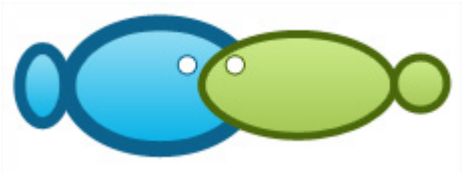

Enzymatic digestion of stomachless fish *Zenarchopterus buffonis*

[1]Diana A. Zainal Abidin, [1]Marina Hashim, [1,2]Simon K. Das, [3]Saleh M. Rahim, [2,4]A. Ghaffar Mazlan

[1] School of Environmental and Natural Resource Sciences, Faculty of Science and Technology, University Kebangsaan Malaysia, 43600 UKM Bangi, Selangor D.E., Malaysia; [2] Marine Ecosystem Research Centre, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor D.E., Malaysia; [3] Department of Biology, Faculty of Education for Pure Sciences, University of Tikrit, 3400 Tikrit Salah Aldeen, Iraq; [4] School of Fisheries and Aquaculture Sciences, University Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia.
Corresponding author: M. A. Ghaffar, magfish05@yahoo.com

Abstract. The absence of a stomach in halfbeaks (Hemiramphidae) does not restrain their capability in digesting food item. Halfbeak (*Zenarchopterus buffonis*) is known to have a wide spectrum of dietary preference. The present study investigates the activity of four digestive enzymes along the gut of halfbeaks to determine how they digest their diets as opportunistic omnivores. A total of 20 halfbeak samples were collected from the coastal waters of Peninsular Malaysia. Overall, we found that the α-amylase shows the highest enzymatic activity, followed by lipase and relatively low activity of protease (trypsin and aminopeptidase) along the alimentary canal, even though all of the enzymes show no significant difference among the gut sections. The increasing activity of *α*-amylase from proximal to distal intestine but with a notably drop-off in rectum zone demonstrates its high consumptions on arthropods and plant materials which are available in the environment. Presence of lipase with uniform distribution along the gut is expected due to insectivorous nature of fish, which gives an indication that there is possibility for halfbeaks to assimilate a wider array of diet nutrients. Our findings in this study is thought to be beneficial for improving knowledge on the biology and nutrition physiology of examined species and may provide valuable information on current model of stomachless digestive system, yet further refinement may be necessary.
Key Words: halfbeak, *α*-amylase, lipase, trypsin, aminopeptidase.

Introduction. Almost all vertebrates possess an alimentary tract that consists of the same basic components, which are the oesophagus, stomach, intestine and rectum, which function to process, digest, absorb and excrete food supported by auxiliary organs such as the liver, pancreas and gallbladder (Stevens & Hume 1995). The vertebrate stomach is derived from a growth of embryonic, undifferentiated alimentary canal (Smith et al 2000; Fukuda & Yasugi 2005). The motor activities of the vertebrate stomach relate its fundamental function in food storage, mechanically process it and initiate digestion by acid denaturation of ingested food and promoting enzymatic hydrolysis of proteins (Greger & Windhorst 1996). The lack of stomach is not only restricted to early developmental stages of fish such as larval fish (Kobegenova 1988), but also in several divergent teleost fish lineages that includes Cyprinidae, Labridae and Gobiidae, the stomach has been secondarily absent (Barton 2007). The Hemiramphidae (halfbeaks) is one of 15 families of stomachless fish. The commercial value of this halfbeak fish (*Zenarchopterus buffonis*) is relatively low especially in Malaysian region, thus less studies has been made and documented information related with their digestive enzyme activities on this particular species is yet to be published. The basic biology of this particular fish in coastal waters of Malaysia has been documented by Abidin et al (2015). Published materials on digestive physiology on halfbeaks are focused mainly in Australian coastal waters (Day et al 2011) but different localities may offer distinctive results based

on food availability. The present study was performed to study the enzymatic activity profile along the segments of alimentary canal of *Z. buffonis* in order to further understand how food is processed in the gut. The data obtained from this study would be useful for better understanding the digestion physiology of this fascinating stomachless fish.

Material and Method. A total of 20 fish samples were collected from coastal waters of Matang, Perak (4°49'0"N, 100°41'0"E) (Figure 1) between December 2012 and February 2013 using fishing rods (fishing hook size 6) on mangrove areas and shady underneath jetties. All fish samples were captured around mid-morning and late afternoon (08:00 and 13:00) to ensure the halfbeaks have fed adequately to fill their guts (Tibbetts & Carseldine 2005). However, due to the irregular nature of feeding opportunities, it is impossible to ensure that all fish had fed. After capture, the fish samples were transferred immediately into a Dewar flask filled with liquid nitrogen (-40°C) and kept frozen, subsequently transported to the laboratory of Universiti Kebangsaan Malaysia for further investigation.

Tissue preparation. The alimentary tract from ten *Z. buffonis* (standard length (SL) range 8.0−13.5 cm and it is measured from the tip of the upper jaw to the posterior end of the last vertebra) were eliminated, cut longitudinally in the mid-ventral region using scissors and contents were emptied and thoroughly rinsed using distilled water. In order to identify the food composition, contents of alimentary tract were analyzed under the light microscope. The guts were then divided into four approximately equal lengths of segments (proximal, middle, distal and rectal) and weighed individually. This is to quantify the difference in enzyme distribution and activity along the gut. Gut segments were homogenized individually using glass Teflon homogenizer (Polytron, Heidolph RZR 1, Germany) in 20 volumes (v/w) of ice cold 50 mMTris–HCl, pH 7.4. Homogenate-buffer solution was centrifuged at 5.500 × g for 5 minutes at 4°C. Supernatant was separated into 500 µL aliquots and stored at -80°C until used in enzyme assays.

Essay conditions. Assays were carried out in duplicate at room temperature (25°C) and absorbance was read with a Thermo Scientific Spectronic GENESYS^{TM} Visible Spectrophotometer 20 (Thermo Fisher Scientific, Inc., Waltham, Massachusetts, USA). All pH values for enzyme assays solutions were taken at room temperature and all reagents were purchased from Sigma-Aldrich (Selangor, Malaysia). Every reaction was run against homogenate and substrate blanks (Skea et al 2005), and all assays were run at saturating substrate concentrations as determined with preliminary optimizations (German et al 2004). Incubation times were also optimized prior to assays to ensure incubation time period was within linear range.

α-amylase. The method to determine α-amylase activity which was described by Bernfeld (1995), modified by Ezeji & Bahl (2007) where the reducing groups released from starch are measured by the reduction of 3,5-dinitrosalicyclic acid. The α-amylase activity was assayed by using 1% starch (Merck) solution in 0.02 M sodium phosphate buffer pH 7.0, containing of 0.006 M sodium chloride as substrate (Worthington 1993). A total of 0.5 mL of substrate solution was added to 0.5 mL enzyme preparation and followed by 5 minutes incubation at room temperature. The mixture was then added with 1 mL of 0.5 dinitrosalicyclic acid colour reagent and heated in a boiling water bath for 5 minutes. A volume of 1.5 mL distilled water was added and then cooled to room temperature. The sample was then read at 540 nm and α-amylase activity was determined from a maltose standard curve. Activity was expressed in U (1 µmol maltose liberated per minute) per wet weight of gut tissue.

Lipase. Lipase (EC 3.1.1.-) activity assay was performed by following the modified Ijima et al (1998) method which was adapted from German et al (2004). A total of 71.5 µL of 7.0 mM sodium cholate bile salt solution in 250 mMTris-HCl buffer solution (pH 9.0) and 2.5 µL of 10 mM 2-methoxyethanol were added to 5 µl of homogenate and incubated for

15 minutes at room temperature. Once the lipase has been triggered by the bile salt solution, 21 µL of 10 mM*p*-nitrophenolmyristate substrate which was dissolved in 95% ethanol, was added into the solution. Absorbance was measured continuously at 405 nm for 15 minutes. Lipase activity was quantified with a *p*-nitrophenol standard curve and results were expressed in U (1 µmol *p*-nitrophenol liberated per minute) per gram wet weight of tissue.

Trypsin. Trypsin (EC 3.4.21.4) activity was determined according to a modified version of the German et al (2004) assay. In a microcentrifuge tube, 12.5 µL of homogenate was added with 95 µL of 2.0 mM Nα- benzoyl-L-tyrosine-*p*-nitroanilide in 100 mMTris-HCl (pH 8.0), and read continuously for 20 minutes at 410 nm. Trypsin activity was measured from a *p*-nitroaniline (pNa) standard curve and expressed in U (1mol*p*-nitroanilide liberated per minute) per gram wet weight of tissue.

Aminopeptidase. Aminopeptidase (EC 3.4.11.2) activity was measured according to Roncari & Zuber (1969) by using 90 µL of 2.04 mML-alanine 4-nitroanilide hydrochloride (HCl dissolved) in 200 mM NaH_2PO_4/Na_2HPO_4 (pH 7.0), following by addition of 20 µL of gut homogenate. The sample was read at 410 nm for 30 minutes continuously and activity was determined with a *p*-nitroaniline (pNa) standard curve. Activity was expressed in U (1µmol*p*-nitroaniline liberated per minute) per gram wet weight of gut tissue.

Statistical analysis. The comparison of specific and total digestive enzyme activities along the gut of *Z. buffonis* was expressed as the mean±SD. All statistical analyses were conducted using One-way ANOVAs followed by a Tukey's HSD multiple comparison test where applicable. Family error rate set at $p \leq 0.05$ were used to perform intraspecific analyses of digestive enzyme activities, compared between each of the four segments of the gut, and interspecific total standardized gut activity, made between each enzyme. All statistics were conducted using Minitab® 17.1.0 for Windows (2013 Minitab Inc).

Results and Discussion. Consequent to dissection and removing the gut contents, it was observed that the alimentary canal of *Z. buffonis* consists of insects, crustaceans, terrestrial plant parts and unidentified particles or detritus (Table 1), which approves this species consume exclusively on arthropods and parts of plants that dropped from the mangrove tree.

Table 1

Diet items with some of their key chemical components, and hypothesised enzymes required to break them down (adapted from Karasov et al 2011)

Diet item(s)	*Key chemical components*	*Enzyme activities*			
		A-amylase	*Lipase*	*Trypsin*	*Amino-peptidase*
Insects	Cuticle, chitin, Glycogen	x	x	x	x
Crustacea	Chitin, glycogen	x	x	x	x
Terrestrial plant materials	Cellulose, starch, lignin, sucrose,	x	x		
Detritus	Cellulose, lignin, starches, α-glucans	x	x	x	x

This species demonstrated a clear pattern of increment *α*-amylase activity from proximal intestine (85.03 U mg^{-1}), followed by mid intestine (166.57 U mg^{-1}) and reached maximum value of activity at distal intestine (533.30 U mg^{-1}) but decreases at the rectal segment of intestine (296.97 U mg^{-1}) (Figure 1). The distal intestine displayed the highest level of activity than proximal, mid and rectal intestinal sections. However, no significant α-amylase performance pattern was found between gut segments.

The lipase activity showed not much significant difference from proximal intestine to rectal intestine distally, where proximal intestine (299.69 U mg^{-1}), mid intestine

(210.77 U mg^{-1}), distal intestine (307.22 U mg^{-1}) and rectum (292.98 U mg^{-1}) (Figure 2). Mid zone of intestine showed the lowest lipase activity among the whole regions of intestine.

Based on Figure 3, trypsin activity in the *Z. buffonis* demonstrated an overall trend towards a distally increasing gradient from proximal intestine (23.33 U mg^{-1}), mid intestine (49.97 U mg^{-1}), distal intestine (151.48 U mg^{-1}) and rectum (200.93 U mg^{-1}). The increasing trend in trypsin activity was not found to be significant.

Aminopeptidase activity exposed an increasing levels (37.28 U mg^{-1}, 39.74 Umg^{-1}, 76.58 U mg^{-1}, 76.48 U mg^{-1}) from proximal intestine, mid intestine, distal intestine and rectal of segment intestine respectively (Figure 4). Proximal and middle parts of intestine displayed similar lower level of aminopeptidase activity, while distal and rectal part of intestine has relatively higher levels of aminopeptidase activity, though the difference was again not significant.

As for Figure 5 demonstrated the whole enzyme activity along the gut proved that *a*-amylase activity has the highest activity on the distal intestine compared to other enzyme activity, while aminopeptidase has the lowest enzyme activity compared to other enzyme activities.

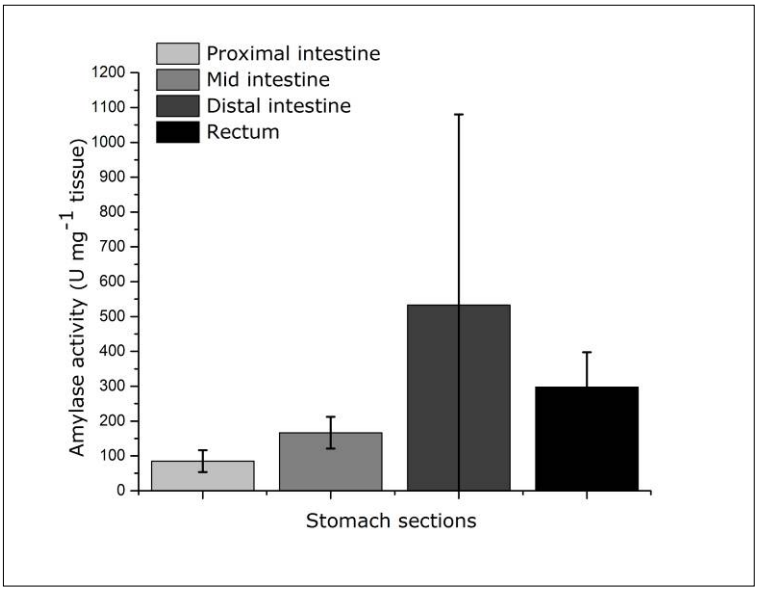

Figure 1. *a*-amylase activity along the gut segment in *Z. buffonis*.

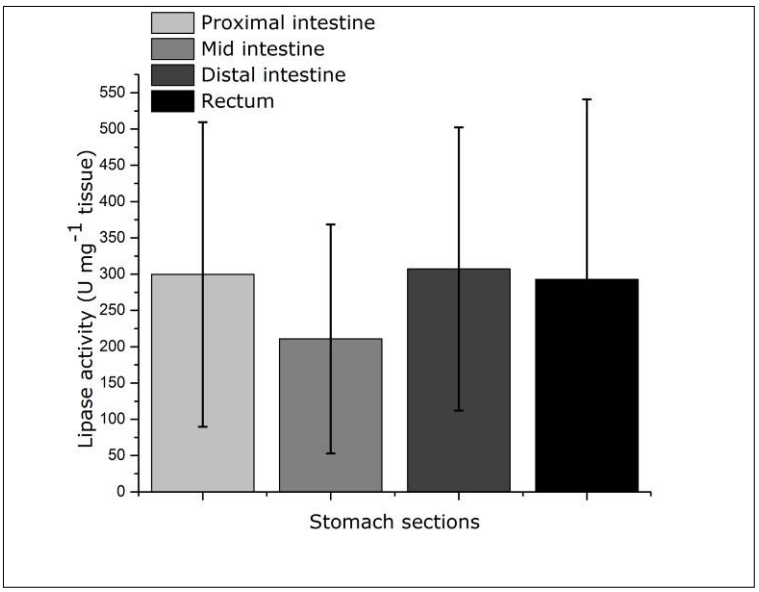

Figure 2. Lipase activity along the gut segment in *Z. buffonis*.

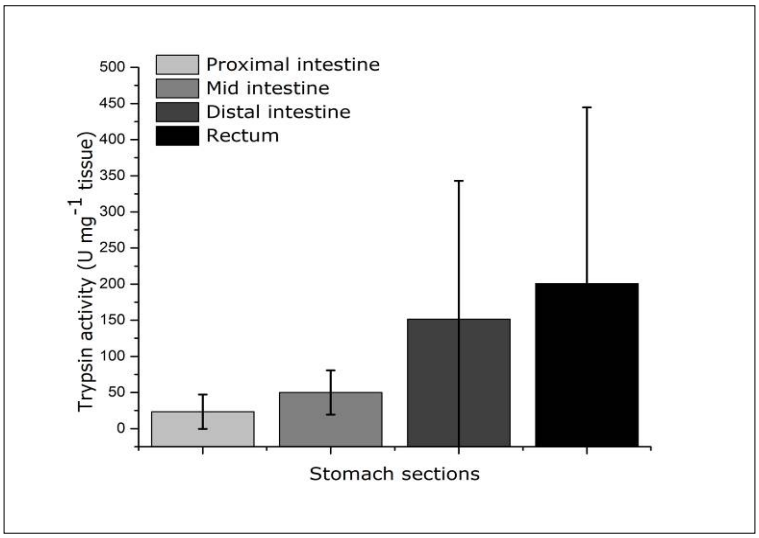

Figure 3. Trypsin activity along the gut segment in *Z. buffonis*.

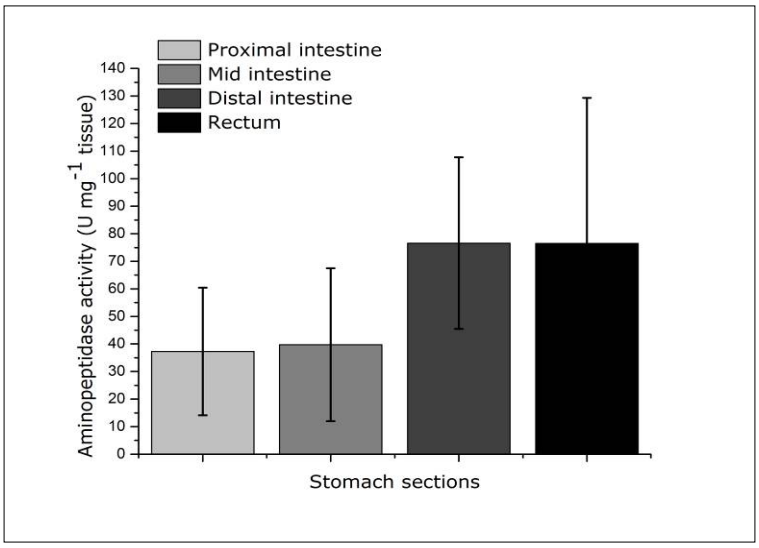

Figure 4. Aminopeptidase activity along the gut segment in *Z. buffonis*.

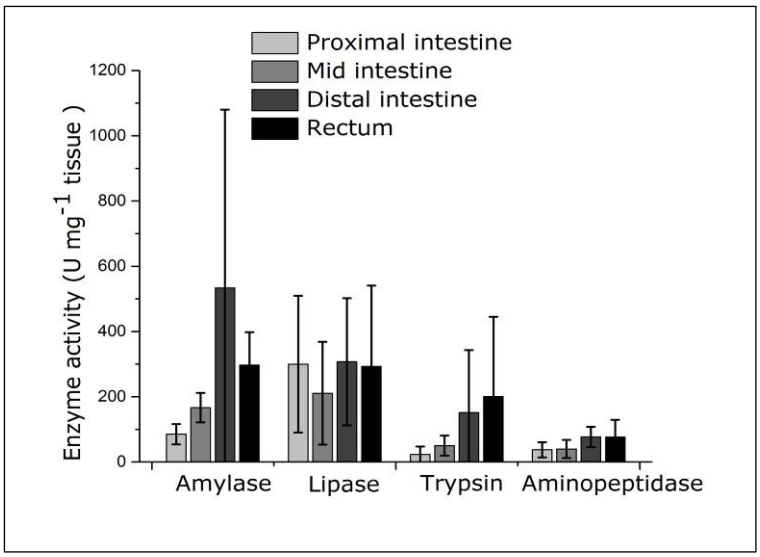

Figure 5. *α*-Amylase, Lipase, Trypsin and Aminopeptiade activity along gut segment in *Z. buffonis*.

Z. buffonis in brackish waters of Matang fed principally on terrestrial insects (Formicidae, Dysticidae, Diptera), crustaceans (shrimp) and parts of mangrove plant parts (Abidin et al 2015) which were found in all guts containing food. This observation has revealed that *Z. buffonis* is an insectivorous and opportunistic omnivore, which may due to vast availability of arthropods on mangrove leaves and trees in the environment.

Despite being a stomachless fish, biochemical digestion for specific enzymes along the alimentary tract reflects the nutritional proficiency of *Z. buffonis*. The digestive enzyme activity profile of *Z. buffonis* followed our predictions, with a prominence on dominant performance of amylase and lipase but low protein diet based on low levels of trypsin and aminopeptidase.

High *α*-amylase activity is an indication that *Z. buffonis* is capable of digesting carbohydrates in its diet. The digestion of starch appears to have initiated in the proximal zone and high *α*-amylase activity in the mid intestine ensured complete digestion of carbohydrates in this region. This is supported by Smoot & Findlay (2000) where some species have displayed enzyme activity design along the gut that range from a relatively consistent distribution to distally increasing (Logothetis et al 2001). This species approves this hypothesis with an increasing pattern of *α*-amylase from proximal to distal intestine, but with a prominent decline in *α*-amlyase activity at the rectal segment of the intestine. However, the rectal decrease is not as conspicuous as expected, suggesting that *Z. buffonis* are capable of digesting plant materials all the way through the intestine, including the rectum. As predicted, *α*-amylase enzyme profile for this species was relatively highest compared to other digestive enzymes. *α*-amylase is a protein enzyme that hydrolyses alpha bonds of alpha-linked polysaccharides such as starch and glycogen, producing glucose and maltose. Glucose or sugars such as sucrose are the photosynthesis product that can be found in plants and are essential storage saccharides in marine plants (Kuiper-Linley et al 2007). Therefore, marine plants such as mangrove fruit petal fragments that were found in food item contents during the gut dissection in this fish suggested that plant materials are a potential target of this omnivorous fish species. Amylase could also be required to digest glycogen, an energy source which is commonly found in animal tissue (Natalia et al 2004). Nevertheless, our result differs from Day et al (2011)'s results where they found that halfbeak fishes *Hyporhamphus regularis ardelio* and *Arrhamphus sclerolepis krefftii* from Queensland, Australia have distally declining of *α*-amylase activity along the gut. This is because habitat and locality differences could perform different adaptive digestive and metabolic strategies to manage with variations in environmental conditions (Duarte et al 2015). Moreover, in the natural environment, carbohydrates are absolutely more predominant than protein (Hari Sankar et al 2014). Therefore, this could be another reason that *Z. buffonis* possess higher α-amylase gradient compared to other digestive enzymes.

Lipase is one of the most important enzymes for digestion (Natalia et al 2004; Jun-Sheng et al 2006). Lipase is an enzyme which has the ability to hydrolyze ester bonds within the triacylglycerol at the hydrophilic-hydrophobic boundary (Park et al 2008; Prim et al 2003). *Z. buffonis* fish demonstrated similar levels of lipase activity profile, as little difference in digestive enzyme activity was showed in the entire gut system. Teleosts with exclusively short guts may absorb nutrients throughout the length of the gut (Ferraris & Ahearn 1984), which may justify the relatively uniform distributions of lipase activity along the gut of this fish. This observation is in agreement with reports from other hemiramphids such as *A. sclerolepis krefftii* (Day et al 2011). *Z. buffonis* requires substantial lipase activity to effectively digest the high dietary fat intake from live insects.

Despite possessing low protein diet, protease (trypsin and aminopeptidase) enzyme has been reported to play a vital role in omnivorous species (Munilla-Moran & Stark 1990; Eshel et al 1993). However, protease activity was a minor constituent of total activity and not as high as expected, which is consistent with reports on *Atherinopsis californiensis* (Horn et al 2006) which is a stomachless silverfish and also possess the same omnivorous diet, indicating that these enzymes are either not preferentially targeted as predicted or are common enough to allow for sufficient

assimilation despite low aminopeptidase activity. It has been claimed that the absorption of amino acids in fish could be due to passive transport. However, amino acid absorption in stomachless fish fed a diet containing a mixture of amino acids led to loss of body weight (Kaushik & Dabrowski 1983) and postulated gill excretion of amino acids. In addition, the chitin and cuticle that form the exoskeleton of the insects may take some time for it to be completely hydrolyzed from proximal intestine to rectum distally, thus the protein that contained in the internal parts of insect body can finally be hydrolyzed by trypsin and aminopeptidase into amino acid, hence elevated the activity of trypsin and aminopeptidase enzyme. Furthermore, elevated activity levels of proteases and lipases have been reported in herbivorous fish and are understood to allow these plant eating fishes to maximise the assimilation of any available proteins and lipids in their food intake, which can be considered limiting nutrients. Hence protein must be utilised efficiently (De Almeida et al 2006; German et al 2010).

Conclusions. This present study explains the existence of several major digestive enzymes functioning in the entire gut of *Z. buffonis* and this reflects their omnivorous nature. Hence they have the efficiency of hydrolyzing and digesting storage polysaccharides, lipid and protein using respective α-amylase, lipase, trypsin and aminopeptidase enzymes. The presence of these important enzymes explains that *Z. buffonis* possesses the ability to digest a wider array of food ingredients, making the development of cost-effective formulated feed for intensive farming of this fish a possibility. Future research into nutrient transport rates and other digestive enzymes on other parts of digestive organs will provide further understanding into how these nutritional demands are met, hence may help clarify a full characterization of the digestive strategy in *Z. buffonis*. Expansion of current knowledge of biochemical digestive mechanism in a variety of stomachless fishes is crucial in order to clarify and improve current model of digestion structure.

Acknowledgements. The authors would like to thank the Ichthyology Laboratory of Universiti Kebangsaan Malaysia for the dedicated field assistance throughout the sample collection. This research was financed and supported by MOSTI Science Fund, 04-01-01-SF0731: STGL-002-2013, AP-2012-013, JSPS-KPT UMT GRANT 53177, and GUP-2015-025.

References

Abidin D. A. Z., Hashim M., Das S. K., Ghaffar A. M., 2015 A description on pharyngeal jaw apparatus and diets of halfbeak fish *Zenarchopterus buffonis* (Valenciennes 1847) in Malaysian waters. AIP Conference Proceedings 1678:020013.

Barton M., 2007 Bond's biology of fishes. 3rd edition, Thomson, Belmont, CA.

Bernfeld P., 1955 Amylases alpha and beta. Methods in Enzymology 1:149-158.

Day R. D., German D. P., Manjakasy J. M., Farr I., Hansen M. J., Tibbetts I. R., 2011 Enzymatic digestion in stomachless fishes: how a simple gut accommodates both herbivory and carnivory. Journal of Comparative Physiology B 181:603-613.

De Almeida L. C., Lundstedt L. M., Moraes G., 2006 Digestive enzyme responses of tambaqui (*Colossoma macropomum*) fed on different levels of protein and lipid. Aquaculture Nutrition 12:443-450.

Duarte S., Silva F. C., Zauli D. A., Nicoli J. R., Araújo F. G., 2015 Gram-negative intestinal indigenous microbiota from two Siluriform fishes in a tropical reservoir. Brazilian Journal of Microbiology 45(4):1283-1292.

Eshel A., Lindner P., Smirnoff P., Newton S., Harpaz S., 1993 Comparative study of proteolytic enzymes in the digestive tracts of the European sea bass and hybrid striped bass reared in freshwater. Comparative Biochemistry and Physiology A 106:627-634.

Ezeji T. C., Bahl H., 2007 Production of raw-starch-hydrolysing alpha-amylase from the newly isolated *Geobacillus thermodenitrificans* HRO10. World Journal of Microbiology and Biotechnology 23:1311-1315.

Ferraris R. P., Ahearn G. A., 1984 Sugar and aminoacid transport in fish intestine. Comparative Biochemistry and Physiology A 77:397-413.
Fukuda K., Yasugi S., 2005 The molecular mechanisms of stomach development in vertebrates. Development, Growth and Differentiation 47:375-382.
German D. P., Horn M. H., Gawlicka A., 2004 Digestive enzyme activities in herbivorous and carnivorous prickleback fishes (Teleostei: Stichaeidae): ontogenetic, dietary, and phylogenetic effects. Physiological and Biochemical Zoology 77:789-804.
German D. P., Nagle B. C., Villeda J. M., Ruiz A. M., Thompson A. W., Contreras Balderas S., Evans D. H., 2010 Evolution of herbivory in a carnivorous clade of minnows (Teleostei: Cyprinidae): effects on gut size and digestive physiology. Physiological and Biochemical Zoology 83:1-18.
Greger R., Windhorst U., 1996 Comprehensive human physiology: from cellular mechanisms to integration. Springer-Verlag, Berlin, Heidelberg.
Hari Sankar H. S., Jose J., Varadarajan R., Bhanu S. V., Joy S., Philip B., 2014 Functional zonation of different digestive enzymes in *Etroplus suratensis* and *Oreochromis mossambicus*. International Journal of Scientific and Research Publications 4(5):1-10.
Horn M. H., Gawlicka A. K., German D. P., Logothetis E. A., Cavanagh J. W., Boyle K. S., 2006 Structure and function of the stomachless digestive system in three related species of New World silverside fishes (Atherinopsidae) representing herbivory, omnivory, and carnivory. Marine Biology 149:1237-1245.
Iijima N., Tanaka S., Ota Y., 1998 Purification and characterization of bile salt-activated lipase from the hepatopancreas of red sea bream, *Pagrus major*. Fish Physiology and Biochemistry 18:59-69.
Jun-Sheng L., Jian-Lin L., Ting-Ting W., 2006 Ontogeny of protease, amylase and lipase in the alimentary tract of hybrid juvenile tilapia (*Oreochromis niloticus* × *Oreochromis aureus*). Fish Physiology and Biochemistry 32:295-303.
Karasov W. H., Martinez del Rio C., Caviedes-Vidal E., 2011 Ecological physiology of diet and digestive systems. Annual Review of Physiology 73:69-93.
Kaushik S. J., Dabrowski K., 1983 Postprandial metabolic changes in larval and juvenile carp (*Cyprinus carpio*). Reproduction Nutrition Developpement 23:223-234.
Kobegenova S. S., 1988 On a possible cause of reduction of the stomach in some teleosts. Journal of Ichthyology 29:61-71.
Kuiper-Linley M., Johnson C. R., Lanyon J. M., 2007 Effects of simulated green turtle regrazing on seagrass abundance, growth and nutritional status in Moreton Bay, south-east Queensland, Australia. Marine and Freshwater Research 58:492-503.
Logothetis E. A., Horn M. H., Dickson K. A., 2001 Gut morphology and function in *Atherinops affinis* (Teleostei: Atherinopsidae), a stomachless omnivore feeding on macroalgae. Journal of Fish Biology 59:1298-1312.
Munilla-Moran R., Stark J. R., 1990 Metabolism in marine flatfish: VI. Effect of nutritional state of digestion in turbot, *Scophtalmus maximus* (L.) Comparative Biochemistry and Physiology B 95:625-634.
Natalia Y., Hashim R., Ali A., Chong A., 2004 Characterization of digestive enzymes in a carnivorous ornamental fish, the Asian bony tongue *Scleropages formusus* (Osteoglossidae). Aquaculture 233:305-320.
Park J., Cho S. Y., Cho S. J., 2008 Purification and characterization of hepaticlipase from *Todarodes pacificus*. BMB Reports 41:254-258.
Prim N., Sánchez M., Ruiz C., Pastor F. I. J., Diaz P., 2003 Use of methylumbeliferyl-derivative substrates for lipase activity characterization. Journal of Molecular Catalysis B: Enzymatic 22:339-346.
Roncari G., Zuber H., 1969 Thermophilic aminopeptidases from *Bacillus stearothermophilus*. I. Isolation, specificity, and general properties of the thermostable aminopeptidase I. International Journal of Protein Research 1:45-61.
Skea G. L., Mountfort D. O., Clements K. D., 2005 Gut carbohydrases from the New Zealand marine herbivorous fishes *Kyphosus sydneyanus* (Kyphosidae), *Aplodactylus arctidens* (Aplodactylidae) and *Odax pullus* (Labridae). Comparative Biochemistry and Physiology B 140:259-269.

Smith D. M., Grasty R. C., Theodosiou N. A., Tabin C. J., Nascone-Yoder N. M., 2000 Evolutionary relationships between the amphibian, avian and mammalian stomachs. Evolution and Development 2:348-359.
Smoot J. C., Findlay R. H., 2000 Digestive enzyme and gut surfactant activity of detritivorous gizzard shad (*Dorosoma cepedianum*). Canadian Journal of Fisheries and Aquatic Sciences 57:1113-1119.
Stevens C. E., Hume I. D., 1995 Comparative physiology of the vertebrate digestive system. Cambridge University Press, Cambridge, 400 pp.
Tibbetts I. R., Carseldine L., 2005 Trophic shifts in three subtropical Australian halfbeaks (Teleostei: Hemiramphidae). Marine and Freshwater Research 56:925-932.
Worthington V., 1993 Alpha amylase. In: Worthington enzyme manual. Worthington Biochemical Corporation Freehold, New Jersey, pp. 36-41.

Received: 31 March 2016. Accepted: 29 May 2016. Published online: 28 June 2016.
Authors:
Diana Atiqah Zainal Abidin, School of Environmental and Natural Resource Sciences, Faculty of Science and Technology, University Kebangsaan Malaysia, 43600 UKM Bangi Selangor, D.E., Malaysia, e-mail: diana.atiqah@gmail.com
Marina Hashim, School of Environmental and Natural Resource Sciences, Faculty of Science and Technology, University Kebangsaan Malaysia, 43600 UKM Bangi Selangor, D.E., Malaysia, e-mail: marinahashimhy@yahoo.com
Simon Kumar Das, School of Environmental and Natural Resource Sciences, Faculty of Science and Technology, University Kebangsaan Malaysia, 43600 UKM Bangi Selangor, D.E., Malaysia; Marine Ecosystem Research Centre, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor D.E., Malaysia, e-mail: skdas_maa@yahoo.com; simon@ukm.edu.my
Saleh Muhammad Rahim, Department of Biology, Faculty of Education for Pure Sciences, University of Tikrit, 3400 Tikrit Salah Aldeen, Iraq, e-mail: saleh_mr57@yahoo.com
Abdul Ghaffar Mazlan, Marine Ecosystem Research Centre, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor D.E., Malaysia; School of Fisheries and Aquaculture Sciences, University Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia, e-mail: magfish05@yahoo.com

How to cite this article:
Abidin D. A. Z., Hashim M., Das S. K., Rahim S. M., Mazlan A. G., 2016 Enzymatic digestion of stomachless fish *Zenarchopterus buffonis*. AACL Bioflux 9(3):695-703.