EXPERIMENTAL WORKS

UDC 594.3:[577.151.64+577.152.3] doi: http://dx.doi.org/10.15407/ubj88.03.005

HYDROLYTIC ENZYMES EXPRESSIVITY IN DIFFERENT PARTS OF THE *RAPANA* DIGESTIVE SYSTEM

V. A. TOPTIKOV, V. N. TOTSKY, T. G. ALIEKSIEIEVA, O. A. KOVTUN

Odesa National Mechnykov University, Ukraine;
e-mail: wat.22@mail.ru

The relevance of comprehensive studies of the Rapana vital functions is determined by its considerable negative impact on the ecosystem of the Black Sea. The aim of the work was to find out the polymorphism and activity of the main hydrolases in the different parts of the digestive system of Rapana. Hydrolases (proteases, amylases, esterases, lipases and phosphatases) in glandular structures of the Rapana digestive system were studied by electrophoresis. It was found that different sets of hydrolytic enzymes are functioning in certain parts of the Rapana digestive tract. The gland of Leiblein and hepatopancreas played the most important role in the digestion of food components. The salivary glands had the significant influence on proteolysis.

K e y w o r d s: Rapana venosa, digestive system, proteinases, amylases, esterases, lipases, phosphatases, expressivity of enzymes.

Adequate monitoring of the environment requires comprehensive studies of lifestyles of all its inhabitants. Currently *Rapana venosa* (Valenciennes, 1846) (*Gastropoda: Neogastropoda: Muricidae*) makes a great contribution to the ecology of the Black Sea. This East Asian predatory mollusk appeared in the Black Sea in 1947 [1]. Due to the high adaptability of this species and the absence of enemies (like starfish), constraining its population at home [2-6], as well as good food base [3, 7-10], rapa whelk has successfully taken root in a new habitat. This mollusk took only a decade before the end of the 60's of the twentieth century to resettle across the Black Sea [3, 11]. Extreme adaptability and plasticity of *Rapana* are proved by its resettlement in different regions of the World Ocean [12]. Rapa whelk has almost completely destroyed natural populations of oysters and substantially reduced the number of mussels settlements in many regions of the Black Sea.

It is known that the meat of *Rapana's* main prey sources (oysters and mussels) in the Black Sea contains all the essential food components – proteins, fats and carbohydrates [13]. Therefore, for the effective and proper digestion in Rapa whelk there must be an adequate set of hydrolytic enzymes. *Rapana's* morphology, anatomy and especially ontogeny have been studied extensively [12], but the number of works on physiology, biochemistry and genetics of this mollusk is extremely small. Mainly these works are devoted to the determination of chemical composition of the mollusk body and calculation of dry matter, glycogen and energy accumulation. A few studies are focused on aspects of *Rapana's* phylogenetics [14-16]. At present, the localization of basic chemicals in the tissues of shellfish has been well detailed in literature and some common morphological and physiological features of various organs and systems of rapa whelk functioning have been identified [5, 17-19]. However, most of genetic and biochemical aspects of these systems, including digestive system, have practically not been studied. In this regard and taking into account the importance of the role of rapa whelk in modern Black Sea ecology, the goal of the present work is to study polymorphism and expression patterns of major hydrolases in different parts of *Rapana's* digestive system.

Materials and Methods

Collection and treatment of molluscs. Samples of *Rapana* were collected manually by divers near Small Fontain in the Odessa Bay 50 meters away from the shore at a depth of 5-7 m. Rapa whelks were collected in August, September and early October 2012, in five independent instances. Collected mollusks were immediately frozen at -28 °C and kept in this state until analysis. Mature specimens of shell height from 65 to 85 were selected for the study. Soft body of rapa whelk was dissected after thawing slightly. The following parts of the digestive system were collected for analysis (Fig. 1): salivary (pharyngeal) glands, accessory salivary glands, Leiblein body (pear-shaped organ) together with the adjacent portion of esophagus, crimson gland together with the adjacent portion of esophagus, gland of Leiblein, hepatopancreas (digestive gland, liver). The cumulated material of the same organ from 6-10 male and female individuals, taken in equal proportions was used for analyses.

Sample preparation, electrophoresis. Tissues were homogenized in extraction buffer of the following composition: 0.05 M Tris-HCl (pH 6.8), 0.01% dithiothreitol, 0.01% ascorbic acid, 0.01% sodium EDTA, 1% Triton X-100, with the ratio of tissue : buffer (weight, mg : volume, μl) being 1 : 1. Samples triturated directly in centrifuge tubes were subjected to freeze-thawing 3-5 times and then centrifuged in the cold (4 °C) for 20 min at 10,000 g. Three volumes of chilled (-28 °C) acetone were added to the supernatant. The protein precipitate was obtained by centrifugation under the aforementioned conditions and treated overnight with butanol cooled at 2-4 °C. After centrifugation, the material was dried for several days at -28 °C and then at room temperature for 2-4 h. The precipitate was dissolved in 1 : 1 (original tissue mg : solution μl) extraction buffer supplemented with 15% sucrose and a small amount of bromophenol blue. The resulting tissue preparations were used for electrophoresis. Vertical native electrophoresis was performed at room temperature in gel plates measuring 130×110×1 mm in apparatus VE-4M (Helicon, Russia). The distribution of proteins was carried out in the system of Davis [20] with or without the use of stacking gel in 10% PAGE (in the study of proteolytic activity) and 7.5% of the gel (the study of other enzymes). Electrophoresis was performed at 15 mA and 110 V until dye front moved from the start to distance of about one third of gel, after which the power and the voltage were increased to 30 mA and 260 V. Electropho-

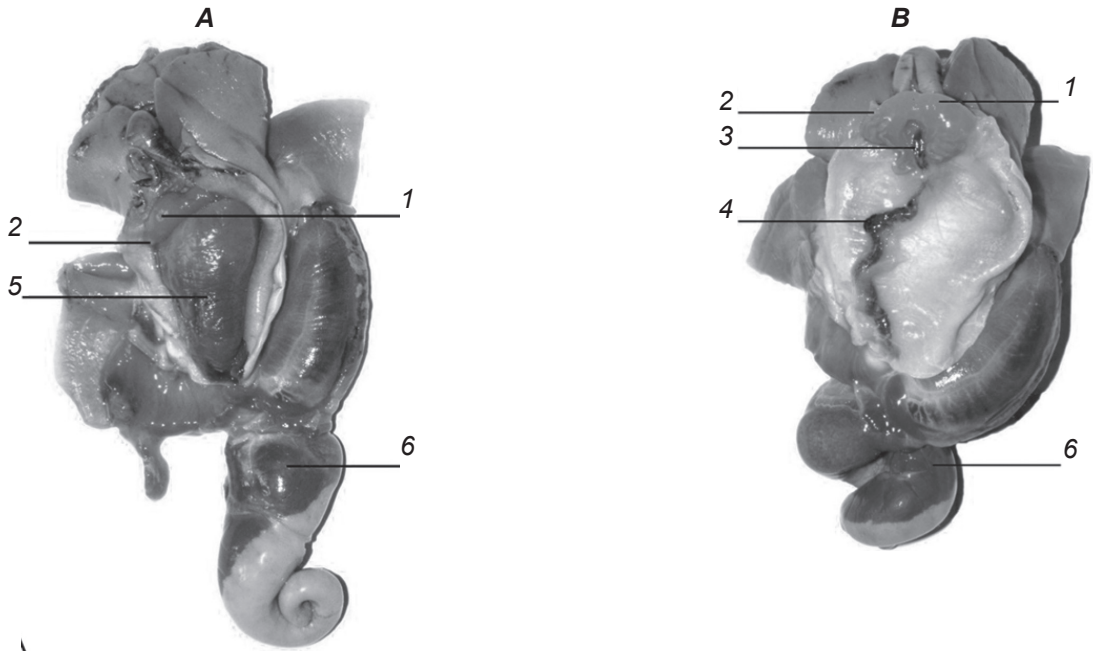

Fig. 1. Portions of the Rapana digestive system, which were used for analysis: 1 –salivary glands, 2 – accessory salivary glands, 3 – Leiblein's body with esophagus, 4 – crimson gland with esophagus, 5 – gland of Leiblein, 5 – hepatopancreas; A – mantle cavity disclosed, mantle diverted to the side, B – gland of Leiblein removed

resis took 4-5 h. Since different tissues had dissimilar enzymatic activity, experimentally determined amounts of starting material were added, which gave the opportunity to detect enzymes in the electrophoregrams even in samples not active enough.

To standardize the data of various biological repetitions, it was decided to use the same conditions of electrophoretic separation, constant values of reagent concentrations, temperature, and gels processing time for the detection of enzymes.

Detection of enzymatic activity. Proteolytic (EC 3.4.21.- – 3.4.24.-) activity was determined by the cleavage of gelatin which was introduced into PAG [21]. Two buffer systems were used: one for the acidic proteinases, 0.1 M sodium acetate buffer pH 4.6, the other for alkaline proteinases, 0.01 M potassium phosphate buffer pH 7.8. Since calcium ions are activators for most of the known proteinases, $CaCl_2$ was added to the medium to the final concentration of 1 mM. After incubation the gels were kept overnight in the solution of 0.1% Coomassie R 250 prepared in the mixture of isopropanol and 9% acetic acid (1 : 1). In the locations of proteinases we observed fading or absence of color: it gives the so-called gelatinase profiles.

To identify the activity of α-amylase (EC 3.2.1.1) the electrophoresis of samples was done in polyacrylamide gel by adding soluble starch to 0.15% [22]. Detection of activity was carried out in two buffer systems - 0.1 M sodium acetate buffer, pH 5.4 or 0.15 M sodium-potassium phosphate buffer pH 7.5. Enzyme activity was determined using solution with acetic acid containing 0.01 M of iodine and 0.014 M KJ. Undyed areas on blue background indicated the presence of amylase activity.

Esterases (EC 3.1.1.-) were manifested in the appropriate buffer solution, which contained the dye Fast blue RR, 1-naphthyl propionate and 2-naphthyl acetate. For esterase activity detection potassium phosphate buffer pH 7.0 or 0.1 M sodium acetate buffer pH 4.6 were used. Esterase zone with specificity for β-isomers of the substrate were dyed red, for α-isomers – brownish-black [22].

It is generally accepted that for the manifestation of amylase activity weak acid medium is optimal, while for esterase activity the medium should be close to neutral. To determine the characteristics of these enzymes in the rapa whelk digestive tract we also used different pH for the activity of enzymes: alkaline – namely slightly alkaline (pH 7.4) medium for the amylase and acidic (pH 4.6) – for esterases.

Lipases (EC 3.1.1.3) were detected on the scheme of Burstone [23] with modifiations. After electrophoresis, the gels were kept at room temperature for 1 h in a potassium phosphate buffer, pH 7.0, which contained 100 ml of Tween-85 (200 μl), sodium desoxycholate (200 mg) and calcium chloride (200 mg). Then the solution was drained and the gel was thoroughly washed with distilled water (3 changes 15 min each). To identify areas of the enzyme, a solution of Nile blue was used. To obtain this solution 20 mg of dye was dissolved in 10 ml of ethanol and topped up with distilled water to a volume of 100 ml. Gels were incubated in a solution of Nile blue overnight and then washed with water until colorless background.

Alkaline phosphatase (EC 3.1.3.1) was detected at pH 8.9 (0.05 M Tris-HCl buffer), acid phosphatase (EC 3.1.3.2) – at pH 4.6 (0.1 M sodium acetate buffer) by the Manchenko [22] with using Fast blue RR and 1-naphthyl phosphate.

Electrophoregrams analysis and mathematical treatment of the data. Electropherograms were documented by scanning. Quantitative analysis of densitograms was obtained through software AnaIs. The number of enzymes multiple forms, their relative electrophoretic mobility and their ratio in percentage in the overall spectrum were determined. Enzyme activity was assessed by the peak area at the densitograms of the corresponding multiple forms, and calculated in conventional units per 1 mg wet tissue. This method does not show the true level of enzyme activity, but it is informative enough for comparative studies. Research results were processed using Microsoft Excel software.

The arithmetic mean of the spectra obtained in each repetition of the experiment and standard error of the arithmetic mean were calculated. The typical spectra that well reflect the obtained average results are shown in this paper. The significance of differences proved in accordance with the Student's *t*-test was calculated for the cosets.

Informational complexity of electrophoretic spectra of the enzymes was evaluated by our proposed factor [24].

Results and Discussion

Analysis of enzymatic activity was performed by means of polyacrylamide gel electrophoresis, which allowed identifying not only the presence of certain enzymes, but also their isozyme composition and activity of some multiple forms.

Typical electrophoregram of proteolytic activity is shown in Fig. 2 (*A*, *B*).

Proteinases were found in all investigated structures of the *Rapana* digestive system. Quantitative treatment showed that the highest proteinase activity for both pH values was observed in the salivary glands. The major part of the proteolytic activity of the *Rapana* digestive system was concentrated in these glands (Fig. 2, *C*, *D*). The presence of proteinases in the salivary glands of *Rapana* had been previously shown by histochemical methods [2], but a comparative analysis of the hydrolytic activity in various glandular structures of the *Rapana* digestive tract was attempted in this study for the first time.

The presence of maximum proteinase activity in the anterior parts of *Rapana* digestive system is a basic difference between these mollusks and vertebrates, in which the process of proteins digestion mainly occurs in the middle part of the digestive system. These features of the proteolytic enzymes distribution in predatory gastropods bring them together with predatory Arthropods, particularly spiders. Besides the poison, they inject into the victim's body a set of hydrolases which help soften the food substrate [25].

The salivary glands are characterized by not only the high level of proteinase activity, but also the largest number of molecular forms of this enzyme group: about 4 molecular forms of proteinase for pH 4.6 were determined and about 10 forms – for pH 7.8. The lowest proteolytic activity and minimum number of isoforms were observed in Leiblein's body and hepatopancreas (3 molecular forms at pH 4.6 and 4 forms at pH 7.8). Various glandular structures of the rapa whelk digestive system also differed in medium pH, in which enzymatic activi-

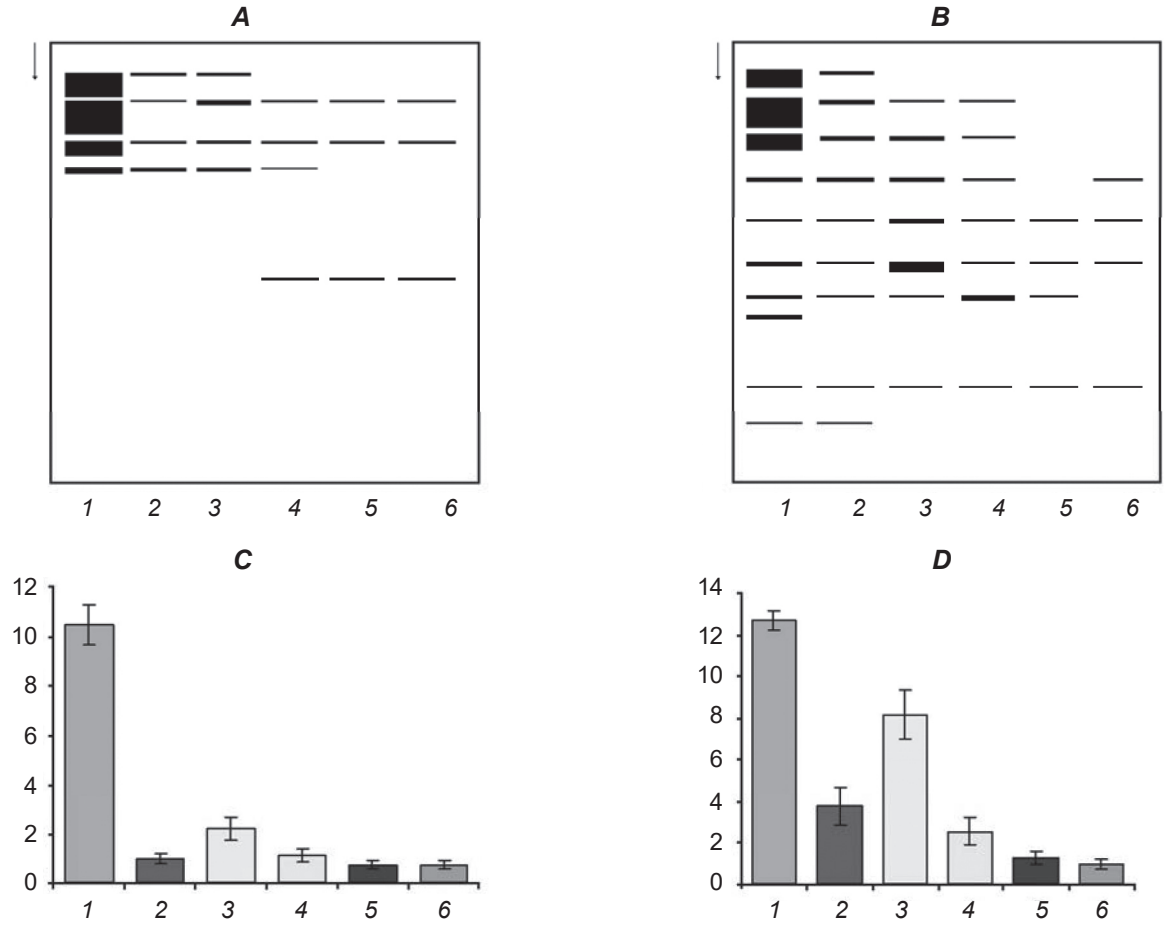

Fig. 2. Gelatinase profiles (A, B) of extracts and proteinase activity (in conventional units per 1 mg wet weight of tissue) (C, D) in the studied parts in digestive system of Rapana: A, C – at pH 4.6, B, D – at pH 7.8; 1 – salivary glands, 2 – accessory salivary glands, 3 – Leiblein's body with esophagus, 4 – crimson gland with esophagus, 5 – gland of Leiblein, 6 – hepatopancreas; the amount of introduced material: 1, 2 – 10 µl of the extract, 3, 4 – 15 µl of extract, 5, 6 – 25 µl of the extract

ty was greater. For example, proteinases of salivary glands showed approximately the same activity in both acidic and alkaline solutions. Proteolytic enzymes of accessory salivary glands, crimson gland and Leiblein gland were active in alkaline medium, and contrary to this, enzymes of Leiblein body and hepatopancreas were active in acidic medium. This is probably motivated by both proteinase form and type of digestion (cisternal for alkaline or acidic for intracellular enzymes).

Based on these data we can assume that in complex and differentiated *Rapana* digestive system hydrolysis of proteins starts outside and in anterior part of the digestive tract in the process of extra-intestinal and cisternal digestion. Processing of proteins in the following segments is likely to occur as a result of cisternal digestion and by intracellular digestion. The fact that acidic proteinases are most active in the salivary glands confirms a general for all living beings pattern, i.e. the initial digestion occurring in acidic medium, whilst subsequent hydrolysis and absorption in neutral [26].

In contrast to proteolytic enzymes amylases were distributed in the parts of the rapa whelk digestive system in a different way (Fig. 3, *A*, *B*).

Activity of α-amylase was detected only in Leiblein gland and hepatopancreas whereas it was practically not observed in other structures. Leiblein gland had maximum amylase activity (Fig. 3, *C*, *D*). Despite differences in activity, the number of isoforms of the enzyme was similar in both glands. As expected, the amylases of *Rapana* digestive system were more active under mildly acidic conditions.

Esterases are a large group of diverse enzymes that break down various esters. Besides digestive functions, esterases perform regulatory (e.g., ace-

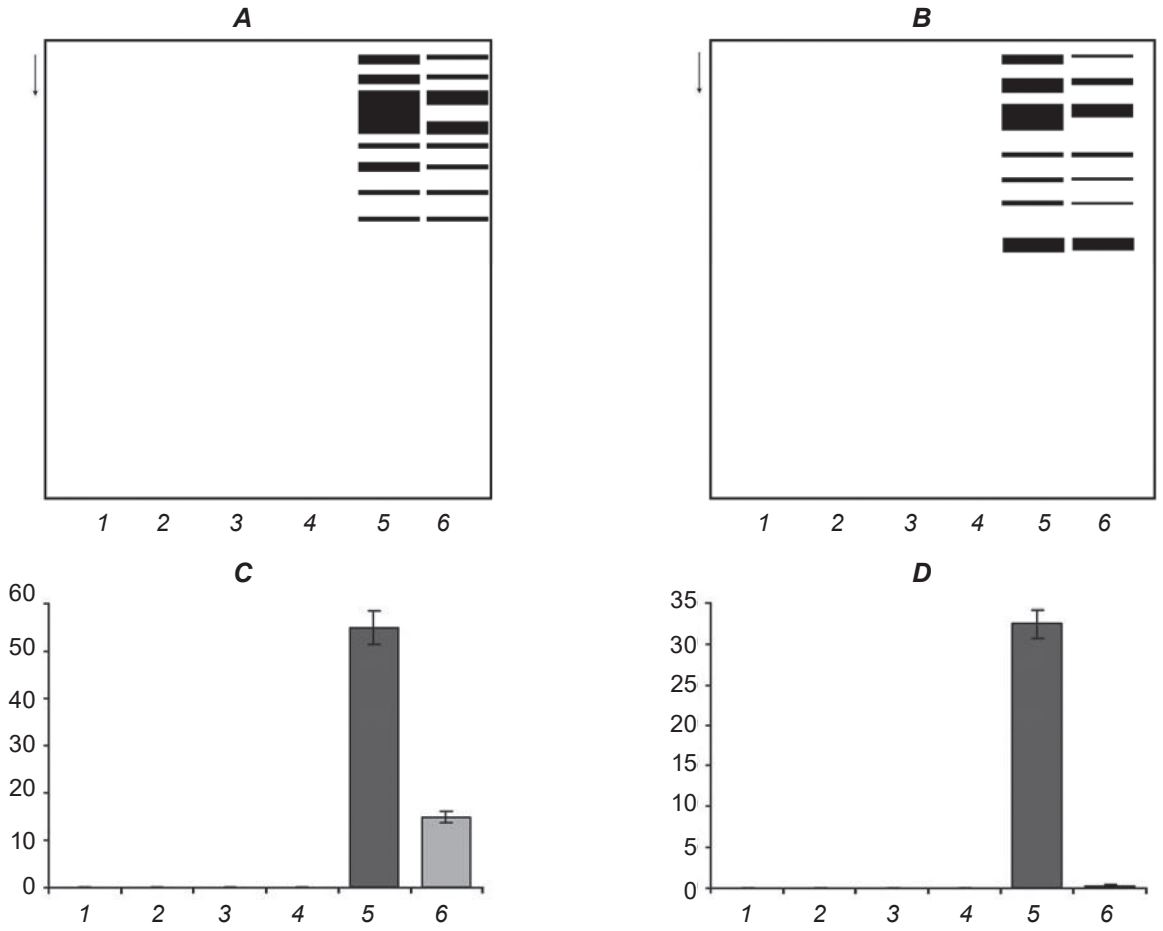

Fig. 3. Electrophoretic profiles of proteins with amylase activity (A, B) and amylase activity (in conventional units per 1 mg wet weight of tissue) (C, D) in the studied parts in digestive system of Rapana: A, C – at pH 5.4, B, D – at pH 7.5; 1 – salivary glands, 2 – accessory salivary glands, 3 – Leiblein's body with esophagus, 4 – crimson gland with esophagus, 5 – gland of Leiblein, 6 – hepatopancreas; the amount of introduced material: 7.5 μl of the extract

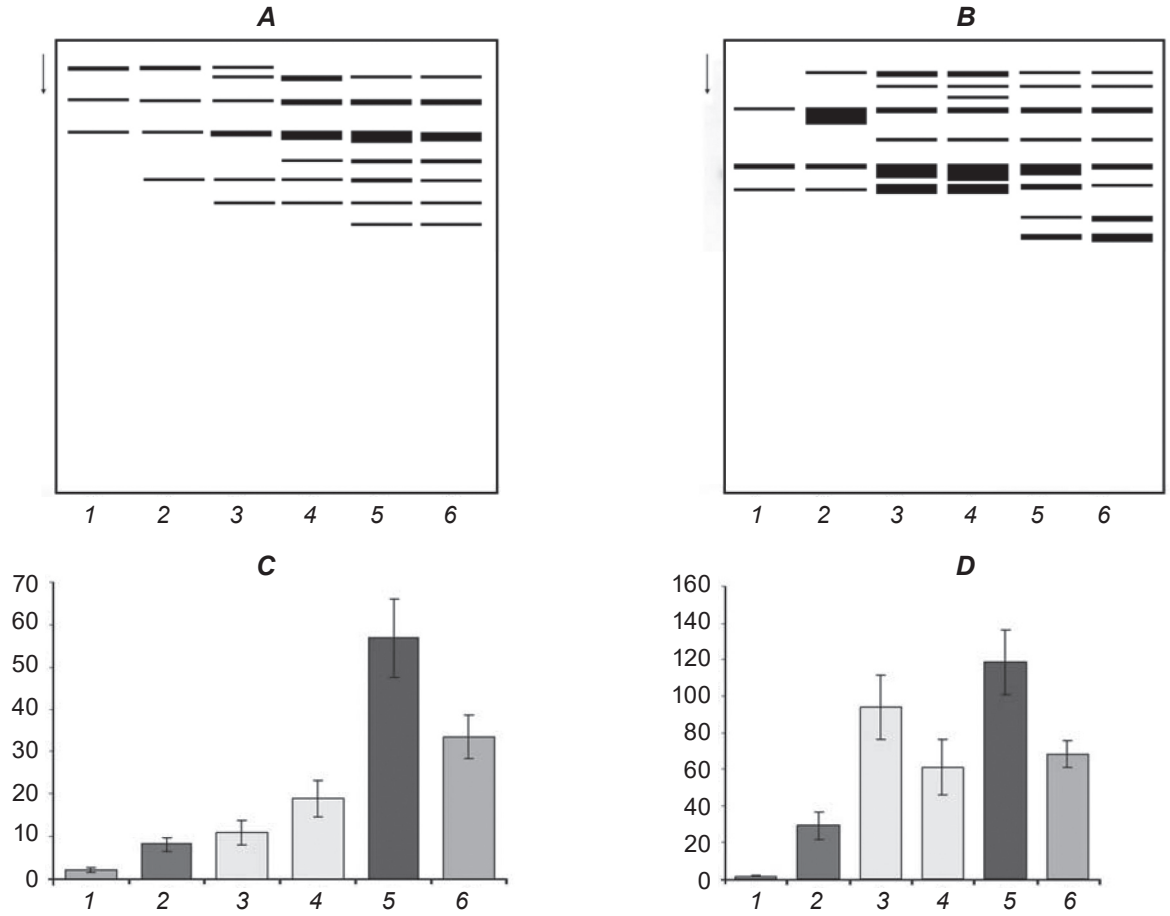

Fig. 4. Electrophoretic profiles of proteins with esterase activity (A, B) and esterase activity (in conventional units per 1 mg wet weight of tissue) (C, D) in the studied parts in digestive system of Rapana: A, C – at pH 4.6, B, D – at pH 7.0; 1 – salivary glands, 2 – accessory salivary glands, 3 – Leiblein's body with esophagus, 4 – crimson gland with esophagus, 5 – gland of Leiblein, 6 – hepatopancreas; the amount of introduced material at pH 4.6: 1 – 20 µl, 2 – 15 µl, 3 – 5 µl, 4 – 10 µl, 5, 6 – 7 µl of the extract, at pH 7.0: 1 – 10 µl, 2, 3, 4 – 5 µl, 5, 6 – 3 µl

tylcholine-acetylhydrolase, or acetylcholinesterase), protective (e.g., acetylcholine-acylhydrolase, or cholinesterase) and various metabolic functions. The important role of this class of enzymes is consistent with the fact that esterases present in all parts of the studied digestive system (Fig. 4, *A*, *B*). For the two values of the medium acidity studied, the highest activity of enzymes with esterase activity was shown in the gland of Leiblein, the lowest – in the salivary glands. In case of esterase activity per 1 mg wet weight of tissue the glandular structure of *Rapana* digestive system under consideration was located in the following order: gland of Leiblein > hepatopancreas ≥ Leiblein's body ≥ crimson gland > accessory salivary glands > salivary gland (Fig. 4, *C*, *D*). Gland of Leiblein, hepatopancreas, Leiblein's body and crimson gland contained the maximum number of esterases isoforms (from 6 to 8). Glands differed by the most favorable for the esterase reaction values of pH: in the salivary glands esterases were active in an acidic environment (pH 4.6), in all other glandular structures the optimal pH was neutral.

There were significant differences in the specificity of esterase isoforms to various substrates between the glandular structures of the rapa whelk digestive system. Salivary glands and accessory salivary glands contained mainly α-targeted enzymes. In gland of Leiblein and hepatopancreas β-specific esterases were largely represented (Fig. 4, *B*). These include multiple forms with electrophoretic mobility *Rf* 0.16, 0.25 and 0.30. Both Leiblein's body and crimson gland isoform with *Rf* 0.20 were characterized by the mixed stereospecificity, while in the gland of Leiblein, salivary glands and hepatopancre-

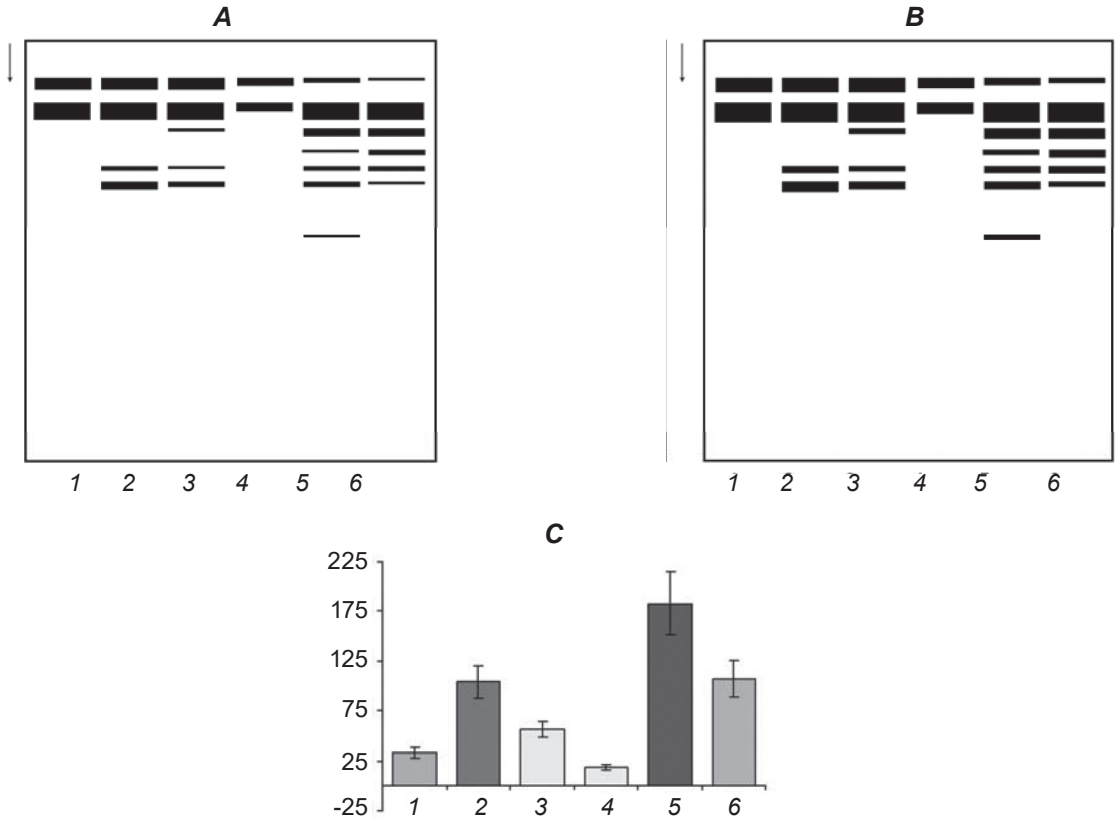

Fig. 5. Electrophoretic profiles of proteins with lipase activity (A, B) and lipase activity (in conventional units per 1 mg wet weight of tissue) (C) in the studied parts in digestive system of Rapana: A – the incubation medium without supplements, B – supplemented with 1 M NaCl before; 1 – salivary glands, 2 – accessory salivary glands, 3 – Leiblein's body with esophagus, 4 – crimson gland with esophagus, 5 – gland of Leiblein, 6 – hepatopancreas; the amount of introduced material: 1 – 10 µl, 2-6 – 5 µl

as this isoform had a higher affinity for substrates with substitution at α-position.

The group of esterases required to digest such important components of food as fats and fatty acids was studied separately. Electrophoretic spectra of lipases are shown in Fig. 5 (*A*, *B*). As in the case of esterase, lipase activity was high in the gland of Leiblein, followed by hepatopancreas and accessory salivary glands (Fig. 5, *C*). The lowest activity of lipases was observed in the crimson gland.

To distinguish triglyceride lipase from the lipoprotein lipases parallel samples were stained with a solution of 1 M NaCl, which is known to selectively inhibit lipoprotein lipases in mammals [27, 28]. Based on the comparison of these two variants of lipase activity manifestations (Fig. 5, *A*, *B*), we can assume that mainly triglyceride lipases were present in the analysed parts of the Rapana digestive system. This conclusion is based on the obvious fact that NaCl did not inhibit lipase activity in experimental conditions.

Phosphatases, like esterases, represent a large group of heterogeneous enzymes – both highly specialized and with a very broad substrate specificity. They play a significant role in the overall metabolism of the cells and have important regulatory and energy functions. Cleaving phosphate residues from a variety of organic phosphate esters, phosphatase promotes assimilation of relevant substances entering the digestive system with food. In the studied tissues of *Rapana* digestive system the ratio of acid and alkaline phosphatases is very different (Fig. 6, *A*, *B*). While acid phosphatases were present in all glandular structures in comparable amounts, alkaline enzymes were mainly observed in the gland of Leiblein and hepatopancreas. In other parts of mollusk digestive system only traces of alkaline phosphatase activity were observed (Fig. 6, *C*, *D*).

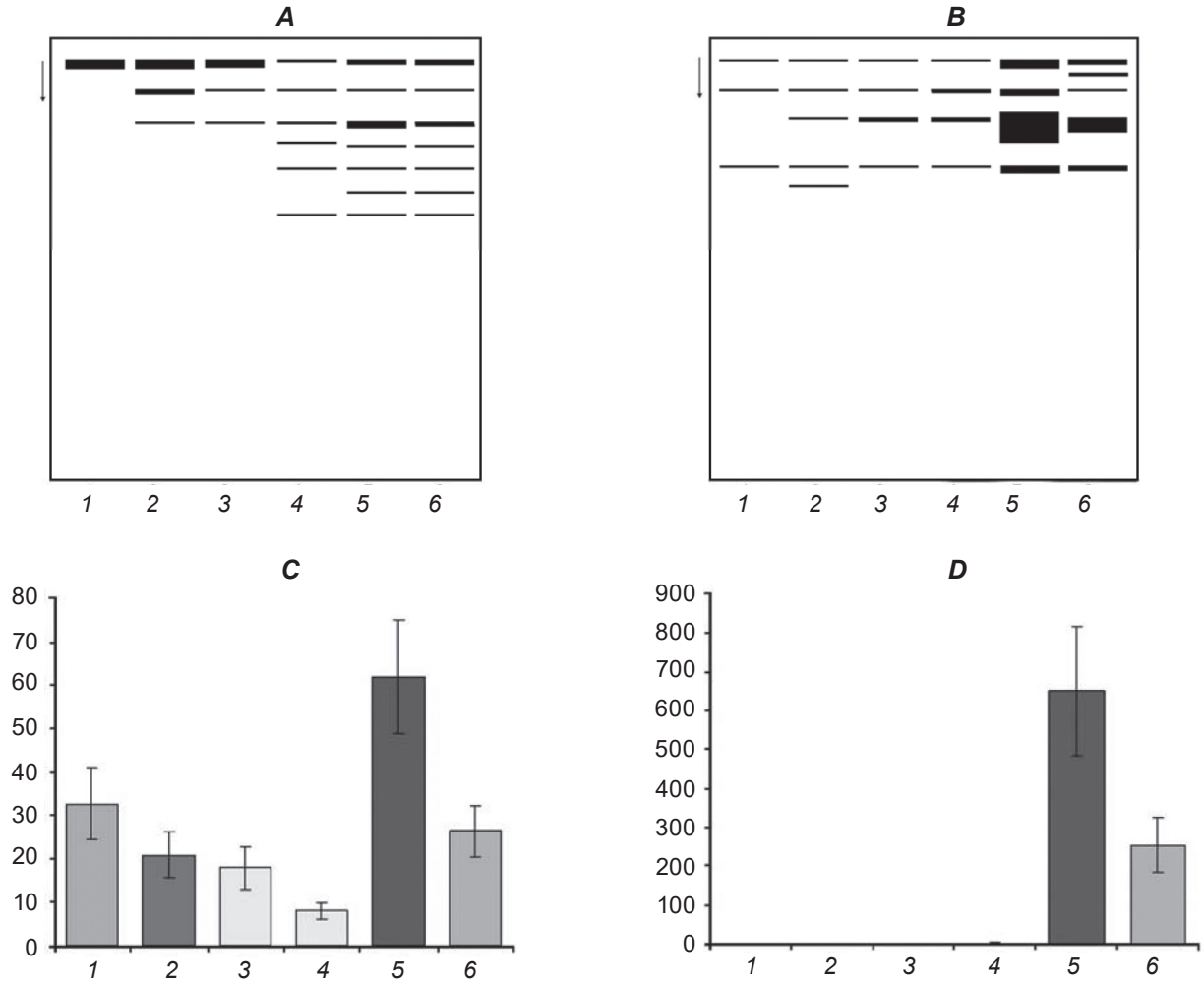

Fig. 6. Electrophoretic profiles of proteins with phosphatase activity (A, B) and phosphatase activity (in conventional units per 1 mg wet weight of tissue) (C, D) in the studied parts in digestive system of Rapana: A, C – at pH 4.6, B, D – at pH 8.9; 1 – salivary glands, 2 – accessory salivary glands, 3 – Leiblein's body with esophagus, 4 – crimson gland with esophagus, 5 – gland of Leiblein, 6 – hepatopancreas; the amount of introduced material at pH 4.6: 1, 2, 4 – 15 µl, 3 – 7.5 µl, 5, 6 – 10 µl of the extract; at pH 8.9: 1, 2, 4 – 15 µl, 3 – 7.5 µl, 5 – 1 µl, 6 – 2 µl of the extract

As it is known, high alkaline phosphatase activity is inherent in organs with significant processes of transport nutrients and secretory activity. This is consistent with the analysis of the gland of Leiblein and hepatopancreas, made on the basis of morphological, functional and histochemical studies, where these organs are treated by providing the mollusk body with products of digestion, as well as carrying out the secretion and excretion of various compounds [2].

Knowing the average weight of the investigated structures, we can estimate their relative contribution to the overall activity of the corresponding hydrolases in all digestive system of *Rapana*. Based on data from the literature [2], *Rapana's* enzymatic activity of the stomach and hindgut can be ignored because of their small values. As resulted from our investigation of 30 rapa whelk specimens the average weight of the studied parts of the digestive system distributed as follows: salivary glands – 301 ± 23 mg, accessory salivary glands– 90 ± 6 mg, Leiblein body with adjoining portion of esophagus – 43 ± 2 mg, crimson gland with adjoining portion of esophagus – 62 ± 4 mg, gland of Leiblein– 2077 ± 148 mg, hepatopancreas – 3695 ± 238 mg.

The calculated results are shown in Table 1.

Table 1. Contribution (%) of different test bodies in general hydrolytic activity of the Rapana digestive system

Hydrolases	The investigated glandular organs of the *Rapana* digestive system					
	salivary glands	accessory salivary glands	Leiblein's body	crimson gland	gland of Leiblein	hepato-pancreas
Acidic proteinases	40	1	1	1	20	36
Alkaline proteinases	36	3	3	1	24	33
Amylases, pH 5.4	0	0	0	0	68	32
Amylases, pH 7.4	0	0	0	0	98	2
Esterases, pH 7.0	< 1	1	1	1	48	49
Esterases, pH 4.6	< 1	< 1	< 1	< 1	48	51
Lipases	1	1	< 1	< 1	48	50
Acidic phosphatases	4	1	< 1	< 1	54	41
Alkaline phosphatases	< 1	< 1	< 1	< 1	59	41

Based on data in Table 1 it can be concluded that all of food components relative to proteolysis gland of Leiblein, hepatopancreas as well as salivary glands play the most important role in the rapa whelk digestion.

As shown in our studies, specific proteolytic activity of the gland of Leiblein and hepatopancreas per unit mass is negligible. However, due to their large mass, the contribution of these glands to the process of proteins assimilation by *Rapana* becomes essential.

As seen from the electrophoregrams, presented in this work, each part of *Rapana* digestive system differs from each other not only in their hydrolases activity, but also in the number of multiple forms of enzymes, their location and the specific gravity of the spectrum. The data are shown in Table 2. On the basis of the obtained data it can be supposed that all the genetic determinants controlling expression of certain isoforms of enzymes do not actively operate in various organs of the *Rapana* digestive system. That is supported by the different number of enzymes multiple forms in the spectra, which were observed in the electrophoretic analysis of hydrolases in the most parts of digestive system (Table 2). However, given the equal number of isoforms, it is difficult to make substantiated conclusions about the features of the genetic control of enzymes expression in individual organs. In these cases, important information can be obtained using the coefficient of internal diversity (informational complexity) of spectra KD [24].

This indicator reflects the complexity of genetic system which determines the formation of the trait. It is considered that complexity of genetic system could be understood as follows: 1) the number and expressivity of structural genes, 2) the number and expression of regulatory genes that control the expression of the structural genes, and 3) the type and degree of interaction of certain genetic system genes. Table 2 shows that in some cases, despite the same number of multiple forms of the enzymes and the similarity of their location in the spectra, which indicates the same structural genes functioning, there is a difference between the values of the coefficient KD. The aforesaid may indicate the differences in the regulation of relevant genetic systems in various organs. Such differences between tissues were observed for the spectra of acidic proteinases, amylases and alkaline phosphatases.

The research indicates that the *Rapana* digestive system is complex and diverse not only morphologically, but also in the biochemical sense. Its different parts synthesize a set of hydrolases, provides efficient digestion of food through various pathways. Intense proteolytic activity of the salivary glands may provide extra-intestinal digestion as well as cisternal proteopepsis together with proteinases of other glands. Amylases from the gland of Leiblein and hepatopancreas contribute to the absorption of

Table 2. Number of isoforms and information complexity of hydrolases spectra of investigated organs in the Rapana digestive system

Hydrolases	The investigated glandular organs					
	salivary glands	accessory salivary glands	Leiblein's body	crimson gland	gland of Leiblein	hepatopancreas
Acidic proteinases	4 (4.20±0.55)†	4 (3.14±0.35)†	4 (4.34±0.41)	4 (6.46±0.67)†	3 (2.03±0.29)	3 (2.25±0.36)
Alkaline proteinases	10 (13.37±3.28)	9 (10.10±2.51)	7 (9.28±2.02)	7 (9.39±1.81)	4 (5.63±0.85)	4 (5.38±0.95)
Amylases, pH 5.4	0 –	0 –	0 –	0 –	8 (12.28±1.60)*	8 (6.72±1.10)*
Amylases, pH 7.4	0 –	0 –	0 –	0 –	7 (8.44±0.83)	7 (9.66±0.69)
Esterases, pH 7.0	3 (2.60±0.42)	4 (2.45±0.20)	6 (3.92±0.31)	6 (3.91±0.29)	7 (11.15±2.00)	7 (9.57±1.61)
Esterases, pH 4.6	3 (1.26±0.29)	4 (3.28±0.39)	6 (5.19±0.68)	7 (6.70±0.48)	8 (14.07±1.08)	8 (13.05±1.84)
Lipases	2 (1.68±0.12)	4 (5.44±0.21)	5 (7.16±0.62)	2 (1.83±0.11)	8 (14.81±1.61)	7 (13.99±0.64)
Acidic phosphatases	1 (0.00)	3 (2.50±0,39)	3 (2.79±0.37)	5 (6.10±0.73)	7 (9.78±0.57)	7 (9.58±0.80)
Alkaline phosphatases	3 (1.01±0.28)	5 (2.21±0.37)**	4 (2.17±0.31)^	4 (2.82±0.47)	4 (5.05±0.65)^	5 (4.00±0.52)**

Note: The top line of each cell of the table shows the number of isoforms in this variant; the bottom line in parentheses are values of the coefficient of internal diversity spectrum KD; †, *, ^, ** – the difference between the corresponding values of KD is reliable.

carbohydrates. Various forms of lipases, esterases and phosphatases from all glandular structures perform the digestion of triglycerides and compounds with ester bonds. Acidic esterases, acidic phosphatases and acidic proteinases may also participate in intracellular digestion. Thus, *Rapana* fully reveals the conveyor principle digestion type (according to I. P. Pavlov), which is understood as a chain of successive stages of grinding (physical breakdown) and food softening, degradation of nutrients, their absorption and transport to other tissues of the body.

The obtained enzyme electropherograms and their analysis make it possible to suggest that the digestive system of *Rapana* is differentiated in genetic terms. During ontogenesis of molluscs individuals the specific systems of hydrolytic enzymes are formed in the different parts of the digestive system.

ЕКСПРЕСИВНІСТЬ ГІДРОЛІТИЧНИХ ЕНЗИМІВ У РІЗНИХ ВІДДІЛАХ ТРАВНОЇ СИСТЕМИ РАПАНИ

В. А. Топтіков, В. М. Тоцький,
Т. Г. Алєксєєва, О. О. Ковтун

Одеський національний університет
імені І. І. Мечникова, Україна;
e-mail: wat.22@mail.ru

Проблема природного та штучного вселення видів у нові ареали завжди актуальна і являє собою великий науковий і практичний інтерес. Чужорідні види можуть бути серйозною загрозою для біорізноманіття, сталого розвитку та охорони довкілля. *Rapana venosa* становить загрозу не тільки для Чорного моря, вона набула статус виду світового розповсюдження. Це вимагає всебічного дослідження особливостей життєдіяльності цього молюска. Більшість генетико-біохімічних аспектів діяльності систем рапани, в тому числі травної, практично не досліджувались. Метою роботи було з'ясувати поліморфізм та особливості експресії основних гідролаз в різних відділах травної системи рапани. Досліджували електрофоретичні спектри протеїназ, амілаз, естераз, ліпаз, фосфатаз у залозистих структурах травної системи рапани. Встановлено, що в окремих відділах травного тракту рапани функціонують різні комбінації гідролітичних ензимів. Найважливішу роль у перетравленні всіх компонентів їжі відіграють лейбленівська залоза і гепатопанкреас, а для розщеплення протеїнів – слинні залози.

Ключові слова: *Rapana venosa*, травна система, протеїнази, амілази, естерази, ліпази, фосфатази, експресивність ензимів.

ЭКСПРЕССИВНОСТЬ ГИДРОЛИТИЧЕСКИХ ЭНЗИМОВ В РАЗНЫХ ОТДЕЛАХ ПИЩЕВАРИТЕЛЬНОЙ СИСТЕМЫ РАПАНЫ

В. А. Топтиков, В. Н. Тоцкий,
Т. Г. Алексеева, О. А. Ковтун

Одесский национальный университет
имени И. И. Мечникова, Украина;
e-mail: wat.22@mail.ru

Проблема естественного и искусственного вселения видов в новые ареалы всегда актуальна и представляет большой научный и практический интерес. Чужеродные виды могут быть серьезной угрозой для биоразнообразия, устойчивого развития и охраны окружающей среды. *Rapana venosa* представляет угрозу не только для Черного моря, она приобрела статус вида мирового распространения, что требует всестороннего исследования особенностей жизнедеятельности этого моллюска. Большинство генетико-биохимических аспектов деятельности систем рапаны, в том числе пищеварительной, практически не исследовались. Целью работы было выяснить полиморфизм и особенности экспрессии основных гидролаз в разных отделах пищеварительной системы рапаны. Исследовали электрофоретические спектры протеиназ, амилаз, эстераз, липаз, фосфатаз в железистых структурах пищеварительной системы рапаны. Установлено, что в отдельных отделах пищеварительного тракта рапаны функционируют различные комбинации гидролитических энзимов. Наиважнейшую роль в переваривании всех компонентов пищи играют лейбленовская железа и гепатопанкреас, а в расщеплении протеинов – слюнные железы.

Ключевые слова: *Rapana venosa*, пищеварительная система, протеиназы, амилазы, эстеразы, липазы, фосфатазы, экспрессивность энзимов.

References

1. Drapkin EI. The new mollusk in the Black Sea. *Priroda*. 1953; (9): 92-95. (In Russian).
2. Chukhchin VD. Functional morphology of Rapana. K.: Naukova Dumka, 1970. 138 p. (In Russian).
3. Zolotarev V. The Black Sea ecosystem changes related to the introduction of new mollusk species. *Marine Ecology.* 1996; 17(1-3): 227-236.
4. Mann R, Harding JM. Salinity tolerance of larval Rapana venosa: implications for dispersal and establishment of an invading predatory gastropod on the North American Atlantic coast. *Biol Bull.* 2003; 204(1): 96-103.
5. Gaevskaya AV. Parasites, diseases and pests of mussels (Mytilus, Mytilidae). II. Mollusca. Sevastopol: EKOSI-Gidrofizika, 2006. 100 p. (In Russian).
6. Zaika V, Sergeeva N, Kolesnikova E. Alien species in bottom macrofauna of the Black Sea: their distribution and influence on benthic communities. *Marine Ecolog J.* 2010; IX(1): 5-7. (In Russian).
7. Chukhchin VD. The ecology of gastropods of the Black Sea. K.: Naukova Dumka, 1984. 176 p. (In Russian).
8. Kantor YuI. Biological and Historical Mysteries of Rapa. *Priroda.* 2003; (5): 32-34. (In Russian).
9. Savini D, Occhipinti-Ambrogi A. Consumption rates and prey preference of the invasive gastropod Rapana venosa in the Northern Adriatic Sea. *Helgol Mar Res.* 2006; 60(2): 153-159.
10. Shadrin NV, Afanasyev TA. Nutrition and the distribution of Rapana venosa (Vallenciennes, 1846) in the waters of Opuksky Reserve (Eastern Crimea, the Black Sea). *Marine Ecolog J.* 2009; 8(2): 24. (In Russian).
11. Snigirov S, Medinets V, Chichkin V, Sylantyev S. Rapa whelk controls demersal community structure off Zmiinyi Island, Black Sea. *Aquatic Invasions.* 2013; 8(3): 289-297.
12. International Council for the Exploration of the Sea. Alien Species Alert: Rapana Venosa (veined whelk). Edited by Roger Mann, Anna Occhipinti, and Juliana M. Harding. ICES Cooperative Research Report, 2004; 264: 14 p.
13. The chemical composition of foods. Bk. 2: Reference table of amino acids, fatty acids, vitamins, macro- and micronutrients, organic acids and carbohydrates. Ed.: Skurikhin IM, Volgarev MN. 2nd ed., Rev. and add. M.: Agropromizdat, 1987. 360 p. (In Russian).
14. Chandler EA, McDowell JR, Graves JE. Genetically monomorphic invasive populations of the rapa whelk, Rapana venosa. *Mol Ecol.* 2008; 17(18): 4079-491.
15. Zou S, Li Q, Kong L. Additional gene data and increased sampling give new insights into the phylogenetic relationships of Neogastropoda, within the caenogastropod phylogenetic framework. *Mol Phylogenet Evol.* 2011; 61(2): 425-435.
16. Zou S, Li Q, Kong L. Multigene barcoding and phylogeny of geographically widespread muricids (Gastropoda: Neogastropoda) along the coast of China. *Mar Biotechnol* (NY). 2012; 14(1): 21-34.
17. Studenikina EI, Pavlenko LN. Reserves and characteristics Rapana thomassiana in the north-eastern part of the Black Sea. Abstracts of the VI All-Russian Conference of commercial invertebrates. M.: VNIRO, 2002. P. 177-188. (In Russian).
18. Mihailov VV, Litvinenko NM. The features of the reserves and distribution of Rapana thomasiana near the Crimean coast of the Kerch Strait. Proc. of Int. Workshop (Murmansk, 19-21 March 2003). Murmansk: Murmansk Marine Biological Institute), 2003. P. 54-56. (In Russian).
19. Saenko EM. Dynamics of biochemical parameters of the Rapana (Rapana thomasiana) tissues in different periods of its annual cycle. *Probl Fisher.* 2008; 9(4(36)): 788-796. (In Russian).
20. Davis BJ. Disc elektrophoresis. II. Method and application to human serum proteins. *Ann N Y Acad Sci.* 1964; 121(2): 404-427.
21. Pena LB, Tomaro ML, Gallego SM. Effect of different metals on protease activity in sunflower cotyledons. *Electronic J Biotechnol.* 2006; 9(3): 258-262.
22. Manchenko GP. Handbook of detection of enzymes on electrophoretic gels. CRC Press LLC, 2003. 592 p.

23. Burstone MS. Enzyme histochemistry and its application in the study of neoplasms. New York–London: Academic Press, 1962. 621 p.
24. Toptikov VA, Diachenko LF, Totsky VM. Estimation of enzyme multiple molecular forms spectra using the index of system diversity level. *Tsitol Genet.* 2010; 44(1): 46-53. (In Russian).
25. Barnes RSK, Calow PP, Olive PJW, Golding DW. The Invertebrates: A New Synthesis. M.: Mir Publishers. 1992. 583 p. (In Russian).
26. Ugolev AM. Natural technologies of biological systems. Leningrad: Nauka, 1987. 317 p. (In Russian).
27. Korn ED. Clearing factor, a heparin-activated lipoprotein lipase. I. isolation and characterization of the enzyme from normal rat hear. *J Biol Chem.* 1955; 215(1): 1-14.
28. Fielding CJ, Fielding PE. Mechanism of salt-mediated inhibition of lipoprotein lipase. *J Lipid Res.* 1976; 17(3): 248-256.

Received 11.01.2016