Aquaculture Reports 9 (2018) 1–9

Contents lists available at ScienceDirect

Aquaculture Reports

journal homepage: www.elsevier.com/locate/aqrep

Temperature and diet effect on the pepsin enzyme activities, digestive somatic index and relative gut length of Malabar blood snapper (*Lutjanus malabaricus* Bloch & Schneider, 1801)

Sabuj Kanti Mazumder[a,e], Simon Kumar Das[a,b,*], Saleh M. Rahim[c], Mazlan Abd Ghaffar[b,d]

[a] School of Environmental and Natural Resource Sciences, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, Malaysia
[b] Marine Ecosystem Research Center, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, Malaysia
[c] Department of Biology, Faculty of Education for Pure Sciences, University of Tikrit, Iraq
[d] Institute of Oceanography and Environment, University Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia
[e] Department of Aquatic Resource Management, Faculty of Fisheries, Sylhet Agricultural University, Sylhet, 3100, Bangladesh

ARTICLE INFO

Keywords:
Digestive enzyme
Digestive somatic index
Relative gut length
Pepsin
Temperature
Diet

ABSTRACT

An integrated experiment was performed on juvenile Malabar blood snapper (*Lutjanus malabaricus*) to investigate the effect of temperature and diet in their pepsin activities in relation with digestive somatic index (I_{DS}) and relative gut length (RGL). One hundred twenty *L. malabaricus* juvenile (13–15 cm) were equally distributed among four exposed temperature treatments (22, 26, 30 and 34 °C) representing their seasonal range and to account for end of century predicted temperatures, and two diets as commercial pellet and natural shrimp. After 7 days of acclimation period fish were reared for 30 days in twenty four 400 l glass aquaria at a stocking density of 5 fish tank^{-1}. All treatments were three replications. The result showed that, I_{DS} and RGL gradually decreased with increasing temperature up to 30 °C and again increase at 34 °C. And the values were also higher in pellet feeding fish than shrimp feeding fish at all the temperatures. Alternatively, in pepsin activity, an increased activity was seen between 26 °C to 30 °C and this activity was significantly higher than the 22 °C and 34 °C ($P < 0.05$). In general, highest pepsin activity was observed among fish which fed on a natural shrimp diet reared at temperature 30 °C (5.47 ± 1.60 U mg protein^{-1}), followed by those at 26, 34 and 22 °C ($P < 0.05$) at both diet however, no mortalities were observed. These results could be used as a basis for selecting a suitable diet for maximizing the growth and sustainable aquaculture coping with global warming.

1. Introduction

Feed taken in by fish undergoes through several mechanical and chemical processes. Once chewed and broken down into small pieces, feed is exposed to various enzymes like proteases, carbohydrases and lipases (Caruso et al., 2009). Studies on digestive secretions in fish can explicate certain aspects of its nutritional physiology and help resolve dietary problems, such as the matching of a diet to the nutritive competences of fish (German et al., 2004, 2010; German and Bittong, 2009; Skea et al., 2005, 2007). Although the array of digestive enzymes in bony fishes is the same as that in other vertebrates (Hidalgo et al., 1999; Stevens and Hume, 2004), digestive enzymes of fish are less studied. As essential digestive enzymes, pepsins are most prominently involved in the protein digestion in fish feeds. In fish, the levels of pepsin enzymes may be influenced by different exogenous and indigenous factors like age of the fish (Il'ina and Turetskiy, 1987), type of feed (Hofer et al., 1982; Hofer and Schiemer, 1981; Jónás et al., 1983), season and/or temperature of acclimatization (Kuzmina, 1991) etc. However, one of the most important difficulties in understanding the exerted action (the cumulative conversion catalyzed by enzymes) is that enzyme activity highly depends on temperature (Tijskens et al., 2001) as well as by the quantity and composition of diet (German et al., 2004; Péres et al., 1998).

The aquatic environmental temperature is changing globally. Temperature has profound effects on the structure and catalytic functions of enzymes embedded within metabolic pathways. All reactions are faster at a higher temperature. However, enzyme-catalyzed reactions become slower or stop if the temperature becomes too high, because enzymes become denatured at high temperatures. Therefore, enzymes have an optimum temperature that corresponds to maximum

* Corresponding author at: School of Environmental and Natural Resource Sciences, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 UKM Bangi Selangor, D.E., Malaysia.
E-mail addresses: skdas_maa@yahoo.com, simon@ukm.edu.my (S.K. Das).

https://doi.org/10.1016/j.aqrep.2017.11.003
Received 26 July 2017; Received in revised form 23 October 2017; Accepted 26 November 2017

activity. Increase in temperature, up to optimum level, favours aquaculture by reducing the time required to produce marketable size animals and producing more number of generations per year. Alternatively, at decreasing temperatures the rates of enzyme-catalysed reactions are reduced by the low heat content of the cellular environment (Q_{10} effect) that effects the weight ligands, and therefore the formation of the enzyme-substrate complex (Kita et al., 1996; Mazumder et al., 2015a,b).

Food preferences of the fishes were established in the course of adaptive radiation and colonization of these habitats, and these required a broad array of different digestive mechanisms to exploit successfully the variety of food available in the ever-changing environment (López-Vásquez et al., 2009). Several previous studies (Tengjaroenkul et al., 2000) have shown that the distribution and activity of digestive enzymes within the gut is affected by feeding habits. An understanding of the functioning of the digestive machinery helps determine the best digestibility of nutrients (Furné et al., 2008; Kolkovski, 2001). Studies on activity of digestive enzymes in fish thus can elucidate some aspects of fish nutritive physiology and contribute to resolving nutritional problems, such as the suitability of a diet and the nutritive capacity of a fish (Furné et al., 2008; Hidalgo et al., 1999; Kolkovski, 2001).

Another important aspect in new species farming is to measure the digestive tract indexes, such as the digestive somatic index (I_{DS}) and relative gut length (RGL). This information could assist in determining feeding type, especially for a species little studied.

Due to the decline in wild fisheries and sustained consumer demand, Malabar blood snapper (*Lutjanus malabaricus*) has been identified as promising candidates for coastal and marine aquaculture (Morais et al., 2001; Nanton et al., 2001; Rosenlund and Skretting, 2006). This species has good characteristics to be considered an adequate candidate for commercial aquaculture with high growth rate, excellent meat quality, and tolerance to overcrowding. The species is carnivorous active feeders, with a well-defined stomach and pyloric ceca, and a very short intestine divided into proximal, middle and distal regions. To our knowledge there is no information is available with respect to the digestive physiology of this specie under culture conditions.

In order to evaluate the possible involvement of temperature and diet in the regulation of digestion in *L. malabaricus,* it was decided to investigate the effect of rearing temperature and diet variation on the digestive somatic index, relative gut length and extractable activities of pepsin enzyme of *L. malabaricus*.

2. Materials and methods

2.1. Sampling and experimental setup

This assay was taken with a homogeneous group (13–15 cm) of *L. malabaricus* (n = 120) collected from local hatcheries of Pulau Ketam (03° 01′ 20” N and 101° 15′ 20” E), Selangor, peninsular Malaysia and transported to the marine science laboratory of UKM Bangi, Malaysia (Fig. 1). The fish were housed in stocking tanks with equal dimensions (1.96 m × 1.02 m × 0.61 m, 1200 l) for up to 7 days with a sustained environmental temperature (~26 °C), salinity (30 psu), pH (~7.5) and NH_3 (< 0.25 mg L^{-1}) and a diet consisting of formulated pellets (protein: 45–47%, lipid: 8%, and carbohydrate: 7%). The acclimation procedure followed the earlier investigations on various fishes (Chatterjee et al., 2004; Debnath et al., 2006). Once the fish started feeding and excreting wastes, they were transferred to 24 experimental tanks where the experiment was carried out for a period of 30 days. The tanks have equal dimensions (1.2 m × 0.5 m × 0.58 m, 400 l). Tanks were randomly divided into eight treatments (four temperatures × two diets). At four different temperatures, twelve tanks were subjected to pellet diet, and the other twelve tanks were subjected to shrimp diet. Under each temperature condition, three replicates were used. A flow-through system was used, and the water exchange rates were 0.5 l min^{-1}.

Each experimental tank contained five fish. The fish were manually fed with a commercial diet pellet (protein: 45–47%, lipid: 8%, and carbohydrate: 7%) or natural shrimp diet (Acetes sp.; protein: 57.55%, lipid: 7.56%, and carbohydrate: 7.54%) twice a day (09.00 and 16.00 h) until apparent satiation was observed (Donelson et al., 2010). Satiation was determined as the point when fish stopped feeding actively and feeds settled at the bottom of the tanks for more than 2 min. Leftover food was collected, dried, and weighed (the weight of dried left over food was about 71% of the wet food); and the total consumption was determined accordingly.

The temperature changes for the experimental groups were increased at a rate of 2 °C day-1 using a thermostat (E-JET heater 200 W, Penang, Malaysia) and a chiller (HS–28 A, 250–1200L/H, Guangdong Hailea Grouph Co. Ltd.) until the experimental temperature that started at the minimum temperature of 22 °C reached the maximum of 34 °C (22, 26, 30, and 34 °C) (De et al., 2016). The fish were then deprived of food for 2 days and anesthetized with α-methyl quinoline (Transmore^{R}; Nika Trading, Puchong, Malaysia) (0.22 ml L-1 in 3 l of sea water as an anasthetic medium for 10–15 min) prior to the initial measurement of body weight (g) and total length (cm). The total length was measured to the nearest 0.01 cm with a measuring board and weighed to the nearest 0.01 g using an electronic balance [Model: KD-300KC] (Simon et al., 2012). During the 30-day experimental period, the water quality parameters viz., temperature, salinity, and pH were monitored daily, whereas the total ammonia nitrogen ((NH_3-N) and total hardness were measured weekly. All measurements were taken at 0900 h. Temperature, pH, and salinity were monitored using a YSI 59 Multiparameter Water Quality Probe (Yellow Springs Instrument Company OH, USA). NH_3-N was measured using the salycilate method (Hach™ method 8155), and total hardness was measured by titration (La Motte Chemical test kit, model WAT-DR). Aeration was provided continuously except during feeding to maintain dissolved oxygen above 6 mg L^{-1}. During the experimental period, samples were maintained on a 12 h light:12 h dark photoperiod.

2.2. Digestive somatic index and relative gut length

After rearing 30 days of different temperature and diet treatments, fish were starved for 48 h prior to sampling in order to ensure their guts were empty. The samples were mildly anesthetized with α-methyl quinoline (Transmore^{R}; Nika Trading, Puchong, Malaysia) (0.22 ml L^{-1} in 3 l of sea water as an anaesthetic medium for 10–15 min) prior to the measurement of body weight (g) and total length (cm) (Simon et al., 2012). To eliminate any effect from differences in individual body mass, the digestive somatic index measured (I_{DS}) and relative gut length (RGL) were measured. For calculation of the I_{DS}, fish were weighed (M) and their entire gut (from oesophagus to anus) were removed by an incision at the oesophagus and cloaca. It was then cut longitudinally and washed thoroughly in ice-cold 0.1 mol L^{-1} phosphate-buffered saline (PBS, pH 7.4). After rinsing, the gut was blotted dry with filter paper, weighed (M_G) and stored at −20 °C until analysed. The I_{DS} (g) was calculated as: $I_{DS} = 100\, M_G\, M^{-1}$ (Abolfathi et al., 2012; Bélanger et al., 2002; Furné et al., 2008). The relative gut length was estimated by dividing the gut length by total length of the body (Al-Hussaini, 1949; Gupta, 2004). All efforts were made to minimize suffering. All animal holding and experimental protocols complied with Universiti Kebangsaan Malaysia ethics regulations (permission number: FST/2016/SIMON/27-JULY/763-JULY-2016-MAY-2017).

2.3. Homogenization

After careful unfreezing, the stomachs were isolated using a preparation needle and weighed. Stomachs were homogenized individually using glass and electric Teflon homogenizer (Polytron, Heidolph RZR 1, Germany) in 20 vols (v/w) of ice cold 50 mM Tris-HCl buffer solution with pH 7.4. The process was performed on ice. For the homogenization, a 50 mM Tris-HCl buffer solution with pH 7.4, was

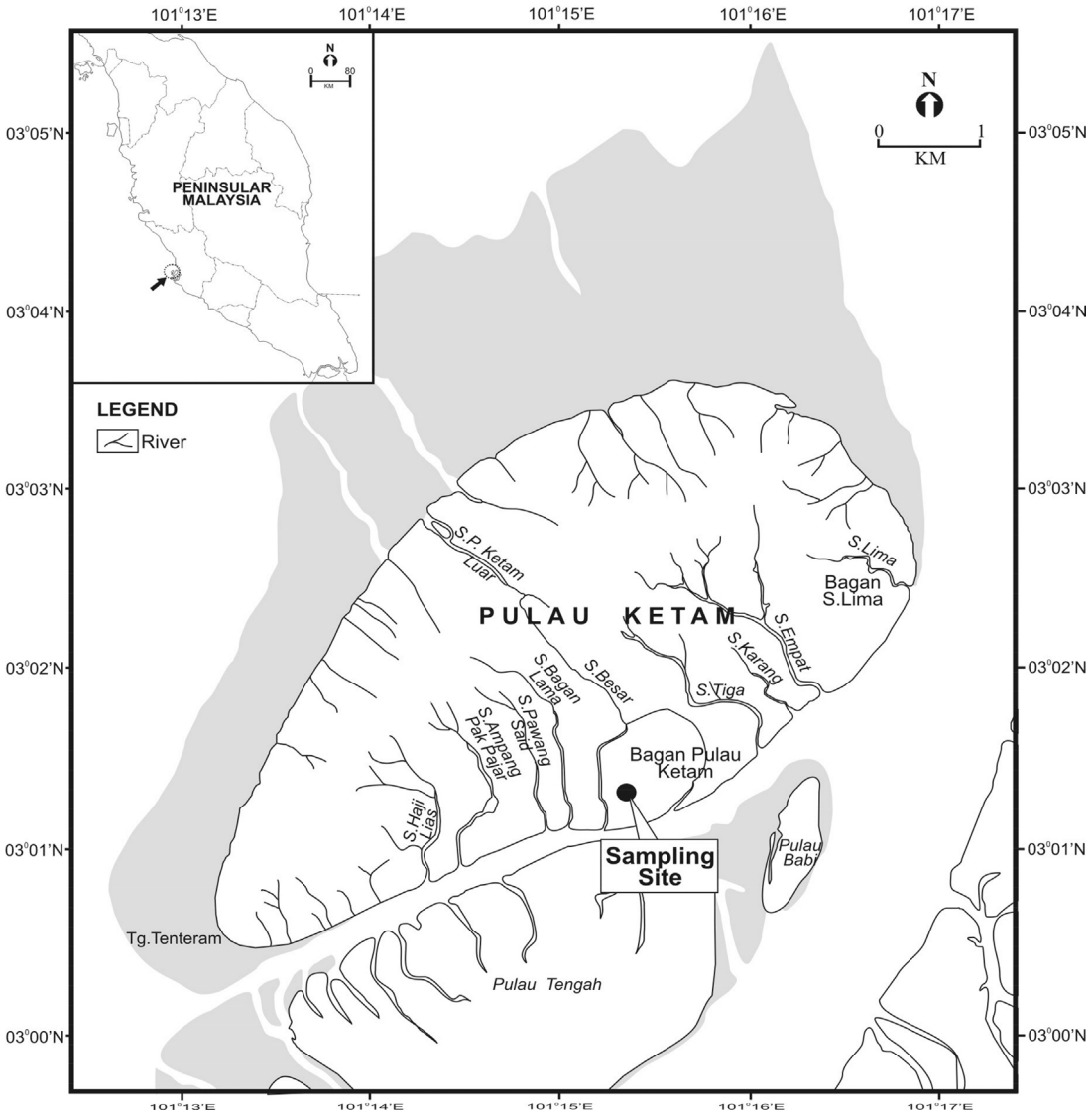

Fig. 1. Map depicting sampling site.

used at a proportion of 1 g tissue in 20 ml of buffer. The homogenates were then centrifuged at 4 °C at 10,000*g* for 15 min in a Kontron centrifuge model Centrikon H-401. After centrifugation, the supernatant was collected and frozen at −20 °C until used in enzyme assays.

2.4. Essay conditions

Assays were carried out in duplicate at room temperature (25 °C) and absorbance was read with a Thermo Scientific Spectronic GENESYS™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. Total protein content of stomach supernatant was determined using Bradford assay utilizing the BioRadR assay kit (Bradford, 1976).

2.5. Pepsin assay

The activity of pepsin was assayed in relation to soluble protein in the extracts (specific activity). Activity was assayed using 2% of haemoglobin in 0.06 N HCl as a substrate and activity was determined based on Natali et al. (Natalia et al., 2004). The assay is based on the stop-point assay of haemoglobin degradation developed by Anson (Anson, 1938). The buffer used was Tris-HCl (pH 7.4). The mixture consisted of 2% (w/v) solution of 0.5 ml haemoglobin, 1 ml TCA and 0.1 ml of crude enzyme. The process involved in two steps, one for unknown samples and another for blank tubes. The samples and blanks were assayed in duplicate. First taken 100 µl crude enzyme extract into each tube following 500 µl substrate (2% bovin haemoglobin). And for blank tubes taken 500 µl substrate (2% bovin haemoglobin). All mixtures were incubated for 10 min in hot air oven at 37 °C and the reaction stopped by adding 1 ml of 5% trichloroacetic acid (TCA) and in blank tube added 100 µl of crude enzyme extract. All the samples and blanks were left at room temperature for 5 min. The samples were then centrifuged at 12000 *g* for 5 min and measurements were carried out by UV/VS spectrophotometer (Ultro Spec 2000 Pharmacia Biotech) and absorbance was carried out at 280 nm. The specific enzyme activity was expressed as unit per milligram of protein (U mg protein^{-1}) as:

[Absorbance value at 280 (supernatant) − Absorbance value at 280

(blank)] × 1000/(10 min × mg protein)

2.6. Statistical analysis

No significant differences were found among any of the replicate means ($P > 0.05$), and the data for the different replicates were therefore averaged (Dean and Voss, 1999). Homogeneity of variance was assessed with Bartlett's test and the Kolmogornov-Smirnov (K-S) test was used to test normality to ensure the requirements of ANOVA were met (Sokal and Rohlf, 1995). Two way ANOVA was used to compare I_{DS}, RGL and specific pepsin enzyme activities at the different water temperatures and diet. Pairwise comparison of means was conducted with Tukey test to identify significant differences (Zar, 1984). Values in the text are expressed as mean ± standard error of the mean (SE), unless otherwise stated and $P < 0.05$ was used as the level of statistical significance. All measurements were carried out in duplicate. A nonlinear polynomial cubic model was fitted to express the relationship of specific pepsin activity with I_{DS} and RGL.

3. Results

3.1. Water quality parameters

Mean values ± SE of physico-chemical parameters recorded in experimental tanks during this study are presented in Table 1. They were adequately stable at the nominal treatment levels of 22, 26, 30, and 34 °C and there was no significant difference ($P > 0.05$) in all the parameters except TH which is significantly different between 34° C and 22° C respectively. These values are within suitable ranges for culture of this species (Marković et al., 2009).

3.2. Digestive somatic index and relative gut length

The biometric parameters for *L. malabaricus* at different temperature and diet are presented in Table 2. Results showed that body mass increased significantly ($P < 0.05$) with increasing temperature from 22 °C to 30 °C and slowed down again at 34 °C. At 30 °C the body mass increased markedly whereas the lowest was observed at 22 °C in pellet and shrimp diet respectively. At all temperatures the values were higher in shrimp diet than that of pellet diet but they are not significantly different from each other at all the temperature ($P > 0.05$, Table 2). Conversely, the digestive tract weight and length decrease with increasing temperature. The highest digestive tract weight and length were observed at 22 °C feeding with pellet diet and lowest observed at 30 °C feeding with shrimp diet. The values of I_{DS} & RGL also showed decreasing trend. At 22 °C the values were observed to the maximum at both diets. When the exposed temperature increased from 22 to 30 °C the values of I_{DS} & RGL gradually decreased and again raised with further increase to 34 °C. The minimum I_{DS} & RGL values observed at 30 °C in both diets. However, the values are not changed significantly with diet variations ($P > 0.05$) except at 22 °C in I_{DS} and 22 °C and 26 °C in RGL.

The two-way ANOVA analysis showed that temperature affected significantly on fish weight (*g*) but diet and the interaction between temperature and diet did not affect significantly on final weight of the *L. malabaricus*. But in case of digestive tract weight (*g*) diet did not affect significantly ($P > 0.055$). Whereas, I_{DS} changed significantly ($P < 0.05$) when changing the temperature and diet but the interaction between temperature and diet did not show any significant effect ($P > 0.05$) on I_{DS}. The digestive tract length and RGL affected significantly ($P < 0.05$) by temperature and diet but their interaction did not affect markedly ($P > 0.05$).

3.3. Pepsin enzyme activity

Analyses of variance (Table 3) indicate that pepsin activity was affected by water temperature and diet ($P < 0.001$). However, no significant effect was recorded for the interacting effect of temperature and diet ($P > 0.05$). When an interaction effect is present, the interpretation of the main effects can be misleading. Therefore, pairwise comparisons of the cell means were performed instead, as this approach is more useful when it is of interest to find the combination of factors that produces the most desirable results (Table 4).

Temperature profiles of pepsin activities were assayed at a range of 22–34 °C. When fish were offered commercial pellet diet the pepsin enzymatic activity revealed an increasing profile from 22 °C to 30° and differ significantly ($P < 0.05$, Table 4). The same pattern also observed when fish were feeding shrimp diet and showed the minimum pepsin activities at lower (22 °C) and higher temperature (34 °C) and maximum activity showed at 30 °C. Overall, the optimal temperature of pepsin activity in the stomach of *L. malanaricus* was 30 °C and was greater in natural shrimp feeding fish than in commercial pellet feeding fish (Table 4).

Fig. 2 showed the relationship between digestive somatic index (I_{DS}) and pepsin enzyme activity in the stomach of *L. malabaricus*. A polynomial cubic model was fitted ($r^2 = 1$) to make the relationship between I_{DS} and pepsin enzyme activity and showed a negative correlation between them. At 30 °C the pepsin activity was highest whereas the I_{DS} value was the lowest at same temperature and followed a decreasing trend of pepsin enzyme activities with increasing the values of I_{DS}. At 22 °C the pepsin activity was minimum whereas the I_{DS} value was maximum (Fig. 2a). In case of shrimp diet the relationship also showed the opposite trend at different temperatures and follow the similar polynomial cubic model though the pattern is different to pellet diet (Fig. 2b).

The pepsin activity also showed the opposite relation to relative gut length; that is, pepsin enzyme activity slowed down with increasing relative gut length (Fig. 3a–b). A polynomial cubic model fitting was also done to correlate their relationship ($r^2 = 1$). Similar to the I_{DS}, the RGL was highest at 22 °C while the pepsin activity was lowest. The lowest RGL observed at 30 °C while pepsin activity was highest feeding on pellet and shrimp diet (Fig. 3).

However, for an understanding of the digestive processes not only the enzymatic activity but also the retention time of food in the gut is relevant. Unfortunately, little information is available on the gut passage time in tropical fish. From gut passage data of *L. malabaricus* obtained from our previous experiment found a very close relationship with pepsin enzymatic activity (Mazumder et al., 2015a,b). At 30 °C for

Table 1
Physico-chemical parameters measured during the period of experiment.

Parameters	22 °C	26 °C	30 °C	34 °C
Temperature (°C)	22 ± 0.56	26 ± 0.14	30 ± 0.29	34 ± 0.29
Salinity (psu)	30 ± 0.45^{a}	30 ± 0.34^{a}	30 ± 0.36^{a}	30 ± 0.12^{a}
TH (mg L^{-1})	119.33 ± 22.33^{a}	108.07 ± 18.41^{ab}	125.4 ± 16.49^{a}	97 ± 3.89^{b}
NH_3-N (mg L^{-1})	0.25 ± 0.20^{a}	0.25 ± 0.20^{a}	0.33 ± 0.24^{a}	0.42 ± 0.24^{a}
pH	7.47 ± 0.09^{a}	7.07 ± 0.31^{a}	7.27 ± 0.17^{a}	6.87 ± 0.17^{a}

*TH: total hardness and NH_3-N: ammonical nitrogen, values in rows followed by different letters differ significantly.

Table 2
Biometric parameters for *Lutjanus malabaricus* at different temperature and diets experiment.

Temperature (°C)	Diet	Fish weight (g)	Digestive tract weight (g)	Digestive tract length (cm)	I_{DS}	RGL
22	Pellet	56.38 ± 1.96^{d}	2.18 ± 0.18^{a}	19.94 ± 0.95^{a}	3.86 ± 0.19^{a}	1.46 ± 0.04^{a}
	Shrimp	59.99 ± 2.65^{d}	1.91 ± 0.10^{ab}	19.60 ± 1.19^{ab}	3.19 ± 0.18^{b}	1.41 ± 0.05^{a}
26	Pellet	69.47 ± 3.95^{bc}	1.78 ± 0.24^{b}	17.20 ± 0.57^{c}	2.57 ± 0.33^{cd}	1.18 ± 0.04^{cd}
	Shrimp	70.86 ± 5.83^{ab}	1.77 ± 0.19^{b}	16.88 ± 0.76^{cd}	2.50 ± 0.13^{cd}	1.15 ± 0.04^{d}
30	Pellet	73.68 ± 2.70^{a}	1.64 ± 0.14^{b}	15.44 ± 0.38d^{e}	2.24 ± 0.24^{d}	1.04 ± 0.03^{e}
	Shrimp	77.16 ± 4.67^{ab}	1.64 ± 0.11^{b}	14.56 ± 0.48^{e}	2.12 ± 0.14^{d}	0.95 ± 0.03^{f}
34	Pellet	62.64 ± 2.14^{cd}	1.84 ± 0.10^{b}	18.14 ± 1.06^{bc}	2.95 ± 0.^{17bc}	1.33 ± 0.06^{b}
	Shrimp	63.18 ± 3.39^{cd}	1.82 ± 0.18^{b}	17.40 ± 0.56^{c}	2.89 ± 0.30^{bc}	1.24 ± 0.01^{c}
Two-way ANOVA *P*-values						
Temperature		0.000**	0.000**	0.000**	0.000**	0.000**
Diet		0.059^{NS}	0.144^{NS}	0.03*	0.003*	0.000**
Tem × Diet		0.724^{NS}	0.233^{NS}	0.811^{NS}	0.011*	0.301 ^{NS}

Values are mean ± SE. Different superscript letters in the same column indicate significant differences ($P < 0.05$) among the treatments in each temperature and diet groups. Asterisk indicates significant effects ($P < 0.05$) on temperature and diet groups. NS indicates not significant ($P > 0.05$). Tem × Diet: Temperature × Diet.

Table 3
Two-way ANOVA tests of significance for the interactions of temperature and diet on specific pepsin activity of *L. malabaricus*.

Source	DF	SS	MS	F	P
Temperature (°C)	3	47.7867	15.928	26.83	0.000**
Diet	1	24.5706	24.5706	41.38	0.000**
Temp × Diet	3	2.9436	0.9812	1.65	0.197^{NS}
Error	112	19.0010	0.5938		
Total	119	94.3020			

* $P < 0.05$; temperature: 22, 26, 30, 34 °C; diet: pellet, shrimp. Asterisk indicates significant effects ($P < 0.05$) on temperature and diet groups. NS indicates not significant ($P > 0.05$). Tem × Diet: Temperature × Diet.

Table 4
Pepsin enzymatic activities in stomach of *L. malanaricus* at different temperature and diet.

Pepsin activity (U mg protein^{-1})				
Diet	Temperature (°C)			
	22	26	30	34
Pellet	0.75 ± 0.14^{c}	1.92 ± 0.78^{b}	3.26 ± 0.08^{a}	1.52 ± 0.38^{bc}
Shrimp	2.34 ± 0.86^{b}	3.67 ± 0.49^{b}	5.47 ± 1.60^{a}	2.90 ± 0.63^{b}

Data are means ± SE (n = 15). Different superscript letters in the same row indicate significant enzymatic differences ($P < 0.05$) among the exposed temperature in each diet.

L. malabaricus the pepsin activity was highest at both diet resulting the minimum time required for complete disappearance of food from the stomach (18 h & 16 h for pellet and shrimp diet respectively). Conversely, maximum time required for complete digestion of food from the stomach at 22 °C at both diet (28 h & 24 h for pellet and shrimp diet respectively) meaning that the increased level of pepsin activity accelerate the digestion process into the stomach of *L. malabaricus* (Fig. 4).

4. Discussion

Acclimation to changed temperature produces morphological change in the gut areas. The I_{DS} tended to be higher during cold and higher temperature and recover initial values in their favourable temperature exposed. The gut of cold and hot-acclimated fish have a larger diameter; this is inferred from the increase in the unmodified cylinder surface area. Cold-acclimation of these fish species has produced villi which are both significantly taller and broader than those of optimum temperature-acclimated fish (Lee and Cossins, 1988). Similar findings have also been reported from *Acipenser transmontanus*, *Acipenser naccarii* Bonaparte 1836, *Oncorhynchus mykiss* and *Salmo salar* L. 1758

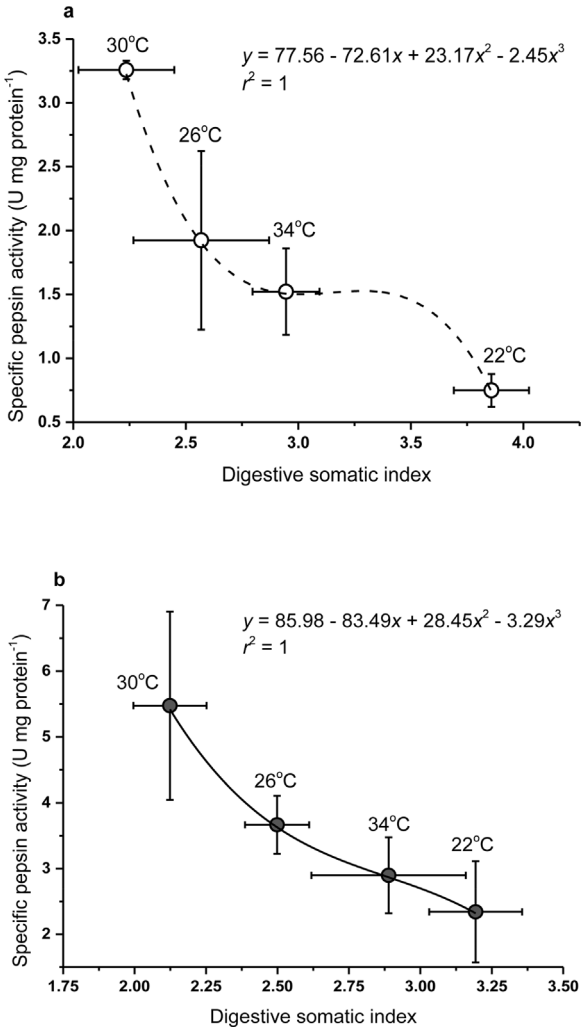

Fig. 2. Correlation between digestive somatic index and specific pepsin activity in the stomach contents of *L. malabaricus* at different temperature and diet: a) pellet diet and b) shrimp diet. Values are means ± SE (n = 15). The open circle with dash line indicates the pellet diet while solid circle with solid line indicates shrimp diet.

(Furné et al., 2008; Hung et al., 1997; Krogdahl and Bakke-McKellep, 2005; Storebakken et al., 1998). The present results revealed that gut weight was related to growth rate through absorption ability. The I_{DS} at 26 and 30 °C exposed fish were lower than that of the lower and higher temperature (22 and 34 °C) exposed fish, which is in accordance with results of Bélanger et al. (2002).

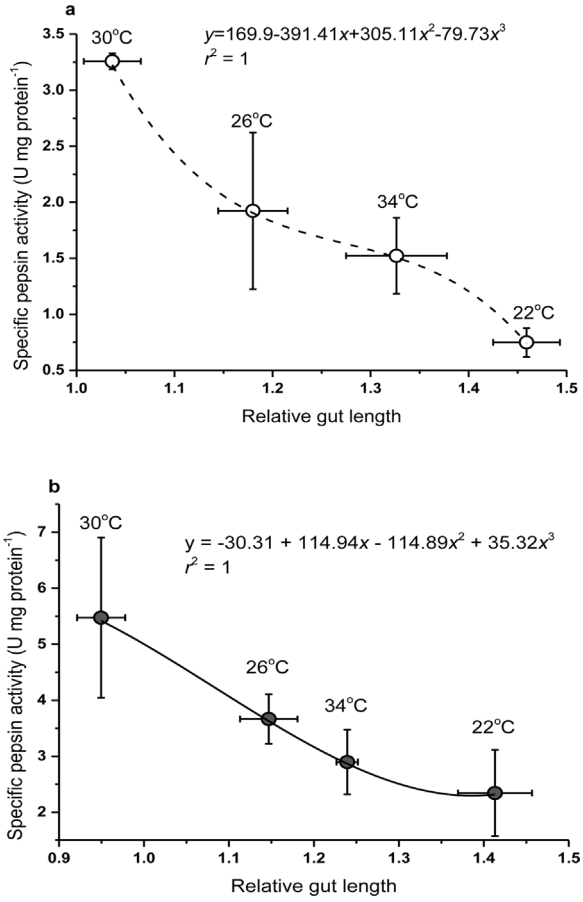

Fig. 3. Correlation between relative gut length and specific pepsin activity in the stomach contents of *L. malabaricus* at different temperature and diet: a) pellet diet and b) shrimp diet. Values are means ± SE (n = 15). The open circle with dash line indicates the pellet diet while solid circle with solid line indicates shrimp diet.

The RGL of sea cucumbers cultivated at a low water temperature of 7 °C was significantly higher than in the other treatment groups during the experimental period (Gao et al., 2009). Sui and Liao (1988) reported that the digestive tract of *A. japonicus* develops rapidly when water temperature is below 8 °C–10 °C. A high RGL was also observed in *A. japonicus* during the winter season from January to March when ambient water temperatures range 3 °C–11 °C (Gao, 2008). The effect of thermal acclimation on the size of the intestine in vertebrates has been the subject of considerable research interest, and the studies have confirmed that the stressed temperature-exposure leads to an increase in intestinal mass (Hammond and Wunder, 1995; Hammond et al., 2001; Nespolo et al., 2002).

However, one of the most important factors other than temperature controlling gut dimension is that of diet. In our present study the *L. malabaricus* taken lower amount of food at lower and higher temperature (22 and 34 °C respectively) and increased their gut mass at these corresponding temperatures. The digestive organ mass shown to be increased at lower ambient temperature in association with low quality diet (Bozinovic et al., 1990). The mass of food eaten strongly determines intestinal mass (Hammond et al., 1994; Konarzewski and Diamond, 1994). Cold and hot-acclimation produces a depression in both appetite and growth (Smith, 1975). It is relevant to ask therefore whether reduced dietary consumption induces the demonstrated intestinal hypertrophy. In the present study, the diet in all treatment groups was the same. Thus, the gut mass increase in *L. malabaricus* at low and high water temperature may also be attributed to decreased digestibility rather than diet quality.

The *L. malabaricus* fish species exhibited enzyme activities able to hydrolyze the protein used as substrate in the assay. Once the food has been swallowed by the fish, it is kept in the stomach where pepsin-like enzymes and, in some instances, HC1 is secreted by the chief cells (Muyan et al., 2006). These cells have been found in all gastric fish species (Munilla-Morán and Saborido-Rey, 1996; Smith, 1989). Therefore, an acidic environment for gastric digestion must be expected. In fish, protein is digested initially in the stomach in breaking down large-chain polypeptides chains by pepsin and acid, and then further degraded into smaller peptides and free amino acids in the intestine by the combined actions of various alkaline proteases (Natalia et al., 2004; Tengjaroenkul et al., 2000).

Determining the optimum temperature for enzyme activity might be interesting for comparative studies. Changing temperature usually radically affects the catalytic performance of enzymes. Report showed that the maximum protease activity of many species could occur in a wide temperature range from 30 to 60 °C (Muyan et al., 2006). The temperature in fish gut lumen is closely linked to that of the environment. Water temperature thus have a manifold effect on fish digestion. When testing the effect of temperature on pepsin enzyme activity, the results showed that pepsin worked best at the temperature 30 °C. When the temperature decreased to 22 °C, the enzyme activity decreased sharply. Turbot and redfish retained almost half of the activity at low temperatures (5 °C). On the contrary, seabream had less than 3.5 times this activity ($P < 0.001$) at the same temperature (Munilla-Morán and Saborido-Rey, 1996). A decrease in the enzymatic activity with declining to the incubation temperature (Table 3), as also observed by several other authors (Kuz'mina and Kuz'mina, 1990; Trofimova, 1973; Uys and Hecht, 1987). Chemical bonds make up the shape of protein and protein's function is related to its shape and therefore activity would decrease or impact the enzyme catalysing a reaction (Reece et al., 2011). When temperature is being increased from lower to higher, the rate of the enzyme activity is high because substrates collide with active sites on the enzyme more frequently as the molecules move rapidly (Reece et al., 2011). The optimum pepsin activity at 30 °C in *L. malabaricus* stomach reveal that the optimal temperature of enzymes was higher than the habitat temperature; however; this phenomenon is consistent with results from studies on other fishes (Chen et al., 1998; Lazo et al., 2007; Xiong et al., 2011). The optimum temperature range of *Scophthalus maximus* is 40–50 °C for protease (Wang et al., 2006). This pattern was also similar to other fish species, such as *Mallotus villosus* and *Gadus morhua*. In this regard, the highest pepsin activity was found at 38 °C (Gildberg, 1983). The optimum temperature range of *Scophthalus maximus* is 40–50 °C for stomach protease (Wang et al., 2006). It has been suggested that the ability of fish to hydrolyse feed may decline with a decrease in the environmental temperature.

In general, the response of the digestive enzyme activity closely linked to the growth performance results reported in Bowyer et al. (2012). In addition, the enzyme activity in fish directly related to feed intake (Eusebio and Coloso, 2002; Hardewig and Van Dijk, 2003). Fish may adapt their metabolic functions to the dietary substrates, through a regulation in enzyme secretion, in order to improve the utilization of feed ingredients (Caruso et al., 2009). Fernandez et al. (2001) pointed out that the adaptations of the digestive system of different species reveal closer correlation with their diet rather than on their taxonomic category was also confirmed by the results of Kuz'mina (1996) who indicated that changes in digestive enzyme activity could be affected by feeding behaviour and biochemical composition of food. In fact, in most studies on tropical freshwater catfish and carps show that excellent results could be achieved if live food is partially or wholly included in the larval diet (Kamarudin et al., 2013).

Pepsin activity increased remarkably in the lower and higher temperature exposed conditions when the fish species taken lower amount of food, showing an opposing trend to that of I_{DS} and RGL. This is supported by the in situ study of Fu et al. (2006) who recorded that pepsin-like activity in *A. japonicas* increased noticeably from June to July (fishes taking lower amount of food or even don't take any food).

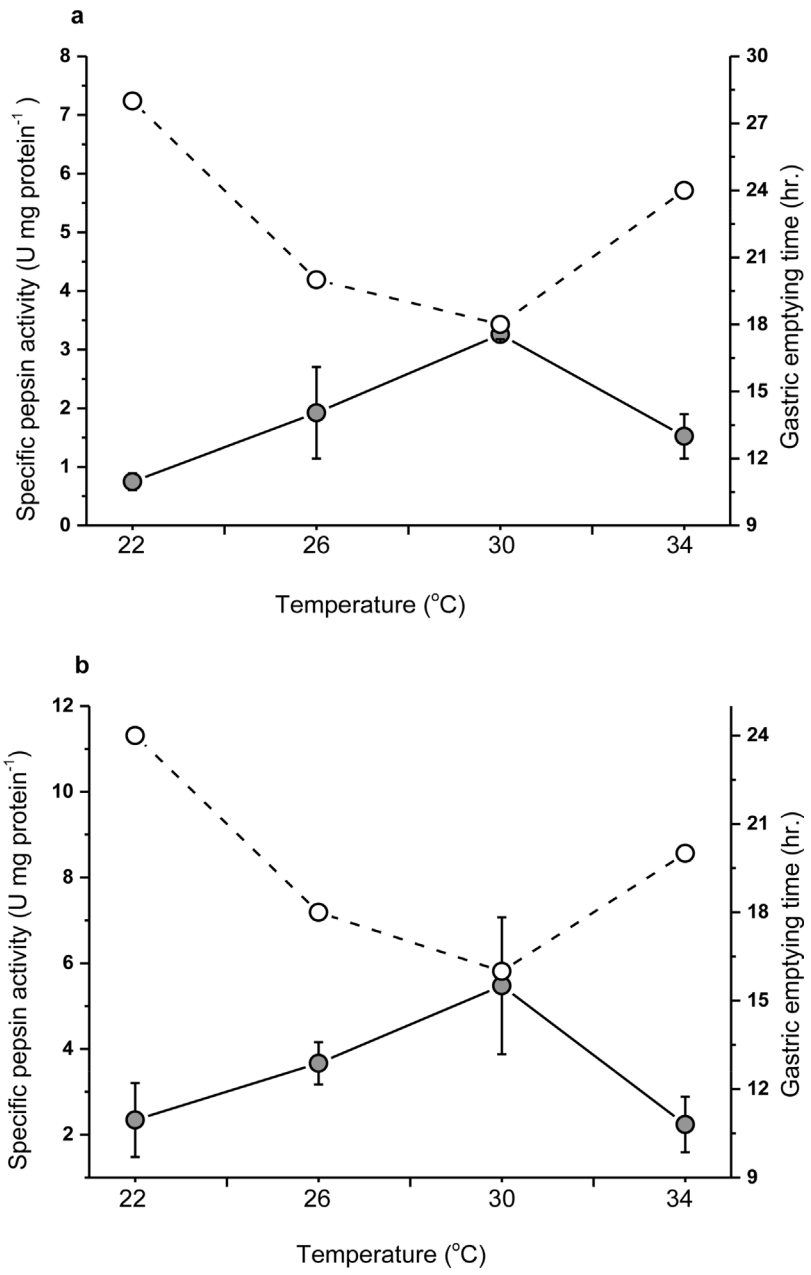

Fig. 4. Relationship between specific activity of pepsin enzyme and gastric emptying time of *L. malabaricus* at different temperature and diet: a) pellet diet and b) shrimp diet. Values are means ± SE (n = 15). Solid circle with solid line denotes enzyme activity while open circle with dotted line denotes gastric emptying time.

Gao (2008) also found that pepsin activity spiked during deep aestivation.

Pepsin activity and hence gastric emptying time is strongly dependent on temperature (Fauconneau et al., 1983; Flowerdew and Grove, 1979; Windell, 1978). Also, fishes acclimated to different temperatures showed distinct activity levels (Hofer, 1979, 1982). And the enzymes involved in pepsin digestion are influenced by temperature both on the enzyme activity or modifying the affinity of the enzyme by its substrate (Wilkison, 1990). In the cold not only is appetite depressed but, more importantly, studies in trout have shown a substantial decrease in gastrointestinal transit rate (Fauconneau et al., 1983). There have been numerous studies of temperature compensation of intestinal transport processes in goldfish and carp (Gibson et al., 1985; Kitchin and Morris, 1971; Smith, 1975; Smith and Ellory, 1971). Marked temperature and diet related differences in the I_{DS}, RGL and pepsin enzyme activity rates of juvenile *L. malabaricus* may have important implications in better aquaculture practices by maintaining the suitable temperature ranges and diet preferences

5. Conclusion

In conclusion, this study primarily reported on the I_{DS}, RGL, and overall activity of pepsin enzyme along the stomach of *L. malabaricus* in various temperature and diets they generally fed in the nature. The findings obtained from this study suggest to culture *L. malabaricus* at 30 °C feeding on shrimp diet as this temperature proliferate the enzymatic activity leading to faster digestion which will ultimately promote faster growth rate of this commercially important fish species. These information can, in turn, be used for future management and conservation issues and research for *L. malabaricus* or any Lutjanidae in Malaysia and any part of the world. Moreover, the results also provide important information for food management to optimize the aquaculture production in snapper aquaculture industry.

Conflict of interest

The authors have declare that no conflict of interests exist.

Acknowledgements

This study was funded by Ministry of Science Technology and Innovation Malaysia (MOSTI) and Institute of Climate Change, Universiti Kebangsaan Malaysia (UKM) through the research grant # 04-01-02-SF1208 and GUP-2017-023 respectively to the corresponding author S.K. Das.

References

Abolfathi, M., Hajimoradloo, A., Ghorbani, R., Zamani, A., 2012. Compensatory growth in juvenile roach *Rutilus caspicus*: effect of starvation and re-feeding on growth and digestive surface area. J. Fish Biol. 81, 1880–1890.

Al-Hussaini, A., 1949. On the functional morphology of the alimentary tract of some fish in relation to differences in their feeding habits: anatomy and histology. Quart. J. Microsc. Sci. 3, 109–139.

Anson, M.L., 1938. The estimation of pepsin, trypsin, papain, and cathepsin with hemoglobin. J. Gen. Physiol. 22, 79–89.

Bélanger, F., Blier, P., Dutil, J.-D., 2002. Digestive capacity and compensatory growth in Atlantic cod (*Gadus morhua*). Fish Physiol. Biochem. 26, 121–128.

Bowyer, J.N., Qin, J.G., Adams, L.R., Thomson, M.J., Stone, D.A., 2012. The response of digestive enzyme activities and gut histology in yellowtail kingfish (*Seriola lalandi*) to dietary fish oil substitution at different temperatures. Aquaculture 368, 19–28.

Bozinovic, F., Novoa, F.F., Veloso, C., 1990. Seasonal changes in energy expenditure and digestive tract of *Abrothrix andinus* (Cricetidae) in the Andes range. Physiol. Zool. 1216–1231.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analy. Biochem. 72, 248–254.

Caruso, G., Denaro, M., Genovese, L., 2009. Digestive enzymes in some Teleost species of interest for Mediterranean aquaculture. Open Fish Sci. J. 2, 74–86.

Chatterjee, N., Pal, A., Manush, S., Das, T., Mukherjee, S., 2004. Thermal tolerance and oxygen consumption of *Labeo rohita* and *Cyprinus carpio* early fingerlings acclimated to three different temperatures. J. Therm. Biol. 29, 265–270.

Chen, P., Wang, C., Lu, H., Gu, L., 1998. Relationship between activity of digestive enzymes of larval *Pagrosomus major* and temperature. J. Xiamen Univ. (Natural Sci.) 37, 931–935.

De, M., Ghaffar, M.A., Bakar, Y., Das, S.K., 2016. EEffect of temperature and diet on growth and gastric emptying time of the hybrid, Epinephelus fuscoguttatus ♀ × E. Aquacult. Rep. 4, 118–124.

Dean, A., Voss, D., 1999. Response surface methodology. Desi. Anal. Exp. 483–529.

Debnath, D., Pal, A., Sahu, N., Baruah, K., Yengkokpam, S., Das, T., Manush, S., 2006. Thermal tolerance and metabolic activity of yellowtail catfish *Pangasius pangasius* (Hamilton) advanced fingerlings with emphasis on their culture potential. Aquaculture 258, 606–610.

Donelson, J., Munday, P., McCormick, M., Pankhurst, N., Pankhurst, P., 2010. Effects of elevated water temperature and food availability on the reproductive performance of a coral reef fish. Marine. Ecol. Prog. Ser. 401, 233–243.

Eusebio, P.S., Coloso, R.M., 2002. Proteolytic enzyme activity of juvenile Asian sea bass, *Lates calcarifer* (Bloch), is increased with protein intake. Aquacult. Res. 33, 569–574.

Fauconneau, B., Choubert, G., Blanc, D., Breque, J., Luquet, P., 1983. Influence of environmental temperature on flow rate of foodstuffs through the gastrointestinal tract of rainbow trout. Aquaculture 34, 27–39.

Fernandez, I., Moyano, F., Dıaz, M., Martınez, T., 2001. Characterization of α-amylase activity in five species of Mediterranean sparid fishes (Sparidae, Teleostei). J. Exp. Mar Biol. Ecol. 262, 1–12.

Flowerdew, M., Grove, D., 1979. Some observations of the effects of body weight, temperature, meal size and quality on gastric emptying time in the turbot, *Scophthalmus maximus* (L.) using radiography. J. Fish Biol. 14, 229–238.

Fu, X., Xue, C., Miao, B., Li, Z., Gao, X., Hirata, T., 2006. Distribution and seasonal activity variation of proteases in digestive tract of sea cucumber *Stichopus japonicus*. Fish. Sci. 72, 1130–1132.

Furné, M., García-Gallego, M., Hidalgo, M.C., Morales, A.E., Domezain, A., Domezain, J., Sanz, A., 2008. Effect of starvation and refeeding on digestive enzyme activities in sturgeon (*Acipenser naccarii*) and trout (*Oncorhynchus mykiss*). Comp. Biochem. Physiol. Part A: Mol. Integr. Physiol. 149, 420–425.

Gao, F., Yang, H., Xu, Q., Wang, F., Liu, G., 2009. Effect of water temperature on digestive enzyme activity and gut mass in sea cucumber *Apostichopus japonicus* (Selenka), with special reference to aestivation. Chin. J. Ocean. Lim. 27, 714–722.

Gao, F., 2008. Seasonal Variations of Nutritional Composition, Food Resources, and Digestive Physiology in Sea Cucumber *Apostichopus Japonicus*. Institute of Oceanology, Chinese Academy of Sciences.

German, D.P., Bittong, R.A., 2009. Digestive enzyme activities and gastrointestinal fermentation in wood-eating catfishes. J. Com. Physiology. B 179, 1025–1042.

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. Physiology. Biochem. Zool. 77, 789–804.

German, D.P., Nagle, B.C., Villeda, J.M., Ruiz, A.M., Thomson, A.W., Balderas, S.C., Evans, D.H., 2010. Evolution of herbivory in a carnivorous clade of minnows (Teleostei: cyprinidae): effects on gut size and digestive physiology. Physiology. Biochem. Zool. 83, 1–18.

Gibson, J., Ellory, J., Cossins, A., 1985. Temperature acclimation of intestinal Na transport in the carp (*Cyprinus carpio* L.). J. Exp. Biology. 114, 355–364.

Gildberg, A., 1983. Purification and characterization of pepsins from the Arctic fish capelin (*Mallotus villosus*). Comp. Biochem. Physiol. Part A: Physiol. 75, 337–342.

Gupta, M.D., 2004. Relative length of the gut of some freshwater fishes of West Bengal in relation to food and feeding habits. Indian J. Fish. 51, 381–384.

Hammond, K., Wunder, B., 1995. Effect of cold temperatures on the morphology of gastrointestinal tracts of two microtine rodents. J. Mammal. 76, 232–239.

Hammond, K.A., Konarzewski, M., Torres, R.M., Diamond, J., 1994. Metabolic ceilings under a combination of peak energy demands. Physiol. Zool. 1479–1506.

Hammond, K., Szewack, J., Król, E., 2001. Effects of altitude and temperature on the morphology of gastrointestinal tracts of two microtine rodents. J. Exp. Biol. 204, 991–992.

Hardewig, I., Van Dijk, P., 2003. Is digestive capacity limiting growth at low temperatures in roach? J. Fish Biol. 62, 358–374.

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

Hofer, R., Schiemer, F., 1981. Proteolytic activity in the digestive tract of several species of fish with different feeding habits. Oecologia 48, 342–345.

Hofer, R., Dalla Via, G., Troppmair, J., Giussani, G., 1982. Differences in Digestive Enzymes Between Cyprinid and Non-Cyprinid Fish. Memorie dell'Istituto Italiano di Idrobiologia, Dr. Marco de Marchi, Verbania Pallanza.

Hofer, R., 1979. The adaptation of digestive enzymes to temperature, season and diet in roach, *Rutilus rutilus* and rudd *Scardinius erythrophthalmus*; Proteases. J. Fish Biol. 15, 373–379.

Hofer, R., 1982. Protein digestion and proteolytic activity in the digestive tract of an omnivorous cyprinid. Comp. Biochem. Physiol. Part A: Physiol. 72, 55–63.

Hung, S.S., Liu, W., Li, H., Storebakken, T., Cui, Y., 1997. Effect of starvation on some morphological and biochemical parameters in white sturgeon, Acipenser transmontanus. Aquaculture 151, 357–363.

Il'ina, I., Turetskiy, V., 1987. Development of the digestive function in fishes. Vopr. Icthyol. 27, 835–845.

Jónás, E., Rágyanszki, M., Oláh, J., Boross, L., 1983. Proteolytic digestive enzymes of carnivorous (*Silurus glanis* L.), herbivorous (*Hypophthalmichthys molitrix* Val.) and omnivorous (*Cyprinus carpio* L.) fishes. Aquaculture 30, 145–154.

Kamarudin, M., Otoi, S., Saad, C.R., 2013. Changes in growth, survival and digestive enzyme activities of Asian redtail catfish, *Mystus nemurus*, larvae fed on different diets. Afr. J. Biotech. 10, 4484–4493.

Kita, J., Tsuchida, S., Setoguma, T., 1996. Temperature preference and tolerance, and oxygen consumption of the marbled rockfish, *Sebastiscus marmoratus*. Mar. Biol. 125, 467–471.

Kitchin, S., Morris, D., 1971. The effect of acclimation temperature on amino acid transport in the goldfish intestine. Comp. Biochem. Physiol. Part A: Physiol. 40, 431–443.

Kolkovski, S., 2001. Digestive enzymes in fish larvae and juveniles-implications and applications to formulated diets. Aquaculture 200, 181–201.

Konarzewski, M., Diamond, J., 1994. Peak sustained metabolic rate and its individual variation in cold-stressed mice. Physiol. Zool. 1186–1212.

Krogdahl, Å., Bakke-McKellep, A.M., 2005. Fasting and refeeding cause rapid changes in intestinal tissue mass and digestive enzyme capacities of Atlantic salmon (*Salmo salar* L.). Comp. Biochem. Physiol. Part A: Mol. Integr. Physiol. 141, 450–460.

Kuz'mina, V., Kuz'mina, Y.G., 1990. Level of total proteolytic activity in some species of fish from the Volga barin. J. Ichthyol. 30, 25–35.

Kuz'mina, V., 1996. Influence of age on digestive enzyme activity in some freshwater teleosts. Aquaculture 148, 25–37.

Kuzmina, V., 1991. Evolutionary features of the digestive-transport function in fish. J. Evol. Biochem. Physiol. 27, 127–134.

López-Vásquez, K., Castro-Pérez, C., Val, A., 2009. Digestive enzymes of eight Amazonian teleosts with different feeding habits. J. Fish Biol. 74, 1620–1628.

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

Lee, J.A., Cossins, A.R., 1988. Adaptation of intestinal morphology in the temperature-acclimated carp, *Cyprinus carpio* L. Cell Tissue Res. 251, 451–456.

Marković, Z., Dulić, Z., Živić, I., Mitrović-Tutundžić, V., 2009. Influence of abiotic and biotic environmental factors on weight gain of cultured carp on a carp farm. Arch. Biol. Sci. 61, 113–121.

Mazumder, S.K., De, M., Ghaffar, M.A., Zaidi, C.C., Rahim, S.M., Simon, K.D., 2015a. Impact of global climate change on fish growth, digestion and physiological status: developing a hypothesis for cause and effect relationships. J. Water Climat. Chang. 6, 200–226. http://dx.doi.org/10.2166/wcc.2014.146.

Mazumder, S.K., Ghaffar, M.A., Das, S.K., 2015b. The effects of temperature on gastric emptying time of malabar blood snapper (*Lutjanus malabaricus*, Bloch & Schneider 1801) using X-radiography technique, The 2015 UKM FST Postgraduate Colloquium. In: The 2015 UKM FST Postgraduate Colloquium: Proceedings of the Universiti Kebangsaan Malaysia, Faculty of Science and Technology 2015 Postgraduate Colloquium. AIP Publishing. (020032).

Morais, S., Bell, J.G., Robertson, D.A., Roy, W.J., Morris, P.C., 2001. Protein/lipid ratios in extruded diets for Atlantic cod (*Gadus morhua* L.): effects on growth feed utilisation, muscle composition and liver histology. Aquaculture 203, 101–119.

Munilla-Morán, R., Saborido-Rey, F., 1996. Digestive enzymes in marine species. I. Proteinase activities in gut from redfish (Sebastes mentella), seabream (Sparus aurata) and turbot (Scophthalmus maximus). Comp. Biochem. Physiol. Part B: Biochem. Mol. Biol. 113, 395–402.

Muyan, C., Xiumei, Z., Tianxiang, G., Chao, C., 2006. Effects of temperature, pH and NaCl on protease activity in digestive tract of young turbot, *Scophthalmus maximus*. Chin. J. Ocean. Lim. 24, 300–306.

Nanton, D., Lall, S., McNiven, M.A., 2001. Effects of dietary lipid level on liver and muscle lipid deposition in juvenile haddock, *Melanogrammus aeglefinus* L. Aquacul. Res. 32, 225–234.
Natalia, Y., Hashim, R., Ali, A., Chong, A., 2004. Characterization of digestive enzymes in a carnivorous ornamental fish, the Asian bony tongue *Scleropages formosus* (Osteoglossidae). Aquaculture 233, 305–320.
Nespolo, R.F., Bacigalupe, L.D., Sabat, P., Bozinovic, F., 2002. Interplay among energy metabolism, organ mass and digestive enzyme activity in the mouse-opossum *Thylamys elegans*: the role of thermal acclimation. J. Exp. Biol. 205, 2697–2703.
Péres, A., Infante, J.Z., Cahu, C., 1998. Dietary regulation of activities and mRNA levels of trypsin and amylase in sea bass (*Dicentrarchus labrax*) larvae. Fish Physiol. Biochem. 19, 145–152.
Reece, J., Urry, L.A., Meyers, N., Cain, M.L., Wasserman, S.A., Minorsky, P.V., Jackson, R.B., Cooke, B.N., 2011. Campbell Biology. Pearson Higher Education AU.
Rosenlund, G., Skretting, M., 2006. Worldwide status and perspective on gadoid culture. ICES J. Mar. Sci.: Journal du Conseil. 63, 194–197.
Simon, K., Bakar, Y., Mazlan, A., Zaidi, C., Samat, A., Arshad, A., Temple, S., Brown-Peterson, N., 2012. Aspects of the reproductive biology of two archer fishes *Toxotes chatareus* (Hamilton 1822) and *Toxotes jaculatrix* (Pallas 1767). Env. Biol. Fish. 93, 491–503.
Skea, G., Mountfort, D., Clements, K., 2005. Gut carbohydrases from the New Zealand marine herbivorous fishes *Kyphosus sydneyanus* (Kyphosidae), *Aplodactylus arctidens* (Aplodactylidae) and *Odax pullus* (Labridae). Comp Biochem. Physiol. Part B: Biochem. Mol. Biol. 140, 259–269.
Skea, G., Mountfort, D., Clements, K., 2007. Contrasting digestive strategies in four New Zealand herbivorous fishes as reflected by carbohydrase activity profiles. Comp. Biochem. Physiol. Part A: Mol. Integr. Physiol. 146, 63–70.
Smith, M., Ellory, J., 1971. Temperature-induced changes in sodium transport and Na +/K+-adenosine triphosphatase activity in the intestine of goldfish (*Carassius auratus* L.). Comp. Biochem. Physiol. Part A: Physiol. 39, 209–218.
Smith, M., 1975. Temperature Adaptation in Fish. Biochemical Society symposium, pp. 43–60.
Smith, L.S., 1989. Digestive functions in teleost fishes. Fish Nutr. 2, 331–421.
Sokal, R.R., Rohlf, F.J., 1995. Biometry: the Principles Andpractice of Statistics in Biological Research. W. H Freeman & Co., San Francisco (SokalBiometry: the principles and practice of statistics in biological research).
Stevens, C.E., Hume, I.D., 2004. Comparative Physiology of the Vertebrate Digestive System. University Press, Cambridge.
Storebakken, T., Shearer, K.D., Refstie, S., Lagocki, S., McCool, J., 1998. Interactions between salinity, dietary carbohydrate source and carbohydrate concentration on the digestibility of macronutrients and energy in rainbow trout (*Oncorhynchus mykiss*). Aquaculture 163, 347–359.
Sui, X., Liao, Y., 1988. Culture and Enhance of Sea Cucumber. Agriculture press, Beijing, China.
Tengjaroenkul, B., Smith, B.J., Caceci, T., Smith, S.A., 2000. Distribution of intestinal enzyme activities along the intestinal tract of cultured Nile tilapia, *Oreochromis niloticus* L. Aquaculture 182, 317–327.
Tijskens, L., Greiner, R., Biekman, E., Konietzny, U., 2001. Modeling the effect of temperature and pH on activity of enzymes: the case of phytases. Biotec. Bioeng. 72, 323–330.
Trofimova, L., 1973. Dynamics of total proteolytic activity along the digestive tract in carp in relation to incubation temperature. Sbornik Nauchi Trudove Vniiprkh 10, 170–181.
Uys, W., Hecht, T., 1987. Assays on the digestive enzymes of sharptooth catfish, *Clarias gariepinus* (Pisces: clariidae). Aquaculture 63, 301–313.
Wang, H.Y., Wang, Y.J., Wang, Q.Y., Xue, C.H., Sun, M., 2006. Purification and characterization of stomach protease from the turbot (*Scophthalmus maximus* L.). Fish Physiol. Biochem. 32, 179–188.
Wilkison, L., 1990. Systat: The System for Statistics. Systat. Inc., Evanston.
Windell, J.T., 1978. Digestion and the daily ration of fishes. Ecol. Freshw. Fish Prod. 159–183.
Xiong, D., Xie, C., Zhang, H., Liu, H., 2011. Digestive enzymes along digestive tract of a carnivorous fish *Glyptosternum maculatum* (Sisoridae, Siluriformes). J. Anim. Physiol. Anim. Nutr. 95, 56–64.
Zar, J.H., 1984. Biostatistical Analysis, 2nd. ed. Prentice Hall, USA.