

Temperature characteristics of peptidase in chironomid larvae, potential fish prey, at various pH values

V. V. Kuz'mina^{*}, E. Y. Chornaya^{**}, E. G. Skvortsova^{***}, E. A. Kulivatskaya^{*}, V. A. Sheptitskiy^{**}

^{*}*I. D. Papanin Institute of Biology of Inland Waters RAS, Borok, Russia*

^{**}*T. G. Shevchenko State University, Tiraspol, Moldova*

^{***}*Yaroslavl State Agricultural Academy, Yaroslavl, Russia*

^{****}*Institute of Physiology and Sanocreatology, Chisinau, Moldova*

Article info

Received 14.06.2018

Received in revised form

02.08.2018

Accepted 08.08.2018

I. D. Papanin Institute of Biology of Inland Waters RAS, Borok, Yaroslavl region, 152742, Russia.

T. G. Shevchenko State University, 25 October st., 128, Tiraspol, 3300, Moldova.

Yaroslavl State Agricultural Academy, Tutaevskoe shosse, 58, Yaroslavl, 150042, Russia.

Institute of Physiology and Sanocreatology, Academiei st., 1, Chisinau, 2028, Moldova. Tel: +373-69-75-37-82. E-mail: septitchi@mail.ru

Kuz'mina, V. V., Chornaya, E. Y., Skvortsova, E. G., Kulivatskaya, E. A., & Sheptitskiy, V. A. (2018). Temperature characteristics of peptidase in chironomid larvae, potential fish prey, at various pH values. Biosystems Diversity, 26(3), 201–205. doi: 10.15421/011830

The temperature dependence of casein- and hemoglobinolytic peptidases functioning in the whole organism of chironomid larvae *Chironomus plumosus*, food objects of adult benthophages and juvenile fish of various ecological groups, was studied within the temperature range of 0–70 °C at different pH values (3.0, 5.0 and 7.4). The method of mixed samples was used to determine the activity and characteristics of enzymes. Homogenates of previously crushed and carefully mixed dozens of larvae were used as enzymatically active preparations. Activity of peptidases was assayed by the increase in tyrosine concentration using the Folin-Ciocalteu reagent. It is shown that the activity of peptidases that function in the tissues of chironomid larvae depends to a considerable extent on temperature and pH, but the pH has a smaller effect on the activity and the temperature dependence of casein- and hemoglobin-lytic peptidases than temperature. The temperature optimum of the studied peptidases of chironomid larvae corresponds to 40 °C. The Q₁₀ values in the zone of vital temperatures are slightly changed. They are, as a rule, increased in the zone of 30–40 °C, and are sharply decreased in the zone of high temperatures. The values of activation energy of the process of hydrolysis of casein and hemoglobin in the zone of low and high temperatures are different. The exact values of the process of hydrolysis of casein and hemoglobin at a temperature not exceeding 20 °C are usually below those in the zone of higher temperatures (except for hemoglobin-lytic peptidases at pH 5.0). The data obtained indicate a significant effect of pH not only on the activity, but also on the temperature characteristics of peptidases that function in the body of chironomid larvae. Differences in the characteristics of casein- and hemoglobin-lytic peptidases in chironomid larvae at different temperatures and pH can influence the digestion in benthophages and fry of all fish species.

Keywords: chironomid larvae; peptidase; temperature dependence; temperature coefficients; activation energy; pH

Introduction

It is known that chironomid larvae play an important role in the trophic relations of hydrobionts (Gerasimov, 2015). In recent years, particular importance has been attached to the study of the characteristics of their digestive hydrolases, since the possibility of the contribution of prey enzymes to the processes of digestion of fish was demonstrated (Dabrowski & Glogowski, 1977; Lauff & Hofer, 1984; Munilla-Moran et al., 1990; Kuz'mina, 2008). The mechanism of induced autolysis plays an important role in this process (Kuz'mina, 2008, 2017). The magnitude of the contribution of food enzymes to the processes of fish digestion depends to a large extent on the species of consumer and prey, temperature and pH of the gastric and enteral media (Kuz'mina, 2008, 2017). When studying the temperature characteristics of peptidases of chyme and intestinal mucosa in a number of species of benthophages, as well as their potential prey, differences in the character of the curves of temperature dependence were revealed (Kuz'mina et al., 2015). In studying the pH dependence of the peptidases of chyme and intestinal mucosa of consumers, the whole body of their potential prey, as well as enteral and associated microbiota, differences were also found that are most significant in the case of microflora (Kuz'mina et al., 2017).

Since the majority of fish species are zoophagous (Gerking, 1994), most attention is usually paid to enzymes that hydrolyse the food proteins, in studies evaluating the effectiveness of their feeding. The activity of trypsin-like and chymotrypsin-like peptidases in many species of marine crustacean (Dendinger, 1987; Fernández-Gimenez et al., 2001;

Kumar et al., 2005; Navarrete del Toro et al., 2006) and molluscs (Reid & Rauchert, 1972; Serviere-Zaragoza et al., 1997) as well as other hydrobionts has been revealed. There is also information about the presence of leucine aminopeptidase, as well as carboxypeptidases A and B in a number of invertebrate species (Dendinger, 1987; Boetius & Felbeck, 1995). However the activity of casein-lytic peptidases in a number of freshwater hydrobionts belonging to the Mollusca, Annelida and Arthropoda phyla (Kuz'mina, 1999) are 5–15 times lower than that of the intestinal mucosa of fish (Kuz'mina, 2008). At the same time, the cathepsins A, B, C, D, E and L are isolated from invertebrate tissues. In a number of crustaceans, cathepsin L is the main enzyme that breaks down proteins (Le Boulay et al., 1996; Butler et al., 2001; Teschke, Saborowski, 2005; Hu & Leung, 2007). However, in the shrimp *Pandalus borealis* (Aoki et al., 2003) and the mollusc *Tresus capax* (Reid & Rauchert, 1976), cathepsin B plays a major role in the cleavage of the protein.

In addition, it was found that the level of peptidase activity in the whole body of hydrobionts (total samples of zooplankton, amphipoda, chironomid larvae, oligochaeta and dreissena) and the associated microbiota largely depends on pH (Kuz'mina et al., 2017). Cysteine-like protease of the hepatic fluke *Fasciola hepatica* exhibited the highest proteolytic activity on casein at pH 5.5 and temperature of 35–40 °C (Hemici et al., 2017). Information on the temperature characteristics of peptidases functioning in the whole body of potential prey of juvenile fish and adult benthophages living in freshwater bodies (Kuz'mina, 1999; Skvortsova et al., 2016) is rare. Information on the effect of pH on peptidase temperature characteristics in the whole body of potential fish prey is

exceptionally scarce (Skvortsova et al., 2016). The aim of the work is to study the temperature characteristics of peptidases that function in the whole body of chironomid larvae, the favourite prey of fish, at different pH values *in vitro*.

Material and methods

The research object was the chironomid larvae *Chironomus plumosus*. The average weight of one larva was 7.5 mg. The method of mixed samples (Egorova et al., 1974) was used to determine the activity and characteristics of enzymes. Homogenates of previously crushed and carefully mixed dozens of larvae were used as enzymatically active preparations. All the operations were conducted in the cold. Aliquots of samples (0.5–1.0 g) were homogenized in a glass homogenizer with a small amount of Ringer's solution for cold-blooded animals (103 mM NaCl, 1.9 mM KCl, 0.45 mM CaCl₂, pH 3.0, 5.0 or 7.4) at a temperature of 2–4 °C. To fulfill this, the glass homogenizer had been placed in a glass with ice. The homogenate was further diluted with Ringer's solution until the final dilution of 1 : 99.

To assess the activity of peptidases in the whole organism of chironomid larvae, 0.5 ml of the homogenate and 0.5 ml of 1% substrate (casein or hemoglobin, pH 3.0, 5.0 or 7.4) prepared in the same Ringer's solution was added to the tubes and the mixture was incubated for another 30 min in special thermostatted chambers. All the operations were carried out at the temperature range of 0–70 °C (0, 10, 20, 30, 40, 50, 60 or 70 °C) and under continuous stirring. The activity of peptidases (the activity of trypsin, EC 3.4.21.4, chymotrypsin, EC 3.4.21.1 and dipeptidases EC 3.4.13 or cathepsins, mainly cathepsin D, EC 3.4.23.5) was assessed by an increasing the tyrosine concentration using the Folin-Ciocalteu reagent (Anson, 1938). The level of enzyme activity was judged by the increment of reaction products per 1 minute of incubation of the substrate and the enzymatically active preparation, taking into account the background (the amount of tyrosine in the original homogenate), calculating per 1 g of wet tissue weight, $\mu\text{mol}/(\text{g}\cdot\text{min})$. The coloration intensity was assessed by means of a photocolorimeter (KFK-2) with a red light filter, $\lambda = 670 \text{ nm}$. The enzyme activity was determined in 5 replications. The temperature coefficients (Q_{10}) were calculated in the traditional way. The values of the activation energy were determined by the Arrhenius' graphical method (according to the temperature dependence). The results are expressed as means \pm SE. One-way ANOVA and Two-Way ANOVA were performed to interpret the significance of the particular enzyme activity variations with the help of the F value.

Results

The temperature dependence of casein-lytic peptidases of the whole body of chironomid larvae at different pH values. The activity of casein-lytic peptidases of all tissues of chironomid larvae on casein at standard temperature 20 °C and at pH 5.0 and 7.4 was significantly different: 0.31 ± 0.01 and $0.81 \pm 0.17 \mu\text{mol}/(\text{g}\cdot\text{min})$, respectively. The study of the peptidase activity at a wide temperature range made it possible to reveal the differences in the shape of the temperature dependence curves of casein-lytic peptidases that function at different pH values (Fig. 1). The relative activity of casein-lytic peptidases in the 0–30 °C zone at 5.0 and 7.4 varied within the following values 21.3–37.9% and 11.7–28.4% of the maximal activity taken as 100, respectively.

The highest pH effect on casein-lytic activity is observed at 10 and 20 °C ($P < 0.05$), at 30 °C it is somewhat lower. In the zone of post-maximal temperatures the effect of pH is negligible (Table 1).

The Two-Way ANOVA analysis of these data showed that the share of pH influence is 2.4% ($P < 0.05$), the share of temperature influence is 55.7% ($P < 0.001$), the share of temperature and pH influence is 4.8%. The influence of the substrate was not taken into account, since temperature dependence is traditionally determined when one substrate only is used.

The temperature dependence of hemoglobin-lytic peptidases of the whole organism of chironomid larvae at different pH values. The activity of hemoglobin-lytic peptidases of all tissues of chironomid larvae at

standard temperature 20 °C and at pH 3.0, 5.0 and 7.4 was 0.63 ± 0.23 , 1.03 ± 0.35 and $0.81 \pm 0.08 \mu\text{mol}/(\text{g}\cdot\text{min})$, respectively. The study of the peptidase activity at a wide temperature range revealed differences in the shape of the temperature dependence curves of hemoglobin-lytic peptidases (Fig. 2). The relative activity of hemoglobin-lytic peptidases in the 0–30 °C range at pH 3.0, 5.0 and 7.4 varied within the following values: 20.6–46.6%, 25.4–72.5% and 23.9–63.6% of the maximal activity taken as 100, respectively.

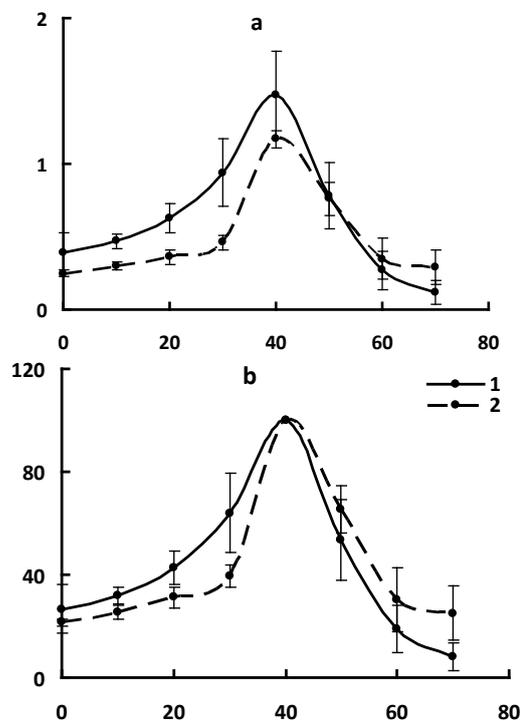


Fig. 1. The temperature dependence of casein-lytic peptidases in the whole body of chironomid larvae at pH 7.4 (1) and pH 5.0 (2): on the abscissa axis – temperature (°C); on the ordinate axis: a – the enzyme activity ($\mu\text{mol}/(\text{g}\cdot\text{min})$), b – the relative enzyme activity (% of the maximum activity taken as 100)

Table 1

Statistical significance of differences for assessment of effects of pH values on casein-lytic peptidase activities in the whole body of chironomid larvae (One-Way ANOVA)

| pH | Temperature | | | | | | | |
|--------------------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| | 0 °C | 10 °C | 20 °C | 30 °C | 40 °C | 50 °C | 60 °C | 70 °C |
| 5.0 | 0.25 ± 0.02 | 0.30 ± 0.03 | 0.36 ± 0.05 | 0.46 ± 0.06 | 1.17 ± 0.07 | 0.25 ± 0.12 | 0.35 ± 0.16 | 0.29 ± 0.14 |
| | 0.39 ± 0.15 | 0.47 ± 0.05 | 0.63 ± 0.11 | 0.94 ± 0.25 | 1.47 ± 0.33 | 0.39 ± 0.26 | 0.28 ± 0.15 | 0.12 ± 0.09 |
| P | 0.34 | 0.02* | 0.04* | 0.08 | 0.36 | 0.34 | 0.70 | 0.27 |
| Share of pH influence, % | 11.6 | 53.3* | 43.2* | 34.4 | 10.8 | 11.6 | 1.9 | 15.2 |

Note: the differences are significant at $P < 0.05$.

There were no significant differences in the temperature dependence of hemoglobin-lytic activity at different pH values (Table 2). The Two-Way ANOVA analysis of these data showed that the share of pH influence is 0.5%, the share of temperature influence is 62.5%, the share of temperature and pH influence is 2.3%. The effect of temperature is significant only ($P < 0.001$).

The temperature coefficients (Q_{10}) of the peptidase activity of the whole body of chironomid larvae. The data on the temperature coefficients of the activity of peptidases of the whole body of chironomid larvae in a wide temperature range indicate that the Q_{10} values range from 1.1 to 2.6 in the temperature range 0–40 °C. In most cases, the highest values of the index were detected in the range of 20–40 °C. These values were much lower than 1.0 in the post-maximal temperature zone (Table 1). The character of the dynamics of the peptidase Q_{10} values largely de-

depends on the pH. So, for hemoglobin-lytic peptidases at pH 5.0 and casein-lytic peptidases at pH 7.4, the similar values of Q_{10} in the 0–40 °C range were observed. At other pH values, an almost twofold increase in the index was observed. It is important to note that in the temperature range of animal vital activity regardless of pH, in most cases, an increase in the Q_{10} values of casein and hemoglobin-lytic peptidases from 10 to 30 °C is characteristic.

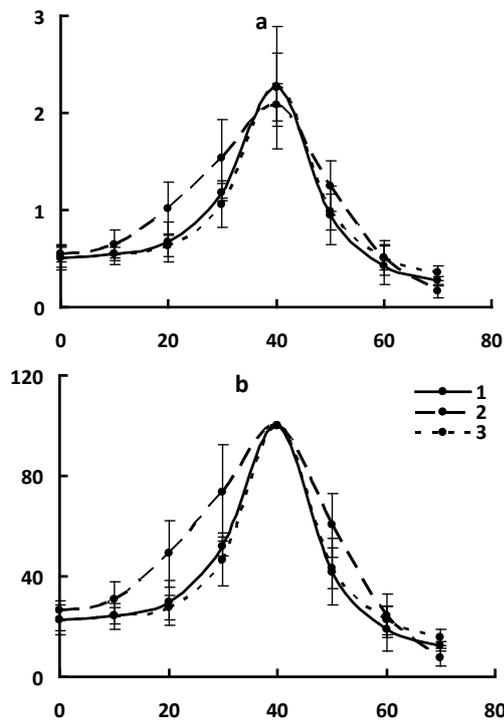


Fig. 2. The temperature dependence of hemoglobin-lytic peptidases in the whole organism of chironomid larvae at pH 7.4 (1), 5.0 (2) and 3.0 (3): note, as in Figure 1

Table 2
Statistical significance of differences for assessment of effects of pH values on hemoglobin-lytic peptidase activities in whole body of chironomid larvae (One-Way ANOVA)

| pH | Temperature | | | | | | | |
|--------------------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| | 0 °C | 10 °C | 20 °C | 30 °C | 40 °C | 50 °C | 60 °C | 70 °C |
| 3.0 | 0.51 ± 0.11 | 0.55 ± 0.13 | 0.63 ± 0.26 | 1.06 ± 0.26 | 2.27 ± 0.40 | 0.98 ± 0.21 | 0.51 ± 0.15 | 0.35 ± 0.08 |
| 5.0 | 0.55 ± 0.09 | 0.65 ± 0.15 | 1.02 ± 0.31 | 1.53 ± 0.45 | 2.08 ± 0.25 | 1.25 ± 0.19 | 0.51 ± 0.20 | 0.16 ± 0.08 |
| 7.4 | 0.51 ± 0.15 | 0.55 ± 0.08 | 0.67 ± 0.23 | 1.18 ± 0.10 | 2.26 ± 0.70 | 0.94 ± 0.33 | 0.43 ± 0.22 | 0.28 ± 0.05 |
| P | 0.96 | 0.78 | 0.37 | 0.47 | 0.94 | 0.64 | 0.93 | 0.15 |
| Share of pH influence, % | 0.71 | 4.10 | 15.4 | 11.83 | 1.04 | 7.07 | 1.17 | 27.53 |

Note: the differences are significant at $P < 0.05$.

Table 3
The Q_{10} values of peptidases of the whole body of chironomid larvae at the temperature range of their living activity

| pH | Substrate | Q_{10} | | | | | | |
|-----|------------|----------|----------|----------|----------|----------|----------|----------|
| | | 0–10 °C | 10–20 °C | 20–30 °C | 30–40 °C | 40–50 °C | 50–60 °C | 60–70 °C |
| 3.0 | Hemoglobin | 1.08 | 1.15 | 1.68 | 2.14 | 0.43 | 0.52 | 0.69 |
| | Casein | 1.20 | 1.20 | 1.28 | 2.54 | 0.65 | 0.46 | 0.82 |
| 5.0 | Hemoglobin | 1.18 | 1.57 | 1.50 | 1.36 | 0.60 | 0.41 | 0.31 |
| | Casein | 1.21 | 1.34 | 1.49 | 1.56 | 0.53 | 0.35 | 0.44 |
| 7.4 | Hemoglobin | 1.08 | 1.22 | 1.69 | 2.00 | 0.43 | 0.46 | 0.63 |

The activation energy of the process of casein and hemoglobin hydrolysis by peptidases of the whole body of chironomid larvae. The data on E_{act} of peptidases functioning in the tissues of chironomid larvae in the temperature range of their living activity (0–30 °C) indicate the pre-

sence of a bend on the Arrhenius plot in all variants of the experiment at 20 °C as well as the dependence of the indicator's value on the substrate and pH (Table 2). At the same time, the values of E_{act} of the process of casein hydrolysis in the range of 0–20 °C at pH 5.0 and pH 7.4 are 3.4 and 1.9 times lower than in the zone of higher temperatures, respectively. When studying hemoglobin as a substrate, the bend on the Arrhenius plot was also detected at 20 °C for all the pH values. At the same time the average values of E_{act} of the process of hemoglobin hydrolysis at pH 3.0 and 7.4 in the range of 0–20 °C are 4.5 and 1.9 times lower than in the zone of higher temperatures, respectively. At pH 5.0, on the contrary, the average values of E_{act} of the process of hemoglobin hydrolysis in the range of 0–20 °C are higher than in the zone of higher temperatures by 1.5 times.

Table 4
The values of the peptidase activation energy in the whole body of chironomid larvae in the temperature range of their life activity

| pH | Substrate | Activation energy, kcal/mol | | Point of break, °C |
|-----|------------|-----------------------------|----------------------|--------------------|
| | | before point of break | after point of break | |
| 3.0 | Hemoglobin | 2.4 | 11.5 | 20 |
| | Casein | 3.1 | 10.5 | 20 |
| 5.0 | Hemoglobin | 9.5 | 6.3 | 20 |
| | Casein | 3.5 | 6.5 | 20 |
| 7.4 | Hemoglobin | 6.8 | 13.2 | 20 |

Discussion

To properly analyze the material obtained, it should be noted that we have studied the integrative index which reflects the activity of a number of enzymes. So, at pH 7.4, the activity of endopeptidases (trypsin, chymotrypsin and elastase) and exopeptidases (various carboxypeptidases, aminopeptidases and dipeptidases) dominates. The most important is the activity of trypsin, mainly hydrolyzing peptide bonds adjacent to lysine or arginine, and also chymotrypsin, which predominantly hydrolyzes peptide bonds adjacent to tyrosine, tryptophan and phenylalanine (Dixon & Webb, 1983). The activity of casein-lytic peptidases primarily reflects the activity of trypsin, that of hemoglobin-lytic peptidases – that of chymotrypsin. At pH 3.0 and 5.0, the activity of various cathepsins dominates (Kuz'mina, 2015).

As indicated in the introduction, the activity of digestive peptidases in marine crustaceans has been studied in the most detailed manner. In such a way, the activity of trypsin-like and chymotrypsin-like enzymes was observed in lobsters, sea crawfish (Kim et al., 1994), crabs (Navarrete del Toro et al., 2006) and shrimps (Van-Wormhoudt et al., 1995). The activity of carboxypeptidases A and B, as well as that of leucine aminopeptidase (Dendinger, 1987; Glass & Stark, 1995) was detected also. In various shrimp tissues cDNA coding cathepsin L (Duan et al., 2013; Zhao et al., 2013), cathepsin B (Stephens et al., 2012; Li et al., 2013) and cathepsin C (Wang et al., 2012) were revealed. These data suggest that chironomid larvae may possess a wide range of peptidases.

The data on the activity of casein- and hemoglobin-lytic peptidases in the whole body of chironomid larvae at a temperature of 20 °C and pH 7.4, as well as the temperature optimum value corresponding to 40 °C, are largely similar to those obtained earlier (Skvortsova et al., 2016). However, when studying the activity of peptidases in the whole body of chironomid larvae from the Kuchurhan reservoir, a significantly higher level of enzymatic activity under the same methodological conditions was revealed: $3.1 \pm 0.16 \mu\text{mol}/(\text{g} \cdot \text{min})$ (Kuz'mina et al., 2017). It is important that the temperature characteristics of peptidases of chironomid larvae differ essentially from those of fish. So, the temperature optimum of casein- and hemoglobin-lytic peptidases of the intestinal mucosa in fish of different species corresponds to 50 or 60 °C (Kuz'mina et al., 2015), and that of chironomid larvae tissues is 40 °C. This may be due to differences in the sources of enzyme activity.

In addition, the range of values of the temperature optimums of different peptidases in crustaceans is quite wide. The specific casein-lytic peptidase from the hepatopancreas of the oriental shrimp *Fenneropenaeus chinensis* (Oh et al., 2000) and the trypsin of the red swamp crawfish *Procambarus clarkii* (Kim et al., 1994) have the highest tem-

perature optimum (70 °C). The maximum activity of trypsin in swimming crabs *Callinectes bellicosus* and *C. arcuatus* was recorded at 55 °C (Diaz-Tenorio et al., 2006). The temperature optimum of the buzzer midge *Chironomus plumosus* larvae is 60 °C. However, the temperature optimum of casein-lytic peptidases of midge *Chaoborus* sp. larvae is only 50 °C (Kuz'mina, 1999).

The temperature optimum of chymotrypsin in the swimming crabs *Callinectes bellicosus* and *C. arcuatus* were recorded at a temperature of 50–55 °C (Diaz-Tenorio et al., 2006). The activity of peptidases in the digestive tract of the Pacific brown shrimp *Farfantepenaeus californiensis* is maximal at a temperature of 50 °C (Vega-Villasante et al., 1995). It has been shown that the differences in the values of the peptidase temperature optimum depend to a large extent on the temperature of the habitat of the species. In this way, the temperature optimum of trypsin-like peptidases of the semi-terrestrial tropical pink ghost crab *Ocypode ryderi* (Dittrich, 1990), the common hermit crab *Pagurus bernhardus* and the hermit crab *Clibanarius striolatus* (Dittrich, 1992) is 50 °C, that of the eurythermal brown crab *Cancer pagurus* is 45 °C. The temperature optimum of the stenothermal Antarctic shrimp *Chorismus antarcticus* is 40 °C (Dittrich, 1990). The temperature optimum of cathepsin D also varies considerably: the minimum values (35 °C) were detected in the Pacific flying squid *Todarodes pacificus* (Sakai et al., 1981), higher values (50 °C) – in larvae of the buzzer midge *Chironomus plumosus* (Kuz'mina, 1999). In all likelihood, the relatively low level of the peptidase maximum activity (40 °C) in the chironomid larvae studied by us is due to the considerable activity of cathepsins. This is also evidenced by the different shape of the temperature dependence curves of casein- and hemoglobin-lytic peptidases in the zone of low and postmaximal temperatures.

Particular mention should be made of the dependence of the temperature effects on pH. Despite the same values of the optimum temperature for different pH values, we found differences in the zone of vital and postmaximal temperatures, especially in the case of casein-lytic peptidases. Actually, despite the lower activity of peptidases at pH 5.0 compared with that at pH 7.4, the relative activity of enzymes in this zone is much higher than at pH 7.4. The observed differences may be due to the fact that different enzymes function at different pH values. This way, in the zone of neutral pH values, both the activity of pancreatic peptidases and the activity of cathepsins the pH optimum of which is in this zone, as well as, apparently, the residual activity of cathepsins whose pH optimum is at pH 5.0, can be detected. Indeed, L-like cathepsin isolated from the hepatopancreas of the white leg shrimp *Litopenaeus vannamei* has a pH optimum of 5.1 (Le Boulay et al., 1996), and that of the brine shrimp *Artemia franciscana* is 5.0–6.5 (Warner et al., 2004). In the common lobster *Homarus gammarus*, one of the peaks of proteolytic activity is at 5.8–6.0 (Glass et al., 1989; Navarrete del Toro et al., 2006). Since different cathepsins have the various characteristics, including pH optimums, different enzymes could function at the pH values studied. At pH 3.0 it can be the cathepsins A, D and E, at pH 5.0 – the cathepsins C and D, at pH 7.0 – the cathepsins B and L (Ashie & Simpson, 1997).

In addition, the associated microbiota, whose pH optimum is 8.0 (Kuz'mina et al., 2017), may affect the temperature characteristics of peptidases of chironomid larvae. At pH 3.0, the activity of the studied peptidases in the tissues of chironomid larvae is determined by cathepsins, the pH optimum of which in invertebrates usually ranges from 2.8 to 4.0 (Gildberg, 1988). In this pH zone, cathepsins D, E and A may function. In the study of the common lobster *Homarus gammarus*, the most significant activity of peptidases was found in the pH 2.5–3.0 zone (Glass et al., 1989; Navarrete del Toro et al., 2006).

The data on the temperature coefficients of peptidases in the tissues of chironomid larvae confirm the classical idea of a sharp decrease in Q_{10} values in the postmaximal temperature zone as a result of denaturation of protein globules of enzymes. In the study of E_{act} of trypsin-like proteinases in crabs, some values similar to ours were obtained: in the tropical hermit crab *Clibanarius striolatus* in the temperature range of 0–40 °C, the E_{act} is 7.64 kcal/mol. In the brown crab *Cancer pagurus*, inhabiting temperate latitudes, it is 5.92 kcal/mol (Dittrich, 1992). The E_{act} of serine peptidases (the casein substrate) of the whole larvae of the midge *Chaoborus* sp. and the buzzer midge *Chironomus plumosus*

in the temperature range of 0–20 °C is high (19.3 and 20.0 kcal/mol), whereas at temperatures above 20 °C, it is considerably lower (5.6 and 11.9 kcal/mol) (Kuz'mina, 1999).

The obtained results indicate a considerable efficiency of the hydrolysis of protein components of the tissues of chironomid larvae in the temperature range most favourable for their vital activity (20–30 °C) at pH 7.0 on casein and pH 5.0 on hemoglobin. However, the pH does not have a significant effect on the temperature dependence of hemoglobin-lytic peptidases. In the hydrolysis of hemoglobin by enzymes of the intestinal mucosa in fish feeding on chironomid larvae, in most cases the bend on the Arrhenius plot is noted at 10 °C. After the point of break, the E_{act} values of peptidases of the mucosa are, as a rule, approximately 1.3 times lower than those in the low temperature zone (Shalygin, 2013). Since benthos- and plankton-eaters practically do not feed at temperatures lower than 10 °C, these data support the idea of sufficient adaptation of the enzymes of both to functioning at temperatures of active fish feeding. The comparison of the activity and temperature characteristics of peptidases of chironomid larvae with those of the digestive tract of various fish species has confirmed the important role of enzymes of their food objects in the processes of digestion of consumers.

Conclusion

The activity of peptidases that function in the tissues of chironomid larvae, food objects of adult benthophages and juvenile fish of various ecological groups, depends to a considerable extent on temperature and pH. However, pH does not have a significant effect on the temperature dependence of casein- and hemoglobin-lytic peptidases. In both cases, the temperature induced the greatest effect on peptidase activity. The temperature optimum of the studied peptidases of chironomid larvae corresponds to 40 °C. The Q_{10} values in the zone of vital temperatures are slightly changed. They are, as a rule, increased in the zone of 30–40 °C, and are sharply decreased in the zone of high temperatures. The values of activation energy of the process of hydrolysis of casein and hemoglobin in the zone of low and high temperatures are different. The E_{act} value at a temperature not exceeding 20 °C is usually below that in the zone of higher temperatures (except for hemoglobin-lytic peptidases at pH 5.0). The data obtained confirmed that the characteristics of casein- and hemoglobin-lytic peptidases in chironomid larvae at various temperatures and pH can influence the digestive processes in benthophages and the fry of all fish species.

The authors would like to thank Mr. Y. Jitari for his exceptional linguistic help. This research was performed in the framework of the state assignment of FASO Russia (theme No. AAAA-A18-118012690102-9).

References

- Anson, M. (1938). The estimation of pepsin, trypsin, papain and cathepsin with haemoglobin. *Journal of General Physiology*, 22, 79–83.
- Aoki, H., Ahsan, M. N., & Watabe, S. (2003). Molecular cloning and characterization of cathepsin B from the hepatopancreas of northern shrimp *Pandalus borealis*. *Comparative Biochemistry and Physiology*, 134B, 681–694.
- Ashie, I. N. A., & Simpson, B. K. (1997). Proteolysis in food myosistem – a review. *Journal of Food Biochemistry*, 21, 91–123.
- Boetius, A., & Felbeck, H. (1995). Digestive enzymes in marine invertebrates from hydrothermal vents and other reducing environments. *Marine Biology*, Berlin, Heidelberg, 122(1), 105–113.
- Butler, A. M., Aiton, A. L., & Wamer, A. H. (2001). Characterization of a novel heterodimeric cathepsin L-like protease and cDNA encoding the catalytic subunit of the protease in embryos of *Artemia franciscana*. *Biochemistry and Cell Biology*, 79, 43–56.
- Dabrowski, K., & Glogowski, J. (1977). The role of exogenic proteolytic enzymes in digestion processes in fish. *Hydrobiologia* (Hagua), 54, 129–134.
- Dendinger, J. E. (1987). Digestive proteases in the midgut gland of the Atlantic blue crab, *Callinectes sapidus*. *Comparative Biochemistry and Physiology*, 88B(2), 503–516.
- Diaz-Tenorio, L. M., Garcia-Carreño, F. L., & Navarrete del Toro, M. Á. (2006). Characterization and comparison of digestive proteinases of the Cortez swim-

- ming crab, *Callinectes bellicosus*, and the arched swimming crab, *Callinectes arcuatus*. *Invertebrate Biology*, 125(2), 125–135.
- Dittrich, B. (1990). Temperature dependence of the activities of trypsin-like proteases in decapod crustaceans from different habitats. *Naturwissenschaften*, 77, 491–492.
- Dittrich, B. (1992). Comparative studies on the thermal properties of a trypsin-like protease in two hermit crabs. *Helgoländer Meeresuntersuchungen. Helgoländer Meeresunters.*, 46, 45–52.
- Dixon, M., & Webb, E. C. (1964). *Enzymes*, 2nd ed. Longmans, Green & Co., London, New York.
- Duan, Y. F., Liu, P., Li, J. T., Li, J., Gao, B. Q., & Chen, P. (2013). Cloning and expression analysis of cathepsin L cDNA of *Exopalaemon carinicauda*. *Dongwuxue Yanjiu*, 34(1), 39–46.
- Egorova, V. V., Izuitova, N. N., Timofeeva, N. M., Tulyaganova, E. K., Guman, E. G., Shcherbakov, G. G., & Ugolev, A. M. (1974). Nekotoryye temperaturnye kharakteristiki i temperaturumye adaptatsii fermentov, obespechivayushchikh membrannoye pishchevarennye u poykilotermnykh i gomoyotermnykh zhivotnykh [Some temperature characteristics and temperature adaptations of enzymes that provide membrane digestion in poikilothermic and homeothermic animals]. *Journal of Evolutionary Biochemistry and Physiology*, 10(3), 223–231 (in Russian).
- Fernández-Giménez, A. V., García-Carreño, F. L., Navarrete del Toro, M. A., & Fenucci, J. L. (2001). Digestive proteinases of red shrimp *Pleoticus muelleri* (Decapoda, Penaeoidea): Partial characterization and relationship with molting. *Comparative Biochemistry and Physiology*, 130B, 331–338.
- Gerasimov, U. V. (Ed.). (2015). *Ryba Rybinskogo vodokhranilishcha: Dinamika chislennosti naseleniya i ekologiya* [Fish of the Rybinsk Reservoir: Population dynamics and ecology]. Filigran', Yaroslavl (in Russian).
- Gildberg, A. (1988). Aspartic proteinases in fishes and aquatic invertebrates. *Comparative Biochemistry and Physiology*, 91B, 425–435.
- Glass, H. J., MacDonald, N. L., Moran, R. M., & Stark, J. R. (1989). Digestion of protein in different marine species. *Comparative Biochemistry and Physiology*, 94B, 607–611.
- Gerking, S. D. (1994). *Feeding ecology of fish*. Academic Press, San-Diego.
- Hemici, A., Benerbahai, R. S., & Bendjedou, D. (2017). Purification and biochemical characterization of a 22-kDa stable cysteine-like protease from the excretory-secretory product of the liver fluke *Fasciola hepatica* by using conventional techniques. *Journal of Chromatography, B. Analytical Technologies in the Biomedical and Life Sciences Journal*, 1068–1069, 268–276.
- Hu, K.-J., & Leung, P.-C. (2007). Food digestion by cathepsin L and digestion-related rapid cell differentiation in shrimp hepatopancreas. *Comparative Biochemistry and Physiology*, 146B, 69–80.
- Kim, H. R., Meyers, S. P., Pyeun, J. H., & Godber, J. S. (1994). Enzymatic properties of anionic trypsins from the hepatopancreas of crayfish, *Procambarus clarkii*. *Comparative Biochemistry and Physiology*, 107B, 197–203.
- Kumar, S., Srivastava, A., & Chakrabarti, R. (2005). Study of digestive proteinases and proteinase inhibitors of *Daphnia carinata*. *Aquaculture*, 243, 367–372.
- Kuz'mina, V. V. (1999). Vliyaniye temperatury na pishchevaritel'nyye gidrolazy vodnykh bespozvonochnykh [Influence of temperature on digestive hydrolases in water invertebrates]. *Journal of Evolutionary Biochemistry and Physiology*, 35(1), 15–19 (in Russian).
- Kuz'mina, V. V. (2008). Classical and modern conceptions of fish digestion. In: Cyrino, J. E. P., Bureau, D. P., & Kapoor, B. G. (Eds.). *Feeding and digestive functions in fishes*. Ch. 4. Science Publications, Enfield (NF), Jersey, Plymouth. Pp. 85–154.
- Kuz'mina, V. (2017). *Digestion in fish. A new view*. Lambert Academic Publishing, Balti.
- Kuz'mina, V. V., Zolotareva, G. V., & Sheptitskiy, V. A. (2017). Proteolytic activity in some freshwater animals and associated microbiota in a wide pH range. *Fish Physiology and Biochemistry*, 43(2), 373–383.
- Kuz'mina, V. V., Skvortsova, E. G., Shalygin, M. V., & Kovalenko, K. (2015). Role of peptidases of the enteral microbiota and preys in temperature adaptations of the digestive system in planktivorous and benthivorous fish. *Fish Physiology and Biochemistry*, 41(6), 1359–1368.
- Lauff, M., & Hofer, R. (1984). Proteolytic enzymes in fish development and the importance of dietary enzymes. *Aquaculture*, 37(4), 335–346.
- Le Boulay, C., Van Wormhoudt, A., & Sellos, D. (1996). Cloning and expression of cathepsin L-like proteinases in the hepatopancreas of the shrimp *Penaeus vannamei* during the intermolt cycle. *Journal of Comparative Physiology*, 166B(5), 310–318.
- Li, X., Meng, X., Kong, J., Luo, K., Luan, S., Cao, B., Liu, N., Pang, J., & Shi, X. (2013). Molecular cloning and characterization of a cathepsin B gene from the Chinese shrimp *Fenneropenaeus chinensis*. *Fish and Shellfish Immunology*, 35(5), 1604–1612.
- Munilla-Moran, R., Stark, J. R., & Babour, A. (1990). The role of exogenous enzymes in digestion in cultured turbot larvae (*Scophthalmus maximus* L.). *Aquaculture*, 88, 337–350.
- Navarrete del Toro, M. A., García-Carreño, F. L., Díaz, L. M., Celis-Guerrero, L., & Saborowski, R. (2006). Aspartic proteinases in the digestive tract of marine decapod crustaceans. *Journal of Experimental Zoology*, 305A, 645–654.
- Oh, E.-S., Kim, D.-S., Kim, J. H., & Kim, H.-R. (2000). Enzymatic properties of a protease from the hepatopancreas of shrimp, *Penaeus orientalis*. *Journal of Food Biochemistry*, 24, 251–264.
- Qiu, L., Jiang, S., Huang, J., Wang, W., Zhang, D., Wu, Q., & Yang, K. (2008). Molecular cloning and mRNA expression of cathepsin C gene in black tiger shrimp (*Penaeus monodon*). *Comparative Biochemistry*, 150A(3), 320–325.
- Reid, R. G. B., & Rauchert, K. (1972). Protein digestion in members of the genus *Macoma* (Mollusca: Bivalvia). *Comparative Biochemistry and Physiology*, 41A, 887–895.
- Reid, R. G. B., & Rauchert, K. (1976). Catheptic endopeptidases and protein digestion in the horse clam *Tresus capax* (Gould). *Comparative Biochemistry and Physiology*, 54, 467–472.
- Sakai, J., Sakaguchi, Y., & Matsumoto, J. J. (1981). Acid proteinase activity of squid mantle muscle: Some properties. *Comparative Biochemistry and Physiology*, 70, 791–794.
- Serviere-Zaragoza, E., Navarrete del Toro, M. A., & Garcia-Carreño, F. L. (1997). Protein hydrolyzing enzymes in the digestive systems of the adult Mexican blue abalone, *Haliotis fulgens* (Gastropoda). *Aquaculture*, 157, 323–332.
- Skvortsova, E. G., Egorova, A. A., & Kuz'mina, V. V. (2016). Vliyaniye temperatury i kislotnosti sredy na aktivnost' peptidaz u lichinok khironomid – potentsial ob'yektov pitaniya ryb-bentofagov [Influence of the temperature and acidity of the medium on the activity of peptidases in larvae of chironomids – potential feeding objects of benthophagous]. *Problems of Biology of Productive Animals*, 4, 46–56 (in Russian).
- Sriket, C., Benjakul, S., & Visessanguan, W. (2011). Characterisation of proteolytic enzymes from muscle and hepatopancreas of fresh water prawn (*Macrobrachium rosenbergii*). *Journal of the Science of Food and Agriculture*, 91(1), 52–59.
- Stephens, A., Rojo, L., Araujo-Bernal, S., Garcia-Carreño, F., & Muhlia-Almazan, A. (2012). Cathepsin B from the white shrimp *Litopenaeus vannamei*: cDNA sequence analysis, tissues-specific expression and biological activity. *Comparative Biochemistry and Physiology*, 161B(1), 32–40.
- Teschke, M., & Saborowski, R. (2005). Cysteine proteinases substitute for serine proteinases in the midgut glands of *Crangon crangon* and *Crangon allmani* (Decapoda: Caridea). *Journal of Experimental Marine Biology and Ecology*, 316, 213–229.
- Van Wormhoudt, A., Sellos, D., Donval, A., Plaire-Goux, S., & Le Moullac, G. (1995). Chymotrypsin gene expression during the intermolt cycle in the shrimp *Penaeus vannamei* (Crustacea; Decapoda). *Experientia*, 51(2), 159–163.
- Vega-Villasante, F., Nolasco, H., & Civera, R. (1995). The digestive enzymes of the Pacific brown shrimp *Penaeus californiensis*. II. Properties of protease activity in whole digestive tract. *Comparative Biochemistry and Physiology*, 112B, 123–129.
- Wang, S., Shi, L. J., Liu, N., Chen, A. J., Zhao, X. F., & Wang, J. X. (2012). Involvement of *Fenneropenaeus chinensis* cathepsin C in antiviral immunity. *Fish and Shellfish Immunology*, 33(4), 821–828.
- Wamer, A. H., Pullumbi, E., Amons, R., & Liu, L. (2004). Characterization of a cathepsin L-associated protein in *Artemia* and its relationship to the FAS-I family of cell adhesion proteins. *European Journal of Biochemistry*, 271, 4014–4025.
- Zhao, W., Chen, L., Zhang, F., Wu, P., Li, E., & Qin, J. (2013). Molecular characterization of cathepsin L cDNA and its expression during oogenesis and embryogenesis in the oriental river prawn *Macrobrachium nipponense* (Palaemonidae). *Genetics and Molecular Research*, 12(4), 5215–5225.