Kasetsart J. (Nat. Sci.) 46 : 217 - 228 (2012)

Digestive Enzyme Activities During Larval Development of Striped Catfish, *Pangasianodon hypophthalmus* (Sauvage, 1878)

Wannapa Rangsin, Nontawith Areechon* and Ruangvit Yoonpundh

ABSTRACT

The specific activities of digestive enzymes of striped catfish, *Pangasianodon hypophthalmus* (Sauvage, 1878) larvae were examined from hatching to 21 days after hatching (DAH) using biochemical techniques. Fertilized eggs of striped catfish hatched after 24 hr and the larval yolk sac was totally absorbed by 3 DAH and then the larvae were provided with artemia throughout the experimental period. The mouth and digestive duct appeared 1 DAH and reached a morphological similarity to the adult 15 DAH. The specific activities of acid and alkaline protease were detected at hatching and 3 DAH, respectively. The specific activities of amylase and lipase were detected 1 DAH and at hatching, respectively. The specific activities of all studied enzymes showed the similar pattern of fluctuation during the first two weeks, and then increased until 21 DAH. These results demonstrated the presence of acid and alkaline proteases, amylase and lipase at a very early life stage, and showed an adaptation period of 2 wk to the first feeding. The existence of these enzymes means that striped catfish larvae have an endogenous ability to digest proteins, carbohydrates and lipids that increases with age. This information can lead to the possibility of developing a cost-effective formulated feed for intensive farming of this fish.

Keywords: Striped catfish, *Pangasianodon hypophthalmus*, protease, amylase, lipase

INTRODUCTION

Striped catfish, *Pangasianodon hypophthalmus* (Sauvage, 1878), also known as the river catfish and sutchi catfish, is a fast growing omnivorous fish and tolerant to high stocking densities (Paripatananont, 2002; Rahman *et al*., 2006). It is an important species in the Asian region due to its high dress-out weight percentage and great value in food processing (Paripatananont, 2002). Vietnam is the largest producer of striped catfish with a production of 1,094,879 t in 2008 (Bui *et al.*, 2010).

Striped catfish is also an economically important freshwater fish in Thailand. In 2005, the production of striped catfish in Thailand was 37,200 t (Department of Fisheries, 2007). However, one major problem in the culture of striped catfish larvae in Thailand is the high mortality rate which is thought to be due to cannibalistic behavior observed 2–3 days after hatching (DAH) (Baras *et al*., 2010; Morioka *et al*., 2010). Islam (2005) reported that cannibalism is evidenced when food is insufficient. The mortality rates could be reduced by using live food and releasing larvae into an earthen pond within a day of hatching. In order to develop mass juvenile production, the feeding schedule of larvae must be improved. Live food presents problems that include large investments in maintaining live food culture,

Department of Aquaculture, Faculty of Fisheries, Kasetsart University, Bangkok 10900, Thailand.
* Corresponding author, e-mail: ffisnwa@ku.ac.th

Received date : 01/08/11 Accepted date : 26/12/11

variable supply and nutritional inconsistencies (Kolkovski, 2001). A potential alternative to the provision of live food is the use of a compound diet which can save production costs as well as providing nutritional consistency and convenience (Kolkovski, 2001). However, the use of these feeds may be seriously limited by several factors, one of the most important of which is the ability of the larvae to efficiently digest the feeds and absorb the nutrients (Alvarez-González *et al*., 2008). Knowledge of the early ontogeny of digestive enzymes is therefore critical for understanding the mechanism pertaining to larval growth and survival strategies. Additionally, it can help to determine the appropriate time for weaning from live food to compound feed, as well as identifying the possibilities and limitations associated with the use of artificial diets (Alvarez-González *et al*., 2006). Digestive enzyme activity is a good indicator of larval digestive capacity; thus, being able to identify the time when high levels of digestive enzyme activity occur generally indicates that the fish larvae have become physiologically ready to assimilate exogenous feed (Gawlicka *et al*., 2000).

During the last decade, there has been an increase in the number of research reports on the development of digestive enzyme activity during the larval growth of cultured fish species (Suzer *et al*., 2007). However, most reports studied marine fish larvae. Proteins and lipids are important components of larval feed; thus, most research has focused on protease and lipase (Lazo *et al*., 2007). Carbohydrate is also important as an inexpensive source of energy which can help to reduce the feed cost but unfortunately, the activity of enzyme carbohydrase has been scarcely investigated (Lazo *et al.*, 2007).

There are a few published reports on the early developmental stages of striped catfish larvae; the focus of the available literature on this subject seems to be on early embryonic development and larval morphology (Islam, 2005; Baras *et al*., 2010; Morioka *et al*., 2010). To the best information of the authors, there are no data available related to the expression of digestive enzymes during early ontogenesis of striped catfish. Therefore, the current investigation was carried out to determine the ontogenetic development of the major digestive enzyme activities of striped catfish larvae fed on live food from hatching to 21 DAH.

MATERIALS AND METHODS

Fish larvae

Striped catfish eggs were obtained from the Pathum Thani Inland Fisheries Research and Development Center, Department of Fisheries. After hatching, larvae were transferred to 250 L fiberglass tanks. Three replicates were maintained for the experiment. Larvae were fed with *Artemia* nauplii after the yolk sac had been totally absorbed from 3–21 DAH. The nauplii were supplied in excess quantities three times daily.

Sample preparation

Larvae were collected every day from hatching to 9 DAH, and every alternate day between 9 and 21 DAH at 08:00 hours before morning feeding in order to minimize the potential effects of exogenous enzymes from undigested live food in the gut. In order to monitor growth, 20 larvae were sampled and weighed. The morphological development of larvae was recorded via microscopic examination. To determine enzyme activity, pooled sample of 400 larvae at hatching to 2 DAH, 200 larvae at 3 and 4 DAH, 100 larvae at 5 and 6 DAH, 50 larvae at 7–9 DAH and 20 larvae from 10 until 21 DAH were collected in triplicate at each sampling time. Following the collection, larvae were maintained in beakers filled with water for 1 hr to allow any remaining food in the gut to be assimilated or excreted. Then, the larvae were frozen at -20 °C until chemical analyses started. The frozen larvae were homogenized in four volumes (v/w)

of ice-cold phosphate buffer at pH 7 (Dawson *et al*., 1986) with a locally made glass pestle tissue grinder , followed by centrifugation at 10,000×*g* for 20 min at 4 °C. Supernatants or crude enzyme extract were stored at -20 °C until analysis. Soluble protein in the crude enzyme was measured by the Lowry method (Lowry *et al*., 1951) using bovine serum albumin as a standard.

Analysis of digestive enzyme activities

Amylase activity

Amylase activity was measured by a starch hydrolysis method modified from Vega-Villasante *et al.* (1999) and Natalia *et al.* (2004). The assay mixture contained 490 µL of phosphate buffer at pH 7 (containing 6.0 mM NaCl), 20 µL of crude enzyme and 500 µL of soluble starch solution (1% (w/v). The mixture was incubated at 37 °C for 60 min. Then, 1.5 mL of dinitrosalicylic acid (DNS) reagent was added and boiled for 5 min. After boiling, 1.5 mL of distilled water was added and the cooled solution was read at 550 nm. Blanks were prepared by replacing the crude enzyme with the buffer. For the Control, the crude enzyme was added after the addition of DNS reagent. All samples were assayed in triplicate. Maltose was used as the standard. One unit (U) of amylase specific activity was defined as 1nmol of maltose liberated per minute per milligram protein of the enzyme extract.

Protease activity

Protease activity was measured using the casein hydrolysis method modified from Vega-Villasante *et al.* (1999) and Hidalgo *et al.* (1999). In the current study, proteases were assessed throughout ontogeny at acid pH 3 and alkaline pH 11, which are the optimum acid and alkaline pH values that showed the highest activity from the previous study in juvenile striped catfish. The buffers used were citric acid-Na_2HPO_4 (McIlvaine) buffer solution, pH 3 and phosphate buffer solution, pH 11 (Dawson *et al*., 1986). The assay mixture contained 20 µL of crude extract, 200 µL of buffer, and 500 µL of azocasein (1% (w/v) in distilled water). The mixture was incubated at 37 °C for 60 min. The reaction was stopped with 500 µL of TCA (trichloroacetic acid, 20% (w/v)) and centrifuged at 15,000 *g* for15 min at 4 °C. Then, 1 mL of the supernatant was added to 1.5 mL of 1 M NaOH and the absorbance measured at 440 nm. The blank was similarly prepared by replacing the crude enzyme with the buffer. For the Control, the crude enzyme was added after the addition of TCA solution. All samples were assayed in triplicate. One unit (U) of protease specific activity was defined as a 0.001 change in the absorbance per minute per milligram protein of the enzyme extract ($\Delta Abs_{440} \times 10^3$ /min/mg protein).

Lipase activity

Lipase activity was measured by the *p*-nitrophenyl palmitate (*p*NPP) hydrolysis method modified from Katsivela *et al.* (1995) and Dosanjh and Kaur (2002). The assay mixture contained 800 µL of glycine-NaOH buffer solution, pH 9 (Dawson *et al*., 1986), 20 µL of crude enzyme and 200 µL of *p*NPP solution (0.01 M in isopropanol). The mixture was incubated at 37 °C for 30 min. The reaction was stopped with 250 µL of 0.1 M Na_2CO_3 and centrifuged at 15,000×*g* for 15 min at 4 °C. The absorbance of supernatant was measured at 410 nm. The blank was similarly prepared by replacing the crude enzyme with the buffer. For the Control, the crude enzyme was added after the addition of 0.1 M Na_2CO_3. All samples were assayed in triplicate. Para-nitrophenol (pNP) was used as the standard. One unit (U) of lipase specific activity was defined as 1µmol of pNP liberated per minute per milligram protein of the enzyme extract.

Statistical analysis

Mean values of each digestive enzyme specific activity between sampling periods were compared by one-way ANOVA, followed by Duncan's new multiple range test. The difference was considered significant at $P < 0.05$.

RESULTS

Larval development

Fertilized eggs of striped catfish were adhesive, round and brownish green in color. The hatching occurred within 24 hr. Growth was measured using the total length and wet weight. The average weight and length of fish larvae were 0.25 ± 0.10 mg and 2.88 ± 0.15 mm at hatching, respectively, and increased to 98.25 ± 19.02 mg and 25.03 ± 3.07 mm, respectively, at 21 DAH (Figure 1).

The important morphological development of striped catfish larvae is presented in Figure 2. The newly hatched larvae were transparent with a large, circular yolk sac and undeveloped mouth (Figure 2A). At 1 DAH, the mouth was formed as a terminal opening and the anus had just opened. The yolk sack was reduced in size and the digestive duct was a simple transparent tube lacking differentiation (Figure 2B). The yolk sack of 2 DAH larvae was further diminished. The alimentary canal had differentiated; a small pouch-like stomach was formed and the intestine became distinct (Figure 2C). In this stage, cannibalism was observed. Three days after hatching, the yolk sac was completely absorbed and the larvae started exogenous feeding (Figure 2D). During 4–7 DAH, the size of the stomach was larger than the intestine and the intestine was convoluted ventrally to the stomach (Figure 2E, 2F). The body of the larvae became more opaque 9 DAH, and at 15 DAH, the larvae were morphologically similar to the adult except for their color patterns (Figure 2G, 2H).

Amylase specific activity

The ontogenic-specific activity profile of amylase is shown in Figure 3. Amylase specific activity was undetectable at hatching, increased slowly to 3.223 ± 0.664 U·mg^{-1} protein·min^{-1} at 1 DAH and two peak activity regions were detected thereafter. The first peak of amylase specific

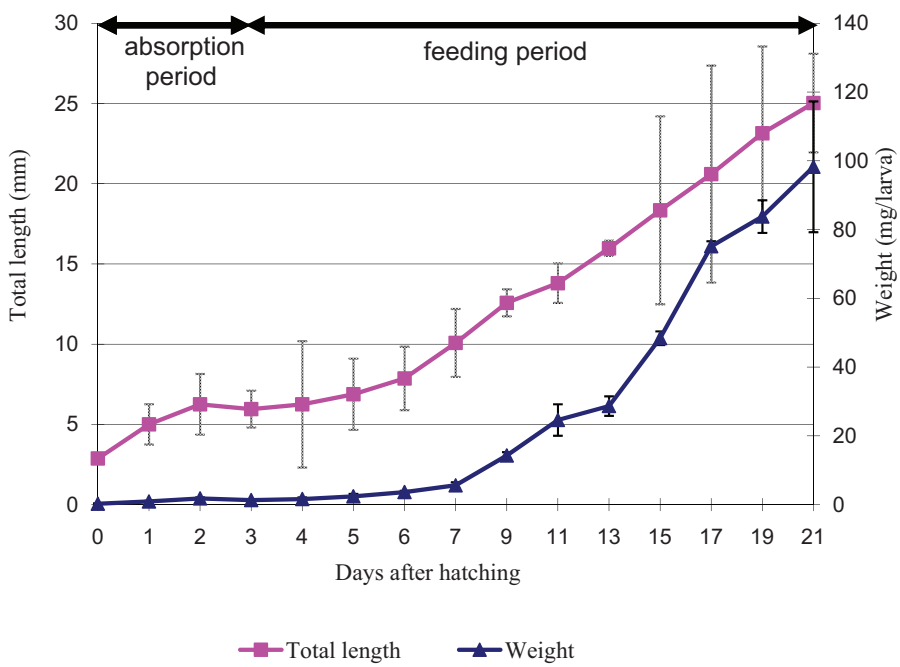

Figure 1 Average weight and length of striped catfish (*Pangasianodon hypophthalmus*) larvae from 0 to 21 days after hatching. (Vertical bars show the standard deviation.)

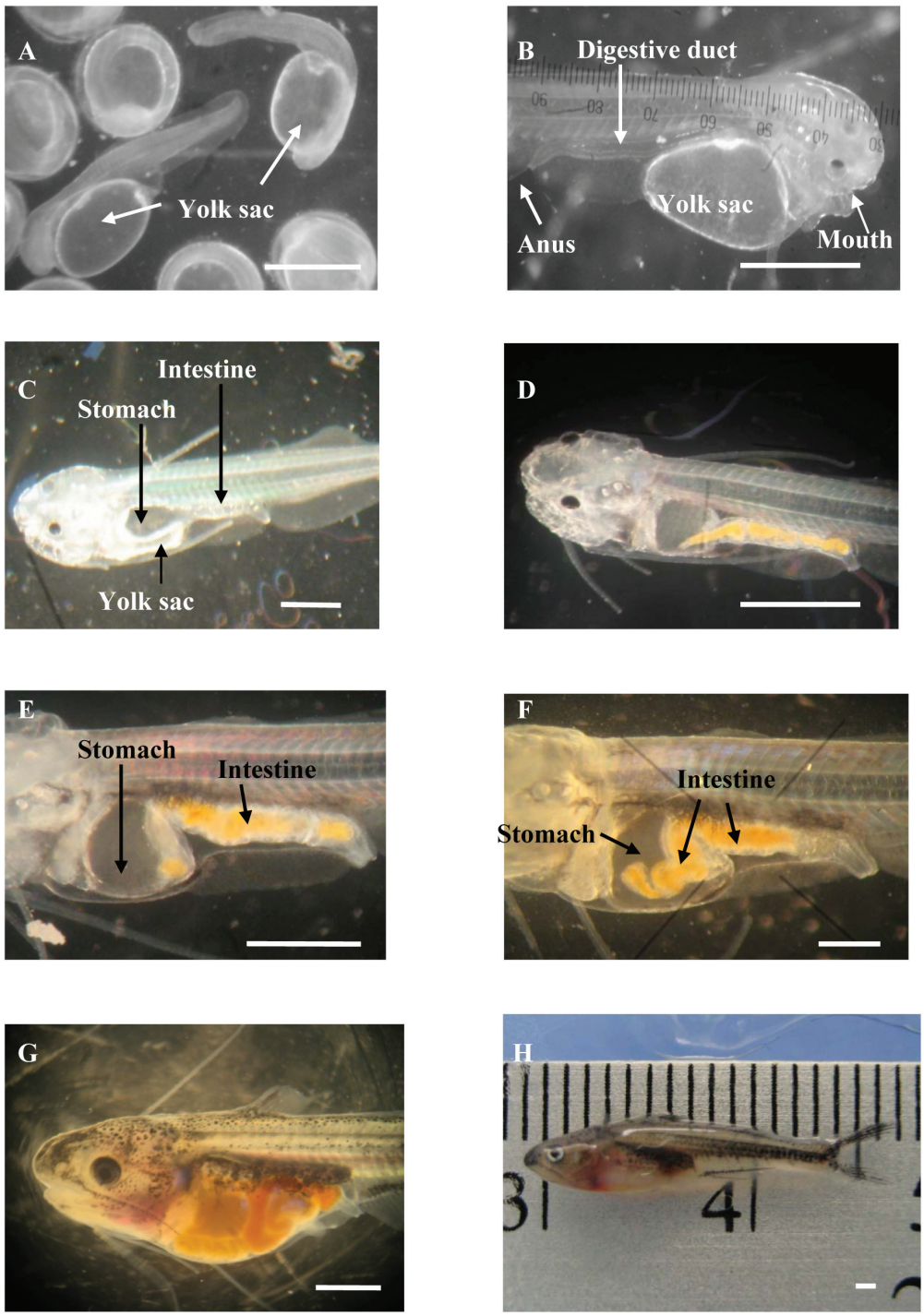

Figure 2 Morphological development of striped catfish larvae. (A) newly hatched larvae; (B) 1 DAH (days after hatching); (C) 2 DAH; (D) 3 DAH; (E) 4 DAH; (F) 7 DAH; (G) 9 DAH; (H) 15 DAH. (Horizontal scale bars = 1 mm.)

activity (37.144 ± 6.866 U·mg^{-1} protein·min^{-1}) was seen 3 DAH ($P < 0.05$). Then, the specific activity of amylase dropped to 10.775 ± 0.774 U·mg^{-1} protein·min^{-1} 5 DAH ($P < 0.05$) and reached a second peak 7 DAH at 86.961 ± 9.590 U·mg^{-1} protein·min^{-1} ($P < 0.05$). The specific activity decreased rapidly to 9.082 ± 0.747 U·mg^{-1} protein·min^{-1} 9 DAH ($P < 0.05$). After 9 DAH, amylase specific activity progressively increased until 21 DAH when it reached 137.173 ± 0.487 U·mg^{-1} protein·min^{-1} ($P < 0.05$).

Protease specific activity

The ontogenic specific activity profile of protease is shown in Figure 4. Acid protease specific activity was detected at hatching and

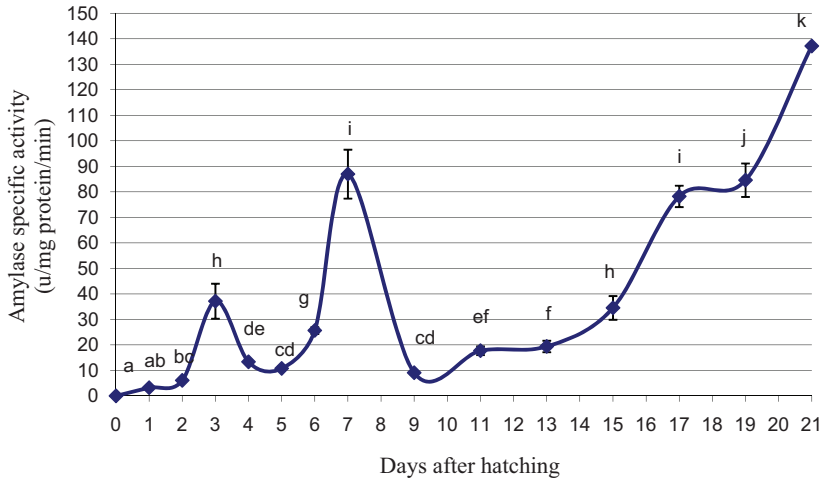

Figure 3 Amylase specific activity during development of *Pangasianodon hypophthalmus* larvae. Means ± SD with different lower case letters are significantly different by multiple comparisons ($P < 0.05$).

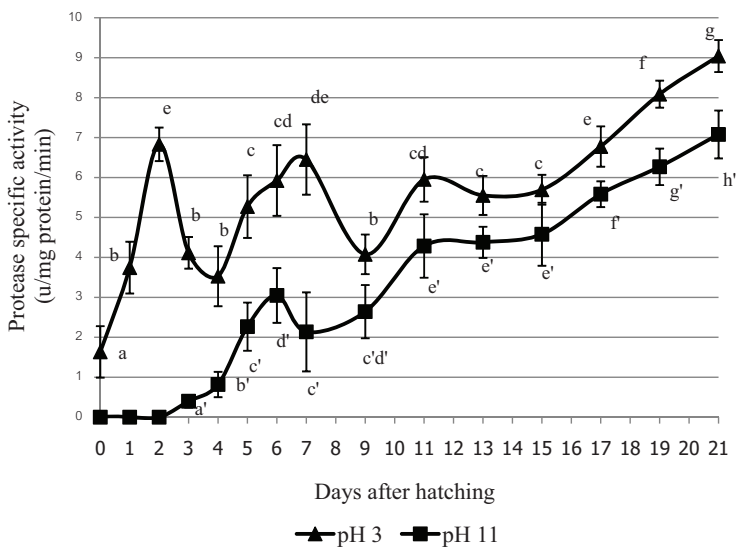

Figure 4 Protease specific activity during development of *Pangasianodon hypophthalmus* larvae at pH 3 and pH 11. Means ± SD with different lower case letters on the same curve are significantly different by multiple comparisons ($P < 0.05$).

showed three peak activity regions. The first peak occurred when acid protease specific activity increased rapidly from hatching at 1.635 ± 0.644 U·mg^{-1} protein·min^{-1} to 6.870 ± 0.410 U·mg^{-1} protein·min^{-1} 2 DAH ($P < 0.05$). Then, the specific activity of protease dropped to 3.503 ± 0.7514 U·mg^{-1} protein·min^{-1} 4 DAH ($P < 0.05$) and reached a second peak 7 DAH at 6.453 ± 0.881 U·mg^{-1} protein·min^{-1} ($P < 0.05$). The specific activity decreased rapidly thereafter to 4.078 ± 0.496 U·mg^{-1} protein·min^{-1} 9 DAH, and reached a third peak at 5.953 ± 0.5576 U·mg^{-1} protein·min^{-1} 11 DAH ($P < 0.05$). Protease specific activity decreased slightly to 5.694 ± 0.374 U·mg^{-1} protein·min^{-1} 15 DAH, and then progressively increased until 21 DAH when it reached 9.043 ± 0.400 U·mg^{-1} protein·min^{-1} ($P < 0.05$).

Alkaline protease specific activity was first detected at 0.399 ± 0.157 U·mg^{-1} protein·min^{-1} 3 DAH and showed two peak activity regions during ontogenesis. The first peak occurred when the alkaline protease specific activity increased rapidly from undetectable levels at hatching to 3.048 ± 0.686 U·mg^{-1} protein·min^{-1} 6 DAH ($P < 0.05$). Then, the specific activity dropped to 2.643 ± 0.667 U·mg^{-1} protein·min^{-1} 9 DAH and increased to 4.286 ± 0.794 U·mg^{-1} protein·min^{-1} on 11 DAH. From then on, the specific activity of the enzyme remained constant until 13 DAH, and then progressively increased until 21 DAH, when it reached 7.079 ± 0.600 U·mg^{-1} protein·min^{-1} ($P < 0.05$).

Lipase specific activity

The ontogenic specific activity profile of lipase is shown in Figure 5. Lipase specific activity was detected at hatching and showed two peak activity regions. The first peak occurred when the lipase specific activity increased rapidly from 8.460 ± 0.363 mU·mg^{-1} protein·min^{-1} at hatching to 12.105 ± 1.323 U·mg^{-1} protein·min^{-1} 2 DAH ($P < 0.05$).Then, the specific activity decreased rapidly 2 DAH to 2.360 ± 0.309 mU·mg^{-1} protein·min^{-1} 6 DAH and increased to the second peak at 8.222 ± 0.897 mU·mg^{-1} protein·min^{-1} 7 DAH ($P < 0.05$). The lipase specific activity then decreased to 2.410 ± 0.247 U·mg^{-1} protein·min^{-1} 11 DAH ($P < 0.05$) followed by a progressive increase until 21 DAH when it reached 7.767 ± 0.255 mU·mg^{-1} protein·min^{-1} ($P < 0.05$).

DISCUSSION

During the early stages, the growth of striped catfish increased steadily and a sharp increase was observed from 7 DAH onwards. The

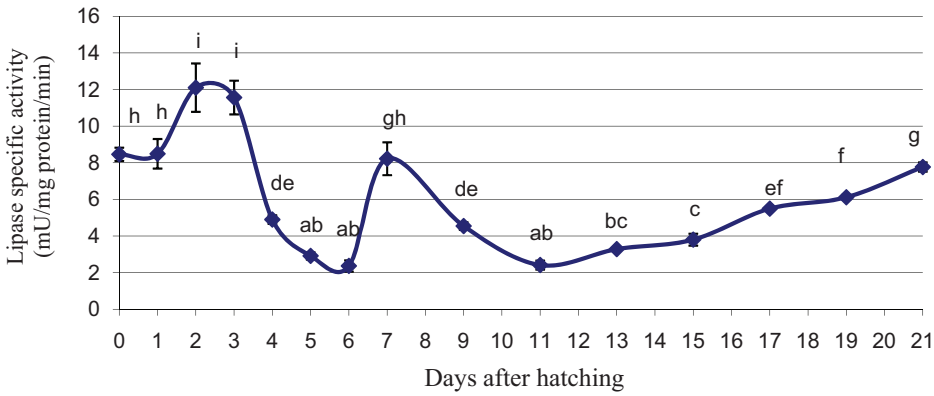

Figure 5 Lipase specific activity during development of *Pangasianodon hypophthalmus* larvae. Means ± SD with different lower case letters are significantly different by multiple comparisons ($P < 0.05$).

ontogenetic development of striped catfish found in this study was similar to other reports on this species (Termvidchakorn, 1997; Hiranwat, 1980; Islam, 2005; Baras *et al*., 2010). In the current study, amylase, protease and lipase specific activities in striped catfish larvae were detected prior to the first feeding. Thus, these findings indicate that these enzymes that were present in the early larval stage (0 –3 DAH) were part of the normal process of ontogenetic development and not the result of induction by exogenous feed which started in the larvae samples 3 DAH. This inference is in line with the findings of Chen *et al*. (2006) and Lazo *et al.* (2007) who suggested that the early development of digestive enzymes was triggered by internal mechanisms, rather than induced by dietary intake.

All the digestive enzyme activity detected during the ontogenesis of the striped catfish larvae exhibited a similar two-phase pattern which could be related to the morphological development of the digestive tract. Fluctuations found in the activity of the enzymes during 0–13 DAH could have been related to the concomitant morphological differentiation taking place in the digestive tract during this early period of growth. However, this view needs to be confirmed. During the next phase of growth—from 13 DAH to 21 DAH—the digestive system was more mature and functional as was reflected in a continuing increase in the activity of the digestive enzymes during this period of development. With regard to fluctuations in the digestive enzyme activity during the first two weeks of growth, Chen *et al*. (2006) suggested that the developmental pattern of enzyme activity from fluctuation to relative stability in the late stages may be related to anatomical and physiological modifications in the larvae during metamorphosis. Oozeki and Bailey (1995) reported that morphological changes (loop formulation) in the digestive tract may guarantee a long retention time and increase the mixing of feed and digestive enzymes in the digestive tract.

Amylase specific activity

The amylase specific activity of striped catfish was detected 1 DAH. Similar results were found by Lazo *et al*. (2000) in red drum (*Sciaenops ocellatus*) and by Gisbert *et al*. (2009) in common dentex (*Dentex dentex*). In other species, the first detection of amylase was 2 DAH for yellowtail kingfish (*Seriola lalandi*) reported by Chen *et al*. (2006) and also for sharpsnout seabream (*Diplodus puntazaa*) reported by Suzer *et al*. (2007).

The pattern of amylase activity in striped catfish larvae was in agreement with that previously reported for common carp (*Cyprinus carpio*) by Rathore *et al*. (2005) and for Indian major carp (*Cirrhinus mrigala*) by Chakrabarti and Rathore (2009). This variation of amylase specific activity can be attributed to the development of the digestive system (Chakrabarti *et al*., 2006), an increase in tissue protein (Rathore *et al*., 2005) and the adaptation of the larva to carbohydrate metabolism (Chakrabarti and Rathore, 2009). The relatively low specific activity of amylase during the first phase possibly reflects a limited ability to digest carbohydrates while the relatively high specific activity of amylase in the second stage suggests the better adaptation of striped catfish to carbohydrate utilization. The presence of amylase indicates that dietary carbohydrates may fill the energetic gap between endogenous and exogenous protein demand for larval fish (Kim *et al*., 2001). Thus, the utilization of carbohydrate as an inexpensive source of energy can provide a possible approach to reduce the cost of feed for fish larvae.

Protease specific activity

The specific activity of acid protease in striped catfish larvae was present at hatching, while the specific activity of alkaline protease was detectable later 3 DAH. Few studies to date have assessed both the acid and alkaline protease activity throughout the ontogenetic growth period. Most of these reported the presence of alkaline protease

before acid protease. Lazo *et al.* (2007) reported that red drum larvae possessed alkaline protease activity 3 DAH and acid protease 22 DAH. In California halibut (*Paralichthys californicus*), alkaline and acid protease specific activities were detected 1 and 15 DAH, respectively (Alvarez-González *et al.*, 2006). In the case of spotted sand bass (*Paralabrax maculatofasciatus*), alkaline protease specific activity was found 1 DAH, whereas acid protease specific activity was detected 12 DAH (Alvarez-González *et al.*, 2008). Guerreiro *et al.* (2010) reported the detection of acid protease activity in white seabream (*Diplodus sargus*) at hatching, when it showed its highest level, followed by a decrease to negligible values 2 DAH. Some papers referred to the acid protease specific activity observed during early stage of fish larvae as cathepsins activity (López-Ramírez *et al.*, 2010; Guerreiro *et al.*, 2010). Cathepsins are involved in yolk degradation and work actively at an acidic pH (Guerreiro *et al*., 2010).

The pattern of acid protease activity observed in striped catfish larvae has only been observed in few species, such as Senegal sole (*Solea senegalensis*) by Martínez *et al*. (1999) and white seabream by Cara *et al.* (2003). In contrast, the acid protease specific activity in several marine species was not detectable in the early stage of development and then increased with age in late stages such as those reported for red drum (Lazo *et al*., 2007) and spotted sand bass (Alvarez-González *et al*., 2008).

The pattern of specific activity of alkaline protease in striped catfish larvae has only been reported in a few freshwater fish species, such as Cuban gar (*Atractosteus tristoechus*) by Comabella *et al*. (2006) and Mayan cichlid (*Cichlasoma urophthalmus*) by López-Ramírez *et al*. (2010). In contrast, alkaline protease specific activity in most marine fish species was high during the early stage of development and then decreased with age such as in Senegal sole (Martínez *et al*., 1999), white seabream (Cara *et al*., 2003) and red drum (Lazo *et al*., 2007).

Both acid and alkaline protease specific activities showed similar patterns, except for the period between 0 and 3 DAH. However, the acid protease specific activity was constantly higher than that of the alkaline enzyme. This pattern has only been observed in a few species such as Cuban gar (Comabella *et al*., 2006). The early development of acid protease as well as the existence of alkaline protease supports the suggestion of the high capability of striped catfish to digest different types of proteins.

Lipase specific activity

In striped catfish larvae, lipase specific activity was detected at hatching. Similar results were also found in California halibut (Alvarez-González *et al*., 2006), red drum (Lazo *et al*., 2007) and common dentex (Gisbert *et al*., 2009). However variations have been reported in the time of first detection of lipase activity, for example 2 DAH in yellowtail kingfish (Chen *et al.*, 2006), 4 DAH in sharpsnout seabream (Suzer *et al*., 2007) and 13 DAH in Mayan cichlid (López-Ramírez *et al.*, 2010)

In the current study, the specific activity of lipase fluctuated from hatching to 11 DAH and then increased with larval development, therefore indicating that striped catfish larvae have a higher capacity to digest and utilize lipid in the second phase in comparison to the first phase of development. The pattern of lipase specific activity was similar to that reported in spotted sand bass (Alvarez-González *et al.*, 2008). In some species, the specific activity of lipase was detected at hatching and increased with growth, then decreased to a minimum at the end of the studied stage, such as in red drum (Lazo *et al*., 2000), California halibut (Alvarez-González *et al*., 2006) and yellowtail kingfish (Chen *et al*., 2006). In other species, like common dentex (Gisbert *et al*., 2009), the specific activity of lipase was high at the early stage and then decreased during larval

development. Oozeki and Bailey (1995) reported the existence of two types of lipase in walleye Pollock (*Theragra chalcogramma*), with the first type activated during the early stage and related to yolk-sac absorption, while the second type of lipase increased with age after day 14 and was related to the digestion of lipid in the live food.

CONCLUSION

The results from this experiment have demonstrated the presence of acid protease, amylase and lipase specific activities at an early stage of striped catfish (*Pangasianodon hypophthalmus*) larvae after the appearance of the mouth. However, the activities of the enzymes in the first two weeks of the larval stage were observed to fluctuate and were relatively low. For this reason, the 0–2 DAH larvae should be fed with live food or an easily digested diet such as boiled egg yolk. At 3 DAH, alkaline protease was detected, which suggested that artificial feed could be provided gradually to induce the enzyme, starting with partial replacement of live food, until total replacement at the end of this early larval stage (11 DAH). In the late stage of development (13–21 DAH), the enzyme activities increased with age which indicated the complete development of digestive enzymes in the striped catfish larvae. At this stage, live food can be totally replaced by artificial diets. Moreover, the existence of these enzymes suggested that striped catfish larvae have the ability to digest protein, carbohydrate and lipid, which can lead to the possibility for the development of cost-effective formulated feed for intensive farming of this fish. However, further research is needed to find a suitable feed formulation for the effective replacement of live food organisms that is easy to digest and provides the essential nutrients optimal for growth and survival of striped catfish during the nursing stage. This can also lead to the potential development of cost-effective formulated feed for the grow-out stage of this species that is very much needed in the aquaculture business.

ACKNOWLEDGMENTS

This research was supported by the Agricultural Research Development Agency (Public Organization), ARDA. The authors thank the Pathum Thani Inland Fisheries Research and Development Center, Department of Fisheries for providing the striped catfish eggs.

LITERATURE CITED

Alvarez-González, C.A., M. Cervantes-Trujano, D. Tovar-Ramírez, D.E. Conklin, H. Nolasco, E. Gisbert and R. Piedrahita. 2006. Development of digestive enzymes in California halibut *Paralichthys californicus* larvae. **Fish Physiol. Biochem.** 31: 83–93.

Alvarez-González, C.A., F.J. Moyano-López, R. Civera-Cerecedo, V. Carrasco-Chávez, J.L. Ortiz-Galindo and S. Dumas. 2008. Development of digestive enzyme activity in larvae of spotted sand bass *Paralabrax maculatofasciatus*. 1. Biochemical analysis. **Fish Physiol. Biochem.** 34: 373–384.

Baras, E., J. Slembrouck, C. Cochet, D. Caruso and M. Legendre. 2010. Morphological factors behind the early mortality of cultured larvae of the Asian catfish, *Pangasianodon hypophthalmus*. **Aquaculture** 298: 211–219.

Bui, T.M., L.T. Phan, B.A. Ingram, T.T.T. Nguyen, G. J. Gooley, H.V. Nguyen, P.T. Nguyen and S.S. De Silva. 2010. Seed production practices of striped catfish, *Pangasianodon hypophthalmus* in the Mekong Delta region, Vietnam. **Aquaculture** 306: 92–100.

Cara, J.B., F.J. Moyano, S. Cárdenas, C. Fernández-Diaz and M. Yúfera. 2003. Assessment of digestive enzyme activities during larval development of white bream. **J. Fish Biol.** 63: 48–58.

Chakrabarti, R., R.M. Rathore and S. Kumar. 2006. Study of digestive enzyme activities and partial characterization of digestive protease in a freshwater teleost, *Labeo rohita*, during early ontogeny. **Aquacult. Nutr.** 12: 35–43.

Chakrabarti, R. and R.M. Rathore. 2009. Ontogenic changes in the digestive enzyme patterns and characterization of proteases in Indian major carp *Cirrhinus mrigala*. **Aquacult. Nutr.** Doi:10.1111/j.1365-2095.2009.00694.x

Chen, B.N., J.G. Qin, M.S. Kumar, W.G. Hutchinson and S.M. Clarke. 2006. Ontogenetic development of digestive enzymes in yellowtail kingfish *Seriola lalandi* larvae. **Aquaculture** 260: 264–271.

Comabella, Y., R. Mendoza, C. Aguilera, O. Carrillo, A. Hurtado and T. García-Galano. 2006. Digestive enzyme activity during early larval development of the Cuban gar *Atractosteus tristoechus*. **Fish Physiol. Biochem.** 32: 147–157.

Dawson, R.M., D.C. Elliott, W.H. Elliott and K.M. Jones. 1986. **Data for Biochemical Research.** 3rd ed. Clarendon Press. New York.

Department of Fisheries. 2007. **Fisheries Statistics of Thailand** (in Thai). Department of Fisheries.Thailand.

Dosanjh, N.S. and J. Kaur. 2002. Biochemical analysis of a native and proteolytic fragment of a high-molecular-weight thermostable lipase from a mesophillic *Bacillus* sp. **Protein Expres. Purif.** 24: 71–75.

Gawlicka, A., B. Parent, M.H. Horn, N. Ross, I. Opstad and O.J. Torrissen. 2000. Activity of digestive enzymes in yolk-sac larvae of Atlantic halibut (*Hippoglossus hippoglossus*): Indication of readiness for first feeding. **Aquaculture** 184: 303–314.

Gisbert, E., G. Giménez, I. Fernández, Y. Kotzamanis and A. Estévez. 2009. Development of digestive enzymes in common dentex *Dentex dentex* during early ontogeny. **Aquaculture** 287: 381–387.

Guerreiro, I., M. de Vareilles, P. Pousão-Ferreira, V. Rodrigues, M.T. Dinis and L. Ribeiro. 2010. Effect of age-at weaning on digestive capacity of white seabream (*Diplodus sargus*). **Aquaculture** 300: 194–205.

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

Hiranwat, S. 1980. **Life History of Pla Sawai (*Pangasius sutchi* Flowler)** (in Thai). Technical paper no.3/1980, National Inland Fisheries, Freshwater Fisheries Division, Department of Fisheries. 58 pp.

Islam, A. 2005. Embryonic and larval development of Thai Pangas (*Pangasius sutchi* Fowler, 1937). **Develop. Growth Differ.** 47: 1–6.

Katsivela, E., F. Kleppe, S. Lang and F. Wagner. 1995. *Ustilago maydis* lipase I. Hydrolysis and ester-synthesis activities of crude enzyme preparation**. Enzyme Microb. Tech.** 17: 739–745.

Kim, B.G., S. Divakaran, C.L. Brown and A.C. Ostrowski. 2001. Comparative digestive enzyme ontogeny in two marine larval fishes: Pacific threadfin (*Polydactylus sexfilis*) and bluefin trevally (*Caranx melampygus*). **Fish Physiol. Biochem.** 24: 225–241.

Kolkovski, S. 2001. Digestive enzymes in fish larvae and juveniles-implications and applications to formulated diets. **Aquaculture** 20: 181–201.

Lazo, J.P., G.J. Holt and C.R. Arnold. 2000. Ontogeny of pancreatic enzymes in larval red drum *Sciaenops ocellatus*. **Aquacult. Nutr.** 6: 183–192.

Lazo, J.P., R. Mendoza, G.L. Holt, C. Aguilera and C.R. Arnold. 2007. Characterization of digestive enzymes during larval development of red drum (*Sciaenops ocellatus*). **Aquaculture** 265: 194–205.

López-Ramírez, G., C.A. Cuenca-Soria, C.A. Alvarez-González, D. Tovar-Ramírez, J.L. Ortiz-Galindo, N. Perales-García, G. Márquez-Couturier, L. Aris-Rodríguez, J.R. Indy, W.M. Contreras-Sánchez, E. Gisbert and F.J. Moyano. 2010. Development of digestive enzymes in larvae of Mayan cichlid *Cichlasoma urophthalmus*. **Fish Physiol. Biochem.** Doi:10.1007/s10695-010-9431-6.

Lowry, O. H., N. J. Rosebrough, A. L. Farr and R. J. Randall. 1951. Protein measurement with the folin phenol reagent. **J. Biol. Chem.** 193: 265–275.

Martínez, I., F.J. Moyano, C. Fernández-Díaz and M.Yúfera. 1999. Digestive enzyme activity during larval development of the Senegal sole (*Solea senegalensis*). **Fish Physiol. Biochem.** 21: 317–323.

Morioka, S., K. Sano, P. Phommachan and B. Vongvichith. 2010. Growth and morphological development of laboratory-reared larval and juvenile *Pangasianodon hypophthalmus*. **Ichthyol. Res.** 57: 139–147.

Natalia, Y., R. Hashim, A. Ali and A. Chong. 2004. Characterization of digestive enzymes in a carnivorous ornamental fish, the Asian bony tongue *Scleropages formosus* (Osteoglossidae). **Aquaculture** 233: 305–320.

Oozeki, Y. and K.M. Bailey. 1995. Ontogenetic development of digestive enzyme activities in larval walleye pollock, *Theragra chalcogramma*. **Mar. Biol.** 122: 177–186.

Paripatananont, T. 2002. Snakehead and pangasius catfish, pp. 396–401 *In* C.D. Webster and C. Lim, (eds.). **Nutrient Requirement and Feeding of Finfish for Aquaculture.** CABI Publishing. New York.

Rahman, M.M., Md. S. Islam and M. Tanaka. 2006. Effects of stocking density and low-cost conventional diet on the growth, yield and profitability in the cage culture of Sutchi catfish, *Pangasius sutchi*. **World Aquac.** 37: 6–13.

Rathore, R.M., S. Kumar and R. Chakrabarti. 2005. Digestive enzyme profile of *Cyprinus carpio* during ontogenic development. **World Aquac.** 36: 37– 41.

Suzer, C., S. Aktülün, D. Çoban, H. O. Kamacı, Ş. Saka, K. Fırat and A. Alpbaz. 2007. Digestive enzyme activities in larvae of sharpsnout seabream (*Diplodus puntazzo*). **Comp. Biochem. Physiol. A.** 148: 470–477.

Termvidchakorn, A. 1997. **The Development and Taxonomy Study of Catfish Fish Larvae Family Pangasiidae** (in Thai). Technical paper 3/1997, Aquatic Natural Resources Museum, Department of Fisheries. 59 pp.

Vega-Villasante, F., I. Fernández, R.M. Preciado, M. Oliva, D. Tovar and H. Nolasco. 1999. The activity of digestive enzymes during the molting stages of the arched swimming *Callinectes arcuatus* Ordway, 1863 (Crustacea:Decapoda:Portunidae). **Bull. Mar. Sci.** 65: 1–9.