even then it was only detected in very small amounts (Cousin et al. 1987). In the same sense, Oozeki and Bailey (1995) suggested the existence of two types of lipase, one related to yolk-sac absorption and the other – in which activity develops later – would be related to the digestion of exogenous lipids. In the present investigation, the high and continuous increment in lipase activity reflects the importance of this pancreatic enzyme in the utilization of lipids, and its significant increase between 12 and 14 DAH could be related to the development of the exocrine pancreas, the mobilization of accumulated lipids, and the increased capacity to digest lipids contained in food items. This conclusion can be made based on the results of adults: lipase activity in the adult Cuban gar was found to be similar to that measured in larvae during the first days of life, indicating the importance of lipase activity during the yolk-sac absorption phase.

In contrast to the activities of the other enzymes studied, amylase activity did not show a clear trend. Early detection of amylase activity has been reported in other fish larvae (Baragi and Lovell 1986; Munilla-Morán et al. 1990; Chakrabarti et al. 2006). However, in the case of Cuban gar the activity did not increase with age but rather oscillated at low levels, similar to those values detected in adult pyloric caeca. In several carnivorous marine fish, amylase expression has been reported to decrease during larval development (Cahu et al. 2004) and has been suggested to be strongly related to specific feeding habits (Horn et al. 1986). In our assays, during the first days of Cuban gar development, amylase activity was low, similar to the pattern reported for *A. spatula* (Mendoza et al. 2002a). This can be explained within the context of the strictly carnivorous food habits of gar larvae. Additional studies are needed to determine if different level of carbohydrates in the diet change amylase activity patterns. Another observation that reinforces the idea of the low amynolitic capacity of this species is when the activities are expressed in relation to the individual. For almost all of the enzymes there was an observed progressive increase in activity, which correlates with the expected improvement in the enzymatic equipment of a larger fish (Cahu and Zambonino 1994; Moyano et al. 1996). The exception was amylase, where this pattern was not found, possibly indicating that the influence of this enzyme is barely significant during all early ontogeny stages.

Besides the luminal digestion by different pancreatic enzymes, there was a significant contribution of aminopeptidases and phosphatases. These enzymes are also indicators of intestinal maturation (Moyano et al. 2005). Aminopeptidases, located in the brush-border membrane of enterocytes, complete luminal digestion by hydrolyzing peptides, and phosphatases are involved in the intracellular digestion of phosphorylated proteins (acid phosphatase), absorption processes, and intestinal transport (alkaline phosphatase) (Letelier et al. 1985).

The decrease in acid phosphatase activity and the concomitant increase in alkaline phosphatase activity during the early stages of development have also been observed by Alvarez (2003) in *Paralabrax maculatofasciatus* and by Aguilera (1999) in *A. spatula*. The high activity of alkaline phosphatase in relation to acid phosphatase and aminopeptidase activities in Cuban gar larvae denotes the significance of intestinal absorption. This result, coupled with a short gut (with a spiral valve to increase absorption area) and lower intestinal proteolytic activity than in the stomach, suggests an active intestinal role in nutrient uptake.

In conclusion, the patterns of activity of the primary enzymes involved in the digestive

processes of larval Cuban gar indicate early functional development of this system and suggest that this species is a good candidate for aquaculture using the artificial diets given to other gar species (Márquez 2002; Mendoza et al. 2002a). However, a multidisciplinary approach should be used to evaluate the effects of artificial food from the first feeding.

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