Characterization and Ontogenic Development of Digestive Enzymes in Blue-Spotted Grouper (*Plectropomus leopardus* Lecepide, 1802) Larvae

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ABSTRACT

This research aimed to provide the basic data for future understanding of feeding habit and nutrient metabolism in blue-spotted grouper (*Plectropomus leopardus* Lecepide, 1802) larvae based on digestive enzyme studies. The pH (2–12) and temperature (25–80^{o}C) characteristics of total protease, amylase and lipase were determined before choosing the optimal conditions for the further study of the ontogenic development of enzymes. The total protease activities had an optimal pH of 2, 7 and 10 with the same optimal temperature at 50^{o}C. Amylase and lipase exhibited the highest activity at pH 7 and 45^{o}C. Specific activities of total proteases and amylase were positively correlated during experimentation ($r = 0.825$, $P < 0.05$). Lipase specific activity was found to be high in fertilized egg (2.20 ± 0.88 mU mg protein^{-1}) and newly hatched larvae (2.55 ± 0.75 mU mg protein^{-1}), and increased again during metamorphosis period (1.59 ± 0.25 mU mg protein^{-1}). The findings from characteristic studies suggest that pH 7 is suitable for evaluating *in vitro* digestibility of protein, carbohydrate and lipid, whereas ontogenic studies support understanding of the feeding habit and nutrient metabolism of blue-spotted grouper larvae.

Keywords: Digestive enzyme, total proteases, amylase, lipase, blue-spotted grouper, *Plectropomus leopardus*

INTRODUCTION

Blue-spotted grouper (*Plectropomus leopardus* Lecepide, 1802) is one of the most important commercial and recreational reef fish species throughout the subtropical and tropical coral areas (Qu *et al*., 2012). It is a candidate species, with high market price and demand, for aquaculture development in Thailand. However, the population of this species is currently declining due to habitat destruction as well as low survival rates from first feeding to grow-out stages (Yoseda *et al*., 2008; Qu *et al*., 2012), when compared with

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other finfish species. In particular, the blue-spotted grouper is considered to be the most difficult grouper species to rear due to early mass mortality from hatching until five days old (Sadovy *et al*., 2003; Yoseda *et al*., 2008). The main cause of early mass mortality in many grouper species, including blue-spotted grouper, is food deprivation. This occurs at the beginning of exogenous feeding when the larvae have a small mouth which restricts food particle size and hunting success (Yufera and Darias, 2007). Therefore, artificial diets with optimized nutritional quality might be used for solving nutritional problems of this species.

Nutrient utilization is driven by activity of digestive enzymes. In larvae of blue-spotted grouper, past research have primarily focused on ecology, physiology, ethology, reproduction and molecular biology (Qu *et al*., 2012). Recently, studies on morphological and histological developments of the digestive system have been reported (Trijuno *et al*., 2002; Qu *et al*., 2012). However, no basic data about digestive enzyme activities during the larval stages have been reported previously. Therefore, the objective of this present study was to characterize the optimal conditions (pH and temperature) and ontogenic study of the main digestive enzymes, including total proteases, amylase and lipase, in blue-spotted grouper larvae. These findings can be used for understanding the feeding habits and nutritional status of this species.

MATERIALS AND METHODS

Broodstock Preparation

The wild-caught broodstock of blue-spotted grouper (*Plectropomus leopardus* Lecepide, 1802) were reared in a 200 m^3 rectangular concrete tank at Trad Coastal Fisheries Research and Development Center, Thailand. The average body weight and total length of males and females were 3.62±0.06 and 2.24±0.2 kg, and 60.00±2.80 and 53.10 ±1.80 cm, respectively. Water temperature and salinity during the rearing period were 28–30^{o}C and 28–32 ppt, respectively. The propagation of blue-spotted grouper using males implanted with methyltestosterone (dose 0.5 mg kg^{-1} body weight) and natural spawning in closed recirculating water system was according to Sriveerachai *et al*. (2004).

Management of Rearing Tanks

Three rearing tanks (1,000 L-round fiberglass) containing 800 L seawater (30 ppt salinity) were prepared for larval stocking. Adult rotifers (*Brachionus rotundiformis* Tschugunoff, 1921) were added (2 individual mL^{-1}) to the rearing tanks for 2 days before the newly hatched larvae were stocked. The larvae were fed daily with rotifers and with a mixture of *Nannochloropsis* sp., *Isochrysis* sp., *Chlorella* sp. and *Tetraselmis* sp. at a final density of 300,000 cell mL^{-1}. Approximately 20–30% of water was exchanged daily by siphoning after 5 days of stocking and then up to 50% after 20 days.

Hatching and Larval Husbandry

Fertilized eggs of blue-spotted grouper were collected from the broodstock tank of Trad Coastal Fisheries Research and Development Center and transferred to Rayong Coastal Fisheries Research and Development Center, Thailand. The floating eggs were incubated in a 1,000 L-fiberglass with 30 ppt salinity and temperature of 28–29^{o}C. Un-hatched eggs were removed by

siphoning and then the water was changed and supplied with 80% new seawater. One day after hatching (1 DAH), the newly hatched larvae were transferred and stocked in a rearing tank at 50 individuals L^{-1}. The feeding techniques for newly hatched larvae were slightly modified from Wuthimethee *et al*. (2005) and Sriveerachai *et al*. (2006).

Briefly, 3 DAH larvae were fed with screened rotifers (80–90 µm lorica length), at 20 individuals mL^{-1}, twice daily at 9.00 and 15.00 h. In addition, the larvae were also fed with boiled chicken egg yolk mixed with liquid feed (Cargill Liqualife Z–M, Cargill, USA), a commercial product of n–3 highly unsaturated fatty acids (HUFA), and distilled water (ratio 5: 3: 1: 50), four times a day (6.00, 10.00, 14.00 and 18.00 h). During 10–16 DAHs, enriched rotifers were added at 10–15 individuals mL^{-1} without screening. From 17–35 DAHs, the larvae were fed with enriched *Artemia* nauplii (5–10 individual mL-1) and then older than 35 DAH were fed with *Artemia* metanauplii and adult *Artemia* (10–20 individual mL^{-1}) until 60 DAH. Rotifers and all stages of *Artemia* were enriched with a HUFA booster about 50 mg L-1 for 6 h before feeding.

Fish Sampling

The fish were randomly collected after feeding for 4 h. Ten larval fish were collected from three rearing tanks to measure their body weights and total lengths. The pooled whole bodies of 1–30 DAH-larvae were collected whereas the pooled digestive parts were used for 40–60 DAHs. All tissues were kept at –80°C until the crude enzymes were extracted.

Digestive Enzyme Study

Enzyme Extraction

The enzyme extraction was modified from Thongprajukaew *et al*. (2110b). Briefly, the whole bodies of 1–30 DAH-larvae and the digestive areas (abdominal region) of 40–60 DAH-larvae were homogenized in 50 mM Tris-HCl buffer pH 8 (1:4 w/v) using micro-homogenizer (THP-220, OMNI International, USA). The homogenate was centrifuged at 10,000 x g for 20 min at 4°C. The supernatant was collected and kept at –80°C for later determination of digestive enzymes. The total protein concentration of crude enzyme extract was determined according to standard method of Lowry *et al*. (1951) using bovine serum albumin as standard protein. All assays were performed in triplicates.

Characterization of Digestive Enzymes

Total protease activity was assayed by measuring the increase in cleaved short-chain polypeptide using azocasein as substrate, according to Areekijseree *et al*. (2004). The buffer solutions were glycine–HCl for pH 2, citrate–phosphate buffer for the pH range 3–5, phosphate buffer for the pH range 6–8, $NaHCO_3$–Na_2CO_3 buffer for the pH range 9–10, Na_2HPO_4–NaOH for pH 11, and KCl–NaOH for pH 12. For pH profile study, the reaction mixture was performed at ambient temperature and at pHs 2–12. For the temperature profile study, the reaction mixture was performed at chosen optimal pHs and at temperatures 25–80°C.

The pH and temperature characteristics of amylase and lipase were studied as described above. Amylase activity was

determined by measuring the increase in sugar reduction from starch solution, using 3, 5-dinitrosalicylic acid method (Areekijseree *et al*., 2004). Lipase activity was assayed by measuring the increase of *p*-nitrophenol from *p*-nitrophenyl palmitate according to Thongprajukaew *et al*. (2010a). The amount of liberated products from hydrolytic reaction of total protease, amylase and lipase was measured spectrophotometrically at 440, 540 and 410 nm, respectively.

Ontogenic Development of Digestive Enzymes

The optimal pH and temperature from characteristic studies were chosen. The specific activity of total protease was expressed as unit (U) mg protein^{-1}, where U is defined as an increase of 1.0 absorbance unit at 440 nm within 1 min (Thongprajukaew *et al*., 2010b). The specific activities of amylase and lipase were also defined as U mg protein^{-1} when giving liberated 1 µmol of maltose and *p*-nitrophenol within 1 min, respectively (Thongprajukaew *et al*., 2010a).

Statistical Analysis

The results are given as mean±SD (n=3). Estimates of the data in triplicate observations were calculated using Microsoft Excel 2007 (Microsoft Corp., Redmond, WA, USA). Regression analysis was carried out on concentration values to obtain standard curves. Analysis of variance (ANOVA) at the 95% significant level was performed, and multiple comparisons were analyzed by Duncan's multiple range test (DMRT) (Zar, 1999).

RESULTS

Growth of Larvae during Experiment

Body weight of blue-spotted grouper larvae before 10 DAH was not measured because they were very tiny. The weight of larvae increased slowly during 10–30 DAHs ($P > 0.05$, Table 1), and increased rapidly after 30 DAHs. However, no difference was observed between the weights of 40 and 50 DAHs. Total length increased differentially during larval development. No significant difference was found during 1–5 DAHs ($P > 0.05$) until after 10 DAH ($P < 0.05$). Condition factor (CF) of larvae increased with age and prominently peaked at 30 and 60 DAHs. The average specific growth rate (SGR) during 10–60 DAHs was 12.83 ± 0.02. The highest SGR was observed during 30–40 DAHs.

Characterization of Digestive Enzymes

Various isoforms of digestive proteases were observed in blue-spotted grouper larvae. Total proteases gave the highest liberated products in alkaline condition (pHs 10–11), followed by equal activities in acidic (pH 2) and neutral conditions (pH 7) (Fig. 1A). The activity of three selected proteases progressively increased in range of 25–45^{o}C and showed the highest value at 50^{o}C (Fig. 1B). However, the activity dramatically decreased at 55^{o}C for alkaline protease whereas probably be stable at 55^{o}C for acidic and neutral isoforms. For higher temperature (60–80^{o}C), all isoforms of total protease continuously decreased and the lowest activity was found at 80^{o}C.

One isoform of amylase was detectable in larvae of blue-spotted grouper. The activity of amylase was very low in both acidic and alkaline conditions (Fig. 2A). The activity was mainly presented at pH 6–7. The highest activity of pH 7 was found at 45^{o}C (Fig. 2B).

Table 1. The changes in weight, length and condition factor of juvenile blue-spotted grouper during 1–60 DAHs. Data were calculated from ten fish in each stage (n = 10).

Age (DAH)	Growth index			
	Weight (mg)	Total length (mm)	CF (g cm^{-3})	SGR (% day^{-1})
1	nd	2.03 ± 0.02^{a}	nd	nd
3	nd	2.15 ± 0.10^{a}	nd	nd
5	nd	2.51 ± 0.05^{a}	nd	nd
10	0.51 ± 0.03^{a}	3.30 ± 0.06^{b}	0.0019 ± 0.0001^{a}	nd
15	0.98 ± 0.04^{a}	4.48 ± 0.27^{c}	0.0090 ± 0.0007^{a}	13.07 ± 0.21^{b}
20	1.59 ± 0.07^{a}	5.90 ± 0.64^{d}	0.0338 ± 0.0040^{a}	11.38 ± 0.09^{a}
30	23.14 ± 2.03^{b}	12.28 ± 1.52^{e}	4.40 ± 0.63^{b}	19.07 ± 0.08^{e}
40	189.89 ± 9.40^{c}	21.70 ± 1.34^{f}	200.46 ± 20.24^{c}	19.73 ± 0.02^{d}
50	199.65 ± 8.99^{c}	22.11 ± 1.04^{f}	220.54 ± 18.74^{c}	14.92 ± 0.02^{c}
60	311.56 ± 13.68^{d}	26.80 ± 1.35^{g}	613.22 ± 56.30^{d}	12.83 ± 0.02^{b}

Mean±SD, nd = not detected. Different superscripts in the same column indicate significant difference ($P < 0.05$). Condition factor (CF) = 100 x [live body weight (g)/total body length $(cm)^3$] Specific growth rate (SGR) = 100 x (ln Wf – ln Wi)/T; Wi = initial average weight (g), Wf = final average weight (g), T = time between weighing (days)

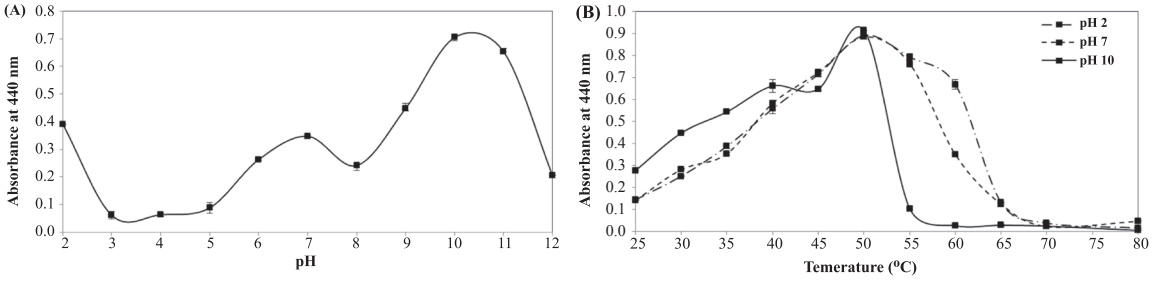

Figure 1. The pH profiles at ambient temperature (A) and temperature profiles at three optimal pH levels (B) of total protease in blue-spotted grouper larvae. Data points were calculated from triplicate samples (n =3).

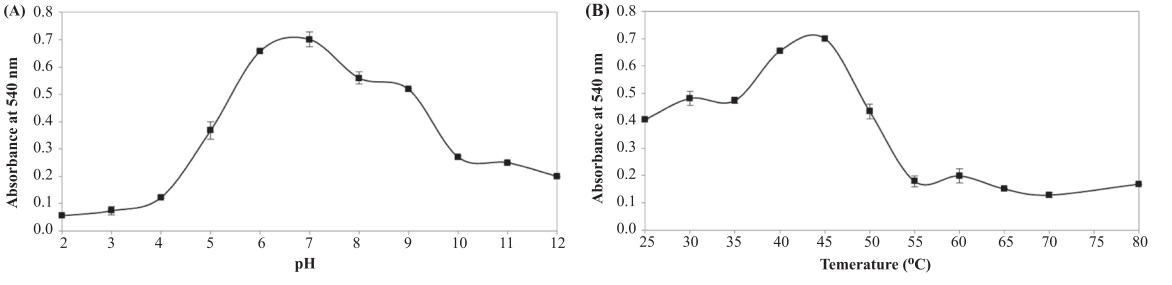

Figure 2. The pH profiles at ambient temperature (A) and temperature profiles at optimal pH (B) of amylase in blue-spotted grouper larvae. Data points were calculated from triplicate samples (n =3).

Lipase activity was prominently found at pH 7 whereas other isoforms were also present at acidic (pH 4) and alkaline conditions (pH 9) (Fig. 3A). The optimal temperature of neutral lipase showed the main peak at 45°C, followed by 30°C and 65°C (Fig. 3B).

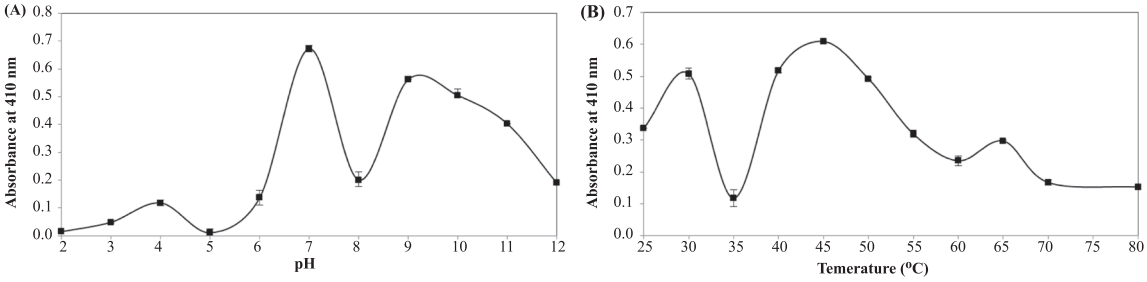

Figure 3. The pH profiles at ambient temperature (A) and temperature profiles at optimal pH (B) of lipase in blue-spotted grouper larvae. Data points were calculated from triplicate samples (n =3).

Ontogenic Development of Digestive Enzymes

Ontogenetic development had a significant effect on the three digestive enzyme activities. Specific activity of total proteases (Fig. 4A and Table 2) changed in a similar pattern with that found in amylase (Fig. 4B and Table 2). Specific activity appeared since the eggs were fertilized, and it progressively increased and showed the highest activity at 15 and 10 DAHs for total proteases and amylase, respectively. However, specific activity dramatically decreased, showing a small peak at 30 DAH and kept decreasing after 30 DAH until 60 DAH. Specific activity of lipase was higher in fertilized eggs and 1 DAH larvae (Fig. 4C and Table 2). Rapid decrease of the activity was observed after 1 to 5 DAHs. However, regained activity was prominently found during 30–40 DAHs (Fig. 4C and Table 2).

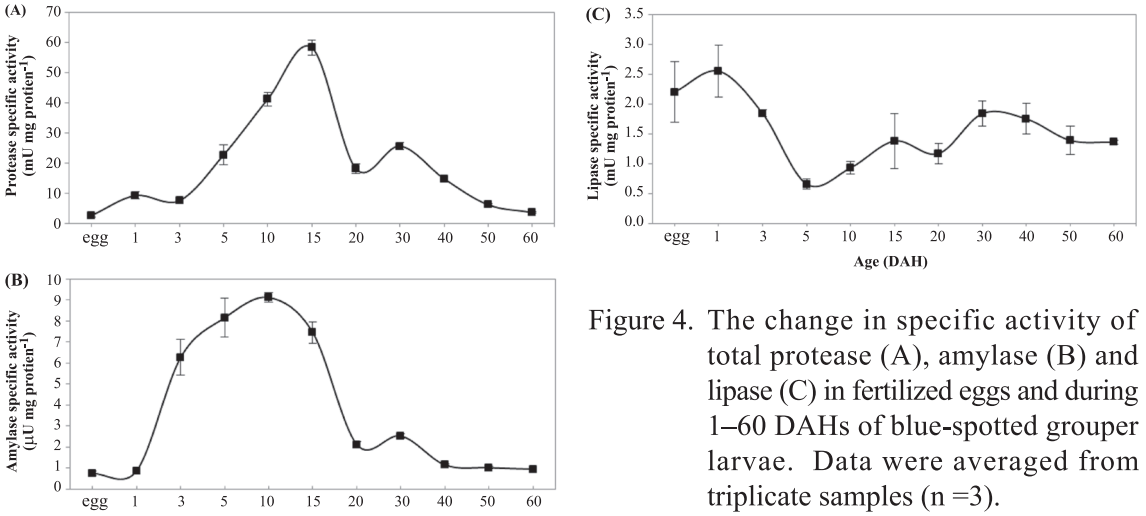

Figure 4. The change in specific activity of total protease (A), amylase (B) and lipase (C) in fertilized eggs and during 1–60 DAHs of blue-spotted grouper larvae. Data were averaged from triplicate samples (n =3).

Table 2. The ontogenetic development of three digestive enzymes in fertilized egg and juvenile blue-spotted grouper during 1–60 DAHs. Data were averaged from triplicate samples (n =3).

Age (DAH)	Total protease specific activity (mU mg protein^{-1})	Amylase specific activity (μU mg protein^{-1})	Lipase specific activity (mU mg protein^{-1})
0 (fertilized egg)	2.73±1.03^{a}	0.74±0.20^{a}	2.20±0.88^{cd}
1	9.35±2.04^{b}	0.87±0.17^{a}	2.55±0.75^{d}
3	7.68±0.47^{ab}	6.87±0.60^{d}	1.84±0.11^{bcd}
5	22.80±5.68^{de}	8.16±1.61^{fg}	0.66±0.14^{a}
9	41.27±3.74^{f}	9.13±0.37^{g}	0.93±0.19^{ab}
15	58.32±4.40^{g}	7.45±0.90^{df}	1.38±0.80^{abc}
20	18.23±2.96^{cd}	2.12±0.26^{bc}	1.17±0.30^{ab}
30	25.70±1.95^{e}	2.53±0.21^{c}	1.84±0.36^{bcd}
40	14.92±0.24^{c}	1.15±0.07^{ab}	1.75±0.46^{bcd}
50	6.30±0.51^{ab}	1.02±0.10^{a}	1.39±0.41^{abc}
60	3.76±0.64^{a}	0.95±0.04^{a}	1.36±0.06^{abc}

Mean±SD Different superscripts in the same column indicate significant difference ($P < 0.05$).

DISCUSSION

Growth of Larvae

Fish growth rate depends on various factors such as species, age, genetic potential, water temperature, health, and food quantity and quality. Low growth performance of newly hatched blue-spotted grouper is probably due to adaptation during first feeding. Qu *et al*. (2012) reported that mouth opening, yolk sac absorption and first feeding of this species occurred around 0–2, 2–3 and 3–19 DAHs, respectively. This finding is in agreement with slow increasing weight and length of the larvae during 1–20 DAHs (CF = 0.0019±0.0001 – 0.0338±0.0040 g cm^{-3}, SGR = 11.38±0.09 – 13.07±0.21 % day^{-1}) observed in this study. On the other hand, higher growth performance during 30–40 DAHs (CF = 4.40±0.63 – 200.46± 20.24 g cm^{-3}, SGR = 19.07±0.08 – 19.73± 0.02 % day^{-1}) might not be due to the less developed digestive tract but to its bigger capacity leading to more food consumption for energy which could be used for growth and complete development. It is assumed that during this stage, because of the less developed digestive system, the limited nutrient and energy intake are preferentially directed to organ development. These reasons correlate with the complete development of digestive glands from larval stage on 19 DAH to juvenile stages in this species, as reported by Qu *et al*. (2012). Therefore, changes in digestive system for contributing growth might be a crucial system for digestion, absorption and utilization of nutrients, like in juvenile Siamese fighting fish, *Betta splendens* (Thongprajukaew, 2011).

The average SGR during experimentation of blue-spotted grouper larvae was greatly higher (12.83% day^{-1}) than reported by Qu *et al*. (2012) (5.69% day^{-1}). This might be because the fast growth in this study was stimulated by higher water temperature (28–30^{o}C) when compared to the previous report

(24–29°C). Similarly, the larval development of Malabar grouper at water temperature of 30°C was faster than 28 and 25°C, respectively (Yosada *et al*., 2006). Moreover, Sabate *et al*. (2009) showed the growth in seven-band group reared in water temperature of 25–26°C was lower than this present study. Additionally, the growth in grouper larvae depends on other rearing factors such as photoperiod, aeration, and light intensity related with initial larval feeding, growth and survival (Yoseda *et al*., 2006, 2008).

Characterization of Digestive Enzymes

Various isoforms of proteolytic enzymes have been reported in many fish species (Chong *et al*., 2002; Castillo-Yanñez *et al*., 2004; Natalia *et al*., 2004; Lazo *et al*., 2007; Klomklao *et al*., 2008; Klahan *et al*., 2009; Thongprajukaew *et al*., 2010b). Prominence of acidic protease (pH 2) in blue-spotted grouper larvae indicated the presence of pepsin-like enzymes. This result is in agreement with histological observation of fully developed gastric gland after 19 DAH in this species (Qu *et al*., 2012). Therefore, the presence of enzyme activity during the larval period might be regarded as a sign of stomach functionality. The finding of suitable condition of the acidic protease in this study was pH 2 and 50°C, which is similar to that found in bolti fish, *Tilapia nilotica* (El-Beltagy *et al*., 2004), Asian bony tongue, *Scleropages formusus* (Natalia *et al*., 2004), grass carp, *Ctenopharyngodon idellus* (Liu *et al*., 2008), Siamese fighting fish (Thongprajukaew *et al*., 2010b), and sardinelle, *Sardinella aurita* (Khaled *et al*., 2011). Occurrences of total proteases from neutral (pH 7) to alkaline (pH 10) conditions designate the digestion of protein within intestinal section. The detected isoforms could probably be classified as serine proteases, trypsin and chymotrypsin, which are dominant in this organ. The optimal conditions in this study was similar to those found in Monterey sardine, *Sardinops sagax caerulea* (Castillo-Yanñez *et al*., 2004), red drum, *Sciaenops ocellatus* (Lazo *et al*., 2007), walleye Pollock, *Theragra chalcogramma* (Kishimura *et al*., 2008), grey triggerfish, *Balistes capriscus* (Jellouli *et al*., 2009), Siamese fighting fish (Thongprajukaew *et al*., 2010b) and goby, *Zosterisessor ophiocephalus* (Nasri *et al*., 2012). The findings from characteristic studies might indicate a carnivorous feeding habit of the larvae as well as protein digestion along the gastrointestinal tract.

The optimal conditions for studying amylase activity in blue spotted grouper larvae were pH 7 and 45°C. This condition is close to the pH range in pancreas and intestine (Chakrabarti *et al*., 1995) and natural habitat. Therefore, the neutral isoform appears to play a major enzyme for carbohydrate digestion in this species. This related condition has been similarly reported, such as pH 7.0–7.5 and 35–45°C in seabream, *Sparus aurata* and turbot, *Scophthalmus maximus* (Munilla-Morán and Saborido-Rey, 1996), pH 7 at 30°C in bouge, *Boops boops* and 45°C in red porgy, *Pagrus pagrus* (Fernández *et al*., 2001), and pH 7 at 40°C in Siamese fighting fish (Thongprajukaew *et al*., 2010a). The results from amylase characteristics study indicated that carbohydrate digestion mainly occurred in the intestine section of larval fish. Therefore, *in vitro* carbohydrate digestibility based on this condition could be used for future development of artificial feed formulation for blue-spotted grouper larvae.

Different isoforms of lipase were detected in blue-spotted grouper larvae. This might be due to the fish requiring substantial lipase to effectively digest the dietary lipid intake. On the other hand, the responses of lipase could be related to the catabolism of lipid reserves in yolk during morphological modification (Martinez *et al*., 1999). Requirements of fatty acid during larval periods vary with fish species (Sargent *et al*., 1999). However, the most common required fatty acids are docosahexaenoic (DHA) and eicosapentaenoic (EPA) acids, which are important for improving survival and fish growth as well as increasing stress tolerance to salinity and dissolved oxygen (Cahu and Zambonino-Infante, 2001; Wu *et al*, 2002; 2003). Therefore, addition of fatty acids to improve the larval quality might be of interest.

Lipase activity found in neutral (pH 7) and alkaline conditions (pH 9) indicated a significant role of lipid breakdown along the intestinal section. The presence of three isoforms at temperatures of 30, 45 and 65^{o}C probably is important for hydrolyzing received lipid with different thermal properties of fatty acids. The suggested optimal conditions of the fish is similar to those found in previous reports, such as pH 7 and 40^{o}C in juvenile Siamese fighting fish (Thongprajukeaw *et al*., 2010a) and pH 7 and 35^{o}C in guppy, *Poecilia reticulata* (Thongprajukaew and Kovitvadhi, 2013).

Ontogenic Development of Digestive Enzymes

The digestive proteases have been reported to play an important role in carnivorous and omnivorous species. Increased specific activity after 3 DAH indicated the functionalityof intestine and accessory organs. The assayed condition in this study (pH 7) was closely related to intestinal enzymes, probably trypsin or chymotrypsin. This finding is in agreement with the segmentation into buccopharynx, foregut, midgut and hindgut after complete yolk absorption, as well as liver and pancreas, which become functional at first feeding (Kolkovski, 2001). The highest specific activity of total protease at 15 DAH is similar to those found in other species, such as trypsin specific activity at 20 DAH of miiuy croaker, *Miichthys miiuy* (Shan *et al*., 2009), and trypsin and chymotrypsin specific activities at 15–20 and 15–25 DAHs, respectively, of common pandora, *Pagellus erythrinus* (Suzer *et al*., 2006).

Amylase activity also varies during development and depends on the nutritional habits (Hidalgo *et al*., 1999; Fernández *et al*., 2001). Development of amylase activity in blue-spotted grouper larvae occurs in a similar pattern as that of other marine finfish. Zambonino-Infante and Cahu (2001) reported that amylase specific activity was very high during young larval stages and markedly decreased during larval development. This correlates with higher amylase mRNA levels in younger than in older sea bass larvae, *Dicentrarchus labrax* (Peres *et al*., 1998). The findings are similar to peak of amylase transcripts at day 20, followed by a decrease during metamorphosis of winter flounder larvae, *Pleuronectes americanus* (Douglas *et al*., 2000). This change may be reflected by different nutrient requirements at different stages of life (Krogdahl and Sundby, 1999). Correlation analysis between specific activity of amylase and total protease was highly positive ($r = 0.825$, $P < 0.05$). This indicates that the protein utilization during larval period is closely linked to carbohydrate metabolism.

Marine fish eggs have high lipid content to fulfill larval requirement after hatching (Sargent *et al*., 1999; Cahu and Zambonino-Infante, 2001). The highest lipase specific activity of grouper larvae in 1 DAH indicates that they can digest the yolk as energy reserve before mouth opening until 3 DAH. Lipase activities were at high levels at 30–40 DAHs, and were almost constant until 60 DAH. This is necessary to meet the higher requirements of some fatty acids, such as EPA, DHA and arachidonic acid, for complete pigmentation and metamorphosis (Sargent *et al*., 1999). Moreover, high specific activity of lipase could be induced by the live preys or compound diets used in this study.

CONCLUSION

Characteristic studies of digestive enzymes in blue-spotted grouper larvae indicate that protein digestion occurs along the gastrointestinal tract. This larval species had a high capacity to digest proteins and lipids. The suitable conditions for studying digestive enzymes are pH 2, 7 and 10 at 50^{o}C for acidic, neutral and alkaline proteases, respectively, and pH 7 at 45^{o}C for amylase and lipase. Development of digestive enzymes during larval stages demonstrates similar activity profiles between total protease and amylase. Lipase specific activity responded during newly hatched and metamorphosis periods. These findings could be used as fundamental data for the development of artificial feed for blue-spotted grouper larvae.

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