Since 2005, the VNIRO specialists have been conducting studies of the red king crab transportation. In 2008–2009, when we started our joint work with the Norway King Crab, our studies attained a new level. The Bugøynes complex of basins allows advancing the known techniques of the crab transportation without water.

Development of scientifically founded methods and techniques of artificial reproduction of red king crab in Russia and identification of optimal parameters of its keeping, rearing and long-distance transportation of alive commercial crab in Norway are promising bases for rational commercial use of the red king crab resources both in Norway, and in Russia.

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A METHOD FOR DERIVING HYDROLYSATES FROM FRESHWATER FISHES (RUFF, SMELT, BLEAK) OF POTENTIAL RESOURCE VALUE IN KARELIA

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One of the ways to get unique biologically active substances from aquatic organisms is recycling of wastes from processing of valuable commercial fish and marine invertebrates, as well as of low-value species that constitute a high proportion in the catches. Quite a number of papers devoted to the issue mainly focus on marine species, whereas studies where the raw material is freshwater aquatic organisms are few. The fact however is that in just one commercial fishery lake of Karelia (Syamozero) these fish species (ruff, bleak, smelt, etc.) contribute up to 70% of the catch. Furthermore, the wastes of processing of more valuable fish species (whitefish, vendace, pike-perch, bream, etc.) are hardly used at all, although they may be utilized in the biotechnology of producing various biologically active substances. Depending on the purpose of deriving a certain hydrolysate, one applies different methods of hydrolysis, but the most promising and convenient one is enzymatic hydrolysis performed using preparations with nuclease and proteinase. In this study, we used the preparation derived from the digestive gland of the king crab, which contains oligonucleotides with a molecular weight of 6–68 kDa and is easily soluble, which makes it more readily available for further utilization, and broadens the range of its applications (Mukhin and Novikov, 2001).

Protein preparations in this study were produced from fish species "of potential resource value" from Republic of Karelia waters: smelt – *Osmerus eperlanus eperlanus* (L.), ruff – *Acerina cernia* (L.), bleak – *Alburnus alburnus* (L.).

Minced tissues and organs of the fishes under study were obtained by homogenizing them in the 1:3 ratio in the chosen extraction medium (distilled water) in Potter-Elvehjem homogenizer (1.200 rpm x 3 min). After the homogenate had been refrigerated for 3 hours, filtered through several layers of gauze, and centrifuged (10.000 g x 30 min, K-24), the activity of proteolytic enzymes was determined in the supernatant fluid (Alekseenko, 1968). The activity of the enzymes was expressed in units of optical density of the solutions containing substrate hydrolysis products. The optical density of the solutions was measured spectrophotometrically at 240 – 320 nm.

Multifactor analysis of variance was used to determine how much various factors (temperature, exposure period, presence/absence of the enzyme) influenced the degree of protein hydrolysis (Korosov and Gorbach, 2007).

Temperature influence on the degree of protein hydrolysis. The studies prove temperature has a significant effect on the degree of protein hydrolysis – this factor accounts for 10% of the total variance of the parameter (F = 8.40; Df = 6).

The influence of the temperature on enzymatic hydrolysis is due, on the one hand, to its effect on the enzyme's protein component, as very high temperature would cause protein denaturation and weakening of the catalytic function, and on the other hand, to its impact on the rate of the enzyme-substrate complex formation and on all further stages of the substrate transformation, resulting in intensification of catalysis.

Relationship between the enzyme catalytic activity and the temperature is visualized by a typical curve (Fig. 1). The catalytic activity grows up a certain temperature value (to 50°C on average), the rate of substrate transformation nearly doubling every 10°C. At the same time, the inactivated enzyme amount gradually increases through denaturation of the enzyme protein component. When the temperature rises over 50°C, denaturation of the enzyme protein intensifies abruptly and, although the rate of the substrate transformation reaction keeps growing, the enzyme activity, expressed through the transformed substrate amount, drops.

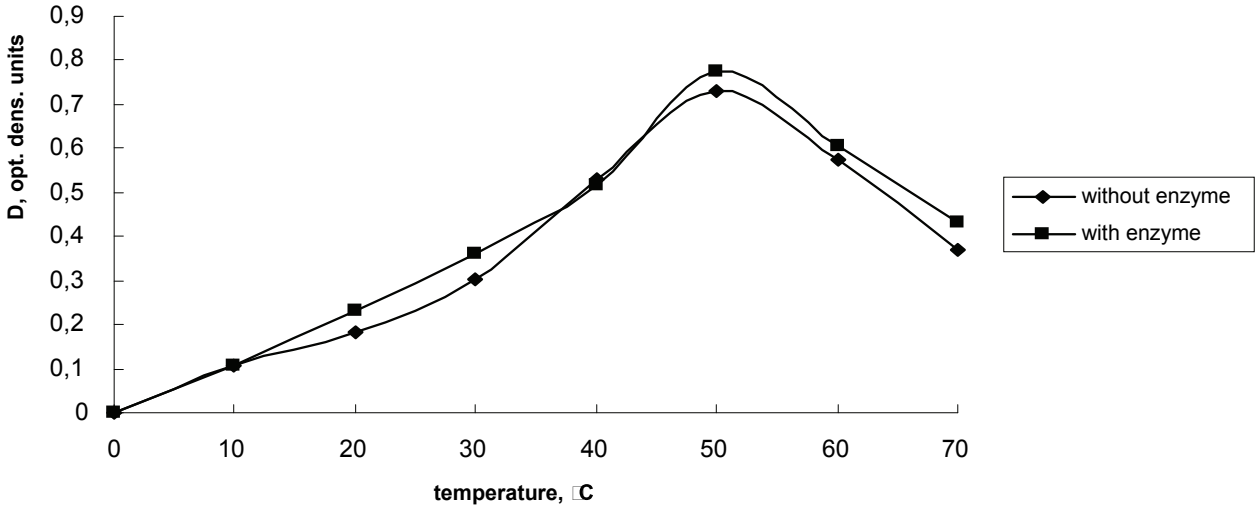

Fig. 1. Temperature dependence of the degree of protein hydrolysis in minced ruff (D_{300})

Data from Figure 1 indicate the degree of protein hydrolysis in ruff homogenates to be maximal at 50°C.

Similar data were gained for minced bleak and smelt.

The studies revealed species-specific distinctions in the degree of protein hydrolysis depending on whether the enzyme was present or absent. Thus, this factor influenced significantly only in hydrolysates from bleak – it accounted for 3% of the total variance of the parameter (F = 3.97; Df = 1).

It was demonstrated that when protein hydrolysis involved proteolytic enzyme the degree of this process in bleak homogenates was 5.5 higher than in the hydrolysis where the proteinase was not added. The absorption of protein hydrolysis products in all study objects was the highest at 300 nm (Fig. 2).

Influence of the exposure period on the degree of protein hydrolysis. No significant effect of the exposure period on the degree of protein hydrolysis was found during the study (Fig. 3).

Like in the experiment with temperature dependence, we detected species-specific distinctions in the degree of protein hydrolysis depending on the presence/absence of the enzyme. Only hydrolysates from bleak were significantly influenced by this factor – it accounted for 15% of the total variance of the parameter (F = 4.11; Df = 1). Addition of the proteolytic enzyme to the procedure of protein hydrolysis in bleak homogenates doubles the rate of the process (Fig. 3).

We also found that absorption of protein hydrolysis products in all the study objects was the highest at 275 nm (Fig. 3).

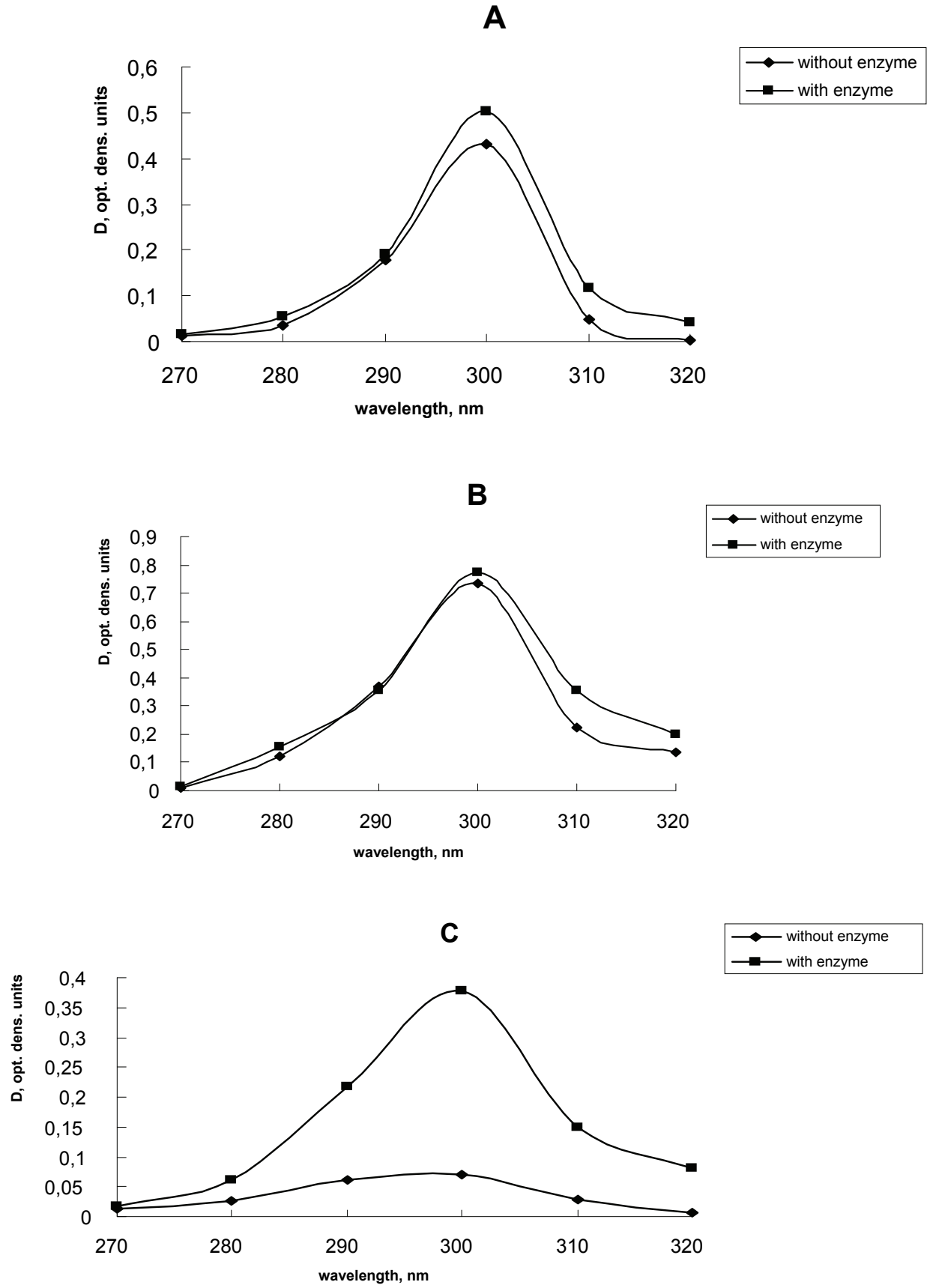

Fig. 2. Dependence of protein hydrolysis on the amount of enzyme added, at constant incubation period (1 h), hydrolysis temperature of 50°C, hydromodulus 1:1 (smelt (A), ruff (B), bleak (C))

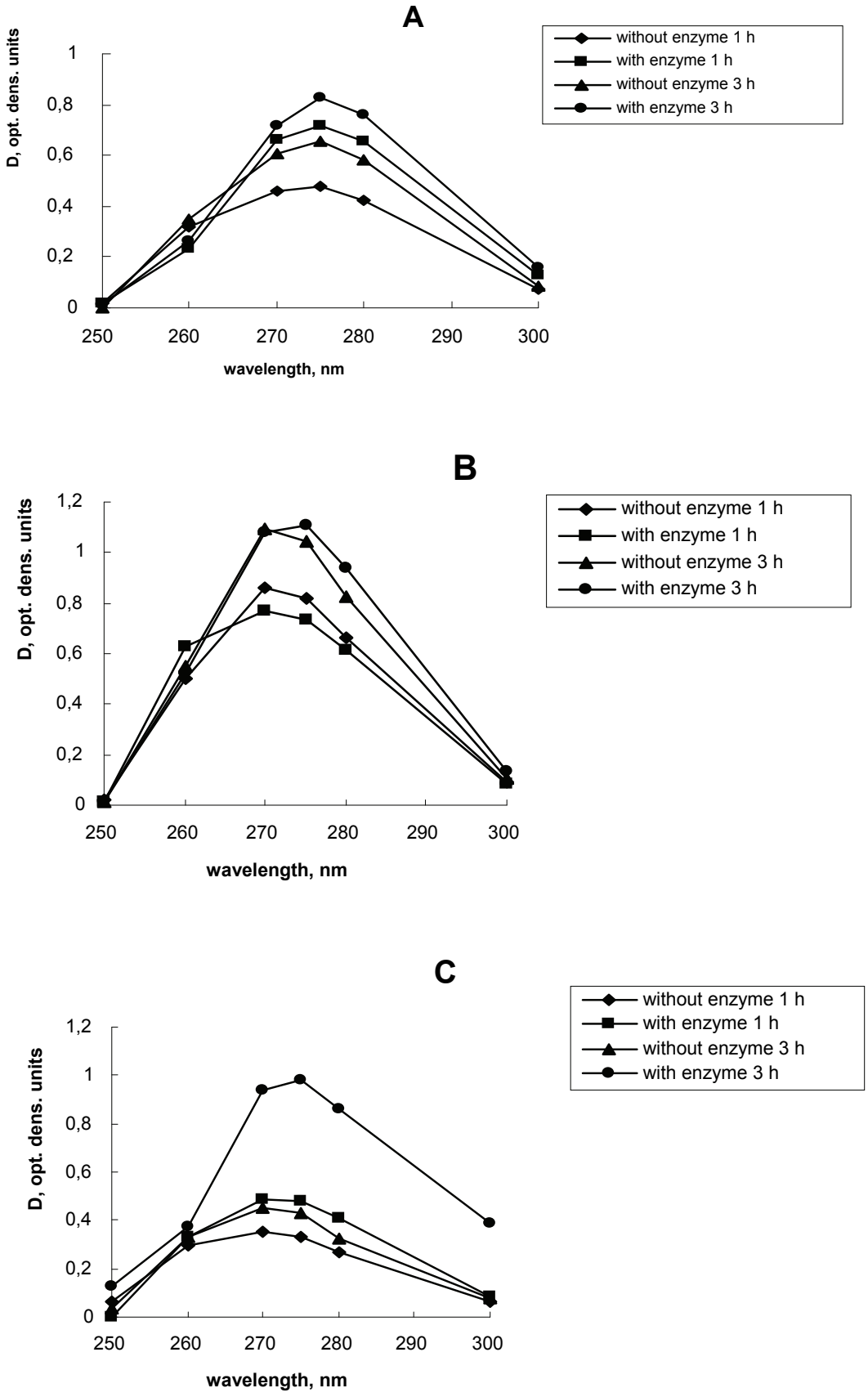

Fig. 3. Effect of the exposure duration and enzyme presence on the degree of protein hydrolysis; smelt (A), ruff (B), bleak (C))

It was thus found that the maximal degree of protein hydrolysis would be reached at the following settings of the enzymatic reaction: temperature 50°C, duration 1 to 3 hours, hydromodulus 1:1.

Absorption of protein hydrolysis products from the low-value freshwater fishes in question (smelt, ruff, bleak) was maximized at 300 nm, whereas earlier results (Mukhin and Novikov, 2001) of selection of optimal conditions for proteolysis in marine invertebrates suggested absorption of protein hydrolysis products peaked at 280 nm. Marine invertebrates are known to have a hypertonic environment (osmotic pressure in their tissues is much lower than the osmotic pressure of sea water) and the range of higher temperatures than our study objects – low-value freshwater fishes, which, on the contrary, feature a hypotonic environment and the range of lower temperatures.

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PROTEINASE COMPLEX FROM FRESHWATER FISHES OF POTENTIAL RESOURCE VALUE IN KARELIA

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People have been aware of nutritional and pharmacologic properties of aquatic organisms since olden times. The uses of biologically active macromolecules derived from aquatic, mainly marine, organisms in human and veterinary medicine, microbiology and various industries, especially in the food and forage industries, are multiple. Freshwater organisms have been less studied in this sense. At the same time, the fish fauna of lakes in Republic of Karelia comprises quite a number of so-called low-value species ("of potential resource value") such as ruff, perch, smelt, stickleback, bleak, which can be viewed as raw material for various biologically active substances, including proteolytic enzymes. Let us stress that available published data on extraction and utilization of proteolytic enzymes from aquatic organisms are mainly concerned with marine organisms (Kuchina et al., 2007; Mukhin and Novikov, 2001; Mukhin, 2003; Stein et al., 2005).

In view of the above, we undertook to separate, purify and describe the proteinase complex from some low-value fishes (smelt – *Osmerus eperlanus eperlanus* (L.), ruff – *Acerina cernia* (L.), bleak – *Alburnus alburnus* (L.) inhabiting waters of Karelia.

Tissues of the organisms in question were homogenized in the 1:2 ratio in the chosen extraction medium (0.1 M KCI) in a MPW-324 homogenizer. After the homogenate had been refrigerated for 3 hours, filtered and centrifuged twice (10,000 g x 30 min, K-24; 30,000 g x 60 min, Optima LE – 80 K), the activity of proteolytic enzymes was determined in the supernatant fluid in the pH range of 2.5 to 8.5. The activity of the enzymes was expressed in units of optical density of the solutions containing the products of hydrolysis of various substrates at 37°C for 1 hour per 1 g of wet weight. The optical density of the solutions was measured spectrophotometrically at 280 nm (protein substrate hydrolysis); at 410 nm (specific elastase substrate hydrolysis); at 525 nm (specific cathepsin B substrate hydrolysis). Proteinase activity was determined following a modified Anson's technique (Alekseenko, 1968): for cathepsin D – through hydrolysis of 1% hemoglobin solution at pH = 3.6; for cathepsin E – through hydrolysis of 1% bovine serum albumin solution at pH = 2.5; for neutral and alkali proteinases – through hydrolysis of 1% casein solution at pH = 7.0 and 8.5, respectively. Cathepsin B activity was determined by decomposition of