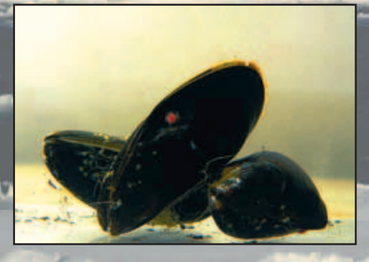

CURRENT PROBLEMS OF PHYSIOLOGY AND BIOCHEMISTRY OF AQUATIC ORGANISMS

VOLUME II

ARCTIC AND SUB-ARCTIC BIOLOGICAL RESOURCES - POTENTIAL FOR BIOTECHNOLOGY

COLLECTED SCIENTIFIC PAPERS OF
THE FIRST INTERNATIONAL SEMINAR
AND PHD WORKSHOP

6-9 SEPTEMBER 2010
PETROZAVODSK, REPUBLIC OF KARELIA, RUSSIA

РОССИЙСКАЯ АКАДЕМИЯ НАУК
Отделение биологических наук РАН
Программа фундаментальных исследований на 2009-2011 гг.:
«Биологические ресурсы России: оценка состояния и фундаментальные основы мониторинга»
Учреждение Российской академии наук
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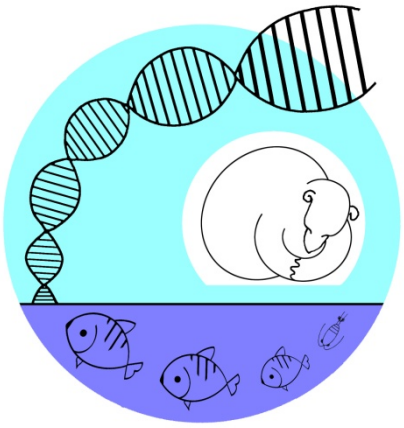
СОВРЕМЕННЫЕ ПРОБЛЕМЫ ФИЗИОЛОГИИ И БИОХИМИИ ВОДНЫХ ОРГАНИЗМОВ

ТОМ II

БИОЛОГИЧЕСКИЕ РЕСУРСЫ АРКТИКИ И СУБАРКТИКИ – ПОТЕНЦИАЛ ДЛЯ БИОТЕХНОЛОГИИ

Сборник научных статей I Международной школы-семинара для молодых ученых
(6–9 сентября 2010 г., Петрозаводск, Республика Карелия, Россия)



Петрозаводск 2010

RUSSIAN ACADEMY OF SCIENCES
The Program “Biological resource of Russia” 2009-2011
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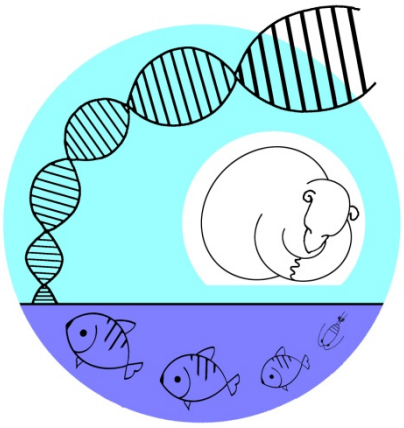

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С 56 **СОВРЕМЕННЫЕ ПРОБЛЕМЫ ФИЗИОЛОГИИ И БИОХИМИИ ВОДНЫХ ОРГАНИЗМОВ**. Том II. Биологические ресурсы Арктики и Субарктики – потенциал для биотехнологии. Сборник научных статей I Международной школы-семинара для молодых ученых (6–9 сентября 2010 г., Петрозаводск, Карелия, Россия). – Петрозаводск: Карельский научный центр РАН, 2010. – 105 с.
ISBN 978-59274-0434-6

В книге представлены статьи участников Международной школы-семинара для молодых ученых «Биологические ресурсы Арктики и Субарктики – потенциал для биотехнологии: исследования и инновации», проведенной Институтом биологии КарНЦ РАН с 6 по 9 сентября 2010 г. Рассмотрены вопросы, связанные с изучением биоресурсов Арктики и Субарктики: биохимические, физиологические и генетические исследования, создание международного банка информации; биотехнологический потенциал белков и пептидов арктических гидробионтов; разработка научных основ и биотехнологий получения продукции пищевого, лечебно-профилактического и медицинского назначения, биологически активных веществ из морских организмов; биотехнология производства кормовых продуктов и биологически активных добавок для объектов аквакультуры и звероводства; исследования по химии и технологии переработки морского и океанического биосырья, разработка безотходных производств переработки водорослей, рыб и морских беспозвоночных.

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CURRENT PROBLEMS OF PHYSIOLOGY AND BIOCHEMISTRY OF AQUATIC ORGANISMS. Volume II. Arctic and Sub-Arctic biological resources – potential for biotechnology: Collected scientific papers of the first International seminar and PhD workshop (6–9 September 2010, Petrozavodsk, Republic of Karelia, Russia). – Petrozavodsk: Karelian Research Centre RAS, 2010. – 105 p.

The edition presented is collected scientific papers of the First International seminar and PhD workshop held on Petrozavodsk, Karelia, Russia in dates 6–9 September 2010. The papers discuss issues of Arctic and sub-Arctic biological resources: biochemical, physiological and genetic researches; biotechnological potential of peptides and proteins of aquatic organisms; development of a scientific and biotechnological basis and technique for making products using in food, medicine, aquaculture, animal breeding and other industries (bioactive substances from aquatic organisms and food-stuff); chemical and technological investigations of marine bio-resources, recycling and development of wasteless-technology.

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CONTENT

QUALITY AND SAFETY OF FLY FISH CAVIAR

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Fish caviar represents not only delicacy, but also a valuable food substance, health-giving and having excellent natural properties. Depending on the fish species, it contains 14–31% of protein, 0.3–15% of oil, and 1.5–2,0% of mineral substances. Fish proteins are almost completely assimilated by a human organism; it is rather unusual for animal proteins. Proteins from the fish caviar contain the full set of nonessential and essential amino acids, biologically active polyunsaturated fatty acids (including essential ones), liposoluble vitamins, and mineral substances, required for the normal metabolism.

The most important fishes, used for the industrial processing of caviar, are sturgeon fishes, which caviar is one of the most health-giving and expensive food products. Its consumption can be considered as a living standard indicator. The caviar of salmon fishes also has a high biological and nutritive value and is a subject of the national pride.

However, other fish species are also used to obtain a valuable caviar production. The output of the caviar of such fishes as codfish, flatfish, mullet, whitefish, carp, perch, ordinary fish, and also nototheniid fishes, whiptail, capelin, sea hen, mackerel, and herring makes thousands of tons.

According to the type of preservation and preparation, the following types of caviar are manufactured: frozen, soft, pasteurized, roe, salted and dried, salted and smoked, etc.

In recent years some products with flying fish caviar (Tobiko), a specific product of the Japan cuisine, appeared on the Russian market. The stock of the flying fish caviar in the World Ocean is very large; only in the Pacific Ocean it makes about 1.5–4 million tons. The flying fish fishery is well developed in some regions, including Japan, the Philippine islands, Indonesia, and Polynesia. In China flying fishes are considered as an anemia-healing product.

The caviar is a traditional sushi component (rolls made from rice, fish, caviar, and other components and wrapped in alga sheets); in addition, it is used in snacks and is prepared in different sauces, imparting unusual smells and tastes.

The technology of the flying fish caviar preparation was developed in Japan more than 500 years ago; this secret was imparted from generation to generation up to our time. The special property of flying fish caviar is the presence of some apophyses on the caviar capsule, which represent thread-like appendices, necessary to fix eggs on plants during the spawning. In many countries people collect flying fish caviar, dry it, and then recover when necessary; this is the main distinction of this technology from other known traditional technologies.

The natural color of this fine crispy caviar is brownish, so it is usually stained in different colors using both natural and artificial staining agents.

Until now flying fish caviar was imported into Russia as the frozen product. However, now the suppliers show interest in the drying-recovery technology of its preparation.

The purpose of our study was to investigate the properties of flying fish caviar for the further development and substantiation of the technology of its preservation, providing the quality and safety of the final product.

The objects of our study were the samples of frozen and dried caviar.

The protein and lipid content was determined according to the State Standard 7636–85. The amino acid composition was studied using a Hitachi A-A-A-835 amino acid analyzer. The fractional composition of lipids was determined by a high-performance thin-layer chromatography, and the fatty acid composition of lipids was analyzed by a gas-liquid chromatography using a Shimadzu 16A. Safety parameters were determined according to the Sanitary Standard 2.3.2.1078-01: microbiological parameters were determined using the common State Standards, the content of toxic substances was determined using a Shimadzu AAS 6701, and the content of chloroorganic pesticides was used by the gas-liquid chromatography using a Carlo Erba HRGC 5300.

The content of water, proteins, lipids, and ash in the dried caviar was 20, 55, 5.5, and 7.11%, respectively. In the case of the frozen caviar, the values of these parameters varied within the following ranges: 7,0–78,0% (water content), 9.0–15.8% (protein content), 1.2–1.6% (lipid content), and 2.97–6.1% (ash content).

The results of our studies showed that the proteins from flying fish caviar are characterized by a full set of essential and nonessential amino acids (the tryptophan content was not analyzed). According to the content of essential amino acids (valine, isoleucine, leucine, lysine, threonine, and the sum of tyrosine and phenylalanine), flying fish caviar proteins even exceed an "ideal" protein. Methionine and cystine represent limiting amino acids. The amino acid composition of caviar proteins is comparable with that of other caviar types, including sturgeon caviar.

The fractional composition of the lipids of flying fish caviar is mainly comparable with that of the caviar of other fish species; however, waxes are absent and the sterol content is twice higher than in the lipids of other caviar kinds.

The content of polyunsaturated fatty acids is rather high in both frozen and dried caviar. In addition, the presence of many fatty acids of ω3 and ω6 classes evidences a high biological value of the lipid fraction of the caviar.

In the most of the examined caviar samples (both frozen and dried) we revealed the presence of bacteria from the colibacillus group and the exceeding of the standard level of a total microbial contamination. At the same time, during the whole storage period we did not revealed any *Salmonella* bacteria, *Staphylococcus aureus*, sulphite-reducing clostridia, yeast, and mould in all examined samples.

The content of lead, arsenic, cadmium, and mercury in the samples of recovered frozen caviar was 0.007, 0.005, 0.005, and 0.02 μg/kg, respectively, which is 100–1000 times lower than the standard values. The content of HCH and isomers did not exceed 0.005 μg/kg, whereas the standardized value makes 0.2 μg/kg; the content of DDT and its metabolites was 0.007 μg/kg, which is significantly lower than the standardized value (2.0 μg/kg).

The revealed inadequacy of the flying fish caviar samples to standard microbiological parameters can indicate, from the one hand, some violations in the sanitary state of the producing facilities and, from the other hand, some breakdowns in the applied technology or its imperfection. Thus, the purpose of our further studies is the substantiation of technological procedures for the recovery of dried flying fish caviar to provide the quality and safety of prepared caviar products.

MARBIO- A MEDIUM/HIGH-THROUGHPUT SCREENING PLATFORM

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Marbio is a medium/high-throughput analytical platform at the University of Tromsø for the identification of novel bioactive compounds from marine organisms. The workflow in Marbio relies heavily on automation of sample handling, preparation and analysis, and this is true for all the three workstations; purification, bioactivity screening, and identification.

Marbio is routinely screening for molecules with these activities:

- Anti-viral
- Anti-bacterial
- Antioxidants
- Anti-diabetes
- Immunomodulatory
- Anti-cancer

In order to extract the widest possible array of compounds from the marine organisms, both aqueous and organic (dichloromethane:methanol) extracts are prepared from freeze-dried biological material. Larger organisms are divided into organ or tissue specific subsamples prior to extraction. The extracts are fractionated by semi-preparative reversed-phase HPLC, and 40 semi-purified fractions from each extract are screened for biological activity. High-resolution mass spectrometry is our primary tool to identify known compounds in active fractions from the bioactivity screening. Accurate mass data acquired by a time-of-flight MS is used to calculate elemental composition of the active compounds. All the available information on the active compound, i.e. elemental composition, biological source and the total bioactivity profile, is used to search databases. If the active compound is known, it is eliminated from further development. Novel compounds are characterized both chemically and biologically in order to evaluate their potential as drug leads. The presentation will give an overview of the workflow and some of the results in the screening program for MabCent-SFI.

THE POSSIBILITIES OF APPLICATION OF SEAL BLUBBER OILS IN PRESCHOOL AND SCHOOL AGE CHILDREN NUTRITION

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Lipids provide about 30% of organism energy and are of great importance for the growth, development and metabolic processes of a child. They are the source of fat-soluble vitamins and polyunsaturated fatty acids (PUFAs), which are an important essential factor of nutrition. Omega-6 and omega-3 PUFAs play a central role in the normal development and functioning of the brain and central nervous system. Various vegetable oils such as sunflower and corn oils are sources of ω-6 PUFAs, whereas omega-3 PUFAs (eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids in particular) are only found in fish and marine mammals oils.

According to the norms of physiological requirements the intake of fat for children at the age from 1 to 14 y.o. should be from 40 to 80 g/day with 5–10% of PUFAs from this quantity. The dietary amounts of ω-6 and ω-3 fatty acids are important: the recommended ratio is around 5÷10 : 1. The best way to provide optimal fatty-acid balance in nutrition of preschool and school age children is the enrichment of their diet with oils of marine species. We suggest to use seal blubber oil in a balanced composition with sunflower oil as various dressings and sauces for salads.

The seal oil, made by cold extraction technology, developed in laboratory of fodder products and biologically active substances, has high quality indicators and less expressed smell in comparison with fish oil. The analysis of physicochemical indicators of oil (Table 1) has shown its conformity to requirements of GOST 8714–72 and SanPiN 2.3.2.1078-01.

Table 1. Seal oil physicochemical parameters

Indicator	Unit of measure	Result of the analysis	Characteristic and norm
Color of oil		light yellow	from yellow to light brown
Oil transparency		transparent	transparent
Saponification number	KOH	184	not normalised
Mass concentration of unsaponifiables	%	0.4	2.5
Peroxide number	$\%J_2$	0.09	10
Mass fraction of moisture	%	0.18	0.5
Acid number	mg KOH/g	0.67	4
Mass fraction of non-oily admixture	%	0.14	0.2

The results of study of fatty acid composition of seal blubber oil are presented in Table 2.

Table 2. Fatty-acid composition of seal blubber oil,%

Fatty acids	Acids content
C 14:0 myristic acid	3.52
C 16:0 palmitic acid	7.55
C 16:1 palmitoleic acid	24.85
C 16:3 hexadecatrienoic acid	1.57
C 18:0 stearic acid	2.16
C 18:1 oleic acid	22.81
C 18:2 linoleic acid ω-6	1.05
C 18:3 linolenic acid ω-3	0.95
C 18:4 octadecatetraenoic acid	0.30
C 20:1 eicosatic acid	2.01
C 20:4 arachidonic acid ω-6	0.15
C 20:5 eicosapentaenoic acid ω-3	10.39
C 22:1 docosenoic acid	1.05
C 22:5 docosapentaenoic acid	6.03
C 22:6 docosahexaenoic acid ω-3	8.57
Sum of saturated acids	13.23
Sum of monounsaturated acids	50.72
Sum of polyunsaturated acids	29.01

It was shown that more than 18% of seal lipids composition is presented by biologically active PUFAs (eicosapentaenoic and docosahexaenoic acids).

Refined deodorized sunflower-seed oil destined for children nutrition was used for manufacturing of compositions with the seal oil. Compositions were designed in laboratory conditions and contained sunflower-seed oil and seal oil in the ratio 1:1, 1:2, 2:1, and 5:1. Taking into account that the vegetable oil expense for salads dressing makes 3,5 g for the portion, degree of satisfaction of daily requirement of children in PUFAs when using salad sauce with the seal oil in various ratios has been defined (Table 3).

Table 3. Polyunsaturated fatty acids content in salad dressing with seal oil, g

Indicator	Average required daily intake for the children from 3 to 7 y.o.	Content in 1 portion with the ratio sunflower seed oil : seal oil			
		1:1	*1:2*	*2:1*	*5:1*
Fat	60	3,5	3,5	3,5	3,5
Saturated fatty acids	-	0,47	0,47	0,48	0,48
PUFAs	10–20	1,55	1,37	1,73	1,91
ω–6	8–18	1,05	0,72	1,39	1,72
ω–3	1,6–2,0	0,35	0,46	0,24	0,12
Ratio ω–6/ω–3	5:1 ÷ 9: 1	3:1	2:1	6:1	14:1

The optimal ratio between sunflower-seed oil and the seal oil is 2:1 since thus the requirement in polyunsaturated fatty acids is satisfied on 12% with a ratio of ω-6 : ω-3 equal 6 that is most near optimal. The degustation evaluation has shown that the composition from sunflower-seed oil and the seal oil in the ratio 2:1 had good organoleptic characteristics.

To improve the composition flavouring characteristics CO_2- extract of fennel in quantity of 0,1% to weight of the sample was offered to use.

On the basis of the carried out researches following requirements to quality and safety indicators of the seal oil and salad dressing on its basis, intended for various groups of children's and dietary products have been offered (Table 4).

Table 4. Quality and safety indicators of seal oil and salad dressing on its basis

Name of an indicator		Seal oil	Salad dressing from sunflower seed and seal oils
Toxic elements, mg/kg			
Lead		0,2	0,2
Arsenic		0,1	0,1
Cadmium		0,1	0,05
Mercury		0,1	0,05
Pesticides, mg/kg			
DDT and its metabolits		0,1	0,1
Hexachlorocyclohexane and its isomers		0,01	0,01
Polychlorinated biphenyls		2,0	1,0
Radionuclides, Bq /kg			
Caesium-137		60	60
Strontium-90		80	80
Oxidising spoilage indicators			
Saponification number, mg KOH/g, less then more		2,0	1,5
Peroxide number, mmole O_2/kg, less then		5,0	4,0
Contents of PUFAs,%, not less then		25	35
Microbiological indicators			
Quantity of mesophilic aerobes and facultative anaerobes, CFU /g, less then		500	100
Weight of a product, g. in which are not supposed	Colon bacillus bacteriums (coliform)		1,0
	S. aureus		1,0
	Pathogenic, including salmonellas		25
	Mould		20
	Yeast		1,0

Biological value and influence of the seal fat and mixture on its basis on indicators of lipid metabolism of experimental animals has been studied. Tests were carried out on white male rats of Vistar line with initial weight of a body 100±5 g. Depending on a diet the animals were divided into 3 groups (each of 10 rats) fed by:

– the seal oil;
– the salad mixture (the seal fat : sunflower-seed oil with a ratio 2:1);
– the control (the lard + sunflower-seed oil 1:1).

At the end of the experiment the animals were subjected to postmortem examination of internal and determination of biochemical and physiological indicators. It was established that the seal oil and the salad mixture did not exert negative influence on the vital functions of the experimental animals.

Inclusion of the seal oil in the diet of experimental animals reduced the cholesterol level in the blood, accompanied by the increase of the general cholesterol in animals' liver. This dependence was less expressed in case of salad mixture.

The contents of ω-3 fatty acids increased with simultaneous decrease of ω-6 fatty acids in liver and erythrocytes of experimental animals under the influence of the seal fat in comparison with the control. The character of these changes was less expressed in the group of the animals fed by the salad mixture.

Erythrocytes hemolytic resistance of rats fed by the salad mixture was increased in comparison with the same of the rats on seal oil diet that testifies the positive influence of the sunflower-seed oil addition in a fatty composition.

In conclusion we consider that inclusion of salad dressings with seals oil in the diet of preschool and school age children, as the balanced product, which can satisfy the daily requirements of the children in the essential fatty acids is highly perspective.

CONCERNING THE SANITARY AND MICROBIOLOGICAL TESTS OF MUSSELS FROM THE WHITE SEA

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In the last decade the issue of studying sanitary and microbiological condition of mussels from the White Sea, which are excellent foodstuff, is becoming urgent, as well as the influence of the mussels on the coastal areas of water.

During the research much attention was paid to studying vibriosis, a dangerous bacterial disease of fish and hydrobiontes in sweet, salty and sea water.

The causative agent of vibriosis in warm water is the culture of strain – Vibrio anguillarum, the causative agent of vibriosis in cold water or the "Hitra" disease is Vibrio salmonicida.

During the research classical microbiological and serological methods were used.

Sampling was made in accordance with State Standard Specification 7631–85 "Fish, sea mammals, sea invertebrates and products of their processing. Rules of acceptance, organoleptic methods of quality rating, methods of sampling for laboratory research" and in accordance with "Methodological recommendations concerning veterinary and sanitary rating of mussels, which are used in food industry".

Sanitary and microbiological researches have been done in accordance with State Standard Specification 51921–2002 "Foodstuffs. Methods of isolation and identification of *Listeria monocytogenes* bacteria", in accordance with the Instructions of sanitary and microbiological control of mussels in areas of their breading and at processing enterprises and of cleaning mussels from bacterial pollution (Puchenkova et al., 1988) and in accordance with the requirements of Sanitary Rules and Standards 2.3.2.1078-01 for alive mussels.

A Russian agglutinating serum, obtained by hyperimmunization of rabbits with antigen from Vibrio anguillarum strain, has been used for diagnosing the causative agent of vibriosis in warm water, for diagnosing the causative agent of vibriosis in cold water *Vibrio salmonicida* strain has been used.

In 1983 scientific and production plantation in Sonostrov's area of the Kandalakshskiy Bay of the White Sea was brought into operation.

Since 1997–2002 "VNIRO" researched sanitary and epizootic condition of *Mytilus edulis* mussels in that area (Melnikova, Bezgachina, Kozitskiy, 1999; Melnikova, Bezgachina, Kozitskiy, 2000; Melnikova, Bezgachina, Kozitskiy, 2000, Bezgachina, Kozitskiy, Melnikova, Storozhuk, Sokolovskaya, 2003).

In 2002–2004 and in 2006–2007 "VNIRO" carried out microbiological research of *Mytilus edulis* mussels and their habitat in the area of Solovetskie Islands of the White Sea (Melnikova, Bezgachina, Biserova, Kozitskiy, 2002). As a result of the researches in the two areas of the White Sea it was stated that even when the quantity of the microorganisms did not exceed the norm of $5x10^4$ colony-forming units per gram, the quality was rather various.

Neisseria, Micrococcus, Aeromonas, Vibrio, Proteus, Pseudomonas genera of strains and *Euterobacteriaceae* family were isolated from water and mussels. The causative agent of vibriosis, a dangerous bacterial disease of fish and hydrobiontes in sweet, salty sea water, is the culture of strain – *Vibrio anguillarum*, which was identified in sea water in Sonostrov's area of the Kandalakshskiy Bay of the White Sea in 2001 (Bezgachina, 2001; Bezgachina, Kozitskiy, 2001).

In 2003–2004, 2006 the causative agent of vibriosis was isolated from *Mytilus edulis* mussels also in the waters of the White Sea in the area of Solovetskie Islands (Bezgachina, Kozitskiy, 2004; Bezgachina, 2005; Bezgachina, 2006; Bezgachina, 2008).

In the autumns of 2006–2007 "VNIRO" isolated *Vibrio salmonicida*, a causative agent of vibriosis in cold water or the "Hitra" disease, from *Mytilus edulis* mussels in Sonostrov's area of the Kandalakshskiy Bay of the White Sea.

As a result of the carried out research it can be stated that alive mussels are unfit for use in food.

It is recommended to do heat treatment of mussels before using them in foodstuffs. Continual sanitary and microbiological control is necessary for safe cultivation of mussels in the White Sea. The use of agglutinating serum for express diagnosing of vibriosis will make it possible to reveal its causative agent at the earliest possible date and prevent the epizootic of disease at farms of microcultures.

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CONSIDERATION OF POSSIBILITY TO PURIFY USED BRINE ON TUBULAR CERAMIC MEMBRANE ELEMENTS

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Processing of secondary raw material resources resulting from making fish products, namely, used brine after salting fish is a live issue nowadays. Annual overall volume of used fish brine is 432700 m^3 and the losses of drinking water and boiled salt from that brine comprise 480500 m^3 and 77000 ton respectively. Its purification with the aim of recycling will increase efficiency of processing secondary raw material resources, cut manufacturing expenses and at the same time will allow to reduce anthropogenic pollution of the environment by cutting dumping of used brine.

Technology for purification of used fish brines was developed in the 1980-s. It is based on pressure driven membrane separation process i.e. ultrafiltration using fluoroplastic membranes F 1 Nowadays, since the advent of inorganic membrane elements characterized by length of service and higher capacity in terms of filtrate, compared to polymeric membranes, further development of brine membrane purification is becoming more feasible.

In 2009 VNIRO has studied a possibility in principle to purify used fish brine after salting herring using ultrafiltering tubular ceramic membrane elements CeRAM INSIDE® which have MWCO equal to 300 and 15 kD. Filtration is performed in a crossflow mode at following parameters: temperature of used brine is 20 – 25 ºC, flow-rate in membrane channel is 5 m/sec at different values of operational pressure. During the tests we defined specific capacity and average capacity of membrane elements with respect to filtrate, as well as peak duration of purification. Moreover, selectivity of membrane elements was studied in terms of solids including lipids, proteins and ashes.

Initially, an optimal membrane element for purification of used brines was selected and after that preliminary parameters of purification process were defined. At that, as results of experiments with used brine, samples of concentrates and filtrates were obtained; besides, the latter were homogeneous transparent light-yellow liquids with characteristic fish odor. During selection it was discovered that application of membrane element with MWCO value 15 kD is inexpedient due to small yield of purified brine (19.8%), insignificant average capacity (24.7 l/m^2 h) and duration of purification process that comprised 2 hours. Technological characteristics of membrane elements with MWCO value 300 kD are favourably compared with the above mentioned: average capacity with respect to filtrate has comprised 75.9 $1/m^2$ h at process duration 3 hours and 91% yield. It was established that it has low selectivity in terms of boiled salt (55%) and overall concentration of salt in purified brine is 12.7% from 13.7% of total

concentration of solids. Considering harmless microbiological index of the obtained brine, use of membrane element with MWCO value 300 kD was accepted expedient for purification of used brine.

During selection of purification mode the experiments on ultrafiltration of brine were conducted at different values of average capacity of the selected membranes in terms of filtrate. The following approximate parameters of used brine purification were established from the experiments: constant average capacity with respect to filtrate is 60 l/m^2 h, variation of operation pressure is from 0.05 MPa to 0.55 MPa at temperature 22 – 25 ºC and flow rate 5m/sec. They ensure expansion of purification cycle from 3 to 3.5 hours with 84% yield value. At that, the purified brine remains with high concentration of salt (11.5%) and it meets the requirements of sanitary regulations and SanPiN 2.3.2.1078-01 in terms of safety microbiological index and can be reused.

Based on the conducted experiments, the production procedures were developed for purification of brine using ultrafiltration at salters.

DEVELOPMENT OF BIOLOGICALLY ACTIVE FOOD ADDITIVE TECHNOLOGY "CRAB OIL"

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Commercial crab is principally a source of raw material for obtaining valuable crab meat. The yield of meat amounts to 25% of the total crab weight; firm wastes (carapace containing residues) and liquid ones, i. e liver (hepatopancreas) reach 75%.

Liquid wastes are proved to be valuable raw material for obtaining crab oil which is a rich source of unique compounds of lipid nature (alkoxyglycerides), polyunsaturated fatty acids (PUFA) – ω^3, fat-soluble vitamins A, D.

For elaborating the method for obtaining crab oil the liver (hepatopancreas) was studied by its chemical composition, microbiological and parasitological indices, as well as by the characteristics of food safety aimed at obtaining crab oil. The result of investigations has shown a high percentage of oil (up to 26%). It was defined by the microbiological, parasitological, toxic indices that the crab liver were in agreement with the requirements SanPiN 2.3.2.1078-01 imposed upon the liver of hydrobionts and can be used for obtaining crab oil.

A method for obtaining crab oil from commercial crab frozen liver (hepatopancreas) was developed at VNIRO in two stages (Patent Nr. 2390274).

At the first stage some 60% of oil from the initial oil content into the raw material is released. Frozen liver is crushed, adding 3% of salt, mixed and heated up to 55 ºC. Two hours later the mixture is cooled and settled, the upper layer of oil is decanted and the left bulk is centrifuged, while the oil is poured out.

At the second stage the remainder matter, where there is some 40% of oil left, is extracted by means of isopropyl alcohol in the ratio of 1:3, respectively, at thorough mixing for an hour and is settled and the resulting extract is filtered. It is liberated from isopropyl alcohol. After both stages the oil is mixed and cleared.

After being separated the oil is to meet the SanPiN requirements 2.3.2.1078-01 imposed upon the oil used for obtaining biologically active food additives. The resultant crab oil was studied for fatty acid and fraction composition which showed that the crab liver lipids were rich in biologically active substances and contain PFA ω^3 content up to 20.0 mg/g; alkoxyglycerides up to 160 mg/g; vitamin A up to 0.16 mg/g and vitamin D up to 1.6 mcg/g.

Thus, the crab oil obtained from crab liver is proved to be valuable and unique raw material for the production of biologically active food additive "Crab meat". Scientific and technical documentation was worked out for this additive and a certificate was obtained on the state registration of this additive, as well as a sanitary and epidemiological conclusion on the documentation.

GLUTATHIONE S-TRANSFERASE ACTIVITY FROM THE NORTHERN FRESH-WATER FISH UNDER MINERAL CONTAMINATION

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Molecular biomarkers attract ever-increasing interest as «early warning» tools for measurement of adverse effects of environment on organisms (de laTorre et al., 2005). Xenobiotic compounds in organisms undergo a series of biotransformation reactions catalyzed by phase I and II detoxification enzymes, the activation of which may point to pollution exposure. According to this detoxification enzymes are being extensively used as molecular biomarkers.

One of the potential biomarkers is glutathione-S-transferase (GST, EC 2.5.1.18) – key phase II detoxification enzyme. The phase II metabolism involves the conjugation of xenobiotics with endogenous substrate, thus facilitating their excretion (Van der Oost et al., 2003). GSTs catalyze binding and inactivation of wide range of both exogenous and endogenous electrophilic compounds and thought to play a significant role in the adaptation to the chemical stress. That is why it is important to study this biomarker in terms of its application and current limitations. But the data available are not consistent, with some reports indicating an increase in the enzyme activity under contamination in contrast with others that do not observe changes or even reported significant decreases (Tuvikene et al., 1999; Van der Oost et al., 2003). GST seemed to be sensitive to both pollution and natural factors, which can hinder the interpretation of the results.

Unlike the marine species, amount of detailed studies of fresh-water fish GST is not sufficient. Among northern ecosystem inhabitants only trout's (Almli et al., 2002; Uguz et al., 2003; Tuvikene et al., 1999; Lindström-Seppä, 1990) and salmon's GSTs have been studied intensively (Nóvoa-Valiñas et al., 2002; Greco et al., 2007; Arukwe A., Nordbø, 2008).

In our study whitefish *Coregonus lavaretus,* pike *Esox lucius* and roach *Rutilus rutilus* were chosen as a test objects. This species are common North-Europe inhabitants which are of commercial interest in the region. Catalytic activity of the GST was determined in fish captured in Karelian Republic lakes in the northwest of Russian Federation.

The contaminated lake Kostomukskoe is located in the source of upper effluent of the river Kem draining into the White Sea. The mining factory releases ore-dressing sewage in this lake leading to abnormally high mineralization (500 mg/l) with K^+, HCO_3^-, SO_4^{2-} and HSO_3^- ions prevalence (table 1). The presence of suspended particles in the water leads to high water feculence. Because the bottom of this lake mainly consists of alkaline rock, water pH level is relatively high (8,5). This could be a basis for natural barrier for heavy metals in this basin. Metal ions precipitate in alkaline water, thus their level is lower than Russian maximum allowable concentrations, except for molybdenum which is ten times higher (*Status of water objects..., 2007*).

The reference lake Kamennoe is situated in the source of the lower effluent of the river Kem on the territory of the Kostomuksha State Natural Reserve. This basin isn't subjected to any anthropogenic inputs and its water is characterized as pure (Table 1).

Mature fish were captured in June 2009 by netting. The animals were measured, weighed and sacrificed within 24 h after capture. After the dissection the tissues were frozen in liquid nitrogen and stored at –80 °C until they were assayed. For extract preparation the tissues were homogenized in 0,125 M PBS buffer (pH 6,5) by Potter homogenizer followed by centrifugation (100000g for 60 min at 4°C). The supernatant was used as an enzymatic solution.

Enzymatic assay was performed on fish livers, kidneys, gills and muscles. The activity was measured spectrophotometrically in cytosolic fractions according to Habig (Habig et al., 1974) using 1-chloro-2,4-dinitrobenzene (CDNB) and reduced glutathione (GSH) as substrates. The final reaction mixture contains 1 mM CDNB and 1 mM GSH.

Proteins were measured in supernatant spectrophotometrically at 205 nm as described by Noble and Bailey (2009), using BSA and reduced glutathione as a standard.

Differences between groups were tested by Manna-Witney's test at a 5% significance level, using the software Statistica 5.0 (Statsoft, Inc., 1995). Correlations between variables (enzymatic activity, age and weight) were examined based on Spearman's index.

Table 1. Water chemical constituents in studied lakes

	Ions concentration in the Kostomukskoe lake, mg/l *	Ions concentration in Kamennoe, mg/l *
Total mineralization	500	17,4
K^+	146	0,45
Na^+	15	0,7
Ca^{2+}	30	1,5
Mg^{2+}	10	1,2
Cl^-	7	1,7
HCO_3^-	145	1–9,7
NO_3^-	9	
NH_4^+	0,05	0,08
NO_2^-	0,03	
N organic	3	0,4–0,7
SO_4^{2-}	172	4,5–6
N total	13	0,3
P total	0,02	0,04
Suspended particles	1,34	0,3–1,8
Fe total	0,3	0,8–1

* (Status of water objects..., 2007).

GST activity was found to positively correlate with age in pike's gills (r_s= –0.62), whitefish kidneys (r_s= –0.63) and roach's kidneys (–0.87) and liver (r_s = –0.87). Concerning to this, this species samples should be chosen very carefully considering its natural variability.

GST activity was found to be sex dependent only in pike's kidneys. It was elevated in female in comparison with males in pikes captured in the Kostomukskoe lake.

The induction of the GST activity was found in pike's gills and kidneys and in whitefish's livers from contaminated lake in comparison with fish from reference site (table 2). GST activity was also altered in whitefish kidneys, there it was significantly higher in the reference than in the contaminated lake.

The altered GST activity in fish from the mining factory lake compared with the unpolluted lake from the same region indicates the bio-protection system response to the mining factory influents. This response in the contaminated site indicates general metabolic stress in water inhabitant under the excessive mineralization. Adverse conditions may modify metabolic processes in tissues, leading to elevated production endogenous reactive GST substrates, for example products of lipids peroxidation.

The absent of roach tissues response indicate its weak adaptive potential to mineral contamination, which can lead to further elimination this species from the contaminated lake. Abnormally low percent of males in population and growth delay of roach from this site can support this assumption.

Table 2. Glutathione S-transferase activity in tissues of fish collected from the lake situated near the mining factory (Kostomukskoe lake) and reference lake (Kamennoe lake)

	pike *Esox lucius*		whitefish *Coregonus lavaretus*		roach *Rutilus rutilus*	
	Kostomu kskoe	Kamennoe	Kostomu kskoe	Kamennoe	Kostomu kskoe	Kamennoe
Liver	699.87	704.62	123.23	75.98*	98.07	71.88
Muscels	7.37	6.35	10.37	7.78	8.22	8.50
Kidneys	31.96	6.58*	16.22	45.71*	3.79	13.55
Gills	819.92	465.95*	9.41	8.94	16.03	29.71

* – groups are significantly different compared to reference lake ($p \leq 0,05$.)
Values are presented as median.

Surprisingly high level of the GST activity was found in pike's liver and gills in comparison with whitefish and roach livers and gills activity, which was on the average 9 and 30 times lover. As obligatory predators pikes seems to have protecting mechanisms against toxicants accumulation during food chain. Elevated biotransformation enzymes level is possibly an adaptation resulting from evolution of this species.

It could be concluded that glutathione S-transferase from pike's kidneys and gills and whitefish's kidneys and liver may be applied as an indicator of fish exposure to non-specific contamination. The GST activity response was observed at polluted lake compared with the control, indicating adverse effect of

elevated mineralization on fish detoxification system. Because whitefish, pike and roach are common in northern European lakes this data may be of importance for ecological monitoring in this region.

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MYOSIN EXPRESSION LEVEL IN WHITE MUSCLE AS A MARKER OF FISH GROWTH

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One of the main questions in biotechnology of aquaculture is the search of convenient markers of fish growth. Methods for growth rate maximizing have been tested for many years in aquaculture. Examples, of such methods are recombinant growth hormone injection, use of transgenic fish, variation in type and regimes of nutrition, etc. That is why it is important to assess accurately the effect of use a new techniques. However, there are numerous ways of assessing growth, from traditional and direct measurements such as length, weight and condition factor, to the newest techniques that measure gene expression. Measuring biological macromolecules has been hypothesized by many investigators to be a more sensitive indicator of overall fish condition than traditional morphometric variables. It is considered that muscle-specific genes (Myogenic regulatory factors, myostatin, myosin) are the most reliable markers for assessment of fish growth. The myosin hard chain (MyHC) gene expression has been tested as possible indicator of muscle growth during the last decade since Overturf and Hardy (2001) demonstrated the

positive correlation of myosin heavy chain expression with nutrient intake in rainbow trout. However, gene and protein expression of myosin has not been widely tested in fish. Furthermore, the limited number of studies in the literature present quite contradictory results (Biga et al., 2004, Johansen and Overturf 2006, Imsland et al., 2006, Dhillon. et al., 2008, Koedijk et al., 2010)

Myosin is the most abundant protein in muscle, comprising as much as 25% of the whole organism protein pool (Baldwin and Haddad, 2001) and up to 50% of the muscle protein pool (Watabe and Ikeda, 2006). The chosen in our study myosin heavy chain isoform belongs to class II of myosins. The class II proteins, referred to as conventional myosins, are expressed in the striated muscles and are directly involved in muscle contractions (Regiani, Bottinelli, 2008). The location and abundance of these proteins in white muscle make myosin an ideal candidate for investigating growth in fish. The gene encoding myosin heavy chain is expressed during the entire life of fish (Regiani, Bottinelli, 2008).

To test the myosin mRNA level as a potential biomarker of fish growth we evaluate the relationship of the MyHC mRNA level in white muscle with body mass and fork length of salmonid fish of different ages. We exmined both farmed fish (rainbow trout, *Parasalmo mykiss* W.) and wild fish (Atlantic salmon, *Salmo salar* L., white-fish, *Coregonus lavaretus* L.)**.** Biological characteristics of fish examined are presented in Table 1.

Table 1. Examined biological characteristics of fishes. The values indicate the mean and standard error with minimal and maximal values in brackets (min-max)

Species	Location	Age	Number of fish	Fork length (mm)	Body mass (g)
Rainbow trout	fish farm Lake Onego (Republic of Karelia)	Two-year-old fish (1+)	20	271.9±4.5	317.76±16.72
				(180–360)	(75.9–755.0)
		Three-year-old fish (2+)	15	470.3±4.9	1789,42±44,88
				(354–524)	(789.9–2210)
Atlantic salmon	River Indera (Kola peninsula)	Two-year-old fish (1+)	20	72.6 ± 1.1	3.64 ± 0.17
				(63–82)	(2.30 – 4.83)
		Three-year-old fish (2+)	10	96.9 ± 2	8.25 ± 0.51
				(86–103)	(5.67–10.20)
Whitefish	Lake Kamennoe (Republic of Karelia)	Three-year-old fish (2+)	9	189.6 ± 2.7	56.8 ± 2.38
				(176 – 197)	(50–67)
		Four-year old fish (3+)	9	205 ± 2.8	84.71 ± 4.9
				(196–241)	(73–110)
	Lake Tumasozero (Republic of Karelia)	Six-year old fish (5+)	15	311.7 ± 1.2	340.23 ± 5.4
				(292–325)	(263–390)

MyHC mRNA level were analyzed by quantitative real-time RT-PCR. Total RNA was extracted from the white muscles according to Chomczynski and Sacchi (1987) using a Yellow Solve kit (Clonogene, St. Petersburg, Russia). Total RNA was treated with DNase (10 U/ml; SibEnzyme, Russia). First strand cDNA was synthesized from the total RNA using MMLV reverse transcriptase and random primers (Sileks, Russia). Amplification was performed in an IQ5 Real-Time PCR Detection System (Bio-Rad, USA) using 2.5x reaction mixture for qRT-PCR in the presence of an intercalating dye, SYBR Green I (Syntol, Russia). Primers for the myosin heavy chain, β-actin and EF1 were selected using the Beacon Designer 5.0 software. Primers used for myosin and reference genes were as follows: rainbow trout *MyHC*: forward 5' – GCTGAGAAGGACGAGGAGATG – 3', reverse 5' – GCCTGCCTGTTGGAGTGG – 3' (GenBank, Z48794); rainbow trout *β-actin*: forward 5' – TGGACTTTGAGCAGGAGATGG – 3', reverse 5' – TCGTGGATACCGCAAGACTC – 3' (GenBank, AJ438158); salmon *MyHC*: forward 5' – TTCAGTGGCGTGCTTCTC – 3', reverse 5' – AAGAGGCTGGAGGATGAGG – 3' (GenBank, DN164736); salmon *EF1*: forward 5' – TGGACTGCCTATCAAACATC – 3', reverse 5' – TCTCACTCGCTATGGAACC – 3' (GenBank AJ427629). To analyse *MyHC* mRNA level in white muscle of whitefish the primers for salmon genes were used. Real-time PCR was conducted under the following conditions: 5 min at 95°C; 50 cycles for 30 s at 95°C, 30 s at 56°C, 30 s at 72°C; and then melting of DNA fragments. The concentration of mRNA was assayed using relative standard curve method for quantification (Gahr et al., 2008). The expression levels of *MyHC* gene were normalized for the expression level of a reference gene, *β-actin* and *EF-1*. The data were expressed as the ratio of *MyHC* mRNA concentration of the studied gene to that of β-actin mRNA.

To examine the relationships between *MyHC* mRNA levels and fork length and body mass of fish, the multiple regression analysis was used. The results are presented as the regression equations relating *MyHC* mRNA level in white muscle to fork length and body mass of fish studied (Table 2 and 3).

Table 2. The regression equations relating *MyHC* mRNA level in white muscle of fish studied to fork lenght. *y*, *MyHC* mRNA level; *x*, fork lenght.

Species	Age	n	Equation	r^2	P
Rainbow trout	1+	20	y = –0.876 + 0.006x	0.34	<0.01
	2+	15	y = –1.849 + 0.006x	0.60	<0.001
Atlantic salmon	1+	20	y = –20.133 + 0.334x	0.48	<0.001
	2+	10	y = –3.545 + 0.508x	0.58	0.01
Whitefish (Lake Kamennoe)	2+	9	y = –0.349 + 0.030x	0.34	<0.05
	3+	9	y = –3.876 + 0.216x	0.81	<0.01
Whitefish (Lake Tumasozero)	5+	15	y = –6.818 + 0.240x	0.40	0.01

Table 3. The regression equations relating *MyHC* mRNA level in white muscle of fish studied to body mass. *y*, *MyHC* mRNA level; *x*, body mass. NS, not significant

Species	Age	n	Equation	r^2	P
Rainbow trout	1+	20	y = 0.245 + 0.002x	0.36	<0.01
	2+	15	y = –0.134 + 0.001x	0.54	0.001
Atlantic salmon	1+	20	y = – 2.659 + 1.819x	0.26	<0.05
	2+	10	y = –0.268 + 0.195x	0.62	<0.01
Whitefish (Lake Kamennoe)	2+	9	y = –0.091 + 0.005x	0.68	0.001
	3+	9	y = –0.701 + 0.015x	0.84	<0.001
Whitefish (Lake Tumasozero)	5+	15	y = –0.265 + 0.003x	0.08	NS

Our results demonstrated the positive correlation of *MyHC* mRNA levels with fork length and body mass of rainbow trout, salmon and white-fish from Lake Kamennoe of all age groups studied. There was significant relationship between MyHC gene expression and fork length of whitefish 5+ from Lake Tumasozero. But no relationship was observed between myosin expression and body mass. The last fact is possible to explain by peculiarities of metabolism of fish at this age. The whitefish 5+ were sexually matured (gonad maturity stage V). It is possible to suppose that body mass is determined by gonad mass and store lipid content, and that only fish length reflects the muscle growth of six-year-old fish.

The relationship between *MyHC* mRNA levels and fork length and body mass is stronger in elder fish of all three species studied. MyHC mRNA levels in white muscle increased whit age of rainbow trout and white-fish (fig 1. A and C).

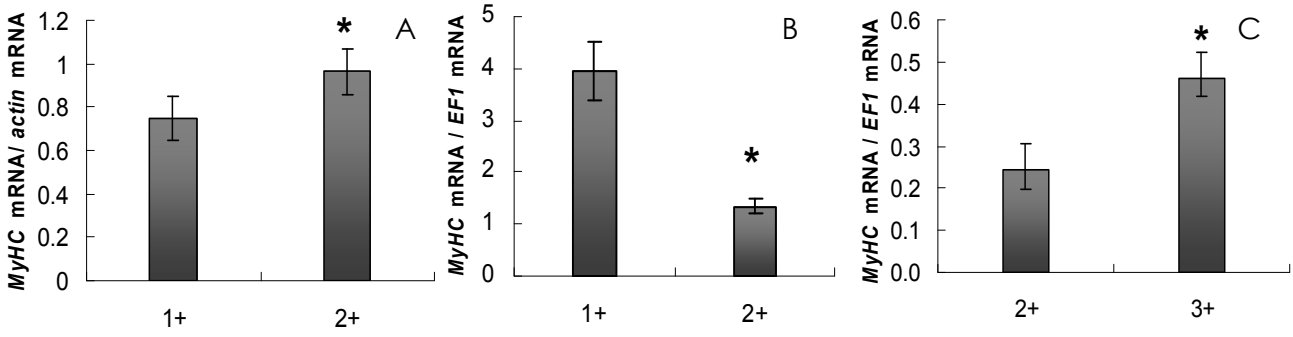

Fig 1. The changes in *MyHC* mRNA levels in white muscle of different age groups of rainbow trout (A), salmon (B), white-fish (C)

That indicates an increase of muscle growth rate with age. However, the level of MyHC gene expression was higher in two-years-old salmon as compared to three-year-old fish (Fig 1. B). It is possible related to switching of myosin isoforms expression. As it is known, in vertebrates, myosin heavy chains are encoded in a family of different genes (Weiss and Leinwand, 1996). For example, in carp, *Cyprinus carpio* L., evidence for at least 28 myosin heavy chain genes have been found (Gerlach et al., 1990). It was

shown, that different isoforms are expressed depending on developmental stage (Ennion et al., 1999; Fukushima et al., 2009). Thus, the reduction of the myosin expression level observed with age in our study may reflect the switch to the expression of a different isoform that was undetected due to the specificity of the primers, or could also reflect an actual reduction of expression of myosin heavy chain as salmon grow. This fact should be studied in more detail, especially in aspects of myogenesis regulation.

Therefore, we studied the features of myosin expression in white muscles of fish of different species and ages. According to our results bigger fish within age cohort have a higher level of myosin expression. This indicates that myosin expression levels may be used as biomarker of fish growth to monitor and predict individual growth both in farmed and in wild fish.

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STUDY OF THE POSSIBILITY TO USE POLYMERIC MATERIALS WITH BARRIER PROPERTIES FOR THE PACKAGING OF FISH PRODUCTS

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In recent years the food safety question became very critical in Russia. One of the main problems facing the fish industry is the retention of the quality and safety of the water bioresources and food, produced from them.

To solve this problem, new technologies are developed and applied in industry; at the same time, scientists improve old traditional technologies, which make it possible to manufacture production, characterizing not only by good organoleptic features, but also by the high food value and stable microbiological parameters during the whole shelf life of the product.

A packaging material, based on polymeric materials with barrier properties, is characterized by low oxygen and carbon dioxide transmission coefficients (cm3/m3 per 24 h); this fact makes it possible to significantly delay and/or suspend the hydrolytic and oxidative processes in proteins and lipids and to prevent the microbiological contamination of a product. In addition, the use of such materials for the packaging not only protects the product against any environmental influence, but also reduces its natural storage losses.

According to statistical data, the use of different kinds of packaging for fish products increased every year. However, comparing to the other branches of the food industry, the percentage of the packaged fish products on the Russian market still remains rather low.

In our opinion, the use of domestic polymeric materials with barrier properties seems to be relevant concerning the retention of the quality and safety of fish products.

The purpose of our study was to investigate the possibility of the use of polymeric materials with barrier properties for the packaging of a fish production in a gas-modified medium.

The object of our study was a filleted Atlantic salmon (*Salmo salar* L.); the weight of pieces did not exceed 0.2 kg.

The fish was packed into a five-layer polymeric co-extrusion film, having a barrier layer. The film was manufactured by the "Polimery XXI veka" Ltd. Company (Russia) and complied with the technical specifications 2245-001–14660125-07. The structure of the film included the following layers: high-density polyethylene, adhesive, co-polymer of ethylene and vinyl alcohol (EVOH), adhesive, and high-density polyethylene. The thickness of the layers was 16.5, 7.0, 18.0, 7.0, and 16.5 μm, respectively. The breaking strength of the polymeric material was 28–32 MPa.

As the components of a gas-modified medium, used for the packaging of samples, we used carbon dioxide and nitrogen in three different volumetric ratios; the first and third variants represented the maximum and minimum CO_2 content, respectively. The use of carbon dioxide as one of the medium components is caused by its high water solubility and inhibitory action on the aerobic microflora, including pathogenic microorganisms. Nitrogen is an inert gas and is characterized by a low water and oil solubility; it is used to prevent the oil oxidation and to displace the rest of oxygen from the packaging.

The samples, packaged without the use of a gas medium and vacuum, were used as the control.

The chilled salmon was stored at +4 ± 0.5º C ($P > 0.95$).

According to the planned program, we took first samples for our study after a 24-h storage, and then at the 7^{th}, 10^{th}, and 14^{th} days. We examined some organoleptic parameters, such as the appearance, color, and consistence, the smell on the surface and inside the product, the smell of the vapor, broth, and boiled product, and the taste of the boiled product, on their compliance to the State Standard 7631–2008. The microbiological parameters (quantity of mesophilic aerobic and optional-anaerobic microorganisms (QMA&OAMO), bacteria from the colibacillus group, *Staphylococcus aureus*, *Salmonella*, *Listeria monocytogenes*, and *Vibrio parahaemolyticus*, regulated by the Sanitary Standard 2.3.2.1078-01), were studied using the common methods.

Since the heightened CO_2 content in a gas medium can stimulate the growth of lactobacteria (Dubrovskaya, 2000), we additionally determined the total amount of lactobacteria according to the State Standard 10444.11–89.

The protein and non-protein nitrogen content were determined using a Kjeltek-2300 analyzer (Foos company). To determine the lipid content, we used the Bligh and Dyer method (Bligh and Dyer, 1959). The oil acidity index was determined according to the State Standard 7636–85.

The result of the organoleptic analysis showed that, during the whole period of the examination, the appearance, color, and consistence of the samples did not change and corresponded to this sort of product. At the 7^{th} (control) and 10^{th} (variant 1) days we registered a foreign smell on the surface and inside the product and in the vapor and broth during the boiling of the fish.

The organoleptic properties of the samples of the variants 2 and 3 (smell inside and on the surface of the product, smell of the vapor and broth, color and transparency of the broth, smell and taste of the boiled fish) corresponded to this sort of product up to 14^{th} day of the storage.

The results of microbiological studies showed the absence of the following microorganisms during the whole experimental period: colibacillus group (in 0.01 and 0.001 g of the product), *Staphylococcus aureus* (in 0.1 and 0.01 g), *Salmonella* (in 25 and 50 g), *Listeria monocytogenes* (in 25 and 50 g), and *Vibrio parahaemolyticus* (in 1.0 g of the product).

In the beginning of the storage, the QMA&OAMO value did not exceed the normalized value and varied from $7.4x10^3$ to $1.3x10^4$ CFU/g (Table 1). In the case of the control samples and samples from the variant 1, on the 7^{th} day we observed the exceeding of the above-mentioned value ($9.6x10^5$ and $7.5x10^5$, respectively). On the 10^{th} and 14^{th} days the samples from the variants 2 and 3 corresponded to the requirements of a normative documentation.

Table 1. Total microbe contamination of the chilled salmon samples during the storage, CFU/g

Variant	Sanitary requirements (SanPiN 2.3.2.1078-01)	Storage period, days			
		Background	7	10	14
1	No more than $1.0x10^5$	$7.4x10^3$	$7.5x10^5$	$3.2x10^5$	$9.9x10^4$
2		$5.3x10^3$	$6.4x10^4$	$5.9x10^4$	$4.0x10^4$
3		$1.3x10^4$	$8.7x10^4$	$7.7x10^4$	$2.8x10^4$
Control		$3.2x10^3$	$9.6x10^5$	$1.2x10^6$	$2.5x10^6$

The obtained results showed that the total quantity of lactobacteria in the samples of the variant 1 (the maximum volume fraction of CO_2) varied from $2.5x10^3$ (1^{st} day) to $8.5x10^4$ CFU/g (14^{th} day). In the case of the variants 2 and 3, where the volume fraction of CO_2 was minimal, the value of this parameter did not exceed $3.2x10^3$ CFU/g during the whole storage period. The maximum CO_2 amount within the packaging stimulates the growth of lactobacteria that corresponds to the data of other authors.

The total protein content in the examined samples varied from 18.1 to 20.5%.

To the end of the first 24 h, the non-protein nitrogen content in the fish products was 1.8–2.0% of the total nitrogen content. During the storage period, we observed a triple increase in the non-protein nitrogen content in the control samples and the samples of the variant 3 (6.1% of the total nitrogen content), whereas in the case of the variants 1 and 2 the value of this parameter increased in 2.0–2.5 times (3.8 and 4.7% of the total nitrogen content).

The lipid content in the chilled salmon samples varied from 20.2 to 26.5%.

The background level of the oil acidity index was 1.1–1.3 mg of KOH/g (Table 2).

The most intensive process of the accumulation of pyrolysis products, or free fatty acids, was observed in the control samples and the samples of the variant 1, where the acidity index exceeded the background value in 2.0–2.5 times to the 14^{th} day of storage. An increase in this index to the 14^{th} day in the samples of the variants 2 and 3 was significantly lower, comparing to the control samples and samples of the variant 1.

Table 2. Acid values of chilled salmon samples, packed in a gas mixture, mg KOH per 1 g of oil

Variant	Storage period, days			
	background	7	10	14
1	1.2	1.9	2.0	2.3
2	1.3	1.4	1.6	1.8
3	1.1	1.4	1.5	1.7
Control	1.21	1.6	2.1	3.11

The obtained results confirmed the reduction of the intensity of hydrolytic processes in proteins and lipids.

Thus, the result of our study made it possible to conclude that the polymeric materials with barrier properties can be used to provide the quality and safety of the chilled salmon production in the gas medium with the maximum volume fraction of nitrogen and minimal volume fraction of CO_2 at the storage temperature equal to 4^0C.

WHITE SEA MUSSELS *MYTILUS EDULIS* L. AS A SOURCE OF N-3 POLYENIC FATTY ACIDS

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The blue mussels, *Mytilus edulis* L. (1758), cultivated on artificial substrates in experimental aquaculture in the Kandalaksha Bay, White Sea, are, like most marine organisms, rich in polyunsaturated fatty acids (PUFA) of the linolenic (n-3) family, originating from phytoplankton. Uptake of various n-3 PUFA with food is known to reduce the risk of cardiac ischemia in humans. Furthermore, the groups of people whose diet is rich in these acids have lower blood coagulability, which is attributed to lower platelet aggregation, blood pressure reduction, weakening of triglyceride and cholesterol circulation (Sergeeva and Varfolomeeva, 2006). PUFA of the n-3 family were found to benefit patients with arthritis, kidney disorders, as well as other inflammatory and immune diseases (Lands, 1992; Mevkh et al., 1996; Bernardi, 1996). At present, one distinguishes the effects of three main fatty acids of the n-3 family: linolenic (18:3), eicosapentaenoic (20:5) and docosahexaenoic (22:6) acids. Eicosapentaenoic acid is the source of products which counteract substances of the arachidonic cascade. Docosahexaenoic acid is essential for the central nervous system (Lauritzen et al., 2000; Jump, 2002).

Comparative analysis of the total lipid fatty acids composition in some species of bivalves from the White Sea, such as *Mytilus edulis* (aged 5–6 years, shell size 65.8 mm), *Hiatella arctica* (shell size 26.6×14.5×12.2 mm) and *Modiolus modiolus* (shell dimensions 63.7×32.7 mm), showed *Mytilus edulis* to contain higher concentrations of both n-3 PUFA and total PUFA (Tab. 1). The proportion of arachidonic 20:4 (n-6) acid was the same in all three species.

Table 1. Fatty acid composition of some bivalves from the White Sea (% of total fatty acids)

Fatty acids (% of total FA)	*Mytilus edulis*	*Modiolus modiolus*	*Hiatella arctica*
Total saturated FA	17.9	22.8	22.8
Total monounsaturated FA	22.1	27.0	24.4
16:4(n-3)	4.6	1.4	1.0
18:3(n-3)	2.1	1.7	1.2
20:5(n-3)	15.4	17.3	10.9
22:6(n-3)	19.4	9.9	16.8
Total n-3 PUFA	44.4	35.8	35.9
20:4(n-6)	2.7	2.7	2.7
Total n-6 PUFA	11.6	10.0	9.6
Total PUFA	60.1	48.3	51.5

Detailed study of the fatty acid composition in cultured *Mytilus edulis* L. mussels from the White Sea showed the content of n-3 PUFA to increase with age (owing to 18:3 (n-3), 20:5 (n-3), 22:6 (n-3) acids), and peak at an age of 4–6 years (Tab. 2).

Interestingly, the content of n-6 PUFA, including the main representative of the series – arachidonic 20:4 (n-6) acid, was about the same, irrespective of the mussels' age (Tab. 2).

Table 2. Fatty acid composition of cultured White Sea mussels *Mytilus edulis* L. of different age (% of total fatty acids)

Fatty acids (% of total FA)	*0+*	*1+*	*2+*	*3+*	*4+*	*5+–6+*
Total saturated FA	18.1	19.0	18.5	19.6	19.1	17.9
Total monounsaturated FA	21.5	24.0	21.4	21.8	20.6	22.1
16:4(n-3)	6.3	4.2	5.4	4.2	5.3	4.6
18:3(n-3)	1.3	1.7	2.0	2.2	1.9	2.1
20:5(n-3)	13.1	13.1	14.8	14.7	16.5	15.4
22:6(n-3)	16.4	17.3	18.8	18.8	19.4	19.4
Total n-3 PUFA	41.0	39.3	43.7	42.6	45.9	44.4
20:4(n-6)	3.6	2.7	2.5	2.3	2.4	2.7
Total n-6 PUFA	14.9	13.1	12.4	11.5	10.9	11.6
Total PUFA	60.4	57.0	60.1	58.6	60.3	60.1

Thus, the fatty acid composition of *Mytilus edulis* L. mussels is noted for high content of n-3 PUFA, represented predominantly by 20:5 (n-3), 22:6 (n-3) acids. Their positive effect at some human diseases has been proven in quite a number of studies. Our results suggest *Mytilus edulis* L is a commendable source of polyunsaturated acids of the n-3 family to be used in manufacturing of medicines.

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IDENTIFICATION AND CHARACTERIZATION OF SMALL REGULATORY RNAS IN THE GRAM-NEGATIVE FISH PATHOGEN *ALIIVIBRIO SALMONICIDA*

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Small RNAs from Bacteria, commonly known as sRNAs, make up a new and interesting group of regulatory RNAs involved in stress responses, central metabolism, quorum sensing, motility and more. Even though data on sRNA function is accumulating, there are still large gaps in our understanding of their biological roles in Bacteria. We use bioinformatic tools, biochemical methods and – omics approaches to identify and characterize sRNAs in the cold-loving fish pathogen *Aliivibrio salmonicida*. Our main goal is to understand the critical roles of sRNAs in virulence, for example by triggering expression of proteins involved in iron uptake, oxidative stress and cell-cell communication. Recent data from these experiments will be presented.

ANALYSIS OF PERFLUORINATED ACIDS (C9-C11) BY USE OF LIQUID PHASE MICROEXTRACTION (LPME) AND UPLC-MS

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The widespread occurrence of perfluorinated organic acids and other perfluorinated compounds in environmental samples are documented. The high water solubility's of these compounds indicate that the water column might be an ultimate recipient for these compounds. There are concerns about the persistence and bio accumulative properties of these compounds, and analysis of water samples might be the first step to understand the persistence and bio accumulative picture of these compounds.

We have developed a sensitive and selective method for perfluorinated carboxylic acids based on the use of LPME and UPLC-MS. In LPME a water immiscible organic solvent is immobilized as a thin supported liquid membrane in pores in the wall of a porous hollow fibre. The lumen of the fibre is filled with a 70 μL acceptor solution, where pH is adjusted to make sure that the analytes are in the ionized state. After extraction, 2 μL the acceptor solution was directly subjected to the final analysis by liquid chromatography-mass spectrometry. The compounds were detected by using the MS-instrument negative Electro Spray ionization mode. For all compounds it was possible to obtain recovery around 60%, which indicated an enrichment factor of around 5000. The least amount on column needed for quantification is around 2 pg. These results indicate that we can quantify perfluorinated compounds from samples containing these substances in the sub-nanogram range.

USING OF PRODUCTS OF MUSSEL MARICULTURE PROCESSING (*MYTILUS* ACID HYDROLYZATE) UNDER MINKS ALEUTIAN DISEASE

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Rational using of the White Sea bioresourses for medical-biological purposes is actual. Some evaluations the influences of mussel hydrolyzate (MIGI-K) on reproduction and physiological condition were made. It was found that the effect of hydrolyzate is dependent from doze, duration of its application, physiological condition and sex of animals. The using of hydrolyzate as food additives for minks infected by Aleutian disease (AD) viruses results in changes in the blood serum protein fractional composition, leukocyte differential count and increasing of weight. The protein spectrum normalization at mink with AD, apparently, reduce disturbances, connected with hyper-γ-globulinaemia. Sex and dose dependent influences of hydrolyzate on liver antioxidant enzymes were established. The question about using of MIGI-K as prophylaxis substances in AD minks was discussed.

Mussel hydrolyzate (MIGI-K) is natural preparation made from mussel meat and containing complex of biologically active substances (amino acids, lipids, trace elements and others). This preparation possesses antiviral and antiradical activity owing to presence of melanoidins. Hydrolyzate demonstrate property of high-performance adaptogen and immunomodulator and is able to normalize homeostasis (Goncharenko et al., 1995).

The problem of sea foods application is of serious interest. Especially it is importance in connection with occurrence Aleutian disease (AD) and another immunodeficiency states finding in farm bread animals. AD, a naturally occurring virus infection in mink, is caused by a parvovirus, ADV. The main features of the chronic and progressive form of AD are virus persistence and hyperglobulinaemia (Bloom

et al., 1994). Therefore search of biologically active substances diminished negative effects of AD is of great interest of fur breeders.

In this study effects of MIGI-K on physiological state of minks infected of AD were investigated.

The study was conducted on mink kits at the fur production farm ("Kondopogskiy zverovod", Karelia, Russia). All the animals were kept in standard farming conditions on a paste-like diet with two meals per day and water *ad libitum* as recommended for the species. Both healthy and minks with AD were divided into 3 groups: control (n=10, sex ratio 1:1) and 2 experimentals (n=10, sex ratio 1:1 in each group). The experimental period lasted from July to October. The control animals were fed the basal diet and the two groups of experimental animals were fed the basal diet supplemented with 0.2 ml/kg and 0.5 ml/kg of *Mytilus* acid hydrolyzate (MIGI-K) accordingly for five-day periods with two-day intervals between them, where animals were fed only the basal diet. This specific design was applied to prevent acquired tolerance of the organism to MIGI-K. The MIGI-K was obtained by method of acid hydrolysis from White Sea mussel.

After killing the animals performed according to European Convention [TA-P (96) 19] the samples of liver were collected and then frozen at –25°C. There were measured the total and specific activities of superoxidedismutase (SOD) and catalase (CAT) and total tissue protein in liver and the protein fractional composition in serum, leukocyte level and leukocyte differential count in peripheral blood.

The total SOD activity was measured by the adrenochromic method based on the spontaneous autooxidation of epinephrine with the formation of end products which have an absorbance peak at 480 nm (Misra, Fridovich, 1972). This reaction depends on the presence of superoxide anions and is specifically inhibited by SOD. The amount of enzyme that caused 50% inhibition of epinephrine autooxidation is defined as 1 unit (U). Catalase activity was evaluated by measuring the decrease in H_2O_2 concentration at 240 nm (Bears, Sizes, 1952). Specific activities of these enzymes were calculated via division total activity of each enzyme by protein content. Total tissue protein level was determined by Lowry method (Lowry, 1951).

Data are presented as% from control. Statistical analysis was performed with Mann-Whitney's test. Differences between samples were considered to be significant when the p value was less than 0.05.

Treatment minks the MIGI-K-supplemented diet resulted in both sex- and dose-dependent changes of the investigated indices. Hydrolyzate (dose 0.5 ml/kg) caused redistribution among serum protein: treatment minks with distinct hyper-γ-globulinaemia the MIGI-K resulted in decrease of γ-fraction content. Hyper-γ-globulinaemia appears as a consequence of immunologic disorder caused by AD (Bloom et al., 1994). The MIGI-K (dose 0.5 ml/kg) stimulated reduction lymphocytes level and rise content of neutrophils within the limits of norm. Obviously hydrolyzate acts as immunostimulant and its effects are realize by way of regulation of both protein synthesis and leucopoesis (Goncharenko et al., 1995).

The results of the determinations of antioxidant enzymes activities are presented in Fig. 1 and 2. MIGI-K had a more pronounced effect on liver antioxidants in male than in female minks. And larger dose (0.5 ml/kg) of MIGI-K caused more changes in studied indices compared with smaller dose.

Minks with AD had higher SOD activities (both total and specific), lower CAT activities (both total and specific) and higher SOD/CAT ratio than healthy animals. In several cases treatment minks the MIGI-K-supplemented diet resulted in opposite to AD changes of parameters. No significant influence of MIGI-K in dose of 0.2 ml/kg on the investigated indices in minks with AD was observed, besides the CAT activity in male mink was elevated. Also it was shown the sex differences in both CAT activities in minks with AD fed the 0.2 ml/kg MIGI-K-supplemented diet: males had the higher activities than females. Apparently it may be connected with different resistance of males and females to AD (Slugin, 1975). Larger dose of MIGI-K caused increase of both CAT activities in female minks with AD compared with control. In male minks with AD it was registered the elevated SOD activities and SOD/CAT ratio compared with both control and group with smaller dose.

In healthy minks it was revealed that MIGI-K in larger dose has contrary effect on the antioxidant enzymes activities than AD. Decreased SOD activities and increased CAT activities were registered in both male and female healthy minks. At the same time influence of smaller dose of MIGI-K on the antioxidant enzymes was not detected.

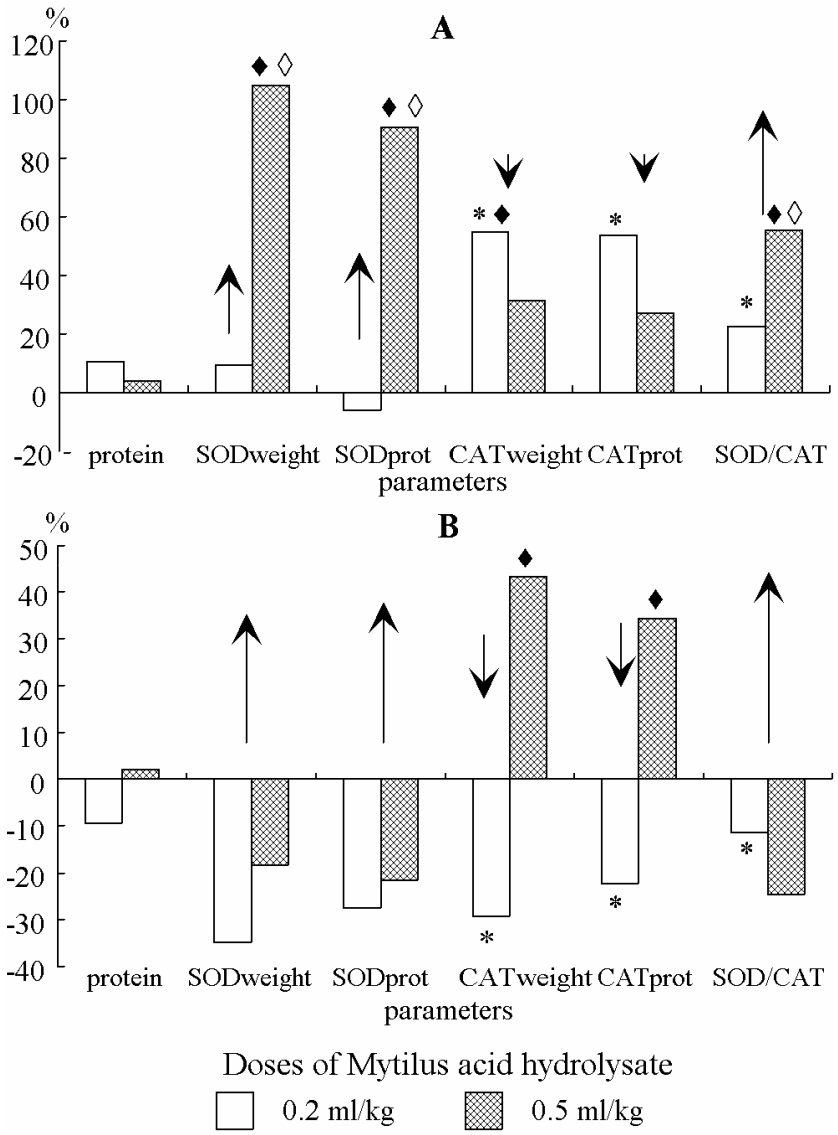

Fig. 1. Effect of mussel hydrolyzate on protein content, SOD and catalase activity in male (A) and female (B) liver of standard mink with AD

* – significant differences between male and female,
♦ – significant differences in comparison with control group,
◊ – significant differences in comparison with doses 0.2 ml/kg,
size and direction of arrow indicate changes of parameters in mink with AD.

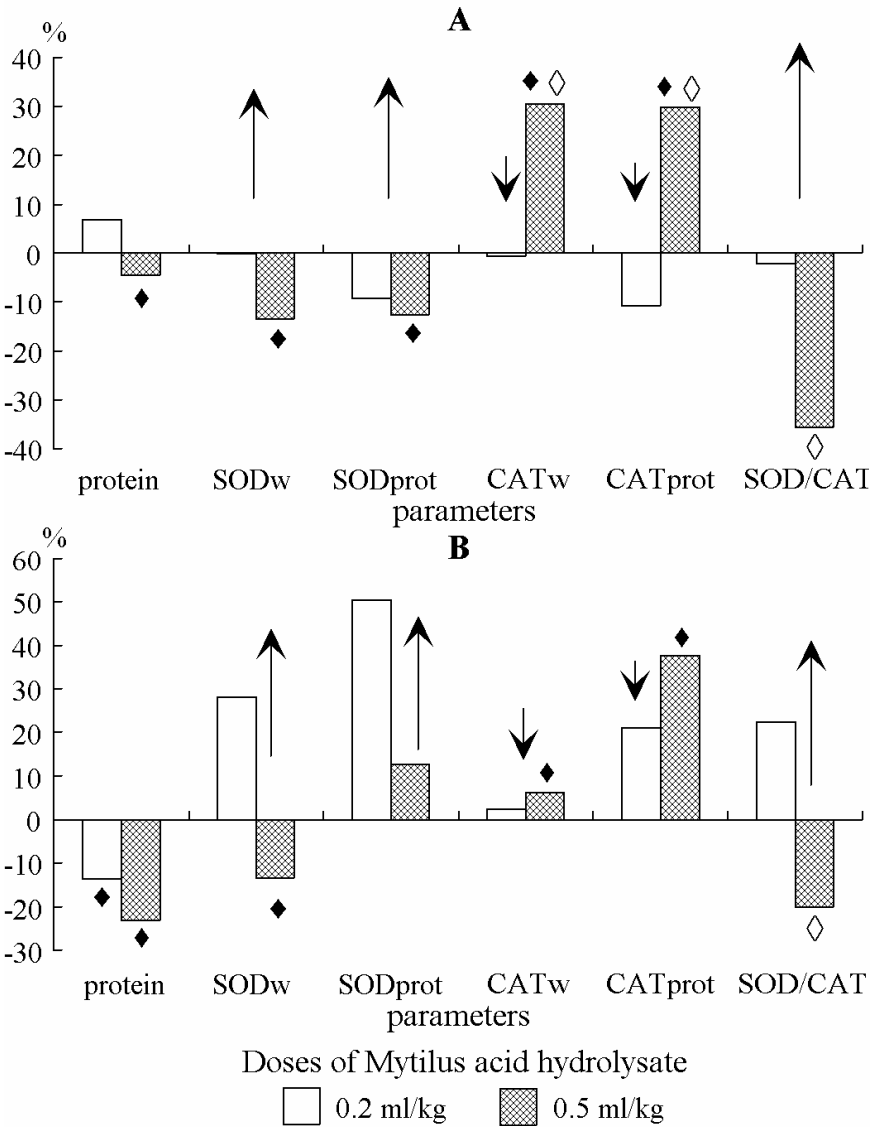

Fig. 2. Effect of mussel hydrolyzate on protein content, SOD and catalase activity in male (A) and female (B) liver of healthy standard mink

* – significant differences between male and female,
♦ – significant differences in comparison with control group,
◊ – significant differences in comparison with doses 0.2 ml/kg,
size and direction of arrow indicate changes of parameters in mink with AD.

Our findings showed that MIGI-K has favourable effect on physiological state of minks with AD. This effect of hydrolyzate was expressed by way of the decreased mortality, increased growth and reproduction rates in animals. Besides that MIGI-K had repairable effect on antioxidant enzymes, parameters of leucopoiesis and protein fractional composition in minks with AD. Larger dose (0.5 ml/kg) of MIGI-K caused more changes in studied indices compared with smaller dose. Hydrolyzate had a more pronounced effect on liver antioxidants in male than in female minks. It was revealed that MIGI-K has contrary influence on investigated indices in minks than AD. In conclusion treatment of MIGI-K (0.5 ml/kg) decreased severity of disease in minks with AD.

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CONTRIBUTION OF NA^+/K^+ ATPASE TO BIOCHEMICAL ADAPTATIONS OF FRESHWATER FISH TO POLLUTED WATER OF THE ORE-DRESSING INDUSTRIAL COMPLEX NEAR KOSTOMUKSHA, KARELIA

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The impact of human activities on water bodies has substantially increased in the past yeas. Therefore, more attention is now given to the existence and survival of the aquatic organisms in contaminated water. Aquatic ecosystems, communities and organisms, including fish, are extremely sensitive to disturbances in the chemical composition of the environment that may inhibit metabolic pathways and reduce the resistance of cells in the organism. Biochemical changes in the cells and tissues of an organism are commonly observed before external signs come to sight (Stroganov et al., 1983; Nemova and Vysotskaya, 2004). The effect of toxicants on the organism is accompanied by the involvement of the cellular and molecular mechanisms that control metabolic homeostasis. It is common knowledge that the ion composition of an organism's internal fluids in the normal condition would always differ to a certain degree from the ion composition of the ambient medium. The state of the mechanisms that maintain the water-salt homeostasis is an essential criterion in assessment of the physiological fitness of an organism. Stress resistance depends not only on the maturity of morphological structures responsible for adaptation to the respective factors, but also on the activity of the constituent enzymes that supply the structures with energy and maintain the ion homeostasis in the cell. The key role in the processes of osmotic and ion regulation is known to belong to active transport enzymes which carry ions against their concentration gradient. Of particular importance among these is the enzyme Na^+/K^+ ATPase. It is the enzyme in the outer membrane of cells in all animal tissues that helps maintain a key property which differentiates living cells from dead cells – asymmetric distribution of sodium and potassium ions on the cell membrane inside and outside. Asymmetric distribution of univalent cations is essential for the formation of the cell's membrane potential, as well as for metabolite transport across the cell membrane, and for regulation of intracellular metabolic reactions (Boldyrev, 1998). Active transport of Na ions from the cell and K ions into the cell, performed by Na^+/K^+-ATPase, is an inseparable property of a living cell.

Investigating the index of active ion transport we can estimate the contribution of Na^+/K^+-ATPase to biochemical mechanisms of freshwater fish (pike, whitefish) adaptation to highly mineralized, particularly rich in potassium ions, technogenic water of Lake Kostomukshskoye, which was impounded to supply the mill with recirculated water. Wastewater from the Kostomuksha mining and ore-dressing complex contains a mixture of metals with high potassium concentration and a high percentage of suspended ore particles. In the contaminated zone, high water mineralization is primarily due to the presence of K^+ (157 mg/l) and sulphate ions (266 mg/l), and the water can be classified into the sulphate-potassium class. Among other elements one may note (Lozovik, 2007) elevated content of Li^+ (83 μg/l) and Ni^+ (11 μg/l). High alkali and alkali-earth metal and hydrocarbonate concentrations in the polluted zone were responsible for the shift of pH toward the alkaline region (8.0). Potassium ion content in Lake Kamennoye water in the clean-environment zone (Kostomukshsky nature reserve territory) is 0.29 mg/l, which is much lower than in the polluted zone. The

increase in Na ion content is insignificant – from 1.0 mg/l in Lake Kamennoye water to 17 mg/l in the polluted zone. The concentrations of elements (Co, Ni, Cr) are a few microgrammes per litre of water in the polluted zone, which slightly exceeds the corresponding values observed in the clean zone (Morozov, 2006).

Research into the activity of Na^+/K^+-ATPase in different tissues (gills, muscles, kidneys, liver, gonads) of fish from the lakes in question revealed tissue-specificity and reduction in the activity of the enzyme in the fish caught in the polluted zone. Since the activity of Na^+/K^+-ATPase is regulated, first of all, by univalent cations, a change in the Na^+/K^+ ratio changes the enzyme activity in a specific way. Suppression of the activity of the active transport enzyme Na^+/K^+-ATPase, which concentrates potassium ions within the cell, is presumably a consequence of the substantial rise (~ 500-fold) in the potassium ion concentration in the ambient environment – the highly mineralized impoundment reservoir, as compared with the normal freshwater habitat. Changes in the composition of the medium, first of all in the content of sodium and potassium ions, as the most variable component, alter the electrolytic composition of an organism (Hlebovich, 1974) and thus trigger adaptive modification of the activity of the membrane enzyme which maintains the intracellular ion homeostasis.

Thus, changes in the activity of Na^+/K^+-ATPase is an example of biochemical adaptation which success depends on the ability of the fish to modify their water-salt metabolism in accord with the environment. The optimal microenvironment of the organism's macromolecules is thus maintained, which is the main principle behind the strategy of biochemical adaptation (Hochachka and Somero, 1977; Nemova and Vysotskaya, 2004).

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ROLE OF THE INSERTION SEQUENCE ELEMENTS IN THE GENOME ORGANIZATION OF THE *ALIIVIBRIO SALMONICIDA*

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Growing evidence show that bacterial genomes actively evolve under pressure of environmental changes. Rearrangements caused by mobile genetic elements and insertion sequence (IS) elements in particular, greatly contribute to the generation of the occasional fitter mutants and thus increase the genetic variability in bacterial populations. IS elements normally encode no functions other than transposases

necessary for their mobility. While sole movement of the IS element normally leads to gene disruption, simultaneous transposition of two IS elements as parts of composite transposon promote relocation, inversion, excision of large DNA regions or even lead to a plasmid fusion as well as new gene acquisition from the environment or horizontal gene transfer. These dramatic events can result in the assembly of new gene clusters providing multidrug resistance or encoding new metabolic pathways. Starting from the first outbreaks of cold-water vibriosis, plasmid pattern investigations had revealed 6 different naturally occurring *Aliivibrio salmonicida* plasmid profiles. Our work is focused on the *A. salmonicida* plasmid profile flexibility and impact of the IS elements to this phenomenon. Our experiments will be carried out on numerous isolates collected from diseased fish and from the water surroundings since the early 1980's and up to present days. Revealing the possible mechanisms of transposition of different isolates of *A. salmonicida* IS elements will bring us closer to understanding the role of this class of mobile genetic elements for the integration/excision of plasmids into the chromosome. Discovering the molecular basis of this process and its impact on virulence of *A. salmonicida* will contribute to the development of revolutionary new types of protective measures against future cold-water vibriosis outbreaks.

SEARCHING OF THE BIOLOGICALLY ACTIVE SUBSTANCES THAT MEDIATE INTRA- AND INTERSPECIFIC COMPETITION BETWEEN EPIBENTHIC ORGANISMS. INVESTIGATION ON THE EXAMPLE OF THE WHITE SEA FOULING COMMUNITIES

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Essential role in itraspecific competition between marine sedentary organisms belongs to allelopathy – negative influence of the one species to another one by chemical agents. Nowadays scientist's interest in allelopathic chemicals rises worldwide. However the White Sea invertebrate's potentials have been studied negligibly in this interest and thus respective biological resources are poorly developed. Actually in the White Sea, just blue mussels (*Mytilus edulis*), brown (*Laminaria saccharina, L. digitata*) and red (*Ahnfeltia plicata*) algae are used as the source of biologically active substances (BAS). Besides of this, there are some prospective producers of chemicals with biological effects or another practicable properties, like a soft coral *Gersemia fruticosa* (prostaglandins), hydroid *Obelia longissima* (photoproteins).

It seems quiet logical to search BAS from organisms possessing allelopathic action. This feature could be a basis for narrowing of BAS screening and increasing of its effectiveness. But such approach implies good knowledgebase of organism's interrelation complex in this or that communities.

From this point of view, fouling communities are the most useful and prospective model for investigations of this kind in the White Sea. Species composition, development dynamics and intraspecific interaction patterns of these communities have been well established by the present time, what could be a reliable basis for directed search of chemical mediated competition among the White Sea sedentary invertebrates.

Therefore, investigations of influence of secretory-excretory products (SEPs), produced by different sedentary organisms, on another hydrobionts were conducted. We tested SEPs of some the most abound species in the White Sea, as follows: bivalve mollusks *Hiatella arctica* and *Mytilus edulis*, sponge *Halichondria panicea*, solitary ascidium *Styela rustica* and starfish *Asterias rubens*. Juvenile or adult mussels (*Mytilus edulis*) were used as a test-object in the work.

On the first stage, competitive relationships among organisms mentioned above were estimated by behavioral reactions. According to the results of field and laboratory experiments, it was shown, that mussels (*M. edulis*) increased byssus production and, thus, movement in response to waterborne cues from sponge *Halichondria panicea* and solitary ascidian *Styela rustica*. Increment of byssus production by mussels in the presence of competitor's SEPs apparently directed to avoidance or even immobilization of a source of stress (Khalaman, Komendantov, 2007; Khalaman et al., 2008a; Khalaman, Lesin, 2008). This reaction is similar to a well known behavioral defense of mussels against carnivorous whelks (Davenport et

al., 1993). In addition, effluent from the sponge affected metamorphosis and caused fate of *S. rustica* (Khalaman et al., 2008b) and *M. edulis* (Khalaman et al., 2009) larvae.

The data obtained instigated biochemical studies of allelopathic action of the principle species in the White Sea fouling communities. In the laboratory experiment, the most changes in mussels metabolism were observed in mollusks treated with SEPs of *A. rubens*, *S. rustica* and *H. panicea*. Water, conditioned with starfish, ascidia and sponge, caused activation of the same enzymes, but to different extend. Foremost among tested enzymes was increment of acid RNase andglycosidases activity in mussel tissues, especially, in the presence of starfish metabolites. But almost all of enzymatic activities tested returned to control level at the end of the experiment in mussel groups treated with SEPs of *A. rubens* and *S. rustica*. This fact could point to compensatory character of metabolic changes observed. In contrast with effect of starfish and ascidian effluents, sponge induced changes in mussel metabolisms were statistically significant at the end of experiment, pointing to slower or different character of biochemical response on *H. panicea* SEPs.

Summarizing results of field and laboratory experiments showed that at least two species pretended to possess allelopathic action. These are sponge *Halichondria panicea* and solitary ascidia *Styela rustica*. In the case of sponge, several known biological activities of chemicals, produced by *Porifera* themselves or their microbial symbionts, may speak well for this suggestion (Althoff et al., 1998; Engel, Pawlik, 2000; Belarbi et al., 2003; Devi at al., 2010; etc.). Ascidian metabolites are not studied as widely as sponge BAS, but there are some with promising clinical application (Kobayashi et al., 1991; McDonald et al., 1994; Ciufolini et al., 1995; Torres et al., 2002; etc.). Thereby, we suggest that *H. panicea* and *S. rustica* from the White Sea could be prospective objects for searching of novel BAS. Additionally, it should be underlined that, if some biologically active compounds with potential application are discovered in these species, they could be cultivated rather easy as they are fouling organisms.

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QUALITY PRESERVATION OF FROZEN SALMON OVARIES

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The salmon caviar refers to the gourmet product with high nutritional and biological value. Volumes of extraction and production of salmon caviar in Russia is enormous and reach 5–6 thousand tons per annum. The amount of caviar produced from frozen salmon ovary has increased in recent years.

As it is known, the quality of caviar products depends on the quality of salmon ovary raw material, processing technology, production sanitary state and conditions of transportation and storage of caviar products.

The preservation of quality is always one of the important issues, so the aim of the work is to study methods to preserve the quality and safety of raw materials.

According to the technical documentations, which serve as a basis for fish processing enterprises, storage life of frozen salmon ovary does not exceed 6 months.

At the present time, the waxed paper is used to preserve the salmon ovary quality with further packaging in waxed boxes and polymer film and bags made of polymer materials, in which salmon ovary are packed under suction or not.

There is another way to preserve the quality of frozen products – icing. We prepared the control samples of salmon ovary packed in waxed film and boxes, and the test samples iced with an antiseptic solution and antioxidants.

Throughout the storage period of the salmon ovary (9 months at the temperature of –18°C) the microbiological properties of the test samples were by an order lower than the properties of the control samples and met hygienic requirements of safety and nutritional value of food products. The content of toxicants – toxic elements, organochlorine pesticides, N-nitrosamines, histamine, in the control and test samples did not exceed the standardized values.

Besides the safety parameters, the parameters responsible for the hydrolytic and oxidative changes in proteins and fats, and also amino-acid composition of proteins and fatty acid composition of lipids were evaluated.

Fat content in the salmon ovary samples ranged from 9.5 to 12.0%. During storage there was a tendency of gradual increase in the value of acid number, while in the non-iced salmon ovary the tendency was expressed in a greater extent than in the iced ones. (Table 1). After 9 months of storage the acid number in the control samples was 9.57, and in the test samples – 7.01 mg KOH/g fat. At the same time the acid number of lipids extracted from the surface of the block of the test samples was higher by 3% than the acid number of lipids extracted from the middle of the block, while in the control samples, this difference was 15%.

Table 1. Acid number content in the control and test samples, mg KOH/g fat

Name	Storage life, months			
	1	5	7	9
control	4.64	6.44	7.19	9.57
test	3.88	6.74	6.92	7.01

We have indentified about 40 fatty acids in lipids of the frozen salmon ovaries (Table 2).

Table 2. The main fatty-acid composition of salmon ovary lipids,% to the amount

Name	Code	Storage life of the control samples, months		Storage life of the test samples, months	
		1	9	1	9
Myristic	14:0	6.39	6.48	3.34	3.99
Pentadecanoic	15:0	0.83	0.81	0.46	0.54
Palmitic	16:0	15.99	16.02	13.69	14.51
Heptadecanoic	17:0	0.47	0.57	0.43	0.44
Stearic	18:0	3.65	4.30	5.53	5.18
Nonadecanoic	19:0	0.34	0.24	0.30	0.33
Heneicosapentanoic	21:0	0.34	0.24	0.20	0.18
Docosanoic	22:0	0.29	0.22	0.31	0.31
Palmitooleic	16:1	10.79	10.82	7.96	8.51
Oleic	18:1	30.98	30.78	28.91	27.19
Eicosenoic	20:1	3.87	5.91	5.04	4.76
Erucic	22:1	2.43	2.55	3.00	2.86
Hexadecadienoic	16:2	0.78	0.58	0.59	0.61
Linoleic	18:2	2.27	2.24	2.23	2.09
Eicosandienoic	20:2	0.53	0.49	0.52	0.50
Docosadienoic	22:2	0.42	0.46	0.32	0.37
Linolenic	18:3	1.46	1.44	1.31	1.23
Eicosatrienoic	20:3	0.82	0.85	0.89	0.83
Octadecatetraenoic	18:4	1.49	1.31	1.30	1.20
Arachidonic	20:4	2.24	2.26	2.57	2.31
Eicosapentaenoic	20:5	7.20	6.99	10.70	9.47
Heneicosapentanoic	21:5	1.16	1.06	0.37	0.37
Docosapentanoic	22:5	1.38	1.28	2.44	2.38
Docosahexaenoic	22:6	2.91	2.67	5.98	5.42

A large proportion belongs to monounsaturated fatty acids – 45.27 – 48.37% to the amount of fatty acids. The following acids dominate among them oleic acid – about 30%, palmitoleic acid – about 10% and eicosane acid – 3.8–5.9%.

The amount of saturated fatty acids ranges from 24.5 to 28.6%. The main saturated acids are palmitic –13.7–15.9%, myristic – 3.3–6.3% and stearic –3.6–5.5%.

The content of polyunsaturated fatty acids in the lipids of salmon ovaries is rather high – from 45.2% to 48.3%, mainly due to two acids: eicosapentaenoic acid, the proportion of which varies from 7.2% to 10.7%, and docosahexaenoic acid, its proportion varies from 2.9% to 5.9%. The amount of essential fatty acids: linoleic, linolenic and arachidonic is approximately 6.0%.

The tendency of insignificant increase in the mass fraction of monounsaturated fatty acids in the non-iced salmon ovary is observed during storage, this fact was confirmed by several authors (Lovern et al., 1959; Olley et al., 1965; Rzhavskaya, 1976).

Despite the relatively high content of polyunsaturated fatty acids (20:5 and 22:6) in the salmon ovaries being initiators of the lipid peroxidation (Vladimirov et al, 1972, Kagan et al, 1983), the degree of hydrolytic changes in the lipids during storage of the control and test samples is expressed in a lesser degree. Apparently, the process of freezing stabilizes the lipids of the salmon ovary hindering the processes of its deterioration during storage, which to some extent can be explained by inactivation of salmon ovary lipases at minus 180C.

One of the nutritional value indices of the caviar is the amino acid composition of its proteins.

The results of study on amino acid composition of the salmon ovary proteins showed that they contain the essential amino acids: isoleucine, leucine, lysine, methionine, cystine, phenylalanine, tyrosine, valine and threonine, the total amount of which varies from 35.5g to 37.5g, which is more than 40% of the sum of all amino acids (Table 3).

Table 3. Amino acid composition of proteins of the control and test sample, g/100 g of protein.

Amino acids	Standard FAO/VOZ 1985	Control		Test	
		1 month	9 months	1 months	9 months
Essential					
Isoleucine	2.8	4.47	4.59	5.09	4.95
Leucine	6.6	8.27	8.48	8.97	8.16
Lysine	5.8	6.59	6.79	6.69	6.76
Methionine + cystine	2.5	2.53	2.65	2.56	2.78
Phenylalanine + tyrosine	6.3	4.26	4.52	4.32	4.45
Threonine	3.4	4.07	3.86	4.12	4.01
Valine	3.5	5.46	5.64	5.74	5.65
Tryptophan *	1.1				
Nonessential					
Glutamic acid		9.97	9.98	10.01	10.03
Tyrosine		3.87	3.96	3.79	3.85
Proline		4.91	5.10	4.85	4.98
Alanine		6.64	7.09	6.75	7.02
Glycine		2.38	2.32	2.42	2.45
Serene		4.52	4.85	4.57	4.71
Aspartic acid		9.52	7.11	9.45	8.36
Arginine		4.95	4.80	4.92	4.85
Histidine		2.11	2.16	2.15	2.24
∑ amino acids		84.52	83.9	86.4	85.25
∑ essential amino acids		35.65	36.53	37.49	36.76
% amounts of essential amino acids		43.4	43.5	43.4	43.1

* not determined

The comparison of amino acid composition of proteins of the control and test samples showed that no changes virtually occur in the amino acid composition of proteins. We note some variations in the content of individual essential amino acids, which occur without a noticeable downward trend. These data indicate the stability of the amino acid composition of proteins of the control and test samples.

As it is seen from the provided data the icing process does not affect the change in the fatty acid composition of lipids and amino acid composition of proteins. However, the icing with application of antioxidants inhibits hydrolytic processes during storage of frozen salmon ovary.

NUTRITIVE VALUE OF STERLET CAVIAR FROM OVULATED EGGS

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Since mid nineties natural stock of sturgeons has been sharply reduced. Only one possibility to maintain the species is their breeding and keeping under control in aquaculture.

Many countries which began cultivation of sturgeons in late seventies now have become big producers of sturgeon meat and caviar.

It's very well known that caviar is the most precious product from both economic and consumer points of view.

Traditional technology of caviar production supposes sturgeon female slaughter, eggs extraction, screening and salting. Thus a sturgeon female which was grown for 5–8 years has to be slaughtered for meat after taking roe only once. It's evident that such a technology is very expensive and doesn't posses high economic efficiency.

Keeping sturgeon female alive gives the possibility to get roe during its whole reproductive period of life, i.e. 10 or even more times.

Till nowadays ovulated raw eggs obtained by intravital method was used only for reproduction as a traditional technology of salting wasn't suitable for food caviar production from ovulated eggs.

A number of principle properties of eggs which differ from that produced by traditional method (slaughtering) didn't allow to use it for food purposes.

We've worked out a technology of food caviar production from sturgeon ovulated eggs which makes it possible to receive high quality ready product and a stable output not less than 90%.

The aim of this work was to study and analyze the food value of caviar products which were produced according to our new technology from ovulated eggs of sterlet.

The object of our study was sterlet caviar produced from ovulated eggs obtained by Burtsev I.A. intravital method (Moscow, Russian Federal Research Institute of Fisheries and Oceanography) at one of fish farms in Moscow Region.

Sensory study of sterlet caviar showed that after salting it has quite a firm capsule (form), tender consistency, “disassembling” eggs and a taste typical for sturgeon caviar. During storage period faulty signs such as sour taste, bitter taste, touch of oxidized fat don't appear. Stability of sterlet caviar taste is proved by absence of microflora growth, absence of autolitical processes in proteins, oxidizing and hydrolitical processes in lipids.

Protein quantity in pasteurized sterlet caviar is 25.8%. Amino acid composition of proteins in sterlet caviar includes non-essential and essential amino acids. The sum of non-essential acids is 43.7% from the total of amino acids. The content of non-essential amino acids in 100 g. of sterlet caviar proteins is higher than in 100 g. of ideal protein (recommended FAO/WHO values, 1985).

During the whole period of storage at the temperature of 2 – 4°C and –18°C no changes appeared in the correlation of non-replaceable and replaceable amino acids.

The content of lipids in caviar products produced from ovulated sterlet eggs fluctuates from 10% to 12%. The results of study of fractional lipids content testify high quantity of triglycerides – 93.5%, share of phospholipids is about 2.1%, content of sterins is not more than 3%. About 1% of free fatty acids appeared in lipids after 13 months of storage.

Fatty acid composition is presented by saturated acids in the amount of 30.5% from the total of fatty acids, mono non-saturated acids –46.9% and poly non-saturated –22.6%. In the group of poly non-saturated fatty acids eicozapentaenoic and docozahexanoic acids dominate, the share of lenoleic, linolenic and arahidonic is about 5%.

Absence of accumulation of proteins and lipids products decay in sterlet caviar during the shelf-life shows that the presented technology suppresses caviar ferment system, stabilizes proteins, prevent oxidation and hydrolitic processes, provides microbiological stability of the product during its storage.

The received results testify that caviar products obtained from ovulated eggs of sterlet according to the technology worked out by the authors are characterized by a high content of proteins, lipids and a full range of non-replaceable and replaceable amino acids, being rich in poly non-saturated fatty acids. Stability of microbiological level during caviar products storage provides their safety.

The elaborated out technology is approved by aquaculturists in the Russian Federation and abroad. The technology is defended by the patent of the Russian Federation #2232523 (priority since 2002, September).

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PERSPECTIVES OF REPRODUCTION AND CULTIVATION OF RED KING CRAB *PARALITHODES CAMTSCHATICUS* IN THE BARENTS SEA (RUSSIA AND NORWAY)

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Significant decline in the red king crab abundance together with sharp fall of the Barents Sea catches (3–4 times), and the expected poor recruitment of the stocks necessitate search for additional measures aimed at conserving this valuable commercial species stocks and reproduction, including techniques of artificial reproduction.

There is no doubt that eventually, we shall have to restore this species stocks in the northern basin. This is obvious from the red king crab exploitation experience in the Far Eastern seas where these fisheries have been long in a depressive state. The stock depletion is apparently caused not only by over fishing but also by low efficiency of natural reproduction. The last made scientists to develop techniques of artificial reproduction and rearing of this species. For many years, Laboratory of crustacean reproduction and cultivation (VNIRO) has been carrying research studies of this problem, both in closed systems with artificial seawater, and in flow-type systems on the coast of the Barents Sea (Kovatcheva et al., 2005; Kovatcheva et al., 2006; Kovatcheva, 2006; Kovatcheva, 2008).

Reproduction and cultivation of red king crab in the coast of the Barents Sea (Russia)

With the aim of optimizing maintenance of the red king crab artificial reproduction, in 2009 the VNIRO specialists developed a design specification for the crab module construction on the Barents Sea coast (Dal'nye Zelentsy Ltd.). This project took account of biological and technological requirements for the red king crab cultivation systems.

The set of basins consists of eight various basins for keeping gravid females, larvae (zoea and glaucothoe), as well as young red king crabs. Two basins designed for gravid females and spawning are made of nontransparent plastic, while six basins for rearing larvae, glaucothoe and juvenile crabs are made of transparent polycarbonate which allows for visual control of the hydrobionts state. The overall area of the basins (bottom) is 8.4 m^2.

The module includes a section for live food (nauplii *Artemia* sp.) production. Thermostatted incubators make adequate daily provision of nauplii for the specified feeding regime.

Basins have a flow water supply system with the overall volume of 4.7 m^3; the seawater intake varies from 0.25 to 5.0 m^3/h. This system provides optimal environment in basins as it has a controlled water intake with mechanical filtration and UV-sterilizer, as well as a separate circuit for the water recirculation with thermostatting to keep the water temperature at the required level. Besides, the module includes a separate water supply system for particular basins to maintain different temperature regimes in them. Thus, the module provides for control of the physiological rhythms of adult and young crabs, as well as larvae through changing of the water temperature.

In March, 2010, after setting up the water supply system and establishment of the experimental module, we started experiments on adaptation and optimization of the artificial reproduction technique in the coast rearing module with the aim to release young crabs into the sea.

Under controlled conditions in March, the module produced 600,000 larvae (zoea I).

During the entire larval period (zoea I – IV) our experiments with zoea were connected with elaboration of some bio technology elements of the red king crab reproduction in natural seawater under constant temperature of 7^o C. Duration of the larvae period was 39 days or 273 degree -days.

At the postlarval stage (glaucothoe), we introduced substrates into the basins for settlement of the hydrobionts and increased the water temperature up to 8^o C. The post larval stage took 19 days or 152 degree -days.

The glaucothoe molt took 10 – 12 days. Mean survival rate of zoea I till the glaucothoe molt ranged from 30 – 60% in various basins and directly depended on the introduced substrates.

On the 9th of June 2010, we released 200,000 juvenile crabs into the sea in presence of officers from the Murmansk state veterinary service, the Murmansk department of Rosselkhoznadzor, the Barents Sea-

White Sea territorial department of the Russian Federal Agency of Fisheries, personnel of Dal'nye Zelentsy Ltd., and the VNIRO specialists.

First results of experiments in the coast module for crab rearing (the Dal'nye Zelentsy Ltd. property), which were supported by the VNIRO specialists, showed good opportunities for successful artificial reproduction of the red king crab under controlled conditions with subsequent release of juveniles into the sea.

For investigation of early stages of the red king crab juveniles cultivated under artificial conditions of the coast rearing complex experiments continue.

In 2010 – 2014, we are planning to conduct the following studies:

1/ Develop a technique for production of the red king crab larvae and juveniles with further rearing under controlled conditions;

2/ Develop a technique for releasing of the red king crab juveniles into the sea (restocking);

3/ Investigate biology and physiology of red king crab at the early life stages.

Proceeding of the red king crab artificial reproduction in the Barents Sea could become a good example of efficient partnership between private and state institutions in addressing of such grand-scale issues as conservation of aquatic biological resources of the Russian Federation. Moreover, the results of these studies will be used to construct the crab rearing complexes with the aim of restocking and maintaining natural populations of red king crab in the Barents Sea and the Far Eastern seas.

Cultivation of the red king crab on the coast of the Barents Sea (Norway)

Red king crab is a valuable fishery resource both for Russia and Norway. Russia has accumulated long traditions of commercial utilization of red king crab. While in Norway, studies and exploitation of this species only started a decade ago in mid-1990s, when natural stocks of this acclimatized species increased in the Barents Sea.

With the object of rational commercial utilization of the red king crab resources came into existence elaborated of technology of the species keeping and cultivation in the Norway region. Therefore, by the end of 2007, the project in this sphere began under the joint Russian-Norwegian project – VNIRO (Moscow, Russia) and Norway King Crab Production AS (Bugøynes, Norway).

***The main goal of the project**:*

1. Establishment of an experimental coast facility for keeping and rearing of red king crab;

2. Optimization of bio technology for prolonged keeping and rearing of red king crab in the basin complex;

3. Improvement of techniques of the long-distance transportation of live red king crab;

4. Development of techniques to control physiological state of red king crab under cultivation conditions.

Studies began with development of the technical design specification for the crab complex construction on the coast of the Barents Sea (Bugøynes, Norway). The project is based on the scientific research outcomes which account of all stages of keeping and artificial rearing of crab under controlled conditions, including a whole range of technical decision.

The complex site is the Barents Sea coast (Bugøynes, Norway).

Since 2008, there are experiments with red king crab in respect of identifying optimal parameters of cultivation and long-distance transportation of live red king crab.

Main activities in the complex under continuous water quality control are as follows:

1. Investigation of feed rations and feed types;

2. Examination of stocking density;

3. Studies of the crab legs filling with muscles;

4. Optimization of the crab rearing during the premolt and molt period under artificial conditions;

5. Studies of physiological state of red king crab under various conditions of keeping and transporting.

Development of techniques of the long-distance transportation of live red king crab is the principal goal of the experiments carried out in the complex.

Live red king crab is the most valuable delicacy. Since 2004, when the Barents Sea fisheries for the acclimatized red king crab started, the demand for live red king crab has been continuously growing.

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.

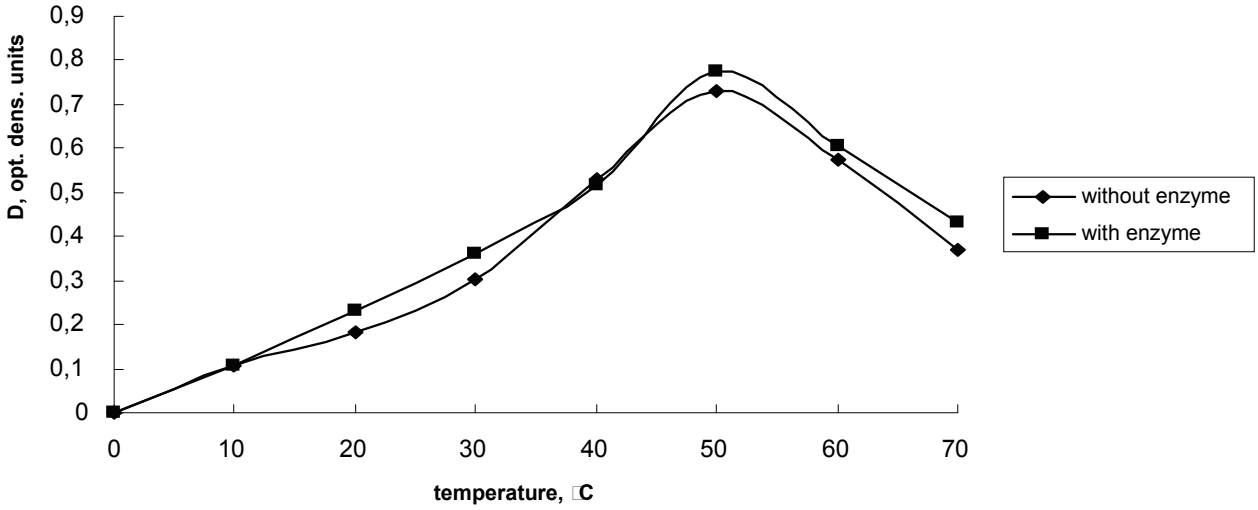

Fig. 1. Temperature dependence of the degree of protein hydrolysis in minced ruff (D_{300})

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).

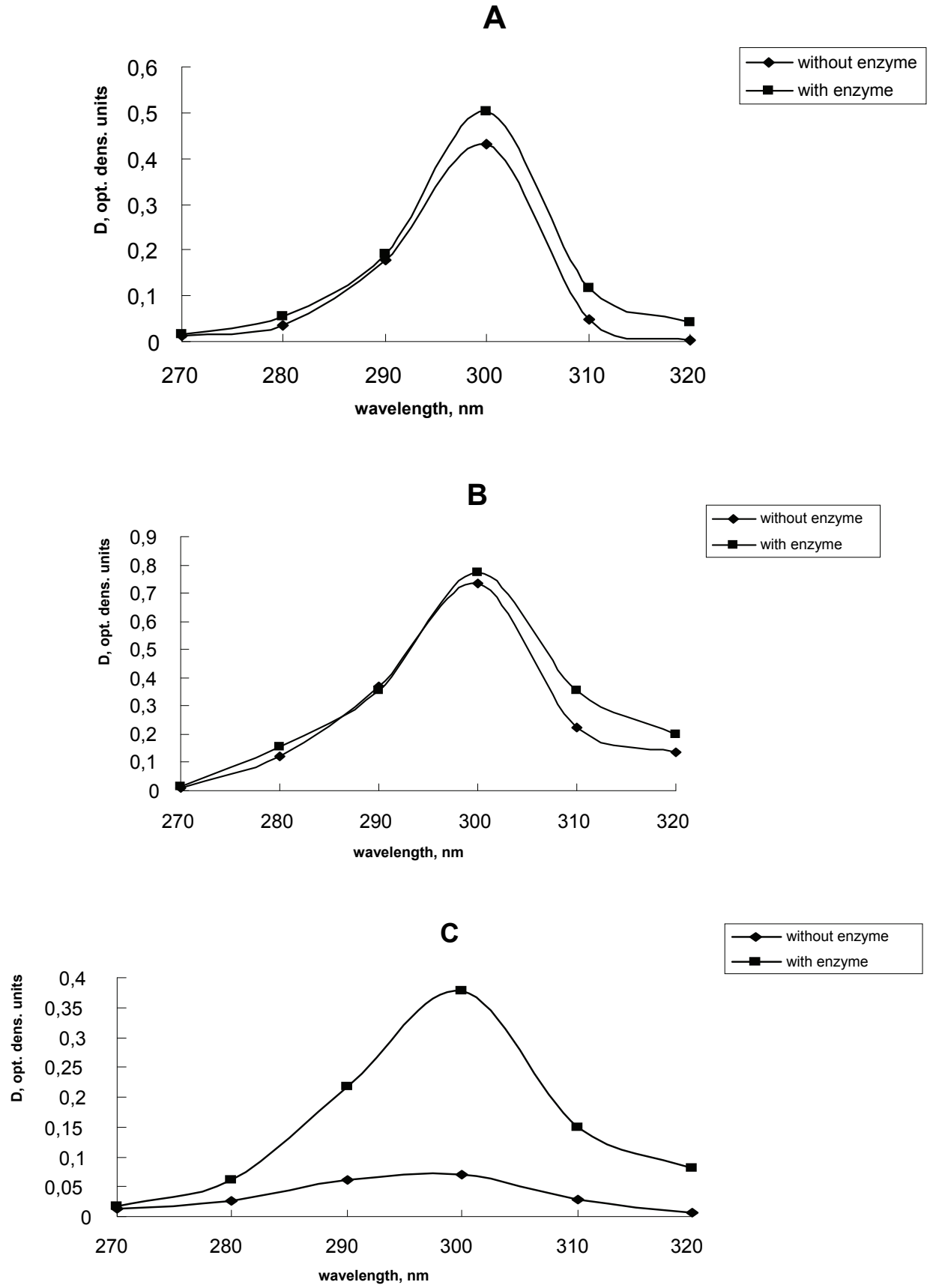

Fig. 2. Dependence of protein hydrolysis on the amount of enzyme added, at constant incubation period (1 h), hydrolysis temperature of 50°C, hydromodulus 1:1 (smelt (A), ruff (B), bleak (C))

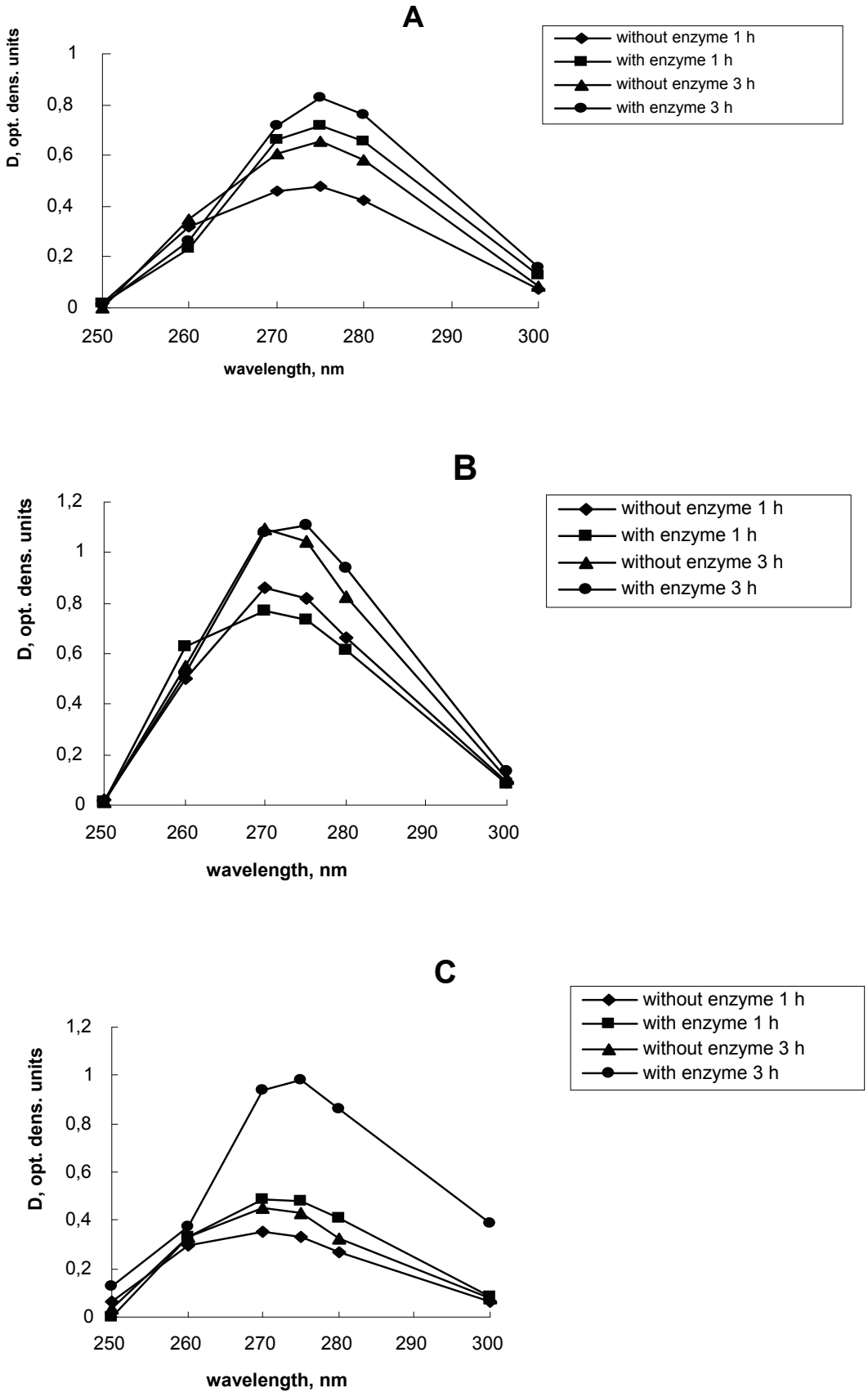

Fig. 3. Effect of the exposure duration and enzyme presence on the degree of protein hydrolysis; smelt (A), ruff (B), bleak (C))

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

the ethyl ester N – benzoyl – DL – arginine, pH 5.0 (Barrett and Heath, 1977). Elastase activity was determined after Feinstein (1973), with Suc – Ala – Ala – Ala – p – NA as the substrate.

Protein concentration in the samples was determined spectrophotometrically using Bradford (1976) assay. We used the resultant data in the process of separation and partial purification of proteinases to estimate specific activity of enzymes, which was referenced to 1 mg of protein.

Gel chromatography. All assaying was done in the cold room (+4°C). Sephadex G-100 and standard equipment by Pharmacia and LKB (Sweden) were used in chromatography. The columns were prepared and packed with Sephadex by the modified Flodin's method, recommended by Pharmacia. Sephadex G-100 swelling and rinsing were performed as described in the manual by Determan (1970).

The resultant data were processed by conventional variation statistics methods (Ivanter and Korosov, 2003). The differences were compared through Pearson's chi-square test using Statgrafics 2.0 software for Windows (Korosov and Gorbach, 2007).

As the result, proteinase activity maxima in all study objects were detected both in the acidic (pH 2.5–5.0) and in the weakly alkaline (pH 7.2–8.0) regions (Fig. 1). Owing to the presence in the cell of two types of hydrolases, active in the neutral and the acidic pH regions, the organism is provided with a wider variety of intracellular enzymes (Nemova, 1991). A known fact is that proteinases which pH optimum is in the acidic region would be found lysosome-like structures, whereas neutral proteinases are located in the intracellular fluid (Mosolov, 1971).

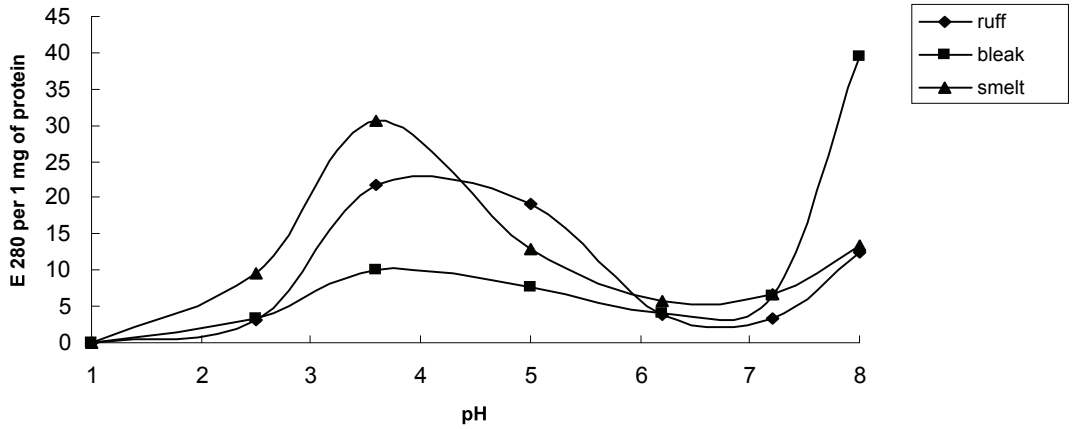

Fig. 1. Total proteolytic activity in minced low-value fishes at different ambient pH values

One can see from Fig. 1 that the proteolytic activity maximum in ruff and smelt homogenates was higher at acid pH values than at a high pH value. The situation with bleak is the opposite: the contribution of alkali proteinases to total activity is greater than that of acid proteinases. These distinctions may be due to eco-physiological characteristics of the fish species. Thus, bleak, in contrast to ruff and smelt, mainly stays in upper water layers, spawns quite late (June-July), and has different feeding habits (does not predate like ruff or smelt). The sample processing procedure might have influenced also: unlike ruff and smelt, which were assayed almost immediately after capture, bleak was stored frozen for a while, and the process of autolysis might have begun in the material. We plan to continue this work and choose certain comparable conditions for storage of the study material.

The graphs in Fig. 2 demonstrate the activity of proteolytic enzymes in ruff homogenate at an ambient pH of 2.5 to 7.7. The proteolytic activity maximum at pH 3.6 (hemoglobin as the substrate) appears to be connected with the lysosomal proteinase cathepsin D (Pohl et al., 1981; Press et al., 1960). The differences are significant (Pearson's chi-square test). The results of gel chromatography, where albumin, casein and immunoglobulin G were used as the markers, indicate the molecular weight of the enzyme protein in this fraction was about 40 kDa. Similar data were obtained for bleak and smelt homogenates.

The fractions with maximal hemoglobin hydrolyzing activity at pH 3.6 were then collected, concentrated in cells with UM-10 membrane (Amicon, USA) and used as the enzyme preparation which properties were further investigated.

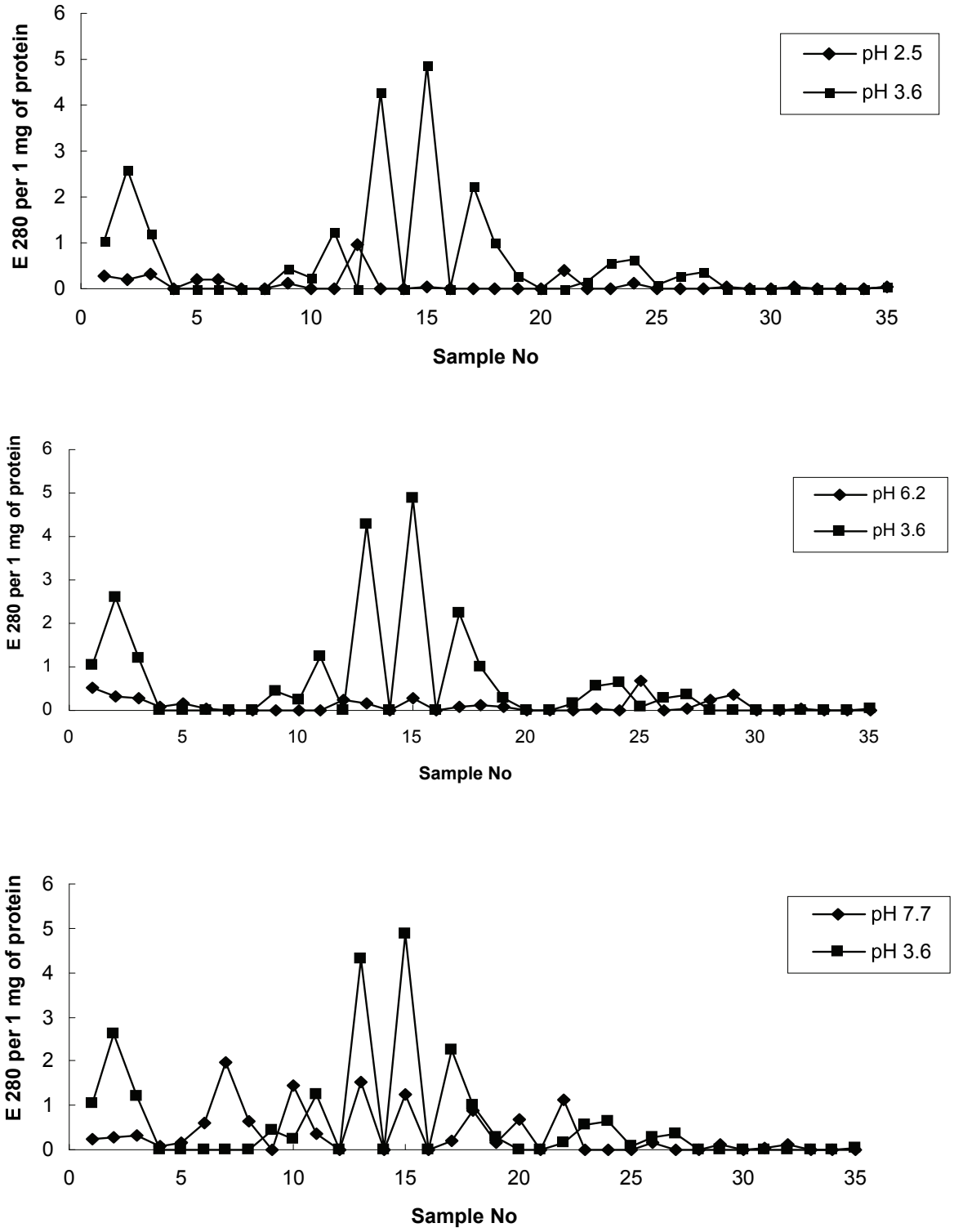

Fig. 2. Activity of proteolytic enzymes in ruff homogenate at pH 2.5–7.7

The results of application of proteinase-specific inhibitors (iodoacetate, Na_2 EDTA, Zn_2SO_4, parachloromercury benzoate (PCMB), phenylmethylsulfonyl fluoride (PMSF), Hg_2SO_4, pepstatin) indicate the range of proteolytic enzymes from the minced tissues of the fish species studied is represented at pH 2.5–3.6 by aspartyl proteinases (100% inhibition of activity by the inhibitor specific to active-site carboxyl groups – pepstatin), metalloproteinases (55–100% inhibition of activity by the inhibitors of metal ions – Na_2 EDTA and Zn_2SO_4), thiol-dependent (100% inhibition of activity by the inhibitors specific to active-

site thiol groups – mercury, iodoacetate and PCMB) and serine (100% inhibition of activity by the inhibitor specific to active-site serine groups – PMSF) proteinases (Mosolov, 1971; Barrett, 1980; Barrett and Heath, 1977).

It was found that at pH 5.0 pepstatin, iodoacetate and mercury sulphate activate the enzyme preparation (Table).

Table. Effect of various inhibitors on the activity of proteinases from smelt and bleak homogenates (% of the control)

pH	Control	iodoacetate	Na_2 EDTA	Zn_2SO_4	PCMB	PMSF	Hg_2SO_4	pepstatin
smelt								
2.5	1.99	–76	–100	–81	–87	–100	–100	–100
3.6	12.64	–80	–45	–20	–55	–31	–65	–95
5.0	6.69	50	–8	25	13	29	105	38
bleak								
2.5	0.98	–100	–100	–59	–98	–100	–100	–100
3.6	3.29	0	–33	–17	–43	–34	–80	–90
5.0	3.91	21	–63	–52	–30	–32	35	97

Thus, the characteristics of inhibition indicate the enzyme preparation produced within the study is a complex of aspartyl, serine, thiol proteinases and metalloproteinases.

Let us note in conclusion that so-called low-value fish species from lakes of Karelia can be viewed as potential raw material for composite preparations with high proteolytic activity, which can be utilized as bioactive additives to high-quality forage in fish and animal farming.

The study was supported by the RF Presidential Programme "Leading Scientific Schools of RF" grant NSh-3731.2010.4, Russian Foundation for Basic Research grant № 08-04-01140-a, RAS Presidium programme "Biological resources of Russia: 2009–2011".

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COLD ADAPTED MARINE ENZYMES

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The world's oceans cover more than 70% of the earth's surface and therefore a large proportion of life on earth is contained in these oceans and includes the largest range of habitats. The marine environment ranges from nutrient rich areas to nutrient sparse areas. In addition high salinity, high pressure, different light conditions and low/high temperature add to the complexity of the marine environment. This may contribute to significant differences between enzymes from marine organisms and homologous enzymes from terrestrial organisms.

Organisms that thrive in cold environments are referred to as psychrophiles or cold-adapted organisms. In order to survive and proliferate in the harsh cold environment, marine organisms must possess a capacity to synthesize cold-adapted enzymes. Cold-adapted enzymes have evolved a range of structural features that are necessary to perform their action at low temperatures and are in general more catalytically efficient and possess usually a lower thermal stability compared to enzymes from organisms adapted to warmer climate. These characteristics make cold-adapted marine enzymes very interesting for biotechnological and industrial purposes.

LIPID CONTENT OF MUSSELS, *MYTILUS EDULIS*, AS A BIOMARKER OF MARINE ENVIRONMENT HEAVY METAL POLLUTION

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The blue mussel, *Mytilus edulis*, is a unique bioresource of the White Sea (Bioresources of the White Sea, 2008). An important area of applied science use of these bivalves is biomonitoring and biotesting of water quality. *Mytilus edulis* satisfies a variety of criteria of a model object. One of them is the ability to accumulate high pollutant concentrations in tissues (Gudimova, 2002). Changes at the level of lipids are an essential strategy organisms employ to protect themselves against various stress factors (Kurashvili and Vasilkov, 2003). A characteristic response at the lipid composition level to adverse impact of pollutants, including heavy metals, is storage of neutral lipids, primarily triglycerides (Capuzzo and Leavitt, 1988; Chetty and Indira, 1994; Bergen et al., 2001). *Mytilus edulis* from the White Sea have also been shown to undergo a reduction in cholesterol content in response to oil pollution (Bakhmet et al., 2009). Heavy metals are high hazard ecotoxicants (Isidorov, 1999), wherefore optimization of the lipid metabolism and mobilization of its reserves notably promotes the organism's adaptation to the adverse environmental conditions. In this study we investigated the effect of various concentrations of such heavy metals as cadmium and copper on the lipid composition in gill and digestive gland from White Sea mussels, *Mytilus edulis*.

To study how the lipid composition in *Mytilus edulis* L. was modified in response to heavy metal pollution, we carried out an experiment in which the animals were kept for 24 and 72 hours in aquaria with different concentrations of copper and cadmium ions in seawater: 5, 50 and 250 μg/l and 10, 100 and 500 μg/l, respectively. The values of 5 (for copper) and 10 μg/l (for cadmium) are maximum allowable concentrations (MAC). Mussels kept under the same regime in the laboratory, but in unmodified seawater, were used as the control. Lipids were extracted by the chloroform/methanol mixture (2:1 by volume) following Folch et al. (1957). The qualitative and quantitative composition of total lipids was determined by thin-layer chromatography, as well as using spectrophotometric techniques (Sidorov et al., 1972; Endelbrecht et al., 1974).

Cholesterol level in digestive gland of *Mytilus edulis* from the White Sea decreased significantly after 24 h exposure to 10, 100, 500 μg/l and 250 μg/l of cadmium and copper ions, respectively. More prolonged (72 h) exposure of the mussels to water with heavy metals similarly influenced cholesterol content at 10 μg/l of cadmium ions and at 250 μg/l of copper ions. A reduction in cholesterol level in *Mytilus edulis* gills was confidently observed at a cadmium concentration of 10 μg/l (24 h exposure). The reduction in cholesterol content observed in digestive gland and gill of *Mytilus edulis* under the impact of various concentrations of cadmium ions is likely to be related to regulation of the lipid bilayer permeability and the activity of membrane enzymes. Cadmium inhibits the cell's ion transport systems, namely, it can replace calcium in calmodulin thus disturbing the process of cytoplasmic Ca-ATPase activation (WHO Cadmium, 1992; Kutsenko, 2004). The cholesterol decline in the mussels' digestive gland under the impact of 250 μg/l of copper ions may indicate not only the process to maintain optimal microviscosity of the bilayer and activity of membrane enzymes, but also development of a pathological process induced by irreversible degradation of lipids through peroxidation (Burlakova, 1976; Khlusov, 2003). Note however that the impact of copper ions in 5 and 50 μg/l concentrations resulted in opposite changes in cholesterol content in the gills of mussels. The rise in the cholesterol level observed in *Mytilus edulis* gills is presumably an indication protective mechanisms of lipid peroxidation (LP) have been launched, for we know that when LP intensifies the synthesis and re-synthesis of the lipid fractions (mainly cholesterol) enhancing membrane microviscosity in the cell is activated (Burlakova, 1976; Khlusov, 2003).

The digestive gland of mussels exposed for 24 h to 500 μg/l of cadmium ions exhibited a reduced content of storage lipids, mainly triacylglycerols (TAG). Contrastingly, TAG content in *Mytilus edulis* gills under the impact of copper ions in 5 and 50 μg/l concentrations (72 h exposure) rose. The changes in the content of storage lipids (namely TAG) in the tissues assayed presumably indicate a misbalance in the energy metabolism processes. Thus, the reduction in storage lipids in *Mytilus edulis* digestive gland is, in all appearance, related to energy starvation of the cell caused by suppression of the tricarboxylic acid enzyme cycle, electron transport chain, as well as ATP-dependent enzyme systems by cadmium ions (Aksyonova, 2000). The rise in storage lipid content in mussel gills is probably due to a decrease in the rate of TAG metabolism in *Mytilus edulis* as the result of adenylate cyclase inhibition by copper ions (Shpakov and Derkach, 1994). Hence, fluctuations of TAG level in the tissues of *Mytilus edulis* are targeted at mobilization of lipid stores and replenishment of the energy deficit caused by the adverse impact of heavy metals.

The resultant data on modifications of the content of cholesterol and storage lipids (mainly TAG) in *Mytilus edulis* from the White Sea under the impact of various concentrations of cadmium and copper ions suggest that fluctuations of these lipid components, alongside with transformations of the protein and carbohydrate metabolism, reflect the organism's response to the stressful environmental impact, and can be used as a biomarker indicating the degree of to which the environment is contaminated with these pollutants.

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STUDIES OF ANTIMICROBIAL PEPTIDES IN THE GREEN SEA URCHIN *STRONGYLOCENTROTUS DROEBACHIENSIS*

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Sea urchins are interesting animals to study as sources for novel compounds that might have promising activities and properties. For the first time from sea urchins, we have recently isolated and characterized two novel families of antimicrobial peptides (AMPs) from the green sea urchin, *Strongylocentrotus droebachiensis*.

The first family of AMPs, named strongylocins, is the cysteine-rich peptides isolated from extracts of the coelomocytes (blood cells). Strongylocins contain 6 cysteines and have a novel cysteine arrangement pattern when compared to other cysteine-rich peptides in the Antimicrobial Peptide Database (http://aps.unmc.edu/AP/main.php). Their putative precursor peptides contain a signal peptide, a prosequence and a native region. Their gene sequences indicate that strongylocins (except strongylocin 1b) have three introns and four exons. The mature strongylocins are active against both Gram-positive and Gram-negative bacteria. Similar genes were found in the sister species, *S. purpuratus*. Recombinant products of these genes inhibited growth of bacteria by a nonlytic, presumably intracellular mechanism.

The other family of AMPs, named centrocins, has a heterodimeric structure (a heavy chain and light chain linked by a disulfide bridge). The gene sequences of centrocins code for a signal peptide, two prosequences and a native region. These genes contain one intron and two exons. The native peptides were highly potent against bacteria. A synthetically made heavy chain had anti-fungi and anti-yeast properties in addition to being active against bacteria.

All together, these two families of AMPs from *S. droebachiensis* have promising antibacterial properties for development and future exploitation.

SEISMOCONDITIONALITY OF REPRODUCTION AND DEVELOPMENT OF FISH CONGESTIONS IN NORTHERN WATERS

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In the 16th–19th centuries already, fishermen noted the impact of earthquakes on fish: "fish try to hide away and many of them throw themselves out of the water. Sometimes fish get injured: their bladder explodes because of fast changes in pressure". This is associated with lithospheric emissions of methane, to which many aerophilic aquatic organisms have not become adapted evolutionally. In places with massive discharge of methane during earthquakes, juveniles and older fish die. The survivors disperse for several weeks and up to 1.5 months depending on the intensity of mixing, and migrate from seismic-active waters or stick to remote water flanks or "liquid" boundaries of the habitat (temperature and salinity fronts, ice margin, or deep pools). Thus, it may be concluded that after receiving information about observed seismic disturbances one should not go fishing in the affected areas because the catch will be much lower than forecasted, and economically inefficient. One should seek for the margins of the seismic-pressure areas, where survivor fish concentrate. Survived juvenile become unviable. Reproductive functions fail in the breeding part of the population, i.e. a decline in the number of fingerlings, down to the collapse, would follow in spite of any biotic and abiotic conditions. Simultaneously, the catches of mollusks also decrease, whereas the catches of crustaceans increase.

For over 50 years hydrobiologists have tried to explain the development of congestions of the majority of commercial marine organisms from the "aquarium" point of view. Spawning stock, water temperature, oxygen, availability and abundance of food items, predation pressure are considered to be the main factors limiting reproduction and survival rate. For specific organisms, ice conditions, sea level, waves, and benthic vegetation are also taken into account. When decrease in reproduction or massive mortalities are inexplicable from the "aquarium" point of view, pollution, undocumented fishery, poor-quality statistics, invader species feeding on native species, genetically dependent periodicity in the development of the organisms are referred to. Hydrobiologists are immensely perplexed when there is no recruitment in well-fed fish stocks with plentiful supply of food and heat, whereas in the situation of very low food availability fish are exceptionally fat; when the declared "genetic" periodicity in reproduction changes; and genetically dependent post-spawning mortality of fish gets no confirmation in control series.

The fact that hydrobiologists do not budget the costs of questionnaires among local people concerning the behavior of aquatic organisms adds to this situation. E.g., there is no information about extraordinary behaviors on the eve of the Gazli, Parakar, and Paravani earthquakes, when fish stuck to the shore opposite the earthquake centers, and small fish beached themselves (Lushvin, 2008). Such situations are not new and quite explainable. Since olden times people have noticed that peatland gas (mostly made up of methane) was destructive for aerophilic fish. It is manifest in unusually excited fish behavior during floods, in shelf and slope sea areas in the vicinity of earthquake centers and active faults of the Earth's crust. Economically, this is used by Melanesians on the Fiji Islands where saline lakes are inhabited by a mackerel species, yellow eye. They strictly follow a ceremony when on a certain day all inhabitants of the village enter the lakes and stir the bottom silt in every possible way. Natural gas mixed with hydrogen sulfide comes out. The half-poisoned fish emerge and are killed. The rite is in agreement with ichthyologic requirements: it takes place after spawning. After that, oxygen re-appears in the water, and the suspended water silt provides the young with first food [http://botinok.co.il/node/48542].

Methane emissions on days of earthquakes and floods are always displayed through outbreaks of methane content in bottom sediments and lower troposphere over the subsurface fields of hydrocarbons and the soft sedimentary sheath (Fig.1).

In the present study we analyze typical situations of development of aquatic organism congestions in northern aquatories in periods of seismic stress.

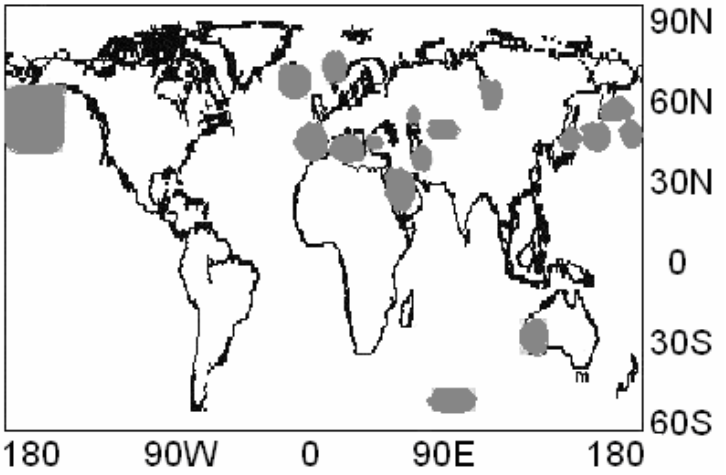

Fig.1. Local maxima of methane content in the atmosphere on the 681-hPa surface, according to the AIRS data obtained at the time of earthquakes in 2004–2006 (ftp://l4ftl01.larc.nasa.gov/TES/TL3CH4D.002/)

Comparison of aquatic organism congestion patterns with seismic activity in the Norwegian and the western Barents Seas

The absence of correlation of Atlantic cod reproduction with water temperature and zooplankton biomass (Karammushko, Mukhina, 2007) is associated with the fact that the bulk of cod spawning takes place on the inner side of the Lofoten Islands, which is inaccessible to Russian hydrobiologists, as well as with the presence of a soft sedimentary sheath with high content of methane, emitted into the water at earthquakes, off the coast of Norway (Fig. 2). The latter is indirectly confirmed by the presence of local methane maxima in the atmosphere over the region after earthquakes. The consequences of seismic stresses are disturbances of reproductive functions in 20÷100% of generations of earlier spawned fish (resorption of eggs, longer ripening) (Oganesyan, 1993). Nevertheless, hydrobiologists are very reluctant to take these facts into account, and persist in forecasting yield proceeding from the population fecundity only (Bondarenko et al., 2003).

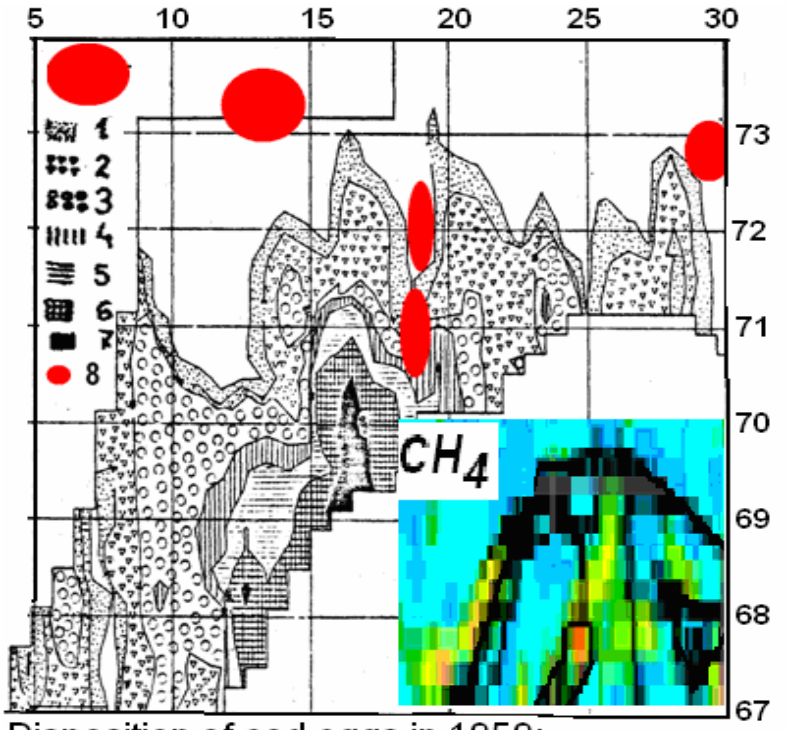

Fig. 2. Markers of the centers of presumed oil and oil-and-gas fields [http://geolib.narod.ru/OilGasGeo/1996/11/Stat/stat06.html] against the distribution of cod eggs in 1959. The insert shows methane content in the atmosphere (a.a.tronin@ecosafety-spb.ru)

Capelin suffocations have been recorded in the sea dozens of times, but neither histological analyses were made nor samples for methane determination were taken. It was assumed that capelin is genetically programmed to post-spawning death. However, the group taken out demonstrated no loss for 90 days. Cod and capelin reproduction in the region declines sharply after intensification of earthquakes in the winter and spring (spawning and post-spawning) seasons (Lushvin, 2008; Lushvin, Sapozhnikov, 2006). Specialists from PINRO specialists (Boitsov, Kovrova, 1993) believe that "the roles of biotic and abiotic factors in formation of the productivity of the Barents Sea flounder generations, including factors such as population fecundity and brood stock numbers" are yet unclear (Fig. 3). The vulnerability of flounder to the impact of lithosphere-generated toxicants was, however, mentioned in paper (Patin, 1997) already. The survival rate of flounder in the Barents Sea is low on the spawning years with increased seismic activity and vice versa. The catches of sprat and ling in the Norwegian Sea are also limited by seismic stress (Fig. 4).

Comparison of aquatic organisms and marine mammals congestion patterns with seismic activity in the White Sea

Herring reproduction in the White Sea is limited by water temperature and seismic stresses, because food and oxygen reserves are sufficient (Fig. 5). In earthquake years, and sometimes in the subsequent year, herring yield is always low! As energy consumption for reproduction decreases, the fish weight grows (Lushvin, 2008). The catches (reproduction) of navaga in the White Sea are also limited by seismic activity (Fig. 6). One or two years after earthquakes navaga catches exhibit local minima. In years with low seismic activity, 70 to 100% of individuals are ready to spawn at an age of 2^{+}. In seismoactive years, their proportion decreases to 30%. Six-eight years after intensification of seismic activity, the take of seals collapses as a consequence of decline in the food resources (navaga) during the periods of puppying, nursing, and feeding of the young (Yakovenko, 1963).

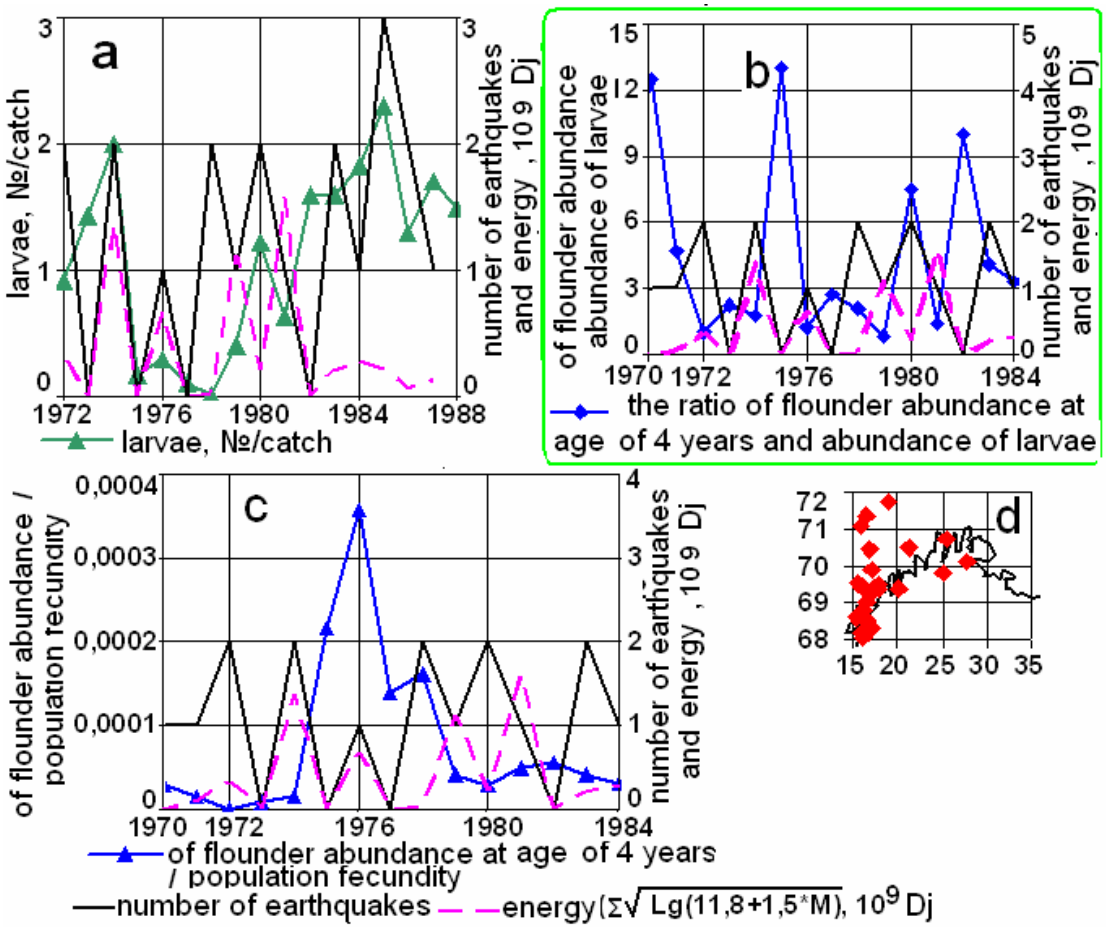

Fig.3. Comparison of seismic activity in the region (with the 4-year lag) with the flounder abundance at an age of 4 years/larvae abundance ratio. The insert shows earthquake epicenters

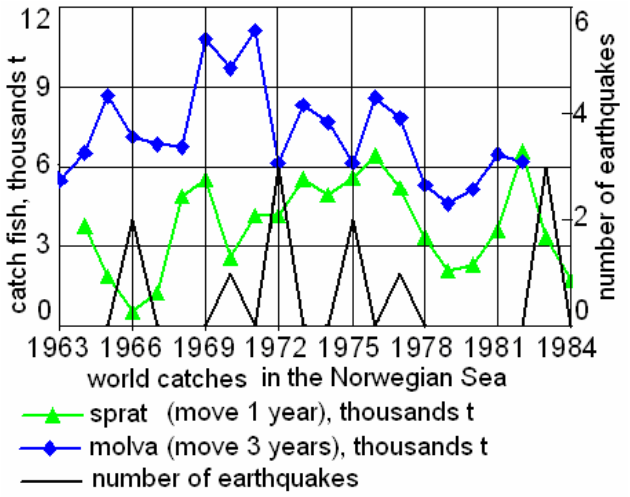

Fig.4 Comparison of world catches of ling and sprat in the Norwegian Sea with regional seismic activity

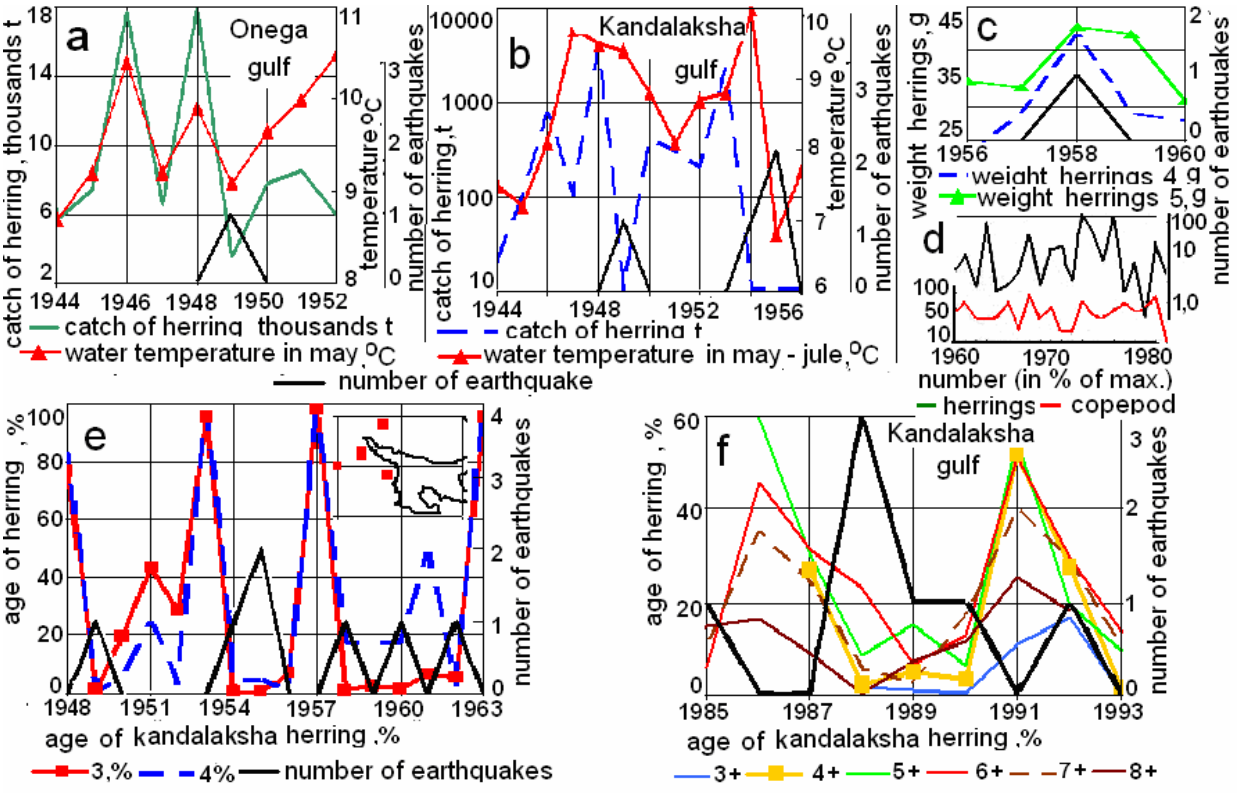

Fig. 5. Comparison of herring catches (a, b), herring weight, and copepod abundance (c), as well as the age structure of herring (d, e) with the number of earthquakes

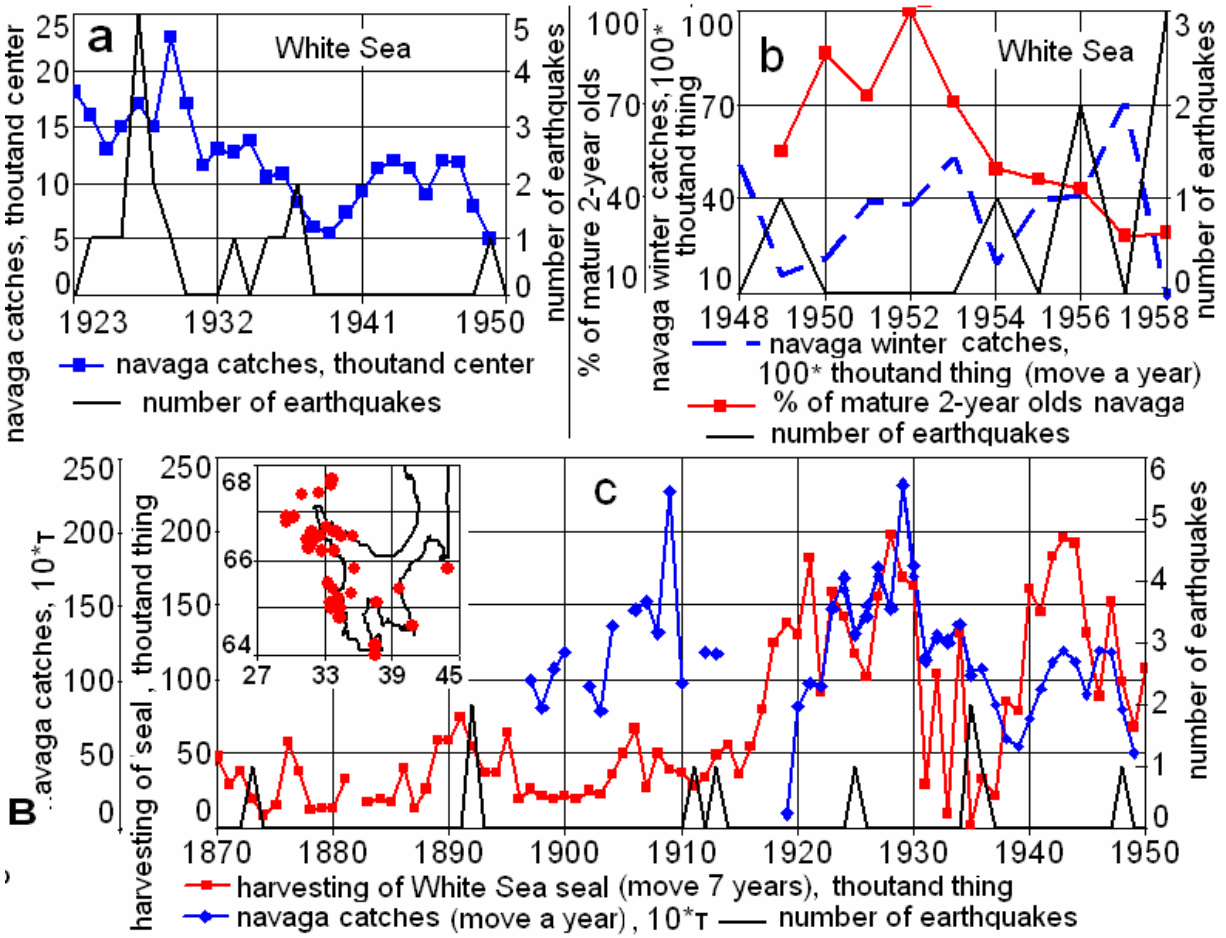

Fig. 6. Comparison of the number of earthquakes with navaga catches,% of mature 2-year olds, and harvesting of the White Sea seal (insert shows epicenters of recorded earthquakes)

Seismogenic emission of methane causes replacement of salmonids with coregonids, and further with less aerophilic osmerids and cyprinids (Klyashtorin, 1982; Reshetnikov, 1986). Peak discharges from water engineering facilities also promote this process because, carried out on dates not justified by the nature, abruptly raising the level and accelerating the flow, they stir up floodplain silts and methane contained there is carried into the reservoirs. Comparison of benthos biomass with methane content indicated the extremely negative response of zoobenthos to methane (Fig. 7) (Sergeeva, Gulin, 2007). In water areas with temporarily impoverished benthos, fatness of benthophage fishes decreases, their visits to these places become fewer, even though they may be the traditional feeding areas, and hypoxia tends to develop in the near-bottom water layers (Lushvin, Karpitsky, 2009).

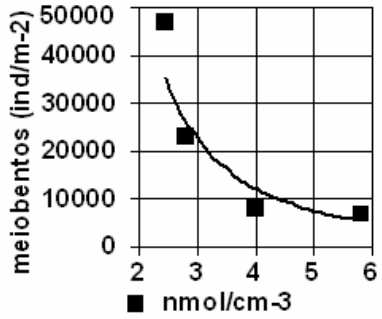

Fig.7. Ratio between meiobenthos (0.1÷1 mm) abundance in the top 5-cm layer of sediments and methane content in the top 25-cm layer of sediments in the paleobed of the Dnieper Canyon (eam of the Donuzlav Bay)

Comparison of aquatic organisms congestion patterns with seismic activity in the Pechora region

In the Pechora region, reproduction, survival rate and catches of polar cod, Atlantic salmon and navaga are determined, alongside with thermal conditions, by seismic stresses (Fig. 8a). In his presentation at the Azov Conference G. Matishov described rather unusual behavior of polar cod on days of the Novaya Zemlya earthquakes – the fish huddled up to brackish Pechora waters (Lushvin, Sapozhnikov, 2006) (other fishery actors also mentioned this fact). The percentage of 3-year old navaga under low seismicity conditions is higher than a year later, and vice versa (Fig. 8b). In years with earthquakes, Atlantic salmon reproduction is below the average, irrespective of the water temperature, and its catches decrease subsequently (Fig. 8c). Fish catches in the downstream of the Pechora River are also in many respects determined by seismic stresses. The catches of aerophilic grayling, whitefish, and pike decrease after a seismic stress, while the catches of crucian carp and vendace, on the contrary, increase in the absence of predators (Fig. 8d).

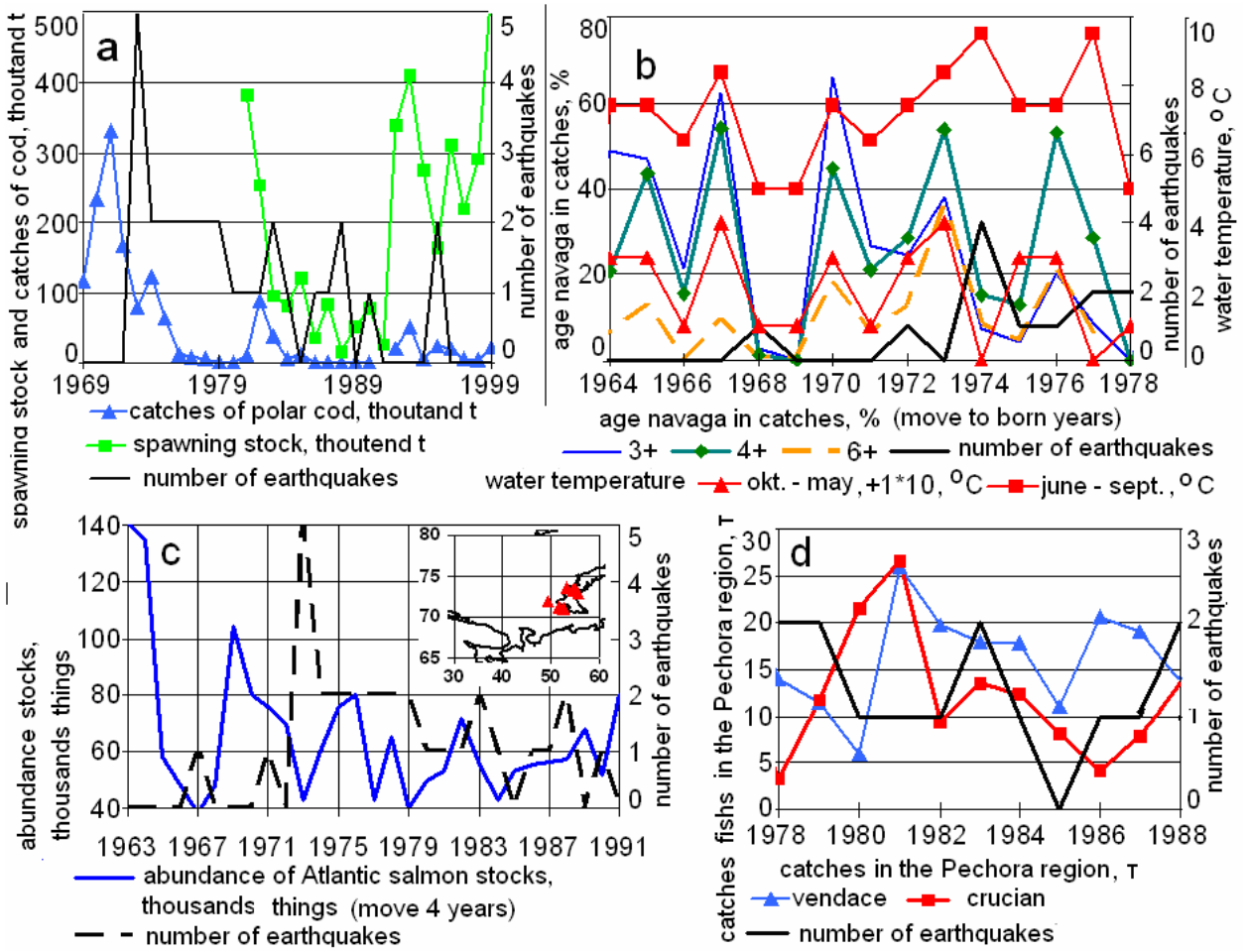

Fig. 8. Comparison of the recorded number of earthquakes with catches of polar cod, navaga, abundance of Atlantic salmon stocks, catches of vendace and crucian carp in the Pechora region (a-d)

Current redfish catches

Current redfish catches in the Norwegian and Barents Seas after seismic stresses decline relative to the monthly average by 20–60%. Such "undercatches" occur either in the beginning (1997) or at the end (2001) of the fishing season. Seismic stresses even "split" the usually unimodal peak of catches into a bimodal one (1999, 2002, 2003), thus extending the fishing season from 3 to 4–5 months (Fig. 9). March and April earthquakes are the most harmful for fishery.

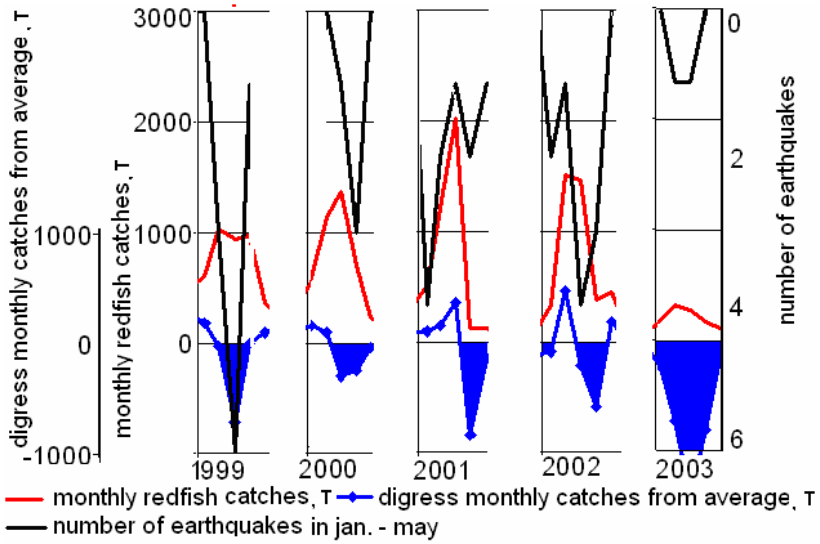

Fig. 9. Comparison of monthly redfish catches by Russian vessels in the Barents and Norwegian Seas with the number of earthquakes from February-March to May-July

Conclusions

In waters subject to massive discharge of lithospheric fluids from seismic activity:

1. Juveniles of many aerophilic commercial species die.

2. Whatever the biotic and abiotic conditions are, the reproductive functions in survivor juveniles are disturbed (decrease in juvenile resilience, sharp decline in fingerling abundance).

3. Commercial congestions of fish temporarily disappear. This implies that after receiving information that an earthquakes has happened, the fishery in the "infected" areas should not be halted because the catches will be much lower than forecasted, and economically unprofitable (or else, one should find areas differing in temperature, salinity or depth, and otherwise not wonted by fish, where the fish had migrated).

4. After anomalous seismicity years, it is necessary to plan potential catches for years ahead, taking the collapse of fish reproduction into account.

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WATER ORGANISMS AS A SOURCE OF PROTEASES AND ITS INHIBITORS. CALCIUM-DEPENDENT PROTEASES (CALPAINS)

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Proteases are enzymes that are essential to all life. They are biology's version of Swiss army knives (Seife, 1997) that cut up biological polymers. Proteases regulate the fate, localization, and activity of many proteins, modulate protein-protein interactions, create new bioactive molecules, contribute to the processing of cellular information, and generate, transduce, and amplify molecular signals. As a direct result of these multiple actions, proteases influence DNA replication and transcription, cell proliferation and differentiation, tissue morphogenesis and remodeling, heat shock and unfolded protein responses, angiogenesis, neurogenesis, ovulation, fertilization, wound repair, stem cell mobilization, hemostasis, blood coagulation, inflammation, immunity, autophagy, senescence, necrosis, and apoptosis (López-Otín and Bond, 2008). Proteases are also essential in viruses, bacteria and parasites for their replication and the spread of infectious diseases, as well as for effective transmission of disease, and in animal hosts for the mediation and sustenance of diseases (Turk, 2006).

The recent availability of the genome sequence of different organisms has allowed the identification of their entire protease repertoire (termed degradome) (Quesada et al., 2009) (table). Thus, the human degradome contains over 500 human proteases (561 known proteases, 175 putative proteases and pseudogenes, 400 inactive homologues), accounting for 2% of structural genes in humans.

Table. Complexity of degradomes (data from *MEROPS* and *Degradome* Databases)

Organisms	Representative species	Number of proteases
Mammals	Homo sapiens	561
	Rattus norvegicus	646
	Mus musculus	656
	Ornithorhynchus anatinus (platypus)	>500
Birds	Gallus gallus	382
Fish	Danio rerio	503
Amphibia	Xenopus tropicalis	278
Insects	Drosophila melanogaster	558
Nematodes	Caenorhabditis elegans	403
Plants	Arabidopsis thaliana	723
	Populus trichocarpa	955

Many families of human proteases are also clearly recognizable in the genomes of *D. melanogaster*, *C. elegans* and *A. thaliana*. This indicates the existence of universal proteolytic routines in these organisms, although they are frequently expanded in vertebrates. It has become evident that, in addition to highly conserved proteolytic routines, there are also specific roles played by unique proteases in different species. These comparative genomic studies have also provided valuable insights into the conservation, evolution, and functional relevance of this group of enzymes.

Proteases likely arose at the earliest stages of protein evolution as simple destructive enzymes necessary for protein catabolism and the generation of amino acids in primitive organisms. Through evolution, proteases have adapted to the wide range of conditions found in complex organisms

(variations in pH, reductive environment and so on). Despite proteases share a common biochemical function, their catalytic domains exhibit high sequence diversity. Thus catalytic core of proteases characterizing mechanisms of action classifies them as either serine, cysteine or threonine proteases (*N*-terminal nucleophile hydrolases), or as aspartic, metallo and glutamic proteases (with glutamic proteases being found only in fungi) (Rawlings et al., 2010). Protease diversity is further increased by the frequent attachment of ancilliary, non-proteolytic domains to the catalytic moieties (López-Otín and Overall, 2002). A variety of specialized functional modules that provide substrate specificity, guide their cellular localization, modify their kinetic properties, and change their sensitivity to endogenous inhibitors. These non-catalytic domains include archetypal sorting signals that direct these enzymes to their proper location, autoinhibitory prodomains that prevent premature activation, and ancillary domains that facilitate homotypic interactions or heterotypic contacts with other proteins, substrates, receptors, or inhibitors. It is very likely that the substantial combinatorial activity observed in protease genes has been a driving force in the protease transition from nonspecific primitive enzymes to highly selective catalysts responsible for subtle proteolytic events that are at the heart of multiple biological processes.

The complexity of proteases is further increased through post-transcriptional events such as alternative splicing and differential polyadenylation of genes encoding proteases (Freije et al., 1994; Mitsui et al., 2008), by the occurrence of gene copy number variations or polymorphic variants that may contribute to the modification of protease functions or alter their regulatory mechanisms (Masson et al., 2008), or by post-translational modifications. Finally, proteases act in the context of complex cascades, pathways, circuits, and networks, comprising many protein partners that dynamically interact to form the so-called protease web (auf dem Keller et al., 2007).

All known endogenous protease inhibitors are proteins, although some microorganisms produce small non-protein inhibitors. To date, the number of identified endogenous inhibitors is considerably lower than that of proteases. As an illustrative example, a total of 105 genes encoding protease inhibitors have been annotated in the human genome, and there are only 1–2 inhibitors per prokaryote genome. Nature inhibitor specificity varies from one target protease (such as calpain for calpastatin) to numerous proteases of several catalytic types (such as plasma α_2-macroglobulin).

Consistent with the essential roles of proteases in cell behavior, survival and death, alterations in spatiotemporal patterns of expression of proteases or abnormal levels of natural inhibitors/activators underlie multiple pathological conditions such as cancer, neurodegenerative disorders, and inflammatory and cardiovascular diseases. Furthermore, mutations in protease genes result in over 80 hereditary diseases in human (Puente et al., 2003). The key role of proteases and protease inhibitors in many physiological and pathophysiological processes makes them attractive targets for pharmaceutical industry as potential drug targets or as diagnostic and prognostic biomarkers (Leung et al., 2000; Turk, 2006). Their best-known representatives include angiotensin-converting enzyme (ACE) inhibitors and HIV protease inhibitors. Finally, proteases are also important tools of the biotechnological industry because of their usefulness as biochemical reagents or in the manufacture of numerous products (Saeki et al., 2007).

Calpains (EC 3.4.22.17) or Ca^{2+}-dependent cysteine neutral proteases along with cathepsins and proteasome are one of the main proteolytic systems present in any cell of various organisms – from protozoan to humans. Total protein degradation in cells is probably the result of the synergistic proteolytic action of proteases indicated above (Ouali et al., 1992; Goll et al., 2003), even if only calpains are sometimes described to mediate the proteolysis or the early stage of this process (Ladrat et al., 2000). Calpains are constitutive enzymes, but as noted by Cottin et al. (1994), they should not be considered as housekeeping enzymes because the transcription of the calpain gene could be regulated. Although definitive physiological roles are not yet clearly identified, calpains, having regulatory or signaling function in cells rather than a digestive function such as the lysosomal proteases or the proteasome (Goll et al., 2003), are believed to participate in numerous cellular and physiological processes: proliferation, differentiation, cell migration, signal transduction, skeletal muscle growth, metabolic disorders or degenerative diseases, and cell death (via necrosis or apoptosis) (Goll et al., 2003; Tidball and Spencer, 2000; Wang, 2000).

Calpains constitute divergent protease family C2 (cysteine protease clan CA) (*MEROPS* database) consisting of 643 nucleotide sequences (14 in human), 26 identifiers, including 3 proteins of known tertiary structures. Typical members are composed of four domains, including prodomain, catalytic domain of

cysteine protease (catalytic triad $Cys^{105}/His^{262}/Asn^{286}$), C2-like domain, and calmoduline-like domain with five EF-hand Ca^{2+}-binding motifs. Catalytic core are highly conservative among family. Atypical calpains contain ancillary functional domains instead of EF-hand-containing domain IV. Despite the absence of Ca^{2+}-binding EF-hand motifs all known calpains are Ca^{2+}-dependent (Croall and Ersfeld, 2007). Ubiquitously expressed calpains 1 and 2 form heterodimers with regulatory small subunit, other calpains are monomeric proteins expressed mainly in tissue-specific manner (Goll et al., 2003). Some calpain-dependent human diseases, so-called calpainopathies, are described. Some of them are hereditary, such as limb-girdle muscular dystrophy type 2A associated with single-nucleotide mutation in calpain 3 gene, whereas other depends on regulatory defects such as calcium inbalance.

The calpain proteolytic system in vertebrates consists of at least three components: 1) the form of the protease that is fully active at micromolar concentration of calcium (μ-calpain), 2) the form of the proteinase that is fully active at millimolar concentration of calcium (m-calpain), and calpastatin, which inhibits the activity of both μ- and m-calpains at their respective calcium requirement. However the structural and biochemical features of invertebrate calpains and contribution of calpain-mediated proteolysis in metabolic response reactions due to variable factors have not defined satisfactory yet (Mykles, 1998).

We studied calpain system in numerous freshwater and marine invertebrates and fish: Annelida (*Stylodrilus heringianus*, *Herpobdella octoculata*), Crustacea (*Asellus aquaticus*, *Gammarus* spp., *Polyphemus pediculus, Daphnia pulex*), Mollusca (*Limnaea intermedia, L. polustris, Viviparius viviparius, Planorbis planorbis, Dreissena polymorpha, D. bugensis*, *Mytilus edulis* L., *Unio longirostris*), Insecta (larvae of *Erythromma najas, Limnephilus stigma*, *Siphlonurus linneanus*, *Acilius* spp., *Chaoborus* spp.), Esocidae (pike *Esox lucius* L.), Cyprinidae (roach *Rutilus rutilus* L., crucian carp *Carassius carassius* L.), Percidae (perch *Perca fluviatilis* L.), Coregonidae (whitefish *Coregonus lavaretus*, *C. albula*), Gadidae (navaga *Eleginus navaga* Pall.), Salmonidae (Atlantic salmon *Salmo salar* L., rainbow trout *Salmo trutta* L.). Calpain-like activity was detected in all studied organisms have atypical calpains. The highest level of calpain activity was observed in the most primitive organisms (annelid worms) (Kantserova et al., 2010). Marine invertebrates (mussels and crustacean) are also a good source of calpains due to high expression and more simple purification procedure in the absence of endogenous inhibitors. It was confirmed that genes of calpastatine *CAST* and regulatory small subunit *CSS* are found only in vertebrates (fish). So invertebrates contain only monomeric calpains which are inherently not susceptible to calpastatin regulation. Fish contain both typical and atypical calpains as well as non-proteolytic homologues. Analysis of structural composition of fish and invertebrate calpains has shown that all known ancillary domains (T, PalB, Zn-finger, SOL, additional C2-like) are presented (Goll et al., 2003; Bondareva et al., 2008). Besides invertebrates calpains are known to have wider substrate specificity than vertebrate calpains. Thus myofibrillar proteins actin and myosin are substrates for crustacean calpains (Mykles, 1998) but not for vertebrate calpains. Due to easier degradome composition, protease abundance, lack of some regulatory mechanisms and wider substrate specificity invertebrates (especially marine invertebrates – crustacean and mussels) are a rich source for calpains. Possessing all human calpain homologs fish and invertebrates are a good model to study basic calpain-mediated processes such as proliferation, cell cycle, biogenesis of organelles, autophagy as well as inducible calpain-dependent disorders: muscular dystrophy, cataractogenesis, necrosis, etc.

Despite indisputable academic interest there are some practical aspects of calpain-calpastatine study. Pharmaceutical industry needs: (1) drug design on the basis of calpastatin inhibitory sequences, (2) recombinant tissue-specific calpains to treat hereditary deficit. Consider role of calpain-calpastatine system in the postmortem tenderization process (Koohmaraie, 1992) the genetic modification of cattle and fish breeds is a tool to enhance quality of meat and fish fillet. There are some successful examples such as cattle and fish breeds characterized by μ-calpain overexpression, specific SNPs in μ-calpain gene (*CAPN1*) or reduced calpastatine expression (Chéret et al., 2007; Salem et al., 2007). Furthermore, a net increase in the calpain/CAST mRNA ratio with a corresponding increase in calpain catalytic activity induced by starvation was shown in rainbow trout (Salem et al., 2005).

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PROTEOLYTIC ACTIVITY OF SQUID PROTEINS

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Formation of the current market of food products satisfying various demands is impossible without development and introduction of underused but nutritive valuable industry objects into processing.

The promising raw material but little used in the fishery industry are Commander squid (Berryteuthis magister) and Humboldt squid (Dosidicus gigas). Lack of recommendations on the fishing period, types of cutting and manufacturing of squid leads to the situation that the product yield might not exceed 30% (of frozen raw material) when processing squid, that is the main cause of underuse of this type of raw material. According to the data from literature such low yield values can be explained by high proteolytic activity of squid enzyme systems.

Data on the chemical composition show that squid is a protein product with low fat content. Results of the studies on amino-acid protein composition and fatty acid lipid composition of squid muscular tissue show its high biological value. It all makes squid a promising product for children's food.

Conclusion to the interconnections between proteinase activity, squid fishing period and types of cutting will be made on the basis of the received data on the integrated study of squid. It will permit to develop scientifically based recommendations on the terms of manufacturing of squid which provide producing the goods of guaranteed quality with high customer properties, met all requirements including those for children's food products.

The objects of the present study were the following samples of Commander squid – males and females of three age group: young species, species of prespawning season and species of spawning season, and also sample of Humboldt squid mantle.

The squid mantle weighing 20–30 gram was thoroughly chopped then 50 ml of buffer (0.05 M Трис-HCl, pH 8.0, containing 10mM NaCl) was poured over, repeatedly homogenized and kept while agitation for 2 hours at the temperature of 7^{o}C. Further the samples were centrifugated for 25 minutes at 3000 rpm; supernatant fluid was sampled and filtered for producing homogeneous solutions, herein after referred to as extracts.

The determine proteolytic activity the following chromogenic substrates were applied: Bz-Arg-pNa (BApNa); Z-Ala-Phe-Arg-pNa (Arg); Suc-Ala-Pro-Phe-pNa (Phe). 500 mcl of buffer (100mM Tris-HCl, pH=7.5), 400 mcl of water and 10 mcl of protein extract solution were introduced into test tubes to carry out enzymatic reaction.

After preliminary incubation of extracts in thermostat for 1 minute at 37^{o}C, 10 mcl of substrate was added, then the extracts were kept for 10 minutes more.

Further the reaction was stopped by introducing 80 mcl of 50% solution of trichloracetic acid into all test tubes (except check test tubes). Final volume of reaction mixture was 1 ml.

Optical density of the reaction mixture was measured at the wave length of 405nm against the relevant control. At the same time the unit of activity was considered to be such amount of enzyme which decomposes 1 micromole of substrate in one minute at the said conditions. We consider specific activity to be a unit of enzyme activity referred to 1 mg of protein in the sample.

The specific activity was evaluated by the formula:

Specific activity, mole/mg*min = $A_{405}/\varepsilon*c*t$, where

A_{405} – absorption at the wave length of 405 nm;

ε – extinction coefficient, equal to 8900 $M^{-1}*cm^{-1}$;

c – protein concentration, mg/ml;

t – reaction time, min.

Electrophoresis was carried out in denaturant non-reducing condition (with 0.1% solution of sodium dodecylbenzenesulfonate, SDS) in plates of polyacrylamide gel (PAAG) with separating 12% PAAG and stacking 4% according to the method of Lammy.

The method of denaturing electrophoresis was applied when carrying out zymography, the gel was copolymerized with gelatin in concentration of 0.05% at the stage of preparation. Set of standard protein-markers of the known molecular mass was used as markers both at the electrophoresis and zymography.

The gel was colored with solution of Kumassi R-250. After electrophoresis the gel with gelatin was immediately washed in 2.5% Triton X-100 (30 minutes) and water (3x10 minutes) then it was kept in tris buffer to carry out enzymatic reaction at the various pH for 1 hour at 37^0C. Further it was colored with the solution of Kumassi R-250, at the same time, the colorless areas were appeared at the places of gelatin hydrolysis (substrate) as a result of decomposition of gelatin by enzymes.

The obtained results show that the highest protein content is observed in the extract produced from commander squid of spawning season (male) as well as in the extract of humboldt squid. The data can indicate that tissues of different species destruct in the different way depending on the squid type, its gender and maturity stage.

When determining of activity of proteinases of trypsin and chemotrypsin type by the method of electrophoresis, zymograms and also when applying low-molecular substrates (pH ranging from 4,0 to 8,0) it was found that all the extracts were active on the most available substrates – BapNa, on which all further studies were carried out.

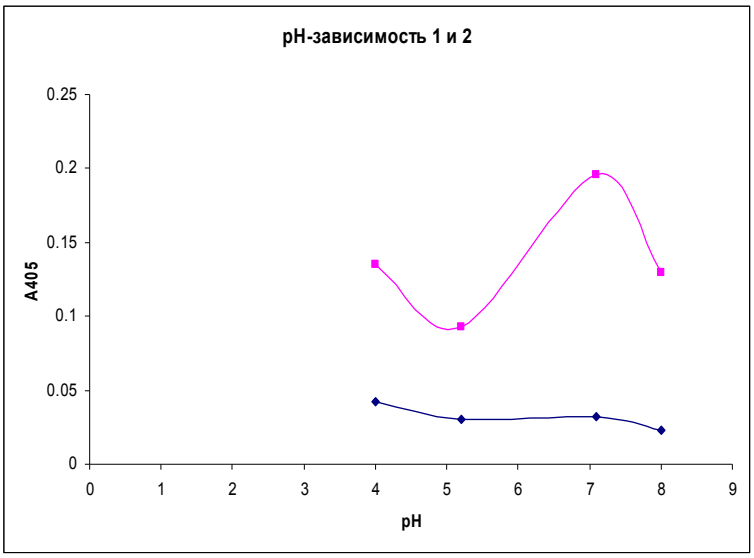

Fig. 1. pH-dependence of proteolytic protein activity of the squid of prespawning season (■ – ♀, ▼ – ♂)

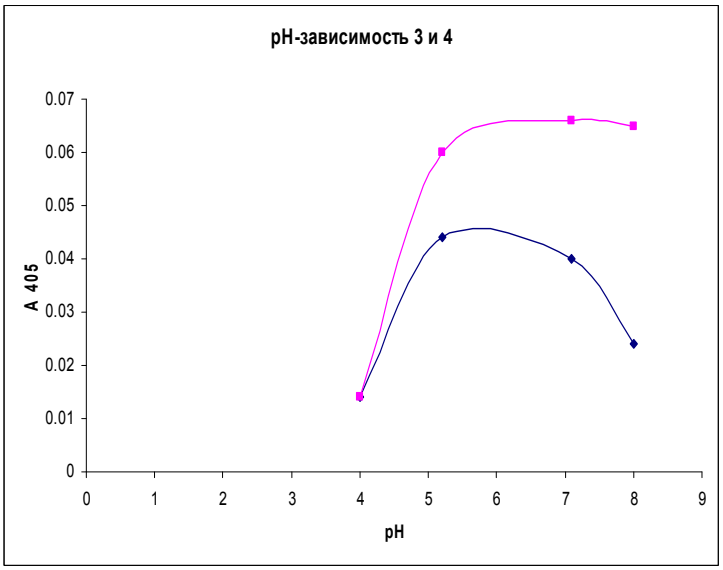

Figure 2. pH-dependence of proteolytic protein activity of the young squid (■ – ♀, ▼ – ♂)

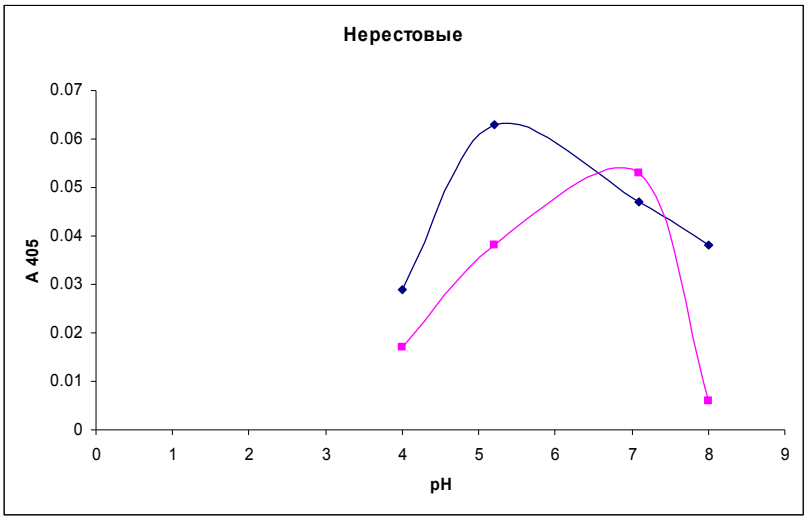

Fig. 3. pH-dependence of proteolytic protein activity of the squid of spawning season (■ – ♀, ▼ – ♂)

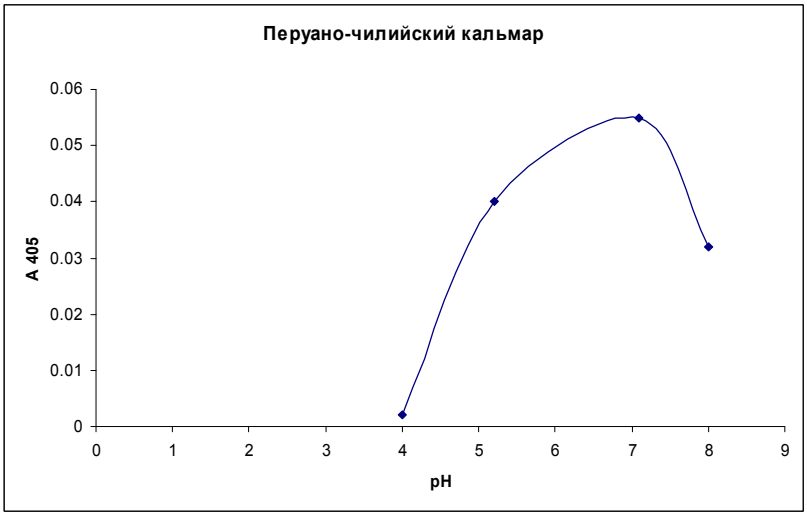

Figure 4. pH-dependence of proteolytic protein activity of Humboldt squid

As it is evident from the results presented in Figures 1–4, regardless of the gender, the young squids have protein-enzymes in mantle showing proteolytic activity in a wide range of pH values – from 4.5 to 8.0 (Fig. 2). In prespawning maturity stage pH-optimum for activity of the extracts of squid female specimen is 7.5 and 4.0 (Fig. 1), and pH-optimum of proteolytic enzyme activity of male squid specimen is shifted in the acidic region 4.0. The proteinase pH-optimum of female specimen of spawning period is 7.5, and male specimen – 5.2 (Fig. 3). pH optimum for the activity of the extracts from a sample of the mantle Humboldt squid is 6,0–7,0.

The enzymes of all extracts in the range of 55–60 kDa show proteolytic activity. At the same time the enzymatic activity depending on pH values are not appeared. Probably it is connected with the fact that proteases with different pH-optimum values have the same or sufficiently close molecular masses.

When analyzing pH-stability of squid proteinases the trend of loss of activity is seen during incubation with increase in pH, which persists regardless of the pH-optimum of the protease activity and the squid type. If the pH value (at which the enzymes were preincubated) coincided with its pH-optimum, then about 20–25% of enzyme activity was remained.

Conclusions

The specific activity of squid proteases increases from young species to prespawning maturity stage species at pH 4.0, and then decreases significantly to the spawning stage species. At pH = 8.0, this activity is similar for young and pre-spawning species, but in the extracts of spawning squid also reduced in 2.5–3 times.

Upon squid maturing the pH-optimum of proteinases varies over a wide range – from 5.0 to 8.0 for the young species, in a narrow range – for the squids of spawning maturity stage, distinguishing by the gender: the pH-optimum of females – 7.5, males – 5.2.

By the method of zymogram it is found that chiefly enzymes with molecular mass of 55–60 kDa have gelatinous activity.

The study of the stability of squid proteinases at different pH values was carried out and a quite rapid loss of activity when increasing pH value to the alkaline region was shown.

PALE ARCTIC COMMON TAIMEN *HUCHO TAIMEN* WITHIN THE REPUBLIC OF SAKHA (YAKUT) – SPAWNING BIOLOGY AND ARTIFICIAL REPRODUCTION

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There are two species of taimen – common taimen *Hucho taimen* (Pallas, 1773) and Sakhalin one *H. perryi* (Brevoort, 1856), inhabiting rivers of the Russian Federation. Common taimen (or taimen) is one of the largest members of family Salmonidae that reaches 2.1 m in length, 105 kg in weight, and 60 years old in age. Its valid Latin name is *Hucho taimen* (Pallas, 1773) which has undergone five revisions made by P.S. Pallas himself. As a result this fish periodically changed its generic, specific or sub specific nomenclature. Therefore, the junior synonym is considered to be *Salmo fluviatilis*, the senior one – *Hucho hucho taimen,* other synonyms are *Salmo taimen* and *Salvelinus taimen* (Froese, Pauly, 2010).

In Russia, taimen is known under different local names: Russian – krasulya, len', talmen, Yakut – beal, Evenki – chonkchur (Sivtseva, Mikodina, 2009); in the English literature it is met as Siberian taimen and Siberian salmon. This species is an object of commercial fishing, game fishing and amateur one (Kirillov F.N., 1972; Kirillov A.F., 2002, 2009; Kirillov A.F. et al., 2007; Sidorov, Tyaptirgyanov, 2004) and aquaculture (Zelyonkin, Fedorova, 1997; Korablina, Ivanova, 2001; Mikodina, Lyubaev, 2005; Kouřil et al., 2009). Being a predator, taimen has delicacy meat and red caviar.

In the territory of the Republic of Sakha (Yakut) as part of Pale Arctic, taimen inhabits the rivers of the Arctic Ocean Basin running to the Laptev Sea. These are large rivers of different length – Undyulyung (*syn* Yundyulyun) R. (414 km), Omoloy R. (593 km), Yana R. (906 km), Anabar R. (939 km), Olenyok R. (2292 km), Lena R. (4400 km), and also south Lena River tributaries: left – Vilyui R. (2650 km), and right – Aldan R. (2273 km). Taimen habitats in Pale Arctic of the Yakut are extremely severe. For instance, the lower Lena R. with its tributaries is located not only beyond the North Polar Circle, but it is also found within the permafrost zone.

In Russia, taimen is included in the Red List Data of the Republic of Altay and Altay territory, Krasnoyarsk territory, Republic of Tyva, and Republic of Buryatiya. Its stock in the Yakut water systems steadily decreases, though it has not reached the critical level yet. In this connection, in this Republic, unlike other Russian Federation territories, it has not included in the Regional Red List Data yet. The stock reduction of taimen in the Yakut water systems is proved by the dynamics of catches (excepting the Great Patriotic War of 1941–1945): in 1940 – 25 tons, 1943 – 179 t, 1945 – 71 t, 1950 – 27 t, 1960 – 26 t, 1970 – 9 t, 1980 – 16 t, 1990 – 10 t, 2000 – 3 t. Since 1999, commercial fishing of taimen in the Republic is banned, and its fishery is allowed only as a by-fishing (10%) (see Sidorov, Tyaptirgyanov, 2004). According to the official statistics, in the zero years of XXI century its catches did not exceed 6 t. Thus, in 2006 the catches of taimen were 5 t, in 2007 – 3.9 t, in 2008 – 5.7 t, in 2009 – 5.98 t. By July, 2010 it has been caught 26.2 t, the Total Allowable Catch (TAC) for 2011 is estimated to be 28 t. Slightly less than half the TAC is allocated for recreational requirements, including the Lena R. – 8.5 t, in the Anabar and Olenyok Rs. together – 2.1 t.

The biology of taimen as an economically valuable species in the Yakut rivers is actively studied. It is a freshwater benthopelagic species. By the ecological classification it is considered to be a potamodromous fish, i.e. its migrations are carried out completely in fresh water (Froese, Pauly, 2010). It does not form great aggregations in the Lena R., being caught in this water basins only in summer (during the post spawning season) in the site between the settlement of Zhigansk and the settlement Jordjan, while in autumn – on the water sand grounds of Joldjongo and Searey-Kumakh (Sivtseva, Mikodina, 2008). During spring migration taimen comes for spawning into some Lena R. tributaries of the first and second orders. The most known sites of taimen spawning are found in the left (Motorchuna and Moona Rs.) and the right (Undyulyung R.) tributaries of the Lena R.

The limit age of the Yakut taimen, according to Kirillov (2002), is not less than 13 years old, average weight at this age is 8.1 kg; but individuals more than 80 kg in weight are met in Yakut. By our data, taimen females reach the age of 16+. Taimen becomes mature at the age of 7–8 years. In the wild it spawns in the late May-early June, its fecundity reaches 20 thousand eggs. More comprehensive and specified data on the reproductive biology taimen from the Anabar R. are shown in the monograph by Kirillov (2007): at the age of 7+ and 8+ years the taimen body length averages 617 and 687 mm, and its weight – 1850 and 2442 g, respectively.

We have studied the taimen biological and reproductive parameters from Motorchuna and Moona Rs. These Arctic Yakut rivers freeze in the end of September-October, and the ice in these rivers is broken up in the late May–first half of June. The taimen from the Motorchuna R. is larger, than that from the Moona R., but the age range of the fishes under study, according to our data, in the Moona R. was narrower (Table 1), that could be due to non-representative quantities of the individuals measured by us. In spite of this fact, in these rivers taimen is larger, than in other Siberian rivers (Reshetnikov, 2002).

Table 1. Age, Fork Length (AC) and Weight (W) of Common Taimen *Hucho taimen* from the Moona (*n* = 141) and Motorchuna (*n* = 24) Rs. during the Spawning Periods

Age, years	Moona R.						Motorchuna R.	
	2003		2004		2005		2008	
	AC, cm	W, kg	AC, cm	W, kg	AC, cm	W, kg	AC, cm	W, kg
5	71.0	3.0	–	–	–	–	–	–
6	74.5	3.0	89.7	7.1	–	–	94.0	6.0
7	83.0	5.2	96.7	7.8	94.2	8.3	93.3	8.6
8	105.0	9.8	103.6	9.5	100.0	9.3	103.1	9.3
9	102.0	9.6	110.5	10.9	109.7	11.2	111.8	16.3
10	106.0	9.8	117.5	13.3	113.0	13.0	–	–
11	108.6	10.7	–	–	119.7	14.5	115.0	22.0
12	113.8	11.5	–	–	–	–	–	–
13	113.0	11.9	–	–	–	–	–	–
14	115.0	14.0	–	–	127.0	17.8	–	–
15	116.0	14.0	–	–	–	–	–	–
16	121.0	17.0	–	–	–	–	–	–
17	124.0	24.0	–	–	–	–	–	–

The extention of taimen spawning migrations and location of it spawning grounds depend both of water regime of the spawning river, and of the Lower Lena R. basin as a whole. According to our data, taimen begins its upstream spawning migration to the Moona R. just after its cleaning from ice usually in May or in early June. For example, in 2003–2004 spawning taimen entered this river on the same days – June 4–5, when water temperature varied from 0.5 to 2.0°C, but in 2005 its run was very early (first half of May). As a rule, its mass spawning occurs in the second half of May. The taimen individuals caught during the spawning period have a nuptial dress: their abdomen and edges of fins are of intensive red color. Their sex ratio on the spawning grounds in different years varies, for example in 2003 in the Moona R. it was equal to 1:1, in 2004 – 0.6:1, in 2005 – 1:3. The condition coefficient (CC) (Table 2) of spawning fishes in the 2000ies fluctuated from 0.65 to 1.3%, that less, than in the middle of the 1980ies. On the spawning grounds the taimen groups are mixed and consist of immature and mature individuals. Thus, in the Moona R. during spawning season females and males with gonads of different maturity stages from II up to VI-II are caught, with predominance of prespawning migrants (51.1%) whose gonads are in the IV maturity

stage, including 36.9% of females and 14.2% of males. There is a slight part of spontaneously spawned females (1.4%).

Table 2. Taimen Condition Coefficient (CC) during Spawning in the Moona R.,%

Parameters	Years			
	1986 **	2003	2004	2005
CC*	1.03 (0.84–1.18)	0.86 (0.65–1.3)	0.91 (0.8–1.1)	0.91 (0.75–1.05)
n	13	89	31	20

Notes. * – by Fulton, ** – Zhigansk Fish Inspection data.

Individual Absolute Fecundity (IAF) of taimen (Table 3) is rather high, on the average it is over 15 thousand eggs, the relative one is 1.7 eggs per kg. The fecundity increases with the age. In the first time spawning females the IAF varies from 7.5 up to 16 thousand eggs. In the repeated spawners in the age of 9 +, this reproductive coefficient increases. Yakut taimen produces large eggs (diameter is 5–9 mm) of dark-amber or orange color, which is laid in self-made redds located on the stone-pebbly and pebble-sandy but no oozed bottom at a depth about 0.5 m. According to the data by Venglinsky et alter (1987), its juveniles downstream migration from spawning grounds begins in the middle of June.

Table 3. Absolute Fecundity of Taimen in the Moona R., thousands of eggs

Age, years	Year					
	2003			2004		
	n	IAF		*n*	IAF	
		M	lim		M	lim
8 +	–	–	–	3	11.9	9.3–13.4
9 +	6	9.2	7.5–10.9	2	12.9	9.9–16.0
10 +	7	13.7	11.3–5.2	2	21.5	20.0–23.0
11 +	9	14.8	10.1–8.4	–	–	–
12 +	1	16.3	–	–	–	–
13 +	3	15.2	11.8–7.8	–	–	–
14 +	2	19.6	18.8–20.5	–	–	–
15 +	–	–	–	–	–	–
16 +	1	20.6	–	–	–	–
	29	15.6±1.4	7.5–20.5	7	15.4±3.1	9.3–23.0

Because of taimen catch reduction in Yakut in more than 70 times during 1943–2010, efforts are taken for increasing the stocks of this species by artificial reproduction. So the Government of the Republic of Sakha (Yakut) issued the decrée "On actions for artificial reproduction of aquatic living resources on the terrain of the Republic Sakha (Yakut) for 2007–2011", including taimen. This document was prepared by experts of the Yakut Ministry for Nature Protection and Yakut branch of Gosrybcenter, including one of present paper authors. Besides, the Regional Program "Reproduction of aquatic living resources in terrain of Yakut for 2011–2015" is being developed. According to this Program, release of common taimen juveniles from hatcheries to natural river basins by 2015 is to reach 200 thousand individuals (Sivtseva, Ivanova, Makarov, 2010).

To realize artificial reproduction of taimen there are all prerequisites in Yakut. Among them, biological and spawning data of this species, experience in artificial reproduction with the use of wild spawners are available, as well as the "Chernyshevsky" Hatchery at present being modernized. In 2009–2010 a legal procedure of this Hatchery transfer into the Federal property is carried out. Just at this hatchery, experimental studies on the elaboration of biotechnics of taimen artificial reproduction have been performed; they will be continued in future.

So far, foundations for artificial reproduction, on-growing and some aquaculture details have been created only for one of Russian taimen species – the Sakhalin one (Korablina, Ivanova, 2001; Mikodina, Lyubaev, 2005; Zelyonkin, Fedorova, 1997; Kouril et al., 2009). Nevertheless, taimen biotechnics artificial reproduction were based on two sources: instruction on artificial reproduction of Pacific salmons of genus *Oncorhynchus* (Smirnov, 1963, 1975), and Sakhalin taimen one.

During 2003–2005, taimen mature spawners were caught in the Moona R. wild spawning grounds, they were transported to temporary fish-breeding site and kept till ovulation. Here eggs and sperm were

obtained for the first time without hormonal stimulation, 30 thousand eggs were inseminated, share of fertilized eggs reached 80%. Early stages of egg incubation was made on the net trays placed in the shallow quite river sites (Tyaptirgyanov, Ivanova, Sivtseva, 2008; Sivtseva, Mikodina, 2009). After that the developing taimen eggs from the Moona R. to the Hatchery "Chernyshevsky" (Mirninsky ulus), the unique in Yakut, were transported (in the beginning by air, then by motor and water transport). The Hatchery is specialized on cultivating cisco, peled, whitefish and their juveniles release to natural water bodies. The final incubation stages of developing taimen eggs were completed at the above Hatchery. Besides, the hatched prelarvae were ongrown in tanks during 30 days with the use of artificial feeds up to the weight of 500 mg. For the first time the taimen juveniles received by aquaculture methods were released to the Lena R.

The results of our Pale Arctic taimen studies on its reproductive biology and artificial reproduction under control conditions in the Republic of Sakha (Yakut) terrain, in our opinion, are an innovative trend in the Russian aquiculture. The accumulated experience makes it possible to rely on success in the maintenance and increase of common taimen population stocks using the methods of artificial reproduction not only in the Lena R., but also in other water systems.

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COMPARATIVE CHARACTERISTIC OF HYDROLYTIC ENZYMES OF THE BARENTS SEA INTRODUCED CRABS *CHIONOECETES OPILIO* AND *PARALITHODES CAMTSCHATICUS*

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Nowadays one of the key problems of the fish-processing industry in Russia is the development of new fishery objects and their rational use. Due to the increase of snow crab abundance in the Barents Sea, it is possible to predict the potential of this species for the industrial fishing. According to the data obtained by PINRO scientists, in 2009, the total stock of snow crab in the Barents Sea was more than 10 million individuals (Pavlov, 2010).

For several decades, hepatopancreas of the red king crab (*Paralithodes camtschaticus)* has been successfully used for production of complex enzymatic preparations applied in medicine and cosmetology, and also in food and microbiological branches of industry in order to obtain the protein hydrolyzates (Klimova et al., 1990; Mukhin and Novikov, 2001). The snow crab (*Chionoecetes opilio*) hepatopancreas, which has been insufficiently studied in this respect, is also of interest.

The objects of research were the enzymatic preparations derived from hepatopancreas of crustaceans *Paralithodes camtschaticus* and *Chionoecetes opilio*, caught in the different areas of the Barents Sea in 2008–2009.

To obtain enzymatic preparations (EP) the comminuted hepatopancreas was processed with acetone and n-butanol in order to remove lipids and low-molecular compounds (Sakharov et al., 1988).

The fractional composition of proteins in samples was determined by the method of low pressure gel-chromatography using «Pharmacia LKB Biotechnology» equipment. Sephadex G-100 Superfine was used as a stationary phase in a column (1,6x70 sm), 0.15 n NaCl (pH 7) – as an eluent buffer. The protein fractions were registered applying photometry at 280 nm (the optical path length – 2 mm). The molecular weight of proteins (MW) was determined using the calibration curves built after the run of the proteins with known MW through the column: thyroglobulin (670 kDa), g-globulin (158 kDa), ovalbumin (44 kDa), myoglobin (17 kDa) as well as vitamin B12 (1,35 kDa) (Laurent and Killander, 1964).

Proteolytic activity was estimated using the Anson's method**,** by the hydrolysis of 1% sodium caseinate solution (Alekseenko, 1968). The temperature and pH values at which the protease activity was maximum were also determined.

Exochitinase activity was calculated through the release of N-acetylglucosamine (GlcNAc) generated by the chitin hydrolysis, the content of GlcNAc in a hydrolyzate solution was determined by the reaction with 4-dimethylaminobenzaldehyde (Decleire et al., 1996).

In the enzymatic preparations obtained from hepatopancreas of two crab species, *Paralithodes camtschaticus* and *Chionoecetes opilio,* chitinolythic and proteolytic activity has been determined. Seasonal dependence of protease activity of these two crustacean species has been found (Fig. 1). Thus, the activity level of EP in winter-spring period exceeded that one in summer-autumn for both crabs. The obtained results agree with the data of other researchers (Nemova, 1996; Mukhin and Novikov, 2002) and indicate the influence of seasonal rhythms on the activity of proteases. It may be caused by both the feeding pattern and the effect of the environment abiotic factors.

Considerable seasonal fluctuations of proteolytic activity in hepatopancreas of crabs indicate high adaptive abilities of the latter ones.

The molecular-weight composition of proteins in obtained EP for both crab species is quite similar (Fig. 2, Table). The high-molecular fraction makes up a considerable part of the total number of proteins in both samples.

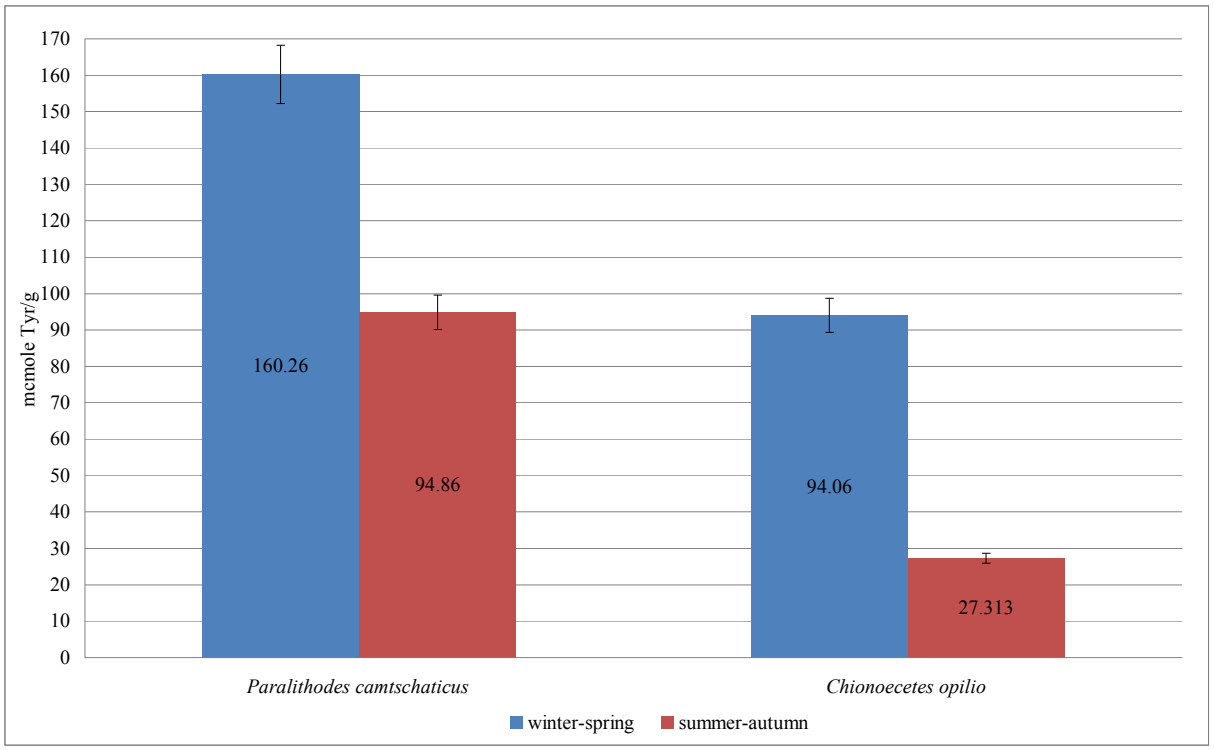

Fig. 1. Seasonal variations of the proteolytic activity of EP, derived from snow and red king crabs

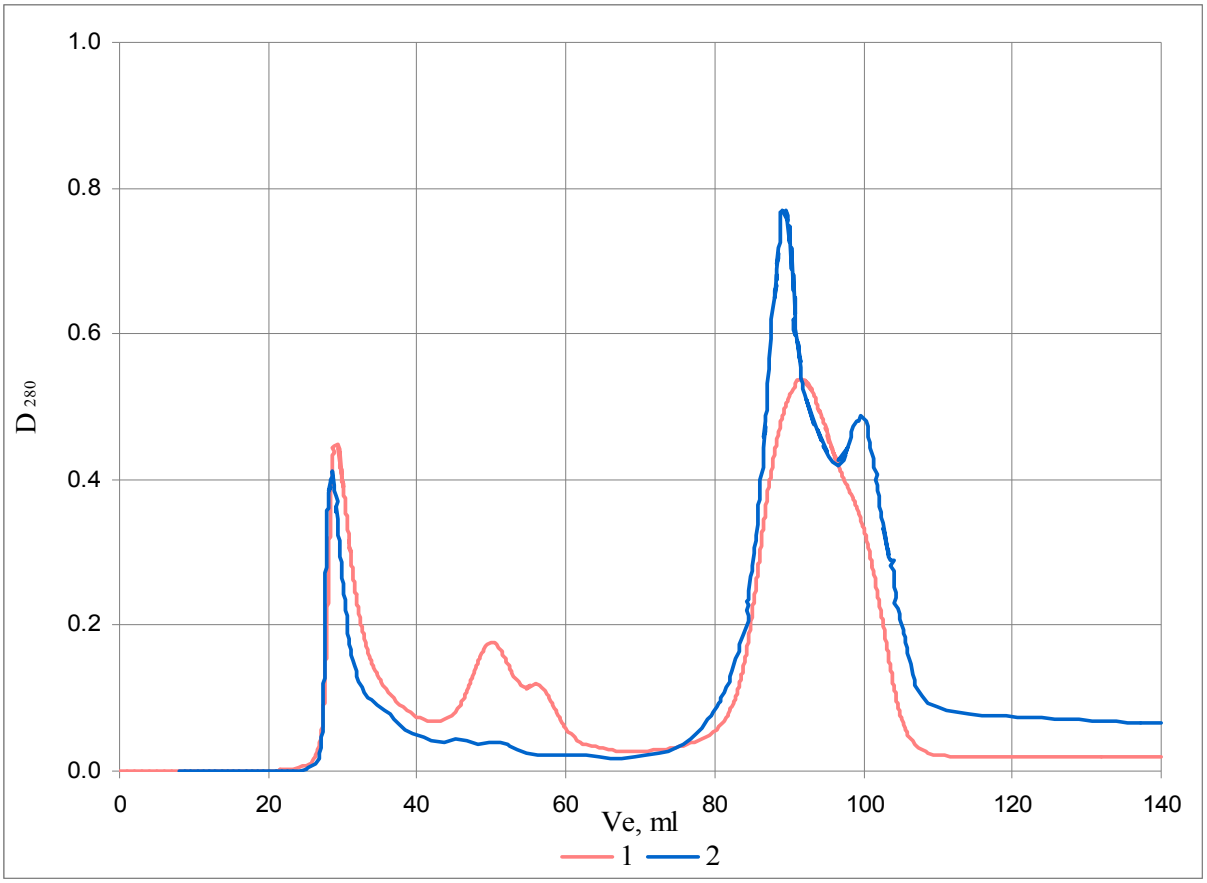

Fig. 2. The elution profile of proteins of hepatopancreas EP: 1 – *Paralithodes camtchaticus*; 2 – *Chionoecetes opilio*. Column Sephadex G-100 Superfine (1.6x70 sm), elution speed 12 ml/hour

Table. Fractional composition of proteins in studied EP

The source of EP	Protein fractions with MW (kDa),%		
	<4	10–70	>100
The red king crab *Paralithodes camtchaticus*	68.12±0.3	16.09±0.1	15.79±0.1
The snow crab *Chionoecetes opilio*	82.47±0.4	1.38±0.1	16.15±0.1

According to the available data, the protein fraction with MW from 10 to 40 kDa provides the basic proteolytic activity of proteinase preparations from the red king crab hepatopancreas (Mukhin and Novikov, 2002). The percentage of proteins with intermediate MW in EP obtained from the red king crab hepatopancreas is 10 times more than that one in the snow crab EP. Nevertheless, the proteotytic activity values are of the same order of magnitude in summer-autumn, and differ less then twice in winter-spring. Then, it is possible to assume that the specific activity of intermediate protein fractions of snow crab hepatopancreas EP is higher. Therefore, with the appropriate EP processing it is possible to predict higher activity of proteinases in it.

A considerable portion of low-molecular fractions in the examined samples can be explained by the drawbacks of the method to derive EP applying which a certain amount of low-molecular ballast substances is not removed from the preparation. On the other hand, some proteins with higher MW can get to the last fraction, due to the insufficiently complete chromatographic division caused by the interaction of the carrier and the examined sample.

When determining the conditions (pH, temperature) for the maximal EP hydrolytic activity the following data have been obtained: the activity peaks were observed at pH of 4.0 and 7.0, the maximum activity was noted at 50 °C. At the temperature of 5–10 °C the EP activity was minor. These data are similar to the characteristics obtained in researches on the proteolytic activity of EP from hepatopancreas of the red king crab (Mukhin and Novikov, 2002). Thereby, the hydrolysis for the industrial purposes can be carried out without the change of conditions for both EPs.

The average exochitinase activity for the red king crab is 2.16 mg of GlcNAc/ml, for the snow crab – 2.03 GlcNAc/ml, i.e. the given values are quite similar. According to the data obtained by the other researchers, the enzymes responsible for exochitinase activity, have a slightly higher MW than proteases, but their MW falls within the same range (10–70 kDa) (Rysakova, 2008). Then, in compliance with the data obtained during the fractionation of EPs (Figure 2), it is possible to speak about higher specific exochitinase activity of the snow crab hepatopancreas EP.

During the carried out researches some characteristics of *Chionoecetes opilio* hydrolases have been determined, and they turned to be similar to those ones of *Paralithodes camtchaticus* as a whole. The obtained data allow speaking about the prospects of utilization of *Chionoecetes opilio* hepatopancreas as an accessible raw material for the commercial purposes in the given area. Obviously, the data available at the moment are not enough to make the concrete recommendations, but, taking into account high adaptive abilities of the snow crab and its increasing abundance, it seems to be necessary to continue researches in this direction.

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SHELF LIVES OF CANNED FOOD "NATURAL PACIFIC SAURY"

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Saury fish is an important item in the realm of fishing. Products from this fish at the Russian market are being in much demand; particularly, the demand for canned goods from saury has grown because they are inexpensive and available for all strata of the population.

The canned food is produced under State standard (GOST) No. 7452–97 from fresh, cooled and frozen glazed saury that has been stored for no more than 2 months. The shelf life of such canned food is 2 years while the American and European manufacturers' analogs have generally the shelf life of five years and over [5].

The aim of this work is to substantiate shelf lives of the canned food "Natural Pacific Saury" having high quality and nutritive value.

As the object of research, a batch of canned fish produced at the Preobrazhenskaya Base of Trawling Fleet, JSC, from frozen saury fish gazed with sea water and stored for 2 months at –25°C was employed.

The canned fish quality was characterized in terms of microbiological, organoleptic, and physicochemical characteristics as well as nutritive value.

Microbiological studies were carried out pursuant to "Hygienic Requirements for Safety and Nutritive Value of Foodstuff" and methodological instructive regulations on the determination of foodstuff shelf lives.

The contents of nonprotein nitrogen and volatile base nitrogen as well as acid-degree value and peroxide number were determined according to State standard (GOST) No. 76–36–85; the amino acid composition of proteins was determined on an AAA-835 Hitachi amino analyzer; the fraction composition of lipids was studied by HPLC; the lipid fraction was analyzed by GLC on a Shimadzu 16A gas chromatograph. Parameters responsible for the variation of protein substances and lipids were determined in the dense portion, liquid (broth) and middle samples of the canned fish.

The organoleptic component was appraised by the method of rating.

The results of the microbiological studies showed that the canned food "Natural Pacific Saury" complied with the industrial sterility requirements over a period of 3.5 years.

The content of nonprotein nitrogen in the canned fish after the fabrication thereof varies from 0.16–0.23% in the dense portion, 0.32–0.38% in the middle sample up to 0.92–0.96% in the broth. After 2 years of storage, the nonprotein nitrogen content amounted to 0.37% and by the 3d year it became 0.52% (in the middle sample); in the dense portion it was 0.28% after 2 years and 0.41% after 3 years; in the broth it constituted 0.92% after 2 years and 0.98% after 3 years. When the storage time is increased by 1 year, the nonprotein nitrogen content rises by approx. 30% in the middle sample and dense portion and by 6% in the broth.

The nonprotein nitrogen accumulation by the end of 3.5 years of storage is to a greater extent characteristic for the dense and middle samples than for the broth.

The content of volatile base nitrogen after 2 years is 43 mg% in the middle sample and 48 mg% by the 3d year; 36 mg% in the dense portion and 42 mg% by the 3d year; 60 mg% in the broth and 66 mg% by the 3d year, that is, the volatile base nitrogen content increases by 10, 14 and 9%, respectively.

Some decrease in the percentage of nonprotein substances at the certain stages of the canned fish storage can be explained by the participation thereof in the formation of protein-lipid complexes.

The amino acid composition of proteins of the natural canned saury comprises a full set of nonessential and essential amino acids. A comparison by the content of essential amino acids of the "ideal" protein (scale of the FAO/WHO, 1975) and the canned fish proteins shows that 100 g of the latter contains more essential acids than 100 g of the "ideal" protein, except for valine. The limiting acids are methionine and cystine.

From among essential amino acids, one should point out a very high content of lysine in the canned fish proteins, which is 50% higher than that of the "ideal" protein, and a sufficiently high percentage of phenylalanine and tyrosine. From among nonessential acids, the predomination is observed for glutamic acid (approx. 14 g/100 g protein), asparaginic acid (approx. 9 g/100 g protein), and arginine (6 g/100 g protein).

The amino acid composition of proteins of the canned saury is stable throughout storage. Differences in the contents of essential and nonessential amino acids after 2 and 3 years have not been observed, as is consistent with data from Z.P. Shvidskaya and Yu.G. Blinov [2].

The fat acidity value in the broth after 2 years, in the middle sample and dense portion as well as in the broth is 3.5, 6.6 and 5.8, respectively; 4.5, 6.9 and 6.5 mg/g fat after 3 years. When stored over a period of 2 and 3 years, the fat acidity value in the dense portion and middle sample has a slight increment (by 4.3 and 10.8%), and in the broth the increment is more significant (by 22%), as is evidently connected with the redistribution of the oxidation products between the dense and liquid portions of the canned fish.

Thus, in process of maturation and storage of the canned fish the acid-degree value of lipids grows in the dense portion, middle sample, and broth. These data are correlated with those from literature wherein the conclusion is drawn regarding the variation in the fat acidity value when stored, which has a linear character and is associated with the oxidation of lipids [4].

The alteration in the peroxide number of lipids of the canned fish has a multiextremal character, and after 2 years of storage the peroxide number has a minimal quantity of 0.001 as well as after 3 years, as is in agreement with data from N.A. Fonarev [1].

The fraction composition comprised of lipids, monoglycerides, diglycerides, sterols, free fatty acids, and triglycerides remains practically unchanged in the course of 2 year storage and persists when stored up to 3.5 years. In this case, the percentage of free fatty acids has a tendency to rise, which may be indicative of the influence thereof upon the organoleptic properties of the canned fish.

The canned fish quality was evaluated from the fatty-acid composition of lipids. The total content of diene acids is not in excess of 4.0%, that of triene acids – 2.5%, tetraene acids – 5.94%, pentaene acids – 5.28%, and essential acids – 4.95%.

In natural canned saury fish when stored during 2 years there are observed no significant variations in the fatty-acid composition of lipids. The content of essential fatty acids changes to a little degree. When the natural canned fish is further stored for 3.5 years, no variations in the fatty-acid composition of lipids are observed.

The composition and ratio of fatty acids of the canned fish lipids in the course of 3 year storage do not practically change and remain the same as for 2 year storage. These data are consistent with the literature ones [4].

The organoleptic properties of the canned saury fish in process of storage were evaluated by the method of rating. The coefficients of significance were determined by the method of expert estimations using the literature recommendations available.

The canned fish fabricated one month ago was evaluated by experts to have 4.5 points; however, some samples had still a taste of an unripened product. When stored for 36 months, the canned fish is evaluated to have 4.9–5.0 points.

The organoleptic assessments of the canned fish stored for 2 and 3 years coincide, according to tasters' data.

In summary, the author has shown that characteristics responsible for quality and safety of the canned fish (microbiological studies) had no considerable changes throughout tests.

The storage times of the canned food "Natural Pacific Saury" are substantiated to be 3 years inclusive at 0°C up to 20°C with a relative humidity below 75%. On the basis of data obtained, technical documentation has been elaborated (TU 9271–118-00472124 and technological instruction thereto).

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LIPIDS AND FATTY ACIDS IN PELAGIC ARCTIC AND SUB-ARCTIC FOOD WEBS (*CALANUS GLACIALIS*, *LEPTOCLINUS MACULATUS*)

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The White Sea is considered as sub-Arctic sea with Arctic flora and fauna. In the era of rapid climate change, the life cycle and adaptations of White sea hydrobionts such as *Calanus glacialis*, fishes from family Stichaedaied – *Leptoclinus maculatus*, *Lupmpenus fabricii* – remains largely unstudied compare to same species from high Arctic region (such as, Svalbard). *Calanus glacialis, Leptoclinus maculatus, Lumpenus fabricii* and economically valuable White sea herring are associated together in food web in the White sea. *Calanus glacialis* – one of the most important Arctic species in the White Sea. Fishes feeding on *Calanus* ssp. diet in Arctic and sub-Arctic ecosystems store a lot amounts of lipids from zooplankton diet during short summer productivity season. Lipids are very important for Arctic and sub-Arctic organisms. Storage of high amount of lipids, specific phospholipids and fatty acids profile might be considered as distinctive characteristics of high Arctic and sub-Arctic aquatic organisms. *Calanus glacialis* – one of the most important Arctic species in the White Sea and provide fishes fed on Calanus diet by lipids, essential fatty acids and specific fatty acids which in fish metabolized to needful fatty acids. Trophic relationships in pelagic sub-Arctic food webs (the White Sea): lipid distribution, transformation and dynamics in food web "phytoplankton – zooplankton (*Calanus glacialis*) – planktivorous fish (*Leptoclinus maculatus*) – economical value fish (White Sea herring)" is a main aim of research work kept in Institute of biology KarRC RAS.

Using classic lipid detection methods such as TLC, LC and GC we determined lipid classes, phospholipids and fatty acids staff in *Calanus glacialis*, *Leptoclinus maculatus*, *Lumpenus fabricii* and White Sea herring caught from the White Sea seasonally. Lab obtained data are under discussion notable that new results renew "history" of White Sea herring and collect new data about source, distribution and transformation of lipid components in White Sea food chain (*Calanus glacialis – Leptoclinus maculatus, Lumpenus fabricii – White Sea herring*).

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COMPARATIVE ANALYSIS OF FATTY ACID COMPOSITION IN FORMULA FEEDS OF DIFFERENT TRADEMARKS USED IN RAINBOW TROUT CULTURE

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Successful application of formula feeds depends on the production process biotechnology, feedstock composition, and the ratio initial components. Analysis of the feed fatty acid composition helps evaluate its

quality, which is predetermined by utilization of lipid-rich feedstock in feed production biotechnology. The composition and concentration of fatty acids (FA) in the feed influence the living functions of the fish (Hansen et al., 2008) since FA in phospholipids are responsible for biomembrane fluidity and, hence, for the cellular metabolism, being at the same time an essential energy substrate (Tocher, 2003; Chentsov, 2004). FA comprised in triglycerides are deposited in rainbow trout muscles, influencing the taste and quality of the product (Okumuú and Mazlum, 2002).

The study object was feeds of four trademarks (1, 2, 3 & 4), in which the content of total lipid fatty acids was determined. Methyl esters of fatty acids were prepared by transesterification with methanol according to the method of Tsyganov (1971). The FA methyl esters derived were separated using the chromatographer Kristall 5000 (Chromotek, Russia), FA were identified through comparison of logarithmic indices against tabular values (Jamieson, 1975). The data were processed by the conventional variation statistics technique (Korosov and Gorbach, 2010).

Analysis of the fatty acid composition of formula feeds of different trademarks demonstrated they were of similar quality (Table). The main component of the feeds was fish flour made of commercial sea fish. Thus, fatty acid composition in a feed should correspond to the fatty acid distribution in fish tissues (Leaver et al., 2006). However, the quantitative distribution of fatty acids in the feeds varied depending on the manufacturing technology and various additives. The content of saturated FA was the highest in feed 4. Saturated FA are a key energy substrate in fish metabolism (Sargent et al., 2002). The content of monounsaturated FA (MUFA) was higher in feeds 1 and 2. MUFA are actively oxidized, are mostly of exogenous genesis, are deposited in the muscles, and influence the taste of the final product (Tocher, 2003). Polyunsaturated fatty acids (PUFA) contribute to major physiological processes and adaptations in fish by influencing biomembrane fluidity and the activity of proteins, including enzymes (Sargent et al, 2002). Significant prevalence (over 30% of total FA) of ω6 PUFA was found in feed 3. Feed 2 contained a fairly high proportion of ω3 PUFA, presumably due to addition of corn oil, which is rich in ω3 PUFA. According to some studies (Zhao, 2008; López et al., 2009), high ω3 PUFA content in a feed retards fish growth and development. Among essential FA supplied to the fish organism with food only (Tocher, 2003) we found the highest level of 18:2ω6 in feed 3, and 18:3ω3 – in feeds 1 and 2. Analysis of the lipid composition revealed high 22:1ω11 concentration in feeds 1 and 2. According to the literature (Bauermeister, 1979; Sargent and Henderson, 1995), high content of the 22:1ω11 acid is characteristic of crustaceans, wherefore we conclude the feeds were most probably produced from feedstock rich in such organisms.

Table. Fatty acid content of feeds (% of total)

Fatty acid		Feed 1	Feed 2	Feed 3	Feed 4
16:0	palmitic acid	13.23	12.49	16.04	21.88
Total saturated fatty acid		23.6	23.43	26.06	36.31
18:1ω9	oleic acid	25.08	24.72	14.14	16.48
22:1ω11		4.56	4.48	0.93	0.49
Total monounsaturated fatty acid		43.18	42.35	26.02	33.74
18:2ω6	linoleic acid	8.44	8.14	30	5.77
20:4ω6	arachidonic acid	0.6	0.58	0.42	0.49
ω6 polyunsaturated fatty acid		10.42	10.44	31.56	8.12
18:3ω3	linolenic acid	3.7	3.61	1.32	1.99
20:5ω3	eicosapentaenoic acid	6.09	6.38	5.63	6.17
22:6ω3	docosahexaenoic acid	6.13	6.29	3.61	3.56
ω3 polyunsaturated fatty acid		20.18	20.75	13.6	16.56
Total polyunsaturated fatty acid		33.22	34.22	49.92	29.96
ω3 / ω6 PUFA		1.94	1.99	0.43	2.04

Thus, analysis of the FA composition enables the manufacturer to choose lipid-rich feedstock to optimize the biotechnology of feed production. The customer, in turn, has the possibility to select the feeds of the trademark best suited to promote muscular gain in trout, accelerate its growth and enhance its taste.

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PROSPECTS FOR CREATING POLYCOMPONENTAL PRODUCTS OF THE GERODIETARY PURPOSES ON FISH AND VEGETABLE RAW MATERIAL BASIS

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One of the most important and actual problems today is a health preservation, increasing a person life and the country population as a whole.

Many scientists believe the main factor that has a direct impact on health and, consequently, on life expectancy is food (Sukhanov B.P.,Korolev A.A., 1991; Kasianov G.I., Zaporozhsky A.A., 1999; Tutelian V.A., Spirichev V.B., Shatnyuk L.N., 1999; Tutelian V.A., Knyazhev V.A., 2000; Monastyrsky K., 2002). The nutritional status of most elderly people under the prevailing socio-economic conditions in Russia cannot be considered rational and balanced.

It is necessary to observe a number of special requirements concerning food and energy value as well as amino acid, fatty acid, carbohydrate composition, a mass weight of food fibres, minerals, vitamins and other biologically active compounds When developing recipes and diets for the elderly. There is a number of functional and metabolic changes in all systems of body functioning coming with ageing: slow metabolism, decreased adaptability of the organism to changing environmental conditions, its resistance to infection and the ability to cell regenerate (Samsonov M.A., 1997; Yudina S.B., 1997).

Modern ideas about different ways of creation new generations of gerodietary profile products taking into account medical and dietary requirements are directed at the following:

– creation of completely balanced polycomponental products, most fully and adequately meeting requirements of elderly and old people's organisms;

– developing technology of the products designed for the improvement of food, i. e. enriched with one or more nutrients;

– creation of food modules (premixes) allowing purposefully to control synergetic properties of separate components of the product and to correct both a disposable meal and a daily meal as a whole;

– obtaining products enriched with biologically active components able to strengthen or to give the product certain properties;

– development of products that contribute to the prevention or treatment of geriatric diseases i. e. focused on specialised nutrition in elderly and old age.

Creating polycomponental products for older people is a rather complicated task due to the need to provide a full balance of a wide range of components. The important role in solving this problem is played by a rational choice of a raw material base. As a rule, gerodietary purpose products include a combination of animal and vegetable raw materials. It improves the biological properties of the product and reduces its cost. It is known that fish raw materials are a source of compounds needed for a human body. Furthermore fish proteins are digested and assimilated better than meat proteins. When using fish raw materials, wastes from their cutting as well as vegetable components computer design to create functional products that meet the medical and dietary requirements seems appropriate (Studentsova N.A., 2003).

Development of technologies for polycomponental products of gerodietary food on fish and vegetable raw materials basis will allow to reach their nutritional and biological balance and to expand a range of affordable and available products to disadvantaged groups of people. The problem of designing of delicious food analogues which look no different from our traditional products based on the use of available natural resources can be simultaneously solved. Such approach will allow to regulate the composition, properties and the degree of digestibility of received analogues. Using special technology makes it possible to recreate the structure, appearance, taste, smell, colour and all the other properties simulating the usual product.

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DISCOVERY OF NATURAL ANTI-DIABETIC DRUG CANDIDATES FROM ARCTIC MARINE ORGANISMS

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The Barents Sea and its seabed contain a diversity of vertebrates, invertebrate and algae species that have so far been utilized by humans mainly as a source of protein and marine lipids. In the last decades, however, researchers have become interested in secondary metabolites from these species, especially from invertebrates. Some of these metabolites demonstrate biological activity that can be utilised as drugs, and a few have advanced into clinical trails and already entered the drug marked. New drugs are needed, however; to replace existing imperfect drugs and to treat emerging life style diseases, such as obesity and its associated disorders (type 2 diabetes mellitus (T2DM), cancer and cardiovascular diseases) that have reached epidemic proportions worldwide.

The aetiology of T2DM is intricate and multifaceted, but insulin deficiency and insulin resistance with resulting hyperglycaemia are the most common symptoms to treat. Additionally, obesity is a predisposing factor for T2DM, which are often associated with a low-grade inflammatory state in adipose tissue. Eating is essential to life, and its episodic nature requires physiological adaptations to avoid excess or insufficiency of circulating fuels, especially glucose and lipids. Our modern lifestyle with an increasing imbalance between energy intake and energy expenditure, often resulting in obesity, is a challenge to this fine-tuned energy adaptation. Chronic disruption of the energy balance causes plasma glucose imbalance, hypertrophy and hyperplasia of adipocytes causing metabolic disorders such as T2DM. A number of potential drug targets have been identified and investigated with respect to treatment. Developed and released drugs have revealed moderate efficiency and many have shown low specificity with adverse effects. The focus of our research activities is on the discovery of bioactive constituents of marine organisms that can be developed into drugs to treat T2DM. The following targets are included in the current screening plan, using both cell based and isolated target assays: 1) The enzyme protein tyrosine phosphatase 1B (PTB-1B), 2) Insulin-stimulated glucose uptake, and 3) Peroxisome proliferator-activated receptors (PPARs) regulating the expression of genes involved in the control of lipid metabolism, glucose homeostasis and inflammatory processes

The protein tyrosine phospatases (PTPs) is an enzyme family that includes about 100 proteins which catalyze dephosphorylation of phosphotyrosine residues in protein substrates. Phosphotyrosine is a central element in cell signalling, and PTP activity is essential for both cellular homeostasis and for appropriate responses to extracellular signals. PTP-1B antagonizes insulin signalling by reducing the activation state of the insulin receptor kinase, thereby inhibiting post-receptor signalling in insulin-responsive tissue. The enzyme has generated a great deal of interest as a potential drug target, and PTP-1B null mice do not accumulate fat when placed on high-fat diet in contrast to their wild-type littermates. Unfortunately, due to the ~80% homology in the catalytic domain of the PTP superfamily, identification of inhibitors that are specific for PTP-1B has so far been proven difficult. Furthermore, the progress towards developing an efficient PTP-1B antagonist for therapeutic use has also been hampered by low bioavailability of inhibitor tested *in vivo*. Our biochemical screening regime, which also includes a counter-screening assay, has so far given us a few active fractions for further testing.

We screen for compounds which can potentiate insulin-stimulated glucose uptake using cell lines (differentiated into adipocyte-like cells) and primary adipocytes isolated from epididymal fat pads of rats. Our aim is to find compounds that interfere with the insulin signalling pathway, promoting translocation of glucose transport molecules (GLUT4) to the cell surface and increased cellular uptake of radioactive glucose (end-point parameter). GLUT4 is expressed only in muscle and fat cells, the major tissues in the body that respond to insulin. Any drug suitable for treatment of T2DM should probably display some potentiating of insulin action together with anti-inflammatory activity.

PPARs are major regulators of glucose and lipid metabolism. Furthermore, PPARs are also involved in the regulation of inflammation and angiogenesis. PPARs were originally named for their ability to induce hepatic peroxisome proliferation in mice in response to xenobiotic stimuli. The expression of three PPAR isoforms, alpha, beta/delta, and gamma, has been described. They share 60%-80% homology in their ligand-binding and DNA-binding domains. Nuclear receptors, to which PPARs belong, are promising targets for T2DM treatment strategies because they act as transcription factors and may produce selective alterations in downstream gene expression. PPAR agonists are used therapeutically in patients with T2DM, but unfortunately, PPAR agonists can have long-term adverse effects, such as increased body weight, fluid retention and increased risk of heart failure. The goal of our screening strategy is to find PPAR agonists that are more selective and have less adverse affects compared to the present PPAR drugs.

Taken together, new drugs to treat T2DM should be more selective with fever adverse effects. Screening strategies and initial results will be presented.

MECHANISM OF ACTION OF ANTIMICROBIAL PEPTIDES ISOLATED FROM INVERTEBRATES

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Antimicrobial peptides (AMPs) use several different strategies to inhibit bacterial growth. While many AMPs are responsible for membrane pore formation and cell lysis, other AMPs manage to permeabilize the membrane without membrane disruption. The aim of our studies is to characterize membrane non-disruptive AMPs, which act by targeting intracellular molecules and thereby inhibit processes vital for bacterial survival. Arasin 1 is a 37 amino acid long proline-rich antimicrobial peptide isolated from the spider crab, *Hyas araneus*. We report the localization of the pharmacophore of arasin 1 to be the proline/arginine-rich NH_2 terminus, whereas the C-terminal cysteine containing part does not seem to have any antimicrobial property. A kinetic killing study of *Escherichia coli* by using a synthetic peptide made of the first 23 NH_2 terminus amino acids, named arasin 1(1–23), revealed that this peptide acts as a bacteriostatic agent. The study implies that arasin 1(1–23) has a different mode of action than membrane lytic peptides like cecropin P1. An *in vivo* bacterial membrane integrity assay, using an *E. coli* strain expressing luciferase, showed that arasin 1(1–23) did not render the cells leaky, indicating that arasin 1(1–23) has intracellular target molecules and inhibits bacterial growth without lysing the cells. Transmission electron microscopy in combination with immunogold staining showed intracellular localization of arasin 1, which could be due to arasin 1 targeting intracellular molecules. Strategies involving peptide tagging together with chromatographic separation and mass spectroscopy identification of intracellular peptide targets will also be presented.

MICROARRAY AS A TOOL TO DISCOVER ENZYMES INVOLVED IN DETOXIFICATION OF OXYGEN-DERIVED OR IRON LIMITATION STRESS AND THAT MAY HAVE POTENTIAL IN BIOTECHNOLOGY

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In general, enzymes have potential in biotechnology and can be used in for instance medicine and life-science industry. Our group utilise the fish pathogenic bacterium *Vibrio (Aliivibrio) salmonicida* as a model organism for various studies including gene regulation, bacterial communication networks and virulence mechanism. *V. salmonicida* causes "cold-water vibriosis" (or "Hitra disease") in fish, including marine-reared Atlantic salmon. Here, we have studied genes and proteins involved in stress management in this bacterium.

Generally, stress to microorganisms can be defined as any deviation from optimal growth conditions that result in a reduced growth rate. In their natural habitat, bacteria can meet various types of stresses such as nutrient availability, radiation, reactive oxygen species or iron limitation. Pathogenic bacteria, for instance, may be subjected to oxidative stress through the oxidative burst from phagocytic cells.

Faced with stress or stressors, bacteria will induce regulatory networks that control the expression of selected gene responses. These regulatory networks are called "stress responses" as the level of the response is highest during a stress condition.

By studying differentially regulated genes and proteins involved in different stress conditions, we get insight into the molecular mechanisms of *V. salmonicida.* In this work, we have studied the response of this bacterium to oxidative-derived free radical (O_2^-, H_2O_2 and $OH^\cdot$) stress and stress caused by iron limitation. Hydrogen peroxide, paraquat or iron chelator was added to the growth media and microarray was used as a tool to examine the response of the bacteria to these stress conditions.

THE USE OF BROWN ALGAE OF THE LAMINARIALES FOR MANUFACTURING OF DIETARY JAMS ENRICHED WITH SELENIUM AND CHROMIUM

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Balanced nutriton is one of the factors determining the health of the population of Russia. The topical issues now are the development and manufacturing application of the healthy dietary products. Laminaria is one of the best raw material for such products. It is a natural selective absorbent of macro- and micronutrients accumulated in tissues, contains a large amount of polysaccharides and organic iodine. Biologically active substances of laminaria can reduce blood pressure, increase resistance to infectious diseases, as well as have a positive effect in treatment of cardiovascular diseases, anemia, osteoporosis (Sovershaeva S. N. et al, 2002).

Useful properties of products based on kelp can be enhanced by the addition of micronutrients required for healthy people, and for people suffering from alimentary-dependent diseases.

Cardio-vascular diseases have the leading role among the pathologies associated with poor diet. One of the effective approaches to this socially important issue is the development and manufacturing of new dietic products intended for use in nutrition of persons suffering from such diseases as coronary heart disease and hypertension.

Epidemiological studies have shown that consumption of selenium not only reduced risk of developing of cardiovascular disease, but also contributed to an increase of life expectancy.

An achievement of the national biotechnology was to develop and commercialize the method of cultivation of baker's yeast Saccharomices cerevisiae with a high content of organic forms of selenium (Zolotov P. A. et al, 1998). Broad use of selenium yeast in the diet of the population, however, was

prevented by the presence of poorly digestible cell membrane. The membrane significantly reduced the absorption of the contents of yeast cells and was potentially allergenic. In contrast the water-soluble fraction of yeast autolysate enriched with selenium, was produced by hydro-processing, then the autolysis at 50 ˚C and removal of damaged cell membranes by centrifugation (Mazo V. K. et al, 2002). Under the title "Selenium-Vitasil" this additional source of food selenium is used in a number of dietary supplements.

Cardiovascular diseases are often accompanied by diabetes mellitus – a disease associated with dismetabolism. The high frequency of this disease in our country determines the special interest in the medical community to the problems of its prevention and treatment. Besides diabetes, along with abdominal obesity, hypertension and other metabolic disorders is an indicator of patients with metabolic syndrome (Chaplin S., 2005; Ushakova T. I., 2007).

A big role in the regulation of carbohydrate and lipid metabolism plays a trace element chromium, which supports normal glucose tolerance and forms a complex compound with insulin, more effective than the free insulin. It is known that chromium in foods is present as inorganic salts and complex compounds with organic ligands, which is the active form of chromium and has a marked effect on the absorption and blood level of glucose. This compound is considered as the glucose tolerance factor. Assimilation of inorganic salts of chromium is extremely low – 0.5 – 0.7% from food quantity. Absorption of the glucose tolerance factor in the intestine is much more intensive, and may reach 25% of dietary chromium.

Chromium is also involved in the regulation of cholesterol metabolism and during the treatment in some cases caused reduction of cholesterol in the blood. Ability to synthesize the glucose tolerance factor in humans is limited, and this determines the need of the intake of the compound with food (Tutelian V.A. et al, 2002).

We suggested the technology of the dietary products – Laminaria jams enriched with selenium and chromium for the nutrition of persons with alimentary-dependent diseases.

The objects of investigation were commercial samples of kelp harvested in various regions of Russia and China, and Laminaria jams with the addition of selenium or chromium. The contents of dry matter, alginic acid, iodine and ash was determined by GOST 26185–84, protein – according to GOST 7636–85 by avtoazotanalyzer «FOSSTecator» 2300 (Sweden) by the Kjeldahl method. Chromium content was determined by mass spectrometry with inductively coupled plasma. The content of selenium – by mikrofluorimetric method by measuring the fluorescence of hexane extract. The composition and amount of carbohydrates was determined by high performance liquid chromatography. Determination of insoluble and soluble fractions of dietary fibers was performed by the method of Gordon and Ohkuma, 2004, through the enzymatic hydrolysis of protein and starch materials in the products and subsequent filtering. Authors thank very much the colleagues from the Institute of Nutrition, RAMS for the help in determination of the content of selenium, dietary fibers and carbohydrates.

Analysis of raw materials has shown that its chemical composition was greatly influenced by the primary processing and harvesting area (Table1).

Table 1. The chemical composition of the commercial samples of kelp

Sample	Origin	Contents (% of dry matter)			
		alginic acid	protein	ash	iodine
Salted kelp (blades)	Sakhalin, Russia	33.33±3.33	7.30± 0.73	31.47±1.57	0.200±0.020
Salted kelp (shredded)	Sakhalin, Russia	26.90±2.69	8.19±0.82	38.23±1.75	0.170±0.017
Dried kelp premium grade	White Sea, Russia	29.74±3.02	8.39±0.94	28.18±1.91	0.179±0.009
Dried kelp first grade	White Sea, Russia	31.86±3.32	9.42±1.16	19.02±0.93	0.062±0.007
Washed shredded kelp	China	49.53±4.95	13.46±1.35	19.52±0.98	0.001±0.0001

The samples of shredded kelp from China were poor of mineral substances and iodine because of the primary treatment – additional washing and drying at high temperatures. Stable and high content of iodine in the White Sea kelp allowed recommending it for making jams, enriched with chromium or selenium.

Various berries and fruits (cranberry, dried apricots, apples, lemon) were added to Laminaria Jams for taste improvement. Selenium was added in organic form (Selenium-Vitasil). As a sweetener for the anti-diabetic jam synthetic sweetener sucralose, derived from sugar was selected. Sucralose has no effect on carbohydrate metabolism, has a pleasant sweet taste, easily soluble in water and is stable for cooking. Chromium was added to the product in a complex with a milk protein hydrolysate.

Laminaria Jams were manufactured in a hydrodynamic rotary cooker which provided simultaneous grinding, mixing and pasteurization at a constant temperature of 90–95 °C.

Analysis of chemical composition and nutritional value of different samples of jams showed that they were of low caloricity (50 – 81 kcal) ; 20 g of jam satisfied up to 30% of the daily requirements of an adult in iodine, selenium or chromium. They were also rich in dietary fibers, which played an important role in the process of digestion: improved intestinal motility, had a prebiotic effect, reduced the rate of mono-and disaccharides absorption, and thus protected the body from high blood glucose and increased insulin synthesis.

Selenium was tested in the department of cardiovascular pathology of Clinic of medical nutrition for the study of the effectiveness of its use in the diet of patients with coronary heart disease (CHD), hypertension and obesity. The study showed that the product had a beneficial effect on the dynamic of clinical and instrumental parameters that characterized the functional state of the cardiovascular and nervous system. Also jam intake contributed significantly to the availability of selenium to patients with initially low level of the element in serum.

Chromium-enriched Laminaria Jam has passed the clinical trials among the patients with insulin-dependent diabetes mellitus type in the therapy department of the Ryazan State Medical University, where the possibility of dietary and medical treatment of metabolic syndrome on an outpatient basis was explored. Fifteen patients aged from 33 to 63 years suffering from arterial hypertension, dismetabolism of carbohydrates and lipids, diseases of the gastrointestinal tract took part in the study.

According to preliminary data, the use of dietary jam enriched with chromium was associated with improved overall health for all patients, normalization of the defecation, weight loss and reduction in waist circumference in some cases. Throughout the study neither of the patients observed adverse reactions. The improvement in lipid and glucose profile indicators was shown. The patients with increased glucose tolerance after 3 months of the product intake showed normal results. A rapid decrease in total cholesterol in the blood of all patients was observed.

Laminaria Jam enriched with selenium can be recommended for use as a dietary product in the nutrition of people with low selenium level and with the pathology of the cardiovascular system (coronary heart disease, hypertension, hyperlipoproteinaemia).

Inclusion of Laminaria jam enriched with chromium in a complex dietary therapy can reduce the manifestations of the metabolic syndrome, namely, improve lipid and carbohydrate metabolism, decrease body weight and waist circumference and subjectively improve the patients life.

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EXPRESSION SYSTEMS FOR PRODUCTION OF RECOMBINANT PROTEINS

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The discovery of the restriction enzymes and the development of the polymerase chain reaction (PCR) made it possible to amplify a gene of interest and to clone it into an appropriate vector.

Both are common and well established techniques in research labs and the basis for gene expression of recombinant proteins.

For structure-determination methods like X-ray crystallography or for biochemical characterization, e.g. of newly discovered cold-adapted enzymes, a rather elevated amount of pure protein (> 95%) is needed. Unfortunately the production of recombinant proteins in prokaryotic or eukaryotic systems is not always straightforward. Some time has to be spent on optimization to get a sufficient amount of protein, preferably in soluble form to get around the longsome process of protein refolding. In some research labs it is already common practice to generate several constructs per protein and thus to test for better solubility in parallel, e.g. using different fusion tags or host systems.

An overview of available host systems, vectors and fusion tags will be presented. A number of examples will be given showing how each of these can be applied to increase the expression yield and/or solubility of the protein.

SUBSTANTIATION OF THE SHELF LIFE FOR THE FROZEN SOFT SALMON CAVIAR

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Salmon caviar, which has high taste and nutrient properties, represents one of the most valuable food substances.

It is known that the caviar quality during its storage depends on the observance of technological conditions during its preparation, sanitary conditions on the factory, type of packaging, storage conditions, and some other factors.

The salting and freezing are the traditional methods of the caviar preservation. However, until now the freezing of salmon caviar was not widely used. Therefore, in our opinion, the technology of freezing of the salmon caviar became relevant.

The purpose of our study was to substantiate the shelf life for the salmon caviar at –18ºC in the absence of any preservatives.

The object of our study was hunchback salmon caviar, prepared according to the State Standard 1629–97 (Soft salmon caviar) without any preservatives.

The sampling, preparation of the average sample, and determination of quality and safety parameters were carried out using the corresponding standard methods of study.

Caviar samples (1000 g each) were packed into polymeric bags, approved for the use with fish products, and stored at minus 18 ºC. The shelf life was determined according to the methodical recommendations of the State Sanitary and Epidemiological Inspection “Sanitary and epidemiological assessment of the substantiation of the shelf life and storage conditions for foodstuffs” (Methodical recommendations 4.2.1847-04).

The dynamics of caviar quality parameters during the storage was characterized by changes in the content of nitrogenous compounds (nonprotein nitrogen, nitrogen from volatile bases (NVB)) and oxyacids and in the values of lipid hydrolysis and lipid oxidation indices.

The organoleptic evaluation was carried out during the whole storage period by at least 2 experts, experienced in the field of the certification of fish, non-fish objects, and the corresponding food substances.

In all samples during the whole storage period we observed the absence of bacteria from the colibacillum group, *Salmonella* genus, *Staphylococcus aureus*, sulphite-reducing clostridia, and also the mould. The microbial contamination of caviar was stable during the whole experiment and was equal to 7.0×10^3 CFU/g to the 13th month of the storage. The yeast number was lower than the normalized value, being equal to 5.0×10^1 CFU/g.

The organoleptic analysis showed that during the storage the eggs remained elastic and had a slightly wet surface; individual eggs could be easily separated from others. The caviar had nice smell and taste, typical for this product. We did not observe any detractive features of the product.

Two weeks after the caviar preparation, the content of nonprotein nitrogen was only 0.16% of the total nitrogen content; to the end of storage (13 months) the value of this parameter gradually increased up to 0.29% (Table 1). The most intensive NVB accumulation was observed during the first 6 months (from 22.06 to 25.13 mg%); during the next 7 months the NVB content slightly increased up to 27.01 mg%.

Table 1. Nonprotein nitrogen, nitrogen from volatile bases (NVB) in caviar

Parameter	Storage time, months					
	1	2	6	8	12	13
Nonprotein nitrogen,%	0.16	0.19	0.23	0.25	0.28	0.29
NVB,%	22.06	23.51	25.13	25.93	26.36	27.01

During the storage time, we observed an increase in the acidity index value from 3.1 to 4.6 mg KOH per 1 g of oil; the oxyacid content increased from 0.3 to 0.6%. The most intensive increase in the number of free fatty acids was observed during the first months of the storage (from 1.3 to 2.6 mg of KOH per 1 g of oil). At the same time, we did not reveal any regularity in the peroxide number changes (Table 2).

Table 2. Acidity index, oxyacid content and peroxide number in caviar

Parameter	Storage time, months					
	1	2	6	8	12	13
Acidity index, mg KOH/1 g of oil	3,1	3,6	3,9	4,2	4,4	4,6
Oxyacid content,%	0,3	0,4	0,4	0,6	0,5	0,6
Peroxide number,% J_2	0,11	0,68	0,23	0,19	0,93	0,48

The results concerning peroxide numbers do not correlate with the observed organoleptic and microbiological changes in the examined samples.

The peroxide number does not reflect the level of oxidative damages, arising during the storage of a product; therefore, this parameter can not be used to characterize such damage of products. This fact is confirmed by publications of other authors and by the data, obtained in the VNIRO-TEST research laboratory during the study of the oxidative damage of fish oil.

The analysis of such parameters as nonprotein nitrogen content, NVB content, oxyacid content, and oil acidity index showed the absence of any explicit hydrolytic and oxidative processes during the storage of salmon caviar; this fact is also confirmed by the organoleptic analysis.

Thus, the results of microbiological, physicochemical, and organoleptic studies confirmed the quality of frozen caviar, stored at minus 18°C in a polymeric packaging, corresponded to the requirements of the State Standard 1629–97 and Sanitary Standard 2.3.2.1078-01 during the whole storage period.

The freezing of soft salmon caviar without any preservatives makes it possible to keep its quality for 12 months in the case of its storage at minus 18 ºC. The results of our studies, conducted jointly with the TINRO-Center state unitary company and devoted to the storage of the frozen salmon caviar, became the basis for the development of the State Standard R 53353–2009 «Frozen soft salmon caviar».

BIOTECHNOLOGICAL ASPECTS OF SEAWEEDS PROCESSING OF ARCTIC SEAS

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Seas of Arctic zone – White Sea and Barents Sea – are practically a perennial source of algae. At present time to seaweed as to perspective raw material for receiving various on the properties and functions of biologically active substances and foodstuff interest of scientists and experts became more active. Seaweeds and products of their processing are widely used in medicine, biochemistry, genetic, microbiology and a lot of other fundamental and applied sciences.

Commercially important brown seaweeds of Arctic Russian Seas are Laminariales – *Laminaria saccharina* and *Laminaria digitata* and Fucales – *Fucus vesiculosus* and *Ascophyllum nodosum*. Considerable volumes of them are caught and processed annually for the purpose of producing a various production.

Analysis of Laminariales and Fucales chemical composition has shown that the content of mineral substances decreases and the quantity of organic components increases in tissue of seaweed to the end of a summer season.

The most appreciable changes with minerals substances in tissue at Laminariales. The content of minerals decreases from 28–30% in June to 18–19% in September that naturally leads to increase of the general content of organic substances in seaweed from 70 up to 82% (Repina et al., 2004; Podkorytova, 2005; Podkorytova et al., 2007).

The content of mineral components in *Fucales* decreases from 19–21.5% from June down to 17.9-18.2% in September. In the sum of organic substances the greatest share belongs to alginic acid which is structural polysaccharide of great importance at processing of algae.

Changes in contents of alginic acid in Laminariales are insignificant, and approximately equal 2–3%. In *Fucales*, especially in *A.nodosum,* this changes are strongly pronounced and reaches to 7%. Such information corresponds common opinion on biosynthesis of alginic acid which collects in brown seaweeds at summer and autumn seasons (Podkorytova, 2005). Exactly in this time seaweeds of White and Barents Seas are the most valuable raw materials for alginate receive.

At the process of biosynthesis brown seaweeds accumulates significant value of low-molecular carbohydrates, generally, mannitol. The content of mannitol in *L. saccharina* and *L. digitata* is a rather stable at summer season date which slightly grows by September up to 20–23% in *L. digitata* and to 19–20% in *L. saccharina*. Fluctuations in contents of mannitol in Fucales of the White Sea are insignificantly – 1–4% on extend of the summer season. In *F. vesiculosus* the content of mannitol hesitates between 5.7 and 9.9%. At the same time in *A. nodosum* fluctuations approximately equal 5.1–7.1%. In general *Laminariales* differs for their higher content of mannitol (4–5 times higher) compared to *Fucales*. The tendency of mannitol content increase is marked in *Laminariales* at the beginning of July and further – some decrease at the middle of July, which is probably related with usage of mannitol by seaweed for synthesis of structural polysaccharides (Repina et al., 2004).

At the process of biosynthesis of an organics in brown seaweeds the laminaran, which plays role of a spare substance, collects. The content of this low-molecular polysaccharide varies between 2% and 20% depending on a species of seaweeds (Zvyagintseva et al., 2002). As for laminaran of brown seaweeds of White Sea – its dynamics for Laminariales and Fucales simultaneously shows growth in 2–3 times by the end of summer season (Repina et al., 2004).

At this time Fucales are intensively investigated and used to receiving probiotics since they synthesize of polysaccharides sulphat known as fucoidan (Painter, 1983; Usov et al., 2001). These biopolymers show different biological activity: anticoagulant, antivirus, anticlerical, anti-inflammatory, antineoplastic etc. As a main source of this valuable polysaccharide we treat *F. vesiculosus,* which received its highest content form 13,4 to 16,5% with some growth by the autumn season. In *A. nodosum* noticed stable in content of this polysaccharide with some limits between 10.0–11.5%. Information about content of fucoidan reveals ability of complex usage of Fucales for the purpose of receiving extracts containing other probiotics – fucoidan and others (Repina et al., 2004; Podkorytova et al., 2007).

Laminariales and Fucales just slightly differ by content of protein. At the time the whole tendency of changing protein content – maximum in June and minimum in September – still traced. The content of Iodine in seaweeds is an indicator of value of this raw material as a natural source of iodine for normal functioning of human organism (Podkorytova et al., 2005). Content of Iodine in Laminariales is 2–3 times higher compared to *Fucales* and equals 0.23% in *L. digitata* at the middle of June. In *F. vesiculosus* maximum takes place at July and in *A. nodosum* at August.

Thus, system knowledge about of seaweed chemical composition, maximums of accumulation of important biocomponents and the biotechnological approach to their processing allows to carry out consecutive extraction of biologically active substances with receiving not only them in the allocated condition, but also drinks, the foodstuff possessing treatment-and-prophylactic properties and well influencing a human body.

On the basis of the researches certain patterns in accumulation of biologically active substances by brown seaweed of Arctic Seas in the process of their growth are determined and the new complex technology of brown seaweed processing of *Fucales* and *Laminariales* species with receiving of functional foodstuff (Repina et al., 2004) and probiotics to nutrition is developed (Usov et al., 2001).

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PROPERTIES AND FUNCTIONS OF VERY LONG POLYENOIC FATTY ACID CHAINS OF MEMBRANE LIPIDS (COMPUTER SIMULATION STUDY)

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Biological membranes are heterogeneous: they consist of various lipid molecules with various head groups and fatty acid (FA) chains, biomembranes include proteins as well as other molecules. Membrane lipids, being organized into a bilayer structure, serve as a basic matrix for other constituents. The most commonly occurring biomembrane FA chains have 12 – 22 carbons; they may contain 1 to 6 *cis* double bonds in various positions, i.e., the chains may be saturated, unsaturated or polyunsaturated (PU). As a rule, the double bonds of natural FAs are methylene-interrupted. Phospholipids (in particular phosphatidylcholine, PC) of some tissues were found to contain a series of unusial FAs with a chain longer than 22 carbon atoms, the so-called 'very long chain' (VLC) FAs. These chains (VLC FAs or VLC PUFAs) are important components of different classes of lipids in all organisms from bacteria to man (Řezanka, Sigler, 2009), in spite of the fact that VLC PUFAs are rare, they represent a minor component of the total fatty acids (~1 – 5%). For instance, marine sponges contain VLC PUFAs 26:3(n-7)*cis*, 28:3(n-9)*cis*, 30:3(n-7)*cis*, 30:4(n-6)*cis* and 30:5(n-3)*cis* (Litchfield et.al., 1979); see also data of the other authors (Joseph, 1979; Řezanka, 1989; Djerassi, Lam, 1991; Řezanka, Sigler, 2009). Marine dinoflagellates

Prorocentrum mexicanum, P. micans, Scrippsiella sp., Symbiodinium microadriaticum, Gymnodinium sp., G. sanguineum, Fragilidium sp. were found to contain VLC PUFAs 28:7(n-6)*cis*, 28:8(n-3)*cis* (Mansour et. al., 1999), dinoflagellates *Cryptecodinium cohnii* were found to contain 28:8(n-3)*cis* (Van Pelt et. al., 1999). A set of VLC PUFAs including 36:8(n-3)*cis* was identified in *Amphidinium carterae* (Řezanka et.al., 2008; Řezanka et.al., 2008a) and other cells (Řezanka, Sigler, 2009). More than 50 VLC PUFAs were identified in freshwater crustacean species *Bathynella natans*, *B. baicalensis*, *Baicalobathynella magna*, predominantly 26:5(n-6)*cis*, 28:7(n-6)*cis*, 30:7(n-3)*cis* and 40:7(n-6)*cis* (Řezanka, 2000).

Various lipid membranes are extensively studied by a variety of experimental and theoretical methods. Nevertheless experimental data concerning physical-chemical properties of VLC PUFAs are scarce. On the theoretical side, atomic-scale computer simulations have become nowadays a standard tool for studying biomolecular systems. The study of molecular models of various lipids and lipid chains by computer simulation methods can well complement experimental techniques. Analysing the properties of such molecules is of great importance both from the scientific and technological points of view. Namely, besides leading to a deep understanding one of the fundamental properties of natural membranes and their constituents (which are surface active substances), it can also help in designing new, potentially environmentally friendly or even biodegradable but effective materials for the chemical, biotechnological, medical (and, perhaps, washing, cosmetic, pharmaceutical) industry.

In this paper, Monte Carlo computer simulations of VLC PUFAs (more correctly, hydrocarbon chains) with methylene-interrupted cis double bonds, N:4(n-6)*cis*, N:4(n-3)*cis*, N:5(n-6)*cis*, N:5(n-3)*cis*, N:6(n-6)*cis*, N:6(n-3)*cis* have been carried out. Here N is carbon atom number, N = 24, 26, 28, …, 38. This computer simulation method was described earlier (Rabinovich, 1991). Variations of all torsion angles of the chains were considered to be continuous from 0 to 360 deg. The conformational energy of a chain was represented as a sum of the structural unit energies. A scheme of pairwise interdependence of torsions was used. The units reproduce precisely the chain structure. The energy of nonbonded interactions, torsional and electrostatic terms were taken into account. The method is applied to an investigation of the flexibility and other characteristics of the FA chains.

According to statistical averaging formulas of classical statistical physics, thermodynamic averages of any observables of a chain molecule are given by mathematical expressions with multidimensional definite integrals (Flory, 1969). Unfortunately, these integrals cannot be evaluated analytically for more than a handful of nontrivial models of the molecule, and conventional methods of integration are also not feasible. The basic idea of the Monte Carlo method is to calculate the integrals numerically: to generate a large number of trial conformations and replace the integrations by summations over a finite number of conformations of the molecule. In this work, 300000 – 1000000 conformations at temperature 298 K (25 ºC) were generated for each of the above-mentioned FA chains. Mean end-to-end (carbon – carbon) distance $\langle h_0 \rangle$ and mean-square end-to-end distance $\langle h^2_0 \rangle$ of each chain were calculated in theta-conditions (Flory, 1969). Then values of $\langle h_0 \rangle / L$ and $\langle h^2_0 \rangle / L^2$ were obtained, where L is contour length of the chain. It is clear that the smaller ratio $\langle h_0 \rangle / L$ (or $\langle h^2_0 \rangle / L^2$) of a chain, the more flexible the chain is.

The calculated values of $\langle h^2_0 \rangle / L^2$ are shown in Fig.1. To compare the calculated ratio $\langle h^2_0 \rangle / L^2$ of hydrocarbon chains of different unsaturation the value X should be used; X is the arithmetical mean of the numbers of carbon atoms of the double bonds (location of the "center" of the double bonds of the given molecule), see Table 1.

Fig.1 shows that chain flexibility depends on (*i*) the number N of carbons, (*ii*) the number of double bonds in the chain, and (*iii*) their locations in the chain. In other words, the number of double bonds and their position in the chain determine the flexibility, other things (N) being equal. Let us compare also the values of $\langle h_0 \rangle / L$ of the chains (Table 2). It is seen from Fig.1 and Table 2 that flexibilities of (n-3)-hexaenes coincide with flexibilities of corresponding (n-6)-pentaenes (pentaenes of equal length N). The same rule is obtained for (n-3)-pentaenes and (n-6)-tetraenes. As a matter of fact, the general rule for modification of VLC PUFA structures is **saturation** of one double bond located in the third carbon of the chain: in that case there is no difference in flexibility between initial and final chains at N=Const. Two different chains of equal length N have the same flexibility, therefore contributions of both chains to membrane fluidity coincide very closely.

On the other hand, fluidity of the lipid bilayer (which is affected by flexibility of the lipid chains) is a necessary but not sufficient condition for biomembrane functionality, especially because the temperatures of the lamellar gel – liquid crystalline phase transition in fully hydrated PCs of different *sn-2* chain

unsaturation (Koynova, Caffrey, 1998) show that increased chain unsaturation above a certain number of double bonds does not necessarily translate into increased membrane fluidity. It is easily seen from the experimental data collected by Koynova and Caffrey that at physiological temperatures, a fluid lipid bilayer could be attained by lipids having less unsaturated fatty acids than VLC PUFAs (for instance, only 18:1 and 18:2 chains).

Hence the influence of usual PUFA chains and unusual VLC PUFA chains of lipids is much more than the simple 'fluidization' of the matrix of lipid membrane. Another data seem to be essential in that case. Namely, it is known that an increase in the number of methylene-interrupted *cis* double bonds to the maximum in a linear hydrocarbon chain, results in a sharp decline of the absolute magnitude of the chain temperature coefficient | $d \ln\langle h_0\rangle/dT$ | (Rabinovich, Ripatti, 1994; Rabinovich, 2008). For instance, the size temperature sensitivity coefficient $|d \ln\langle h_0\rangle/dT|$ of PU molecule 22:6(n-3)*cis* is ten times lower than that of saturated 22:0 molecule.

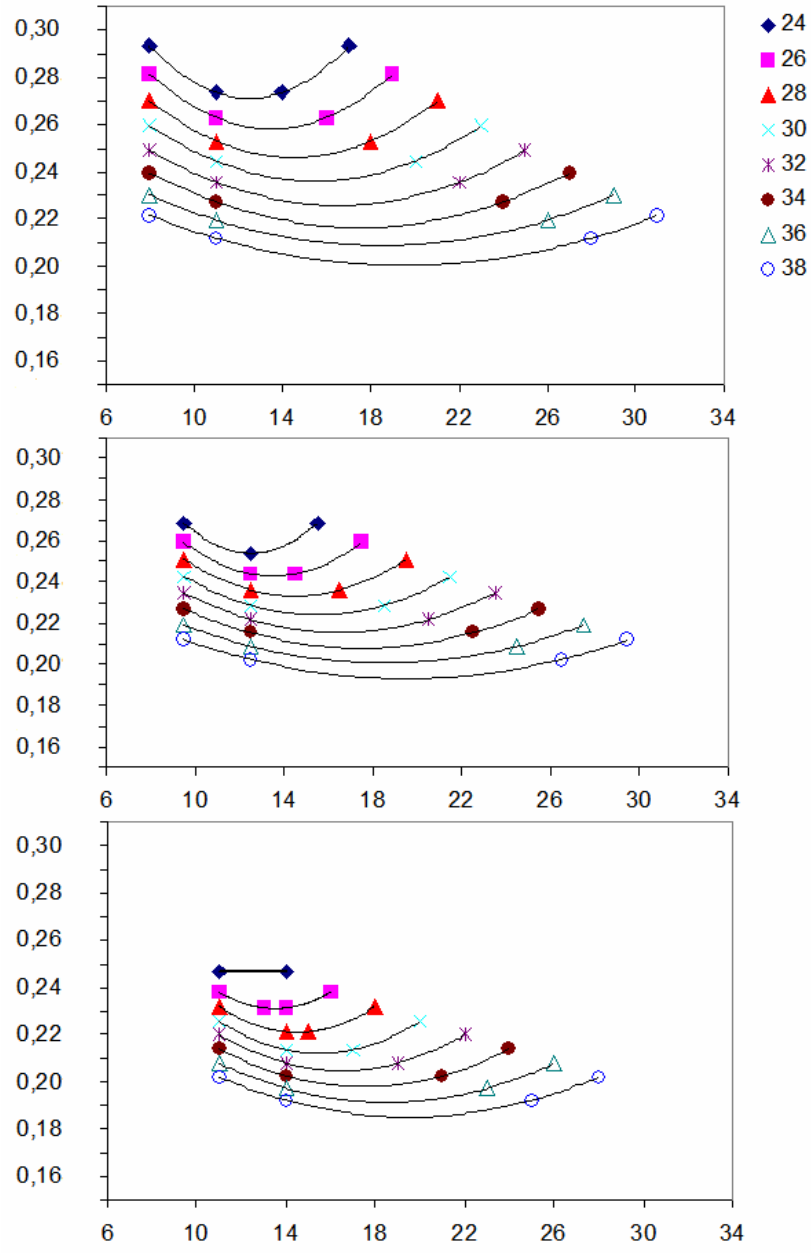

Fig.1. Monte Carlo computer simulation results, T = 25 ºC.
Vertical axes: flexibility $\langle h^2_0\rangle/L^2$ of chains
N:4(n-3)*cis* and N:4(n-6)*cis* (above);
N:5(n-3)*cis* and N:5(n-6)*cis* (in the centre);
N:6(n-3)*cis* and N:6(n-6)*cis* (below).
Each number in the column of symbols is the chain length (it is the number of carbon atoms N).
Horizontal axes:
The arithmetical mean of the numbers of the double bonded carbon atoms, X

Table 1. Correspondence of the X value to the location of methylene-interrupted double bonds in polyunsaturated hydrocarbon chains

Position of double bonds, **tetraenes**	X	Position of double bonds, **pentaenes**	X	Position of double bonds, **hexaenes**	X
3, 6, 9, 12	**8**	3, 6, 9, 12, 15	**9.5**	3, 6, 9, 12, 15, 18	**11**
4, 7, 10, 13	**9**	4, 7, 10, 13, 16	**10.5**	4, 7, 10, 13, 16, 19	**12**
5, 8, 11, 14	**10**	5, 8, 11, 14, 17	**11.5**	5, 8, 11, 14, 17, 20	**13**
6, 9, 12, 15	**11**	6, 9, 12, 15, 18	**12.5**	6, 9, 12, 15, 18, 21	**14**
7, 10, 13, 16	**12**	7, 10, 13, 16, 19	**13.5**	7, 10, 13, 16, 19, 22	**15**
8, 11, 14, 17	**13**	8, 11, 14, 17, 20	**14.5**	8, 11, 14, 17, 20, 23	**16**
9, 12, 15, 18	**14**	9, 12, 15, 18, 21	**15.5**	9, 12, 15, 18, 21, 24	**17**
10, 13, 16, 19	**15**	10, 13, 16, 19, 22	**16.5**	10, 13, 16, 19, 22, 25	**18**
11, 14, 17, 20	**16**	11, 14, 17, 20, 23	**17.5**	11, 14, 17, 20, 23, 26	**19**
12, 15, 18, 21	**17**	12, 15, 18, 21, 24	**18.5**	12, 15, 18, 21, 24, 27	**20**
13, 16, 19, 22	**18**	13, 16, 19, 22, 25	**19.5**	13, 16, 19, 22, 25, 28	**21**
14, 17, 20, 23	**19**	14, 17, 20, 23, 26	**20.5**	14, 17, 20, 23, 26, 29	**22**
15, 18, 21, 24	**20**	15, 18, 21, 24, 27	**21.5**	15, 18, 21, 24, 27, 30	**23**
16, 19, 22, 25	**21**	16, 19, 22, 25, 28	**22.5**	16, 19, 22, 25, 28, 31	**24**
17, 20, 23, 26	**22**	17, 20, 23, 26, 29	**23.5**	17, 20, 23, 26, 29, 32	**25**
18, 21, 24, 27	**23**	18, 21, 24, 27, 30	**24.5**	18, 21, 24, 27, 30, 33	**26**
19, 22, 25, 28	**24**	19, 22, 25, 28, 31	**25.5**	19, 22, 25, 28, 31, 34	**27**
20, 23, 26, 29	**25**	20, 23, 26, 29, 32	**26.5**	20, 23, 26, 29, 32, 35	**28**
21, 24, 27, 30	**26**	21, 24, 27, 30, 33	**27.5**		
22, 25, 28, 31	**27**	22, 25, 28, 31, 34	**28.5**		
23, 26, 29, 32	**28**	23, 26, 29, 32, 35	**29.5**		
24, 27, 30, 33	**29**				
25, 28, 31, 34	**30**				
26, 29, 32, 35	**31**				

Table 2. Values of $<h_0>/L$ for several FA chains*

chain	$<h_0>/L$	chain	$<h_0>/L$
38:5(n-6)*cis*	**0.423**	**38:6(n-3)*cis***	**0.423**
36:5(n-6)*cis*	**0.430**	**36:6(n-3)*cis***	**0.429**
34:5(n-6)*cis*	**0.436**	**34:6(n-3)*cis***	**0.435**
32:5(n-6)*cis*	**0.442**	**32:6(n-3)*cis***	**0.441**
30:5(n-6)*cis*	**0.449**	**30:6(n-3)*cis***	**0.447**
28:5(n-6)*cis*	**0.456**	**28:6(n-3)*cis***	**0.452**
26:5(n-6)*cis*	**0.463**	**26:6(n-3)*cis***	**0.457**
24:5(n-6)*cis*	**0.472**	**24:6(n-3)*cis***	**0.465**
chain	$<h_0>/L$	chain	$<h_0>/L$
38:4(n-6)*cis*	**0.434**	**38:5(n-3)*cis***	**0.434**
36:4(n-6)*cis*	**0.441**	**36:5(n-3)*cis***	**0.441**
34:4(n-6)*cis*	**0.449**	**34:5(n-3)*cis***	**0.449**
32:4(n-6)*cis*	**0.458**	**32:5(n-3)*cis***	**0.458**
30:4(n-6)*cis*	**0.466**	**30:5(n-3)*cis***	**0.465**
28:4(n-6)*cis*	**0.474**	**28:5(n-3)*cis***	**0.473**
26:4(n-6)*cis*	**0.482**	**26:5(n-3)*cis***	**0.480**
24:4(n-6)*cis*	**0.491**	**24:5(n-3)*cis***	**0.487**

*Errors of the calculations are within ±0,004 value.

It was shown experimentally that VLC PUFAs are concentrated in unusual dipolyunsaturated molecular species of PC and are attached to the *sn-1* position of the glycerol backbone, whereas 22:6(n-3)*cis* is mainly esterified at the *sn-2* position. Dipolyunsaturated boundary lipids with VLC PUFAs seem to provide the proper conditions of the proteins for their optimal functioning at different temperatures, similar to usual PUFAs (Rabinovich, Ripatti, 1994; Rabinovich, 2008). Clearly, further research is necessary to elucidate fully the properties of this unique VLC PUFA lipid tails.

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COMPUTER SIMULATION STUDY OF PROPERTIES OF UNSATURATED LIPID MEMBRANES

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Biomembranes surround cells: a membrane separates the interior of a cell from the outside environment. Being selectively permeable, membranes participate in control of the movement of various compounds (substances) into and out of cells. Biomembranes are very complex heterogeneous systems consisting of many different types of lipids, sterols, proteins, carbohydrates and various membrane associated molecules which are involved in a variety of cellular processes. Consequently, membranes play an active part in the life of the cell, they exist as dynamic structures. Lipid molecules differ with respect to the type of hydrophilic head-group and occur with a wide variety of hydrophobic hydrocarbon chains of fatty acids (FAs). Usually the most abundant phospholipid in animal and plants is phosphatidylcholine (PC), it is the key building block of membrane bilayers. Knowledge of physical-chemical properties of lipid bilayers is a key element of our general understanding of biomembrane functioning, which is one of the greatest challenging problems in biochemical, biophysical and biomedical sciences. This information is also relevant and essential in new biotechnological and biomedical applications.

Experimental measurements of structural and dynamical properties are obtained as averages over a large number of lipids and over a certain time interval, which not always can give an unambiguous picture of individual lipids and their interactions. During the last decades computer simulations have become a well established tool of modern investigations of molecular structure. Monte Carlo (MC) or molecular dynamics (MD) can provide three-dimensional real-time imaging of the system with atomistic-level resolution, and hence can give essential structural and dynamical information which otherwise is hardly accessible by any experimental method. The rapid development of the accessible computer power has made simulations of more and more complicated systems feasible, and allowed also increase the size of the simulated systems. The amount of works on simulations of lipid membrane systems has increased tremendously, and a number of reviews appeared accounting for this in the past decade (Damodaran, Merz, 1994; Mouritsen, Jørgensen, 1994; Pastor, 1994; Mouritsen et al., 1996; Jacobsson, 1997; Merz, 1997; Tieleman et al., 1997; Tobias et al., 1997; Berendsen, Tieleman, 1998; Feller, MacKerell, 2000; Feller, 2000; Forrest, Sansom, 2000) and more recently (Feller, 2001; Tobias, 2001; Scott, 2002; Hansson, 2002; Saiz, Klein, 2002; Saiz et.al., 2002; Vigh et. al., 2005; Chan, Boxer, 2007; Vermeer et al., 2007; Feller, 2008; Marrink et al., 2009; Pandit, Scott, 2009).

It was mentioned that a typical biological membrane contains many species of lipid molecules, with different head groups and hydrocarbon tails. The most commonly occurring FA chains may contain 1 – 6 carbon – carbon double bonds of the cis configuration in different positions. In most cases, at least half of the FA chains are unsaturated. The double bonds of polyunsaturated (PU) chains are, as a rule, methylene-interrupted. The PUFA tails of lipids are of great importance for the structure and functioning of biomembranes (Dratz, Deese, 1986; Rabinovich, Ripatti, 1994; Gawrisch et al., 2003; Stillwell, Wassall, 2003; Rabinovich et al., 2003; Valentine, Valentine, 2004; Feller, Gawrisch, 2005; Gawrisch et al., 2008; Stillwell, 2008; Wassall, Stillwell, 2009). Docosahexaenoic acid, 22:6(n-3)cis, is the longest and most unsaturated FA commonly found in nature. It is known that membranes that are active metabolically have high levels of PU chains. PU chains have been linked to the great number of biochemical processes, to an enormous variety of human afflictions. Evidently the basis of these phenomena is the specific chemical structure of PU chains, which results in their specific physical properties, which are in its turn cause their specific functioning in living organisms. Full understanding of the effects of lipid unsaturation on various physical properties of membranes at the molecular level, affecting their functioning, is not yet achieved. The mechanisms of many biological functions of PUFAs remain a subject of much debate.

We have carried out series of MD simulations of 16 hydrated liquid crystalline phase PC bilayers consequently changing the number of double bonds in the *sn*-2 chain of phospholipids with *sn*-1 saturated and *sn*-2 unsaturated chains: 18:0/18:1(n-9)cis PC, 18:0/18:2(n-6)cis PC, 18:0/18:3(n-3)cis PC, 18:0/18:4(n-3)cis PC, 18:0/18:5(n-3)cis PC, 18:0/20:4(n-6)cis PC, 18:0/20:5(n-3)cis PC, 18:0/22:6(n-3)cis PC, 16:0/18:1(n-9)cis PC, 16:0/18:2(n-6)cis PC, 16:0/18:3(n-3)cis PC, 16:0/18:4(n-3)cis PC, 16:0/18:5(n-3)cis PC, 16:0/20:4(n-6)cis PC, 16:0/20:5(n-3)cis PC, 16:0/22:6(n-3)cis PC. The main goal was to study their physical properties and the features of PU bilayers.

The main idea of MD simulations of a many-particle system is the solution of the Newton's equations of motion for a set of particles (atoms or molecules) that comprises the system. This procedure includes a model description of the atomic system, atom-atom interaction potentials, boundary conditions of the system, and an approximate step-by-step technique for solving the classical equations of motion. Bilayer system setup and simulation details were as follows: each bilayer was simulated in a rectangular periodic box within NPT ensemble, i.e., with constant number of lipid molecules N, pressure P (1 atm) and temperature T (303 K); 128 PC lipids per bilayer (64 lipids in each leaflet) with explicit hydrogens were used; the two hydrocarbon tails, the glycerol section and the head group of the lipid molecules were treated in accordance with their known chemical structure; the lipids were hydrated by 3840 water molecules (30 waters per lipid) which were approximated by anharmonic flexible SPC water model; the electrostatic interactions were treated by the Ewald summation method. To calculate the energy of the bilayer systems CHARMM27 force field with a scaling factor for electrostatic interactions between 1...4 neighbours and corrections of partial atom charges were used (Hőgberg et al., 2008). The double time step algorithm (Tuckerman et al., 1992) was used to treat separately fast (covalent bonds, angles, torsions, collision Lennard-Jones forces within 5 A distance) and slow forces: 0.25 fs time step for the fast, 2 fs – for the slow forces.

The average area per lipid defined in constant pressure – zero tension simulations, is a parameter which is most often used to define the quality of the force field used in the simulations. Area per lipid molecule A_p_m is one of the most fundamental properties of a lipid bilayer and one of the most common ways to determine whether the bilayer system has reached equilibrium. When the area per lipid reaches a stable value, other structural properties (density distributions, NMR order parameters) do not change either. Simulated area per lipid can be also compared with experimental values available from X-ray or neutron diffraction and volumetric data. A collection of average lipid areas for several bilayers composed from different lipids and computed from different force fields, as well as experimental areas, is available in Table 1 of paper (Poger, Mark, 2010). More reliable validation of a force field can be done by comparison of simulated and experimental structure factors as it was shown in paper (Benz et al., 2005). Additional important source of data for validation of a force field used in lipid bilayer simulations is NMR bond order parameters.

The temporal behavior of the A_p_m ($Å^2$) of the bilayers is shown in Fig.1(*a* and *b*). It is clear from this figure that MD simulations of the bilayers should be at least of the order of tens of nanoseconds to reach equilibrium and surpass the longest characteristic timescales for A_p_m fluctuations. For this reason, the first 20 ns of the total 60 ns were considered as equilibration, and the last 40 ns were used for analysis and calculations. Consecutive configurations of the bilayers were sampled every 1 ps.

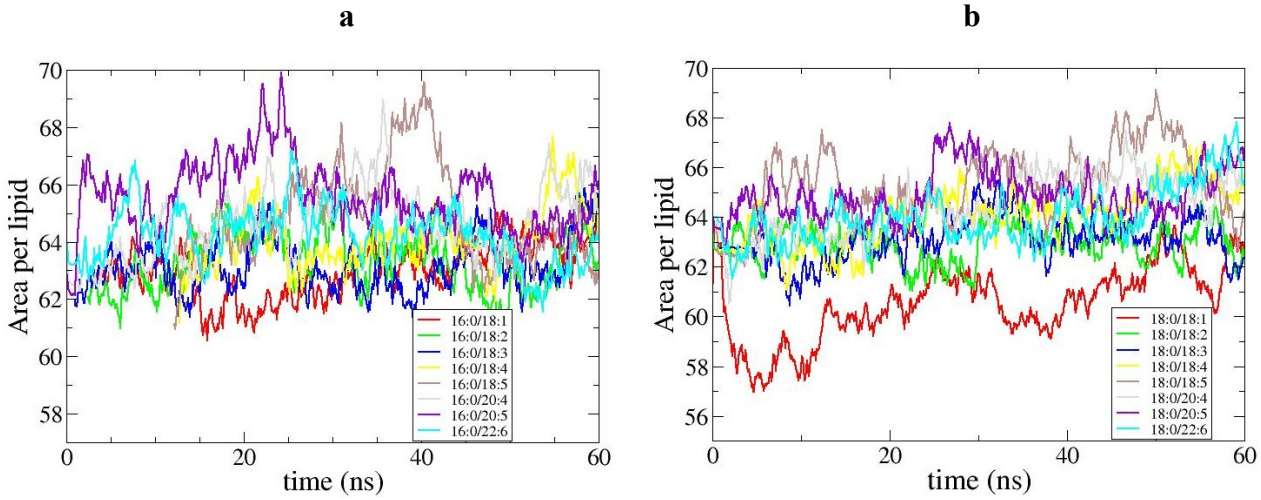

Fig.1. The temporal behavior of the average area per lipid A_p_m ($Å^2$) of the PC bilayers with 16:0 (*a*) and 18:0 (*b*) *sn*-1 chains

Different equilibrium structural and dynamic parameters of the bilayers were defined, such as the average area per lipid head group and its fluctuations, bond order parameters of lipid molecules with respect to the bilayer normal, the orientational fluctuations of the bond vectors, the root mean square values of the positional fluctuations of all lipid atoms relative to the average atomic coordinates, mass density distributions of various atoms of lipids relative to the bilayer middle plane, interpenetration of lipid tails of the opposite monolayers into each other, etc. In this paper the main goals are the C-C bond order parameters to characterize the order in lipid bilayers. These quantities are sensitive to the degree of unsaturation in the chains. The order parameter for each C-C bond of the chains may be defined as:

$$S_{CC} = (3\cdot\langle\cos^2\beta_{CC}\rangle - 1)/2,$$

where β_{CC} is the angle between a C-C-bond and the bilayer normal. Fig. 2 (*a, b, c, d*) shows the C-C bond order profiles for the sixteen bilayers and marked differences in the S_{CC} order parameter between odd and even running numbers for hydrocarbon chains *sn*-1 and *sn*-2 of the bilayers.

The 'ordinary' odd-even effect for saturated *sn*-1 chains 16:0 and 18:0 (Fig.2) is well known: this is because the rotations of the CH_2 groups about their local axes are anisotropic. The main difference in behavior and properties of unsaturated lipid molecules is that the S_{CC} order parameters of single C-C-bonds next to the cis double bond C=C in all unsaturated *sn*-2 chains are lower than that for the double bond C=C and for the corresponding single bond C-C (i.e. C-C bond **with the same number**) in the saturated chains *sn*-1.

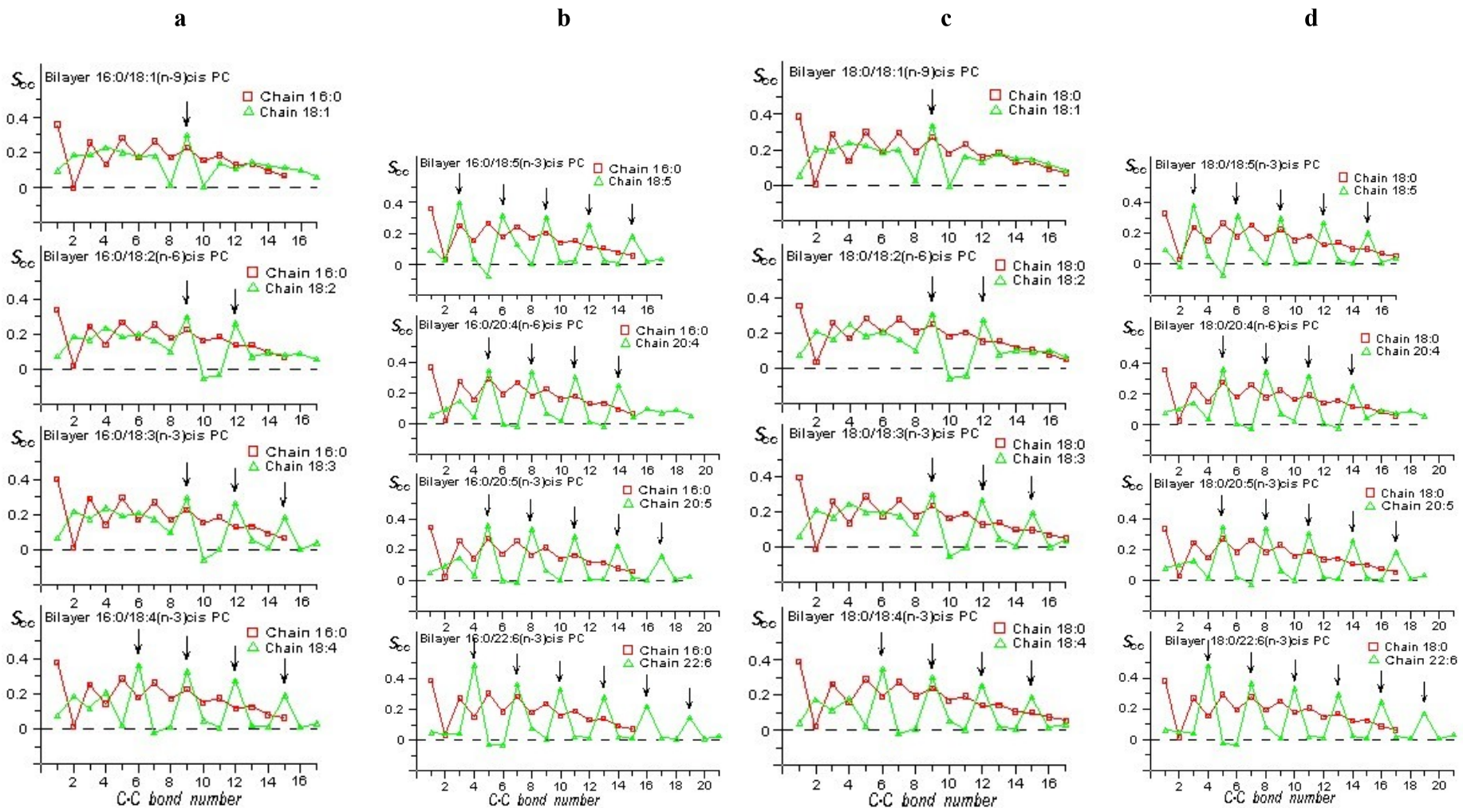

Fig. 2. Order parameter S_{CC} as a function of the C-C bond number of the lipid hydrocarbon chains of the eight PC bilayers with *sn-1* chain 16:0 (*a,b*) and the eight PC bilayers with *sn-1* chain 18:0 (*c,d*) as determined from the MD simulations. The arrows indicate the double bonds

It should be noted that the main qualitatively characteristic features of the profiles of S_{CC} for a given acyl chain (*sn*-2) of the bilayers are highly characteristic for the level of unsaturation. It means that a close relationship between the investigated order parameters and the structure of the chains is elucidated. The number of the chain carbons, the number of *cis* double bonds and their position in the chain determine principally the calculated order properties: they are similar both for the bilayers with 16:0 and 18:0 chains *sn*-1. The understanding of the molecular basis of the physical properties of the lipids allows one to narrow down the list of hypotheses under consideration about the possible function of various acyls in lipid membranes.

The temperatures of the lamellar gel – liquid crystalline phase transition in fully hydrated PCs of different *sn-2* chain unsaturation (Koynova, Caffrey, 1998) show that increased chain unsaturation above a certain number of double bonds does not necessarily translate into increased membrane fluidity: a fluid lipid bilayer could be attained by lipids having only mono- and diunsaturated FAs (e.g., 18:1 and 18:2) and hence the influence of PU chains of lipids is much more than the simple 'fluidization' of the matrix of lipid membrane. The effects of PU chains are more profound than would be observed by solely controlling the temperature. The results obtained in this work supplement the information about PU lipids, these data can be used for the analysis of the relations between properties and functions of PU lipids that play a key role in functioning of biomembranes (Rabinovich, Ripatti, 1994; Rabinovich, 2008).

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MULTI-DOMAIN ANTIMICROBIAL PEPTIDES – PURELY ANTIMICROBIALS OR MULTI-FUNCTIONAL MOLECULES

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Antimicrobial peptides (AMPs) are short proteins which have an *in vitro* inhibitory activity against microorganisms. Whereas the majority of AMPs are short peptides, an increasing number of AMPs are longer and contain regions within the molecule having clearly different characteristics. In our lab we have isolated several multi-domain AMPs from the crustacean *Hyas araneus*. Two of these belong to the well defined crustin family within *Crustacea*, which are cysteine-rich AMPs containing a whey acidic protein domain. This domain has been shown to have a multi-faceted role. In addition, we have isolated AMPs with novel primary structures, but which consist of domains with some similarities to domains found in already known AMPs. Arasin 1 has two distinctly different regions, with an N-terminal end enriched in proline and arginine residues, and a C-terminal end containing two disulphide bridges. Hyastatin is a 115 amino acid long AMP, containing three distinctly different regions. Both arasin 1 and hyastatin show high affinity to chitin, which enable them to contribute in wound healing and the molting process. All of these AMPs have been isolated from apparently healthy animals and they are constitutively and highly expressed in both stimulated and non-stimulated experimental animals, which strengthen the hypothesis that AMPs have additional roles than purely acting as antimicrobials during microbial invasion.

THE USE OF NATURAL BIOACTIVE PEPTIDES

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Short bioactive peptides are responsible for different functions in nature and have activities and properties that might be developed and exploited for commercial purposes. Different challenges and strategies will be focused on in the presentation.

The activities of peptides in animals are very broad and varied. The potential is huge as the activity can expand from antimicrobial and antitumoral activities, immune regulating properties, enzymatic or enzyme-inhibiting activities to mention some. The peptides might be constitutively produced or produced by induction by stimuli that might be working – locally, like for instance by the invasion of microbes.

Important questions are how the different activities can be exploited commercially in drug development or for other commercial purposes? What are the limitations and how can these be dealt with to overcome unwanted effects? Limitations can be characteristics in relationship to the ability to transverse membranes to reach sites where they are indented to act, or unwanted properties like being toxic to normal cells, or proteolytic instability.

In science it is also important to study bioactive peptides and reveal their mechanism of action to discover new potential drug targets, like networks of interacting proteins, receptors and ligands. Furthermore, performing investigations to overcome or limit unwanted properties is essential. For a drug it is important to resist degradation by proteases long enough to have an effect. New methods can for instance be used to create “tailor made” or designed proteins with improved pharmacological properties. Also a range of other modification can be made to the peptides.

EXCRETION OF TOTAL AMMONIUM BY SOME MARINE CRUSTACEANS

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At the present time in the world, including in Russia, developing trade in live aquatic animals. The main problem faced by employers is the creation and maintenance necessary base for aquatic animals pre-sale kept in large cities, which are the main market. Red King crab (*Paralithodes camtschaticus, Tilesius*, 1815) and the American lobster (*Homarus americanus*) is one of the main objects of commercial interest.

The most important condition necessary for the maintenance of aquatic habitat parameters is the correct design and further use of closed recycling water supply systems (CRWS). It should be borne in mind that the water temperature in the habitats of the red king crab is in the range from 0 to 14°C, American lobster – from 0 to 20°C, which is a deterrent to the process of biological treatment of recycled water by nitrifying bacteria inhabiting the biofilter. Low water temperature explain the relatively low level of metabolism.

The level of excretion ammonia is one of the major factors considered when designing CRWS. Therefore, the study of this process at different animal species and depending on those or other conditions is the key. Red king crab and american lobster are ammoniotelium species, i.e. excreting ammonia as the main product of nitrogen metabolism. Most part of the ammonia they have released through the gill epithelium. Ammonia (NH_3) is acutely toxic substance and its permissible concentration in circulating water in CRWS for aquatic animals is only 0.05 mg/L. Free ammonia reacts with water to form a less toxic compound – ammonium (NH_4OH or in the ionized form – NH_4^+). Its permissible concentration for marine crustaceans during prolonged keeping and rearing in CRWS is already 0.25–0.5 mg/L (Kovatcheva N. P. et al, 2006). Value of ammonium and free ammonia in total ammonium depends on pH and water temperature (at 5–10°C and pH 8–8.3 ammonium is 90% or more).

In scientific papers this question raised in terms of biochemical mechanism for the excretion of ammonia (Kormanik, Cameron, 1981; Weihrauch and oth., 2002), but not the quantitative aspect, which is more important for the production. In earlier studies in the laboratory of crustacean reproduction and cultivation (VNIRO, Moscow) have been investigated the excretion of total ammonium by red king crab larvae and postlarvae (Shakula L. A. et al, 2008).

The purpose of this study was determination of daily total ammonium excretion amount by market sized red king crab and american lobster in a CRWS per 1 kg of body weight (BW) at different water temperatures – 6 and 12°C.

Experiment was carried out in 2010 in the storage complex with CRWS «LaMareè» Ltd. (Moscow, Russia) under a contract with the laboratory. For the experiment, were selected 20 males red king crabs and 20 males american lobsters. Before landing in the experimental aquatron (fig. 1) animals were kept in the common industrial CRWS with planting density 50–60 kg/m^3 for red king crab, and 60–75 kg/m^3 – for American lobster. The concentration of ammonium in the water in this CRWS was an average of 0.155 mg/L, nitrites – 0.092 mg/L, nitrates – 25.744 mg/L and pH was an average 7.22. The total duration of animals' exposure in the CRWS was 2 – 5 days after catching from the sea. In the aquatron was installed a circulating pump and chiller, but there was no water purification unit. Aquatron were filled with 150 liters of artificial seawater with salinity 32–35‰, prepared from tap water and dry salt produced by "Marine Aquarium" (Russia).

Each crab or lobster was kept in aquatron 1 day without feeding. Before landing, the crabs were measured (total body weight and carapace width). The water sample was taken for hydrochemical analysis. Before boarding the animal aquatron was washed with fresh water. Concentration of ammonium was carried out on a photometer by Sedji-Solorzano method using phenol-hypochlorite reaction. Also measured water temperature and the content of dissolved oxygen (multiparameter instrument «YSI-85», USA), salinity (refractometer), pH (pH-meter «Hanna Instruments pH211», USA), consumption of circulating water (instrumental method). After 1 day crab or lobster was returned to a common CRWS, were taken samples from water, then filled again by new prepared artificial sea water and planted the following animal.

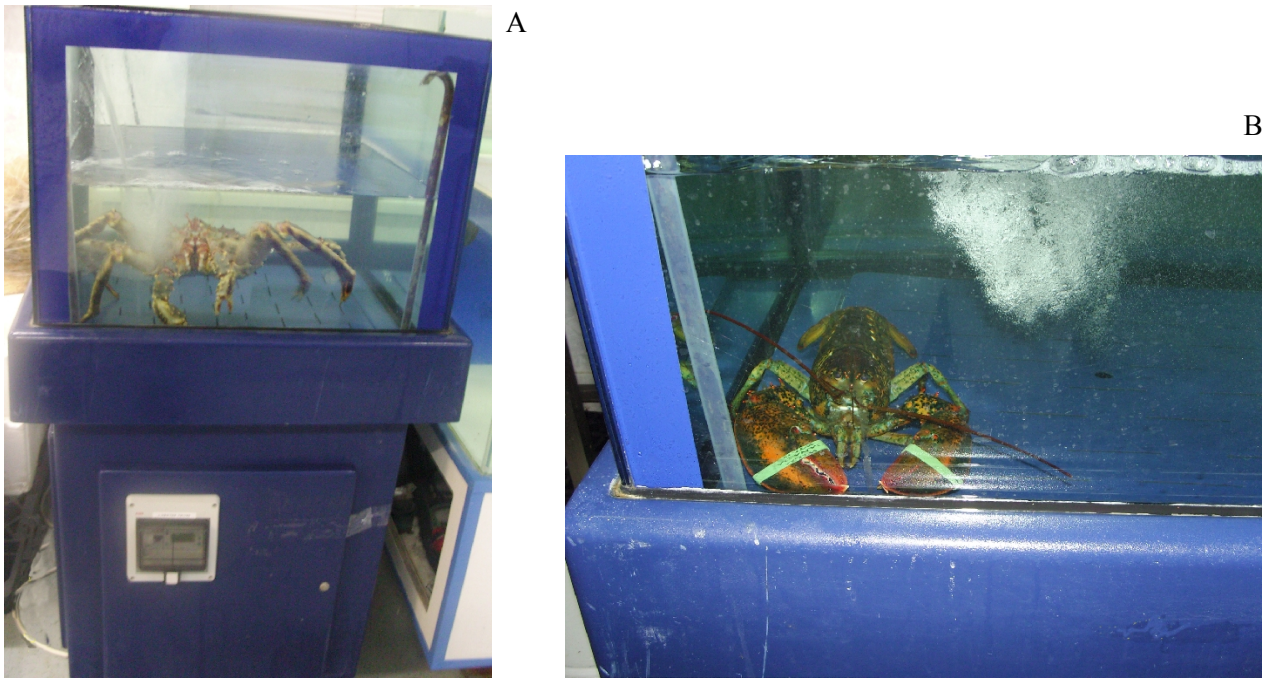

Fig. 1. Aquatron with red king crab (A – left) and american lobster (B – right)

Ranges, average values of the measured indicators and the brief bioassay are summarized in the Table 1. The results of hydrochemical analysis of water are presented in the Tables 2 and 3.

Table 1. Hydrochemical parameters in the experimental aquatron and bioassay indicators of animals

Indicators (an average)	Temperature, °C	
	5.9–6.9 (6.5)	12–12.5 (12.3)
Body weight, kg (BW): red king crabs american lobsters	 1.66–2,68 (2.108) 0.58–0.79 (0.703)	 1.95–2.74 (2.436) 0.66–0.78 (0.739)
Carapace width of crabs, mm	149–171 (160)	155–174 (167)
Planting density, specimens per m^3	6.7	
Biomass, kg/m^3: red king crabs american lobsters	 11,1–17,9 (14.1) 3,9–5,3 (4.6)	 13,0–18,3 (16.2) 4,4–5.2 (4,8)
The average specific water consumption, l/h per 1 kg BW: red king crabs American lobsters	 207.47 556.86	 176.03 518.31
Dissolved oxygen in water, mg / l: red king crabs American lobsters	 9.7–12.2 (10.6) 10.3–12.4 (11.4)	 8.8–9.6 (9.2) 8.4–9.6 (9)
pH of water: red king crabs American lobsters	 7.63–8.02 (7.83) 7.42–7.73 (7.56)	 7.72–8.29 (7.96) 7.64–7.86 (7.8)

Table 2. Changes in the ammonium concentrations in water (Red King crab)

Number of crab	Initial concentration of NH_4^+, mg/L	Final concentration of NH_4^+, mg/L	Δ, mg/L
	6°C		
1	0.293	0.535	0.242
2	0.237	0.319	0.082
3	0.310	0.473	0.163
4	0.230	0.371	0.141
5	0.250	0.338	0.088
6	0.365	0.636	0.271
7	0.280	0.335	0.055
8	0.277	0.397	0.120
9	0.258	0.327	0.069
10	0.443	0.580	0.137
Average	0.294	0.431	0.137

12**°C**			
1	0.481	0.821	0.340
2	0.531	0.833	0.302
3	0.506	0.865	0.359
4	0.529	1.028	0.499
5	0,547	1.014	0.467
6	0.496	1.016	0.520
7	0.407	0.787	0.380
8	0.373	0.870	0.497
9	0.307	1.084	0.777
10	0.503	1.098	0.595
Average	0.468	0.942	0.474

In the experiment with red king crab at the water temperature 12°C mean concentrations of ammonium was 3.46 times higher than at 6°C.

Table 3. Changes in the ammonium concentrations in water (american lobster)

Number of lobsters	Initial concentration of NH_4^+, mg/L	Final concentration of NH_4^+, mg/L	Δ, mg/L
6°C			
1	0.389	0.42	0.031
2	0.419	0.435	0.016
3	0.447	0.454	0.007
4	0.251	0.256	0.005
5	0.223	0.231	0.008
6	0.202	0.213	0.011
7	0.187	0.192	0.005
8	0.129	0.131	0,002
9	0.164	0.21	0.046
10	0.13	0.157	0.027
Average	0.25	0.27	0.02
12°C			
1	0.16	0.234	0.074
2	0.141	0.161	0.02
3	0.115	0.134	0.019
4	0.133	0.143	0.01
5	0.072	0.128	0.056
6	0.097	0.118	0.021
7	0.116	0.12	0.04
8	0.08	0.089	0.09
9	0.068	0.077	0.09
10	0.048	0.058	0.1
Average	0.1	0.13	0.02

In the experiment with american lobster at both water temperatures average difference of ammonium concentrations was approximately the same level.

However, the difference between the ammonium concentrations is only an intermediate result. Daily excretion of the total ammonium was calculated by dividing the difference between final and initial concentration of total ammonium in water, expressed in milligrams per kilogram of BW (Fig. 3, 4).

For a day, on average crabs were excreted 9.73 mg total ammonium per kilogram of BW at 6°C and 29.16 mg total ammonium per kilogram of BW at 12°C. That way, at 12°C red king crab was excreted total ammonium at 3.04 times more than 6°C. This is due to accelerated metabolism at the water temperature 12°C and increasing in general activity of crabs, because 4°C and 12°C – average water temperature in the Norwegian Sea in winter and summer respectively.

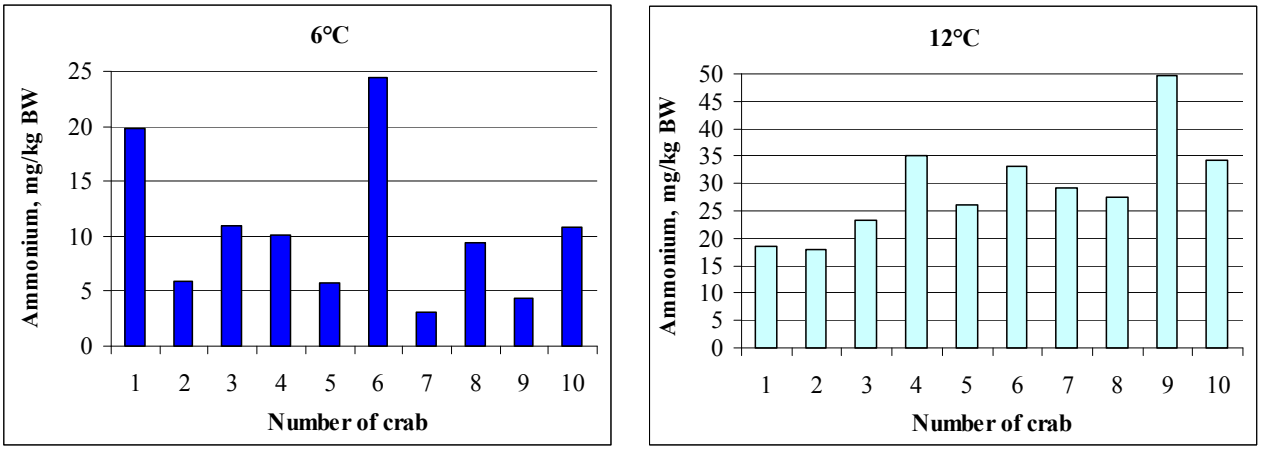

Fig. 3. Daily excretion of total ammonium by red king crab

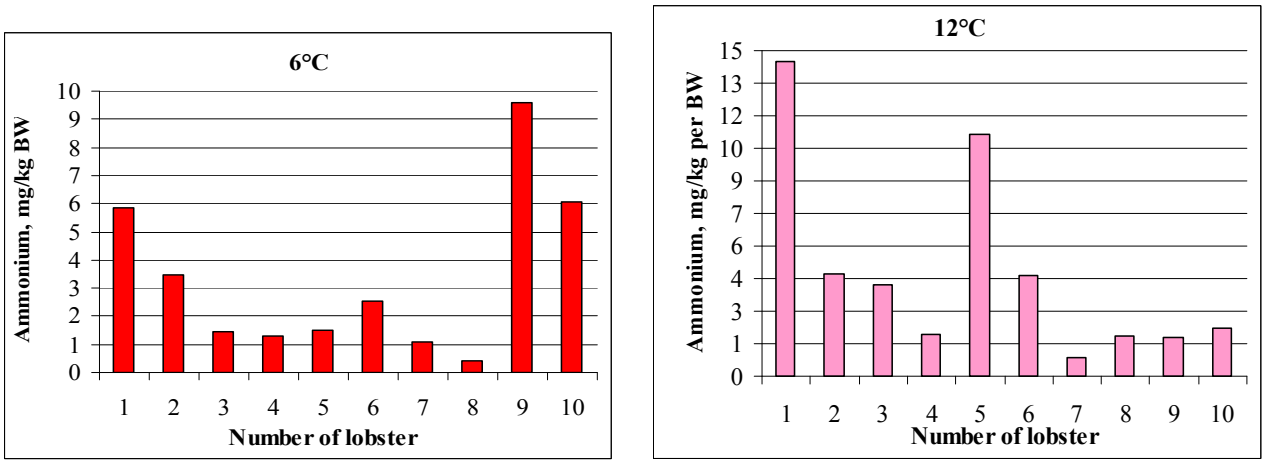

Fig. 4. Daily excretion of total ammonium by american lobster

At the water temperature 6°C american lobsters excreted on average 3.32 mg ammonium per 1 kg BW, and 4.63 mg/kg BW at 12°C respectively. A slight difference may indicate better adaptive ability of american lobster (it is known that lobster is much more stable to changes in water temperature than red king crab). Peaks of concentrations which are demonstrate on diagrams probably due to their different exposure after catching, since the storage complex was carried out continuously acceptance of new parties.

Conclusions

1. At the water temperature 6°C red king crab excreted on average 9.73 mg of total ammonium per kilogram of BW, and 29.16 mg at the water temperature 12°C respectively.

2. At the water temperature 6°C american lobster excreted on average 3.32 mg of total ammonium per kilogram of BW, and 4,63 mg at the water temperature 12°C respectively.

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SALTWATER MUSSELS (FAMILY MYTILIDAE) – PROSPECTIVE SOURCE OF HIGH-ACTIVE HYDROLYTIC ENZYMES

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An economically and environmentally pressing challenge of today is waste-free sustainable utilization of natural biological resources, a substantial part of which is extracted from the World Ocean. Researchers' attention to marine macro- and microorganisms remains high, since they are a source of physiologically active substances used in human and veterinary medicine, food industry, agriculture and other sectors. Marine flora and fauna supply substances employed in genetic engineering, as fungicides, immunity stimulators, anti-radiation products, diagnostic and health food preparations. Widely used alongside with antibiotics, toxins, pigments, polysaccharides, and proteins are enzymatic preparations, including hydrolases, which decompose natural biopolymers to easily digestible and metabolisable components. Particular focus is on proteolytic enzymes (Mukhin and Novikov, 2001). Little studied in terms of applied enzymology are glycosidases from marine organisms. Since hereditary deficit of acid glycosidases causes lysosomal storage disorders, such studies would promote development of means and methods to fight this disease.

The present study aimed to investigate the activity of lysosomal glycosidases in different organs of the bivalve *Mytilus edulis* L. Mussels are typical inhabitants of the littoral and sublittoral zones of the White Sea. Besides, they are widely used in mariculture, both in Russia and in many European countries.

The material for the study was collected at the Kartesh facility of the RAS Zoological Institute's White Sea Biological Station. Mussels *M. edulis* were captured from the sublittoral zone in the Chupa Bay, Gulf of Kandalaksha. Mussels of one size were placed in 16 litre aquaria with salt water, natural temperature and light regimes, and forced aeration. After the mussels had grown acclimated to laboratory conditions they were subjected to biochemical study. Mussel organs were frozen, taken to the laboratory and stored at – 80°C until analysis. The material was then homogenized in 0.25 M sucrose solution with 0.001 M EDTA and 0.1% triton X-100 detergent, pH 7.4. The tissue/sucrose ratio was 1:9. The homogenates were clarified by centrifugation in K-24 centrifuge at 12 000 rpm. Lysosomal enzyme activity and protein content were determined in the supernatant fluid using techniques adjusted to the study object (Vysotskaya and Nemova, 2008). β-glycosidase (EC 3.2.1.21) activity was determined using the sodium para-nitrophenyl-β, D-glucopyranoside substratum with 0.15 M citrate-phosphate buffer (pH 5.0). β-galactosydase (EC 3.2.1.23) activity was determined by its reaction with para-nitrophenyl-β, D-glucopyranoside with citrate buffer (pH 4.0).

Enzyme activity was calculated as μM of para-nitrophenol formed through the reaction per 1 g of wet weight of tissue per minute and per 1 mg of protein.

The study showed the activity of lysosomal glycosidases in organs of mussels from the White Sea to be quite high (Table). Notably high is the β-glycosidase activity in the mussels' digestive gland. We have never observed such high glycosidase activity in our studies of lysosomal enzyme activity in other aquatic organisms. Absolute values of acid glycosidase and galactosydase in the digestive gland of the mussels are an order of magnitude higher than in fish, even in lysosome-rich organs such as kidneys and liver (Vysotskaya and Nemova, 2008).

Table. Glycosidase activity in organs of the mussel *Mytilus edulis* (μM para-nitrophenol / g wet weight / min), n = 7

Organ	β-glycosidase	β-galactosidase
Digestive gland	1.48–1.57	0.474–0.640
Gills	0.022–0.041	0.123–0189
Foot	0.012–0.013	0.040–0.048

The data obtained have to do with specific characteristics of the biochemical organization of carbohydrate and energy metabolism in mussels inhabiting the tidal zone – one of the most stressful environments. Being exposed to frequent and abrupt alternations of ambient conditions, the enzyme systems of saltwater mussels have evolved to acquire a number of features (such as low sensitivity to changes in the microenvironment, high activity, thermal stability, etc.) maintaining their catalytic ability in a wide range of conditions. Lysosomal enzymes, including glycohydrolases, perform many physiological functions connected not only with substrate destruction but also with secretion processes and metabolism regulation (Vysotskaya and Nemova, 2008). Thus, glycosidases are involved in these processes as they hydrolyse glucosidic bonds and through transglycolysation reactions. Among glycohydrolases, acid β-glycosidase (human β-glucocerebrosidase) is the most common enzyme, present in all living organisms from bacteria to humans. It can be used in various fields of biotechnology: from enzyme replacement therapy to cellulolysis for renewable energy production (Turan, 2008). β-glucocerebrosidase deficiency leads to glucosylceramide storage in lysosomes and triggers the inherited disorder – Gaucher disease. Other lysosomal storage disorders, as well as neurodegenerative diseases, ageing processes and many pathological states are attributed to the deficiency of other acid glycosidases. Injections of the deficient enzymes are widely practiced in the therapy of human lysosomal storage diseases (Pupyshev, 2006). As a rule, preparations made of human cells are used. Research now focuses on finding cheaper sources of the enzymes, as well as on investigating the mechanisms to deliver the injected enzymes to the destination, i.e. lysosomes (Aerts et al., 2003; LeBowitz et al., 2004). In addition to the enzyme replacement therapy one is developing gene therapy methods based on most recent scientific findings (Pupyshev, 2006; Desnick, 2004). A prerequisite for success in this undertaking is comprehensive study of the properties and distinctive patterns of lysosomal glycosidase functioning in different taxa and different ecological circumstances (Barkalova and Yershova, 2009; Xie and Chen, 2004; Turan, 2008). A vivid example of the mussels' adaptation to the ambient conditions is the high activity of carbohydrate metabolism enzymes, including lysosomal glycosidases.

Thus, our studies demonstrated high β-glycosidase and β-galactosidase activity in the digestive gland of the White Sea mussels, wherefore this object is commendable as a source of acid glycosidases. The hydrolases can be used both in the research meant to reach a deeper understanding of these important lysosomal enzymes, and to address various applied tasks involving hydrolysis of glucosidic bonds.

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APPLYING TRANSCRIPTOME PROFILING TO EXPLORE THE QS-SYSTEMS IN THE FISH PATHOGEN *ALIIVIBRIO SALMONICIDA*

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Bacteria coordinate activities as a population, which likely provides a selective advantage in the natural environment by allowing them to alter their morphology and physiology quickly to adapt to environmental changes. In order to adapt, bacteria need a wide variety of mechanisms for sensing and responding to these changes. Recent work has clarified many aspects of how bacteria communicate and synchronize cell behaviour through an elegant process known as quorum sensing (QS). QS mediated by signal molecules, referred to as Autoinducers (AI), enable bacteria to control and synchronize behaviour such as motility, biofilm formation, virulence factor production and bioluminescence under different environmental conditions. Although some details of QS are known for a few model organisms, the understanding of the broader role of QS in gene regulation and the diversity of adaptive responses and how these responses are linked to virulence remains fragmented.

To address the lack of knowledge of the diversity of adaptive stress responses, such as intra- and inter-species communication, population-level cooperation, and the principles underlying signal transduction and information processing during infection *Aliivibrio salmonicida*, the causative agens of cold-water vibriosis has been used as a model system. The observed phenotypic variability due to gene knockouts in the sensing and responding systems (QS-systems), using transcriptome profiling (microarray), will be presented.

ACHIEVEMENTS AND PROSPECTS IN LONG-DISTANCE TRANSPORTATION OF LIVE RED KING CRAB *PARALITHODES CAMTSCHATICUS*

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Red King Crab (*Paralithodes camtschaticus*) is one of the most valuable and expensive seafood delicacies. It comes to the market in different forms: cooked-frozen legs, raw-frozen legs, boiled meat, etc... The most valuable product, providing the best preservation of food quality, is live red king crab. But there are some problems associated with operational live crab transportation from place of catch to the final consumer. Crabs habitats are far away from major economic centers and transportation hubs. That's the Sea of Okhotsk in Russia, where the catch of crab in the declining population has virtually closed, the coast of Alaska in the United States, the Barents Sea in Russia and northern Norway. Experiments show that crabs can be kept for a long time out of water and are transportable by air. But when transportation takes more then 24 hours, there is significant mortality. Works on developing new and improving existing methods of life crabs transportation are actual and are aimed at increase in duration of transport and reducing mortality.

Experiments were conducted on the land based water tank complex Norway King Crab (Byugoynes, Norway) and storage complex with closed recirculation water system (CRWS) – «La Maree» LTD. (Moscow, Russia). In addition, were analyzed the outcome of life crabs sending from the northern part of Norway to Belgium, France, Italy, Korea, Japan and China. Crabs for experiments were caught in the Varanger Fjord (Norway).

Polystyrene boxes with wet or dry material inside were used for crab's transportation. Low temperature maintained using frozen gel-ice.

Fig. 1. Polystyrene transport box with crabs

The monitoring of physiological condition was carried out using the method of noninvasive pulsometry. To read the heartbeat parameters and transmit information to the computer an optic fiber sensor was fastened on crab's carapace. As a result crab's heart rate and stress-index were recorded.

To establish the maximum crabs are still alive out of water, as well the impact of various parameters on the physiological condition of the crab, the experimental group was formed. Crabs from this group were subjected to simulate transport without water. Crabs from this group were put in same transport boxes with frozen gel-ice and different materials inside.

The influence effect of humidity level inside the container on the transport duration was studied on a group of 14 crabs. Half of them were put inside the transport boxes with wet foam. Other crabs were kept out of water for 5–10 minutes to remove moisture from the gill chamber and after that were set inside the boxes with dry material.

Crabs mortality data and general physical condition of the surviving animals after the transport from northern part of Norway to the cities of Europe and Asia was collected. For estimation of crabs activity after transportation visual census method was used.

Before and after transport the washings from crab's gills were carried and the hemolymph samples were taken for determination of the quantity of substances. Ammonia content in the washings was quantified by Sedghi- Solorzano Method.

In the proccess of experiments it was found that crabs can stay alive in polystyrene containers without water for up to 81 hours at a temperature up to 7 degrees. The heart rate of four different crabs presented in Figure 2.

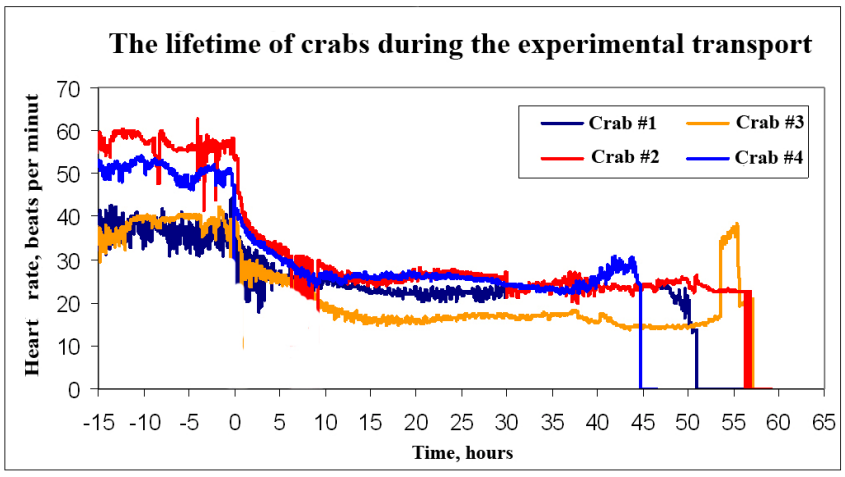

Fig. 2. The lifetime of crabs during the experimental transport determined through monitoring of cardiac activity

Using dry material inside containers and pre-transportation exposure of crabs out of water show significantly not worse results than the traditionally used method where the crabs are covered by wet foam ore paper. General results presented in the Table1.

Table1. The results of crab's life expectancy in boxes with wet and dry material

	Wet material	Dry material
Number of crabs	7	7
Minimum lifetime, hours	27	41
Maximum lifetime, hours	59	61
Average lifetime, hours	43	53
Standard deviation, ± hours	10	8,3

Consequently average lifetime was 43 hours for crabs in wet boxes and 53 hours for dry transport.

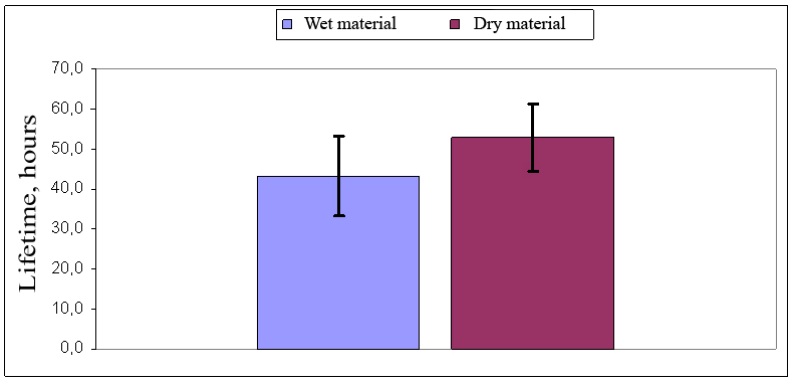

Fig. 3. The average lifetime of crabs during the dry and wet transport

It was established that the minimum mortality of crabs observed when the duration of transport was up to 36 hours.

A correlation between the average crabs mortality during the transportation, pre-transportation storage period and the season was found. When the transportation time was up to 36 hours and crabs were kept in tanks with sea water for one week, mortality was 2.5%. When crabs overexposure lasted less than 2 days – mortality was 10%. In autumn and winter, when air and water temperature is low, mortality does not exceed 3%. In warm season when temperature is high mortality of crabs increases sharply and can reach 20% during transport up to 24 hours and 50% during longer transport.

The high content of NH_4^+ ions in the washings from crab's gills after transportation was found. The content of ammonia on the gills after 36-hour staying out of water was 8–14 mg/kg body weight.

Transportation of crabs in containers without water is the most efficient and cost-effective way to deliver live crabs to consumers. This method does not require complicated technical devices. For reducing mortality and increasing transport duration have to use some innovations. Thus we have proved that high humidity inside the container is not a factor in the success of transportation. An obvious necessity of overexposure crabs in the tanks or cages without food for removing metabolic products. Influence of season on the success of transport is very high. An important reason of high mortality in summertime is not only high temperature but also weakened crabs condition during first 3–5 months after molting in January – April. Starting from September mortality after transport up to 24 hours is significantly reduced compared with the summer period. After the molting, calcium in crabs shell is not enough, and it becomes more fragile and in addition, falling meat content in legs, which reduces the viability of individuals in extreme conditions. Requires further study the possibility of reducing mortality by using materials absorbent ammonium inside the containers. Deceleration of metabolic processes in crabs by reducing the water temperature before transportation can be effective.

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