1871
ИнБЮМ – IBSS

Морской биологический журнал
Marine Biological Journal
2022, vol. 7, no. 1, pp. 55–64
https://doi.org/10.21072/mbj.2022.07.1.05

UDC 597.5-113.32(282.253.11.05)

ACTIVITY OF PEPTIDASES AND GLYCOSIDASES OF THE DIGESTIVE TRACT IN SOME SPECIES OF BONY FISH OF VIETNAM

© 2022 **V. V. Kuz'mina[1], E. E. Slynko[1,2], E. A. Kulivatskaya[1], E. P. Karpova[2], and Dinh Cu Nguyen[2]**

[1]Papanin Institute for Biology of Inland Waters Russian Academy of Sciences, Borok, Russian Federation
[2]Southern Branch of the Joint Russian–Vietnamese Tropical Research and Technological Center, Ho Chi Minh City, Vietnam
E-mail: *elena.slynko.76@mail.ru*

Received by the Editor 16.12.2020; after reviewing 11.08.2021; accepted for publication 24.12.2021; published online 22.03.2022.

For the first time, the activity and pH dependence of digestive enzymes were studied in fish inhabiting the Mekong Delta: duskyfin glassy perchlet *Parambassis wolffii*, smallscale croaker *Boesemania microlepis*, catfish *Pangasius macronema*, and representatives of the family Cyprinidae. Significant interspecific differences were revealed in the level of peptidase and glycosidase activity providing hydrolysis of protein and carbohydrate food components. The greatest interspecific differences are characteristic of glycosidases: the level of enzymatic activity in Cyprinidae fish exceeds that in *P. wolffii* by 13.6 times. The differences in the level of peptidase activity in fish of different species are lower: in the case of the activity of stomach enzymes in *P. wolffii*, the values are 1.8 times higher than those in *P. macronema*, and in the case of total activity of stomach and intestinal enzymes in the same species, the values are 1.5 times higher. The data obtained confirm the concept that the digestive hydrolase activity depends on the fish feeding spectrum. The activity of intestinal enzymes decreases more significantly in the acidic pH zone than in the basic one. Consequently, acidification of the intestinal environment will negatively affect the digestive processes in these fish species.

Keywords: Vietnam, digestive enzymes, *Parambassis wolffii*, *Boesemania microlepis*, *Pangasius macronema*, Cyprinidae

In the Mekong Delta, there are two large ecosystems – freshwater and estuarine ones. To date, due to reduction in river flow, climate changes, and several other natural and anthropogenic factors, an extreme salinization of water in the Mekong Delta occurs (Tuan et al., 2007). Therefore, the analysis of various aspects of biology and physiology of freshwater indicator species is of great interest. As one of such fish species inhabiting the Mekong River, the duskyfin glassy perchlet *Parambassis wolffii* (Bleeker, 1850) is suggested: the fish regularly migrate from spawning and feeding spots in the floodplain to deeper areas in the main riverbed. These fish are ichthyophages – facultative benthophages, with the diet including small pelagic fish, crustaceans, and insects (Rainboth, 1996 ; Tran et al., 2013).

Unlike the duskyfin glassy perchlet *P. wolffii*, the smallscale croaker *Boesemania microlepis* (Bleeker, 1858) is a non-migratory species and a constant inhabitant of freshwater areas. It feeds mainly on crustaceans (shrimps), fish, and insects (Baird et al., 2001). The catfish *Pangasius macronema* is an euryphage, with the diet including benthic species, *inter alia* molluscs, as well as zooplankton,

algae, small fish, and detritus (Kottelat & Widjanarti, 2005 ; Taki, 1978). Amidst the most abundant migratory species of the Mekong River, representatives of the Cyprinidae family stand out: *Henicorhynchus lobatus* Smith, 1945, *Henicorhynchus siamensis* (Sauvage, 1881), and *Barbonymus gonionotus* (Bleeker, 1849) – a species performing regional migrations (Jasmine & Begum, 2016). *Henicorhynchus* fish feed on benthic species and, to a lesser extent, on zooplankton (Baird et al., 2003). *B. gonionotus* prefers vegetation and, to a lesser extent, small invertebrates (Mohsin & Ambak, 1983).

Importantly, such significant differences in fish feeding spectrums affect the digestion processes. In fish, the digestion processes are traditionally assessed by the level of enzyme activity in the mucous membrane of the stomach and intestines (Kuz'mina, 2018 ; Ugolev & Kuz'mina, 1993 ; Bakke et al., 2011 ; Fange & Grove, 1979 ; Kapoor et al., 1975). At the same time, the mucous membrane of the stomach and intestines includes not only the monolayer epithelium, but also the submucosa or stroma, which is based on a collagen scaffold (Verigina & Zholdasova, 1982 ; Kapoor et al., 1975). When comparing the enzyme activity in the epithelium and stroma, their levels were close; in the case of dipeptidases, the enzyme activity could be higher in the stroma than in the epithelium (Ugolev & Kuz'mina, 1992). Initially, it was assumed that stromal enzymes perform a protective function (Kuz'mina, 1995); subsequently, that they are also involved in the processes of post-epithelial digestion (Kuz'mina, 2018).

As known, enzymes of a prey are involved in the induced autolysis and play a significant role in fish gastric digestion, while enzymes of the enteric microbiota are important for intestinal digestion (Kuz'mina, 2018 ; Ugolev & Kuz'mina, 1993). At the same time, lysosomal enzymes of various tissues of a prey play the crucial role in the induced autolysis, in particular cathepsins which hydrolyze protein components (Vysotskaya & Nemova, 2008 ; Ashie & Simpson, 1997 ; Wang et al., 2000). Moreover, there is much evidence that different strains of the enteric microbiota have enzymes similar to those of fish. Specifically, bacteria of the genera *Pseudomonas*, *Aeromonas*, *Bacillus*, *Vibrio*, *Acinetobacter*, and *Enterobacter* have proteolytic activity (Askarian et al., 2012 ; Austin, 2006 ; Belchior & Vacca, 2006 ; Esakkiraj et al., 2009 ; Ganguly & Prasad, 2012 ; Hoshino et al., 1997 ; Ray et al., 2012), while representatives of the genera *Acinetobacter*, *Bacillus*, *Pseudomonas*, *Moraxella*, and *Micrococcus* have amylolytic activity (Austin, 2006 ; Ganguly & Prasad, 2012 ; Izvekova & Plotnikov, 2011 ; Ray et al., 2012 ; Sugita et al., 1997). Since there are no data on the status of the enzyme systems of the digestive tract of Vietnamese fish, it seemed reasonable to evaluate the integral characteristics of enzymes providing hydrolysis of protein and carbohydrate food components in the stomach and intestines of fish from the area of Vietnam.

The aim of the work was to assess the activity of peptidases and glycosidases providing hydrolysis of protein and carbohydrate food components in the stomach and intestines of Vietnamese fish within a wide range of pH values.

MATERIAL AND METHODS

The material was sampled in the Bassac River (a distributary of the Mekong River) during the flood period, 12 to 17 October, 2019. Stomach fish were studied – three species representing different families:

1) Pangasiidae – the catfish *Pangasius macronema* (4 ind., 15.6–16.5 g);
2) Sciaenidae – the smallscale croaker *Boesemania microlepis*, the only monotypic species of the genus *Boesemania* (7 ind., 29.5–34.1 g);
3) Ambassidae – the duskyfin glassy perchlet *Parambassis wolffii* (18 ind., 39.5–74.8 g).

Stomachless fish of the family Cyprinidae were studied as well – mainly representatives of the genera *Barbonymus* and *Henicorhynchus* (13 ind., 6.9–10.7 g).

Cyprinidae fish and the catfish *P. macronema* were sampled in the An Giang province, Long Xuyên region. The trawling coordinates were as follows (start – end): N10.48851°, E105.34119° – N10.47775°, E105.35133°. The duskyfin glassy perchlet and smallscale croaker were sampled in the Cần Thơ province, Thốt Nốt region. For the first species, the trawling coordinates were the following: N10.29886°, E105.52441° – N10.25485°, E105.57977°. For the second species, the coordinates were as follows: N10.26297°, E105.54821° – N10.21951°, E105.58443°. The bottom water temperature was of +30.3…+34.1 °C.

In the fish groups studied, the intestinal mucous membrane and chyme (in total) were used as enzyme preparations. The mucous membrane and chyme were thoroughly mixed; to prepare a homogenate, an aliquot was taken and weighed. The intestines of each fish, except for *P. wolffii* and Cyprinidae representatives, were examined individually. In the case of *P. wolffii*, the material was divided into 7 samples, 2–3 individuals in each. In the case of Cyprinidae fish, it was divided into 4 samples, 3–4 individuals in each. Each total sample was considered as one biological sample.

The analysis was carried out at a temperature of +25 °C within a wide range of pH values: in the case of the stomach, 2.0 to 4.0 with an interval of 1.0; in the case of the intestines, 5.0 to 11.0 with an interval of 1.0. Proteolytic activity (total activity of trypsin, EC 3.4.21.4; chymotrypsin, EC 3.4.21.1; and dipeptidases, EC 3.4.13.1 – EC 3.4.13.11) was assessed by an increase in the tyrosine concentration using the Folin–Ciocalteu reagent (Kuz'mina et al., 2019). Amylolytic activity (total activity of α-amylase, EC 3.2.1.1; γ-amylase, EC 3.2.1.3; and maltase EC 3.2.1.20) was determined by an increase in hexoses using an arsenic-molybdenum reagent (Ugolev & Iezuitova, 1969). When assessing pH dependence of enzymes, the activity was determined in 5 replicates for each point (considering the initial amounts of tyrosine or hexoses in the sample) and expressed in $\mu mol \cdot g^{-1} \cdot min^{-1}$.

The results were statistically processed using a standard software package (Microsoft Excel) and presented as follows: mean value ± standard error of the mean (*SE*). The distribution of the studied parameters did not differ from the normal one (the Shapiro–Wilk test). Therefore, the significance of the differences was assessed using the Student's *t*-test for small samples at $p < 0.001$; $p < 0.01$; and $p < 0.05$.

RESULTS

Enzyme activity. Peptidase activity in the stomach differs significantly in fish of the species studied (Table 1).

Table 1. Activity of peptidases and glycosidases in the digestive tract of fish inhabiting the Mekong Delta

Fish species	Enzyme activity, $\mu mol \cdot g^{-1} \cdot min^{-1}$			P/G
	Stomach	Intestines		
	Peptidases	Peptidases	Glycosidases	
Pangasius macronema	9.73 ± 2.09*	5.35 ± 1.04*	2.80 ± 0.10**	1.9
Boesemania microlepis	13.86 ± 0.42*	8.05 ± 0.94	1.85 ± 0.32**	4.4
Parambassis wolffii	17.82 ± 0.61	4.93 ± 0.77*	0.88 ± 0.17***	5.6
Cyprinidae sp.	–	7.88 ± 0.26	12.0 ± 0.83	0.7

Note: gastric enzymes were studied at pH 3.0; intestinal enzymes, at pH 7.4. P/G is the ratio of peptidases and glycosidases in the intestines. The differences between the maximum and other values in the column are statistically significant at $p < 0.05$ (*); $p < 0.01$ (**); and $p < 0.001$ (***).

Specifically, peptidase activity in the stomach of the duskyfin glassy perchlet *P. wolffii* was 1.3 times higher than that of the smallscale croaker *B. microlepis* and 1.8 times higher than that of the catfish *P. macronema*. In its turn, peptidase activity in the stomach of the smallscale croaker was 1.4 times higher than that of the catfish. The maximum peptidase activity in the intestines was registered for the smallscale croaker *B. microlepis*, and the minimal one – for the duskyfin glassy perchlet *P. wolffii*. At the same time, the total activity of peptidases in the stomach and intestines in this species was the highest – 22.75 $\mu mol \cdot g^{-1} \cdot min^{-1}$. The total activity of peptidases of the stomach and intestines in the smallscale croaker *B. microlepis* from the same province (Cần Thơ) was extremely close to that of the duskyfin glassy perchlet *P. wolffii* – 21.91 $\mu mol \cdot g^{-1} \cdot min^{-1}$; in the catfish *P. macronema*, the value was lower – 15.08 $\mu mol \cdot g^{-1} \cdot min^{-1}$. Glycosidase activity was significantly higher in stomachless fish than in stomach ones. The maximum differences (by 13.6 times) were revealed when comparing the level of enzyme activity in Cyprinidae fish and in the duskyfin glassy perchlet *P. wolffii*.

The ratio of peptidases and glycosidases in the intestines (P/G) was quantified. With activity of all peptidases of the gastrointestinal tract in Cyprinidae fish taken into account, the value remained equal to 0.7. In stomach fish, P/G value was significantly higher: 25.9 in the duskyfin glassy perchlet *P. wolffii* (the maximum one); 5.4 in the catfish *P. macronema* (the minimum one); and 11.8 in the smallscale croaker *B. microlepis*. When comparing the ratio of glycosidase activity to peptidase activity, it turned out that the shift in the parameter is of the opposite nature: 0.2; 0.08; and 0.04 in these three species, respectively.

pH dependence of enzymes. The maximum activity of stomach peptidases in the smallscale croaker *B. microlepis* and duskyfin glassy perchlet *P. wolffii* was recorded at pH 3.0: (12.85 ± 0.26) and (14.44 ± 0.12) $\mu mol \cdot g^{-1} \cdot min^{-1}$ for the first and second species, respectively (Fig. 1). However, at pH 2.0, the level of activity in both cases was lower than the maximum one only by 1.1 times. At pH 4.0, greater differences were revealed: in the first species, the activity decreased by 2.4 times compared with that at pH 3.0; in the second species, it decreased only by 1.4 times. The nature of pH dependence of digestive enzymes differs significantly from that of the stomach. The maximum activity of intestinal peptidases in the smallscale croaker *B. microlepis* and duskyfin glassy perchlet *P. wolffii* was registered at pH 7.0: (4.08 ± 0.3) and (5.20 ± 0.4) $\mu mol \cdot g^{-1} \cdot min^{-1}$, respectively. At the same time, the level of activity in both cases decreased sharply in the acidic zone and smoothly in the basic one. Importantly, at pH 6.0 and 5.0, there were no statistically significant species differences in the level of peptidase activity; in the basic zone, the level of peptidase activity was higher in the duskyfin glassy perchlet *P. wolffii* than in the smallscale croaker *B. microlepis*. In this case, the degree of differences sequentially increased from 1.2 at pH 8.0 to 2.2 at pH 11.0.

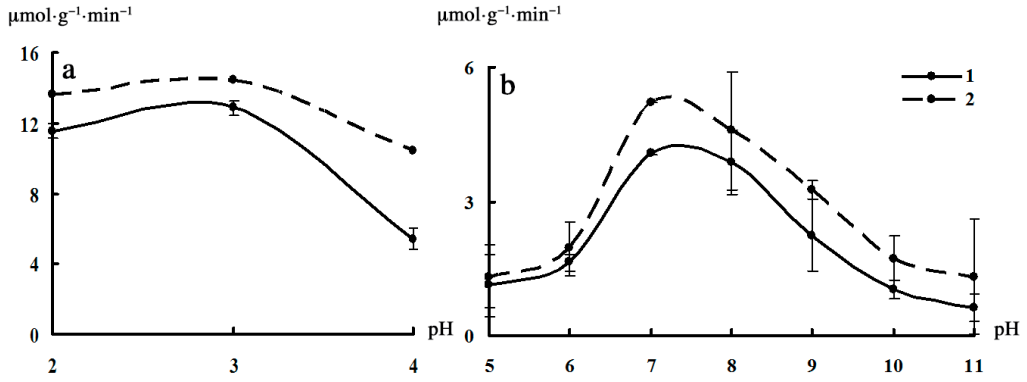

Fig. 1. Effect of pH on peptidase activity in the stomach (a) and intestines (b) in *Boesemania microlepis* (1) and *Parambassis wolffii* (2) from the Mekong Delta

Since the amount of sampled material was small, pH dependence of glycosidases was determined in the representatives of the family Cyprinidae alone (Fig. 2).

At pH 7.0, the maximum activity was recorded (see Fig. 2): (13.46 ± 0.69) μmol·g^{-1}·min^{-1}. At pH 8.0, the level of enzymatic activity was close to that at pH 7.0: (12.88 ± 0.56) μmol·g^{-1}·min^{-1}. In the zone of acidic pH values, glycosidase activity decreases more sharply than in the zone of basic pH values. At pH 5.0, the minimum activity was registered: (1.88 ± 0.20) μmol·g^{-1}·min^{-1}. At pH 11.0, the level of enzymatic activity was slightly higher than that at 5.0: (2.11 ± 0.40) μmol·g^{-1}·min^{-1}.

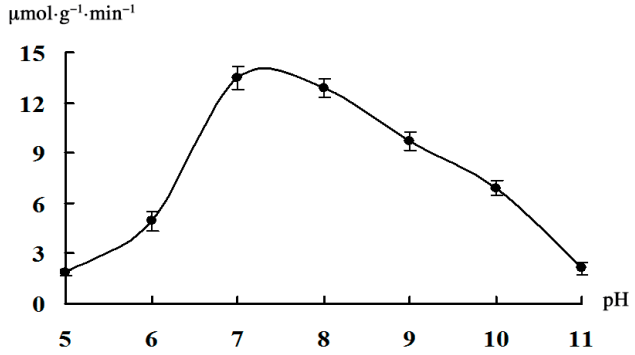

Fig. 2. Effect of pH on intestinal glycosidase activity in Cyprinidae fish from the Mekong Delta

DISCUSSION

The obtained results indicate significant species differences in the levels of peptidase and glycosidase activity, and this corresponds with the data on fish feeding spectrums. Specifically, in the duskyfin glassy perchlet *P. wolffii*, the activity of stomach peptidases is 1.3 times higher than in the smallscale croaker *B. microlepis* and 1.8 times higher than in the catfish *P. macronema*. However, the differences in the level of the total activity of peptidases in the stomach and intestines are much lower. In the first species, the activity exceeds that in the third species only by 1.5 times; between the first and second species, the differences are practically absent. These data indicate the following: in the feeding spectrums of the duskyfin glassy perchlet and smallscale croaker, fish prevail, while the feeding spectrum of the catfish is not limited by fish alone (there are other food items, in particular invertebrates).

Glycosidase activity is significantly higher in stomachless fish than in stomach ones. At the same time, P/G value in stomachless fish of the family Cyprinidae is below 1 (0.7), while in stomach fish, it is above 1 (1.9–5.6). All this corresponds with numerous data obtained in studies of other fish species (Kuz'mina, 2018 ; Ugolev & Kuz'mina, 1993 ; Bakke et al., 2011 ; Fange & Grove, 1979). Importantly, the group of stomach fish is characterized by the following peculiarity: the maximum glycosidase activity in the catfish *P. macronema* is 1.5 times higher than in the smallscale croaker *B. microlepis* and 3.2 times higher than in the duskyfin glassy perchlet *P. wolffii*. The value of the ratio of the total activity of peptidases in the stomach and intestines to the activity of glycosidases, which is maximum in the duskyfin glassy perchlet *P. wolffii*, exceeds that in the smallscale croaker *B. microlepis* by 2.2 times and that in the catfish *P. macronema* by 4.8 times. These data confirm that in the diet of the duskyfin glassy perchlet *P. wolffii*, the amount of fish is higher than in the diets of the smallscale croaker *B. microlepis* and especially catfish *P. macronema*.

The results obtained on pH dependence of enzymes correspond with the literature data (Kuz'mina, 2018 ; Ugolev & Kuz'mina, 1993 ; Bakke et al., 2011). Indeed, in most fish species, optimum pH values of stomach acid peptidases are in the range 2 to 4 (Gawlicka et al., 2001 ; Natalia et al., 2004). Optimum pH value of stomach peptidases being 3.0 for the species studied, close values of enzymatic activity at pH 2.0, and a slight decrease at pH 4.0 indicate the following: in fish, in addition to pepsin, other peptidases synthesized by gastrocytes are functioning, as well as various cathepsins of prey tissues. Enzymes of food items can also play a certain role.

In most fish species, optimum pH values of pancreatic intestinal peptidases (mainly trypsin and chymotrypsin) range 7.5 to 10 (Castillo-Yáñez et al., 2005 ; García-Carreño et al., 2002 ; Hau & Benjakul, 2006 ; Hidalgo et al., 1999 ; Kishimura et al., 2008 ; Krogdahl et al., 2015 ; Kumar et al., 2007 ; Kuz'mina et al., 2011, 2017 ; Natalia et al., 2004). In the smallscale croaker *B. microlepis* and duskyfin glassy perchlet *P. wolffii*, optimum pH value of intestinal peptidases is 7.0; apparently, this is due to the activity of pancreatic peptidases and eponymous hydrolases of the enteric microbiota (the activity of the enteric microbiota results from high abundance of bacteria in the Mekong Delta). Low activity of peptidases in the zone of acidic pH values seems to be due to the fact that trypsin is not stable at pH < 6 in most fish species (Hau & Benjakul, 2006 ; Pavlisko et al., 1999).

Optimum pH values of glycosidases, in particular α-amylase providing the initial stages of hydrolysis of polysaccharides, are in a narrower range compared to that of peptidases: 6.5 to 8.5 (Ushiyama et al., 1965) or 7.0 to 8.0 (when used for preparation of homogenate and substrate of balanced salt solutions) (Ugolev & Kuz'mina, 1993). Optimum pH value of glycosidases of the enteric microbiota is 7.0 (Kuz'mina et al., 2011). In the absolute majority of fish species investigated by us under the same methodological conditions, optimum pH value of glycosidases corresponded to 7.0. Accordingly, it can be expected that in fish inhabiting freshwater basins of Vietnam, the nature of pH dependence is close to that revealed in our work.

Conclusion. The studied fish species inhabiting the Mekong Delta are characterized by high activity of peptidases and glycosidases providing hydrolysis of protein and carbohydrate food components. The greatest interspecific differences are revealed when analyzing glycosidase activity. In Cyprinidae fish, the level of enzymatic activity exceeds that in the duskyfin glassy perchlet *Parambassis wolffii* by 13.6 times. In the level of peptidase activity, the differences in the investigated species are lower. Specifically, in the case of the activity of stomach enzymes in *P. wolffii*, the values are 1.8 times higher than those in *Pangasius macronema*. In the case of total activity of stomach and intestinal enzymes in the same species, the values are 1.5 times higher. The obtained data correspond with the information on the fish feeding spectrums. The material concerning pH dependence of intestinal enzymes indicate that the activity of enzymes of both chains decreases more significantly in the acidic pH zone than in the basic one. Consequently, significant acidification of the enteric environment will negatively affect the digestion processes in these fish species.

The work was carried out within the framework of the state research assignments "Systematics, variety, biology, and ecology of aquatic and seaboard invertebrates; the structure of populations and communities in continental waters" (No. 121051100109-1) and "Population, morphological, structural, and physiological adaptations of parasites of freshwater hydrobionts in changing environmental conditions" (No. 121051100100-8), as well as within the framework of the Ekolan E-3.4 project "The Mekong River ecosystem under global climate changes and anthropogenic load".

REFERENCES

1. Verigina I. A., Zholdasova I. M. *Ekologo-morfologicheskie osobennosti pishchevaritel'noi sistemy kostistykh ryb*. Tashkent : FAN, 1982, 154 p. (in Russ.)
2. Vysotskaya R. U., Nemova N. N. *Lizosomy i lizosomal'nye fermenty ryb*. Moscow : Nauka, 2008, 284 p. (in Russ.)
3. Kuz'mina V. V. Zashchitnaya funktsiya pishchevaritel'nogo trakta ryb. *Voprosy ikhtiologii*, 1995, vol. 35, no. 1, pp. 86–93. (in Russ.)
4. Kuz'mina V. V. *Protsessy pishchevareniya u ryb. Novye fakty i gipotezy*. Yaroslavl : Filigran', 2018, 300 p. (in Russ.)
5. Ugolev A. M., Iezuitova N. N. Opredelenie aktivnosti invertazy i drugikh disakharidaz. In: *Issledovanie pishchevaritel'nogo apparata u cheloveka* / A. M. Ugolev (Ed.). Leningrad : Nauka, 1969, pp. 192–196. (in Russ.)
6. Ugolev A. M., Kuz'mina V. V. Distribution of digestive hydrolases activity in epithelial, submucosal and musculoserous layers of fish intestine. *Doklady Akademii nauk*, 1992, vol. 326, no. 3, pp. 566–569. (in Russ.)
7. Ugolev A. M., Kuz'mina V. V. *Pishchevaritel'nye protsessy i adaptatsii u ryb*. Saint Petersburg : Gidrometeoizdat, 1993, 238 p. (in Russ.)
8. Ashie I. N. A., Simpson B. K. Proteolysis in food myosystems – A review. *Journal of Food Biochemistry*, 1997, vol. 21, iss. 5, pp. 91–123. https://doi.org/10.1111/j.1745-4514.1997.tb00218.x
9. Askarian F., Zhou Z., Olsen R. E., Sperstad S., Ringo E. Culturable autochthonous bacteria in Atlantic salmon (*Salmo salar* L.) fed diets with or without chitin. Characterization by 16S rRNA gene sequencing, ability to produce enzymes and *in vitro* growth inhibition of four fish pathogens. *Aquaculture Research*, 2012, vols 326–329, pp. 1–8. https://doi.org/10.1016/j.aquaculture.2011.10.016
10. Austin B. The bacterial microflora of fish, revised. *The Scientific World Journal*, 2006, vol. 6, pp. 931–945. https://doi.org/10.1100/tsw.2006.181
11. Bakke A. M., Glover Ch., Krogdahl A. Feeding, digestion and absorption of nutrients. In: *The Multifunctional Gut of Fish* / M. Grosell, A. P. Farrell, C. J. Brauner (Eds). Amsterdam ; Boston : Academic Press, 2011, pp. 57–110. (Series: Fish Physiology ; vol. 30). https://doi.org/10.1016/S1546-5098(10)03002-5
12. Baird I. G., Flaherty M. S., Phylavanh B. Rhythms of the river: Lunar phases and migrations of small carp (Cyprinidae) in the Mekong River. *Natural History Bulletin of the Siam Society*, 2003, vol. 51, pp. 5–36.
13. Baird I. G., Phylavanh B., Vongsenesouk B., Xaiyamanivong K. The ecology and conservation of the smallscale croaker *Boesemania microlepis* (Bleeker, 1858–1859) in the mainstream Mekong River, Southern Laos. *Natural History Bulletin of the Siam Society*, 2001, vol. 49, pp. 161–176.
14. Belchior S. G. E., Vacca G. Fish protein hydrolysis by a psychrotrophic marine bacterium isolated from the gut of hake (*Merluccius hubbsi*). *Canadian Journal of Microbiology*, 2006, vol. 52, no. 12, pp. 1266–1271. https://doi.org/10.1139/w06-083
15. Castillo-Yáñez F. J., Pacheco-Aguilar R., García-Carreño F. L., Navarrete-Del Toro M. Á. Isolation and characterization of trypsin from pyloric caeca of Monterey sardine *Sardinops sagax caeruleus*. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, 2005, vol. 140, iss. 1, pp. 91–98. http://dx.doi.org/10.1016/j.cbpc.2004.09.031

16. Esakkiraj P., Immanuel G., Sowmya S. V., Iyapparaj P., Palavesam A. Evaluation of protease-producing ability of fish gut isolate *Bacillus cereus*. *Food and Bioprocess Technology*, 2009, vol. 2, pp. 383–390. https://doi.org/10.1007/s11947-007-0046-6
17. Fange R., Grove D. Digestion. In: *Bioenergetics and Growth* / W. S. Hoar, D. J. Randall, J. R. Brett (Eds). New York : Academic Press, 1979, pp. 161–260. (Book series: Fish Physiology ; vol. 8).
18. Ganguly S., Prasad A. Microflora in fish digestive tract plays significant role in digestion and metabolism. *Reviews in Fish Biology and Fisheries*, 2012, vol. 22, pp. 11–16. https://doi.org/10.1007/s11160-011-9214-x
19. García-Carreño F. L., Albuquerque-Cavalcanti C., Navarrete del Toro M. A., Zaniboni-Filho E. Digestive proteinases of *Brycon orbignyanus* (Characidae, Teleostei): Characteristics and effects of protein quality. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, 2002, vol. 132, iss. 2, pp. 343–352. https://doi.org/10.1016/S1096-4959(02)00038-6
20. Gawlicka A., Leggiadro C. T., Gallant J. W., Douglas S. E. Cellular expression of the pepsinogen and proton pump genes in the stomach of winter flounder as determined by *in situ* hybridization. *Journal of Fish Biology*, 2001, vol. 58, iss. 2, pp. 529–536. https://doi.org/10.1111/j.1095-8649.2001.tb02271.x
21. Hau P. V., Benjakul S. Purification and characterization of trypsin from pyloric caeca of bigeye snapper (*Pricanthus macracanthus*). *Journal of Food Biochemistry*, 2006, vol. 30, iss. 4, pp. 478–495. https://doi.org/10.1111/j.1745-4514.2006.00089.x
22. Hidalgo M. C., Urea E., Sanz A. Comparative study of digestive enzymes in fish with different nutritional habits. Proteolytic and amylase activities. *Aquaculture*, 1999, vol. 170, iss. 3–4, pp. 267–283. https://doi.org/10.1016/S0044-8486(98)00413-X
23. Hoshino T., Ishizaki K., Sakamoto T., Kumeta H., Yumoto I., Matsuyama H., Ohgiya S. Isolation of a *Pseudomonas* species from fish intestine that produces a protease active at low temperature. *Letters in Applied Microbiology*, 1997, vol. 25, iss. 1, pp. 70–72. https://doi.org/10.1046/j.1472-765x.1997.00183.x
24. Izvekova G. I., Plotnikov A. O. Hydrolytic activity of symbiotic microflora enzymes in pike (*Esox lucius* L.) intestines. *Inland Water Biology*, 2011, vol. 4, no. 1, pp. 72–77. https://doi.org/10.1134/S1995082911010081
25. Jasmine S., Begum M. Biological aspects of *Barbonymus gonionotus* (Bleeker, 1849) in the Padma River, Bangladesh. *International Journal of Fisheries and Aquatic Studies*, 2016, vol. 4, pp. 661–665.
26. Kapoor B. G., Smit H., Verighina I. A. The alimentary canal and digestion in teleosts. *Advances in Marine Biology*, 1975, vol. 13, pp. 109–239.
27. Kishimura H., Klomklao S., Benjakul S., Chun B.-S. Characteristics of trypsin from the pyloric ceca of walleye pollock (*Theragra chalcogramma*). *Food Chemistry*, 2008, vol. 106, iss. 1, pp. 194–199. https://doi.org/10.1016/j.foodchem.2007.05.056
28. Kottelat M., Widjanarti E. The fishes of Danau Sentarum National Park and the Kapuas Lakes area, Kalimantan Barat, Indonesia. *Raffles Bulletin of Zoology – Supplement*, 2005, vol. 13, pp. 139–173.
29. Krogdahl Å., Sundby A., Holm H. Characteristics of digestive processes in Atlantic salmon (*Salmo salar*). Enzyme pH optima, chyme pH, and enzyme activities. *Aquaculture*, 2015, vol. 449, pp. 27–36. https://doi.org/10.1016/j.aquaculture.2015.02.032

30. Kumar S., Garcia-Carreno F. L., Chakrabarti R., Toro M. A. N., Cordova-Murueta J. H. Digestive proteases of three carps *Catla catla*, *Labeo rohita* and *Hypophthalmichthys molitrix*: Partial characterization and protein hydrolysis efficiency. *Aquaculture Nutrition*, 2007, vol. 13, iss. 5, pp. 381–388. https://doi.org/10.1111/j.1365-2095.2007.00488.x
31. Kuz'mina V. V., Skvortsova E. G., Zolotareva G. V., Sheptitskiy V. A. Influence of pH upon the activity of glycosidases and proteinases of intestinal mucosa, chyme and microbiota in fish. *Fish Physiology and Biochemistry*, 2011, vol. 37, no. 3, pp. 345–357. https://doi.org/10.1007/s10695-010-9426-3
32. Kuz'mina V. V., Komov V. T., Tarleva A. F., Sheptitskiy V. A. Effect of dietary metal exposure on the locomotor reactions and food consumption in common carp *Cyprinus carpio* (L.). *Inland Water Biology*, 2019, vol. 12, no. 3, pp. 356–364. https://doi.org/10.1134/S1995082919030106
33. Kuz'mina V. V., Zolotareva G. V., Sheptitskiy V. A. Proteolytic activity in some freshwater animals and associated microflora in a wide pH range. *Fish Physiology and Biochemistry*, 2017, vol. 43, iss. 2, pp. 373–383. https://doi.org/10.1007/s10695-016-0293-4
34. Mohsin A. K. M., Ambak M. A. *Freshwater Fishes of Peninsular Malaysia.* Serdan : Penerbit University Pertanian Malaysia, 1983, 284 p.
35. Natalia Y., Hashim R., Ali A., Chong A. Characterization of digestive enzymes in a carnivorous ornamental fish, the Asian bony tongue *Scleropages formosus* (Osteoglossidae). *Aquaculture*, 2004, vol. 233, iss. 1–4, pp. 305–320. https://doi.org/10.1016/j.aquaculture.2003.08.012
36. Pavlisko A., Rial A., Coppes Z. Purification and characterization of a protease from the pyloric caeca of menhaden (*Brevoortia* spp.) and mullet (*Mugil* spp.) from the southwest Atlantic region. *Journal of Food Biochemistry*, 1999, vol. 23, iss. 2, pp. 225–241. https://doi.org/10.1111/j.1745-4514.1999.tb00016.x
37. Rainboth W. J. *Fishes of the Cambodian Mekong.* Rome : FAO, 1996, 265 p. (FAO species identification field guide for fishery purposes).
38. Ray A. K., Ghosh K., Ringø E. Enzyme-producing bacteria isolated from fish gut: A review. *Aquaculture Nutrition*, 2012, vol. 18, iss. 5, pp. 465–492. https://doi.org/10.1111/j.1365-2095.2012.00943.x
39. Sugita H., Kawasaki J., Deguchi Y. Production of amylase by the intestinal microflora in cultured freshwater fish. *Letters in Applied Microbiology*, 1997, vol. 24, iss. 2, pp. 105–108. https://doi.org/10.1046/j.1472-765x.1997.00360.x
40. Taki Y. *An Analytical Study of the Fish Fauna of the Mekong Basin as a Biological Production System in Nature.* Tokyo : Research Institute of Evolutionary Biology, 1978, 77 p. (Research Institute of Evolutionary Biology special publications ; no. 1).
41. Tran D. D., Shibukawa K., Nguyen P. T., Ha H. P., Tran L. X., Mai H. V., Utsugi K. *Fishes of the Mekong Delta, Vietnam.* Can Tho : Can Tho University Publishing House, 2013, 174 p.
42. Tuan L. A., Hoanh C. T., Miller F., Sinh B. T. Flood and salinity management in the Mekong Delta, Vietnam. In: *Challenges to Sustainable Development in the Mekong Delta: Regional and National Policy Issues and Research Needs: Literature Analysis* / T. T. Be, B. T. Sinh, F. Miller (Eds). Bangkok : The Sustainable Mekong Research Network (Sumernet), 2007, pp. 15–68.
43. Ushiyama H., Fujimori T., Shibata T., Yoshimura K. Studies on carbohydrases

in the pyloric caeca of the salmon *Oncorhynchus keta*. *Bulletin of the Faculty of Fisheries Hokkaido University*, 1965, vol. 16, no. 3, pp. 183–188.

44. Wang B., Wang C., Mims S. D., Xiong Y. L. Characterization of the proteases involved in hydrolyzing paddlefish (*Polyodon spathula*) myosin. *Journal of Food Biochemistry*, 2000, vol. 24, iss. 6, pp. 503–515. https://doi.org/10.1111/j.1745-4514.2000.tb00719.x

АКТИВНОСТЬ ПЕПТИДАЗ И ГЛИКОЗИДАЗ ПИЩЕВАРИТЕЛЬНОГО ТРАКТА У НЕКОТОРЫХ ВИДОВ КОСТИСТЫХ РЫБ ВЬЕТНАМА

В. В. Кузьмина[1], Е. Е. Слынько[1,2], Е. А. Куливацкая[1], Е. П. Карпова[2], Динь Ку Нгуен[2]

[1]Институт биологии внутренних вод имени И. Д. Папанина Российской академии наук, пос. Борок, Российская Федерация

[2]Южное отделение Совместного российско-вьетнамского тропического научно-исследовательского и технологического центра, Хошимин, Вьетнам

E-mail: *elena.slynko.76@mail.ru*

Впервые исследованы активность и pH-зависимость пищеварительных ферментов у рыб, обитающих в дельте р. Меконг: стеклянного окуня *Parambassis wolffii*, мелкочешуйного горбыля *Boesemania microlepis*, пангасиуса *Pangasius macronema* и представителей семейства Cyprinidae. Выявлены значительные межвидовые различия в уровне активности пептидаз и гликозидаз, обеспечивающих гидролиз белковых и углеводных компонентов пищи. Наибольшие межвидовые различия характерны для гликозидаз: уровень ферментативной активности у рыб сем. Cyprinidae превышает таковой у стеклянного окуня *P. wolffii* в 13,6 раза. Различия в уровне активности пептидаз у рыб разных видов ниже: в случае активности ферментов желудка у стеклянного окуня *P. wolffii* значения выше таковых для пангасиуса *P. macronema* в 1,8 раза, в случае суммарной активности ферментов желудка и кишечника у тех же видов — в 1,5 раза. Полученные данные подтверждают представления о зависимости активности пищеварительных гидролаз от спектра питания рыб. Активность ферментов кишечника значительнее снижается в кислой зоне pH, чем в щелочной. Следовательно, закисление энтеральной среды будет негативно влиять на процессы пищеварения у этих видов рыб.

Ключевые слова: Вьетнам, пищеварительные ферменты, *Parambassis wolffii*, *Boesemania microlepis*, *Pangasius macronema*, Cyprinidae